Germ Stem Cell Literatures

Mark H Smith

Queens, New York 11418, USA
mark20082009@gmail.com

Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on germ stem cell.
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Abbreviations: ESC, embryonic stem cell; MSC, mesenchymal stem cell; PGC, primordial germ cell; SC, stem cell; SSC, somatic stem cell

1. Introduction

Life is a physical and chemical process (Hongbao Ma 2005). To the life, 2 points are most important: live and die. Conventionally, everybody of us thinks that all the life has a beginning as the birth and the end as the die. All plants and animals, including all the people must die. It is the stem cell (SC) gives the hope for life to maximum extend the living time arrangement and finally target the eternal (Ma Hongbao, 2009).

The definition of SC is an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell. Embryonic stem cell (ESC) is derived from the inner cell mass of blastocyst stage embryos. Somatic stem cell (SSC) is generally believed to differentiate only into cells characteristic of the tissue wherein they reside. SC is the original of life. All cells come from SCs (Hongbao Ma, 2005).

SCs are undifferentiated cells that have the potential to self-replicate and differentiate into all kinds of cells, which are unique in their ability to undergo both self-renewal and differentiation. (Lester et al., 2004). ESCs are derived from the inner cell mass of the preimplantation embryo and retain their pluripotency. SCs have the capacity to develop into any cell type of somatic ectodermal, mesodermal, or endodermal lineages. ESCs can develop into the germ cell. The studies of germ cell development in culture provide unprecedented opportunities to understand the basis of the propagation of life. Advances in SC research have opened new perspectives for regenerative and reproductive medicine and the life sciences (Weissman, 2005).

Human mesenchymal stem cell (MSC) isolated from bone marrow stroma, peripheral blood, dermis, muscle and adipose tissue have the advantage of potential autologous transplantation ability. They can be differentiated into chondrogenic, osteogenic, adipogenic and myogenic lineages. The isolation of MSCs from adipose tissue can be as an alternative source. Adipose tissue is derived from embryonic mesoderm and contains a heterogeneous stromal cell population. Inductions of the cells into multiple mesenchymal lineages resulted in the expression of several lineage-specific genes, proteins and specific metabolic activity. The potential benefit of the multi-germline capacity of human MSCs seems to be a promising approach for allogenic cell therapy and human tissue engineering (Pansky et al., 2007). SCs can be obtained from the embryo at cleavage or blastocyst stages, but also from extra-embryonic tissues such as the umbilical cord blood obtained at birth, the placenta and the amniotic fluid. SC can also be obtained in adult body. These somatic SCs can be found in a wide range of tissues including bone marrow, blood fat, skin and also testis. Human umbilical cord blood is an excellent primitive source of noncontroversial SCs for clinical applications. It was reported that in situ characterization to identify and localize a panel of some markers expressed by mesenchymal stromal cells (CD44, CD105, CD73, CD90) and CD146 in UC (Schugar et al., 2009). The predominant expression by UC-MSCs of genes engaged in the osteogenic differentiation and their tendency to differentiate into osteoblasts (Ciavarella et al., 2009). ESCs exposed to appropriate and specific conditions differentiate into cell types of all three

germ layers and also into germline cells (Iwayama et al., 2005).

In males, sperm are produced continuously during the adult life. Male germ cells, the repository cells of the genome, comprise several successive developmental stages starting in the embryo and ending up with the spermatozoon. Gonocytes represent the fetal and neonatal stages preceding the formation of spermatogonial SCs. The option of storing mature sperm prior to treatment is a common practice (Ahringer and Kimble, 1991; Culty, 2009). SCs can be obtained from the embryo at cleavage or blastocyst stages, but also from extra-embryonic tissues such as the umbilical cord blood obtained at birth, the placenta and the amniotic fluid. SC can also be obtained in adult mammals from specific niches. These somatic SCs can be found in a wide range of tissues including bone marrow, blood, fat, skin and also the testis. The differentiated somatic cells could be reprogrammed into pluripotent state. Consequently, the reprogrammed somatic cells recapitulate the capacity to differentiate into specific cell lineages under appropriate culture conditions (Huang et al., 2009).

There are two pathways for germ cell fate: (1) preformation, to be embryonic cells that inherit maternal determinants from the egg go on to form the germ cell lineage; (2) epigenesis, pluripotent cells formed early in development are induced by signals from adjacent tissues to form the germ cell. The germ cell is the only lineage that makes the genetic information across the generations in most multicellular organisms perpetuation. Germ cell produces primordial germ cell (PGC) and PGS becomes the oocytes and spermatozoa. In non-mammalian species of fruitflies, nematodes and frogs, germ cells of both males and females are specified via the inheritance of germ plasm. Specification of germ cell fate is vital to development and heredity. In mice, germ cell fate is induced in a subset of pluripotent epiblast cells during early gastrulation. Reflecting the function of the germ line as the transducer of genetic information, germ cell specification integrates at least three key events: repression of the somatic program, re-acquisition of potential pluripotency, and ensuing genome-wide epigenetic reprogramming (Saitou, 2009).

Consistent with the function of germ cell lineage as the transducer of genetic information, PGC specification is an integration of at least 3 key events: (1) repression of the somatic program; (2) re-acquisition of potential pluripotency; (3) an ensuing genome-wide epigenetic reprogramming. More detailed knowledge of the mechanism of PGC specification will provide a critical foundation for induction of the germ cell lineage from pluripotent stem cells *in vitro* not only in mice but also in other mammals, including humans. Immature sperm cells derived from ESCs in culture could generate live offspring. Nuclear transfer-derived SCs may also be selected and directed into differentiation pathways leading to the production of specific cell types, tissues and, eventually, even organs for research and transplantation (Tecirlioglu and Trounson, 2007). The ESC technology offers great potential for new types of reproductive investigations including epigenetic modifications of the germline (Moore and Aflatoonian, 2007).

According to the World Health Organization, infertility is considered a disease affecting millions of people in Europe, where prevalence is 14% of couples in reproductive age. Based on the 2005 National Survey on Family Growth American report, there was a 20% increase in American couples experiencing impaired fecundity between 1995 and 2002. This may be related to a tendency to delay motherhood to the third decade of life due to professional and social reasons. As a consequence, oocyte quality in females is reduced. This article summarizes the current knowledge of germ cells.

2. General Description of Germ Stem Cells

Germ cells carry the genome onto the next generation. In females, the gonocyte surrounded by a cortical interstitial layer initiates meiosis and becomes a primary oocyte and follicle, thereby ending precursor proliferative potential. In males, the gonocyte surrounded by the fetal sex cord of the gonadal ridge arrests in G0/G1 of mitosis as a prospermatogonium, but retains a proliferative precursor potential. After birth, prospematogonia migrate to the basement membrane of the seminiferous tubule and differentiate into spermatogonial tem cells. Like adult SCs, SSCs can both self-renew and provide daughter cells, which differentiate into one or more terminal cell types. In the testis, the continuous production of sperm is maintained by a small population of SCs (Wei Leng, 2006). Fragilis is a transmembrane protein and part of a larger interferon-inducible family of genes that is evolutionarily conserved and has human homologues. Interferon-inducible proteins such as fragilis have an anti-proliferative function and may serve to increase the length of the cell cycle in PGCs. The genes fragilis and stella have key roles in germ cell competency and development (Lange et al., 2008). Expression of some of these genes is wide spread in a variety of mouse immune tissues while others appear to be much more restricted (Smith et al., 2006).

Mammalian development commences with the totipotent zygote which is capable of developing into all the specialized cells that make up the adult

animal. As development unfolds, cells of the early embryo proliferate and differentiate into the first two lineages, the pluripotent inner cell mass and the trophectoderm. Pluripotent cells can be isolated, adapted and propagated indefinitely in vitro in an undifferentiated state as ESCs. ESCs retain their ability to differentiate into cells representing the three major germ layers: endoderm, mesoderm or ectoderm or any of the 200+ cell types present in the adult body. Since many human diseases result from defects in a single cell type, pluripotent human ESCs represent an unlimited source of any cell or tissue type for replacement therapy thus providing a possible cure for many devastating conditions. Pluripotent cells resembling ESCs can also be derived experimentally by the nuclear reprogramming of somatic cells. Reprogrammed somatic cells may have an even more important role in cell replacement therapies since the patient's own somatic cells can be used for reprogramming thereby eliminating immune based rejection of transplanted cells. ESCs are pluripotent and they differentiate along somatic cell lineages. ESCs are totipotent as they can spontaneously generate trophoblast and trophoblast-SCs. ESCs can form germ cells which have a totipotent potential (Mitalipov and Wolf, 2009). Regenerative medicine and tissue engineering are searching for a novel SC based therapeutic strategy that will allow for efficient treatment or even potential replacement of damaged organs. The pluripotent stem cell, which gives rise to cells from all three germ lineages, seems to be the most ideal candidate for such therapies. Pluripotent SC could be extracted from developing embryos. However, since this source of SCs for potential therapeutic purposes remains controversial, SC researchers look for pluripotent SC that could be isolated from the adult tissues or generated from already differentiated cells. True pluripotent SC should possess both potential for multilineage differentiation in vitro and, more importantly, also be able to complement in vivo blastocyst development (Ratajczak et al., 2008).

During human ESC culture, markers of female germ cells are expressed in both XX and XY cell lines. Both male and female ESC lines to display female germ cell markers, since culture conditions may be sub-optimal and lack meiosis inhibition. When human ESCs are cultured the process of sex determination seems even more dysregulated as markers of both male and female germ cell development have been detected regardless of the sex of the cell line. Testis, for example, displays no intrinsic circadian rhythmicity and the molecular mechanisms of clock gene activation in male germ cells appear to differ from other tissues. Germ cells may develop according to an intrinsic clock (Cermakian et al., 2003). In 2007, Kucia et al identified a population of CXCR4(+) lin(-) CD45(-) cells that express SSEA, Oct-4 and Nanog in adult bone marrow. These cells are very small and display several features typical for primary ESCs such as: i) a large nuclei surrounded by a narrow rim of cytoplasm; ii) open-type chromatin; and iii) high telomerase activity. Germ lineage, in order to pass genes on to the next generation, has to create soma and thus becomes a 'mother lineage' for all somatic cell lineages present in the adult body. Germ potential is established after conception in a totipotent zygote and retained subsequently during development in blastomers of morula, cells form the inner cell mass of blastocyst, epiblast and population of PGCs. The authors envision that VSEL-SC are epiblast-derived pluripotent SCs and could potentially become a less-controversial source of SCs for regeneration (Kucia et al., 2007).

3. Endogenous Oocyte Development

Totipotent SCs have the potential to differentiate into every cell type. Renewal of totipotent SCs in the germline and cellular differentiation during early embryogenesis rely upon posttranscriptional regulatory mechanisms (Pagano et al., 2009). The mammalian germline is specified through inductive signaling during gastrulation. Mammalian germ cells lack definitive germ plasm but still contain homologs of germ plasm components such as *Nanos* and *Pumilio*. These components, in addition to epigenetic regulation, are thought to similarly suppress somatic development and activate germ cell-specific programs. In nonmammalian species, the germline is specified through germ plasm inheritance from the maternal oocyte just after fertilization (Saitou et al., 2003). A hallmark of germline cells throughout the animal kingdom is their ability to execute meiosis (Mukai et al., 2007). Mammalian germ cells have additional mechanisms to preserve germ cell identity in the midst of somatic cell embryonic development (Pagano et al., 2009).

After specification, PGCs migrate from their extragonadal origin to the developing gonad, or genital ridge, where they interact with gonadal somatic cells that will later determine their commitment to a sex-specific developmental program and maturation into functional gametes. It is a central dogma in reproductive biology that oogenesis is completed before or just after birth and that the postnatal ovary is endowed by a fixed and non-renewing number of oocytes in mammals (Oktem and Oktay, 2009).

Germ cells in the fetal testis are protected from retinoic acid exposure and are inhibited from entering meiosis due to factors expressed by somatic cells in the fetal testis. Male germ cells do not enter

meiosis until puberty. In the developing ovary, oocytes are required for follicle formation, and an absence of oocytes results in an absence of follicles. The lack of meiotic oocytes in the ovary can result in ovarian sex reversal, the formation of testicular-like tubules, and differentiation of Sertoli-like cells from granulosa cells. Meiotic oocytes are required to direct ovarian follicular maturation. Pluripotent cells can be isolated from the mammalian inner cell mass of the embryo at the blastocyst stage, and maintained in culture as undifferentiated. During normal embryo development, PGCs migrate into the aorta-gonad-mesonephros region (De Miguel et al., 2009). Apoptosis is prominent during follicular endowment, and apoptosis regulatory genes are involved in its regulation (Greenfeld et al., 2007).

To accomplish its future fate as an oocyte or sperm, a developing germ cell must erase the epigenetic program obtained in early embryogenesis, remove the genomic imprints inherited from the previous generation, and reestablish parental imprints in a sex-specific manner during spermatogenesis or oogenesis. This erasure and remodeling of epigenetic marks is termed epigenetic reprogramming and refers to DNA modifications that do not alter the sequence of DNA. Three main types of epigenetic mechanisms are known to exist and include DNA methylation, histone modification, and RNA-mediated silencing. Epigenetic information undergoes extensive reprogramming in the germline between generations (Katz et al., 2009).

DNA methylation is a vital epigenetic mark that participates in establishing and maintaining chromatin structures and regulates gene transcription during mammalian development and cellular differentiation. Differences in epigenetic patterns between individuals may contribute to phenotypic variation and disease susceptibility. Of the different epigenetic mechanisms, DNA methylation is the most widely studied and best-characterized epigenetic modulator. The genomic context determines the developmentally regulated epigenetic status at most nonimprinted regions of mammalian genomes (Schilling et al., 2009). Two waves of erasure and establishment of DNA methylation patterns occur during early embryogenesis. At the morula stage of development, the methylation of DNA is almost completely erased, with the exception of maternal and paternal DNA imprints. DNA methylation is subsequently reestablished during a period of *de novo* methylation, which takes place at the time of implantation. DNA methylation could indicate preferred sites for recombination, or methylation following recombination could inhibit further recombination, perhaps by being part of the enigmatic molecular pathway mediating crossover interference (Sigurdsson et al., 2009).

Histones are the main protein components of chromatin and are comprised of two classes, core histones and linker histones. Core histones are unique in that they have the capacity to undergo posttranslational modifications, including methylation, acetylation, phosphorylation, ubiquitination, sumoylation, citrullination and ADP-ribosylation (Lin et al., 2009). Although chromatin remodeling in female germ cells is not as dramatic as the DNA-protein structure changes that occur during the final stages of male germ cell development, certain histone modifications are known to be essential for achieving meiotic and developmental competency in the oocyte. Although phosphorylation is important for histone regulation during spermatogenesis, acetylation appears to be the predominant type of histone modification in oogenesis. Post-translational modification of chromatin is emerging as an increasingly important regulator of chromosomal processes. In particular, histone lysine and arginine methylation play important roles in regulating transcription, maintaining genomic integrity, and contributing to epigenetic memory (Ng et al., 2009).

Methylation of DNA might occur in a sequence-specific manner. Many organisms have a mechanism for down regulating the expression of non-synapsed chromosomes and chromosomal regions during meiosis (She et al., 2009; Sugimoto et al., 2007). Significant evidences have brought new insights on the mechanisms by which epigenetic machinery proteins regulate gene expression, leading to a redefinition of chromatin regulation in terms of modification of core histones, DNA methylation, RNA-mediated silencing pathways, action of methylation-dependent sensitive insulators and Polycomb/Trithorax group proteins. The possibility of reversing epigenetic marks, in contrast to genetic code, may provide new pharmacological targets for emerging therapeutic intervention (Santos-Reboucas and Pimentel, 2007).

Centrosomes had been discovered in germ cells and germ cells continue to provide excellent but also challenging material in which to study complex centrosomal dynamics. Asymmetric centrosome dynamics are also critical for SC division to maintain one daughter cell as a SC while the other daughter cell undergoes centrosome growth in preparation for differentiation (Schatten and Sun, 2009). Mammalian oogenesis is characterized by three main developmental stages: the initiation of meiosis in the fetus, follicle formation during the perinatal period, and oocyte growth and maturation in the adult (Kimble and Crittenden, 2007). During the development of multicellular organisms, the processes of growth and differentiation are kept in balance to generate and maintain tissues and organs of the

correct size, shape and cellular composition. The primitive oocytes begin to undergo atresia shortly after their entry into meiosis (Crittenden et al., 2003).

Oocytes secrete soluble factors that regulate the growth and differentiation of follicular cells, including maintenance of the distinctive cumulus cell phenotype (Gilchrist et al., 2001). Oogenesis is a complex process that is tightly regulated by both endocrine and locally produced factors. From follicle formation to ovulation, extensive cross-talk between the arrested meiotic oocyte and the granulosa cells is required for proper oogenesis. The oocyte directs granulosa cell proliferation, whereas the granulosa cells regulate oocyte maturation. During fetal development, the oocytes develop in groups, or cysts, and are connected, as in spermatogenesis, by intercellular bridges. The intercellular bridges enable the sharing of RNAs and proteins to facilitate oocyte maturation. The oocyte cysts are then surrounded by pregranulosa somatic cells during meiotic prophase and later break down at birth in mice. This breakdown allows for the infiltration of pregranulosa cells and formation of primordial follicles consisting of a single oocyte surrounded by a single layer of flattened granulosa cells. TNF α signaling is thought to be important for cyst break down and primordial follicle assembly, whereas estrogen and progesterone inhibit follicle assembly (Sun et al., 2008). The steroidogenic enzymes expressed in theca cells convert cholesterol into androgen, and androgen is then used by granulosa cells expressing aromatase to produce estrogen. Oocytes reach maturity during the antral follicle stage, and mature mouse oocytes of approximately 70–80 μm in size are capable of resuming meiosis upon ovulation. Although follicle-like aggregates expressing steroidogenic enzymes and producing estrogen have been generated from differentiating ESCs, the characterization of stepwise primordial to antral follicle maturation has not been reported (Kolle et al., 2007). The gap junction proteins connexin37 and connexin43 are required for ovarian folliculogenesis in the mouse. The oocytes do not need to express connexin43 in order to develop into meiotically competent, fertilizable gametes, but must express connexin37 for communication with granulosa cells, a requirement for oogenesis (Gittens and Kidder, 2005).

Prenatal oogenesis produces hundreds of thousands of oocytes, most of which are discarded through apoptosis before birth (Cushman et al., 2000; Hartshorne et al., 2009). It is possible that the recruitment signal may actually originate from the oocyte itself. The newborn ovary contains only a small fraction of the total oocytes that entered meiosis in the fetal ovary (Anderson et al., 2007). It is the remaining sister chromatids, together with the chromosomes contributed by the sperm that form a zygote. homologous chromosomes pair very efficiently, but the high frequency of complete, premature homologue separation found at diplotene suggests that mechanisms other than the pairing process could be more likely to lead to the high aneuploidy rate observed in human oocytes (Roig et al., 2005).

Spermatogonial SC ensures continuous production of mammalian male gametes. In rodents, the SSC are Asingle spermatogonia (Grisanti et al., 2009). It appears that precommitted and premeiotic fetal ovarian germ cells are unable to completely mature *in vitro* and require transplantation to produce mature oocytes (Nagano, 2007).

Pluripotent SCs are of paramount therapeutic interest, since they could be utilized: as in vitro models of disease, for pharmaceutical screening purposes, and for the regeneration of damaged organs. Over the years, pluripotent cells have been cultured from teratomas, the inner cell mass, and PGCs. Accumulating informations have partially decrypted the molecular machinery responsible for the maintenance of a very primitive state, permitting the reprogramming of differentiated cells. Although the debate is still open, an extreme excitement is arising from two strictly related possibilities: pluripotent cells could be obtained from adult tissues with minimal manipulations or very rare pluripotent cells could be identified in adult tissues (Beltrami et al., 2009). Oocytes can be fertilized to produce offspring, form teratomas in humans and mice after parthenogenetic activation, and reprogram terminally differentiated somatic nuclei after somatic cell nuclear transfer. Mature gametes are ultimately totipotent, but nascent PGCs require reprogramming during germ cell development to regain pluri/totipotency. It appears that postspecification mammalian germ cells may possess the potential to be artificially reprogrammed throughout the entirety of germ cell development until the naturally reprogrammed totipotent state of mature gametes is restored (Feng and Chen, 2009; Korkola et al., 2009; Mitalipov and Wolf, 2009).

4. Oocyte Differentiation from ESCs

The isolation of human ESC in 1998 has created the hope that SCs will one day be used to regenerate tissues and organs, even though it is obvious that a number of hurdles will need to be overcome for such therapies to become reality. The cloning of "Dolly" in 1997, more than 40 years after the first frogs were cloned, combined with the very fast progress made in our understanding of the molecular processes that govern the pluripotency of ESC has lead to the ability of scientists to recreate a pluripotent state in fibroblasts and other cells from

mouse, rat and man, named induced pluripotent SC (Verfaillie, 2009).

Global gene expression profiling has shown human ESC lines to be distinct from the inner cell mass. In fact, human ESC lines may be more closely related to epiblast because they are similar to mouse epiblast-derived SCs, and human ESCs may have different germ cell developmental potential than mouse ESCs (Machaca, 2007; Theurkauf, 1997).

The therapeutic potential for human ESC drives intense public and scientific interest (Gavrilov et al., 2009). The clinical utility of SC-derived oocytes would require autologous SCs for the treatment of non-germ cell autonomous causes of infertility and a combination of this with gene therapy for germ cell autonomous causes. Additionally, oocyte differentiation from autologous SCs would be useful for drug screening and toxicity testing. In contrast to men who have testicular spermatogonial SCs into adulthood that can yield pluripotent autologous SCs, it is thought that women do not have functional adult germline SCs as evidenced by oocyte depletion and limited reproductive potential. This dogma was recently challenged, but the existence of functional adult female mouse or human germline SCs in the bone marrow or peripheral blood remains controversial (Fu et al., 2009; Gavrilov et al., 2009; Hough et al., 2009; Smith and Wagner, 2009).

Pluripotency can be artificially maintained *in vitro* by culturing mouse ESCs with human ESCs. The artificial nature of this system poses a significant challenge for generating germ cells considering that endogenous PGCs may be reprogrammed when removed from their somatic niche *in vivo* and cultured *in vitro* on feeders with similar factors (Elkabetz and Studer, 2008; Rippon et al., 2008; Verfaillie, 2009).

The integration of X-inactivation with development is a crucial aspect of this classical paradigm of epigenetic regulation. During early female mouse development, X-inactivation reprogramming occurs in pluripotent cells of the inner cell mass of the blastocyst and in pluripotent PGCs (Do and Scholer, 2009; Navarro and Avner, 2009).

Application of embryonic and adult SCs in regenerative medicine will require efficient protocols for directing SC differentiation into well-defined lineages. The use of exogenous cytokines, growth factors, or extracellular matrix substratum, will obviously require extended durations of in vitro culture. With autologous adult SCs, this could delay transplantation to the patient, as well as alter the immunogenicity of the cultured autologous cells. Genetic modulation to direct SC differentiation would obviate prolonged durations of in vitro culture; but there are overwhelming safety concerns with regards to the application of recombinant DNA technology in human clinical therapy (Heng and Cao, 2005). SCs contain an intrinsically predefined, unidirectional differentiation program. This means that the developmental fate of a SC is dependent on the general potential of the cell as well as on microenvironmental cues, such as stimuli from growth factors (Banas et al., 2007; Bruserud et al., 2000; Piller, 2009; Schaeffer et al., 2009).

Undifferentiated ESCs grow as colonies with defined borders and are compact cells with prominent nucleoli. ESC differentiation is routinely achieved by withdrawing the pluripotency-maintaining factors from the culture media, removing the feeder layer, and allowing the ESCs to spontaneously differentiate into a heterogeneous mixture of cell types representing the three somatic germ layers and the germline. ESCs are primarily differentiated as either embryoid bodies in suspension, using hanging drops or untreated low-attachment plates, or as adherent monolayer cultures (Ko and Scholer, 2006; Saiti and Lacham-Kaplan, 2007). Spontaneous differentiation of ESCs into a diverse mixture of cell types is poorly understood and is most likely related to the unknown mixture of factors in the serum and media. To help direct the differentiation of a specific cell type, factors can be added to the differentiation media, with or without serum, to generate more homogeneous cultures (Hubner et al., 2006; Ko and Scholer, 2006; Nicholas et al., 2009; Sasaki et al., 2009; Vadivelu et al., 2005).

Germ cells were originally identified in human ESC-derived cultures by assessing the expression of germ cell-specific markers during differentiation (Brevini et al., 2007; Fadare and Zheng, 2009; Matsumoto et al., 2009; Walakira et al., 2008).

ESC technology should enable the generation of specific cell types for the study and treatment of human diseases. Therapeutic cloning provides a way to generate ESCs genetically matched to diseased individuals through nuclear reprogramming of the somatic genome. An alternative approach to reprogram the somatic genome involves fusion between somatic and pluripotent cells. Potential fusion partners with reprogramming activities include embryonal carcinoma cells, embryonic germ cells, and ESCs (Pralong et al., 2006). Cells isolated from both the embryoid bodies and genital ridge positive controls expressed germ cell markers and are viable, suggesting that density gradient isolation might represent a feasible and clinically relevant approach for obtaining ESC-derived germ cells (Brevini et al., 2009; Lin et al., 2007; Mise et al., 2008; Nicholas et al., 2009).

ESCs, derivatives of totipotential cells of early mammalian embryos, have proven to be one of the most powerful tools for studying developmental

and SC biology. When injected into embryos, ESCs can contribute to tissues derived from all three germ layers and to the germ line (Hubner et al., 2006).

Many challenges need to be overcome to achieve robust and functional oocyte differentiation from ESCs. The low efficiencies of ESC-derived oocyte-like cell maturation reported to date may be unavoidable because most endogenous oocytes undergo atresia during development. However, the optimization of methods to direct ESC-derived germ cell specification, oocyte commitment, and oocyte maturation may increase efficiencies and enable functional oocyte production. After endogenous germ cell specification in the early embryo, germ cells are set aside from the embryo proper and are isolated from initial somatic cell differentiation. Considering that meiotic progression to diplotene arrest may be required for subsequent follicle formation, it will be of utmost importance to optimize ESC-derived germ cell specification and/or oocyte commitment protocols to overcome the current obstacles to proper meiotic entry and progression during ESC differentiation (Mendenhall et al., 2001; Simonian, 2003).

Given the success of ESC-derived sperm and endogenous PGC maturation via transplantation and the particular importance of ovarian somatic cells in supporting endogenous oocyte development, appropriately staged ovarian granulosa cells may be required for the maturation of committed ESC-derived oocytes using coculture and/or transplantation methods (Hsieh et al., 2009; Kato et al., 2009; Lenartowicz et al., 2008; Nicholas et al., 2009; Whitten and Miller, 2007).

5. PGC Description

PGCs in mice have been shown to originate from some of the most proximal epiblast cells, to become identifiable during early gastrulation by their characteristic alkaline phosphatase activity. (de Las Heras et al., 2009; Houston and King, 2000; Rangan et al., 2009; Tani et al., ; Yabuta et al., 2006).

Most notably, PRDM1 has been found to be one of the most critical regulators for PGC specification. In the absence of PRDM1 activity (Kurimoto et al., 2008; Yabuta et al., 2006; Yamano et al.).

Specification of germ cell fate is fundamental in development and heredity. Recent evidence indicates that in mice, specification of PGC), the common source of both oocytes and spermatozoa, occurs through the integration of three key events: repression of the somatic program, reacquisition of potential pluripotency and ensuing genome-wide epigenetic reprogramming. Here we provide genetic evidence that Prdm14, a PR domain-containing transcriptional regulator with exclusive expression in the germ cell lineage and pluripotent cell lines, is critical in two of these events, the reacquisition of potential pluripotency and successful epigenetic reprogramming (Yamaji et al., 2008). Germ cell specification in mice, which generates PGCs, the common source of the oocytes and spermatozoa, from the epiblast, integrates three key events: repression of the somatic program, re-acquisition of potential pluripotency, and genome-wide epigenetic reprogramming The launch of the germ cell lineage in mice, therefore, is orchestrated by two independently acquired, PR domain-containing transcriptional regulators (Kurimoto et al., 2008). PGCs have been shown to undergo extensive epigenetic reprogramming, including genome-wide DNA demethylation, erasure of parental imprints, and re-activation of the inactive X-chromosome (Hayashi and Surani, 2009, Chuva de Sousa Lopes et al., 2008; Hayashi and Surani, 2009; Yabuta et al., 2006).

The formation of germ cells during embryogenesis bears the ultimate importance for the continuation of every species. It becomes evident that mechanisms governing germ cell fate specification are not well conserved across the animal kingdom. In most of the invertebrate and nonmammalian vertebrate species, certain maternally derived factors are key to the establishment of germ cell lineage. In contrast, mouse PGCs are induced from the pluripotent epiblast cells before and during gastrulation by the extraembryonic cell-derived signals (Ying et al., 2002). There have been several reports regarding the derivation of gametes from ES cells in culture (Fukunaga et al., ; Hiller et al., ; Yabuta et al., 2006).

In the last decade of intensive research, a major part of the framework for the signaling and transcription in PGC specification in mice has been established (Kimura and Brenner, 1997; Mistry and Cresci, ; Soodeen-Karamath and Gibbins, 2001).

6. Related Techniques

Mammalian spermatogenesis consists of three biologically significant processes: SC self-renewal and differentiation, meiosis, and haploid cell morphogenesis. Understanding the molecular mechanisms behind these processes might provide clues to the puzzle of species preservation and evolution, and to treatments for male infertility. However, few useful in vitro systems exist to investigate these processes at present. To elucidate these mechanisms, in vivo electroporation of the testis might be a convenient option (Bowles et al., ; Kanatsu-Shinohara et al., 2004; Yomgogida, 2008).

(1) Specification of germ cells in mammals

Germ cells are the biological route for genetic transmission from one generation to the next.

These cells constitute a very different cell population from somatic cells, with unique characteristics, and display a haploid chromosomal number after a delicate process of meiosis. PGCs in the female embryo do not complete meiosis, and are arrested at prophase I of the first meiotic cycle. Progress in meiosis occurs post-natally just prior to ovulation followed by another arrest in meiosis II, which is completed just after fertilization (Morimoto et al., 2009; Park et al., 2009; Van Haute et al., 2009; Yang et al., 2009).

(2) *In vitro* germ cells differentiation from ESCs

ESCs represent permanent cell lines that can be maintained in an undifferentiated state. In an environment that induces differentiation, they form derivatives of the three embryonic germ layers: mesoderm, ectoderm, and endoderm. The pups showed growth abnormalities, and most died during the first months of life, emphasizing the crucial concept that imprinting status of the derived gametes from SCs must be carefully studied (Aflatoonian et al., 2009; Teramura et al., 2009; Zovoilis et al., 2009). Taking a step back, scientists need to reconsider their strategies when related to *in vitro* gametogenesis by looking at the *in vivo* processes. During murine and human ESCs *in vitro* differentiation, markers of both male and female germ cells have been detected in culture regardless of the sex of the cell line. The cells may be responding to an external signal instead to an intrinsic program to enter meiosis. It is possible that retinoic acid present in the culture conditions propitiate meiosis commencement and gamete determination. (Hwang et al., 2008; Micallef et al., 2007; Vaags et al., 2009). Attempts to derive germ cells from human ES resulted in similar findings as described in mice (Lin et al., 2007; Mai et al., 2007; Teramura et al., 2007; Wianny et al., 2008).

(3) *In vitro* germ cells differentiation from somatic SCs

Spermatogenesis is a complex process that cannot be modeled in vitro. The somatic Sertoli cells within the seminiferous tubules perform a key role in supporting maturation of germ cells. intra-testicular injection of adenoviral vectors disturbs SC function in vivo and future work will therefore focus on the use of lentiviral delivery systems (Hooley et al., 2009). Germline SCs and mature gametes can be derived *in vitro* from multipotent SCs other than ESCs. In mice, teratocarcinoma and MSC and induced iPS are considered multipotent cells, which are able to differentiate into various cell types representative of all three germ layers. With the exception of iPS cells, the other cell types have been shown to differentiate into either germline SCs or early germ cells *in vitro* and to advanced male gametes *in vivo* following transplantation. Embryonal carcinoma cells derived from teratocarcinomas, like ESCs, are pluripotent and able to differentiate through embryoid bodies to germline SCs. When transplanted into sterile mice, EC-derived germline SCs are able to complete meiosis and spermatogenesis (Marques-Mari et al., 2009).

(4) Demanding biological descent: how to obtain patient-specific gametes

The growing demand for biological offspring for patients with impaired fertility has put its hope in scientific research and the obtaining of patient-specific differentiated gametes. Nuclear transfer can be defined as the creation of somatic embryos using the oocyte cytoplasm as the reprogramming conductor following transfer of somatic nuclei into enucleated mature oocytes (Galende et al., 2009; Kim et al., 2009a; Lee et al., 2009a; Utikal et al., 2009). Somatic cells can be reprogrammed to a totipotent state through nuclear transfer or cloning, because it has been demonstrated that the oocyte has the ability to reprogram an adult nucleus into an embryonic state that can initiate the development of a new organism. Therapeutic cloning, whereby nuclear transfer is used to derive patient-specific ESCs, embraces an entire new opportunity for regenerative medicine (Chang et al., 2009a).

(5) Epigenetic modifications in the germline

Genomic imprinting is a DNA modification pattern which is common and unique to all cells of an individual. This imprinting can be found at both genomic areas without a specific function and areas where the expression of specific genes is controlled. Thereby imprinting can control the proper expression levels of genes required for normal embryonic development and cell function, without changing the genome itself. In an organism the imprinting pattern is partially inherited from both paternal and maternal genomes. After fertilization the imprinting marks and epi-mutations are erased by the epigenetic machinery of the zygote, and then re-established in a new and unique pattern depending on the sex of the new individual. Germ cells constitute the vehicles of genetic information and thereby of inheritance through generations. The epigenetic control mechanisms that govern maintenance and reprogramming of the germline in diverse organisms have gained increasing interest as they reveal essential regulatory pathways implicated in health and disease (Mishra and Sassone-Corsi, 2008). These epigenetic marks, which define the imprinting, include modifications in DNA and histones, especially methylation, acetylation, phosphorylation and ubiquinitation, DNA methylation being the best

characterized of these mechanisms, which has been shown to have essential functions in the germline and the embryo development. These chromatin modifications provide a mechanism for the adequate expression and repression of genes and hence for their temporal or permanent inactivation. Epigenetic modifications are sequentially established and erased in the germinal lineage. PGCs undergo DNA demethylation of the imprinted loci as soon as they reach the gonadal ridge and the parental patterns are erased. Re-establishment of different imprints in germ cells of both sexes with new patterns according to the gender of the new individual is initiated during male and female gamete differentiation. During spermatogenesis, de novo methylation occurs before the onset of meiosis, while in oogenesis it occurs after the onset of meiosis. For the zygote to acquire totipotency extensive epigenetic reprogramming occurs. Shortly after fertilization the paternal genome undergoes rapid demethylation, while the maternal genome is slowly demethylated during the first cleavages. Then, around the time of implantation of the blastocyst there is a wave of de novo methylation to establish an individual specific pattern. Parthenogenesis, a mechanism by which an oocyte is activated without fertilization by a spermatozoid, has been described in some studies after differentiation of oocyte-like cells from SC in culture (Ambe et al., 2008; Devaux et al., 1989; Van den Bergh et al., 1995).

(6) *In vitro* differentiation of gametes from SC

Meiotic and post-meiotic markers are more reliable markers, but it has been demonstrated that the progression through the meiotic process is still a challenge in the *in vitro* differentiation of gametes. The transfection of ESC lines with marked or fluorescent proteins linked to specific gene promoters enables the visualization of the cells in which the specific gene of interest is expressed during the differentiation process (Maurin et al., 2009; Muruvi et al., 2009; Netzel-Arnett et al., 2009; Pires et al., 2009; Xu et al., 2009).

(7) Human stem cell culture

Information regarding the human ES cell lines, HSF-6, HSF-1 and H9 (NIH codes UCO6, UC01 and W-9, respectively) can be obtained at http://stemcells.nih.gov/stemcell/. Undifferentiated human ES cell colonies are cultured on irradiated CF1 mouse embryonic fibroblast feeder cells at 5% CO_2 in supplemented DMEM medium as previously described (Caldwell et al., 2009; Kim et al., 2009c; Oyer et al., 2009; Rodriguez et al., 2009; Slack-Davis et al., 2009).

(8) RNA and cDNA production

At days 0, 3, 7, 14 and 22, embryoid bodies are collected, centrifuged and resuspended in 600 ml RLT buffer for RNA extraction. Total RNA is extracted via the RNeasy system from three independently isolated samples from each of the three different human ES cell lines. cDNA is generated from 3 µg of total RNA using 250 ng of random hexamers under standard conditions with MLV reverse transcriptase. PCR is performed with 50 ng of the first strand cDNA reaction. Three inner cell masses are isolated from blastocysts by immunosurgery using antibody against human choriocarcinoma cells and guinea pig complement according to standard procedures. Total RNA from the three independently isolated mammalian inner cell mass is extracted using the PicoPure RNA isolation system followed by reverse transcription and cDNA production. Mammalian inner cell mass cell cDNA is concentrated using DNA Clean and Concentrator and resuspended in 15 µl water. cDNA from each mammalian inner cell mass is split into eight equal aliquots and used to assay expression of NCAM1, OCT4, NANOS, STELLAR, DAZL, VASA, SCP1 *and* GAPDH (Jones et al., 2009; Junttila et al., 2009).

(9) Quantitative PCR

Quantitative PCR reactions on human ES cell cDNA are performed in duplicate on each sample in the presence of 4.5 mM $MgCl_2$, 10 mM dATP, dGTP, dCTP and dTTP, 2 µM primers, 0.25 U Platinum *Taq*, 1×SYBR green, 1×fluorosceine and 2% DMSO. SYBR green PCR amplifications are initiated at 95°C for 5 min followed by 35 cycles of 95°C, 30 s; 60°C, 30 s; and 72°C, 30 s. Quantitative PCR on human mammalian inner cell mass cell cDNA is performed using the Assay-on-Demand technology for NCAM1, OCT4, VASA, DAZL, SCP1 and GAPDH and Assay-by-Design for NANOS *and* STELLAR according to manufacturer's specifications using FAM-490. Results are analyzed using an iCycler iQ^{tm}. For SYBR green, equal reaction efficiencies are verified via serial dilution of testis cDNA over a 100-fold range. All experiments included negative controls with no cDNA for each primer pair. Primers are designed to span exons to distinguish cDNA from genomic DNA products on human ES cell cDNA (de la Lastra and de la Fuente, 2008; Hu et al., 2009; Page et al., 2007; Xie et al., 2009).

(10) Western blot analysis

Western blot analysis is performed according to standard procedures.

(11) Hypotonic immunohistochemistry for meiotic markers

Germ cells arise from a small group of cells that express markers of pluripotency including OCT4. In humans formation of gonadal compartments takes

place during the 1st trimester (6-8 weeks gestation) (Anderson et al., 2007). Human ES cells at day 0 are digested for 15 min at 37°C with 1 mg/ml collagenase type IV/1 mg/ml dispase in knockout DMEM high glucose containing 20% knockout serum replacer, 1 mM glutamine, non-essential amino acids, 0.1 mM β-mercaptoethanol and 4 ng/µl FGF2. Colonies are gently dislodged, centrifuged at 1000 rpm, 5 min, and then resuspended in freshly prepared hypoextraction buffer pH 8.2 (30 mM Tris pH 8.2/50 mM sucrose/17 mM citric acid/5 mM EDTA/0.5 mM DTT/0.5 mM Pefabloc). Embryoid bodies and ES cell colonies are then lightly teased apart before further incubation in hypoextraction buffer for 30 min. ES cell colonies and embryoid bodies are then collected into 100 mM Sucrose before teasing apart using 20 gage needles to form a single cell suspension. The single cell suspensions are pippetted onto glass slides, previously treated with 1% paraformaldehyde in PBS pH 9.2 containing 0.25% Tween 20. The slides are then in 0.04% photoflo in distilled H_2O followed by incubation for 30 min in Antibody Dilution Buffer containing 10% Normal Donkey Serum/3% BSA/0.5% Tween 20. Slides are incubated overnight at 37°C with rabbit anti-human SCP3 and mouse anti-rat MLH diluted in ADB at 1/500 and 1/25, respectively. Slides are washed for 10 min in ADB followed by 24 h in ADB at 4°C. Slides are incubated with secondary antibodies (rhodamine-conjugated anti-mouse for MLH; FITC-conjugated anti-rabbit for SCP3; 1/100 dilution), 45 min at 37°C. Slides are washed four times in PBS and sealed under a coverslip with anti-fade mounting media (Borodin et al., 2009; Togni et al., 2009; Viana-Pereira et al., 2009).

(12) Immunohistochemistry on paraffin-embedded tissue sections

Contemporary pathology involves an emerging role for molecular diagnostics (van Maldegem et al., 2008). Human testis specimens are fixed by immersion in Bouin's fixative; embryoid bodies are fixed in 4% paraformaldehyde in PBS (pH 7.4), 1 h, processed to paraffin, and cut at 5 µm serial sections onto slides. Slides are blocked by incubation in PBS/0.1% BSA/0.3% Tween 20/10% normal goat serum as appropriate for 30 min. Slides are incubated with primary antibody overnight at 4°C (rabbit anti-human DAZL; 1/100; rabbit anti-human STELLAR: 1/1000 [antibodies made against peptide sequence RESVGAAVLREIEDE of human STELLAR; chicken anti-human VASA, 1/1000] in PBST before washing twice for 10 min in PBS. Sections are then incubated twice for 10 min in PBS before mounting under a glass coverslip with anti-fade mounting media. For avidin biotin immunohistochemistry, following incubation in primary antibodies, sections are incubated for 30 min at room temperature, with either biotin conjugated anti-rabbit to detect DAZL and STELLAR according to manufacturer's instructions, or biotin conjugated anti-chicken (1/200) to detect VASA. Sections are washed twice for 10 min in PBS followed by incubation for 30 min in ABC reagent (Lu et al., 2009; Shi et al., 2007).

(13) Whole mount immunohistochemistry

Whole-mount in situ hybridization is a technique used to localize and visualize specific gene transcripts in whole embryos by hybridizing labeled RNA probes complementary to the sequence of interest. A digoxigenin (DIG)-labeled riboprobe synthesized during in vitro transcription through the incorporation of DIG-labeled UTP is hybridized to the target sequence under stringent conditions, and excess unhybridized probe is removed during a series of washes. The location of the labeled riboprobe, and thus the mRNA sequence of interest, is then visualized by immunohistochemistry. This protocol outlines the steps involved in preparing Hawaiian bobtail squid embryos, hybridizing a DIG-labeled riboprobe in whole-mount embryos, and visualizing the labeled RNA colorimetrically using an alkaline-phosphatase-conjugated anti-DIG antibody (Lee et al., 2009b).

Undifferentiated human ES cells are grown on feeder layers for at least 4 days before removing the media and fixing in 4% paraformaldehyde in PBS (pH 7.4) for 15 min. Colonies are then washed twice for 10 min in TBS+0.05% Tween 20, followed by incubation in 0.1% Triton X in PBS for 10 min. Colonies are washed as above, incubated for 30 min with 4% normal donkey serum, and incubated with primary antibodies diluted in PBS (1/100, DAZL; 1/100, STELLAR; 1/1000, VASA; 1/30 SSEA1; 1/30 SSEA3; 1/30 SSEA4; 1/30 TRA-1-81; 1/30 TRA-1-60; 1/50 NCAM1; 1/50 KDR and neat AFP for immunofluorescence). For Avidin–biotin immunohistochemistry antibodies are diluted (1/500, DAZL; 1/1000, STELLAR; 1/1000 VASA in PBS). All primary antibodies are incubated for 1 h at room temperature. Colonies are then washed twice in PBS and incubated with secondary antibodies as described above. Colonies are viewed using a Leica microscope fitted for immunofluorescence after mounting with anti-fade mounting media or PBS (Akhtar et al., 2009; Rasweiler et al., 2009; Sawada et al., 2008).

7. Clinical Significance, applications and future prospects of Germ Stem Cell

SC research offers great promise for understanding basic mechanisms of human development and differentiation, as well as the hope for new treatments for diseases such as diabetes, spinal cord injury, Parkinson's disease, and myocardial infarction. However, human SC research

also raises sharp ethical and political controversies. The derivation of pluripotent SC lines from oocytes and embryos is fraught with disputes about the onset of human personhood. The reprogramming of somatic cells to produce induced pluripotent SCs avoids the ethical problems specific to ESC research. In any human SC research, however, difficult dilemmas arise regarding sensitive downstream research, consent to donate materials for human SC research, early clinical trials of human SC therapies, and oversight of human SC research. These ethical and policy issues need to be discussed along with scientific challenges to ensure that SC research is carried out in an ethically appropriate manner (Lo and Parham, 2009). If SC-derived oocytes could someday be safely used for reproductive purposes, a large and diverse group of infertile women would have the opportunity to bear genetically related offspring. Common causes of infertility that could be treated using SC-derived oocytes include premature ovarian failure, reproductive aging associated with delayed childbearing, and poor oocyte quality, which can occur even in young women (Revazova et al., 2008).

Mouse ESCs can differentiate into female and male germ cells in vitro. Primate ES cells can also differentiate into immature germ cells in vitro (Yamauchi et al., 2009). Seemingly, the germ cell differentiation process is dependent on spatial distribution of differentiating cells. Embryoid bodies represent a three-dimensional structure with a microenvironment that propitiates differentiation, although other cell types besides germ cells arise from them. Currently, one of the most critical steps after differentiation is selection and isolation of differentiated germ cells. Since ESCs and PGCs share some common markers, detection of post-migratory and meiotic markers is a useful method, as well as the use of transgenes with fluorescent reporter genes under the control of specific promoters of male and female germ cells. These have been the most employed strategies in the studies of germ cell differentiation developed to date. The problem is that methods based on gene modification and the use of retroviral vectors limit the use of gamete-like cells in future clinical treatments. Future translational application of these ESC-derived gametes in ART when functional gametes are not available from patients require still further investigation into gamete differentiation. Ultimately, the process needs to be reproducible and efficient. The focus of current and future studies should be on meiotic completion to avoid unwanted aneuploidies and the determination and establishment of accurate epigenetic modifications and imprinting status to provide reproductive hope to humans lacking gametes (Berruti, 2006).

Premature ovarian failure is typically defined by amenorrhea, hypoestrogenism, and hypergonadotropinism in a woman who is under 40 yr of age at the time of onset. Although the incidence of premature ovarian failure is not known with certainty, it has been estimated to occur at a frequency of approximately 1%. Because some women with apparent premature ovarian failure may ovulate or occasionally conceive after the diagnosis is established, the term "primary ovarian insufficiency" has been suggested to describe the condition more accurately. Primary ovarian insufficiency is a heterogeneous disorder that may result from a decrease in the initial primordial follicle number, an increase in follicle destruction, or a failure of the follicle to respond to gonadotropin stimulation. Known genetic causes include absence or structural abnormalities of the X chromosome, Fragile X syndrome, structural rearrangements of autosomes, point mutations of autosomal genes that are required for oogenesis and folliculogenesis, and abnormalities of pleiotropic Mendelian genes that may disturb ovarian function along with perturbation of other organ systems. Multiple autoimmune etiologies for primary ovarian insufficiency have also been described, and environmental causes are just beginning to be explored as well. For example, in the mouse, *in utero* exposure to the environmental contaminant bisphenol A has been convincingly associated with oocyte aneuploidy in female offspring. Ovarian function may also be lost via surgical removal of the ovaries due to ovarian cancer or as a result of chemotherapy for nongynecological malignancy. Many causes of ovarian failure remain idiopathic. Research involving SC-derived oocytes may not only provide a potential reproductive option for women with primary ovarian insufficiency, but may also lead to a better understanding of its causes (Guo et al., 2009).

The rise in life expectancy over the last century, together with higher maternal and paternal ages and have highlighted the issue of reduced fertility with advancing age. Aging of the male reproductive system is incited by multi-factorial changes at molecular, cellular and regulatory levels, and individual characteristics are highly variable, although strongly influenced by lifestyle and environmental factors. Damage accumulated with age leads to progressive deregulation of the hypothalamic-pituitary-gonadal axis and of local auto/paracrine interactions, thereby inducing changes in target organs such as the testis, penis and prostate. Elderly human males produce less testosterone, have fewer motile sperm and a higher incidence of erectile dysfunction and prostate disorders, all of which contribute to lower fertility. Cellular aging can manifest itself at

several levels. Aging cells progressively accumulate "waste" products, resulting in a decreased functionally. Changes to mitochondria are among the most remarkable features observed in aging cells and several theories place mitochondria at the hub of cellular events related to aging, namely in terms of the accumulation of oxidative damage to cells and tissues, a process in which these organelles may play a prominent role, although alternative theories have also emerged. Furthermore, mitochondrial energy metabolism is also crucial for male reproductive function and mitochondria may therefore constitute a common link between aging and fertility loss (Amaral and Ramalho-Santos, 2009). Childbearing at an advanced maternal age is strongly associated with an increase in the risk of fetal aneuploidy. An age-associated increase in aneuploidy has also been noted in human oocytes and in biopsied human embryos. Multiple factors could potentially account for genetic abnormalities in the oocyte, and it is likely that abnormalities may arise during multiple stages of oogenesis. SC-derived oocytes may eventually allow a better understanding of age-associated aneuploidy as well as a potential option for the creation of euploid oocytes (Atsalis and Margulis, 2008).

Assessment of oocyte maturity and quality at the time of retrieval is difficult as the egg is obscured by a large cumulus mass that hinders adequate scoring (Ebner et al., 2008). Fertility specialists commonly encounter infertile women with poor oocyte quality and resulting poor embryo development, particularly in couples who are characterized as having "unexplained" infertility before *in vitro* fertilization. In some cases, the problem with oocyte quality may be defined by recurrent low rate of oocyte maturation. In other cases, the recurrent problems with oocyte quality are characterized on the basis of abnormal morphological appearance of the oocytes and resulting embryos that develop with high rates of fragmentation and arrest before the blastocyst stage. There are no suitable techniques for improving oocyte quality either in the clinic or in the embryology laboratory. Problems with oocyte quality are poorly understood, and it is hoped that SC-derived oocytes may offer some understanding and eventually a treatment option for these infertile couples as well (Nikolaou, 2004).

Oocyte donation is very expensive due to the need for extensive screening of the donor, ovarian stimulation with injections of gonadotropin, oocyte retrieval, and financial compensation given to the donor. As an alternative to oocyte donation, some couples may be willing to consider embryo donation. Research utilizing oocytes derived from SCs could also provide valuable insight into the processes of both normal and abnormal oocyte development. It is quite plausible that this research will not only enhance our ability to address infertility, but will also help us to understand better some of the causes of abnormal offspring in spontaneous conceptions occurring among the general population. The clinical relevance of research involving SC-derived oocytes is unquestionable (Egashira et al., 2009; Tao and Del Valle, 2008). In diverse model organisms that include species of flies, worms, frogs, fish, salamanders, mice and non-human primates, as well as in humans, the expression of *DAZ* gene family homologs has been shown to be restricted entirely to the germ cell lineage (Chang et al., 2009b).

Embryoid bodies represent a three-dimensional structure with a microenvironment that propitiates differentiation, although other cell types besides germ cells arise from them. Currently, one of the most critical steps after differentiation is selection and isolation of differentiated germ cells. Since ESCs and PGCs share some common markers, detection of post-migratory and meiotic markers is a useful method, as well as the use of transgenes with fluorescent reporter genes under the control of specific promoters of male and female germ cells. These have been the most employed strategies in the studies of germ cell differentiation developed to date. The problem is that methods based on gene modification and the use of retroviral vectors limit the use of gamete-like cells in future clinical treatments. Future translational application of these ESC-derived gametes in ART when functional gametes are not available from patients require still further investigation into gamete differentiation (Chomez et al., 1996).

Human ES cell lines are the *in vitro* manifestation of cells of the mammalian inner cell mass derived from blastocysts. Previous reports have demonstrated that human ES cells are pluripotent and capable of differentiating into a wide variety of somatic cell types during EB formation. However, germ cell differentiation from human ES cells has never been reported. In this study, we used a panel of germ cell-specific and germ cell-enriched markers, together with markers of somatic cell lineages, to assess the ability of three lines of undifferentiated human ES cells to form germ cells *in vitro*.

Directing embryonic stem (ES) cells to differentiate into functional motoneurons has proven to be a strong technique for studying neuronal development as well as being a potential source of tissue for cell replacement therapies involving spinal cord disorders (Soundararajan et al., 2007). The genetic similarity of human ES cells to the mammalian inner cell mass has never been addressed. It is difficult to determine *a priori* whether it would be likely or not for human ES cells and mammalian inner

cell mass to differ significantly in gene expression (Inanc et al., 2008).

VASA expression during ES cell differentiation allows for identification of more mature stages of germ cells in humans (Eguizabal et al., 2009; Lavagnolli et al., 2009).

Previously, two independent studies demonstrated that mouse ES cells could form germ cells *in vitro*. Both groups took advantage of the ease of genetic manipulation of mouse ES cells and generated undifferentiated ES cell lines that could be sorted for a fluorescent GFP marker under the control of germ cell specific promoters (Denker et al., 2007). The derivation of germ cells from pluripotent SCs in vitro could provide an invaluable model system to study both the genetic and epigenetic programming of germ cell development in vivo (Eguizabal et al., 2009).

Discussion

Exactly how ESC cultures may mimic the somatic environment that encapsulates either developing oogonia (the follicle) or the sperm SC (seminiferous epithelium) is unclear. The appropriate growth factor and hormonal microenvironment required to support and sustain these complex niches probably depends to some extent on the type of culture system adopted for the differentiation process. ESCs are cultured usually in two basic ways. Monolayer adherent cultures of ESCs can be allowed to differentiate directly to form an appropriate niche or ESCs can be induced to aggregate to form embryoid bodies that form a more three-dimensional microenvironment. In conjunction with these two culture methods, ESCs can be initially co-cultured with feeder layers or conditioned-medium from feeder cells and various growth factor and serum supplementation (Falender et al., 2005; Goel et al., 2008; Nistal et al., 2006; Sakai et al., 2004).

In the mouse, it has been claimed that germ cells can be derived from bone marrow and peripheral blood cells and bone marrow-derived germ cells can repopulate the ovarian follicular reserve. Many studies in both mice and humans have shown that ES cells have the capacity to proliferate and to differentiate to numerous cell lineages. Using *in vitro* studies, mouse ES cells can differentiate into lineages that include hematopoietic, vascular, pancreatic, neural, muscular and germ cells. Likewise, human ES cells can also differentiate to several lineages *in vitro*, including neural, pancreatic, muscular, endothelial, trophoblast and hematopoietic cells (Choi et al., 2009; Hester et al., 2009; Inanc et al., 2008; Martinez-Lorenzo et al., 2009).

Since undifferentiated human ES cells expressed a number of markers indicative of putative premeiotic germ cells, we tested whether germ cell specific proteins are also expressed in undifferentiated human ES cells. Western blot analysis of human fetal and adult testis tissue was used as a positive control since the transcriptional and translational profiles of these tissues have been well documented (Faipoux et al., 2008; Kuypers et al., 2009; Nervi et al., 2007).

The culture of spermatogonial cells under well-defined conditions would be an important method for elucidating the mechanisms involved in spermatogenesis and in establishing tissue regeneration in vivo (Creemers et al., 2002). The promiscuous nature of spontaneous ESC differentiation in culture makes it especially difficult to determine at present whether PGC and germ cell development follow similar program to those occurring *in vivo* or whether the conditions merely provide suitable conducive conditions that permit an intrinsic developmental process but not necessarily the same as the gonadal environment (Kim et al., 2009b). From their formation in the early embryo to their ultimate acquisition of functionality in the adult, germ cells must complete a long journey with many genetic and epigenetic requirements (Arany et al., 2009; Hsieh et al., 2009).

Literatures

Abrahamson, S., K. Houtchens, et al. (1983). "Germ cell comparative Drosophila mutagenesis: sensitivity and mutation pattern in chemically treated stem cells." Environ Mutagen **5**(6): 891-905.

Mutagenesis studies on Drosophila oogonial cells with methylnitrosourea, dimethylnitrosamine, and diethylnitrosamine revealed unexpectedly high rates of sex-linked recessive lethals relative to other male and female germ cell stages. Indeed, the oogonial mutation rates with chemicals are higher than with massive X-ray or neutron exposures of oogonia. Analysis of the distribution of lethals per treated female suggests most of the mutations recovered are of independent origin, with very small levels of clustering of identical mutations. In the male stem cell population (spermatogonia) on the other hand, the distribution of lethals is primarily nonrandom and highly clustered. The nature of the mutational endpoint and the different pattern of germ cell development in the two sexes are the probable causes of this difference. The oogonial sensitivity to chemical mutagens may have important bearing on strategies for assessing human hazard.

Aflatoonian, B., L. Ruban, et al. (2009). "In vitro post-meiotic germ cell development from human embryonic stem cells." Hum Reprod **24**(12): 3150-9.

BACKGROUND: Investigating the mechanisms of human primordial germ cell (PGC) and gamete development are important for

understanding the causes of infertility and effects of environmental chemicals on reproductive development. However, there are practical and ethical difficulties associated with obtaining human tissue in early development. The aim of this study was to investigate whether human embryonic stem cell-hESC-generated germ cells could provide an in vitro model of gamete development. METHOD: Human ESCs were differentiated as embryoid bodies (EBs) in vitro. Gene and protein marker expression profiles of EBs in different periods of culture were analysed by quantitative polymerase chain reaction (Q-PCR) and immunolocalization to monitor germ cell development. Secretion of hormones involved in germ cell maturation was measured, to detect the existence of a germ cell niche within EBs. RESULTS: Q-PCR revealed gene expression profiles consistent with PGC formation and germ cell development. A small population of post-meiotic spermatid cells were identified using sperm-specific antibodies (Protamine 1 and 1.97). Although gene expression profiles characteristic of oocyte development and follicle-like structures were detected, a committed oocyte with extra-cellular zona pellucida was not recognized with zona pellucida-specific monoclonal antibody. CONCLUSIONS: hESCs can form PGCs and post-meiotic spermatids in vitro, however, there remains doubt about oocyte development. Levels of steroid hormones produced by EBs were significant when compared with known values for a similar quantity of human testis, suggesting that hESC may intrinsically create a favourable hormonal niche for spermatogenesis.

Agarwal, R., C. C. Dvorak, et al. (2009). "High-dose chemotherapy followed by stem cell rescue for high-risk germ cell tumors: the Stanford experience." Bone Marrow Transplant **43**(7): 547-52.

Germ cell tumors carry an excellent prognosis with platinum-based therapy upfront. The patients who either relapse or demonstrate refractoriness to platinum pose a challenge. There exist many reports in the literature on the use of high-dose chemotherapy and stem cell rescue improving the outcome in patients with relapsed germ cell tumors. However, the reports have great variability in the patient selection, prior treatments, the choice of the conditioning regimen and variability of the doses within the same regimen. In this report, we present 37 patients who underwent a uniform protocol of high-dose chemotherapy with stem cell rescue. Stem cell mobilization was performed with high-dose CY (4 g per m(2)) and we were able to collect adequate cells for marrow rescue in all patients. Patients received a high-dose regimen with etoposide (800 mg/m(2) per day) days -6, -5 and -4 as a continuous infusion, carboplatin (667 mg/m(2) per day) on days -6, -5 and -4 as a 1 h infusion, and CY (60 mg/kg per day) on days -3 and -2. In this high-risk group of patients, high-dose chemotherapy with autologous stem cell rescue led to a 3-year overall survival of 57% and a 3-year event-free survival of 49%. The results are reflective of a single procedure. No tandem transplants were performed. The treatment-related mortality was low at 3% in this heavily pretreated group.

Ahmad, I., K. Haider, et al. (2004). "Autoimmune thrombocytopenia following tandem autologous peripheral blood stem cell transplantation for refractory germ cell tumor." Bone Marrow Transplant **34**(3): 279-80.

Akamatsu, W., B. DeVeale, et al. (2009). "Suppression of Oct4 by germ cell nuclear factor restricts pluripotency and promotes neural stem cell development in the early neural lineage." J Neurosci **29**(7): 2113-24.

The earliest murine neural stem cells are leukemia inhibitory factor (LIF)-dependent, primitive neural stem cells, which can be isolated from embryonic stem cells or early embryos. These primitive neural stem cells have the ability to differentiate to non-neural tissues and transition into FGF2-dependent, definitive neural stem cells between embryonic day 7.5 and 8.5 in vivo, accompanied by a decrease in non-neural competency. We found that Oct4 is expressed in LIF-dependent primitive neural stem cells and suppressed in FGF-dependent definitive neural stem cells. In mice lacking germ cell nuclear factor (GCNF), a transcriptional repressor of Oct4, generation of definitive neural stem cells was dramatically suppressed, accompanied by a sustained expression of Oct4 in the early neuroectoderm. Knockdown of Oct4 in GCNF(-/-) neural stem cells rescued the GCNF(-/-) phenotype. Overexpression of Oct4 blocked the differentiation of primitive to definitive neural stem cells, but did not induce the dedifferentiation of definitive to primitive neural stem cells. These results suggested that primitive neural stem cells develop into definitive neural stem cells by means of GCNF induced suppression of Oct4. The Oct4 promoter was methylated during the development from primitive neural stem cell to definitive neural stem cell, while these neural stem cells lose their pluripotency through a GCNF dependent mechanism. Thus, the suppression of Oct4 by GCNF is important for the transition from primitive to definitive neural stem cells and restriction of the non-neural competency in the early neural stem cell lineage.

Allard, E. K. and K. Boekelheide (1996). "Fate of germ cells in 2,5-hexanedione-induced testicular injury. II. Atrophy persists due to a reduced stem cell mass and ongoing apoptosis." Toxicol Appl Pharmacol **137**(2): 149-56.

The Sertoli cell toxicant 2,5-hexanedione (2,5-HD) causes irreversible testicular atrophy in rats. After toxicant exposure, only Sertoli cells, stem cells, and a few spermatogonia remain in the seminiferous epithelium. In this study, the number, type, and fate of the remaining germ cells were determined. Male Sprague-Dawley rats were exposed to 1% 2,5-HD in drinking water for 5 weeks and then sacrificed 12 or 40 weeks after the start of exposure. Cell counts determined that the stem cell population was diminished in size, but made up a significant portion of the remaining germ cells. The remaining germ cells were primarily type A spermatogonia. Modeling of spermatogonial divisions suggested that most spermatogonia undergo degeneration at the level of type A3 spermatogonia after 2,5-HD-induced atrophy. Apoptosis was demonstrated to occur in the remaining germ cells by nuclear morphology and in situ analysis of DNA fragmentation. Quantitation indicated that apoptosis occurred in a majority of stem cell progeny. We conclude that the irreversibility of 2,5-HD-induced testicular injury results from the reduced size of the stem cell population as well as a block in germ cell development at the level of type A spermatogonia.

Almstrup, K., S. B. Sonne, et al. (2006). "From embryonic stem cells to testicular germ cell cancer--should we be concerned?" Int J Androl **29**(1): 211-8.

Since the discovery of testicular carcinoma in situ (CIS) -- the precursor cell for the vast majority of germ cell tumours -- it has been proposed that CIS cells could be derived from transformed primordial germ cells or gonocytes. Here, we review recent discoveries not only substantiating that initial hypothesis but also indicating that CIS cells have a striking phenotypic similarity to embryonic stem cells (ESC). Many cancers have been proposed to originate from tissue-specific stem cells [so-called 'cancer stem cells' (CSC)] and we argue that CIS may be a very good example of a CSC, but with exceptional features due to the retention of embryonic pluripotency. In addition, considering the fact that pre-invasive CIS cells are transformed from early fetal cells, possibly due to environmentally induced alterations of the niche, we discuss potential risks linked to the uncontrolled therapeutic use of ESC.

Anderson, P. D., V. R. Nelson, et al. (2009). "Genetic factors on mouse chromosome 18 affecting susceptibility to testicular germ cell tumors and permissiveness to embryonic stem cell derivation." Cancer Res **69**(23): 9112-7.

Despite strong heritability, little is known about the genetic control of susceptibility to testicular germ cell tumors (TGCT) in humans or mice. Although the mouse model of spontaneous TGCTs has been extensively studied, conventional linkage analysis has failed to locate the factors that control teratocarcinogenesis in the susceptible 129 family of inbred strains. As an alternative approach, we used both chromosome substitution strains (CSS) to identify individual chromosomes that harbor susceptibility genes and a panel of congenic strains derived from a selected CSS to determine the number and location of susceptibility variants on the substituted chromosome. We showed that 129-Chr 18(MOLF) males are resistant to spontaneous TGCTs and that at least four genetic variants control susceptibility in males with this substituted chromosome. In addition, early embryonic cells from this strain fail to establish embryonic stem cell lines as efficiently as those from the parental 129/Sv strain. For the first time, 129-derived genetic variants that control TGCT susceptibility and fundamental aspects of embryonic stem cell biology have been localized in a genetic context in which the genes can be identified and functionally characterized.

Bauman, J. G., P. de Vries, et al. (1988). "Purification of murine hemopoietic stem cells and committed progenitors by fluorescence activated cell sorting using wheat germ agglutinin and monoclonal antibodies." Acta Histochem Suppl **36**: 241-53.

Two procedures to purify and separate pluripotent hemopoietic stem cells (PHSC) and committed progenitor cells from mouse bone marrow cells, and a three colour stem cell staining procedure are described. Visser et al., (J. Exp. Med. 59, 1576-1590, 1984) described the purification of PHSC by metrizamide density gradient centrifugation followed by wheat germ agglutinin-FITC (WGA-FITC) and light scatter sorting using a fluorescence activated cell sorter (FACS). The light density, WGA-positive, high forward (FLS) and low perpendicular light scatter (PLS) blast cells, after removal of the lectin from the sorted cells by the competing sugar, are restained with biotinylated anti-H-2K plus avidin-FITC. The H-2K positive cells proved to be pure pluripotent stem cells. Bauman et al. (J. Cell. Physiol. 128, 133-142, 1986) started by sorting the 6% most positive fluorescent cells with low PLS and high FLS from WGA-FITC stained normal bone marrow. After lectin removal the sorted cells are restained with anti-GM-1.2. Sorted, GM-1.2 negative cells are almost exclusively PHSC plus committed colony forming cells. A three colour staining procedure was designed to measure stem cells

in bone marrow samples by flow cytometry. Cells are simultaneously stained with anti-H-2K-biotin plus avidin-phycoerythrin, a rat monoclonal antibody detecting a cell surface antigen plus goat anti rat Ig-FITC and 1-butyryl-pyrene-WGA. Analysis and sorting on the two laser Rijswijk Experimental Light-Activated Cell Sorter (RELACS II) using selected windows indicated that these windows contained high frequencies of PHSC. This multicolour analysis and sorting therefore is equivalent to the multistep sorting procedures.

Bokemeyer, C., M. A. Kuczyk, et al. (1996). "Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours." J Cancer Res Clin Oncol **122**(5): 301-6.

The proto-oncogene c-kit and its ligand stem-cell factor (SCF) may play an important role in the development of normal and malignant testicular tissue. This study investigates the presence of SCF and c-kit protein in 32 orchiectomy specimens of patients with testicular cancer, in 5 specimens of normal testicular tissue and in three established non-seminomatous germ-cell cancer cell lines (H12.1, H32, 577ML) by an immunohistochemical approach. Out of 9 testicular cancer specimens classified as pure seminomas, 7 (78%) showed a strong immunohistochemical reaction for both SCF and c-kit protein on the surface of the tumour cells. Fourteen non-seminomatous germ-cell tumours composed of embryonal carcinoma were completely negative for both SCF and c-kit proteins and only faint positivity was found in 6 tumours (26%). Differentiated teratomatous structures within the specimens on non-seminomatous tumours showed a strong immunohistochemical reaction for SCF and c-kit protein in 8 of 11 (73%) cases. All three testicular cancer cell lines showed only faint staining reactions for c-kit protein and none for SCF. No secretion of SCF by the three lines in vitro was detected. The addition of high concentrations of SCF (100 ng/ml) to the testicular cancer cell lines in culture conditions without fetal calf serum resulted in a 1.4 to 3-fold growth stimulation compared to cell growth in serum-free medium alone. This effect was not detectable when the cells were cultured in serum-containing media. In the normal testicular tissue the germ-cells displayed a strong immunohistochemical reaction for c-kit protein while SCF positivity was found at the tubular membrane and on the surface of Sertoli cells. The SCF/c-kit system may possess a regulatory function in normal testicular tissue by possibly providing the microenvironment necessary for spermatogenesis. With the development of testicular cancer, this regulatory system seems to be lost, particularly in non-seminomatous germ-cell tumours. A growth-stimulatory effect of high concentrations of SCF on non-seminomatous testicular cancer cell lines can be detected only in culture conditions with serum-free media. The effects achievable by the combination of SCF with other growth factors need to be further studied, as well as the role of the c-kit/SCF regulatory system for normal spermatogenesis and its possible implications for the understanding and treatment of male infertility.

Bokemeyer, C. and H. J. Schmoll (1993). "Treatment of advanced germ cell tumours by dose intensified chemotherapy with haematopoietic growth factors or peripheral blood stem cells (PBSC)." Eur Urol **23**(1): 223-9; discussion 230.

Dose intensification of the most active drugs may improve the treatment results of patients (pts.) with far advanced metastatic testicular cancer. At Hannover University pts. with advanced testicular cancer (Indiana University criteria) were entered in a phase I/II study of a step-wise dose-intensified regimen of platinum, etoposide, and ifosfamide (PEI) with s.c. GM-CSF (5-10 micrograms/kg/day). Due to thrombocytopenia and mucositis the MTD was reached at dose level 3 with P 30 mg/m2, E 200 mg/m2, and I 1.6 g/m2 (d 1-5, q 21 d, 4 cycles). Overall, 49 of 75 pts. (65.3%) achieved CR/NED. 11 pts. (14.6%) failed PEI therapy, but 4 of these achieved CR/NED by salvage treatment. After a median follow-up of 22 months 66% of pts. are alive and free of disease. The dose intensity was 1.42 compared to a standard PEI regimen. A further increase of dose-intensity will require additional haematopoietic support, e.g. peripheral blood stem cells (PBSC). PBSC following dose-intensified PEI-chemotherapy plus G- or GM-CSF are used in the current study of the German Testicular Cancer Study Group for the treatment of advanced germ cell tumours.

Braydich-Stolle, L., C. Nolan, et al. (2005). "Role of glial cell line-derived neurotrophic factor in germ-line stem cell fate." Ann N Y Acad Sci **1061**: 94-9.

The overall goal of this study is to unravel the role(s) played by glial cell line-derived neurotrophic factor (GDNF) in the fate of spermatogonial stem cells. There is great interest in the biology of spermatogonial stem cells, or A(single) spermatogonia, because of their importance in the treatment of infertility, the development of contraceptives, and the understanding of the etiology of testicular cancer, particularly seminoma. In the mouse, spermatogonial stem cells express GFRalpha-1, the receptor for GDNF, and respond to this growth factor in vivo and in vitro. GDNF is produced by the

adjacent Sertoli cells, which are part of the germ-line stem cell niche in vertebrates. We specifically isolated GFRalpha-1-positive spermatogonia using an immunomagnetic bead technique. We then stimulated the cells with 100 ng/mL of rGDNF for 10 hours; unstimulated cells served as negative controls. Microarray analysis, immunocytochemistry, and Western blotting revealed that Numb, a regulator of the Notch pathway, is upregulated by GDNF in spermatogonial stem cells. There are indications that in rats, mice, and humans, the Notch pathway promotes spermatogonial differentiation. We observed that an increase in Numb expression is concomitant with Notch degradation in these cells. Thus, through Numb, GDNF might inhibit differentiation and allows the maintenance of the stem cell pool in the mouse seminiferous epithelium.

Brehm, A., K. Ohbo, et al. (1999). "Synergism with germ line transcription factor Oct-4: viral oncoproteins share the ability to mimic a stem cell-specific activity." Mol Cell Biol **19**(4): 2635-43.

Activation of transcription by Oct-4 from remote binding sites requires a cofactor that is restricted to embryonal stem cells. The adenovirus E1A protein can mimic the activity of this stem cell-specific factor and stimulates Oct-4 activity in differentiated cells. Here we characterize the Oct-4-E1A interaction and show that the E1A 289R protein harbors two independent Oct-4 binding sites, both of which specifically interact with the POU domain of Oct-4. Furthermore, we demonstrate that, like E1A, the human papillomavirus E7 oncoprotein also specifically binds to the Oct-4 POU domain. E7 and Oct-4 can form a complex both in vitro and in vivo. Expression of E7 in differentiated cells stimulates Oct-4-mediated transactivation from distal binding sites. Moreover, Oct-4, but not other Oct factors, is active when expressed in cells transformed by human papillomavirus. Our results suggest that different viruses have evolved oncoproteins that share the ability to target Oct-4 and to mimic a stem cell-specific activity.

Bremer, S. and R. Vogel (1999). "Pluripotent stem cells of the mouse as a potential in vitro model for mammalian germ cells. Sister chromatid exchanges induced by MMC and ENU in undifferentiated cell lines compared to differentiated cell lines." Mutat Res **444**(1): 97-102.

We tried to develop an in-vitro test system which could serve as a model for mammalian germ cells in vivo. Two pluripotent cell types were used, because they express some germ cell specific immunological and biochemical markers: (1) Embryonal carcinoma cells (ECC) of the line P19 had been isolated from a teratocarcinoma of murine primordial germ cells (PGC). (2) Embryonal stem cells (ESC) are obtained from the inner cell mass of mouse blastocysts. Sister chromatid exchanges (SCE) induced by mitomycin C and ethylnitrosourea (ENU) were analysed in the two undifferentiated cell lines, ECC and ESC, to detect differences in their sensitivity compared with differentiated cell lines of the mouse. Neither of the model cell lines have shown a greater sensibility after exposure to MMC and ENU. In contrary, the carcinoma cell line was able to tolerate higher concentrations of these genotoxicants. Therefore, SCE analysis in the ECC and ESC lines used does not provide a suitable model for genotoxicity testing on mammalian germ cells.

Buaas, F. W., A. L. Kirsh, et al. (2004). "Plzf is required in adult male germ cells for stem cell self-renewal." Nat Genet **36**(6): 647-52.

Adult germline stem cells are capable of self-renewal, tissue regeneration and production of large numbers of differentiated progeny. We show here that the classical mouse mutant luxoid affects adult germline stem cell self-renewal. Young homozygous luxoid mutant mice produce limited numbers of normal spermatozoa and then progressively lose their germ line after birth. Transplantation studies showed that germ cells from mutant mice did not colonize recipient testes, suggesting that the defect is intrinsic to the stem cells. We determined that the luxoid mutant contains a nonsense mutation in the gene encoding Plzf, a transcriptional repressor that regulates the epigenetic state of undifferentiated cells, and showed that Plzf is coexpressed with Oct4 in undifferentiated spermatogonia. This is the first gene shown to be required in germ cells for stem cell self-renewal in mammals.

Buhr, N., C. Carapito, et al. (2007). "Proteome analysis of the culture environment supporting undifferentiated mouse embryonic stem and germ cell growth." Electrophoresis **28**(10): 1615-23.

The therapeutical interest of pluripotent cells and ethical issues related to the establishment of human embryonic stem cell (ESC) or embryonic germ cell (EGC) lines raise the understanding of the mechanism underlying pluripotency to a fundamental issue. Establishing a protein pluripotency signature for these cells can be complicated by the presence of unrelated proteins produced by the culture environment. Here, we have analyzed the environment supporting ESC and EGC growth, and established 2-D reference maps for each constituent present in this culture environment: mouse embryonic fibroblast feeder cells, culture medium (CM) and gelatin. The establishment of these reference maps is essential

prior to the study of ESC and EGC specific proteomes. Indeed, these maps can be subtracted from ESC or EGC maps to allow focusing on spots specific for ESCs or EGCs. Our study led to the identification of 110 unique proteins from fibroblast feeder cells and 23 unique proteins from the CM, which represent major contaminants of ESC and EGC proteomes. For gelatin, no collagen-specific proteins were identified, most likely due to difficulties in resolution and low quantities. Furthermore, no differences were observed between naive and conditioned CM. Finally, we compared these reference maps to ESC 2-D gels and isolated 17 ESC specific spots. Among these spots, proteins that had already been identified in previous human and mouse ESC proteomes were identified but no apparent ESC-specific pluripotency marker could be identified. This work represents an essential step in furthering the knowledge of environmental factors supporting ESC and EGC growth.

Chang, M. C., S. O. Vargas, et al. (2009). "Embryonic stem cell transcription factors and D2-40 (podoplanin) as diagnostic immunohistochemical markers in ovarian germ cell tumors." Int J Gynecol Pathol **28**(4): 347-55.

SUMMARY: The embryonic stem cell transcription factors SOX2, NANOG, and OCT3/4 are involved in the regulation of germ cell tumor growth and differentiation. They, and D2-40 (podoplanin), an antigen expressed in seminomas, are emerging as useful diagnostic markers in testicular germ cell tumors. This study evaluates the use of these markers in ovarian tumors. Ovarian germ cell tumors (n=31) have distinct immunostaining profiles, depending on the type of differentiation as follows: dysgerminoma (SOX2-, NANOG+, OCT3/4+, D2-40+), embryonal carcinoma (SOX2+, NANOG+, OCT3/4+, D2-40-), immature teratomas (SOX2+, NANOG-, OCT3/4-, D2-40-), yolk sac tumors, and choriocarcinoma (SOX2-, NANOG-, OCT3/4-, D2-40-). In immature teratomas, SOX2 positivity was limited to neural and epithelial tissues, and OCT3/4 was positive only in scattered epithelial cells (<10% of cells). Nongerm cell tumors (n=57, including surface-epithelial stromal tumors and sex-cord stromal tumors) were negative for NANOG and D2-40. OCT3/4 was positive in 4 of 9 adult granulosa cell tumors (15% to 85% of cells). In a small number of surface-epithelial stromal tumors, SOX2 and/or OCT3/4 were variably positive (20% to 90% of cells). Of the markers, SOX2 and D2-40 discriminated between dysgerminoma and embryonal carcinoma. NANOG distinguished between either of these 2 tumors and nongerm cell tumors. The inclusion of these markers should therefore be considered in cases of pure or mixed ovarian germ cell tumors that are difficult to classify, and to exclude nongerm cell tumor mimics.

Chaudhary, U. B., L. E. Damon, et al. (2005). "High-dose etoposide, thiotepa, and dose-adjusted carboplatin (TVCa) with autologous hematopoietic stem cell rescue as treatment of relapsed or refractory germ cell cancer." Am J Clin Oncol **28**(2): 130-7.

A phase I/II trial with high-dose etoposide, thiotepa, and dose-adjusted carboplatin (TVCa) with autologous hematopoietic stem cell rescue (AHSCT) as treatment for patients with relapsed or refractory germ cell cancer was investigated. The phase I portion involved a dose escalation schema for carboplatin and thiotepa while keeping the dose of etoposide constant. The intended carboplatin dose was adjusted for renal function based on the glomerular filtration rate. The phase II portion of the trial evaluated the efficacy, feasibility, and safety of tandem TVCa with AHSCT. Twenty-four patients with relapsed or refractory germ cell cancer were treated in this phase I/II trial. Nine of 24 (38%) achieved a complete response. With a median follow up of 71 months (range,1-108 months), all 9 of 24 (38%) are alive and continuously disease-free. There were 2 (7%) treatment-related deaths. The median time to an absolute granulocyte count greater than 0.5 x 10/L was 11 days (range, 9-20 days) on phase I and 10 days (range, 9-13 days) on phase II therapy. The median time to a platelet count greater than 20 x 10/L was 15 days (range, 12-40 days) on phase I and 14 days (range, 13-27 days) on phase II therapy. Nonhematologic toxicity was mild to moderate. A significant correlation was seen between intended carboplatin dose and actual AUC. TVCa high-dose chemotherapy is active and well tolerated in patients with relapsed or refractory germ cell cancer.

Chen, H. F., H. C. Kuo, et al. (2007). "Derivation, characterization and differentiation of human embryonic stem cells: comparing serum-containing versus serum-free media and evidence of germ cell differentiation." Hum Reprod **22**(2): 567-77.

BACKGROUND: This study was designed to establish human embryonic stem cell (hESC) lines, to identify the differences when maintained in serum-containing versus serum-free medium and to test their potential of in vitro differentiation. METHODS: Procedures including immunosurgery were performed on 11 donated human blastocysts to establish hESC lines. The cell lines were characterized and maintained using either serum-free or serum-containing media to compare their morphology, Oct-4 expression, apoptosis and growth speed. Differentiation of these lines was evaluated by the morphology and the expression of genes belonging to the three embryonic germ layers and the germ cell

lineage. RESULTS: Three hESC lines were established, and they grew at similar speed in both media (serum-containing or serum-free), but hESC cultured in serum-containing medium yielded significantly higher percentages of morphologically good colonies and cells expressing Oct-4. These cell lines differentiated spontaneously in vitro into cells expressing markers belonging to all three embryonic germ layers and germ cell markers, including c-Kit, STELLA, VASA and growth differentiation factor 9 (GDF9), in directly adherent culture. CONCLUSIONS: Three hESC lines with Taiwanese ancestry have been established, and they retain the in vitro differentiation potential with or without embryoid body (EB) formation. The data support that hESC may be capable of differentiation into germ cells although further confirmation is needed. It is also suggested that strategies such as stepwise adaptation will be needed before implementing a serum-free culture condition for hESC lines that have previously been derived in a medium containing serum.

Chen, Y. M., Z. W. Du, et al. (2005). "Molecular cloning and functional analysis of ESGP, an embryonic stem cell and germ cell specific protein." Acta Biochim Biophys Sin (Shanghai) **37**(12): 789-96.

Several putative Oct-4 downstream genes from mouse embryonic stem (ES) cells have been identified using the suppression-subtractive hybridization method. In this study, one of the novel genes encoding an ES cell and germ cell specific protein (ESGP) was cloned by rapid amplification of cDNA ends. ESGP contains 801 bp encoding an 84 amino acid small protein and has no significant homology to any known genes. There is a signal peptide at the N-terminal of ESGP protein as predicted by SeqWeb (GCG) (SeqWeb version 2.0.2, http://gcg.biosino.org:8080/). The result of immunofluorescence assay suggested that ESGP might encode a secretory protein. The expression pattern of ESGP is consistent with the expression of Oct-4 during embryonic development. ESGP protein was detected in fertilized oocyte, from 3.5 day postcoital (dpc) blastocyst to 17.5 dpc embryo, and was only detected in testis and ovary tissues in adult. In vitro, ESGP was only expressed in pluripotent cell lines, such as embryonic stem cells, embryonic caoma cells and embryonic germ cells, but not in their differentiated progenies. Despite its specific expression, forced expression of ESGP is not indispensable for the effect of Oct-4 on ES cell self-renewal, and does not affect the differentiation to three germ layers.

Clark, A. T. (2007). "Establishment and differentiation of human embryonic stem cell derived germ cells." Soc Reprod Fertil Suppl **63**: 77-86.

Germ cells are absolutely essential for fertility. Aberrant germ cell development can result in abnormal gonadal function, incomplete embryogenesis and infertility, or germ cell tumors. Our understanding of the molecular regulation of normal germ cell development in mammals has progressed significantly due to the utility of the mouse as a genetic model system. However, the molecular regulation of human germ cell development is almost completely unknown due to the historical lack of a malleable model. The purpose of this review is to compare the cell-based events leading up to the specification of the germ cell lineage in both mice and humans and to discuss some of the key signaling pathways that have recently been identified, which regulate germ cell specification. In addition, the new cell-based models for differentiating germ cells from both mouse and human embryonic stem cells (ESCs) will be summarized.

Clark, A. T. and R. A. Reijo Pera (2006). "Modeling human germ cell development with embryonic stem cells." Regen Med **1**(1): 85-93.

There has previously been no robust cell-based model for examining the genetic and epigenetic mechanisms of human germ cell formation. Human embryonic stem cells (hESCs) could potentially fill this need, as all cell types analyzed to date (including mature germ cells) can be identified by marker analysis during hESC differentiation. Furthermore, hESCs could also be used to differentiate mature female germ cells (oocytes) in culture as an alternate reprogramming cell for somatic cell nuclear transfer. However, to differentiate and isolate a functional germ cell from hESCs, the mechanisms that regulate germ cell formation need to be understood. The purpose of this review is to summarize the current understanding of the earliest events in human germ cell formation and to describe some of the known genetic pathways that regulate germ cell specification and development in the mouse. Finally, the current literature on the formation of germ cells from ESCs will be described.

Connolly, R. M. and J. A. McCaffrey (2009). "High-dose chemotherapy plus stem cell transplantation in advanced germ cell cancer: a review." Eur Urol **56**(1): 57-64.

CONTEXT: High-dose chemotherapy (HDCT) with stem cell transplantation (SCT) has been investigated as a treatment strategy for advanced germ cell cancer (GCC) for >2 decades. In an effort to improve on the overall cure rates of 80% achievable

with conventional chemotherapy, researchers have investigated this therapeutic option as a first-line therapy for those with poor-prognosis disease and as a salvage therapy for those with relapsed or refractory disease. OBJECTIVE: The primary objective of this review is to define the role of HDCT plus SCT in advanced GCC. Prognostic indicators for this group of patients are also presented. EVIDENCE ACQUISITION: A Medline search of English-language literature was performed to identify studies published in the last 20 yr relating to the use of HDCT plus SCT in advanced GCC. Phase 1, phase 2, and phase 3 trials were included, as were retrospective reviews and meta-analyses. EVIDENCE SYNTHESIS: Phase 2 trials investigating HDCT plus SCT as a therapeutic option for advanced germ cell cancer have indicated a survival advantage over conventional chemotherapy. This has not been confirmed in the phase 3 setting. Alternative chemotherapeutic strategies and options following failure of HDCT plus SCT are discussed. CONCLUSIONS: Studies to date have not indicated a survival advantage for the use of HDCT plus SCT in advanced germ cell cancer. Many questions, however, remain unanswered, and further research is required to identify whether optimising the strategy of HDCT plus SCT will improve outcome in this predominantly young group of patients.

Damjanov, I. (2004). "From stem cells to germ cell tumors and back." Verh Dtsch Ges Pathol **88**: 39-44.

Germ cell tumors originate from ovarian, testicular and extragonadal germ cells. Tumor stem cells can retain most of the features of germ cells and form seminomas or dysgerminomas or, transform into developmentally pluripotent embryonic stem cells and give rise to teratomas or teratocarcinomas. Similar tumors can be experimentally produced in mice from early mouse embryos transplanted to extrauterine sites. The malignant stem cells of teratocarcinomas, called in analogy with their human counterparts embryonal carcinoma (EC), can be isolated and grown in culture and or propagated indefinitely by isotransplantation in syngeneic inbred mice. When injected into the blastocyst, i.e., the embryonic environment from which they have been originally isolated, EC cells lose their malignancy and become benign, participating in the normal development of the injected blastocyst. Injection of EC cells into blastocysts has been used to generate transgenic mice. Developmentally pluripotent non-neoplastic embryonic stem (ES) cells can be produced from mouse blastocysts cultured in vitro. These cells are developmentally similar to EC cells. In contrast to EC cells, ES cells injected into adult mice do not produce teratocarcinomas but only teratomas. Similar ES cells were produced from human blastocysts cultured in vitro. Human ES injected into nude mice produce teratomas composed of various somatic tissues. Human ES cells resemble mouse ES cells, but differ from human EC cells. Like their mouse equivalents, human ES cells could be used for generating experimental models of various diseases and, hopefully, for cell therapy in not so distant future.

Davies, E. L. and M. T. Fuller (2008). "Regulation of self-renewal and differentiation in adult stem cell lineages: lessons from the Drosophila male germ line." Cold Spring Harb Symp Quant Biol **73**: 137-45.

The ability to identify stem cells and trace their descendants in vivo has yielded insights into how self-renewal, proliferation, and differentiation are regulated in adult stem cell lineages. Analysis of male germ-line stem cells in Drosophila has revealed the importance of local signals from the microenvironment, the stem cell niche, in controlling stem cell behavior. Germ-line stem cells physically attach to the niche via localized adherens junctions that provide a polarity cue for orientation of centrosomes in interphase and the spindle in mitosis. As a result, stem cells divide asymmetrically: One daughter inherits attachment to the niche and remains within its embrace, whereas the other is displaced away and initiates differentiation. Strikingly, much as leukemia inhibitory factor (LIF) and transforming growth factor-beta (TGF-beta) signaling maintain mouse embryonic stem (ES) cells, maintenance of stem cell state in the Drosophila male germ line is regulated by cytokine-like signals from hub cells that activate the transcription factor STAT (signal transducer and activator of transcription) and TGF-beta class signals from surrounding support cells that repress expression of a key differentiation factor. Surprisingly, transit-amplifying cells can revert to the stem cell state if they reoccupy the niche. Upon cessation of mitosis and the switch to terminal differentiation, germ cells express cell-type- and stage-specific transcription machinery components that drive expression of terminal differentiation genes, in part by removing Polycomb transcriptional silencing machinery.

De Felici, M., A. Di Carlo, et al. (1996). "Role of stem cell factor in somatic-germ cell interactions during prenatal oogenesis." Zygote **4**(4): 349-51.

De Giorgi, U., G. Rosti, et al. (2002). "The status of high-dose chemotherapy with hematopoietic stem cell transplantation in germ cell tumor patients." Haematologica **87**(1): 95-104.

BACKGROUND AND OBJECTIVES: Germ cell tumors (GCTs) are very chemosensitive

cancers, in which high-dose chemotherapy (HDCT) has been investigated as salvage therapy or as first-line treatment in poor prognosis patients. This paper presents an update of available information in order to define the status of HDCT in GCT patients. INFORMATION SOURCES: The authors have been working in this field, contributing to international clinical trials and to peer-reviewed journals with original papers. The material examined in this review includes articles published in journals covered by MedLine, reviews from journals with high impact factor, and unpublished data from the European Group for Blood and Marrow Transplantation (EBMT) registry. STATE OF THE ART AND PERSPECTIVES: The delineation of prognostic factors associated with a poor probability of survival after HDCT contributed to the selection of patients who are likely to get an advantage from HDCT and those who should be spared from dose-intensive treatment. HDCT as first-line therapy for poor prognosis GCT (IGCCCG classification), and in a salvage setting in good risk GCT (prognostic index from Beyer et al.77), has been associated with a very high rate of complete remissions and long-term disease-free survivors. However, it is important to wait for the results of ongoing randomized trials for the validation of these findings. Other strategies are required for patients with refractory GCTs. Several new treatment options are currently emerging for this subset of patients.

de Jong, J. and L. H. Looijenga (2006). "Stem cell marker OCT3/4 in tumor biology and germ cell tumor diagnostics: history and future." Crit Rev Oncog **12**(3-4): 171-203.

The transcription factor OCT3/4 (also known as POU5F1 and Oct4) is regarded as one of the key regulators of pluripotency. Expression in nonmalignant cells is restricted to the pluripotent cells in the embryo and the primordial germ cells that will pass pluripotency to future generations via the gametes. Although major progress has been made in successfully identifying other players in the pluripotency network by using high-throughput screening methods, the exact mechanisms involved in regulation of OCT3/4 in vivo remain largely to be elucidated. In human tumors, OCT3/4 is the most informative marker in germ cell tumors diagnostics and is expressed in the precursor lesions gonadoblastoma and carcinoma in situ, as well as in invasive embryonal carcinoma and seminomatous tumors. Currently, the application of OCT3/4 for screening in high-risk patient populations is the novel focus of study. This article reviews OCT3/4 expression in normal development and germ cell tumors. In addition, expression in adult tissues and nongerm cell tumor malignancies in relation to splice variants and pseudogenes is discussed. An overview of the upstream and downstream factors in the OCT3/4 pathway as well as the epigenetic regulation of the gene is summarized and the possible role in oncogenesis considered.

Dolci, S., L. Levati, et al. (2002). "Stem cell factor activates telomerase in mouse mitotic spermatogonia and in primordial germ cells." J Cell Sci **115**(Pt 8): 1643-9.

The discovery of sterility in the descendants of telomerasenull mutant mice, owing to the lack of spermatogonia proliferation, has drawn attention to the role of telomerase activity in mouse spermatogenesis. Since spermatogonia proliferation is under Kitl control, we explored its possible role in the regulation of telomerase activity. We show that Kitl induces telomerase activity in mitotic spermatogonia and increases the mRNA levels of both the catalytic subunit form and the telomerase RNA template. The increase of telomerase activity by Kitl is blocked by the presence of the PI3K inhibitor LY294002. Kit-positive proliferating male primordial germ cells (PGCs) show low levels of telomerase activity, but they increase telomerase activity upon Kitl stimulation. Diplotene-arrested growing oocytes that reexpress Kit do not increase telomerase activity upon Kitl stimulation. Our data suggest that the induction of telomerase by Kitl may contribute to the self-renewing potential of male germ cells and of PGCs.

Dolci, S., M. Pesce, et al. (1993). "Combined action of stem cell factor, leukemia inhibitory factor, and cAMP on in vitro proliferation of mouse primordial germ cells." Mol Reprod Dev **35**(2): 134-9.

In the present paper we investigated the effects of stem cell factor/mastocyte growth factor (SCF/MGF), leukemia inhibitory factor/differentiating inhibitory activity (LIF/DIA) (two growth factors known to affect primordial germ cell growth in vitro) and forskolin (FRSK) (an activator of adenylate cyclase in many cell types) alone or in combination on the survival and proliferation of primordial germ cells (PGCs) obtained from 8.5, 10.5, and 11.5 days post coitum (dpc) mouse embryos and cultured without pre-formed cell feeder layers. The results showed that both at 1 and 3 days of culture the addition of 100 ng/ml SCF, 20 microM FRSK, or in some instances 20 ng/ml LIF alone caused a significant increase of PGC number as compared with controls. The highest effects were obtained when SCF and/or LIF were used together with FRSK. Moreover, we found that FRSK elevated cAMP levels in purified 11.5 dpc PGCs and that this compound, but not SCF and LIF, stimulated PGC proliferation, as assessed by 5-bromo-2'-

deoxyuridin (BrdU) incorporation. These results suggest a mechanism of combined action of cAMP with SCF and/or LIF in the control of proliferation of mouse PGCs in vitro.

Donovan, P. J. (1998). "The germ cell--the mother of all stem cells." Int J Dev Biol **42**(7): 1043-50.

The germline, uniquely amongst the lineages of the embryo, carries the genome from generation to generation and is therefore the only lineage which retains true developmental totipotency. Paradoxically, when mouse primordial germ cells (PGCs) are introduced into a host blastocyst, they do not contribute to either the germline or the soma, suggesting that they are restricted in developmental potency. Conversely, in vivo PGCs give rise to embryonal carcinoma (EC) cells, the pluripotent stem cells of teratomas, benign tumors containing derivatives of the three primary germ layers. Similarly, PGCs can be converted in vitro into embryonic germ (EG) cells, pluripotent stem cells capable of giving rise to somatic and germline chimeras. The ability of PGCs to form EC cells in vivo and EG cells in vitro suggests that developmental potency of PGCs is regulateable. The molecular mechanisms controlling PGC growth and differentiation are gradually being elucidated through the characterization of sterile mutants and through the use of in vitro culture systems. Understanding how a PGC can give rise to a pluripotent stem cell could give significant insights into the regulation of developmental totipotency as well as having important implications for male fertility and the etiology of testicular cancer.

Ebata, K. T., X. Zhang, et al. (2005). "Expression patterns of cell-surface molecules on male germ line stem cells during postnatal mouse development." Mol Reprod Dev **72**(2): 171-81.

Spermatogonial stem cells (SSCs) are stem cells of the male germ line. In mice, SSCs are quiescent at birth but actively proliferate during the first postnatal week, while they rarely divide in adult, suggesting an age-dependent difference in SSC characteristics. As an approach to evaluate this possibility, we studied the expression pattern of cell-surface molecules on neonatal, pup, and adult mouse SSCs. Using immunomagnetic cell sorting, testis cells were selected for the expression of alpha(6) integrin, alpha(v) integrin, c-kit receptor tyrosine kinase (Kit), or a binding subunit of glial-cell-line-derived neurotrophic factor (GDNF) receptor, GFRalpha1. Selected cells were assayed for their stem cell activity using spermatogonial transplantation. The results showed that SSCs expressed alpha(6) integrin, but not alpha(v) integrin and Kit, regardless of age. The SSC activity in pup GFRalpha1(+) cells was higher than that in adult and neonatal cells, indicating that the expression pattern of GFRalpha1 varied age-dependently. To evaluate if SSCs show an age-dependent difference in their response to GDNF, we cultured highly enriched pup and adult SSCs with GDNF: we could not observe such an age-dependent difference in vitro. In addition, we failed to immunologically detect the expression of two types of GDNF receptor signaling subunits on SSCs. These results indicate that SSCs may change the expression patterns of cell-surface molecules during postnatal development, and suggest that GDNF receptor molecules may not be abundantly or specifically expressed in the in vivo population of mouse SSCs.

Einhorn, L. H., S. D. Williams, et al. (2007). "High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors." N Engl J Med **357**(4): 340-8.

BACKGROUND: Metastatic testicular tumors that have not been successfully treated by means of initial chemotherapy are potentially curable with salvage chemotherapy. METHODS: We conducted a retrospective review of 184 consecutive patients with metastatic testicular cancer that had progressed after they received cisplatin-containing combination chemotherapy. We gave 173 patients two consecutive courses of high-dose chemotherapy consisting of 700 mg of carboplatin per square meter of body-surface area and 750 mg of etoposide per square meter, each for 3 consecutive days, and each followed by an infusion of autologous peripheral-blood hematopoietic stem cells; the other 11 patients received a single course of this treatment. In 110 patients, cytoreduction with one or two courses of vinblastine plus ifosfamide plus cisplatin preceded the high-dose chemotherapy. RESULTS: Of the 184 patients, 116 had complete remission of disease without relapse during a median follow-up of 48 months (range, 14 to 118). Of the 135 patients who received the treatment as second-line therapy, 94 were disease-free during follow-up; 22 of 49 patients who received treatment as third-line or later therapy were disease-free. Of 40 patients with cancer that was refractory to standard-dose platinum, 18 were disease-free. A total of 98 of 144 patients who had platinum-sensitive disease were disease-free, and 26 of 35 patients with seminoma and 90 of 149 patients with nonseminomatous germ-cell tumors were disease-free. Among the 184 patients, there were three drug-related deaths during therapy. Acute leukemia developed in three additional patients after therapy. CONCLUSIONS: Testicular tumors are potentially curable by means of high-dose chemotherapy plus hematopoietic stem-cell rescue, even when this

regimen is used as third-line or later therapy or in patients with platinum-refractory disease.

el Badri, N. S. and R. A. Good (1993). "Lymphohemopoietic reconstitution using wheat germ agglutinin-positive hemopoietic stem cell transplantation within but not across the major histocompatibility antigen barriers." Proc Natl Acad Sci U S A **90**(14): 6681-5.

Nonadherent (NA), low density (LD), wheat germ agglutinin-positive (WGA+) murine hemopoietic stem cell-enriched preparations (HSCPs) were tested for the capability to reconstitute lymphohemopoietic elements in lethally irradiated mice. HSCPs from BALB/c mice reconstituted lethally irradiated, major histocompatibility complex (MHC)-matched DBA/2 mice to normal histology of the thymus and spleen and normal humoral and cellular immune functions. By contrast, lethally irradiated B6 mice could not be reconstituted after transplantation with NA, LD, WGA+ cells from MHC-mismatched BALB/c mice. We previously observed frequent survival, stable chimerism, and normally vigorous functioning immune systems in B6 mice transplanted with T-cell-depleted bone marrow from both BALB/c and B6 donors. To extend these findings to a stem cell transplantation system, lethally irradiated B6 mice were transplanted with NA, LD, WGA+ cells from both BALB/c and B6 mice. These mixed stem cell-enriched preparations did not reconstitute the lethally irradiated, MHC-mismatched mice. By contrast, such HSCPs from BALB/c plus DBA/2 into DBA/2 mice reconstituted the hematologic and lymphoid tissues and functional immune systems when the donor and the recipient pairs were matched at MHC and mismatched at multiminor histocompatibility barriers. These purified blood progenitors thus appear to lack certain cells/factors essential for engraftment and reconstituting recipients in a fully allogeneic environment.

El-Helw, L. M., J. D. Naik, et al. (2006). "High-dose chemotherapy with haematopoietic stem-cell support in patients with poor prognosis, relapsed or refractory germ cell tumours." BJU Int **98**(3): 519-25.

OBJECTIVE: To report our experience of high-dose chemotherapy (HDC) with haematopoietic stem-cell support (HSC) in patients with poor risk, relapsed or refractory germ cell tumours (GCTs), as this treatment might offer effective salvage for patients with disseminated GCTs. PATIENTS AND METHODS: We retrospectively reviewed the medical records and database for 33 patients with GCT who were treated with HDC with HSC in our centres. RESULTS: Thirty-three patients were treated with either one or two cycles of carboplatin and etoposide-based HDC with HSC support, between March 1990 and October 2003. Twenty-six patients (79%) had nonseminomatous GCT, six seminoma (18%), and one (3%) a combined seminoma and teratoma. Twenty patients (60%) had previously had a clinical complete response after previous chemotherapy +/- surgery for residual disease. Most patients were treated with HDC for relapsing (49%) or relative refractory disease (30%), but seven (21%) had HDC in the first partial remission. The complete response rate to HDC was 58%. There were two treatment-related deaths (6%). As of April 2005, 18 patients were alive and disease-free with a median (range) follow-up of 72 (0.5-174) months. The 5-year overall and progression-free survival probabilities were 57% and 56%, respectively. The median (range) times to absolute neutrophil count recovery (> or = 500/microL) were 13 (9-24) and 12 (10-15) days, and for platelet count recovery (> or = 20,000/microL) were 16 (7-50) and 13 (11-17) days, in the first and second cycles, respectively. CONCLUSION: The role of HDC with HSC support in metastatic GCTs remains controversial, and data from randomized controlled trials are needed. Our experience suggests that, in selected patients, this approach might be a useful form of salvage therapy.

Erickson, B. H. and M. J. Blend (1976). "Response of the Sertoli cell and stem germ cell to 60Co gamma-radiation (dose and dose rate) in testes of immature rats." Biol Reprod **14**(5): 641-50.

Flechon, A., P. Biron, et al. (1999). "High-dose chemotherapy with hematopoietic stem-cell support in germ-cell tumor patient treatment: the French experience." Int J Cancer **83**(6): 844-7.

Germ-cell tumors (GCTs) are very chemosensitive and highly curable cancers. For the small proportion of patients who fail conventional chemotherapy (CT), high-dose CT (HDCT) was introduced in France and elsewhere in 1982-1984. We report here on the French experience with HDCT in GCTs. At the Centre Leon Berard, 75 patients were treated with HDCT between 1982 and 1996. Patients received HDCT in 2 different settings: 46 in consolidation of first-line treatment or in incomplete response, 29 in salvage of relapse or refractory disease. The most common regimens of HDCT were the combination of etoposide, double-dose cisplatin and either ifosfamide (VIC regimen, n = 46) or cyclophosphamide (PEC regimen, n = 9) and the combination of carboplatin, etoposide and cyclophosphamide (Carbo-PEC regimen, n = 17). Seven patients died of toxicity. The median follow-up was 42 months. Forty-five of 75 patients are alive and

free of disease at long term, 2 of whom had refractory disease. The median time to recovery of a granulocyte count > or = 0.5 x 10(9)/l and a platelet count > or = 25 x 10(9)/l was 14 and 11 days, respectively. The French development was based on double-dose cisplatin until the results of the French randomized trial, which showed no advantage of HDCT in the first-line treatment of poor-risk group patients. Then carboplatin was associated with etoposide and cyclophosphamide in a phase I trial. A European randomized trial, which studies the role of HDCT in the first-line salvage treatment of non-refractory disease, is ongoing. So far, HDCT is not a standard treatment of GCT.

Forstemann, K., Y. Tomari, et al. (2005). "Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein." PLoS Biol **3**(7): e236.

microRNAs (miRNAs) are single-stranded, 21- to 23-nucleotide cellular RNAs that control the expression of cognate target genes. Primary miRNA (pri-miRNA) transcripts are transformed to mature miRNA by the successive actions of two RNase III endonucleases. Drosha converts pri-miRNA transcripts to precursor miRNA (pre-miRNA); Dicer, in turn, converts pre-miRNA to mature miRNA. Here, we show that normal processing of Drosophila pre-miRNAs by Dicer-1 requires the double-stranded RNA-binding domain (dsRBD) protein Loquacious (Loqs), a homolog of human TRBP, a protein first identified as binding the HIV trans-activator RNA (TAR). Efficient miRNA-directed silencing of a reporter transgene, complete repression of white by a dsRNA trigger, and silencing of the endogenous Stellate locus by Suppressor of Stellate, all require Loqs. In loqs(f00791) mutant ovaries, germ-line stem cells are not appropriately maintained. Loqs associates with Dcr-1, the Drosophila RNase III enzyme that processes pre-miRNA into mature miRNA. Thus, every known Drosophila RNase-III endonuclease is paired with a dsRBD protein that facilitates its function in small RNA biogenesis.

Gasser, T. (2007). "Words of wisdom. Re: High-dose chemotherapy and stem-cell rescue for metastatic germ-cell tumors." Eur Urol **52**(6): 1795-6.

Gilboa, L., A. Forbes, et al. (2003). "Germ line stem cell differentiation in Drosophila requires gap junctions and proceeds via an intermediate state." Development **130**(26): 6625-34.

Gap junctions coordinate processes ranging from muscle contraction to ovarian follicle development. Here we show that the gap junction protein Zero population growth (Zpg) is required for germ cell differentiation in the Drosophila ovary. In the absence of Zpg the stem cell daughter destined to differentiate dies. The zpg phenotype is novel, and we used this phenotype to genetically dissect the process of stem cell maintenance and differentiation. Our findings suggest that germ line stem cells differentiate upon losing contact with their niche, that gap junction mediated cell-cell interactions are required for germ cell differentiation, and that in Drosophila germ line stem cell differentiation to a cystoblast is gradual.

Gilboa, L. and R. Lehmann (2004). "Repression of primordial germ cell differentiation parallels germ line stem cell maintenance." Curr Biol **14**(11): 981-6.

In Drosophila, primordial germ cells (PGCs) are set aside from somatic cells and subsequently migrate through the embryo and associate with somatic gonadal cells to form the embryonic gonad. During larval stages, PGCs proliferate in the female gonad, and a subset of PGCs are selected at late larval stages to become germ line stem cells (GSCs), the source of continuous egg production throughout adulthood. However, the degree of similarity between PGCs and the self-renewing GSCs is unclear. Here we show that many of the genes that are required for GSC maintenance in adults are also required to prevent precocious differentiation of PGCs within the larval ovary. We show that following overexpression of the GSC-differentiation gene bag of marbles (bam), PGCs differentiate to form cysts without becoming GSCs. Furthermore, PGCs that are mutant for nanos (nos), pumilio (pum) or for signaling components of the decapentaplegic (dpp) pathway also differentiate. The similarity in the genes necessary for GSC maintenance and the repression of PGC differentiation suggest that PGCs and GSCs may be functionally equivalent and that the larval gonad functions as a "PGC niche".

Goel, S., M. Fujihara, et al. (2008). "Expression of NANOG, but not POU5F1, points to the stem cell potential of primitive germ cells in neonatal pig testis." Reproduction **135**(6): 785-95.

Gonocytes are primitive germ cells that are present in the neonatal testis and are committed to male germline development. Gonocytes differentiate to spermatogonia, which establish and maintain spermatogenesis in the postnatal testis. However, it is unknown whether large animal species have pluripotency-specific proteins in the testis. Nanog and Pou5f1 (Oct3/4) have been identified as transcription factors essential for maintaining pluripotency of embryonic stem cells in mice. Here, we show that NANOG protein was expressed in the germ cells of neonatal pig testes, but was progressively lost with age. NANOG was expressed in most of the lectin

Dolichos biflorus agglutinin- and ZBTB16-positive gonocytes, which are known gonocyte-specific markers in pigs. NANOG was also expressed in Sertoli and interstitial cells of neonatal testes. Interestingly, POU5F1 expression was not detected at either the transcript or the protein level in neonatal pig testis. In the prepubertal testis, NANOG and POU5F1 proteins were primarily detected in differentiated germ cells, such as spermatocytes and spermatids, and rarely in undifferentiated spermatogonia. By using a testis transplantation assay, we found that germ cells from 2- to 4-day-old pigs could colonize and proliferate in the testes of the recipient mice, suggesting that primitive germ cells from neonatal pig testes have stem cell potential.

Gopalan, A., D. Dhall, et al. (2009). "Testicular mixed germ cell tumors: a morphological and immunohistochemical study using stem cell markers, OCT3/4, SOX2 and GDF3, with emphasis on morphologically difficult-to-classify areas." Mod Pathol **22**(8): 1066-74.

Stem cell markers, OCT3/4, and more recently SOX2 and growth differentiation factor 3 (GDF3), have been reported to be expressed variably in germ cell tumors. We investigated the immunohistochemical expression of these markers in different testicular germ cell tumors, and their utility in the differential diagnosis of morphologically difficult-to-classify components of these tumors. A total of 50 mixed testicular germ cell tumors, 43 also containing difficult-to-classify areas, were studied. In these areas, multiple morphological parameters were noted, and high-grade nuclear details similar to typical embryonal carcinoma were considered 'embryonal carcinoma-like high-grade'. Immunohistochemical staining for OCT3/4, c-kit, CD30, SOX2, and GDF3 was performed and graded in each component as 0, negative; 1+, 1-25%; 2+, 26-50%; and 3+, >50% positive staining cells. The different components identified in these tumors were seminoma (8), embryonal carcinoma (50), yolk sac tumor (40), teratoma (40), choriocarcinoma (3) and intra-tubular germ cell neoplasia, unclassified (35). By immunohistochemistry, the staining patterns were OCT3/4 -3+, all seminomas, embryonal carcinomas and intra-tubular germ cell neoplasia; SOX2 -3+, all embryonal carcinomas and -2 to 3+, 11/14 (79%) primitive neuroectodermal components in immature teratomas; GDF3 -2 to 3+, all yolk sac tumors, seminomas and intra-tubular germ cell neoplasia and 1 to 2+, 40/50 embryonal carcinomas. A total of 34/43 (79%) of difficult-to-classify areas stained 3+ for OCT3/4, CD30, and SOX2, similar to embryonal carcinoma. Among these areas, only 'embryonal carcinoma-like high-grade' nuclear details were significantly associated with such an immunophenotype. Thus, SOX2 is expressed in embryonal carcinoma and primitive neuroectoderm of teratoma, and unlike OCT3/4, not in intra-tubular germ cell neoplasia and seminoma. Therefore, it may be useful in the distinction of seminoma from embryonal carcinoma, and potentially in diagnosing early carcinomatous differentiation in seminoma. GDF3 positivity, in the absence of OCT3/4 and CD30, combined with morphological features, is helpful in the diagnosis of yolk sac tumor. 'Embryonal carcinoma-like high-grade' nuclear details are the most important morphological criterion for the diagnosis of embryonal carcinoma in difficult-to-classify areas.

Gordeeva, O., R. Zinovieva, et al. (2005). "Differentiation of embryonic stem cells after transplantation into peritoneal cavity of irradiated mice and expression of specific germ cell genes in pluripotent cells." Transplant Proc **37**(1): 295-8.

Permanent embryonic stem cell lines (ES cells) are considered as one of the most promising cellular sources for regenerative medicine. ES cells have a high proliferative potency and ability to differentiate into all kinds of somatic and germ cells. However, transplantation of undifferentiated ES cells into adult recipient tissue results in the formation of teratomas. To understand the mechanisms underlying self-renewal and determination of pluripotent cells, we investigated differentiation potencies of undifferentiated ES cells and differentiating embryoid bodies (EB). ES cells and EBs growing on acetate-cellulose membranes were transplanted into the peritoneal cavity of irradiated mice. Behavior and differentiation of transplanted cells were studied within 1, 2, 3, and 6 weeks after transplantation. No differences in the cell composition were found in the teratomas formed by ES cells and differentiating EBs. The pattern of expression of the genes specific for pluripotent and germ cells was studied in all types of experimental teratomas. The expression of oct4, stella, fragilis was detected in the teratomas, but nanog was not expressed. We conclude that pluripotent cells are retained in the experimental teratomas formed after transplantation of ES cells and EBs but the pattern of expression of the studied genes underwent changes.

Gordeeva, O. F., N. Y. Krasnikova, et al. (2006). "Analysis of expression of genes specific for pluripotent and primordial germ cells in human and mouse embryonic stem cell lines." Dokl Biol Sci **406**: 115-8.

Hanaoka, K., M. Hayasaka, et al. (1991). "The stem cells of a primordial germ cell-derived

teratocarcinoma have the ability to form viable mouse chimeras." Differentiation **48**(2): 83-7.

A euploid testicular teratocarcinoma line, STT-3, has been established from a tumor spontaneously occurring in the testis of a 129/Sv-ter male. Developmental ability of the STT-3 stem cells was tested by injecting these cells into mouse blastocysts. The frequency and the extent of chimerism were examined in mid-gestational fetuses and in live-born mice. STT-3 stem cells form viable chimeras at a high rate and differentiate into normal tissues. This is the first reported testicular teratocarcinoma-derived stem line with a proven capacity to form viable chimeric mice upon injection into the blastocysts.

Hara, I., H. Miyake, et al. (2006). "Feasibility and usefulness of high-dose chemotherapy (high-dose ifosfamide, carboplatin and etoposide) combined with peripheral blood stem cell transplantation for male germ cell tumor: a single-institute experience." Anticancer Drugs **17**(9): 1057-66.

Although the usefulness of high-dose chemotherapy with peripheral blood stem cell transplantation for advanced germ cell tumor is still under evaluation in phase III randomized controlled studies, this approach is currently used as one treatment option for relapsed or advanced male germ cell tumor. Clinical outcomes of high-dose chemotherapy for a single institute from Japan are presented herein. We administered 63 courses of high-dose ifosfamide, carboplatin and etoposide chemotherapy (1250 mg/m carboplatin; 1500 mg/m etoposide; 7.5 g/m ifosfamide) to 34 men with germ cell tumors. Of these, 27 patients underwent high-dose ifosfamide, carboplatin and etoposide as first-line therapy after 2-3 courses of conventional bleomycin, etoposide and cisplatin chemotherapy, and seven patients underwent high-dose ifosfamide, carboplatin and etoposide for relapsed germ cell tumor. Peripheral blood stem cells were harvested during previous chemotherapy and sufficient CD34 cells were harvested for transplantation. Although all patients experienced grade 4 hemotoxicity, leukocyte counts recovered to above 1000/mul within 8-11 days after peripheral blood stem cell transplantation. No treatment-related deaths occurred. After a mean follow-up of 45 months (range 12-118 months), 23 of 34 patients (67.6%) remained disease-free. High-dose ifosfamide, carboplatin and etoposide could be performed safely, and could offer an effective means of treating advanced or refractory germ cell tumors in men.

Hara, I., Y. Yamada, et al. (2005). "High dose chemotherapy including paclitaxel (T-ICE) combined with peripheral blood stem cell transplantation for male germ cell tumor. Preliminary report." Int J Urol **12**(12): 1074-8.

AIM: To evaluate the feasibility and usefulness of high dose chemotherapy including paclitaxel (T-ICE) combined with peripheral blood stem cell transplantation (PBSCT) for male germ cell tumor. METHODS: Five male patients with advanced germ cell tumor underwent 1-6 courses of high dose chemotherapy including paclitaxel (T-ICE; 175 mg/m2 of paclitaxel, 1250 mg/m2 of carboplatin, 1500 mg/m2 of etoposide and 7.5 g/m2 of ifosfamide) with PBSCT after 2-3 courses of induction chemotherapy (PEB or VIP). RESULTS: In all patients, serum marker levels decreased to within the normal range by T-ICE. Two patients underwent resection of residual tumor. In one patient, viable cancer cells were detected in resected lymph node tissue and adjuvant chemotherapy was then performed. Although the follow-up period was short (7-15.5 months), four of the five patients (80%) showed no evidence of recurrent disease. No significant differences in side-effects were noted between T-ICE and conventional high dose ICE, which was previously performed in 39 patients at the Division of Urology, Kobe University Graduate School of Medicine, Japan. CONCLUSIONS: High dose chemotherapy, including T-ICE, combined with PBSCT showed an almost identical degree of side-effects as seen in previous high dose chemotherapy without paclitaxel. Although 80% of the patients showed no evidence of disease so far, the efficacy of T-ICE should be evaluated with more patients and longer follow up.

Hara, I., Y. Yamada, et al. (2002). "Detection of beta-human chorionic gonadotropin expressing cells by nested reverse transcriptase-polymerase chain reaction in the peripheral blood stem cells of patients with advanced germ cell tumor." J Urol **167**(3): 1487-91.

PURPOSE: We investigated whether peripheral blood stem cells (PBSCs) were contaminated by tumor cells. MATERIALS AND METHODS: A total of 13 patients with advanced testicular cancer underwent PBSC transplantation at our institute. Nested reverse transcriptase-polymerase chain reaction using primers specific for beta-human chorionic gonadotropin (beta-HCG) or alpha-fetoprotein (AFP) detected 1 beta-HCG or AFP producing cell in 1 x 10(6) PBSCs. RESULTS: Although AFP messenger (m) RNA was not identified in any of the 13 patients, beta-HCG mRNA was detected in 7. All patients with beta-HCG mRNA in PBSCs had elevated serum beta-HCG before treatment. Three patients with a normal beta-HCG range before treatment did not have beta-HCG mRNA

in the PBSCs. The serum beta-HCG level in the patients whose PBSCs were contaminated with tumor cells was significantly higher than in patients whose PBSCs contained no tumor cells. Four of the 7 patients with beta-HCG mRNA in PBSCs eventually died of cancer, whereas those whose PBSCs were not contaminated with tumor cells survived without disease. CONCLUSIONS: Patients with elevated serum beta-HCG levels are likely to have PBSCs contaminated by tumor cells. Moreover, the prognosis of patients with tumor cells in the PBSCs is poor.

Hara, I., Y. Yamada, et al. (1999). "Clinical outcome of high-dose chemotherapy combined with peripheral blood stem cell transplantation for male germ cell tumors." Anticancer Drugs **10**(8): 711-8.

Peripheral blood stem cell transplantation (PBSCT) is widely performed currently instead of bone marrow transplantation (BMT) because bone marrow reconstruction is better and the procedure is less invasive. We applied 26 courses of high-dose chemotherapy (1250 mg/m2 of carboplatin, 1500 mg/m2 of etoposide and 7.5 g/m2 of ifosfamide) to 14 male patients with germ cell tumors. Eleven patients underwent high-dose chemotherapy as induction after two to three courses of conventional BEP therapy. The remaining three patients had recurrent disease after conventional chemotherapies. Peripheral blood stem cells were harvested during previous chemotherapy and sufficient CD34+ cells were harvested for transplantation. Although all patients had grade 4 hematotoxicity, the white blood cell count recovered to more than 1000/microl within 8-11 days after PBSCT. No treatment-related death was found. Nine of 14 patients (64.3%) remain disease free at 18 months of median follow up time (range 12-60). We conclude that high-dose chemotherapy is a safe and effective means of treating advanced or refractory germ cell tumors in male patients.

Harrison, N. J., D. Baker, et al. (2007). "Culture adaptation of embryonic stem cells echoes germ cell malignancy." Int J Androl **30**(4): 275-81; discussion 281.

Teratocarcinomas are a subset of tumours that result from the neoplastic transformation of primordial germ cells. Such germ cell tumours (GCT) are histologically heterogeneous, reflecting a capacity for differentiation (pluripotency) of their embryonal carcinoma (EC) stem cells. However, malignant evolution of these tumours may ultimately correlate with a decrease in pluripotency, because this would tend to increase the propensity of EC cells for self-renewal. Human embryonic stem (ES) cells, derived from early blastocysts, closely resemble EC cells and, on prolonged culture in vitro, acquire progressive genetic changes that show striking similarity to those seen in GCT (e.g. gain of material from chromosome 12). In parallel, these abnormal ES cells show enhanced population growth rates and plating efficiencies, indicative of their adaptation to culture conditions. Understanding the mechanisms that drive such culture adaptation of ES cells may also provide insights into the development and progression of GCT.

Hennessy, B., J. A. McCaffrey, et al. (2002). "High dose chemotherapy and stem cell support for poor risk and recurrent nonseminomatous germ cell cancer: initial experience with sequential therapy." Ir J Med Sci **171**(3): 158-60.

BACKGROUND: Approximately 20% of patients with germ cell tumours do not respond fully to standard therapy, or relapse after treatment. The prognosis of these patients is poor with conventional chemotherapy. Preliminary data suggest that they may have a higher durable response rate with high dose chemotherapy and peripheral blood stem cell support. AIMS: To treat a group of testicular cancer patients, either with relapsed disease or with poor prognostic features initially, with high dose chemotherapy and stem cell support, and evaluate their outcome. METHODS: Five patients with testicular cancer were treated with high dose chemotherapy and stem cell support. Of these, four underwent this treatment as salvage therapy and one patient with poor prognostic features was treated as primary treatment. RESULTS: At an average follow-up of 18 months, four patients remain free of disease while one patient has developed relapse. CONCLUSION: This report provides further support for high dose chemotherapy in this setting although randomised, controlled trials are essential to clarify its use.

Hentrich, M., X. Schiel, et al. (2009). "Successful salvage high-dose chemotherapy and autologous stem-cell transplantation in HIV-related germ-cell tumor." Ann Oncol **20**(11): 1900-1.

Hermann, B. P., M. Sukhwani, et al. (2009). "Molecular dissection of the male germ cell lineage identifies putative spermatogonial stem cells in rhesus macaques." Hum Reprod **24**(7): 1704-16.

BACKGROUND: The spermatogonial stem cell (SSC) pool in the testes of non-human primates is poorly defined. METHODS: To begin characterizing SSCs in rhesus macaque testes, we employed fluorescence-activated cell sorting (FACS), a xenotransplant bioassay and immunohistochemical methods and correlated our findings with classical descriptions of germ cell nuclear morphology (i.e. A(dark) and A(pale) spermatogonia). RESULTS:

FACS analysis identified a THY-1+ fraction of rhesus testis cells that was enriched for consensus SSC markers (i.e. PLZF, GFRalpha1) and exhibited enhanced colonizing activity upon transplantation to nude mouse testes. We observed a substantial conservation of spermatogonial markers from mice to monkeys [PLZF, GFRalpha1, Neurogenin 3 (NGN3), cKIT]. Assuming that molecular characteristics correlate with function, the pool of putative SSCs (THY-1+, PLZF+, GFRalpha1+, NGN3+/-, cKIT-) comprises most A(dark) and A(pale) and is considerably larger in primates than in rodents. It is noteworthy that the majority of A(dark) and A(pale) share a common molecular phenotype, considering their distinct functional classifications as reserve and renewing stem cells, respectively. NGN3 is absent from A(dark), but is expressed by some A(pale) and may mark the transition from undifferentiated (cKIT-) to differentiating (cKIT+) spermatogonia. Finally, the pool of transit-amplifying progenitor spermatogonia (PLZF+, GFRalpha1+, NGN3+, cKIT+/-) is smaller in primates than in rodents. CONCLUSIONS These results provide an in-depth analysis of molecular characteristics of primate spermatogonia, including SSCs, and lay a foundation for future studies investigating the kinetics of spermatogonial renewal, clonal expansion and differentiation during primate spermatogenesis.

Hoei-Hansen, C. E. (2008). "Application of stem cell markers in search for neoplastic germ cells in dysgenetic gonads, extragonadal tumours, and in semen of infertile men." Cancer Treat Rev **34**(4): 348-67.

Germ cell tumours (GCTs) are a complex entity. Current areas of attention include early detection and avoidance of unnecessary over-treatment. Novel findings regarding diagnosis of GCTs located in various anatomical sites are described, particularly testicular GCTs and their common progenitor, carcinoma in situ (CIS). Recognition of CIS enables intervention before tumour development, but nevertheless, testicular GCTs are sporadically diagnosed at the pre-invasive stage where minimal treatment is necessary. As presence of CIS is asymptomatic, a simple screening method is needed when CIS is suspected (i.e. in males investigated for infertility). To develop approaches for early detection CIS gene expression studies have been performed showing many similarities with embryonic stem cells with confirmation of established markers (i.e. PLAP, OCT-3/4, KIT) and identification of novel markers (i.e. AP-2 gamma, NANOG). We have reported a very promising new approach of AP-2 gamma (or OCT3/4) based immunocytological semen analysis (specificity 93.6%, sensitivity 54.5%). Comparative studies of gonadal/extragonadal GCTs have revealed resemblance pointing towards similar, but not identical, origins. Moreover, infertility and testicular cancer are connected in the 'Testicular Dysgenesis Syndrome' and 25% of contralateral testes from testicular GCT patients harbour dysgenetic features, including impaired spermatogenesis. Thus, recent data have provided potential diagnostic tools including CIS detection in semen, microarray-based tumour classification, additional serological GCT markers, and novel stem cell markers for immunohistochemical diagnosis of gonadal and extragonadal GCTs. Many CIS candidate genes are yet uninvestigated, and information from these could increase knowledge about CIS tumour initiation/progression and be used for optimisation of a non-invasive detection method.

Hoei-Hansen, C. E., K. Almstrup, et al. (2005). "Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours." Histopathology **47**(1): 48-56.

AIMS: NANOG is a key regulator of embryonic stem cell (ESC) self-renewal and pluripotency. Our recent genome-wide gene expression profiling study of the precursor of testicular germ cell tumours, carcinoma in situ testis (CIS), showed close similarity between ESC and CIS, including high NANOG expression. In the present study we analysed the protein expression of NANOG during normal development of human testis and in a large series of neoplastic/dysgenetic specimens. METHODS AND RESULTS: We detected abundant expression of NANOG in CIS and in CIS-derived testicular tumours with marked differences; seminoma and embryonal carcinoma were strongly positive, differentiated somatic elements of teratoma were negative. We provide evidence for the fetal origin of testicular cancer as we detected strong expression of NANOG in fetal gonocytes up to gestational week 20, with subsequent down-regulation occurring earlier than for OCT-4. We detected no expression at the protein level in normal testis. CONCLUSIONS: NANOG is a new marker for testicular CIS and germ cell tumours and the high level of NANOG along with OCT-4 are determinants of the stem cell-like pluripotency of the preinvasive CIS cell. Timing of NANOG down-regulation in fetal gonocytes suggests that NANOG may act as a regulatory factor up-stream to OCT-4.

Honaramooz, A., S. Megee, et al. (2008). "Adeno-associated virus (AAV)-mediated transduction of male germ line stem cells results in transgene transmission after germ cell transplantation." Faseb J **22**(2): 374-82.

We explored whether exposure of mammalian germ line stem cells to adeno-associated virus (AAV), a gene therapy vector, would lead to stable transduction and transgene transmission. Mouse germ cells harvested from experimentally induced cryptorchid donor testes were exposed in vitro to AAV vectors carrying a GFP transgene and transplanted to germ cell-depleted syngeneic recipient testes, resulting in colonization of the recipient testes by transgenic donor cells. Mating of recipient males to wild-type females yielded 10% transgenic offspring. To broaden the approach to nonrodent species, AAV-transduced germ cells from goats were transplanted to recipient males in which endogenous germ cells had been depleted by fractionated testicular irradiation. Transgenic germ cells colonized recipient testes and produced transgenic sperm. When semen was used for in vitro fertilization (IVF), 10% of embryos were transgenic. Here, we report for the first time that AAV-mediated transduction of mammalian germ cells leads to transmission of the transgene through the male germ line. Equally important, this is also the first report of transgenesis via germ cell transplantation in a nonrodent species, a promising approach to generate transgenic large animal models for biomedical research.

Houck, W., R. Abonour, et al. (2004). "Secondary leukemias in refractory germ cell tumor patients undergoing autologous stem-cell transplantation using high-dose etoposide." J Clin Oncol **22**(11): 2155-8.

PURPOSE: To quantify the risk of secondary leukemias in relapsed testicular cancer patients undergoing autologous stem-cell transplantation with high-dose etoposide. PATIENTS AND METHODS: Single institution, retrospective study of germ cell tumor patients who underwent autologous transplantation using high-dose etoposide from 1987 to 2001. RESULTS: One hundred thirteen patients received high-dose etoposide and carboplatin followed by autologous stem-cell transplantations for germ cell tumors. Follow-up ranged from 12 to 166 months (median, 51 months). Three patients (2.6%; 95% CI, 0.55% to 7.50%) subsequently developed leukemia at an average of 16 months post-autologous transplantation (range, 11 to 21 months). All three had received tandem transplantations and had been heavily pretreated, including at least one prior cycle of etoposide. Following autologous transplantation, all three patients exhibited refractory cytopenias before developing overt leukemia. All leukemias were of myeloid lineage. One patient developed an M2 with a t(8,21) chromosomal translocation; another, an M5 with a t(11,19); and one patient exhibited an unclassified leukemia with cytogenetic abnormalities resulting in monosomy for 7p and partial monosomy of 7q. Treatment of the leukemias involved allogeneic bone marrow transplantation. CONCLUSION: High-dose chemotherapy using high-dose etoposide as therapy for relapsed germ cell tumors was associated with a 2.6% risk of developing a secondary myeloid leukemia. This figure was not significantly different from the expected rate of secondary leukemias when patients receive additional cycles of standard-dose etoposide as salvage chemotherapy for germ cell tumors. Other factors, including the use of platinum agents, may also have a role in leukemogenesis in this patient population.

Hoyer, P. E., A. G. Byskov, et al. (2005). "Stem cell factor and c-Kit in human primordial germ cells and fetal ovaries." Mol Cell Endocrinol **234**(1-2): 1-10.

The distribution of the tyrosine kinase receptor c-Kit and its ligand stem cell factor (SCF) was evaluated by immunohistochemistry in primordial germ cells (PGCs) and human embryonic gonads during weeks 5-8 of prenatal life, and fetal ovaries during weeks 9-36 of prenatal life. Distinct c-Kit and SCF staining was present in primordial germ cells in the wall of the hindgut and in the dorsal mesentery, particularly on level with the 10th thoracic columnar segment. Several PGCs were in close contact with c-Kit-negative but SCF-positive autonomic nerve fibers of the dorsal mesentery. Many fibroblasts and mesothelial cells of the dorsal mesentery were clearly stained for SCF, but not for c-Kit. Prominent c-Kit and SCF staining was present in germ cells of the embryonic gonadal anlage and in oogonia during further ovarian development. However, oocytes were either unstained or faintly stained for SCF. Oocytes not yet enclosed in follicles or present in primordial follicles were either unstained or exhibited faint cytoplasmic c-Kit staining, whereas oocytes of growing preantral follicles again showed distinct cell membrane staining which decreased during further follicular growth. Theca cells did not stain for c-Kit. Some pregranulosa cells and the first formed granulosa cells of primordial follicles were c-Kit stained. Granulosa cells of other follicles were not c-Kit stained. In the inner part of the cortex, SCF immunolabeling was detected in some pregranulosa cells surrounding cords containing germ cells and involved in formation of primordial follicles. Granulosa cells of primordial and growing follicles, including medium-sized antral follicles also revealed SCF staining. In conclusion, this first report on SCF in human PGCs and embryonic and fetal ovaries together with the c-Kit data lend substantial countenance to the notion that c-Kit and SCF play important roles during ascent of primordial germ cells towards the gonadal anlage, and during oogenesis and folliculogenesis in the human fetal ovary. We suggest

that both autocrine and paracrine mechanisms are involved in the proposed anti-apoptotic effect of the c-Kit/SCF duet while PGCs are present in the dorsal mesentery. The SCF-positive autonomic nerve fibers of the dorsal mesentery, mesothelial cells and fibroblasts may nurse and perhaps guide PGCs during their ascent.

Hua, J., S. Pan, et al. (2009). "Derivation of male germ cell-like lineage from human fetal bone marrow stem cells." Reprod Biomed Online **19**(1): 99-105.

Mesenchymal stem cells derived from bone marrow are a well characterized population of adult stem cells that can be maintained and propagated in culture for a long time with the capacity to form a variety of cell types. Reports have shown that murine and human embryonic stem cells can differentiate into primordial germ cells and then to early gametes. Evidence has indicated that some adult stem cells also have the potential to differentiate into germ cells. Currently, there are no reports on directed differentiation of human mesenchymal stem cells into germ cells. This study investigated the ability of retinoic acid and testicular extracts to induce human bone marrow stem cells (hBMSC) to differentiate into male germ cells. It was found that a small population of hBMSC seem to transdifferentiate into male germ cell-like cells. These cells expressed early germ cell markers OCT4, STELLA, NANOG and VASA, and male germ-ceil-specific markers such as DAZL, TH2, c-kit, beta(1)-integrin, ACR, PRMl, FSHR, STRA8 and SCP3, as analysed by reverse transcription-polymerase chain reaction and immunohistochemistry. These results demonstrated that hBMSC may differentiate into male germ cells and the same could be used as a potential source of cells for reproductive toxicological studies.

Hubbard, E. J. (2007). "Caenorhabditis elegans germ line: a model for stem cell biology." Dev Dyn **236**(12): 3343-57.

Like many stem cell systems, the Caenorhabditis elegans germ line contains a self-renewing germ cell population that is maintained by a niche. Although the exact cellular mechanism for self-renewal is not yet known, three recent studies shed considerable light on the cell cycle behavior of germ cells, including a support for significant and plastic renewal potential. This review brings together the results of the three recent cell-based studies, places them in the context of previous work, and discusses future perspectives for the field.

Hubbard, E. J. and R. A. Pera (2003). "A germ-cell odyssey: fate, survival, migration, stem cells and differentiation. Meeting on germ cells." EMBO Rep **4**(4): 352-7.

Ito, M., K. Kizawa, et al. (2004). "Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen." Differentiation **72**(9-10): 548-57.

The lowermost portion of the resting (telogen) follicle consists of the bulge and secondary hair germ. We previously showed that the progeny of stem cells in the bulge form the lower follicle and hair, but the relationship of the bulge cells with the secondary hair germ cells, which are also involved in the generation of the new hair at the onset of the hair growth cycle (anagen), remains unclear. Here we address whether secondary hair germ cells are derived directly from epithelial stem cells in the adjacent bulge or whether they arise from cells within the lower follicle that survive the degenerative phase of the hair cycle (catagen). We use 5-bromo-2'-deoxyuridine to label bulge cells at anagen onset, and demonstrate that the lowermost portion of the bulge collapses around the hair and forms the secondary hair germ during late catagen. During the first six days of anagen onset bulge cells proliferate and self-renew. Bulge cell proliferation at this time also generates cells that form the future secondary germ. As bulge cells form the secondary germ cells at the end of catagen, they lose expression of a biochemical marker, S100A6. Remarkably, however, following injury of bulge cells by hair depilation, progenitor cells in the secondary hair germ repopulate the bulge and re-express bulge cell markers. These findings support the notion that keratinocytes can "dedifferentiate" to a stem cell state in response to wounding, perhaps related to signals from the stem cell niche. Finally, we also present evidence that quiescent bulge cells undergo apoptosis during follicle remodeling in catagen, indicating that a subpopulation of bulge cells is not permanent.

Izadyar, F., F. Pau, et al. (2008). "Generation of multipotent cell lines from a distinct population of male germ line stem cells." Reproduction **135**(6): 771-84.

Spermatogonial stem cells (SSCs) maintain spermatogenesis by self-renewal and generation of spermatogonia committed to differentiation. Under certain in vitro conditions, SSCs from both neonatal and adult mouse testis can reportedly generate multipotent germ cell (mGC) lines that have characteristics and differentiation potential similar to embryonic stem (ES) cells. However, mGCs generated in different laboratories showed different germ cell characteristics, i.e., some retain their SSC

properties and some have lost them completely. This raises an important question: whether mGC lines have been generated from different subpopulations in the mouse testes. To unambiguously identify and track germ line stem cells, we utilized a transgenic mouse model expressing green fluorescence protein under the control of a germ cell-specific Pou5f1 (Oct4) promoter. We found two distinct populations among the germ line stem cells with regard to their expression of transcription factor Pou5f1 and c-Kit receptor. Only the POU5F1+/c-Kit+ subset of mouse germ line stem cells, when isolated from either neonatal or adult testes and cultured in a complex mixture of growth factors, generates cell lines that express pluripotent ES markers, i.e., Pou5f1, Nanog, Sox2, Rex1, Dppa5, SSEA-1, and alkaline phosphatase, exhibit high telomerase activity, and differentiate into multiple lineages, including beating cardiomyocytes, neural cells, and chondrocytes. These data clearly show the existence of two distinct populations within germ line stem cells: one destined to become SSC and the other with the ability to generate multipotent cell lines with some pluripotent characteristics. These findings raise interesting questions about the relativity of pluripotency and the plasticity of germ line stem cells.

Jathavedam, A., D. R. Feldman, et al. (2008). "Infectious complications from high-dose chemotherapy and autologous stem cell transplantation for metastatic germ cell tumors." Biol Blood Marrow Transplant **14**(5): 595-600.

High-dose chemotherapy with autologous stem cell transplantation (ASCT) is increasingly utilized in patients with relapsed and refractory germ cell tumors (GCT). Infectious complications are common after ASCT for hematologic malignancies, but their epidemiology in GCT patients has not been described. To identify infectious complications of ASCT for GCT, we conducted a retrospective study of patients treated at our institution, a tertiary-care cancer center in New York City between 1994 and 2006. Patients received ciprofloxacin prophylaxis but no routine antifungal or antiviral prophylaxis. In addition, patients were housed in shared rooms of 2 with standard precautions during hospitalizations. Overall, 107 patients with relapsed or refractory GCT were treated with 1-2 cycles of paclitaxel/ifosfamide and 1-3 cycles of high-dose carboplatin/etoposide with ASCT. Sixty (56%) of 107 patients developed 95 total infections, including 33 catheter-associated bloodstream infections. Fungal, viral, and nosocomial infections were uncommon. There were no infection-related deaths. In conclusion, serious morbidity from infection is uncommon among GCT patients receiving high-dose chemotherapy with ASCT. Isolation and aggressive antifungal and antiviral prophylaxis is not warranted in these patients.

Johnston, D. S., L. D. Russell, et al. (2001). "Murine germ cells do not require functional androgen receptors to complete spermatogenesis following spermatogonial stem cell transplantation." Endocrinology **142**(6): 2405-8.

The spermatogonial stem cell transplantation technique was employed to determine if murine germ cells require functional androgen receptors to complete qualitatively normal spermatogenesis. Testicular cells from testicular feminized mice were injected into the seminiferous tubules of azoospermic mice expressing functional androgen receptors. Recipient testes were analyzed between 110 and 200 days following transplantation. Multiple colonies of complete and qualitatively normal donor-derived spermatogenesis were seen within the seminiferous tubules of each recipient testis, demonstrating that murine germ cells do not require functional androgen receptors to complete spermatogenesis.

Kanatsu-Shinohara, M., N. Ogonuki, et al. (2003). "Allogeneic offspring produced by male germ line stem cell transplantation into infertile mouse testis." Biol Reprod **68**(1): 167-73.

The testis is one of several immune-privileged organs and is known for its unique ability to support allogeneic or xenogeneic tissue transplants. We investigated the possibility of deriving offspring from mice that underwent transplantation with allogeneic male germ line stem cells in the testis. Although mature adult mice rejected allogeneic germ cells and were infertile, offspring were obtained by intracytoplasmic germ cell injection using partially differentiated donor cells. In contrast, complete spermatogenesis occurred when allogeneic germ cells were transplanted into immature pup testes. Tolerance induction by monoclonal antibody administration allowed the pup transplant recipients to produce allogeneic offspring by natural mating, whereas no spermatozoa were found in the epididymis of untreated recipients. Thus, these results indicate that a histoincompatible recipient can serve as a "surrogate father" to propagate the genetic information of heterologous male donors.

Kasahara, T., N. Hara, et al. (2001). "Sequential detection of alphafetoprotein-bearing cells in blood stem cell fraction of germ cell tumour patients." Br J Cancer **85**(8): 1119-23.

High-dose chemotherapy with peripheral blood stem cell (PBSC) transplantation in advanced germ cell tumour (GCT) patients is widely applied. The aims of this study were: (1) To examine the

presence of alphafetoprotein (AFP) bearing tumour cells in PBSC harvests from advanced GCT patients obtained after multiple cycles of induction chemotherapy. (2) To determine whether induction chemotherapy contributed to in vivo purging of the tumour. We evaluated cryopreserved PBSC samples from 5 patients with advanced stage II/III AFP producing GCT. PBSC were separated after the first, second and third cycles of induction chemotherapy. Those samples were analysed using the nested reverse transcription polymerase chain reaction (RT-PCR) method to detect AFP mRNA. Although, in all patients, AFP mRNA was detected in PBSC samples after the first or second cycle of induction chemotherapy, but was not detected in 3 of 4 samples after the third cycle of chemotherapy. Although it is not clear whether tumour cells contaminating PBSC fraction contribute to disease relapse, PBSC harvested after at least 3 cycles of induction chemotherapy might be recommended to avoid such a possibility.

Kauffman, T., J. Tran, et al. (2003). "Mutations in Nop60B, the Drosophila homolog of human dyskeratosis congenita 1, affect the maintenance of the germ-line stem cell lineage during spermatogenesis." Dev Biol **253**(2): 189-99.

Spermatogenesis in Drosophila is maintained by germ-line stem cells. These cells undergo self-renewing divisions and also generate daughter gonial cells, whose function is to amplify the germ cell pool. Gonial cells subsequently differentiate into spermatocytes that undergo meiosis and generate haploid gametes. To elucidate the circuitry that controls progression through spermatogenic stem cell lineages, we are identifying mutations that lead to either excess germ cells or germ cell loss. From a collection of male sterile mutants, we identified P-element-induced hypomorphic alleles of nop60B, a gene encoding a pseudouridine synthase. Although null mutations are lethal, our P element-induced alleles generate viable, but sterile flies, exhibiting severe testicular atrophy. Sterility is reversed by P-element excision, and the atrophy is rescued by a Nop60B transgene, confirming identity of the gene. Using cell-type-specific markers, we find that testicular atrophy is due to severe loss of germ cells, including stem cells, but much milder effects on the somatic cells, which are themselves maintained by a stem cell lineage. We show that Nop60B activity is required intrinsically for the maintenance of germ-line stem cells. The relationship of these phenotypes to the human syndrome Dyskeratosis congenita, caused by mutations in a Nop60B homolog, is discussed.

Kee, K., J. M. Gonsalves, et al. (2006). "Bone morphogenetic proteins induce germ cell differentiation from human embryonic stem cells." Stem Cells Dev **15**(6): 831-7.

The growth factors bone morphogenetic protein-4 (BMP4), BMP7, and BMP8b are required for specification of primordial germ cells (PGCs) in mice. Disruption of the genes that encode these factors leads to a severe reduction in number, or the complete absence, of PGCs. In addition, several studies have demonstrated that human BMP4 can promote PGC differentiation from mouse embryonic stem (ES) cells and in organ cultures. Here, we sought to determine whether recombinant human BMPs could induce differentiation of germ cells from human (h) ES cells. We found that addition of recombinant human BMP4 increased the expression of the germ cell-specific markers VASA and SYCP3 during differentiation of hES cells to embryoid bodies (EBs). In addition, BMP7 and BMP8b showed additive effects on germ cell induction when added together with BMP4. Finally, we observed that addition of BMPs to differentiating ES cells also increased the percentage of cells that stained positively for VASA. We note that the effects of recombinant BMPs were modest but reproducible and suggest that addition of BMPs to differentiation media increases differentiation of human germ cells from hES cells.

Kee, K. and R. A. Reijo Pera (2008). "Human germ cell lineage differentiation from embryonic stem cells." CSH Protoc **2008**: pdb prot5048.

INTRODUCTIONBiological and ethical constraints hinder studies of human germ cell development despite its importance to reproductive health, including fertility and tumorigenesis. Thus, most of what we know of human germ cell development has been extrapolated from studies in model organisms. Human embryonic stem cells (hESCs) may provide an ideal system for probing the developmental genetics of germ cell formation and differentiation in vitro. The growth factors BMP (bone morphogenetic protein) 4, BMP7, and BMP8b are required for development of primordial germ cells (PGCs) in mice. It has been shown that these BMPs significantly increase germ cell differentiation from hESCs in vitro. This protocol describes a method to induce germ cell differentiation from hESCs by the addition of BMPs to hESC differentiation medium. The protocol can be used to study the basic mechanism of germ cell development in human cells.

Kim, Y., V. Selvaraj, et al. (2006). "Recipient preparation and mixed germ cell isolation for spermatogonial stem cell transplantation in domestic cats." J Androl **27**(2): 248-56.

The loss of genetic diversity poses a serious threat to the conservation of endangered species,

including wild felids. We are attempting to develop spermatogonial stem cell transplantation in the cat as a tool to preserve and propagate male germ-plasm from genetically valuable animals, be they threatened wild species or lines of cats used as models for inherited diseases. In this study, we investigated the use of local external beam radiation treatment to deplete the endogenous germ cells of male domestic cats, a step necessary to prepare them for use as recipients for transplantation. Testes of 5-month-old domestic cats were irradiated with a fractionated dose of 3 Gy per fraction for 3 consecutive days. These cats were castrated at 2, 4, 8, 16, and 32 weeks posttreatment, and progress of spermatogenesis was evaluated histologically and compared against age-matched controls. Even at the latest time points, less than 10% of tubules contained germ cells at any stage of meiosis, showing the efficacy of this protocol. In addition, male germ cells were isolated from the testes of domestic cats using a 2-step enzymatic dissociation to establish a protocol for the preparation of donor cells. The presence and viability of spermatogonia within this population were demonstrated by successful transplantation into, and colonization of, mouse seminiferous tubules. The success of these protocols provides a foundation to perform spermatogonial stem cell transplantation in the domestic cat.

Kobayashi, T., S. Yamamoto, et al. (2002). "Dose-intensive chemotherapy with syngeneic peripheral blood stem cell support for poor risk germ cell tumor of extragonadal origin: a case report." Jpn J Clin Oncol **32**(1): 33-4.

A variety of regimens of high-dose chemotherapy with hematopoietic stem cell support for poor risk germ cell tumors have been established. However, a series of chemotherapy steps carried out prior to the harvest sometimes leads to an insufficient number of peripheral blood stem cells. Here, we report a case of a patient who successfully underwent high-dose chemotherapy for the treatment of poor risk extragonadal germ cell tumor by receiving peripheral blood stem cell transplantation donated from his genomically identical twin brother.

Kohno, A., K. Takeyama, et al. (1995). "Low-dose granulocyte colony-stimulating factor enables the efficient collection of peripheral blood stem cells after disease-oriented, conventional-dose chemotherapy for breast cancer, malignant lymphoma and germ cell tumor." Bone Marrow Transplant **15**(1): 49-54.

Peripheral blood stem cells (PBSCs) were collected from 29 adult patients (median age 42 years, range 14-59 years) with breast cancer, germ cell tumor and malignant lymphoma after disease-oriented, conventional-dose chemotherapy combined with daily subcutaneous injections of low-dose (50 micrograms/m2 or 2 micrograms/kg) granulocyte colony-stimulating factor (G-CSF). The median number of colony-forming units-granulocyte macrophage (CFU-GM) collected in an apheresis was 2.37 (range 0-60.6) x 10(4)/kg body weight. Taking into consideration the minimum number of CFU-GM for hematopoietic reconstitution (at least 1 x 10(5) CFU-GM/kg), it was suggested that sufficient PBSCs could be collected by a few leukaphereses, although the cell yields of PBSCs tended to differ among the chemotherapeutic regimens. Twelve patients subsequently received high-dose chemotherapy followed by peripheral blood stem cell transplantation (PBSCT), including four receiving PBSCT alone and eight both PBSCT and autologous bone marrow transplantation (BMT). When compared with the 20 patients who received high-dose chemotherapy followed by autologous BMT alone, the median day of recovery of a neutrophil count > 0.5 x 10(9)/l and a platelet count > 20 x 10(9)/l was significantly shortened in those who received PBSCT (9 vs 12 days; $P < 0.01$ and 14 vs 30.5 days; $P < 0.001$), resulting in a lower platelet transfusion requirement (4.5 vs 9; $P < 0.001$).(ABSTRACT TRUNCATED AT 250 WORDS)

Kollmannsberger, C., N. Schleucher, et al. (2003). "Analysis of salvage treatments for germ cell cancer patients who have relapsed after primary high-dose chemotherapy plus autologous stem cell support." Eur J Cancer **39**(6): 775-82.

The aim of this study was to identify treatment strategies and therapeutic or clinical factors that predict for response to salvage therapy and survival in patients with metastatic 'Indiana advanced' or International Germ-Cell Cancer Collaborative Group (IGCCCG) poor prognosis' germ cell cancer (GCT) failing first-line sequential high-dose chemotherapy plus autologous stem cell support (HD-CT). A total of 58 'poor prognosis' patients who had relapsed after HD-CT were identified within two large prospective German first-line HD-CT trials (n=286) performed between March 1993 and March 2001. Salvage treatment consisted of the following: cisplatin-based conventional dose CTx+/-resection (19/58; 33%), non-cisplatin based CTx (16/58; 28%) or salvage HD-CT (14/58; 24%)+/-resection; resection (n=3) and/or radiation (n=5) only: 7 patients (12%); no specific therapy: 2 patients. 21 (38%) patients responded favourably (Complete Response (CR)/Partial Response (PR) marker-negative) to salvage therapy. The use of salvage HD-CT (2-year survival 48%; P=0.03, the complete resection of residual masses (2-year survival 42%; P=0.015) as

well as a favourable response to salvage therapy (2-year survival: 31%, P=0.014) were the only variables on univariate analysis associated with an improved survival. The estimated 2-year overall survival rate is 32% (95% Confidence Interval CI: 29-45%). Approximately 30% of patients relapsing after first-line HD-CT will survive>2 years, particularly those patients who can be treated with a second HD-CT +and/or surgical resection. If feasible, complete surgical resection of residual tumours appears to be the most efficient treatment.

Korkola, J. E., J. Houldsworth, et al. (2006). "Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with in vivo differentiation of human male germ cell tumors." Cancer Res **66**(2): 820-7.

Adult male germ cell tumors (GCTs) comprise distinct groups: seminomas and nonseminomas, which include pluripotent embryonal carcinomas as well as other histologic subtypes exhibiting various stages of differentiation. Almost all GCTs show 12p gain, but the target genes have not been clearly defined. To identify 12p target genes, we examined Affymetrix (Santa Clara, CA) U133A+B microarray (approximately 83% coverage of 12p genes) expression profiles of 17 seminomas, 84 nonseminoma GCTs, and 5 normal testis samples. Seventy-three genes on 12p were significantly overexpressed, including GLUT3 and REA (overexpressed in all GCTs) and CCND2 and FLJ22028 (overexpressed in all GCTs, except choriocarcinomas). We characterized a 200-kb gene cluster at 12p13.31 that exhibited coordinated overexpression in embryonal carcinomas and seminomas, which included the known stem cell genes NANOG, STELLA, and GDF3 and two previously uncharacterized genes. A search for other coordinately regulated genomic clusters of stem cell genes did not reveal any genomic regions similar to that at 12p13.31. Comparison of embryonal carcinoma with seminomas revealed relative overexpression of several stem cell-associated genes in embryonal carcinoma, including several core "stemness" genes (EBAF, TDGF1, and SOX2) and several downstream targets of WNT, NODAL, and FGF signaling (FGF4, NODAL, and ZFP42). Our results indicate that 12p gain is a functionally relevant change leading to activation of proliferation and reestablishment/maintenance of stem cell function through activation of key stem cell genes. Furthermore, the differential expression of core stem cell genes may explain the differences in pluripotency between embryonal carcinomas and seminomas.

Kucia, M., B. Machalinski, et al. (2006). "The developmental deposition of epiblast/germ cell-line derived cells in various organs as a hypothetical explanation of stem cell plasticity?" Acta Neurobiol Exp (Wars) **66**(4): 331-41.

The embryo develops from germ cell line (fertilized oocyte) and precursors of primordial germ cells (PGC) are the first population of stem cells that are specified in mice at the beginning of gastrulation in proximal primitive ectoderm (epiblast)--region adjacent to the extraembryonic ectoderm. These founder cells subsequently move through the primitive streak and give rise to several extra-embryonic mesodermal lineages and to germ cells. By day 7.25 of embryonic development, a cluster of PGC is visible at the basis of allantois. Subsequently PGC migrate through the embryo proper and colonize genital ridges, where they finally differentiate into sperm and oocytes. We hypothesize that during early development epiblast/germ line-derived cells including PGC become a founder populations of pluripotent stem cells (PSC). These cells are deposited during embryogenesis in various organs and may persist in these locations into adulthood - for example in bone marrow (BM). To support this, we recently identified in BM a population of very small embryonic-like (VSEL) stem cells that express epiblast/germ line-derived cells transcription factor Oct-4 and several other PGC markers. Similarly, cells expressing Oct-4 were also identified in several adult tissues by other investigators. Thus, pluripotent epiblast/PGC may persist beyond embryogenesis in neonatal and adult tissues. Their fate is defined by several mechanisms which regulate cell proliferation and affect status of somatic imprint on selected genes responsible for pluripotency. We hypothesize that these cells play an important role in tissue/organ regeneration and their presence in adult tissues may explain phenomenon of stem cell plasticity. In pathological situations, however they may undergo malignant transformation and give rise to tumors.

Kumano, M., H. Miyake, et al. (2006). "Stable hematopoietic recovery after peripheral blood stem cell transplantation in patients receiving high-dose chemotherapy for advanced germ cell tumors." Anticancer Res **26**(6C): 4965-8.

AIM: The objective of this study was to evaluate the utility of CD34-positive peripheral blood stem cell transplantation (PBSCT) in the hematopoietic recovery, in patients receiving high-dose chemotherapy (HDCT), for advanced germ cell tumor (GCT). MATERIALS AND METHODS: This study included 41 patients with advanced GCT, who were treated with HTCT combined with PBSCT. PBSCs were harvested after conditioning

chemotherapy followed by the administration of granulocyte colony-stimulating factor. A retrospective analysis of a total of 86 PBSCTs was carried out focusing on the effects of several factors, including age (<35 years versus > or = 35 years), CD34-positive cell dose (<5.0 x10(6)/kg versus > or =5.0 x10(6)/kg), indication of HDCT (first-line versus salvage) and previous history of HDCT before PBSCT (with versus without), on hematopoietic recovery after PBSCT. RESULTS: The median number of CD34-positive PBSCs collected during a single apheresis and the median cumulative number of CD34-positive PBSCs from each patient were 8.3x10(6)/kg and 23.2x10(6)/kg, respectively. The median number of PBSCT performed in each patient was two and the median number of CD34-positive cells transplanted during a single course was 5.7x10(6)/kg. The median recovery times to white blood cells (WBC) greater than 500/microl, 1000/microl and 2000/microl were 8, 9 and 10 days, respectively, following PBSCT, while that to neutrophils greater than 500/microl and that to platelets greater than 50000/microl were 9 and 13 days, respectively, following PBSCT Only the recovery time to platelet count greater than 50000/microl was significantly affected by age; however, there were no significant differences in the recovery of WBC, neutrophils and platelets in relation to several parameters examined. CONCLUSION: These findings suggest that CD34-positive PBSCT may facilitate stable hematopoietic recovery after HDCT in patients with advanced GCT, and that HDCT, if combined with PBSCT, could be performed with comparative safety in such patients, irrespective of their individual characteristics.

Kumano, M., H. Miyake, et al. (2007). "First-line high-dose chemotherapy combined with peripheral blood stem cell transplantation for patients with advanced extragonadal germ cell tumors." Int J Urol **14**(4): 336-8.

BACKGROUND: The objective of this study was to evaluate the efficacy and safety of first-line high-dose chemotherapy (HDCT) combined with peripheral blood stem cell transplantation (PBSCT) for patients with advanced extragonadal germ cell tumors (EGGCT). METHODS: Six male patients with advanced non-seminomatous EGGCT were treated with HDCT combined with PBSCT following 2-3 cycles of conventional-dose induction chemotherapy. The regimens used for HDCT were carboplatin, etoposide and ifosfamide (ICE) in five patients and ICE plus paclitaxel (T-ICE) in one patient, and that for induction therapy was cisplatin, etoposide and bleomycin (PEB) in all patients. As a rule, HDCT was continuously administered until alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin normalized (beta-HCG). RESULTS: Following 1-6 courses of HDCT (median, 4 courses), beta-HCG and AFP were normalized in all patients, and five and one patient were diagnosed as showing partial remission and stable disease, respectively. Five patients underwent surgical resection of residual tumors after HDCT, yielding necrotic tissue in two, mature teratoma in two, and viable cancer tissue in one, and the surgical margin was negative in all patients. At a median follow-up of 36 months, five patients were alive and disease-free, whereas the remaining one died of disease progression. Although all patients had grade 3 hematological toxicity, there was no treatment-related death by combining PBSCT. CONCLUSIONS: First-line HDCT with PBSCT could be safely administered to patients with advanced EGGCT, and the antitumor effect of this treatment was comparatively favorable. First-line HDCT therefore may represent an attractive option for patients with advanced EGGCT.

Labosky, P. A., D. P. Barlow, et al. (1994). "Mouse embryonic germ (EG) cell lines: transmission through the germline and differences in the methylation imprint of insulin-like growth factor 2 receptor (Igf2r) gene compared with embryonic stem (ES) cell lines." Development **120**(11): 3197-204.

Primordial germ cells of the mouse cultured on feeder layers with leukemia inhibitory factor, Steel factor and basic fibroblast growth factor give rise to cells that resemble undifferentiated blastocyst-derived embryonic stem cells. These primordial germ cell-derived embryonic germ cells can be induced to differentiate extensively in culture, form teratocarcinomas when injected into nude mice and contribute to chimeras when injected into host blastocysts. Here, we report the derivation of multiple embryonic germ cell lines from 8.5 days post coitum embryos of C57BL/6 inbred mice. Four independent embryonic germ cell lines with normal male karyotypes have formed chimeras when injected into BALB/c host blastocysts and two of these lines have transmitted coat color markers through the germline. We also show that pluripotent cell lines capable of forming teratocarcinomas and coat color chimeras can be established from primordial germ cells of 8.0 days p.c. embryos and 12.5 days p.c. genital ridges. We have examined the methylation status of the putative imprinting box of the insulin-like growth factor type 2 receptor gene (Igf2r) in these embryonic germ cell lines. No correlation was found between methylation pattern and germline competence. A significant difference was observed between embryonic stem cell and embryonic germ cell lines in their ability to maintain the methylation imprint of the Igf2r gene in

culture. This may illustrate a fundamental difference between these two cell types.

Lavial, F., H. Acloque, et al. (2009). "Ectopic expression of Cvh (Chicken Vasa homologue) mediates the reprogramming of chicken embryonic stem cells to a germ cell fate." Dev Biol **330**(1): 73-82.

When they are derived from blastodermal cells of the pre-primitive streak in vitro, the pluripotency of Chicken Embryonic Stem Cells (cESC) can be controlled by the cPouV and Nanog genes. These cESC can differentiate into derivatives of the three germ layers both in vitro and in vivo, but they only weakly colonize the gonads of host embryos. By contrast, non-cultured blastodermal cells and long-term cultured chicken primordial germ cells maintain full germline competence. This restriction in the germline potential of the cESC may result from either early germline determination in the donor embryos or it may occur as a result of in vitro culture. We are interested in understanding the genetic determinants of germline programming. The RNA binding protein Cvh (Chicken Vasa Homologue) is considered as one such determinant, although its role in germ cell physiology is still unclear. Here we show that the exogenous expression of Cvh, combined with appropriate culture conditions, induces cESC reprogramming towards a germ cell fate. Indeed, these cells express the Dazl, Tudor and Sycp3 germline markers, and they display improved germline colonization and adopt a germ cell fate when injected into recipient embryos. Thus, our results demonstrate that Vasa can drive ES cell differentiation towards the germ cell lineage, both in vitro and in vivo.

Ledermann, B. and K. Burki (1991). "Establishment of a germ-line competent C57BL/6 embryonic stem cell line." Exp Cell Res **197**(2): 254-8.

Embryonic stem (ES) cell lines have been derived from blastocysts of the inbred mouse strain C57BL/6. The highest frequencies of ES cell colonies were observed when blastocysts were explanted directly onto growth-arrested feeder layers of 5637 human bladder carcinoma cells in the presence of conditioned medium. One of the male ES cell lines tested (BL/6-III) was shown to be karyotypically stable and germ-line competent when introduced into BALB/c host blastocysts. These results demonstrate that ES cell lines from inbred mouse strains other than 129/Sv may be used as vectors to introduce selected mutations into the germ-line of mice.

Lee, D. R., K. S. Kim, et al. (2006). "Isolation of male germ stem cell-like cells from testicular tissue of non-obstructive azoospermic patients and differentiation into haploid male germ cells in vitro." Hum Reprod **21**(2): 471-6.

BACKGROUND: The purpose of this study was to establish the culture conditions required to isolate, identify and expand male germ stem cell-like cells (GSC-LC) from the testicular tissue of patients with non-obstructive azoospermia (NOA). METHODS AND RESULTS: Testicular tissues obtained from patients (two with maturation arrest (MA, n = 2) and Sertoli cell-only syndrome (SCOS, n = 11) were dissociated and plated into gelatin-coated dishes. After 2-4 weeks, cultures from both MA patients (100%) and four SCOS patients (36.3%) exhibited multicellular colonies, which proliferated successfully until passage 10. GSC-LC in the colonies displayed alkaline phosphatase activity, as well as Oct-4 and integrin b1 expression after every passage. After the fifth passage, GSC-LC were differentiated by encapsulation in calcium alginate and further cultivation. At 2 and 6 weeks, cells expressed c-Kit, Scp3, testis-specific histone protein 2B (TH2B), and transition protein (TP)-1. Fluorescence in situ hybridization additionally disclosed a few tetraploid and haploid cells at 6 weeks. Human oocytes were activated in the absence of artificial activation and cleaved after the injection of presumptive spermatids. CONCLUSIONS: Our novel culture system may be useful for diagnosing the existence of germ cells and facilitating the treatment of NOA patients.

Li, Y., N. T. Minor, et al. (2009). "Bam and Bgcn antagonize Nanos-dependent germ-line stem cell maintenance." Proc Natl Acad Sci U S A **106**(23): 9304-9.

The balance between germ-line stem cell (GSC) self-renewal and differentiation in Drosophila ovaries is mediated by the antagonistic relationship between the Nanos (Nos)-Pumilio translational repressor complex, which promotes GSC self-renewal, and expression of Bam, a key differentiation factor. Here, we find that Bam and Nos proteins are expressed in reciprocal patterns in young germ cells. Repression of Nos in Bam-expressing cells depends on sequences in the nos 3'-UTR, suggesting that Nos is regulated by translational repression. Ectopic Bam causes differentiation of GSCs, and this activity depends on the endogenous nos 3'-UTR sequence. Previous evidence showed that Bgcn is an obligate factor for the ability of Bam to drive differentiation, and we now report that Bam forms a complex with Bgcn, a protein related to the RNA-interacting DExH-box polypeptides. Together, these observations suggest that Bam-Bgcn act together to antagonize Nos expression; thus, derepressing cystoblast-promoting factors. These findings emphasize the importance of

translational repression in balancing stem cell self-renewal and differentiation.

Lin, D. P., M. Y. Chang, et al. (2003). "Male germ line stem cells: from cell biology to cell therapy." Reprod Fertil Dev **15**(6): 323-31.

Research using stem cells has several applications in basic biology and clinical medicine. Recent advances in the establishment of male germ line stem cells provided researchers with the ability to identify, isolate, maintain, expand and differentiate the spermatogonia, the primitive male germ cells, as cell lines under in vitro conditions. The ability to culture and manipulate stem cell lines from male germ cells has gradually facilitated research into spermatogenesis and male infertility, to an extent beyond that facilitated by the use of somatic stem cells. After the introduction of exogenous genes, the spermatogonial cells can be transplanted into the seminiferous tubules of recipients, where the transplanted cells can contribute to the offspring. The present review concentrates on the origin, life cycle and establishment of stem cell lines from male germ cells, as well as the current status of transplantation techniques and the application of spermatogonial stem cell lines.

Linher, K., P. Dyce, et al. (2009). "Primordial germ cell-like cells differentiated in vitro from skin-derived stem cells." PLoS One **4**(12): e8263.

BACKGROUND: We have previously demonstrated that stem cells isolated from fetal porcine skin have the potential to form oocyte-like cells (OLCs) in vitro. However, primordial germ cells (PGCs), which must also be specified during the stem cell differentiation to give rise to these putative oocytes at more advanced stages of culture, were not systematically characterized. The current study tested the hypothesis that a morphologically distinct population of cells derived from skin stem cells prior to OLC formation corresponds to putative PGCs, which differentiate further into more mature gametes. METHODOLOGY/PRINCIPAL FINDINGS: When induced to differentiate in an appropriate microenvironment, a subpopulation of morphologically distinct cells, some of which are alkaline phosphatase (AP)-positive, also express Oct4, Fragilis, Stella, Dazl, and Vasa, which are markers indicative of germ cell formation. A known differentially methylated region (DMR) within the H19 gene locus, which is demethylated in oocytes after establishment of the maternal imprint, is hypomethylated in PGC-like cells compared to undifferentiated skin-derived stem cells, suggesting that the putative germ cell population undergoes imprint erasure. Additional evidence supporting the germ cell identity of in vitro-generated PGC-like cells is that, when labeled with a Dazl-GFP reporter, these cells further differentiate into GFP-positive OLCs. SIGNIFICANCE: The ability to generate germ cell precursors from somatic stem cells may provide an in vitro model to study some of the unanswered questions surrounding early germ cell formation.

Longo, L., A. Bygrave, et al. (1997). "The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism." Transgenic Res **6**(5): 321-8.

Mouse pluripotent embryonic stem (ES) cells, once reintroduced into a mouse blastocyst, can contribute to the formation of all tissues, including the germline, of an organism referred to as a chimaeric. However, the reasons why this contribution often appears erratic are poorly understood. We have tested the notion that the chromosome make-up may be important in contributing both to somatic cell chimaerism and to germ line transmission. We found that the percentage of chimaerism of ES cell-embryo chimaeras, the absolute number of chimaeras and the ratio of chimaeras to total pups born all correlate closely with the percentage of euploid metaphases in the ES cell clones injected into the murine blastocyst. The majority of the ES cell clones that we tested, which were obtained from different gene targeting knockout experiments and harboured 50 to 100% euploid metaphases, did transmit to the germline; in contrast, none of the ES cell clones with more than 50% of chromosomally abnormal metaphases transmitted to the germline. Euploid ES cell clones cultured in vitro for more than 20 passages rapidly became severely aneuploid, and again this correlated closely with the percentage of chimaerism and with the number of ES cell-embryo chimaeras obtained per number of blastocysts injected. At the same time, the ability of these clones to contribute to the germline was lost when the proportion of euploid cells dropped below 50%. This study suggests that aneuploidy, rather than 'loss of totipotency', in ES cells, is the major cause of failure in obtaining contributions to all tissues of the adult chimaera, including the germline. Because euploidy is predictive of germline transmission, karyotype analysis is crucial and time/cost saving in any gene-targeting experiment.

Looijenga, L. H., H. de Leeuw, et al. (2003). "Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors." Cancer Res **63**(22): 7674-8.

Testicular germ-cell tumors (TGCTs) of adolescents and adults originate from intratubular germ cell neoplasia (ITGCN), which is composed of the malignant counterparts of embryonal germ cells.

ITGCN cells are characterized, among others, by the presence of stem cell factor receptor c-KIT. Once established, ITGCN will always progress to invasiveness. Approximately 2.5-5% of patients with a TGCT will develop bilateral disease and require complete castration, resulting in infertility, a need for lifelong androgen replacement, and psychological stress. To date, the only way to predict a contralateral tumor is surgical biopsy of the contralateral testis to demonstrate ITGCN. We did a retrospective study of 224 unilateral and 61 proven bilateral TGCTs (from 46 patients, in three independently collected series in Europe) for the presence of activating c-KIT codon 816 mutations. A c-KIT codon 816 mutation was found in three unilateral TGCT (1.3%), and in 57 bilateral TGCTs (93%; $P < 0.0001$). In the two wild-type bilateral tumors for which ITGCN was available, the preinvasive cells contained the mutation. The mutations were somatic in origin and identical in both tumors. We conclude that somatic activating codon 816 c-KIT mutations are associated with development of bilateral TGCT. Detection of c-KIT codon 816 mutations in unilateral TGCT identifies patients at risk for bilateral disease. These patients may undergo tailored treatment to prevent the development of bilateral disease, with retention of testicular hormonal function.

Lotz, J. P., B. Bui, et al. (2005). "Sequential high-dose chemotherapy protocol for relapsed poor prognosis germ cell tumors combining two mobilization and cytoreductive treatments followed by three high-dose chemotherapy regimens supported by autologous stem cell transplantation. Results of the phase II multicentric TAXIF trial." Ann Oncol **16**(3): 411-8.

BACKGROUND: High-dose chemotherapy (HD-CT) is able to circumvent platinum resistance of resistant/refractory germ-cell tumors (GCTs), but expectancy of cure remains low. New strategies are needed with new drugs and a sequential approach. MATERIALS AND METHODS: Patients with relapsed poor-prognosis GCTs were scheduled to receive two cycles combining epirubicin and paclitaxel (Taxol) followed by three consecutive HD-CT supported by stem cell transplantation [one course combining cyclophosphamide, 3 g/m(2) + thiotepa, 400 mg/m(2), followed by two ICE regimens (ifosfamide, 10 g/m(2), carboplatin, AUC 20, etoposide, 1500 mg/m(2))]. RESULTS: From March 1998 to September 2001 (median follow-up, 31.8 months), 45 patients (median age, 28 years) were enrolled in this phase II study. Twenty-two patients received the complete course. Twenty-five patients died from progression and five from toxicity. The overall response rate was 37.7%, including an 8.9% complete response rate. The median overall survival was 11.8 months. The 3-year survival and progression-free survival rate was 23.5%. The 'Beyer' prognostic score predicted the outcome after HD-CT. CONCLUSION: Although our results warrant further studies on HD-CT in relapsed poor prognosis GCTs, patients with a Beyer score >2 did not benefit from this approach and should not be enrolled in HD-CT trials. Better selection criteria have to be fulfilled in forthcoming studies.

Madan, B., V. Madan, et al. (2009). "The pluripotency-associated gene Dppa4 is dispensable for embryonic stem cell identity and germ cell development but essential for embryogenesis." Mol Cell Biol **29**(11): 3186-203.

Dppa4 (developmental pluripotency-associated 4) has been identified in several high-profile screens as a gene that is expressed exclusively in pluripotent cells. It encodes a nuclear protein with an SAP-like domain and appears to be associated preferentially with transcriptionally active chromatin. Its exquisite expression pattern and results of RNA interference experiments have led to speculation that Dppa4, as well as its nearby homolog Dppa2, might play essential roles in embryonic stem (ES) cell function and/or germ cell development. To rigorously assess suggested roles, we have generated Dppa4-deficient and Dppa4/Dppa2 doubly deficient ES cells, as well as mice lacking Dppa4. Contrary to predictions, we find that Dppa4 is completely dispensable for ES cell identity and germ cell development. Instead, loss of Dppa4 in mice results in late embryonic/perinatal death and striking skeletal defects with partial penetrance. Thus, surprisingly, Dppa4-deficiency affects tissues that apparently never transcribed the gene, and at least some loss-of-function defects manifest phenotypically at an embryonic stage long after physiologic Dppa4 expression has ceased. Concomitant with targeted gene inactivation, we have introduced into the Dppa4 locus a red fluorescent marker (tandem-dimer red fluorescent protein) that is compatible with green fluorescent proteins and allows noninvasive visualization of pluripotent cells and reprogramming events.

Magin, T. M., J. McWhir, et al. (1992). "A new mouse embryonic stem cell line with good germ line contribution and gene targeting frequency." Nucleic Acids Res **20**(14): 3795-6.

Margolin, K. (2003). "High dose chemotherapy and stem cell support in the treatment of germ cell cancer." J Urol **169**(4): 1229-33.

PURPOSE: The current status of high dose chemotherapy with autologous stem cell support in

patients with germ cell cancer is reviewed. MATERIALS AND METHODS: Advanced germ cell cancer can be cured in most patients using chemotherapy with or without surgery. A small fraction of patients fail to achieve a marker remission, have residual viable carcinoma at post-chemotherapy surgery or have relapse after remission. Phase II trials suggest that autologous stem cell support is more active than standard dose chemotherapy in patients with relapse. A comprehensive literature review, focusing on trials published in the last decade, is followed by a discussion of current trials and recommendations for the use of autologous stem cell support in germ cell cancer. RESULTS: In early trials about 15% of patients with multiple relapsed and refractory disease had durable remission with high dose carboplatin and etoposide. Most regimens now add high dose cyclophosphamide or ifosfamide to carboplatin and etoposide. Together with the use of autologous stem cell support in less heavily-pretreated patients, these regimens have produced durable remissions in 40% to 50% of patients. Multivariate analyses led to the identification of prognostic factors at diagnosis and predictive factors during therapy which were associated with a low rate of durable remission. Ongoing randomized trials of autologous stem cell support early in relapse or as part of initial therapy are designed to study and validate further these prognostic factors. CONCLUSIONS: For patients with poor risk presenting features, the role of autologous stem cell support has not been proven and awaits the results of an ongoing United States intergroup trial. Patients with residual cancer at post-chemotherapy surgery may have a substantial risk of relapse despite additional cycles of the same drugs used to achieve marker remission. For select patients in this category alternatives to additional cycles of the original chemotherapy may include established second line regimens or autologous stem cell support. The role of autologous stem cell support for germ cell tumor in relapse may be challenged by the future discovery of new agents for these diseases.

Margolin, K., J. H. Doroshow, et al. (1996). "Treatment of germ cell cancer with two cycles of high-dose ifosfamide, carboplatin, and etoposide with autologous stem-cell support." J Clin Oncol **14**(10): 2631-7.

PURPOSE: To evaluate the activity of two cycles of high-dose ifosfamide, carboplatin, and etoposide (ICE) with autologous hematopoietic progenitor cell support (aHPCS) in patients with poor-prognosis, chemotherapeutically sensitive germ cell cancer. PATIENTS AND METHODS: Twenty patients with germ cell tumor who had persistent disease or relapse from standard-risk or high-risk presentation were entered on this pilot study. The entry criteria included relapsed gonadal and extragonadal germ cell cancer unlikely to be cured by standard salvage therapy but without proven refractoriness to chemotherapy. Treatment consisted of two cycles of ICE chemotherapy with mesna uroprotection and aHPCS. On the first cycle, ifosfamide (IFX), 2 gm/m2; carboplatin, 400 mg/m2; and etoposide, 20 mg/kg, were administered on days -6, -5, and -4. On the second cycle, the doses and schedule of carboplatin and etoposide were identical, and patients with normal renal function received additional IFX, 2 g/m2 on day -3 and 1 g/ m2 on day -2. Mesna, 600 mg/m2 every 6 hours, was given until 24 hours following the final dose of IFX on each cycle, and autologous bone marrow and/or peripheral stem-cells were infused on day 0. RESULTS: All twenty patients are assessable for toxicity and current disease status. Two patients received only one cycle of therapy, one because of the development of active hepatitis C following cycle 1, and one because of renal insufficiency. No patient died as a result of protocol therapy, and no patient developed debilitating peripheral neuropathy, symptomatic hearing loss, or severe renal insufficiency requiring dialysis. The median time to recovery of > or = 500 neutrophils/microL and platelets > or = 50,000/microL was day +11 and day +15, respectively. The median maximum creatinine was 1.6 mg/dL on each treatment cycle, and there was no other significant organ toxicity. With a median follow-up of 45 months, nine patients are alive and disease-free following protocol chemotherapy. One patient with embryonal cancer developed progressive pulmonary metastases 3 months after completing high-dose therapy, underwent complete resection of lung metastases, and remains disease-free at 63+ months. Eight patients are continuously disease free at 23+ to 70+ months after protocol therapy. Eleven patients died of progressive disease between 4 and 23 months following completion of treatment. CONCLUSION: These results compare favorably to other studies in similarly selected patients undergoing salvage therapy with one or two cycles of chemotherapy containing high-dose carboplatin and etoposide with or without cyclophosphamide (CTX) or IFX. The excellent safety and tolerability profile of this regimen and its encouraging activity in poor-prognosis patients make it worthy of further study as part of initial therapy in randomized protocols for high-risk disease and early in the treatment of relapsed germ cell cancer.

Margolin, K. A., J. H. Doroshow, et al. (2005). "Paclitaxel-based high-dose chemotherapy with autologous stem cell rescue for relapsed germ cell

cancer." Biol Blood Marrow Transplant **11**(11): 903-11.

We evaluated the antitumor activity of tandem cycles of high-dose chemotherapy with autologous peripheral stem cell transplantation (aPSCT) in relapsed germ cell tumors by using high-dose paclitaxel, carboplatin, etoposide, and ifosfamide. Thirty-three patients were entered, and 31 underwent protocol therapy. Paclitaxel 350 mg/m2 (5 patients) or 425 mg/m2 (26 patients) by 24-hour continuous intravenous infusion was followed by 3 daily doses of carboplatin and either etoposide (cycle 1) or ifosfamide/mesna (cycle 2). The carboplatin dose had a calculated area under the curve of 7 mg-min/mL, and the daily dose of etoposide was 20 mg/kg (cycle 1). Ifosfamide 3 g/m2/d for 3 days (with mesna uroprotection) was substituted for etoposide in cycle 2. Each cycle was supported by granulocyte colony-stimulating factor-mobilized peripheral blood stem cells. Thirty-one patients were evaluable for response, toxicity, and long-term disease control. Two patients did not undergo aPSCT because of rapid disease progression. Nineteen patients received both cycles of aPSCT, 8 progressed after cycle 1, 3 refused the second cycle, and 1 died of fungal infection during cycle 1. Twelve patients remain relapse free at a median of 67 months from the initiation of therapy. Whereas the International Germ Cell Cancer Collaborative Group category at the time of initial diagnosis did not seem to predict outcome, the patient's probability of achieving durable remission was significantly associated with the Beyer prognostic score at the time of protocol entry. Regimens containing the most active agents in relapsed nonseminomatous germ cell tumors, including high-dose paclitaxel, are well tolerated and have promising activity even in patients with poor-risk features who do not achieve durable remissions with standard therapy. The Beyer prognostic system is a valuable predictor for patients undergoing aPSCT.

McLean, D. J., L. D. Russell, et al. (2002). "Biological activity and enrichment of spermatogonial stem cells in vitamin A-deficient and hyperthermia-exposed testes from mice based on colonization following germ cell transplantation." Biol Reprod **66**(5): 1374-9.

Spermatogenesis is a complex process in which spermatogonial stem cells divide and subsequently differentiate into spermatozoa. This process requires spermatogonial stem cells to self-renew and provide a continual population of cells for differentiation. Studies on spermatogonial stem cells have been limited due to a lack of unique markers and an inability to detect the presence of these cells. The technique of germ cell transplantation provides a functional assay to identify spermatogonial stem cells in a cell population. We hypothesized that vitamin A-deficient (VAD) and hyperthermically treated testes would provide an enriched in vivo source of spermatogonial stem cells. The first model, hyperthermic treatment, depends on the sensitivity of maturing germ cells to high temperatures. Testes of adult mice were exposed to 43 degrees C for 15 min to eliminate the majority of differentiating germ cells. Treated donor testes were 50% of normal adult testis size and, when transplanted into recipients, resulted in a 5.3- and 19-fold (colonies and area, respectively) increase in colonization efficiency compared to controls. The second model, VAD animals, also lacked differentiating germ cells, and testes weights were 25% of control values. Colonization efficiency of germ cells from VAD testes resulted in a 2.5- and 6.2-fold (colonies and area, respectively) increase in colonization compared to controls. Hyperthermically treated mice represent an enriched source of spermatogonial stem cells. In contrast, the low extent of colonization with germ cells from VAD animals raises important questions regarding the competency of stem cells from this model.

Mego, M. (2006). "Cancer stem cell in relapsed testicular germ cell cancer: embryonic or somatic?" Int J Androl **29**(6): 627.

Miki, T., Y. Mizutani, et al. (2007). "Long-term results of first-line sequential high-dose carboplatin, etoposide and ifosfamide chemotherapy with peripheral blood stem cell support for patients with advanced testicular germ cell tumor." Int J Urol **14**(1): 54-9.

OBJECTIVE: Standard chemotherapy shows relatively low long-term survival in patients with poor-risk testicular germ cell tumor (GCT). First-line high-dose chemotherapy (HD-CT) may improve the result. High-dose carboplatin, etoposide, ifosfamide chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT) was investigated as first-line chemotherapy in patients with advanced testicular GCT. METHODS: Fifty-five previously untreated testicular GCT patients with Indiana 'advanced disease' criteria received three cycles of bleomycin, etoposide and cisplatin (BEP) followed by one cycle of HD-CT plus PBSCT, if elevated serum tumor markers were observed after three cycles of the BEP regimen. RESULTS: Thirty patients were treated with BEP alone, because the tumor marker(s) declined to normal range. Twenty-five patients received BEP and HD-CT. One patient died of rhabdomyolysis due to HD-CT. Three and six (13% and 25%) out of 24 patients treated with BEP and HD-CT achieved marker-negative and marker-

positive partial responses, respectively. The other patients achieved no change. Fifteen (63%) are alive and 14 (58%) are free of disease at a median follow-up time of 54 months. Severe toxicity included treatment-related death (4%). CONCLUSIONS: HD-CT with peripheral stem cell support can be successfully applied in a multicenter setting. HD-CT demonstrated modest anticancer activity for Japanese patients with advanced testicular GCT and was well tolerated. This regimen might be examined for further investigation in randomized trials in first-line chemotherapy for patients with poor-risk testicular GCT.

Modak, S., S. Gardner, et al. (2004). "Thiotepa-based high-dose chemotherapy with autologous stem-cell rescue in patients with recurrent or progressive CNS germ cell tumors." J Clin Oncol **22**(10): 1934-43.

PURPOSE: To evaluate the efficacy and toxicity of high-dose chemotherapy (HDC) followed by autologous stem-cell rescue (ASCR) in patients with relapsed or progressive CNS germ cell tumors (GCTs). PATIENTS AND METHODS: Twenty-one patients with CNS GCTs who experienced relapse or progression despite having received initial chemotherapy and/or radiotherapy were treated with thiotepa-based HDC regimens followed by ASCR. RESULTS: Estimated overall survival (OS) and event-free survival (EFS) rates for the entire group 4 years after HDC were 57% +/- 12% and 52% +/- 14%, respectively. Seven of nine (78%) patients with germinoma survived disease-free after HDC with a median survival of 48 months. One patient died as a result of progressive disease (PD) 39 months after HDC, and another died as a result of pulmonary fibrosis unrelated to HDC 78 months after ASCR without assessable disease. However, only four of 12 patients (33%) with nongerminomatous germ cell tumors (NGGCTs) survived without evidence of disease, with a median survival of 35 months. Eight patients with NGGCTs died as a result of PD, with a median survival of 4 months after HDC (range, 2 to 17 months). Patients with germinoma fared better than those with NGGCTs (P =.016 and.014 for OS and EFS, respectively). Patients with complete response to HDC also had significantly better outcome (P <.001 for OS and EFS) compared with patients with only a partial response or stable disease. There were no toxic deaths because of HDC. CONCLUSION: Dose escalation of chemotherapy followed by ASCR is effective therapy for patients with recurrent CNS germinomas and might be effective in patients with recurrent NGGCTs with a low tumor burden.

Motzer, R. J., C. J. Nichols, et al. (2007). "Phase III randomized trial of conventional-dose chemotherapy with or without high-dose chemotherapy and autologous hematopoietic stem-cell rescue as first-line treatment for patients with poor-prognosis metastatic germ cell tumors." J Clin Oncol **25**(3): 247-56.

PURPOSE: To investigate the role of high-dose chemotherapy (HDCT) as first-line treatment in patients with metastatic germ cell tumor (GCT) and poor-prognostic clinical features. Serum tumor marker decline during chemotherapy was assessed prospectively as a predictor of treatment outcome. PATIENTS AND METHODS: In this randomized phase III trial, previously untreated patients with intermediate- or poor-risk GCT received either four cycles of standard bleomycin, etoposide, and cisplatin (BEP alone), or two cycles of BEP followed by two cycles of HDCT containing carboplatin and then by hematopoietic stem-cell rescue (BEP + HDCT). Serum tumor markers alpha-fetoprotein and human chorionic gonadotrophin were correlated with treatment outcome as a secondary end point. RESULTS: Two hundred nineteen patients were randomly assigned: 108 to BEP + HDCT and 111 to BEP alone. The 1-year durable complete response rate was 52% after BEP + HDCT and 48% after BEP alone (P = .53). Patients with slow serum tumor marker decline (alpha-fetoprotein and/or human chorionic gonadotrophin) during the first two cycles of chemotherapy had a shorter progression-free survival and overall survival compared with patients with satisfactory marker decline (P = .02 and P = .03, respectively). Among 67 patients with unsatisfactory marker decline, the 1-year durable complete response proportion was 61% for patients who received HDCT versus 34% for patients receiving BEP alone (P = .03). CONCLUSION: The routine inclusion of HDCT in first-line treatment for GCT patients with metastases and a poor predicted outcome to chemotherapy did not improve treatment outcome. Frequent serum marker determinations to estimate marker decline during the first two cycles of BEP chemotherapy provide a clinically useful estimate of outcome.

Mueller, D., M. J. Shamblott, et al. (2005). "Transplanted human embryonic germ cell-derived neural stem cells replace neurons and oligodendrocytes in the forebrain of neonatal mice with excitotoxic brain damage." J Neurosci Res **82**(5): 592-608.

Stem cell therapy is a hope for the treatment of some childhood neurological disorders. We examined whether human neural stem cells (hNSCs) replace lost cells in a newborn mouse model of brain damage. Excitotoxic lesions were made in neonatal mouse forebrain with the N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA). QA

induced apoptosis in neocortex, hippocampus, striatum, white matter, and subventricular zone. This degeneration was associated with production of cleaved caspase-3. Cells immunopositive for inducible nitric oxide synthase were present in damaged white matter and subventricular zone. Three days after injury, mice received brain parenchymal or intraventricular injections of hNSCs derived from embryonic germ (EG) cells. Human cells were prelabeled in vitro with DiD for in vivo tracking. The locations of hNSCs within the mouse brain were determined through DiD fluorescence and immunodetection of human-specific nestin and nuclear antigen 7 days after transplantation. hNSCs survived transplantation into the lesioned mouse brain, as evidenced by human cell markers and DiD fluorescence. The cells migrated away from the injection site and were found at sites of injury within the striatum, hippocampus, thalamus, and white matter tracts and at remote locations in the brain. Subsets of grafted cells expressed neuronal and glial cell markers. hNSCs restored partially the complement of striatal neurons in brain-damaged mice. We conclude that human EG cell-derived NSCs can engraft successfully into injured newborn brain, where they can survive and disseminate into the lesioned areas, differentiate into neuronal and glial cells, and replace lost neurons. (c) 2005 Wiley-Liss, Inc.

Muller, A. M., G. Ihorst, et al. (2006). "Intensive chemotherapy with autologous peripheral blood stem cell transplantation during a 10-year period in 64 patients with germ cell tumor." Biol Blood Marrow Transplant **12**(3): 355-65.

Despite gratifying cure rates in germ cell tumors, conventional-dose chemotherapy achieves long-term remissions in less than 50% of patients at high risk. High-dose chemotherapy followed by autologous (auto) peripheral blood stem cell transplantation (PBSCT) has shown impressive remission rates in high-risk and relapsed germ cell tumors. We report on 64 consecutive patients with high- (n=39), intermediate- (n=18), and refractory or relapsed low- (n=7) risk germ cell tumors who underwent auto-PBSCT between January 1993 and February 2003. PBSCTs were performed as a single (n=40) or repeated (n=24) transplantation using either etoposide, ifosfamide, and carboplatin (n=80) or related protocols (paclitaxel, ifosfamide, carboplatin, etoposide [n=7]; carboplatin, etoposide, thiotepa [n=4]). With a median follow-up of 6 years, estimated 2- and 5-year overall survivals were 77.2% (95% confidence interval [CI] 66.7-87.7) and 73.1% (95% CI 61.7-84.5), respectively. We observed unfavorable results in those patients showing refractoriness to cisplatin (hazard ratio 20.36; 95% CI 6.64-62.47) or no response to induction chemotherapy (hazard ratio 10.67; 95% CI 1.37-83.37). Auto-PBSCT was well tolerated, showed objective antitumor activity, and achieved long-term survival in patients at high risk and with relapse. Our data suggest that auto-PBSCT can increase response rates and may improve the outcome in these patients.

Muller, T., G. Fleischmann, et al. (2009). "A novel embryonic stem cell line derived from the common marmoset monkey (Callithrix jacchus) exhibiting germ cell-like characteristics." Hum Reprod **24**(6): 1359-72.

BACKGROUND: Embryonic stem cells (ESC) hold great promise for the treatment of degenerative diseases. However, before clinical application of ESC in cell replacement therapy can be achieved, the safety and feasibility must be extensively tested in animal models. The common marmoset monkey (Callithrix jacchus) is a useful preclinical non-human primate model due to its physiological similarities to human. Yet, few marmoset ESC lines exist and differences in their developmental potential remain unclear. METHODS: Blastocysts were collected and immunosurgery was performed. cjes001 cells were tested for euploidy by karyotyping. The presence of markers for pluripotency was confirmed by immunofluorescence staining and RT-PCR. Histology of teratoma, in vitro differentiation and embryoid body formation revealed the differentiation potential. RESULTS: cjes001 cells displayed a normal 46,XX karyotype. Alkaline phosphatase activity, expression of telomerase and the transcription factors OCT4, NANOG and SOX2 as well as the presence of stage-specific embryonic antigen (SSEA)-3, SSEA-4, tumor rejection antigens (TRA)-1-60, and TRA-1-81 indicated pluripotency. Teratoma formation assay displayed derivatives of all three embryonic germ layers. Upon non-directed differentiation, the cells expressed the germ cell markers VASA, BOULE, germ cell nuclear factor and synaptonemal complex protein 3 and showed co-localization of VASA protein within individual cells with the germ line stem cell markers CD9, CD49f, SSEA-4 and protein gene product 9.5, respectively. CONCLUSIONS: The cjes001 cells represent a new pluripotent ESC line with evidence for enhanced spontaneous differentiation potential into germ cells. This cjes001 line will be very valuable for comparative studies on primate ESC biology.

Mummery, C. L., M. van Rooyen, et al. (1993). "Fibroblast growth factor-mediated growth regulation and receptor expression in embryonal carcinoma and

embryonic stem cells and human germ cell tumours." Biochem Biophys Res Commun **191**(1): 188-95.

FGFs have been implicated in the induction of mesoderm in amphibian development and are present in the mouse embryo at stages that would be appropriate for a similar function in mammals. Primitive ectoderm would then be the target tissue. We have now changes in the expression of receptors for FGFs during the differentiation of embryonal carcinoma (EC) and embryonic stem (ES) cells from the mouse. These cells resemble those of the inner cell mass and later primitive ectoderm. On Northern blots of mRNA from undifferentiated cells, transcripts for FGF R1, R2 and R3 are expressed. All are upregulated during differentiation of ES cells and are upregulated or remain constant as EC cells differentiate. FGF R4 is only expressed after differentiation to derivatives resembling parietal endoderm. By contrast in human EC cells, FGF R2 is downregulated during differentiation, FGF R1 and FGF R3 are unchanged and FGF R4 is expressed before and after differentiation. In both human and mouse EC cells three members of the FGF family (a FGF, b FGF and k FGF, also known as FGFs 1,2 and 4) are mitogenic in serum-free medium and one (KGF or FGF 7) appears to have no effect on growth although cellular morphology is altered. Differences between human and mouse cells are primarily in the effects of heparin on the FGF-induced response.

Muramaki, M., I. Hara, et al. (2003). "Long-term cryopreservation of peripheral blood stem cells in patients with advanced germ cell tumors using the dump-freezing method at -80 degrees C." Oncol Rep **10**(6): 1993-8.

The objectives of this study were to evaluate the hematopoietic activity of peripheral blood stem cells (PBSCs) harvested from patients with advanced germ cell tumors after long-term cryopreservation in a mechanical freezer at -80 degrees C. Forty-one patients with advanced tumors were enrolled in this study. Flow cytometric analysis was performed to determine the total cell viability and the viability of CD34 positive cells of PBSCs stored between January 1995 and December 2001. The 41 patients were divided into two groups according to the duration of cryopreservation. In the 21 patients in whom the duration of storage was greater than 5 years, the total cell number did not change significantly before and after long-term cryopreservation ($p=0.75$). The percentage of viable cells and the total number of viable cells was significantly decreased after long-term cryopreservation ($p<0.001$ and $p<0.05$, respectively). However, the percentage of CD34 positive cells was significantly increased to 2.34% from 1.85% ($p<0.05$), resulting in non-statistically significant change in the total viable CD34 positive cells ($p=0.98$). A similar relation was found in the 20 patients whose storage duration was less than 2 years, although a significant change was found only in the percentage of viable cells. Moreover, pre-PBSCH factors, including age, cumulative dosage of cisplatin and the International Germ Cell Cancer Collaborative Group classification at the diagnosis did not affect the viable CD34 positive cell count after long-term cryopreservation. In conclusion, the present results suggest that CD34 positive cells could be successfully cryopreserved in a mechanical freezer at -80 degrees C for more than 5 years with no relation to pre-PBSCH factors and efficacy of PBSCH itself.

Muramaki, M., H. Miyake, et al. (2003). "Predicting factors for collection of peripheral blood stem cells in patients with advanced germ cell tumors after cisplatin-based combination chemotherapy." Oncol Rep **10**(5): 1545-9.

The objectives of this study were to identify the predictive factors for the mobilization of CD34 positive peripheral blood stem cells (PBSC) and to determine the optimal timing of PBSC harvest in patients with advanced germ cell tumors after cisplatin-based combination chemotherapy. Thirty-eight patients with advanced germ cell tumors were enrolled in this study. We undertook a retrospective analysis of 124 aphereses performed between March, 1994 and December, 1999. The predictive value for the optimal timing of PBSC harvest was determined by the analysis of the correlation factors between the number of harvested CD34 positive cells and several clinicopathological factors. The mean number of CD34 positive cells obtained at a single apheresis was 5.38 x 10(6)/kg, and the mean cumulative number of CD34 positive cells in each patient was 24.07 x 10(6)/kg. The number of CD34 positive cells were significantly correlated to the number of previously performed chemotherapies before PBSC harvest, leukocyte count and the percentage of immature leukocytes (myelocytes plus metamyelocytes) ($p=0.0098$, $p=0.011$, and $p<0.0001$, respectively). Multivariate analysis revealed that the number of chemotherapies and the percentage of immature leukocytes were independent predictors for the number of harvested CD34 positive cells ($p=0.012$ and $p=0.016$, respectively). The present findings suggest that PBSC harvest should be performed during first-line chemotherapy, and that the monitoring of the percentage of immature leukocytes could be a useful predictor for determining the optimal timing of PBSC harvest.

Nagafuchi, S., H. Katsuta, et al. (1999). "Establishment of an embryonic stem (ES) cell line

derived from a non-obese diabetic (NOD) mouse: in vivo differentiation into lymphocytes and potential for germ line transmission." FEBS Lett **455**(1-2): 101-4.

A non-obese diabetic (NOD) mouse-derived embryonic stem (ES) cell line has been stably maintained in an undifferentiated state with a characteristic ES cell-like morphology, expressing the stem cell marker alkaline phosphatase, and displaying a normal diploid karyotype. After injecting the NOD-ES cells into blastocysts, chimeric mice were obtained. Small but significant numbers of lymphocytes expressed the NOD-derived MHC allele. When a chimeric mouse was mated with C57BL/6 mice, an agouti mouse was obtained, having the NOD-derived H-2 I-A(beta)g7 haplotype. Thus, an NOD-ES cell line which can differentiate into lymphocytes with potential for germ line transmission was successfully established.

Nagamatsu, G., M. Ohmura, et al. (2006). "A CTX family cell adhesion molecule, JAM4, is expressed in stem cell and progenitor cell populations of both male germ cell and hematopoietic cell lineages." Mol Cell Biol **26**(22): 8498-506.

Stem cells are maintained in an undifferentiated state by interacting with a microenvironment known as the "niche," which is comprised of various secreted and membrane proteins. Our goal was to identify niche molecules participating in stem cell-stem cell and/or stem cell-supporting cell interactions. Here, we isolated genes encoding secreted and membrane proteins from purified male germ stem cells using a signal sequence trap approach. Among the genes identified, we focused on the junctional adhesion molecule 4 (JAM4), an immunoglobulin type cell adhesion molecule. JAM4 protein was actually localized to the plasma membrane in male germ cells. JAM4 expression was downregulated as cells differentiated in both germ cell and hematopoietic cell lineages. To analyze function in vivo, we generated JAM4-deficient mice. Histological analysis of testes from homozygous nulls did not show obvious abnormalities, nor did liver and kidney tissues, both of which strongly express JAM4. The numbers of hematopoietic stem cells in bone marrow were indistinguishable between wild-type and mutant mice, as was male germ cell development. These results suggest that JAM4 is expressed in stem cells and progenitor cells but that other cell adhesion molecules may substitute for JAM4 function in JAM4-deficient mice both in male germ cell and hematopoietic lineages.

Nayernia, K., M. Li, et al. (2004). "Stem cell based therapeutical approach of male infertility by teratocarcinoma derived germ cells." Hum Mol Genet **13**(14): 1451-60.

Infertility affects 13-18% of couples and growing evidence from clinical and epidemiological studies suggests an increasing incidence of male reproductive problems. There is a male factor involved in up to half of all infertile couples. The pathogenesis of male infertility can be reflected by defective spermatogenesis due to failure in germ cell proliferation and differentiation. We report here in vitro generation of a germ cell line (SSC1) from the pluripotent teratocarcinoma cells by a novel promoter-based sequential selection strategy and show that the SSC1 cell line form mature seminiferous tubule structures, and support spermatogenesis after transplantation into recipient testes. To select differentiated germ cell population, we generated a fusion construct (Stra8-EGFP) harbouring the 1.4 kb promoter region of germ line specific gene Stra8 and coding region of enhanced green fluorescence protein. This region was sufficient to direct gene expression to the germinal stem cells in testis of transgenic mice. The purified cells expressed the known molecular markers of spermatogonia Rbm, cyclin A2, Tex18, Stra8 and Dazl and the beta1- and alpha6-integrins characteristic of the stem cell fraction. This cell line undergoes meiosis and can develop into sperm when transplanted into germ cell depleted testicular tubules. Sperm were viable and functional, as shown by fertilization after intra-cytoplasmic injection into mouse oocytes. This approach provides the basis that is essential for studying the development and differentiation of male germ line stem cell, as well as for developing new approaches to reproductive engineering and infertility treatment.

Neri, Q. V., T. Takeuchi, et al. (2009). "Treatment options for impaired spermatogenesis: germ cell transplantation and stem-cell based therapy." Minerva Ginecol **61**(4): 253-9.

Advances in infertility treatment had the most extraordinary breakthrough with the birth of the first in vitro fertilization baby in 1978. Fourteen years later, intracytoplasmic sperm injection has been introduced for the treatment of male factor infertility. Intra cytoplasmic sperm injection in combination with testicular sperm extraction has allowed men with azoospermia to father children. In fact, as long as a fully developed spermatozoon is identified, it can be utilized or can even be duplicated to inseminate several oocytes while providing information on its genomic content. There are, however, men who are suffering from spermatogenic arrest, where no post-meiotic germ cells are retrieved, and therefore, unable to generate their own offspring. More recently, the successful isolation and cultivation of spermatogonial

stem cells has allowed the exploration of their biological characteristics and their application in therapeutic approaches following transplantation or in vitro maturation. Finally, men diagnosed with germ cell aplasia can only be treated by donor or de novo generated gametes. In the past several years, we have attempted to manufacture gametes by inducing haploidization of somatic cells and more recently, generating sperm-like cells through embryonic stem cell differentiation.

Niki, Y., T. Yamaguchi, et al. (2006). "Establishment of stable cell lines of Drosophila germ-line stem cells." Proc Natl Acad Sci U S A **103**(44): 16325-30.

Each Drosophila ovariole has three independent sets of stem cells: germ-line stem cells (GSCs) and escort stem cells, located at the anterior tip of the germarium, and somatic stem cells (SSCs), located adjacent to the newly formed 16-cell cysts. Decapentaplegic (Dpp) is required to maintain the anterior stem cells, whereas Hedgehog is required for maintenance and cell division of the SCCs. In an effort to establish a new in vitro system to analyze intrinsic and extrinsic factors regulating the division and differentiation of GSCs of Drosophila, we tested various culture conditions for growing GSCs, derived from bag of marbles (bam) mutant ovaries. We have shown that bam(-) GSCs can be maintained and promoted to divide in vitro in media containing Dpp. These cells retain the morphological features of GSCs, i.e., expression of Vasa and Nanos and spectrosomes, even after several months of culture. Somatic cells are induced to grow in culture by the presence of sonic Hedgehog. The somatic cells produce Dpp. GSCs associate with the somatic cells via DE-cadherin, features that are also prominent at the niche of a normal germarium. Finally, we have established stable cell cultures consisting of GSCs and sheets of somatic cells, which are dependent on the addition of fly extract. A somatic cell line, lacking GSCs, has also been established. These cells are thought to be descendants of SCCs. Our in vitro system may provide the opportunity to manipulate GSCs genetically and to analyze the interaction of germ-line stem cells and soma.

Ohmura, M., S. Yoshida, et al. (2004). "Spatial analysis of germ stem cell development in Oct-4/EGFP transgenic mice." Arch Histol Cytol **67**(4): 285-96.

Questions persist regarding male germ stem cells and how they mature during the prespermatogenic period of testicular development. We successfully labeled the prespermatogonia with green fluorescence protein (GFP) by using Oct-4 enhancer/promoter. This study shows that GFP was specifically expressed in prespermatogonia, spermatogonia and spermatids that faithfully reproduce the endogenous expression of Oct-4. Histochemical analysis revealed that most of the TRA98-positive gonocytes are also positive for GFP. However, the frequency of GFP expressing cells out of TRA98 expressing cells decreased together with the maturation of gonocytes in the first week after birth. To compare the stem cell activity between GFP-positive and -negative populations, we performed a transplantation of sorted cells into testes from an individual population. Colonization efficiency of germ cells from a GFP-positive population resulted in a 30-fold increase in colonization compared with a GFP-negative population. Since the expression of Oct-4 in prespermtogonia correlates well with the stem activity, Oct-4 might be a crucial molecule in the stem cell property of spermatogonia but not in cell survival.

Ohta, H., S. Aizawa, et al. (2003). "Functional analysis of the p53 gene in apoptosis induced by heat stress or loss of stem cell factor signaling in mouse male germ cells." Biol Reprod **68**(6): 2249-54.

Apoptosis plays an important role in controlling germ cell numbers and restricting abnormal cell proliferation during spermatogenesis. The tumor suppressor protein, p53, is highly expressed in the testis, and is known to be involved in apoptosis, which suggests that it is one of the major causes of germ cell loss in the testis. Mice that are c-kit/SCF mutant (Sl/Sld) and cryptorchid show similar testicular phenotypes; they carry undifferentiated spermatogonia and Sertoli cells in their seminiferous tubules. To investigate the role of p53-dependent apoptosis in infertile testes, we transplanted p53-deficient spermatogonia that were labeled with enhanced green fluorescence protein into cryptorchid and Sl/Sld testes. In cryptorchid testes, transplanted p53-deficient spermatogonia differentiated into spermatocytes, but not into haploid spermatids. In contrast, no differentiated germ cells were observed in Sl/Sld mutant testes. These results indicate that the mechanism of germ cell loss in the c-kit/SCF mutant is not dependent on p53, whereas the apoptotic mechanism in the cryptorchid testis is quite different (i.e., although the early stage of differentiation of spermatogonia and the meiotic prophase is dependent on p53-mediated apoptosis, the later stage of spermatids is not).

Orwig, K. E., B. Y. Ryu, et al. (2002). "Male germ-line stem cell potential is predicted by morphology of cells in neonatal rat testes." Proc Natl Acad Sci U S A **99**(18): 11706-11.

Gonocytes are a transient population of male germ-line stem cells that are derived from primordial

germ cells in the embryo and give rise to spermatogonial stem cells, which establish and maintain spermatogenesis in the postnatal testis. In contrast to spermatogonial stem cells, gonocytes can be identified easily in neonatal rat testis cell suspensions based on their large size and distinct morphology. Furthermore, histological analysis of testes from neonatal transgenic rats demonstrated that gonocytes are the only cells that express a lacZ reporter transgene. Two gonocyte subpopulations, designated pseudopod and round, were identified and isolated from neonatal (0-4 days postpartum) rat testis cell suspensions. Male germ-line stem cells, identified by their ability to produce and maintain colonies of spermatogenesis upon transplantation into infertile recipient testes, were present almost exclusively in the pseudopod gonocyte subpopulation. In contrast, annexin V staining indicated that the majority of round gonocytes undergo apoptosis. These results indicate that a nearly pure population of male germ-line stem cells can be prospectively identified in neonatal rat testis cell suspensions by morphological criteria. Together, the pseudopod and round gonocyte populations will provide powerful tools for the study of cellular mechanisms that control cell fates and the establishment of spermatogenesis in the postnatal testis.

Park, J. H., S. J. Kim, et al. (2004). "Establishment of a human embryonic germ cell line and comparison with mouse and human embryonic stem cells." Mol Cells **17**(2): 309-15.

Human embryonic stem (ES) cells and embryonic germ (EG) cells are pluripotent and are invaluable material for in vitro studies of human embryogenesis and cell therapy. So far, only two groups have reported the establishment of human EG cell lines, whereas at least five human ES cell lines have been established. To see if human EG cell lines can be reproducibly established, we isolated primordial germ cells (PGCs) from gonadal ridges and mesenteries (9 weeks post-fertilization) and cultured them on mouse STO cells. As with mouse ES colonies, the PGC-derived cells have given rise to multilayered colonies without any differentiation over a year of continuous culture. They are karyotypically normal and express high levels of alkaline phosphatase, Oct-4, and several cell-surface markers. Histological and immunocytochemical analysis of embryoid bodies (EBs) formed from floating cultures of the PGC-derived cell colonies revealed ectodermal, endodermal, and mesodermal tissues. When the EBs were cultured in the presence of insulin, transferrin, sodium selenite, and fibronectin for 1 week, markers of primitive neuroectoderm were expressed in cells within the EBs as well as in cells growing out from the EBs. These observations indicate that our PGC-derived cells satisfy the criteria for pluripotent stem cells and hence may be EG cells.

Perkel, J. (2009). "Comparative proteomics study suggests germ-cell-derived stem cells can stand in for ESCs." J Proteome Res **8**(12): 5407.

Pesce, M., M. G. Farrace, et al. (1993). "Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis)." Development **118**(4): 1089-94.

Proliferating primordial germ cells (PGCs) isolated from mouse embryos soon after their arrival in the genital ridges would only survive in vitro at temperature of less than 30 degrees C (De Felici, M. and McLaren, A. (1983). Exp. Cell. Res. 144, 417-427; Wabik-Sliz, B. and McLaren, A. (1984). Exp. Cell. Res. 154, 530-536) or when co-cultured on cell feeder layers (Donovan, P. J., Stott, D., Godin, I., Heasman, J. and Wylie, C. C. (1986). Cell 44, 831-838; De Felici, M. and Dolci, S. (1991). Dev. Biol. 147, 281-284). In the present paper we report that mouse PGC death in vitro occurs with all the hallmarks of programmed cell death or apoptosis. We found that after 4-5 hours in culture many PGCs isolated from 12.5 dpc fetal gonads assumed a nuclear morphology and produced membrane bound fragments (apoptotic bodies) typical of apoptotic cells. In addition, PGCs in culture accumulated high level of tissue transglutaminase (tTGase; an enzyme that is induced and activated during apoptosis) and showed extensive degradation of DNA to oligonucleosomal fragments, which is characteristic of apoptosis. The physiological relevance of this mechanism of PGC death is supported by the finding that some PGCs undergoing apoptosis, as revealed by the high level of tTGase expression, were detected in the embryo. Most importantly, we show that the addition of stem cell factor (SCF) or leukemia inhibitory factor (LIF) to the culture medium, two cytokines known to favour PGC survival and/or proliferation in vitro, markedly reduced the occurrence of apoptosis in PGCs during the first hours in culture.(ABSTRACT TRUNCATED AT 250 WORDS)

Ploemacher, R. E., J. C. van der Loo, et al. (1993). "Wheat germ agglutinin affinity of murine hemopoietic stem cell subpopulations is an inverse function of their long-term repopulating ability in vitro and in vivo." Leukemia **7**(1): 120-30.

Hemopoietic stem cells show extensive heterogeneity with respect to their proliferative potential and activity. We have recently reported that the accepted technique for sorting stem cells on the

basis of high affinity for the lectin wheat germ agglutinin (WGA) did not select for cells initiating long-term production of new stem cells on a stromal layer in vitro. We have therefore reinvestigated the expression of cell surface sialic acid residues in the hemopoietic stem cell compartment by sorting murine bone marrow cells on the basis of affinity for WGA. Frequency analysis of long-term bone marrow culture initiating stem cells was done using the cobblestone-area-forming cell (CAFC) assay with limiting dilution set-up. In vivo stem cell quality was determined by spleen colony formation, marrow-repopulating ability (MRA) and long-term repopulating ability (LTRA) using sex-mismatched hemopoietic chimerism. The data indicate that MRA and LTRA in vivo and in vitro are among the most WGA-dim cells. In contrast, the enrichment factors for splenic colony-forming units (CFU-S) at day 12 and transient CAFC increase with increasing WGA affinity. These characteristics allowed us to concentrate LTRA cells 590- to 850-fold over their activity in normal bone marrow without significant enrichment of day-12 CFU-S. The data reveal that WGA affinity is an inverse function of the primitiveness of murine hemopoietic stem cells and that long-term production of blood cells in vivo and in vitro is provided for by primitive cells that are physically separable from the vast majority of day-12 CFU-S. In addition the data reveal, that the CAFC frequency at day 28-35 of a graft strongly correlates with the number of cells required to induce 40% donor-type chimerism at 15 months post-transplantation and thus predicts the in vivo LTRA of a graft.

Pont, J., C. Bokemeyer, et al. (1997). "Chemotherapy for germ cell tumors relapsing after high-dose chemotherapy and stem cell support: a retrospective multicenter study of the Austrian Study Group on Urologic Oncology." Ann Oncol **8**(12): 1229-34.

BACKGROUND: In most patients with advanced refractory germ cell tumors undergoing high-dose chemotherapy with stem cell support (HDCT) the disease progresses after HDCT. This study was designed to shed light on the unestablished role of post-HDCT chemotherapy. PATIENTS AND METHODS: In a retrospective multicenter study data of 47 evaluable patients from nine centers subjected to post-HDCT chemotherapy for progression of their germ cell tumors were collected in a questionnaire survey and analyzed for treatment response and survival. RESULTS: Of 191 patients pretreated by HDCT, 48 (25%) were subjected to post-HDCT chemotherapy for disease progression. Remission was achieved in 17 (36%) and marker-negative remission in eight (17%). The median survival time was 26 weeks, 65 weeks for responders and 13 weeks for non-responders. Only one of 47 evaluable patients achieved sustained complete remission. Remissions significantly correlated with the post-HDCT interval, the use of ifosfamide and the combination regimens of cisplatin + etoposide i.v. or ifosfamide and of paclitaxel + ifosfamide or cisplatin. On univariate analysis a longer post-HDCT interval, the use of cisplatin, paclitaxel and ifosfamide and the combined use of paclitaxel + ifosfamide and/or cisplatin significantly improved the chances of survival. On multivariate analysis only treatment with paclitaxel and ifosfamide retained independent prognostic significance for survival. CONCLUSIONS: One third of the patients considered to be candidates for further chemotherapy once progressive after HDCT went into remission with a gain in survival time. Sustained remissions may occur, but are rarely seen. Paclitaxel and ifosfamide appear to be the most effective drugs in these heavily pretreated patients.

Ragone, G., A. Bresin, et al. (2009). "The Tcl1 oncogene defines secondary hair germ cells differentiation at catagen-telogen transition and affects stem-cell marker CD34 expression." Oncogene **28**(10): 1329-38.

Overexpression of the TCL1 gene family plays a role in the onset of T-cell leukemias in mice and in humans. The Tcl1 gene is tightly regulated during early embryogenesis in which it participates in embryonic stem (ES)-cells proliferation and during lymphoid differentiation. Here, we provide evidences that Tcl1 is also important in mouse hair follicle (HF) and skin homeostasis. We found that Tcl1(-/-) adult mice exhibit hair loss, leading to alopecia with extensive skin lesions. By analysing Tcl1 expression in the wild-type (wt) skin through different stages of hair differentiation, we observe high levels in the secondary hair germ (HG) cells and hair bulges, during early anagen and catagen-telogen transition phases. The loss of Tcl1 does not result in apparent skin morphological defects during embryonic development and at birth, but its absence causes a reduction of proliferation in anagen HFs. Importantly, we show the that absence of Tcl1 induces a significant loss of the stem-cell marker CD34 (but not alpha6-integrin) expression in the bulge cells, which is necessary to maintain stem-cell characteristics. Therefore, our findings indicate that Tcl1 gene(s) might have important roles in hair formation, by its involvement in cycling and self-renewal of transient amplifying (TA) and stem-cell (SC) populations.

Rick, O., C. Bokemeyer, et al. (2001). "Salvage treatment with paclitaxel, ifosfamide, and cisplatin plus high-dose carboplatin, etoposide, and thiotepa followed by autologous stem-cell rescue in patients

with relapsed or refractory germ cell cancer." J Clin Oncol **19**(1): 81-8.

PURPOSE: To study feasibility and efficacy of a new salvage regimen in patients with relapsed and/or refractory germ cell tumors. PATIENTS AND METHODS: Between May 1995 and February 1997, 80 patients were entered onto a phase II study. Conventional-dose salvage treatment with three cycles of paclitaxel 175 mg/m(2), ifosfamide 5 x 1.2 g/m(2), and cisplatin 5 x 20 mg/m(2) (TIP) was followed by one cycle of high-dose chemotherapy (HDCT) with carboplatin 500 mg/m(2) x 3, etoposide 600 mg/m(2) x 4, and thiotepa 150 to 250 mg/m(2) x 3 (CET). In 23 patients, one additional cycle of paclitaxel 175 mg/m(2) and ifosfamide 5 g/m(2) (TI) was given immediately before TIP to improve stem-cell mobilization. RESULTS: Fifty-five (69%) of 80 patients responded to TIP, 24 (30%) of 80 patients had stable disease (n = 5) or tumor progression (n = 19), and one patient died. Only 62 (78%) of 80 patients received subsequent HDCT. Among those, 41 (66%) of 62 patients responded and 20 (32%) of 62 patients had stable disease (n = 3) or tumor progression (n = 17). One patient died after HDCT from multiorgan failure. Survival probabilities at 3 years were 30% for overall and 25% for event-free survival. Peripheral neurotoxicity with sensorimotor impairment grade 2 through 4 in 29%, paresthesias grade 2 through 4 in 24%, and skin toxicity grade 2 through 3 in 15% of patients were the most relevant side effects. CONCLUSION: Treatment with TIP followed by high-dose CET is feasible and can induce long-term remissions in 25% of patients with relapsed or refractory germ cell tumors. Peripheral nervous toxicity in approximately one third of patients is a disadvantage of this salvage strategy.

Rohwedel, J., U. Sehlmeyer, et al. (1996). "Primordial germ cell-derived mouse embryonic germ (EG) cells in vitro resemble undifferentiated stem cells with respect to differentiation capacity and cell cycle distribution." Cell Biol Int **20**(8): 579-87.

Embryonic germ (EG) cells of line EG-1 derived from mouse primordial germ cells were investigated for their in vitro differentiation capacity. By cultivation as embryo-like aggregates EG-1 cells differentiated into cardiac, skeletal muscle and neuronal cells accompanied by the expression of tissue-specific genes and proteins as shown by RT-PCR analysis and indirect immunofluorescence. In comparison to embryonic stem (ES) cells of line D3 the efficiency of differentiation into cardiac and muscle cells was comparatively low, whereas spontaneous neuronal differentiation was more efficient than in D3 cells. Furthermore, the distribution of cell cycle phases as a parameter for the differentiation state was analysed in undifferentiated EG cells and ES cells and compared to data obtained for embryonic carcinoma (EC) cells of line P19 and differentiated, epithelioid EPI-7 cells. Flow cytometric analysis revealed similar cell cycle phase distributions in EG, EC and ES cells. In contrast, the somatic differentiated EPI-7 cells showed a longer G1-phase and shorter S- and G2/M-phases. Together, our results demonstrate that the differentiation state and capacity of EG cells in vitro resemble that of totipotent ES cells.

Santagata, S., K. L. Ligon, et al. (2007). "Embryonic stem cell transcription factor signatures in the diagnosis of primary and metastatic germ cell tumors." Am J Surg Pathol **31**(6): 836-45.

The core embryonic stem cell transcription factors (TFs) OCT3/4 (OCT4), NANOG, and SOX2 have shared as well as nonoverlapping roles in stem cell growth and differentiation. These same TFs are also expressed in various types of human germ cell tumors (GCTs), implicating them in regulation of tumor growth and differentiation. Although NANOG and OCT3/4 are sensitive and specific markers for seminoma and embryonal carcinoma, neither factor aids in the clinically important distinction of seminomatous from nonseminomatous tumors. In contrast, expression profiling data suggest that SOX2 may help with this distinction. To determine if a panel of embryonic stem cell TFs (NANOG, OCT3/4, and SOX2) can facilitate the identification and distinction of seminomatous from nonseminomatous GCTs, we evaluated their expression by immunohistochemistry in primary testicular (n=41) and metastatic retroperitoneal (n=43) GCTs. Our results confirm NANOG and OCT3/4 as sensitive and specific markers for primary seminoma and embryonal carcinoma and demonstrate the novel finding that NANOG is a marker for metastatic GCTs. In addition, SOX2 is expressed in embryonal carcinoma but not pure seminoma and is therefore a useful diagnostic marker for distinguishing seminomatous and nonseminomatous GCTs. In summary, we find that the embryonic stem cell TF signature of seminoma is NANOG+, OCT3/4+, and SOX2-, whereas embryonal carcinoma is NANOG+, OCT3/4+, and SOX2+, and expect these immunohistochemical profiles will facilitate the diagnosis of both primary and metastatic GCTs.

Schlatt, S., J. Ehmcke, et al. (2009). "Testicular stem cells for fertility preservation: preclinical studies on male germ cell transplantation and testicular grafting." Pediatr Blood Cancer **53**(2): 274-80.

Spermatogonial stem cells open novel strategies for preservation of testicular tissue and

fertility preservation in boys and men exposed to gonadotoxic therapies. This review provides an update on the physiology of spermatogonial stem cells in rodent and primate testes. Species-specific differences must be considered when new technologies on testicular stem cells are considered. Germ cell transplantation is presented as one novel and promising strategy. Whereas this technique has become an important research tool in rodents, a clinical application must still be regarded as experimental and many aspects of the procedure need to be optimized prior to a safe and efficient clinical application in men. Testicular grafting opens another exciting strategy for fertility preservation. Autologous and xenologous transfer of immature tissue revealed a high regenerative potential of immature testicular tissue. Grafting was applied in rodents and primates and resulted in the generation of sperm. Further research is needed before an application in humans can be considered safe and efficient. Despite the current limitations in regard to the generation of sperm from cryopreserved male germline cells and tissues, protocols for cryopreservation of testicular tissue are available and reveal a promising outcome. Since future improvements of germ cell transplantation and grafting approaches can be assumed, bioptic retrieval and cryopreservation of testicular tissue fragments should be performed in oncological patients at high risk of fertility loss since this is their only option to maintain their fertility potential.

Schmoll, H. J., C. Kollmannsberger, et al. (2003). "Long-term results of first-line sequential high-dose etoposide, ifosfamide, and cisplatin chemotherapy plus autologous stem cell support for patients with advanced metastatic germ cell cancer: an extended phase I/II study of the German Testicular Cancer Study Group." J Clin Oncol **21**(22): 4083-91.

PURPOSE: Patients with disseminated germ cell cancer and poor prognosis (International Germ Cell Cancer Collaborative Group [IGCCCG] classification) achieve only a 45% to 50% long-term survival by standard chemotherapy. First-line high-dose chemotherapy might be able to improve the result. This analysis reports toxicity and long-term results of a large phase I/II study of sequential high-dose etoposide, ifosfamide, and cisplatin (VIP) in patients with advanced germ cell tumors. PATIENTS AND METHODS: Between July 1993 and November 1999, 221 patients with either Indiana "advanced disease" (n = 39) or IGCCCG "poor prognosis" criteria (n = 182) received one cycle of VIP followed by three to four sequential cycles of high-dose VIP chemotherapy plus stem cell support, every 3 weeks, at six consecutive dose levels. RESULTS: Dose limiting toxicity occurred at level 8 (100 mg/m2 cisplatinum, 1750 mg/m2 etoposide, 12 g/m2 ifosfamide) with grade 4 mucositis (three of eight patients), grade 3 CNS toxicity (one of eight patients), grade 4 renal toxicity (one of eight patients), and prolonged granulocytopenia (one of eight patients). After 4-year median follow-up, progression-free survival and disease-specific survival rates in the poor prognosis subgroup were 69% and 79% at 2 years and 68% and 73% at 5 years, with 76% for gonadal/retroperitoneal versus 67% for mediastinal primaries. Severe toxicity included treatment related death (4%), treatment-related acute myeloid leukemia (1%), long-term impared renal function (3%), chronic renal failure (1%), and persistent grade 2-3 neuropathy (5%). CONCLUSION: Repetitive cycles of high-dose VIP with peripheral stem cell support can be successfully applied in a multicenter setting. Dose level 6 with cisplatin 100 mg/m2, etoposide 1500 mg/m2, and ifosfamide 10 g/m2 is recommended for further investigation in randomized trials. An ongoing randomized trial within the European Organization for Research and Treatment of Cancer evaluates this protocol against four cycles of standard cisplatin, etoposide, and bleomycin.

Schulz, C., A. A. Kiger, et al. (2004). "A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the Drosophila male germ-line stem cell lineage." Genetics **167**(2): 707-23.

Male gametes are produced throughout reproductive life by a classic stem cell mechanism. However, little is known about the molecular mechanisms for lineage production that maintain male germ-line stem cell (GSC) populations, regulate mitotic amplification divisions, and ensure germ cell differentiation. Here we utilize the Drosophila system to identify genes that cause defects in the male GSC lineage when forcibly expressed. We conducted a gain-of-function screen using a collection of 2050 EP lines and found 55 EP lines that caused defects at early stages of spermatogenesis upon forced expression either in germ cells or in surrounding somatic support cells. Most strikingly, our analysis of forced expression indicated that repression of bag-of-marbles (bam) expression in male GSC is important for male GSC survival, while activity of the TGF beta signal transduction pathway may play a permissive role in maintenance of GSCs in Drosophila testes. In addition, forced activation of the TGF beta signal transduction pathway in germ cells inhibits the transition from the spermatogonial mitotic amplification program to spermatocyte differentiation.

Seipel, K., N. Yanze, et al. (2004). "The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea." Int J Dev Biol **48**(1): 1-7.

In most animal phyla from insects to mammals, there is a clear division of somatic and germ line cells. This is however not the case in plants and some animal phyla including tunicates, flatworms and the basal phylum Cnidaria, where germ stem cells arise de novo from somatic cells. Piwi-like genes represent essential stem cell genes in diverse multicellular organisms. The cnidarian Piwihomolog Cniwiwas cloned from Podocoryne carnea, a hydrozoan with a full life cycle. CniwiRNA is present in all developmental stages with highest levels in the egg and the medusa. In the adult medusa, Cniwi expression is prominent in the gonads where it likely functions as a germ stem cell gene. The gene is also expressed, albeit at low levels, in differentiated somatic cells like the striated muscle of the medusa. Isolated striated muscle cells can be induced to transdifferentiate into smooth muscle cells which proliferate and differentiate into nerve cells. Cniwi expression is upregulated transiently after induction of transdifferentiation and again when the emerging smooth muscle cells proliferate and differentiate. The continuous low-level expression of an inducible stem cell gene in differentiated somatic cells may underlie the ability to form medusa buds from polyp cells and explain the extraordinary transdifferentation and regeneration potential of Podocoryne carnea.

Sette, C., S. Dolci, et al. (2000). "The role of stem cell factor and of alternative c-kit gene products in the establishment, maintenance and function of germ cells." Int J Dev Biol **44**(6): 599-608.

The c-kit gene plays a fundamental role during the establishment, the maintenance and the function of germ cells. In the embryonal gonad the c-kit tyrosine kinase receptor and its ligand Stem Cell Factor (SCF) are required for the survival and proliferation of primordial germ cells. In the postnatal animal, c-kit/SCF are required for the production of the mature gametes in response to gonadotropic hormones, i.e. for the survival and/or proliferation of the only proliferating germ cells of the testis, the spermatogonia, and for the growth and maturation of the oocytes. Finally, a truncated c-kit product, tr-kit, specifically expressed in post-meiotic stages of spermatogenesis and present in mature spermatozoa, causes parthenogenetic activation when microinjected into mouse eggs, suggesting that it might play a role in the final function of the gametes, fertilization.

Shinohara, T., K. E. Orwig, et al. (2002). "Germ line stem cell competition in postnatal mouse testes." Biol Reprod **66**(5): 1491-7.

Niche is believed to affect stem cell behavior. In self-renewing systems for which functional transplantation assays are available, it has long been assumed that stem cells are fixed in the niche and that ablative treatments to remove endogenous stem cells are required for successful donor engraftment. Our results demonstrate that enriched populations of donor stem cells can produce long-lasting spermatogenic colonies in testes of immature and mature, nonablated mice, albeit at a lower frequency than in ablated mice. Colonization of nonablated recipient testes by neonate, pup, and cryptorchid adult donor spermatogonial stem cells demonstrates that competition for niche begins soon after birth and that endogenous stem cells influence the degree and pattern of donor cell colonization. Thus, a dynamic relationship between stem cell and niche exists in the testis, as has been suggested for hematopoiesis. Therefore, similar competitive properties of donor stem cells may be characteristic of all self-renewing systems.

Siegert, W., J. Beyer, et al. (1994). "High-dose treatment with carboplatin, etoposide, and ifosfamide followed by autologous stem-cell transplantation in relapsed or refractory germ cell cancer: a phase I/II study. The German Testicular Cancer Cooperative Study Group." J Clin Oncol **12**(6): 1223-31.

PURPOSE: This trial evaluated the toxicity and efficacy of high-dose carboplatin, etoposide, and ifosfamide followed by autologous stem-cell transplantation in patients with refractory or relapsed germ cell cancer. PATIENTS AND METHODS: Between August 1989 and September 1992, 74 patients with refractory or recurrent germ-cell tumors received one cycle of escalating doses of carboplatin (1,500 to 2,000 mg/m2), etoposide (1,200 to 2,400 mg/m2), and ifosfamide (0 to 10 g/m2). Before high-dose therapy, two cycles of conventional-dose cisplatin, etoposide, and ifosfamide were administered to assess tumor responsiveness. Seventy-four patients were assessable for toxicity and 68 for response. RESULTS: The doses of carboplatin 1,500 mg/m2, etoposide 2,400 mg/m2, and ifosfamide 10 g/m2 appeared to be safe. At this dosage, we treated 20 patients and observed World Health Organization (WHO) grade 3 and 4 hematotoxicity (100%), nausea (100%), diarrhea (30%), and hepatotoxicity (10%). All patients developed granulocytopenic fever. At carboplatin doses of 1,500 mg/m2, kidney toxicity was mild, with a median maximum creatinine level of 1.4 mg/dL (range, 1.1 to 3.0 mg/dL). However, at carboplatin doses of 1,750 and 2,000 mg/m2, we observed nonacceptable nephrotoxicity and neurotoxicity. Two (3%) patients died of treatment-related complications. Six patients required

hemodialysis, which was temporary in five patients and permanent in one. Objective responses were obtained in 43 of 68 (63%) patients, including 21 (31%) complete remissions (CRs) and 14 (20%) inoperable partial remissions (PRs) with marker normalization. The median observation time of surviving patients was 12 months (range, 2 to 32). The probabilities of overall survival, event-free survival, and the relapse-free survival at 2 years were 44% (SD 8%), 35% (SD 6%), and 67% (SD 9%), respectively. Patients with disease refractory to conventional-dose pretreatment had a poor prognosis, with only one of 23 patients surviving event-free at 7 months after high-dose chemotherapy (HDT). In contrast, 24 of 45 (53.3%) patients with sensitive disease survive event-free with a probability of event-free survival at 2 years of 50% (SD 8%). CONCLUSION: High-dose carboplatin, etoposide, and ifosfamide plus autologous stem-cell transplantation can be used in refractory and relapsed germ cell cancer with acceptable toxicity, and represents an effective, potentially curative salvage treatment.

Siegert, W., O. Rick, et al. (1998). "High-dose chemotherapy with autologous stem cell support in poor-risk germ cell tumors." Ann Hematol **76**(5): 183-8.

High-dose chemotherapy (HDT) and stem cell transplantation is a newer treatment option widely applied in poor-risk germ cell tumor patients. Due to the increasing practical clinical experience and the availability of hematopoietic growth factors, this treatment approach has become a relatively safe procedure. Depending on the drugs used. the most prominent therapy-associated side effects are myelosuppression, infections, mucositis, moderate, mostly reversible neurotoxicity, and renal impairment. Because of their unique pharmacologic characteristics, carboplatin and etoposide have proved to be the most important drugs for HDT. It is not known whether the addition of ifosfamide or cyclophosphamide or other drugs to the combination of carboplatin and etoposide leads to superior results. It is not yet clear if HDT should already be instituted in first-line treatment of poor-risk patients, or later after relapse or incomplete response. Even if precise data on the therapeutic value of HDT are still missing because randomized trials are not yet ready for evaluation, there is good evidence for the effectiveness of HDT. The demonstration of remissions in cisplatin-refractory patients, the inversion of remission durations, and the induction of long-term freedom from disease in multiply relapsed patients who were deemed incurable with conventional-dose procedures argue in favor of HDT. Finally, the delineation of prognostic factors associated with a poor probability of survival after HDT contributes to the selection of patients who are likely to profit from HDT and those who should be spared from dose-intensive treatment.

Silva, C., J. R. Wood, et al. (2009). "Expression profile of male germ cell-associated genes in mouse embryonic stem cell cultures treated with all-trans retinoic acid and testosterone." Mol Reprod Dev **76**(1): 11-21.

Cells that morphologically and functionally resemble male germ cells can be spontaneously derived from ES cells. However, this process is inefficient and unpredictable suggesting that the expression pattern of male germ cell associated genes during spontaneous ES cell differentiation does not mimic the in vivo profiles of the genes. Thus, in the present study, the temporal profile of genes expressed at different stages of male germ cell development was examined in differentiating ES cells. The effect of all-trans retinoic acid (RA) which is a known inducer of primordial germ cell (PGC) proliferation/survival in vitro and testosterone which is required for spermatogenesis in vivo on the expression of these genes was also determined. Each of the 12 genes analyzed exhibited one of four temporal expression patterns in untreated differentiating ES cells: progressively decreased (Dppa3, Sycp3, Msy2), initially low and then increased (Stra8, Sycp1, Dazl, Act, Prm1), initially decreased and then increased (Piwil2, Tex14), or relatively unchanged (Akap3, Odf2). RA-treated cells exhibited increased expression of Stra8, Dazl, Act, and Prm1 and suppressed expression of Dppa3 compared to untreated controls. Furthermore, testosterone increased expression of Stra8 while the combination of RA and testosterone synergistically increased expression of Act. Our findings establish a comprehensive profile of male germ cell gene expression during spontaneous differentiation of murine ES cells and describe the capacity of RA and testosterone to modulate the expression of these genes. Furthermore, these data represent an important first step in designing a plausible directed differentiation protocol for male germ cells.

Sligh, J. E., S. E. Levy, et al. (2000). "Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice." Proc Natl Acad Sci U S A **97**(26): 14461-6.

We report a method for introducing mtDNA mutations into the mouse female germ line by means of embryonic stem (ES) cell cybrids. Mitochondria were recovered from the brain of a NZB mouse by fusion of synaptosomes to a mtDNA-deficient (rho degrees) cell line. These cybrids were enucleated and the cytoplasts were electrofused to rhodamine-6G (R-

6G)-treated female ES cells. The resulting ES cell cybrids permitted transmission of the NZB mtDNAs through the mouse maternal lineage for three generations. Similarly, mtDNAs from a partially respiratory-deficient chloramphenicol-resistant (CAP(R)) cell line also were introduced into female chimeric mice and were transmitted to the progeny. CAP(R) chimeric mice developed a variety of ocular abnormalities, including congenital cataracts, decreased retinal function, and hamaratomas of the optic nerve. The germ-line transmission of the CAP(R) mutation resulted in animals with growth retardation, myopathy, dilated cardiomyopathy, and perinatal or in utero lethality. Skeletal and heart muscle mitochondria of the CAP(R) mice were enlarged and atypical with inclusions. This mouse ES cell-cybrid approach now provides the means to generate a wide variety of mouse models of mitochondrial disease.

Sobecks, R. M. and N. J. Vogelzang (1999). "High-dose chemotherapy with autologous stem-cell support for germ cell tumors: a critical review." Semin Oncol **26**(1): 106-18.

High-dose chemotherapy (HDC) with autologous stem-cell support (ASCS) has been investigated in patients with cisplatin-resistant, relapsed, or poor-prognosis germ cell tumor (GCT). Although some of these patients have benefited from this approach, it is unknown when best to administer such therapy. This review categorizes the HDC/ASCS trials into those performed as (1) salvage therapy for second or greater relapse, (2) salvage therapy for first relapse, and (3) first-line therapy. From the trials performed to date, earlier use of HDC/ASCS (first-line or salvage therapy in first relapse) achieved a higher durable remission rate than when used later as salvage therapy in second or greater relapse (approximately 50% v 15%, respectively). HDC/ASCS is not beneficial for relapsed or cisplatin-resistant primary extragonadal GCT patients, but may have a role in testicular GCT who are not "absolutely" cisplatin-resistant. Trial differences regarding the patients selected and the high-dose transplant preparative regimen used have made precise comparative analyses difficult. There has been only one phase III trial and it did not show a survival advantage to the HDC/ASCS arm, although this trial had significant methodological difficulties. In the future, more definitive treatment recommendations may be made upon completion of two ongoing phase III trials comparing HDC/ASCS with standard chemotherapy in the first salvage and front-line settings.

Sorrentino, E., V. Nazzicone, et al. (2007). "Comparative transcript profiles of cell cycle-related genes in mouse primordial germ cells, embryonic stem cells and embryonic germ cells." Gene Expr Patterns **7**(6): 714-21.

We used cDNA array to compare the relative transcript levels of 96 cell cycle-related genes in mouse primordial germ cells (PGCs), embryonic germ (EG) cells and embryonic stem (ES) cells. Among 38 genes of the G1 phase analysed, Ccnd3 (CyclinD3), Cdkn1c (p57(kip2)), Rb1, and Tceb1l (Skip1-like) were expressed at significantly higher levels in PGCs than in EG and ES cells; Ccnd1 (CyclinD1) was more abundant in EG cells than in PGCs. Except for higher mRNA levels of Ccng (CyclinG1) in EG and ES cells in comparison to PGCs, no difference among 20 genes of the S and 12 genes of G2/M phases was found. Less than half of the 26 genes regarded as DNA damage checkpoint/Trp53/Atm pathway genes showed significant transcript levels in all three cell populations. Among these, the transcript levels of Ube1x and Atm were significantly higher in PGCs than in EG and ES cells while that of Ube3a was higher in these latter. In addition, relatively high mRNA levels of Timp3 characterizes EG cells while transcripts of this gene were very low in PGCs and barely detectable in ES cells. With the exception of Tceb1l, differential transcript levels found in the cDNA array assay were confirmed by real time RT-PCR. Using this method, we also analysed the transcripts of two genes not present in the cDNA array: c-myc, known to be critical for the control of cell cycle in many cell types, and Eras, specifically expressed in ES cells and involved in the control of ES cell proliferation and their tumorigenic properties. While c-myc transcripts were present at similar levels in all three cell types examined, Eras was expressed at high levels in ES cells (10-fold) and even more so in EG cells (almost 40-fold) in comparison to PGCs. Taken together, these results indicate that despite similarities between PGCs and ES or EG cells, their cell cycles are differently regulated. In particular, it appears that PGCs, like most mitotic cells, possess a more regulatable control of G1 phase than EG and ES cells. Moreover, our data provide useful clues for further studies aimed at identifying cell cycle genes critical for PGC growth and their transformation in tumorigenic cells.

Sperger, J. M., X. Chen, et al. (2003). "Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors." Proc Natl Acad Sci U S A **100**(23): 13350-5.

Remarkably little is known about the transcriptional profiles of human embryonic stem (ES) cells or the molecular mechanisms that underlie their

pluripotency. To identify commonalties among the transcriptional profiles of different human pluripotent cells and to search for clues into the genesis of human germ cell tumors, we compared the expression profiles of human ES cell lines, human germ cell tumor cell lines and tumor samples, somatic cell lines, and testicular tissue samples by using cDNA microarray analysis. Hierarchical cluster analysis of gene expression profiles showed that the five independent human ES cell lines clustered tightly together, reflecting highly similar expression profiles. The gene expression patterns of human ES cell lines showed many similarities with the human embryonal carcinoma cell samples and more distantly with the seminoma samples. We identified 895 genes that were expressed at significantly greater levels in human ES and embryonal carcinoma cell lines than in control samples. These genes are candidates for involvement in the maintenance of a pluripotent, undifferentiated phenotype.

Stoop, H., F. Honecker, et al. (2008). "Stem cell factor as a novel diagnostic marker for early malignant germ cells." J Pathol **216**(1): 43-54.

Carcinoma in situ (CIS) of the testis is the pre-invasive stage of type II testicular germ cell tumours (TGCTs) of adolescents and adults. These tumours are the most frequently diagnosed cancer in Caucasian adolescents and young adults. In dysgenetic gonads, the precursor of type II GCTs can be either CIS or a lesion known as gonadoblastoma (GB). CIS/GB originates from a primordial germ cell (PGC)/gonocyte, ie an embryonic cell. CIS can be cured by local low-dose irradiation, with limited side effects on hormonal function. Therefore, strategies for early diagnosis of CIS are essential. Various markers are informative to diagnose CIS in adult testis by immunohistochemistry, including c-KIT, PLAP, AP-2gamma, NANOG, and POU5F1 (OCT3/4). OCT3/4 is the most informative and consistent in presence and expression level, resulting in intense nuclear staining. In the case of maturational delay of germ cells, frequently present in gonads of individuals at risk for type II (T)GCTs, use of these markers can result in overdiagnosis of malignant germ cells. This demonstrates the need for a more specific diagnostic marker to distinguish malignant germ cells from germ cells showing maturation delay. Here we report the novel finding that immunohistochemical detection of stem cell factor (SCF), the c-KIT ligand, is informative in this context. This was demonstrated in over 400 cases of normal (fetal, neonatal, infantile, and adult) and pathological gonads, as well as TGCT-derived cell lines, specifically in cases of CIS and GB. Both membrane-bound and soluble SCF were expressed, suggestive of an autocrine loop. SCF immunohistochemistry can be a valuable diagnostic tool, in addition to OCT3/4, to screen for precursor lesions of TGCTs, especially in patients with germ cell maturation delay.

Sugiura, K., M. Inaba, et al. (1988). "Wheat germ agglutinin-positive cells in a stem cell-enriched fraction of mouse bone marrow have potent natural suppressor activity." Proc Natl Acad Sci U S A **85**(13): 4824-6.

In the present study we have characterized natural suppressor (NS) cells, which nonspecifically suppress mitogen responses and mixed-lymphocyte reaction. The strongest NS activity was found in a fraction of relatively low-density cells (1.063 less than p less than 1.075) obtained by equilibrium density centrifugation. Further purification and characterization of these NS cells by using a fluorescence-activated cell sorter indicated that wheat germ agglutinin (WGA)-positive cells have potent NS activity, whereas WGA-negative cells have no NS activity. Spleen colony-forming unit (CFU-S) assays demonstrated a significant correlation between the number of CFU-S cells and the NS activity in the bone marrow. However, WGA-positive cells obtained from the bone marrow of animals treated with 5-fluorouracil, which cells in turn were exposed to 5-fluorouracil, showed a marked reduction in NS activity. These results suggest that the pluripotent stem cells have NS activity when the cells are in the cycling phase but not when the cells are in the G0 phase. It seems possible that apparently primitive cells play an important role in down-regulation of immune responses.

Tada, T., T. Takizawa, et al. (1999). "Treatment of intracranial nongerminomatous germ-cell tumor by high-dose chemotherapy and autologous stem-cell rescue." J Neurooncol **44**(1): 71-6.

Nongerminomatous germ-cell tumor (NGGCT) in the central nervous system (CNS) is still highly lethal. The present study evaluated the outcome of high-dose chemotherapy followed by autologous stem-cell rescue (ASCR). The patients included three cases of choriocarcinoma, two cases of embryonal carcinoma and one case of yolk sac carcinoma. High-dose cisplatin (200 mg/m2), etoposide (1250 mg/m2) and ACNU (150 mg/m2) were administrated in combination with ASCR to patients at complete remission as a result of surgical removal, irradiation, and from four to seven courses of induction chemotherapy. All the patients treated with this therapy were alive from one to seven years after the diagnosis, living with good performance status. The patients have not required any additional treatments after ASCR. The myelosuppression period,

characterized by fewer than 500/microl peripheral neutrophils, ranged from 8 to 15 days (median, 11.5 days). Within seven days of ASCR, high fever was found in four patients. Although mild liver dysfunction was found in all patients, renal dysfunction was not observed. Hearing disturbance was found in 50% of the patients. This treatment regime will improve long-term survival for patients with NGGCT.

Tamagawa, T., I. Ishiwata, et al. (2004). "Establishment and characterization of a pluripotent stem cell line derived from human amniotic membranes and initiation of germ layers in vitro." Hum Cell **17**(3): 125-30.

OBJECTIVES: Pluripotent stem cells are proposed to be used in regenerative therapy and may exist in the human amniotic membrane. The present article is aimed at establishing a pluripotent stem cell line from human placenta. METHODS: HAM-1 (stem cell line derived from human amniotic membranes) was established by the colonial cloning technique using aMEM culture medium containing 10 ng/ml of EGF, 10 ng/ml of hLIF and 10% fetal bovine serum. RESULTS: HAM-1 cells appeared to maintain a normal karyotype indefinitely in vitro and expressed markers characteristic of stem cells from mice and human, namely alkaline phosphatase. Also, these cells contributed to the formation of chimeric mouse embryoid bodies and gave rise to cells of all germ layers in vitro. CONCLUSIONS: This study demonstrates that human amniotic membranes derived stem cells have a wide developmental capability and might be utilized to regenerate different types of cells or tissues for transplantation therapy.

Teramura, T., Y. Onodera, et al. (2009). "Mouse androgenetic embryonic stem cells differentiated to multiple cell lineages in three embryonic germ layers in vitro." J Reprod Dev **55**(3): 283-92.

The embryos of some rodents and primates can precede early development without the process of fertilization; however, they cease to develop after implantation because of restricted expressions of imprinting genes. Asexually developed embryos are classified into parthenote/gynogenote and androgenote by their genomic origins. Embryonic stem cells (ESCs) derived from asexual origins have also been reported. To date, ESCs derived from parthenogenetic embryos (PgESCs) have been established in some species, including humans, and the possibility to be alternative sources for autologous cell transplantation in regenerative medicine has been proposed. However, some developmental characteristics, which might be important for therapeutic applications, such as multiple differentiation capacity and transplantability of the ESCs of androgenetic origin (AgESCs) are uncertain. Here, we induced differentiation of mouse AgESCs and observed derivation of neural cells, cardiomyocytes and hepatocytes in vitro. Following differentiated embryoid body (EB) transplantation in various mouse strains including the strain of origin, we found that the EBs could engraft in theoretically MHC-matched strains. Our results indicate that AgESCs possess at least two important characteristics, multiple differentiation properties in vitro and transplantability after differentiation, and suggest that they can also serve as a source of histocompatible tissues for transplantation.

Tesar, P. J. (2005). "Derivation of germ-line-competent embryonic stem cell lines from preblastocyst mouse embryos." Proc Natl Acad Sci U S A **102**(23): 8239-44.

The first differentiation event of the mammalian embryo is thought to occur during blastulation and results in two populations of cells, the inner cell mass (ICM) and the trophectoderm. Most embryonic stem (ES) cell lines have been derived from the ICM or a further subset of ICM cells known as the epiblast. There appears to be a limited period of embryonic development during which pluripotent ES cells can be adapted from the cells of the blastocyst to culture. A method is presented here that allows ES cell lines to be isolated from preblastocyst mouse embryos. These lines were derived from 129S2/SvHsd mouse morulae and earlier cleavage stages with high efficiency. The lines expressed genes and antigens characteristic of pluripotent ES cells. XY cell lines remained karyotypically stable through extensive passaging and produced germ-line-competent chimeras upon blastocyst injection. These results suggest that true ES cells can be derived from embryos explanted at any stage of preimplantation development in the mouse. This finding raises the interesting question of whether ES cell lines derived from embryos at different stages of preimplantation development possess the same potential.

Thomson, J. A. and J. S. Odorico (2000). "Human embryonic stem cell and embryonic germ cell lines." Trends Biotechnol **18**(2): 53-7.

Undifferentiated human embryonic stem (ES) cells and embryonic germ (EG) cells can be cultured indefinitely and yet maintain the potential to form many or all of the differentiated cells in the body. Human ES and EG cells provide an exciting new model for understanding the differentiation and function of human tissue, offer new strategies for drug discovery and testing, and promise new therapies

based on the transplantation of ES and EG cell-derived tissues.

Unhavaithaya, Y., Y. Hao, et al. (2009). "MILI, a PIWI-interacting RNA-binding protein, is required for germ line stem cell self-renewal and appears to positively regulate translation." J Biol Chem **284**(10): 6507-19.

The Argonaute/PIWI protein family consists of Argonaute and PIWI subfamilies. Argonautes function in RNA interference and micro-RNA pathways; whereas PIWIs bind to PIWI-interacting RNAs and regulate germ line development, stem cell maintenance, epigenetic regulation, and transposition. However, the role of PIWIs in mammalian stem cells has not been demonstrated, and molecular mechanisms mediated by PIWIs remain elusive. Here we show that MILI, a murine PIWI protein, is expressed in the cytoplasm of testicular germ line stem cells, spermatogonia, and early spermatocytes, where it is enriched in chromatoid bodies. MILI is essential for the self-renewing division and differentiation of germ line stem cells but does not affect initial establishment of the germ line stem cell population at 7 days postpartum. Furthermore, MILI forms a stable RNA-independent complex with eIF3a and associates with the eIF4E- and eIF4G-containing m7G cap-binding complex. In isolated 7 days postpartum seminiferous tubules containing mostly germ line stem cells, the mili mutation has no effect on the cellular mRNA level yet significantly reduces the rate of protein synthesis. These observations indicate that MILI may positively regulate translation and that such regulation is required for germ line stem cell self-renewal.

Vadivelu, S., M. M. Platik, et al. (2005). "Multi-germ layer lineage central nervous system repair: nerve and vascular cell generation by embryonic stem cells transplanted in the injured brain." J Neurosurg **103**(1): 124-35.

OBJECT: To restore proper function to a damaged central nervous system (CNS) through transplantation, it is necessary to replace both neural and nonneural elements that arise from different germ layers in the embryo. Mounting evidence indicates the importance of signals related to vasculogenesis in governing neural proliferation and differentiation in early CNS development. Here, the authors examined whether embryonic stem cell (ESC)-derived progenitors can selectively generate both neural and endothelial cells after transplantation in the damaged CNS. METHODS: Injections of 20 nmol N-methyl-D-aspartate created a unilateral striatal injury in 7-day-old rats. One week postinjury, murine ESCs, neural-induced with retinoic acid, were transplanted into the injured striatum. Histological staining, laser confocal microscopy, and transmission electron microscopy of grafted ESCs were performed 1 week posttransplantation. CONCLUSIONS: Transplanted ESCs differentiated into neural cells, which segregated into multiple pools and formed neurons that conformed to host cytoarchitecture. The ESCs also generated endothelial cells, which integrated with host cells to form chimeric vasculature. The combination of ESC pluripotentiality and multiple germ layer differentiation provides a new conceptual framework for CNS repair.

Wakayama, S., S. Kishigami, et al. (2005). "Propagation of an infertile hermaphrodite mouse lacking germ cells by using nuclear transfer and embryonic stem cell technology." Proc Natl Acad Sci U S A **102**(1): 29-33.

Animals generated by systematic mutagenesis and routine breeding are often infertile because they lack germ cells, and maintenance of such lines of animals has been impossible. We found a hermaphrodite infertile mouse in our colony, a genetic male with an abnormal Y chromosome lacking developing germ cells. We tried to clone this mouse by conventional nuclear transfer but without success. ES cells produced from blastocysts, which had been cloned by using somatic cell nuclear transfer (ntES cells) from this mouse, were also unable to produce offspring when injected into enucleated oocytes. Although we were able to produce two chimeric offspring using these ntES cells by tetraploid complementation, they were infertile, because they also lacked developing germ cells. However, when such ntES cells were injected into normal diploid blastocysts, many chimeric offspring were produced. One such male offspring transmitted hermaphrodite mouse genes to fertile daughters via X chromosome-bearing sperm. Thus, ntES cells were used to propagate offspring from infertile mice lacking germ cells.

Wei, W., T. Qing, et al. (2008). "Primordial germ cell specification from embryonic stem cells." PLoS One **3**(12): e4013.

BACKGROUND: Primordial germ cell (PGC) specification is the first crucial step in germ line development. However, owing to significant challenges regarding the in vivo system, such as the complex cellular environment and potential problems with embryo manipulation, it is desirable to generate embryonic stem (ES) cells that are capable of overcoming these aforementioned limitations in order to provide a potential in vitro model to recapitulate the developmental processes in vivo. METHODOLOGY AND PRINCIPAL FINDINGS: Here, we studied the

detailed process of PGC specification from stella-GFP ES cells. We first observed the heterogeneous expression of stella in ES cells. However, neither Stella-positive ES cells nor Stella-negative ES cells shared a similar gene expression pattern with either PGCs or PGC precursors. Second, we derived PGCs from ES cells using two differentiation methods, namely the attachment culture technique and the embryoid body (EB) method. Compared with PGCs derived via the attachment culture technique, PGCs derived via the EB method that had undergone the sequential erasure of Peg3 followed by Igf2r resulted in a cell line in which the expression dynamics of T, Fgf8 and Sox17, in addition to the expression of the epiblast markers, were more similar to the in vivo expression, thus demonstrating that the process of PGC derivation was more faithfully recapitulated using the EB method. Furthermore, we developed an in vitro model of PGC specification in a completely chemically defined medium (CDM) that indicated that BMP4 and Wnt3a promoted PGC derivation, whereas BMP8b and activinA had no observable effect on PGC derivation. CONCLUSIONS AND SIGNIFICANCE: The in vitro model we have established can recapitulate the developmental processes in vivo and provides new insights into the mechanism of PGC specification.

Wiles, M. V. and B. M. Johansson (1997). "Analysis of factors controlling primary germ layer formation and early hematopoiesis using embryonic stem cell in vitro differentiation." Leukemia **11 Suppl 3**: 454-6.

Differentiation and subsequent development are intricately interwoven processes operating as an integrated whole to form the organism. As an approach to examine these interactions in early mammalian development, we used embryonic stem (ES) cell in vitro differentiation. ES cells can, depending upon the environment differentiated to neuroectoderm, mesoderm and hematopoietic cells. We developed a serum-free, chemically defined medium (CDM) in which ES cells survive and differentiate. In CDM, in the absence of exogenous factors, ES cells form neuroectoderm, upregulating the early neural marker Pax-6. This is consistent with the view that neuroectoderm development can represent a default state, where the absence or sequestration of mesoderm inducing factors permits neuroectoderm formation. In contrast, if CDM is supplemented with bone morphogenetic protein (BMP) 2 or 4 a process resembling primitive streak formation, least at the molecular level occurs, with the formation of mesoderm and subsequently endothelial and hematopoietic cells. If used with care, ES cell in vitro differentiation can act as a guide in understanding the environment which controls early differentiation events in mammals.

Wu, J., P. F. Mu, et al. (2005). "Parental experience of family stress during hematopoietic stem cell transplantation of pediatric patients in germ-free isolation in Taiwan." Cancer Nurs **28**(5): 363-71.

This qualitative inquiry study used convenience sampling through in-depth interviews to obtain life experiences and feelings of parents while their children underwent hematopoietic stem cell transplantation. Eleven parents of children younger than 18 years undergoing hematopoietic stem cell transplantation in a medical center in north Taiwan consented to participate in this study and provide oral and written responses in Mandarin or Taiwanese. Semistructured interviews lasting 60 minutes were conducted privately and audiotaped. Verbatim transcriptions of the interviews were examined by the content analysis method. The trustworthiness of the data was examined by Lincoln and Guba (Naturalistic Inquiry. Newbury Park, Calif: Sage; 1985) principles. Results indicated that family stress experiences of parents of children undergoing hematopoietic stem cell transplants include 4 themes: parental psychological distress, family lifestyle disturbances, parents' coping patterns, and family resources. The results of this study provide evidence of the nature of parents' experiences while their children undergo hematopoietic stem cell transplantation and provide guidelines for family-centered nursing care.

Yamashita, Y. M. and M. T. Fuller (2005). "Asymmetric stem cell division and function of the niche in the Drosophila male germ line." Int J Hematol **82**(5): 377-80.

The balance between stem cell and differentiating cell populations is critical for the long-term maintenance of tissue renewal for cell types derived from adult stem cell lineages such as blood, skin, intestinal epithelium, and sperm. To keep this balance, stem cells have the potential to divide asymmetrically, producing one daughter cell that maintains stem cell identity and one daughter cell that initiates differentiation. In many adult stem cell systems, the maintenance, proliferation, and number of stem cells appear to be controlled by the microenvironment, or niche. The Drosophila male and female germ line provide excellent model systems in which to study asymmetric stem cell divisions within the stem cell niche. In addition to signals from the niche that specify stem cell self-renewal, the stem cells themselves have elaborate cellular mechanisms to ensure the asymmetric outcome of cell division.

Yamauchi, K., K. Hasegawa, et al. (2009). "In vitro germ cell differentiation from cynomolgus monkey embryonic stem cells." PLoS One **4**(4): e5338.

BACKGROUND: Mouse embryonic stem (ES) cells can differentiate into female and male germ cells in vitro. Primate ES cells can also differentiate into immature germ cells in vitro. However, little is known about the differentiation markers and culture conditions for in vitro germ cell differentiation from ES cells in primates. Monkey ES cells are thus considered to be a useful model to study primate gametogenesis in vitro. Therefore, in order to obtain further information on germ cell differentiation from primate ES cells, this study examined the ability of cynomolgus monkey ES cells to differentiate into germ cells in vitro. METHODS AND FINDINGS: To explore the differentiation markers for detecting germ cells differentiated from ES cells, the expression of various germ cell marker genes was examined in tissues and ES cells of the cynomolgus monkey (Macaca fascicularis). VASA is a valuable gene for the detection of germ cells differentiated from ES cells. An increase of VASA expression was observed when differentiation was induced in ES cells via embryoid body (EB) formation. In addition, the expression of other germ cell markers, such as NANOS and PIWIL1 genes, was also up-regulated as the EB differentiation progressed. Immunocytochemistry identified the cells expressing stage-specific embryonic antigen (SSEA) 1, OCT-4, and VASA proteins in the EBs. These cells were detected in the peripheral region of the EBs as specific cell populations, such as SSEA1-positive, OCT-4-positive cells, OCT-4-positive, VASA-positive cells, and OCT-4-negative, VASA-positive cells. Thereafter, the effect of mouse gonadal cell-conditioned medium and growth factors on germ cell differentiation from monkey ES cells was examined, and this revealed that the addition of BMP4 to differentiating ES cells increased the expression of SCP1, a meiotic marker gene. CONCLUSION: VASA is a valuable gene for the detection of germ cells differentiated from ES cells in monkeys, and the identification and characterization of germ cells derived from ES cells are possible by using reported germ cell markers in vivo, including SSEA1, OCT-4, and VASA, in vitro as well as in vivo. These findings are thus considered to help elucidate the germ cell developmental process in primates.

Yan, W., J. Suominen, et al. (2000). "Involvement of Bcl-2 family proteins in germ cell apoptosis during testicular development in the rat and pro-survival effect of stem cell factor on germ cells in vitro." Mol Cell Endocrinol **165**(1-2): 115-29.

A large part of germ cells die apoptotically during testicular development in rodents. In the present study, a wave of germ cell apoptosis was observed between days 10 and 30 of postnatal life by in situ 3'-end labeling and DNA fragmentation analysis. To explore the potential involvement of Bcl-2 family members in this process, the expression and localization of some Bcl-2 family proteins (Bcl-2, Bcl-xL, Bcl-w, Bak, Bax, and Bad) and p53 were analyzed during testicular development in the rat by Western blotting and immunohistochemistry. The dynamic changes in the expression profiles of Bcl-2 family proteins are consistent with a model in which germ cells are primed for apoptosis during the first cycle of spermatogenesis by de novo expression of the death effectors Bax and Bad in a p53-dependent manner and these proteins are prevented from triggering further apoptosis after the first spermatogenic cycle has been set up by anti-apoptotic Bcl-2 family proteins Bcl-xL and Bcl-w. To examine whether the pro-survival effect of stem cell factor (SCF) on germ cells in vitro is mediated by Bcl-2 family proteins, the correlation between the pro-survival effect of SCF on germ cells and the expression of the above-mentioned apoptosis-related gene products in the seminiferous tubules at stage XII of the epithelial cycle were also investigated using a tubular culture system. The data suggest that SCF supports germ cell survival during spermatogenesis by up-regulating pro-survival Bcl-2 family proteins, Bcl-w and Bcl-xL, and down-regulating pro-apoptosis Bcl-2 family proteins, e.g. Bax.

Yan, W., J. Suominen, et al. (2000). "Stem cell factor protects germ cells from apoptosis in vitro." J Cell Sci **113 (Pt 1)**: 161-8.

Stem cell factor (SCF) plays an important role in migration, adhesion, proliferation, and survival of primordial germ cells and spermatogonia during testicular development. However, the function of SCF in the adult testis is poorly described. We have previously shown that, in the presence of SCF, there were more type A spermatogonia incorporating thymidine at stage XII of rat seminiferous tubules cultured in vitro than in the absence of SCF, implying that the increased DNA synthesis might result from enhanced survival of spermatogonia. To explore the potential pro-survival function of SCF during spermatogenesis, the seminiferous tubules from stage XII were cultured in the presence or absence of SCF (100 ng/ml) for 8, 24, 48, and 72 hours, respectively, and apoptosis was analyzed by DNA laddering and in situ 3'-end labeling (ISEL) staining. Surprisingly, not only spermatogonia, but also spermatocytes and spermatids, were protected from apoptosis in the presence of SCF. Apoptosis took place much later and

was less severe in the SCF-treated tubules than in the controls. Based on previous studies showing that FSH prevents germ cells from undergoing apoptosis in vitro, and that SCF level is increased dramatically in response to FSH stimulation, we also tested if the pro-survival effect of FSH is mediated through SCF by using a function-blocking monoclonal antibody, ACK-2, to block SCF/c-kit interaction. After 24 hours of blockade, the protective effect of FSH was partially abolished, as manifested by DNA laddering and ISEL analyses. The present study demonstrates that SCF acts as an important survival factor for germ cells in the adult rat testis and FSH pro-survival effect on germ cells is mediated partially through the SCF/c-kit pathway.

Yang, Z. H., X. J. Zhang, et al. (2009). "Apical tooth germ cell-conditioned medium enhances the differentiation of periodontal ligament stem cells into cementum/periodontal ligament-like tissues." J Periodontal Res **44**(2): 199-210.

BACKGROUND AND OBJECTIVE: Limitations of current periodontal regeneration modalities in both predictability and extent of healing response, especially on new cementum and attachment formation, underscore the importance of restoring or providing a microenvironment that is capable of promoting the differentiation of periodontal ligament stem cells (PDLSCs) towards cementoblast-like cells and the formation of cementum/periodontal ligament-like tissues. The aim of this study was to investigate the biological effect of conditioned medium from developing apical tooth germ cells (APTG-CM) on the differentiation and cementogenesis of PDLSCs both in vitro and in vivo. MATERIAL AND METHODS: Using the limiting dilution technique, single-colony-derived human PDLSCs were isolated and expanded to obtain homogeneous populations of PDLSCs. Morphological appearance, cell cycle analysis, bromodeoxyuridine incorporation, alkaline phosphatase (ALP) activity, mineralization behavior, gene expression of cementoblast phenotype and in vivo differentiation capacities of PDLSCs co-cultured with APTG-CM were evaluated. RESULTS: The induced PDLSCs exhibited several characteristics of cementoblast lineages, as indicated by the morphological changes, increased proliferation, high ALP activity, and the expression of cementum-related genes and calcified nodule formation in vitro. When transplanted into immunocompromised mice, the induced PDLSCs showed tissue-regenerative capacity to produce cementum/periodontal ligament-like structures, characterized by a layer of cementum-like mineralized tissues and associated periodontal ligament-like collagen fibers connecting with the newly formed cementum-like deposits, whereas control, untreated PDLSCs transplants mainly formed connective tissues. CONCLUSION: Our findings suggest that APTG-CM is able to provide a cementogenic microenvironment and induce differentiation of PDLSCs along the cementoblastic lineage. This has important implications for periodontal engineering.

Yu, J., Z. Deng, et al. (2006). "Differentiation of dental pulp stem cells into regular-shaped dentin-pulp complex induced by tooth germ cell conditioned medium." Tissue Eng **12**(11): 3097-105.

Investigations of the odontoblast phenotype are hindered by obstacles such as the limited number of odontoblasts within the dental pulp and the difficulty in purification of these cells. Therefore, it is necessary to develop a cell culture system in which the local environment is inductive and can promote dental pulp stem cells (DPSCs) to differentiate into odontoblast lineage. In this study, we investigated the effect of conditioned medium from developing tooth germ cells (TGCs) on the differentiation and dentinogenesis of DPSCs both in vitro and in vivo. DPSCs were enzymatically isolated from the lower incisors of 4-week-old Sprague-Dawley rats and co-cultured with TGC conditioned medium (TGC-CM). The cell phenotype of induced DPSCs presents many features of odontoblasts, as assessed by the morphologic appearance, cell cycle modification, increased alkaline phosphatase level, synthesis of dentin sialoprotein, type I collagen and several other noncollagenous proteins, expression of the dentin sialophosphoprotein and dentin matrix protein 1 genes, and the formation of mineralized nodules in vitro. The induced DPSC pellets in vivo generated a regular-shaped dentin-pulp complex containing distinct dentinal tubules and predentin, while untreated pellets spontaneously differentiated into bone-like tissues. To our knowledge, this is the first study to mimic the dentinogenic microenvironment from TGCs in vitro, and our data suggest that TGC-CM creates the most odontogenic microenvironment, a feature essential and effective for the regular dentinogenesis mediated by DPSCs.

Yu, Z., P. Ji, et al. (2009). "Dazl promotes germ cell differentiation from embryonic stem cells." J Mol Cell Biol **1**(2): 93-103.

It has been demonstrated that through the formation of embryoid bodies (EBs) germ cells can be derived from embryonic stem (ES) cells. Here, we describe a transgene expression approach to derive germ cells directly from ES cells in vitro without EB formation. Through the ectopic expression of Deleted in Azoospermia-Like (Dazl), a germ cell-specific RNA-binding protein, both motile tailed-sperm and

oocytes were induced from mouse ES (mES) cells in culture. Furthermore, transient overexpression of Dazl led to suppression of Nanog but induced germ cell nuclear antigen in mES cells. Dazl knockdown resulted in reduction in the expression of germ cell markers including Stella, MVH and Prdm1. Our study indicates that Dazl is a master gene controlling germ cell differentiation and that ectopic expression of Dazl promotes the dynamic differentiation of mouse ES cells into gametes in vitro.

Zhang, D., H. Fouad, et al. (2008). "Expression of stem and germ cell markers within nonfollicle structures in adult mouse ovary." Reprod Sci **15**(2): 139-46.

Recent studies have suggested that germline stem cells may generate new follicles in the adult murine ovary. In this study, the authors use a pou5f1-enhanced green fluorescent protein (EGFP) transgenic mouse model to study the expression of stem and germ cell markers in adult murine ovaries. Immunohistochemical analyses and reverse transcription polymerase chain reaction were performed to detect the expression of mouse vasa homologue, stem cells factor receptor, stage-specific embryonic antigen 1, synaptonemal complex proteins, disrupted meiotic, and growth differentiation factor-9 in GFP+ ovarian tissues. GFP+ cell aggregates of nonfollicle structures were identified and isolated from adult B6.CBA-Tg(pou5f1-EGFP)2Mnn/J transgenic mouse ovaries. This study shows the presence of cell aggregates that are distinct from ovarian follicles and are coexpressing germline and stem cell surface markers in adult murine ovaries. These cell aggregates may represent a mixed population of germ cells and germline stem cells. Further research is necessary to evaluate the plasticity of the potential stem cell population in these cell aggregates.

Zwaka, T. P. and J. A. Thomson (2005). "A germ cell origin of embryonic stem cells?" Development **132**(2): 227-33.

Because embryonic stem (ES) cells are generally derived by the culture of inner cell mass (ICM) cells, they are often assumed to be the equivalent of ICM cells. However, various evidence indicates that ICM cells transition to a different cell type during ES-cell derivation. Historically, ES cells have been believed to most closely resemble pluripotent primitive ectoderm cells derived directly from the ICM. However, differences between ES cells and primitive ectoderm cells have caused developmental biologists to question whether ES cells really have an in vivo equivalent, or whether their properties merely reflect their tissue culture environment. Here, we review recent evidence that the closest in vivo equivalent of an ES cell is an early germ cell.

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