The octamer-binding transcription factor 4 (Oct-4) and stem cell literatures

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Abstract: The octamer is made of eight units. The octamer-binding transcription factor 4 (Oct-4) is a protein also known as POU5F1 (POU domain, class 5, transcription factor 1) encoded by the *POU5F1* gene. Oct-4 is a homeodomain transcription factor of the POU family. Oct-4 is replated to animal self-renewal of undifferentiated embryonic stem cells. It is used as a marker for undifferentiated cells. The octamer in this family of transcription factors is the DNA nucleotide sequence "ATTTGCAT", the etymology for the naming of the octamer transcription factor.

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Key words: life; stem cell; octamer-binding transcription factor 4 (Oct-4)

Introduction

The octamer is made of eight units. The octamer-binding transcription factor 4 (Oct-4) is a protein also known as POU5F1 (POU domain, class 5, transcription factor 1) encoded by the *POU5F1* gene. Oct-4 is a homeodomain transcription factor of the POU family that is replated to animal self-renewal of undifferentiated embryonic stem cells. It is used as a marker for undifferentiated cells. The octamer in this family of transcription factors is the DNA nucleotide sequence "ATTTGCAT", the etymology for the naming of the octamer transcription factor.

Oct-4 transcription factor is initially active as a maternal factor in the oocyte but remains active in embryos throughout the preimplantation period and its expression is associated with an undifferentiated phenotype and tumors. Gene knockdown of Oct-4 promotes differentiation, thereby demonstrating a role for these factors in human embryonic stem cell self-renewal. Oct-4 can form a heterodimer with Sox2 and the 2 proteins bind DNA together. The Oct-4 expression level is related to the regulating of pluripotency and early cell differentiation and it has been implicated in tumorigenesis of adult germ cells. It's ectopic expression possibly causes the formation of dysplastic lesions of the skin and intestine. The intestinal dysplasia could be resulted from the increase of progenitor cell population.

The precise Oct-4 level governs 3 distinct fates of ES cells. The Oct-4 repression induces pluripotency loss and dedifferentiation to trophectoderm. The transcription factors Oct-4, Sox2 and Nanog are capable of inducing the expression of each other, and are essential for maintaining the self-renewing undifferentiated state of the inner cell mass of the blastocyst, as well as in embryonic stem cells. Oct-4 is one of the transcription factors used to create induced pluripotent stem cells, together with Sox2, Klf4 and c-Myc. Normally, Oct4 and Klf4 or Oct-4 individually are sufficient to reprogram mouse adult neural stem cells. For embryonic stem cells the Oct-4 can be used as a marker of stemness, as differentiated cells show reduced expression of this marker.

Oct3/4 can both repress and activate the Rex1 promoter. In cells Oct3/4 are not actively expressed, and an exogenous transfection of Oct3/4 will lead to the activation of Rex1, which implies a dual regulatory ability of Oct3/4 on Rex1. The Rex1 promoter is activated at low levels of the Oct3/4 protein while the Rex1 promoter is repressed. at high levels of the Oct3/4 protein.

There are many methods to deliver the transcription factors into target cells to generate iPSCs. The first method is retrovirus or lentivirus transduction. The problem of this technique is the genome integration of virus DNA which could possibly alter differentiation potential or other malignant transformation. The second method is adenoviral vectors to induce iPSC. The advantage of adenovirus vector based expression is that the transgenes will not integrate into the house genome, thus reduces the risk of tumorogenesis. The third one is a plasmid based transfection that can avoid the genome integration also. Recently, the Cre-recombinase excisable systems are used in iPSC induction and subsequent transgene removal making the iPSC technology closer to clinic applications.

Literatures

The following gives some recent reference papers on this topic:

Ahn, B. C., N. Parashurama, et al. "Noninvasive Reporter Gene Imaging of Human Oct4 (Pluripotency) Dynamics During the Differentiation of Embryonic Stem Cells in Living Subjects." Mol Imaging Biol. 2014 May 21.

PURPOSE: Human pluripotency gene networks (PGNs), controlled in part by Oct4, are central to understanding pluripotent stem cells, but current fluorescent reporter genes (RGs) preclude noninvasive assessment of Oct4 dynamics in living subjects. PROCEDURES: To assess Oc4 activity noninvasively, we engineered a mouse embryonic stem cell line which encoded both a pOct4-hrluc (humanized renilla luciferase) reporter and a pUbi-hfluc2-gfp (humanized firefly luciferase 2 fused to green fluorescent protein) reporter. RESULTS: In cell culture, pOct4-hRLUC activity demonstrated a peak at 48 h (day 2) and significant downregulation by 72 h (day 3) (p=0.0001). Studies in living subjects demonstrated significant downregulation in pOct4-hRLUC activity between 12 and 144 h (p = 0.001) and between 12 and 168 h (p = 0.0003). pOct4-hRLUC signal dynamics after implantation was complex, characterized by transient upregulation after initial downregulation in all experiments (n = 10, p = 0.01). As expected, cell culture differentiation of the engineered mouse embryonic stem cell line demonstrated activation of mesendodermal, mesodermal, endodermal, and ectodermal master regulators of differentiation, indicating potency to form all three germ layers. CONCLUSIONS: We conclude that the Oct4-hrluc RG system enables noninvasive Oct4 imaging in cell culture and in living subjects.

Bhuvanalakshmi, G., F. Arfuso, et al. "Multifunctional Properties of Chicken Embryonic Prenatal Mesenchymal Stem Cells-Pluripotency, Plasticity, and Tumor Suppression." Stem Cell Rev. 2014 Jun 13.

The chick embryo represents an accessible and economical in vivo model, which has long been used in developmental biology, gene expression analysis, and loss/gain of function experiments. In the present study, we assessed and characterized bone marrow derived mesenchymal stem cells from prenatal day 13 chicken embryos (chBMMSCs) and determined some novel properties. After assessing the mesenchymal stem cell (MSC) properties of these cells by the presence of their signature markers (CD 44, CD 73, CD 90, CD 105, and vimentin), we ascertained a very broad spectrum of multipotentiality as these MSCs not only differentiated into the classic tri-lineages of MSCs but also into ectodermal, endodermal, and mesodermal lineages such as neuron, hepatocyte, islet cell, and cardiac. In addition to wide plasticity, we detected the presence of several pluripotent markers such as Oct4, Sox2, and Nanog. This is the first study characterizing prenatal chBMMSCs and their ability to not only differentiate into mesenchymal lineages but also into all the germ cell layer lineages. Furthermore, our studies indicate that prenatal chBMMSCs derived from the chick provide an excellent model for multi-lineage development studies because of their broad plasticity and faithful reproduction of MSC traits as seen in the human. Here, we also present evidence for the first time that media derived from prenatal chBMMSC cultures have an anti-tumorigenic, anti-migratory, and pro-apoptotic effect on human tumors cells acting through the Wnt-ss-catenin pathway. These data confirm that chBMMSCs are enriched with factors in their secretome that are able to destroy tumor cells. This suggests a commonality of properties of MSCs across species between human and chicken.

Carelli, S., F. Messaggio, et al. "Characteristics and Properties of Mesenchymal Stem Cells Derived from Micro-fragmented Adipose Tissue." Cell Transplant. 2014 May 6.

The subcutaneous adipose tissue provides a clear advantage over other mesenchymal stem cells sources due to the ease with which it can be accessed as well as to the ease of isolating the residing stem cells. Human adipose derived stem cells (hADSCs) localize in the stromal-vascular portion and can be ex vivo isolated using a combination of washing steps and enzymatic digestion. In this study we report that microfragmented human lipoaspirated adipose tissue results as a better stem cell source compared to normal lipoaspirated tissue. The structural composition of microfragments is comparable to the original tissue. Differently however, this procedure activates the expression of antigens such as beta-tubulin III. The hADSCs derived from microfragmented lipoaspirate tissue have been systematically characterized for growth features, phenotype and multi-potent differentiation potential. They fulfill the definition of mesenchymal stem cells although with a higher neural phenotype profile. These cells also express genes that constitute the core circuitry of self-renewal such as OCT4, SOX2, NANOG and neurogenic lineage genes such as NEUROD1, PAX6 and SOX3. Such findings suggest further studies by evaluating Microfrag-AT hADSCs action in animal models of neurodegenerative conditions. Key words. mechanical fracturation; gene activation; neurospheres; neural phenotype; fat particles; mesenchymal stem cells.

Dudakovic, A., E. Camilleri, et al. "High-resolution molecular validation of self-renewal and spontaneous differentiation in clinical-grade adipose-tissue derived human mesenchymal stem cells." J Cell Biochem. 2014 Oct;115(10):1816-28. doi: 10.1002/jcb.24852.

Improving the effectiveness of adipose-tissue derived human mesenchymal stromal/stem cells (AMSCs) for skeletal therapies requires a detailed characterization of mechanisms supporting cell proliferation and multi-potency. We investigated the molecular phenotype of AMSCs that were either actively proliferating in platelet lysate or in a basal non-proliferative state. Flow cytometry combined with high-throughput RNA sequencing (RNASeq) and RT-qPCR analyses validate that AMSCs express classic mesenchymal cell surface markers (e.g., CD44, CD73/NT5E, CD90/THY1, and CD105/ENG). Expression of CD90 is selectively elevated at confluence. Self-renewing AMSCs express a standard cell cycle program that successively mediates DNA replication, chromatin packaging, cyto-architectural enlargement, and mitotic division. Confluent AMSCs preferentially express genes involved in extracellular matrix (ECM) formation and cellular communication. For example, cell cycle-related biomarkers (e.g., cyclins E2 and B2, transcription factor E2F1) and histone-related genes (e.g., H4, HINFP, NPAT) are elevated in proliferating AMSCs, while ECM genes are strongly upregulated (>10-fold) in quiescent AMSCs. AMSCs also express pluripotency genes (e.g., POU5F1, NANOG, KLF4) and early mesenchymal markers (e.g., NES, ACTA2) consistent with their multipotent phenotype. Strikingly, AMSCs modulate expression of WNT signaling components and switch production of WNT ligands (from WNT5A/WNT5B/WNT7B to WNT2/WNT2B), while upregulating WNT-related genes (WISP2, SFRP2, and SFRP4). Furthermore, post-proliferative AMSCs spontaneously express fibroblastic, osteogenic, chondrogenic, and adipogenic biomarkers when maintained in confluent cultures. Our findings validate the biological properties of self-renewing and multi-potent AMSCs by providing high-resolution quality control data that support their clinical versatility. J. Cell. Biochem. 115: 1816-1828, 2014. (c) 2014 Wiley Periodicals, Inc.

Duenas, F., V. Becerra, et al. "Hepatogenic and neurogenic differentiation of bone marrow mesenchymal stem cells from abattoir-derived bovine fetuses." BMC Vet Res. 2014 Jul 10;10:154. doi: 10.1186/1746-6148-10-154.

BACKGROUND: Mesenchymal stem cells (MSC) are multipotent progenitor cells characterized by their ability to both self-renew and differentiate into tissues of mesodermal origin. The plasticity or transdifferentiation potential of MSC is not limited to mesodermal derivatives, since under appropriate cell culture conditions and stimulation by bioactive factors, MSC have also been differentiated into endodermal (hepatocytes) and neuroectodermal (neurons) cells. The potential of MSC for hepatogenic and neurogenic differentiation has been well documented in different animal models; however, few reports are currently available on large animal models. In the present study we sought to characterize the hepatogenic and neurogenic differentiation and multipotent potential of bovine MSC (bMSC) isolated from bone marrow (BM) of abattoir-derived fetuses.

RESULTS: Plastic-adherent bMSC isolated from fetal BM maintained a fibroblast-like morphology under monolayer culture conditions. Flow cytometric analysis demonstrated that bMSC populations were positive for MSC markers CD29 and CD73 and pluripotency markers OCT4 and NANOG; whereas, were negative for hematopoietic markers CD34 and CD45. Levels of mRNA of hepatic genes alpha-fetoprotein (AFP), albumin (ALB), alpha1 antitrypsin (alpha1AT), connexin 32 (CNX32), tyrosine aminotransferase (TAT) and cytochrome P450 (CYP3A4) were up-regulated in bMSC during a 28-Day period of hepatogenic differentiation. Functional analyses in differentiated bMSC cultures evidenced an increase ($P < 0.05$) in albumin and urea production and glycogen storage. bMSC cultured under neurogenic conditions expressed NESTIN and MAP2 proteins at 24 h of culture; whereas, at 144 h also expressed TRKA and PrPC. Levels of MAP2 and TRKA mRNA were up-regulated at the end of the differentiation period. Conversely, bMSC expressed lower levels of NANOG mRNA during both hepatogenic and neurogenic differentiation processes. CONCLUSION: The expression patterns of linage-specific markers and the production of functional metabolites support the potential for hepatogenic and neurogenic differentiation of bMSC isolated from BM of abattoir-derived fetuses. The simplicity of isolation and the potential to differentiate into a wide variety of cell lineages lays the foundation for bMSC as an interesting alternative for investigation in MSC biology and eventual applications for regenerative therapy in veterinary medicine.

Em, S., M. Kataria, et al. "Expression profile of developmentally important genes between hand-made cloned buffalo embryos produced from reprogramming of donor cell with oocytes extract and selection of recipient cytoplast through brilliant cresyl blue staining and in vitro fertilized embryos." J Assist Reprod Genet. 2014 Aug 21.

PURPOSE: To compare the expression profile of developmentally important genes between hand-made cloned buffalo embryos produced from reprogramming of donor cell with oocyte extracts and selection of recipient cytoplast through brilliant cresyl blue staining and in vitro fertilized (IVF) embryos. METHODS: Hand-made cloned embryos were produced using oocyte extracts treated donor cells and brilliant cresyl blue (BCB) stained recipient cytoplasts. IVF embryos were produced by culturing 15-20 COCs in BO capacitated sperms from frozen thawed buffalo semen and the mRNA expression patterns of genes implicated in metabolism (GLUT1), pluripotency (OCT4), DNA methylation (DNMT1), pro- apoptosis (BAX) and anti-apoptosis (BCL2) were evaluated at 8- to16- cell stage embryos. RESULTS: A significantly ($P < 0.05$) higher number of 8- to16- cell and blastocyst stages (73.9 %, 32.8 %, respectively) were reported in hand-made cloning (HMC) as compared to in vitro fertilization (49.2 %, 24.2 %, respectively). The amount of RNA recovered from 8- to 16- cell embryos of HMC and in vitro fertilization did not appear to be influenced by the method of embryo generation (3.76 +/- 0.61 and 3.82 +/- 0.62 ng/mul for HMC and in vitro fertilization embryos, respectively). There were no differences in the expression of the mRNA transcripts of genes (GLUT1, OCT4, DNMT1, BAX and BCL2) were analysed by real-time PCR between hand-made cloned and IVF embryos. CONCLUSIONS: Pre-treatment of donor cells with oocyte extracts and selection of developmentally competent oocytes through BCB staining for recipient cytoplast preparations may enhance expression of developmentally important genes GLUT1, OCT4, DNMT1, BAX, and BCL2 in hand-made cloned embryos at levels similar to IVF counterparts. These results also support the notion that if developmental differences observed in HMC and in vitro fertilization produced foetuses and neonates are the results of aberrant gene expression during the pre-implantation stage, those differences in expression are subtle or appear after the maternal to zygotic transition stage of development.

Ennen, M., C. Keime, et al. "Single-cell gene expression signatures reveal melanoma cell heterogeneity." Oncogene. 2014 Aug 18;0. doi: 10.1038/onc.2014.262.

It is well established that tumours are not homogenous, but comprise cells with differing invasive, proliferative and tumour-initiating potential. A major challenge in cancer research is therefore to develop methods to characterize cell heterogeneity. In melanoma, proliferative and invasive cells are characterized by distinct gene expression profiles and accumulating evidence suggests that cells can alternate between these states through a process called phenotype switching. We have used microfluidic technology to isolate single melanoma cells grown in vitro as monolayers or melanospheres or in vivo as xenografted tumours and analyse the expression profiles of 114 genes that discriminate the proliferative and invasive states by quantitative PCR. Single-cell analysis accurately recapitulates the specific gene expression programmes of melanoma cell lines and defines subpopulations with distinct expression profiles. Cell heterogeneity is augmented when cells are grown as spheres and as xenografted tumours. Correlative analysis identifies gene-regulatory networks and changes in gene expression under different growth conditions. In tumours, subpopulations of cells that express specific invasion and drug resistance markers can be identified amongst which is the pluripotency factor POUF51 (OCT4) whose expression correlates with the tumorigenic potential. We therefore show that single-cell analysis can be used to define and quantify tumour heterogeneity based on detection of cells with specific gene expression profiles.Oncogene advance online publication, 18 August 2014; doi:10.1038/onc.2014.262.

Faucon, P. C., K. Pardee, et al. "Gene networks of fully connected triads with complete auto-activation enable multistability and stepwise stochastic transitions." PLoS One. 2014 Jul 24;9(7):e102873. doi: 10.1371/journal.pone.0102873. eCollection 2014.

Fully-connected triads (FCTs), such as the Oct4-Sox2-Nanog triad, have been implicated as recurring transcriptional motifs embedded within the regulatory networks that specify and maintain cellular states. To explore the possible connections between FCT topologies and cell fate determinations, we employed computational network screening to search all possible FCT topologies for multistability, a dynamic property that allows the rise of alternate regulatory states from the same transcriptional network. The search yielded a hierarchy of FCTs with various potentials for multistability, including several topologies capable of reaching eight distinct stable states. Our analyses suggested that complete auto-activation is an effective indicator for multistability, and, when gene expression noise was incorporated into the model, the networks were able to transit multiple states spontaneously. Different levels of stochasticity were found to either induce or disrupt random state transitioning with some transitions requiring layovers at one or more intermediate states. Using this framework we simulated a simplified model of induced pluripotency by including constitutive overexpression terms. The corresponding FCT showed random state transitioning from a terminal state to the pluripotent state, with the temporal distribution of this transition matching published experimental data. This work establishes a potential theoretical framework for understanding cell fate determinations by connecting conserved regulatory modules with network dynamics. Our results could also be employed experimentally, using established developmental transcription factors as seeds, to locate cell lineage specification networks by using auto-activation as a cipher.

Fereydouni, B., C. Drummer, et al. "The neonatal marmoset monkey ovary is very primitive exhibiting many oogonia." Reproduction. 2014 Aug;148(2):237-47. doi: 10.1530/REP-14-0068. Epub 2014 May 19.

Oogonia are characterized by diploidy and mitotic proliferation. Human and mouse oogonia express several factors such as OCT4, which are characteristic of pluripotent cells. In

human, almost all oogonia enter meiosis between weeks 9 and 22 of prenatal development or undergo mitotic arrest and subsequent elimination from the ovary. As a consequence, neonatal human ovaries generally lack oogonia. The same was found in neonatal ovaries of the rhesus monkey, a representative of the old world monkeys (Catarrhini). By contrast, proliferating oogonia were found in adult prosimians (now called Strepsirrhini), which is a group of 'lower' primates. The common marmoset monkey (Callithrix jacchus) belongs to the new world monkeys (Platyrrhini) and is increasingly used in reproductive biology and stem cell research. However, ovarian development in the marmoset monkey has not been widely investigated. Herein, we show that the neonatal marmoset ovary has an extremely immature histological appearance compared with the human ovary. It contains numerous oogonia expressing the pluripotency factors OCT4A, SALL4, and LIN28A (LIN28). The pluripotency factor-positive germ cells also express the proliferation marker MKI67 (Ki-67), which has previously been shown in the human ovary to be restricted to premeiotic germ cells. Together, the data demonstrate the primitiveness of the neonatal marmoset ovary compared with human. This study may introduce the marmoset monkey as a non-human primate model to experimentally study the aspects of primate primitive gonad development, follicle assembly, and germ cell biology in vivo.

Frankenberg, S. R., D. Frank, et al. "The POU-er of gene nomenclature." Development. 2014 Aug;141(15):2921-3. doi: 10.1242/dev.108407.

The pluripotency factor POU5F1 (OCT4) is well known as a key regulator of stem cell fate. Homologues of POU5F1 exist throughout vertebrates, but the evolutionary and functional relationships between the various family members have been unclear. The level to which function has been conserved within this family provides insight into the evolution of early embryonic potency. Here, we seek to clarify the relationship between POU5F1 homologues in the vertebrate lineage, both phylogenetically and functionally. We resolve the confusion over the identity of the zebrafish gene, which was originally named pou2, then changed to pou5f1 and again, more recently, to pou5f3. We argue that the use of correct nomenclature is crucial when discussing the degree to which the networks regulating early embryonic differentiation are conserved.

Galiger, C., S. Kostin, et al. "Phenotypical and ultrastructural features of Oct4-positive cells in the adult mouse lung." J Cell Mol Med. 2014 Jul;18(7):1321-33. doi: 10.1111/jcmm.12295. Epub 2014 Jun 3.

Octamer binding trascription factor 4 (Oct4) is a transcription factor of POU family specifically expressed in embryonic stem cells (ESCs). A role for maintaining pluripotency and self-renewal of ESCs is assigned to Oct4 as a pluripotency marker. Oct4 can also be detected in adult stem cells such as bone marrow-derived mesenchymal stem cells. Several studies suggest a role for Oct4 in sustaining self-renewal capacity of adult stem cells. However, Oct4 gene ablation in adult stem cells revealed no abnormalities in tissue turnover or regenerative capacity. In the present study we have conspicuously found pulmonary Oct4-positive cells closely resembling the morphology of telocytes (TCs). These cells were found in the perivascular and peribronchial areas and their presence and location were confirmed by electron microscopy. Moreover, we have used Oct4-GFP transgenic mice which revealed a similar localization of the Oct4-GFP signal. We also found that Oct4 co-localized with several described TC markers such as vimentin, Sca-1, platelet-derived growth factor receptor-beta C-kit and VEGF. By flow cytometry analyses carried out with Oct4-GFP reporter mice, we described a population of EpCAM(neg) /CD45(neg) /Oct4-GFP(pos) that in culture displayed TC features. These results were supported by qRT-PCR with mRNA isolated from lungs by using laser capture microdissection. In addition, Oct4-positive cells were found to express Nanog and Klf4 mRNA. It is concluded for the first time that TCs in adult lung mouse tissue comprise Oct4-positive cells, which express pluripotency-related genes and represent therefore a population of adult stem cells which might contribute to lung regeneration.

Garg, R. K., R. C. Rennert, et al. "Capillary force seeding of hydrogels for adipose-derived stem cell delivery in wounds." Stem Cells Transl Med. 2014 Sep;3(9):1079-89. doi: 10.5966/sctm.2014-0007. Epub 2014 Jul 18.

Effective skin regeneration therapies require a successful interface between progenitor cells and biocompatible delivery systems. We previously demonstrated the efficiency of a biomimetic pullulan-collagen hydrogel scaffold for improving bone marrow-derived mesenchymal stem cell survival within ischemic skin wounds by creating a "stem cell niche" that enhances regenerative cytokine secretion. Adipose-derived mesenchymal stem cells (ASCs) represent an even more appealing source of stem cells because of their abundance and accessibility, and in this study we explored the utility of ASCs for hydrogel-based therapies. To optimize hydrogel cell seeding, a rapid, capillary force-based approach was developed and compared with previously established cell seeding methods. ASC viability and functionality following capillary hydrogel seeding were then analyzed in vitro and in vivo. In these experiments, ASCs were seeded more efficiently by capillary force than by traditional methods and remained viable and functional in this niche for up to 14 days. Additionally, hydrogel seeding of ASCs resulted in the enhanced expression of multiple stemness and angiogenesis-related genes, including Oct4, Vegf, Mcp-1, and Sdf-1. Moving in vivo, hydrogel delivery improved ASC survival, and application of both murine and human ASC-seeded hydrogels to splinted murine wounds resulted in accelerated wound closure and increased vascularity when compared with control wounds treated with unseeded hydrogels. In conclusion, capillary seeding of ASCs within a pullulan-collagen hydrogel bioscaffold provides a convenient and simple way to deliver therapeutic cells to wound environments. Moreover, ASC-seeded constructs display a significant potential to accelerate wound healing that can be easily translated to a clinical setting.

Ghasemzadeh-Hasankolaei, M., M. B. Eslaminejad, et al. "Male and female rat bone marrow-derived mesenchymal stem cells are different in terms of the expression of germ cell specific genes." Anat Sci Int. 2014 Aug 5.

Recent studies have shown that mesenchymal stem cells (MSCs), under appropriate conditions, can differentiate into cell types including germ cells (GCs). These studies also show that MSCs without any induction express some GC-specific genes innately. Moreover, one report suggests that female MSCs have a greater tendency to differentiate into female instead of male GCs. Therefore, for the first time, this study attempts to assay and determine the differences between the expression levels of some important GC-specific genes (Stra8, Vasa, Dazl, Stella, Piwil2, Oct4, Fragilis, Rnf17 and c-Kit) in male and female bone marrow (BM)-MSCs of rats. BM sampling of the rate was performed by a newly established method. We cultured rat BM samples, then characterized male and female MSCs according to their adhesion onto the culture dish, their differentiation potential into bone, cartilage and fat cells, and phenotype analysis by flow cytometry. The expression of GC-specific genes and their expression levels were evaluated with reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR. Our results showed that Dazl and Rnf17 did not express in the cells. The majority of examined genes, except Piwil2, expressed at almost the same levels in male and female MSCs. Piwil2 had higher expression in male MSCs which was probably related to the more prominent role of Piwil2 in the male GC development process. Male BM-MSCs appeared more prone to differentiate into male rather than female GCs. Additional research should be performed to determine the exact role of different genes in the male and female GC development process.

Goh, P. A. and P. J. Verma "Generation of induced pluripotent stem cells from mouse adipose tissue." Methods Mol Biol. 2014;1194:253-70. doi: 10.1007/978-1-4939-1215-5_14.

The discovery that embryonic stem (ES) cell-like cells can be generated by simply over-expressing four key genes in adult somatic cells has changed the face of regenerative medicine. These induced pluripotent stem (iPS) cells have a wide range of potential uses from drug testing and in vitro disease modeling to personalized cell therapies for patients. However, prior to the realization of their potential, many issues need to be considered. One of these is the low-efficiency formation of iPSC. It has been extensively demonstrated that the somatic cell type can greatly influence reprogramming outcomes. We have shown that adipose tissue-derived cells (ADCs) can be easily isolated from adult animals and can be reprogrammed to a pluripotent state with high efficiency. Here, we describe a protocol for the high-efficiency derivation of ADCs and their subsequent use to generate mouse iPSC using Oct4, Sox2, Klf4, and cMyc retroviral vectors.

Gonzalez-Munoz, E., Y. Arboleda-Estudillo, et al. "Cell reprogramming. Histone chaperone ASF1A is required for maintenance of pluripotency and cellular reprogramming." Science. 2014 Aug 15;345(6198):822-5. doi: 10.1126/science.1254745. Epub 2014 Jul 17.

Unfertilized oocytes have the intrinsic capacity to remodel sperm and the nuclei of somatic cells. The discoveries that cells can change their phenotype from differentiated to embryonic state using oocytes or specific transcription factors have been recognized as two major breakthroughs in the biomedical field. Here, we show that ASF1A, a histone-remodeling chaperone specifically enriched in the metaphase II human oocyte, is necessary for reprogramming of human adult dermal fibroblasts (hADFs) into undifferentiated induced pluripotent stem cell. We also show that overexpression of just ASF1A and OCT4 in hADFs exposed to the oocyte-specific paracrine growth factor GDF9 can reprogram hADFs into pluripotent cells. Our Report underscores the importance of studying the unfertilized MII oocyte as a means to understand the molecular pathways governing somatic cell reprogramming.

Groen, J. N., D. Capraro, et al. The emerging role of pseudogene expressed non-coding RNAs in cellular functions, Int J Biochem Cell Biol. 2014 Sep;54C:350-355. doi: 10.1016/j.biocel.2014.05.008. Epub 2014 May 16.

A paradigm shift is sweeping modern day molecular biology following the realisation that large amounts of "junk" DNA", thought initially to be evolutionary remnants, may actually be functional. Several recent studies support a functional role for pseudogene-expressed non-coding RNAs in regulating their protein-coding counterparts. Several hundreds of pseudogenes have been reported as transcribed into RNA in a large variety of tissues and tumours. Most studies have focused on pseudogenes expressed in the sense direction, but some reports suggest that pseudogenes can also be transcribed as antisense RNAs (asRNAs). A few examples of key regulatory genes, such as PTEN and OCT4, have in fact been reported to be under the regulation of pseudogene-expressed asRNAs. Here, we review what are known about pseudogene expressed non-coding RNA mediated gene regulation and their roles in the control of epigenetic states. This article is part of a Directed Issue entitled: The Non-coding RNA Revolution.

Guzzo, R. M., V. Scanlon, et al. "Establishment of Human cell Type-Specific iPS cells with Enhanced Chondrogenic Potential." Stem Cell Rev. 2014 Jun 25.

The propensity of induced pluripotent stem (iPS) cells to differentiate into specific lineages may be influenced by a number of factors, including the selection of the somatic cell type used for reprogramming. Herein we report the generation of new iPS cells, which we derived from human articular chondrocytes and from cord blood mononucleocytes via lentiviral-mediated delivery of Oct4, Klf4, Sox2, and cMyc. Molecular, cytochemical, and cytogenic analyses confirmed the acquisition of hallmark features of pluripotency, as well as the retention of normal karyotypes following reprogramming of both the human articular chondrocytes (AC) and the cord blood (CB) cells. In vitro and in vivo functional analyses formally established the pluripotent differentiation capacity of all cell lines. Chondrogenic differentiation assays comparing iPS cells derived from AC, CB, and a well established dermal fibroblast cell line (HDFa-Yk26) identified enhanced proteoglycan-rich matrix formation and cartilage-associated gene expression from AC-derived iPS cells. These findings suggest that the tissue of origin may impact the fate potential of iPS cells for differentiating into specialized cell types, such as chondrocytes. Thus, we generated new cellular tools for the identification of inherent features driving high chondrogenic potential of reprogrammed cells.

Hahn, S. and H. Hermeking "ZNF281/ZBP-99: a new player in epithelial-mesenchymal transition, stemness, and cancer." J Mol Med (Berl). 2014 Jun;92(6):571-81. doi: 10.1007/s00109-014-1160-3. Epub 2014 May 18.

Epithelial-mesenchymal transition (EMT) represents an important mechanism during development and wound healing, and its deregulation has been implicated in metastasis. Recently, the Kruppel-type zinc-finger transcription factor ZNF281 has been characterized as an EMT-inducing transcription factor (EMT-TF). Expression of ZNF281 is induced by the EMT-TF SNAIL and inhibited by the tumor suppressive microRNA miR-34a, which mediates repression of ZNF281 by the p53 tumor suppressor. Therefore, SNAIL, miR-34a and ZNF281 form a feed-forward regulatory loop, which controls EMT. Deregulation of this circuitry by mutational and epigenetic alterations in the p53/miR-34a axis promotes colorectal cancer (CRC) progression and metastasis formation. As ZNF281 physically interacts with the transcription factors NANOG, OCT4, SOX2, and c-MYC, it has been implicated in the regulation of pluripotency, stemness, and cancer. Accordingly, ectopic ZNF281 expression in CRC lines induces the stemness markers LGR5 and CD133 and promotes sphere formation, suggesting that the elevated expression of ZNF281 detected in cancer may enhance tumor stem cell formation and/or function. Here, we review the functional and organismal studies of ZNF281/ZBP-99 and its close relative ZBP-89/ZFP148 reported so far. Taken together, ZNF281 related biology has the potential to be translated into cancer diagnostic, prognostic, and therapeutic approaches.

Itoh, F., T. Watabe, et al. "Roles of TGF-beta family signals in the fate determination of pluripotent stem cells." Semin Cell Dev Biol. 2014 Aug;32:98-106. doi: 10.1016/j.semcdb.2014.05.017. Epub 2014 Jun 6.

Members of the transforming growth factor-beta (TGF-beta) family have been implicated in embryogenesis as well as in the determination of the cell fates of mouse and human embryonic stem (ES) cells, which are characterized by their self-renewal and pluripotency. The cellular responses to TGF-beta family signals are divergent depending on the cellular context and local environment. TGF-beta family signals play critical roles both in the maintenance of the pluripotent state of ES cells by inducing the expression of Nanog, Oct4, and Sox2, and in their differentiation into various cell types by regulating the expression of master regulatory genes. Moreover, multiple lines of evidence have suggested the importance of TGF-beta family signals in establishing induced pluripotent stem (iPS) cells. Since ES and iPS cells have great potential for applications in regenerative medicine, it is critical to figure out the mechanisms underlying their self-renewal, pluripotency, and differentiation. Here, we discuss the roles of TGF-beta family ligands and their downstream signaling molecules, Smad proteins, in the maintenance of the pluripotency and lineage specification of mouse and human ES and iPS cells.

Jamaladdin, S., R. D. Kelly, et al. "Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells." Proc Natl Acad Sci U S A. 2014 Jul 8;111(27):9840-5. doi: 10.1073/pnas.1321330111. Epub 2014 Jun 23.

Histone deacetylases 1 and 2 (HDAC1/2) form the core catalytic components of corepressor complexes that modulate gene expression. In most cell types, deletion of both Hdac1 and Hdac2 is required to generate a discernible phenotype, suggesting their activity is largely redundant. We have therefore generated an ES cell line in which Hdac1 and Hdac2 can be inactivated simultaneously. Loss of HDAC1/2 resulted in a 60% reduction in total HDAC activity and a loss of cell viability. Cell death is dependent upon cell cycle progression, because differentiated, nonproliferating cells retain their viability. Furthermore, we observe increased mitotic defects, chromatin bridges, and micronuclei, suggesting HDAC1/2 are necessary for accurate chromosome segregation. Consistent with a critical role in the regulation of gene expression, microarray analysis of Hdac1/2-deleted cells reveals 1,708 differentially expressed genes. Significantly for the maintenance of stem cell self-renewal, we detected a reduction in the expression of the pluripotent transcription factors, Oct4, Nanog, Esrrb, and Rex1. HDAC1/2 activity is regulated through binding of an inositol tetraphosphate molecule (IP4) sandwiched between the HDAC and its cognate corepressor. This raises the important question of whether IP4 regulates the activity of the complex in cells. By rescuing the viability of double-knockout cells, we demonstrate for the first time (to our knowledge) that mutations that abolish IP4 binding reduce the activity of HDAC1/2 in vivo. Our data indicate that HDAC1/2 have essential and pleiotropic roles in cellular proliferation and regulate stem cell self-renewal by maintaining expression of key pluripotent transcription factors.

Johnsson, P., K. V. Morris, et al. "Pseudogenes: a novel source of trans-acting antisense RNAs." Methods Mol Biol. 2014;1167:213-26. doi: 10.1007/978-1-4939-0835-6_14.

While long thought to represent only "junk" DNA, several recent studies support a functional role for pseudogenes. Several hundreds of pseudogenes have been reported as transcribed into RNA in a large variety of tissues and tumors. Most studies have focused on pseudogenes expressed in the sense direction, but some reports suggest that pseudogenes can be also transcribed as antisense RNAs (asRNAs). A few examples of key regulatory genes, such as PTEN and OCT4, have in fact been reported to be under the regulation of pseudogene-expressed asRNAs. Here, we review what is known about pseudogene-expressed asRNAs and we discuss the functional role that these transcripts may have in gene regulation. Finally, we discuss some technical challenges when characterising the function of pseudogene asRNAs.

Jouni, F. J., P. Abdolmaleki, et al. "An in vitro study of the impact of 4mT static magnetic field to modify the differentiation rate of rat bone marrow stem cells into primordial germ cells." Differentiation. 2014 Jul 15. pii: S0301-4681(14)00036-X. doi: 10.1016/j.diff.2014.06.001.

This investigation was performed to evaluate the differentiation capacity and alteration in genes expression patterns during in vitro differentiation of bone marrow stem cells into primordial germ cells using static magnetic field (4mT) and BMP-4 (25ng/ml). The rate of differentiation was investigated using the Real Time-PCR method for tracing expression of differentiation markers (Oct-4, Nanog, C-Myc, Fragilis, Mvh and Stella). Then, immunocytochemical reaction was carried out for detection of marker proteins (Oct4 and Mvh). Increasing of the exposure time of the 4mT SMF (24 and 48h) and treatment time with 25ng/ml BMP4 (48 and 96h) indicated a marked decrease in expression of pluripotency genes (Oct-4, Nanog and C-Myc) and Oct4 protein and increase in primordial germ cell-specific genes (Fragilis, Mvh and Stella) and Mvh protein compared with the control group. Final results showed that in a synergistic manner, the combination of SMF with BMP4 exaggerates the differentiation potential of BMSCs to PGCs by activating the MAPK pathway and altering the Ca2+ concentration.

Kapinas, K., H. Kim, et al. "microRNA-Mediated Survivin Control of Pluripotency." J Cell Physiol. 2014 Jun 2. doi: 10.1002/jcp.24681.

Understanding the mechanisms that sustain pluripotency in human embryonic stem cells (hESCs) is an active area of research that may prove useful in regenerative medicine and will provide fundamental information relevant to development and cancer. hESCs and cancer cells share the unique ability to proliferate indefinitely and rapidly. Because the protein survivin is uniquely overexpressed in virtually all human cancers and in hESCs, we sought to investigate its role in supporting the distinctive capabilities of these cell types. Results presented here suggest that survivin contributes to the maintenance of pluripotency and that post-transcriptional control of survivin isoform expression is selectively regulated by microRNAs. miR-203 has been extensively studied in human tumors, but has not been characterized in hESCs. We show that miR-203 expression and activity is consistent with the expression and subcellular localization of survivin isoforms that in turn modulate expression of the Oct4 and Nanog transcription factors to sustain pluripotency. This study contributes to understanding of the complex regulatory mechanisms that govern whether hESCs proliferate or commit to lineages. (c) 2014 Wiley Periodicals, Inc.

Kimura, T., Y. Kaga, et al. "Pluripotent stem cells derived from mouse primordial germ cells by small molecule compounds." Stem Cells. 2014 Sep 3. doi: 10.1002/stem.1838.

Primordial germ cells (PGCs) can give rise to pluripotent stem cells known as embryonic germ cells (EGCs) when cultured with basic fibroblast growth factor (bFGF), stem cell factor (SCF), and leukemia inhibitory factor (LIF). Somatic cells can give rise to induced pluripotent stem cells (iPSCs) by introduction of the reprogramming transcription factors Oct4, Sox2, and Klf4. The effects of Sox2 and Klf4 on somatic cell reprogramming can be reproduced using the small molecule compounds, transforming growth factor-beta receptor (TGFbetaR) inhibitor and Kempaullone, respectively. Here we examined the effects of TGFbetaR inhibitor and Kempaullone on EGC derivation from PGCs. Treatment of PGCs with TGFbetaR inhibitor and/or Kempaullone generated pluripotent stem cells under standard embryonic stem cell (ESC) culture conditions without bFGF and SCF, which we termed induced EGCs (iEGCs). The derivation efficiency of iEGCs was dependent on the differentiation stage and sex. DNA methylation levels of imprinted genes in iEGCs were reduced, with the exception of the H19 gene. The promoters of genes involved in germline development were generally hypomethylated in PGCs, but three germline genes showed comparable DNA methylation levels among iEGs, ESCs, and iPSCs. These results show that PGCs can be reprogrammed into pluripotent state using small molecule compounds, and that DNA methylation of these germline genes is not maintained in iEGCs. Stem Cells 2014.

Klincumhom, N., T. Tharasanit, et al. "Selective TGF-beta1/ALK inhibitor improves neuronal differentiation of mouse embryonic stem cells." Neurosci Lett. 2014 Aug 22;578:1-6. doi: 10.1016/j.neulet.2014.06.001. Epub 2014 Jun 9.

The transforming growth factor-beta1 (TGF-beta1), a polypeptide member of the TGF-beta superfamily, has myriad cellular functions, including cell fate differentiation. We hypothesized that suppression of TGF-beta1 signaling would improve the efficacy of neuronal differentiation during embryoid body (EB) development. In this study, mouse embryonic stem cells (ESCs) were allowed to differentiate into their neuronal lineage, both with, and without the TGF-beta1 inhibitor (A83-01). After 8 days of EB suspension culture, the samples were examined by morphological analysis, immunocytochemistry and

immunohistochemistry with pluripotent (Oct4, Sox2) and neuronal specific markers (Pax6, NeuN). The alteration of gene expressions during EB development was determined by quantitative RT-PCR. Our results revealed that the TGF-beta1/ALK inhibitor potentially suppressed pluripotent gene (Oct4) during a rapidly up-regulation of neuronal associated genes including Sox1 and MAP2. Strikingly, during EB development, the expression of GFAP, the astrocyte specific gene, remarkably decreased compared to the non-treated control. This strategy demonstrated the beneficial function of TGF-beta1/ALK inhibitor that rapidly and uniformly drives cell fate alteration from pluripotent state toward neuronal lineages.

Koka, P. S. "Biomarker discovery and biotherapeutics applications of photosynthetic light-harvesting and bioluminescence light-emitting chromophore-protein complexes in stem cell biology and regenerative medicine." J Stem Cells. 2014;9(3):127-33. doi: jsc.2014.9.3.127.

We have since the 1970's embarked on the development of biologically derived fluorophore-protein complexes that will find applications in the communicable and non-communicable disease etiology processes and their cures. We have since then become largely successful in these endeavors along with interspersed contributions also from investigators who have generally restricted to working in confined disciplines. Their encompassment with our works as this investigator has traversed his definitely chosen and not merely a circumstantial, coincidental, or accidental step-wise multi-disciplinary scientific path from biophysics to regenerative medicine spanning these lines of investigations for last four decades have finally yielded the much necessitated disease related applied biological interventions for human benefits. Taking a cue from our early investigations and findings on which we call attention to the identification and characterization of the use of the primary light-emitting lumazine precursor of riboflavin which is 6,7-dimethyl-8-ribityl lumazine-protein complex which we had derived from the bioluminescence bacterium (Photobacterium phosphoreum) wherein it functions as a naturally occurring fluorescence light emitter (LumP). These in vivo phenomena have been a precursor to the subsequent developments in vitro. This in vivo to in vitro investigation path of ours has been also comprised among others of binding of photosynthetic light-harvesting marine dinoflagellate algae (Glenodinium sp.) derived peridinin-chlorophyll a-protein (PerCP) complex-labeled monoclonal antibodies useful in the development of flow cytometry. These fluorescence labeled antibodies bound antigens which include those of communicable infectious diseases (HIV/AIDS - env-gp160, gag-p24), non-communicable but also potential hereditary and malignant disorders (Cancer/Tumor Markers - Melan-A/Mart-1 of melanoma), normal immune response cells (Human/Mouse/species cellular MHC/TCR/CD45/CD33/CD56/CD19/CD41), and of types of stem cells (CD34/CD38/c-Mpl/Oct4/Neuropilin-1/SOX17). Such antigens have been analyzed by us and other investigators by fluorescence-activated cell sorting (FACS - cell surface and intracellular binding), confocal fluorescence microscopy, or/and immunohistochemistry, to determine qualitative and quantitative antigen expression levels and their mechanistic implications. We have followed stem cell differentiation patterns and signaling mechanisms through marker antigen-antibody binding wherein the antibodies are labeled with covalently linked fluorophore-protein complexes or fluorescence emitting chromophores. These complexes among others also have included PerCP.

Koo, B. S., S. H. Lee, et al. "Oct4 is a critical regulator of stemness in head and neck squamous carcinoma cells." Oncogene. 2014 Jun 23. doi: 10.1038/onc.2014.174.

Cancer stem cells (CSCs) have been suggested as responsible for the initiation and progression of cancers. Octamer-binding transcription factor 4 (Oct4) is an important regulator of embryonic stem cell fate. Here, we investigated whether Oct4 regulates stemness of head and neck squamous carcinoma (HNSC) CSCs. Our study showed that ectopic expression of Oct4 promotes tumor growth through cyclin E activation, increases chemoresistance through ABCC6 expression and enhances tumor invasion through slug expression. Also, Oct4 dedifferentiates differentiated HNSC cells to CSC-like cells. Furthermore, Oct4high HNSC CSCs have more stem cell-like traits compared with Oct4low cells, such as self-renewal, stem cell markers' expression, chemoresistance, invasion capacity and xenograft tumorigeneity in vitro and in vivo. In addition, knockdown of Oct4 led to markedly lower HNSC CSC stemness. Finally, there was a significant correlation between Oct4 expression and survival of 119 HNSC patients. Collectively, these data suggest that Oct4 may be a critical regulator of HNSC CSCs and its targeting may be potentially valuable in the treatment of HNSC CSCs.Oncogene advance online publication, 23 June 2014; doi:10.1038/onc.2014.174.

Kraus, P., S. V, et al. "Pleiotropic functions for transcription factor zscan10." PLoS One. 2014 Aug 11;9(8):e104568. doi: 10.1371/journal.pone.0104568. eCollection 2014.

The transcription factor Zscan10 had been attributed a role as a pluripotency factor in embryonic stem cells based on its interaction with Oct4 and Sox2 in in vitro assays. Here we suggest a potential role of Zscan10 in controlling progenitor cell populations in vivo. Mice homozygous for a Zscan10 mutation exhibit reduced weight, mild hypoplasia in the spleen, heart and long bones and phenocopy an eye malformation previously described for Sox2 hypomorphs. Phenotypic abnormalities are supported by the nature of Zscan10 expression in midgestation embryos and adults suggesting a role for Zscan10 in either maintaining progenitor cell subpopulation or impacting on fate choice decisions thereof.

Lacerda, S. M., G. M. Costa, et al. "Biology and identity of fish spermatogonial stem cell." Gen Comp Endocrinol. 2014 Jun 23. pii: S0016-6480(14)00251-2. doi: 10.1016/j.ygcen.2014.06.018.

Although present at relatively low number in the testis, spermatogonial stem cells (SSCs) are crucial for the establishment and maintenance of spermatogenesis in eukaryotes and, until recently, those cells were investigated in fish using morphological criteria. The isolation and characterization of these cells in fish have been so far limited by the lack of specific molecular markers, hampering the high SSCs biotechnological potential for aquaculture. However, some highly conserved vertebrate molecular markers, such as Gfra1 and Pou5f1/Oct4, are now available representing important candidates for studies evaluating the regulation of SSCs in fish and even functional investigations using germ cells transplantation. A technique already used to demonstrate that, different from mammals, fish germ stem cells (spermatogonia and oogonia) present high sexual plasticity that is determined by the somatic microenvironment. As relatively well established in mammals, and demonstrated in zebrafish and dogfish, this somatic environment is very important for the preferential location and regulation of SSCs. Importantly, a long-term in vitro culture system for SSCs has been now established for some fish species.

Leten, C., V. D. Roobrouck, et al. "Controlling and monitoring stem cell safety in vivo in an experimental rodent model." Stem Cells. 2014 Aug 21. doi: 10.1002/stem.1819.

Adult stem cells have been investigated increasingly over the past years for multiple applications. Although they have a more favorable safety profile compared to pluripotent stem cells, they are still capable of self-renewal and differentiate into several cell types. We investigated the behavior of Oct4 positive (Oct4+) and Oct4 negative (Oct4-) murine or rat bone marrow (BM) derived stem cells in the healthy brain of syngeneic mice and rats. Engraftment of mouse and rat Oct4 positive BM-derived hypoblast like stem cells (m/rOct4+ BM-HypoSC's) resulted in yolk-sac tumor formation in the healthy brain which were monitored longitudinally using magnetic resonance imaging (MRI) and bioluminescence imaging (BLI). Contrast enhanced MRI confirmed the disruption of the blood brain barrier (BBB). In contrast, m/r Oct4 negative BM derived multipotent adult progenitor cells (m/rOct4- BM-MAPC's)

did not result in mass formation after engraftment into the brain. mOct4+ BM-HypoSC's and mOct4- BM-MAPC's were transduced to express enhanced green fluorescent protein (eGFP), firefly luciferase (fLuc) and herpes simplex virus - thymidine kinase (HSV - tk) to follow up suicide gene expression as a potential 'safety switch' for tumor forming stem cells by multi - modal imaging. Both cell lines were eradicated efficiently in vivo by ganciclovir (GCV) administration indicating successful suicide gene expression in vivo, as assessed by MRI, BLI and histology. The use of suicide genes to prevent tumor formation is in particular of interest for therapeutic approaches where stem cells are used as vehicles to deliver therapeutic genes. Stem Cells 2014.

Luhrig, S., I. Siamishi, et al. "Lrrc34, a Novel Nucleolar Protein, Interacts with Npm1 and Ncl and Has an Impact on Pluripotent Stem Cells." Stem Cells Dev. 2014 Aug 5.

The gene Lrrc34 (leucine rich repeat containing 34) is highly expressed in pluripotent stem cells and its expression is strongly downregulated upon differentiation. These results let us to suggest a role for Lrrc34 in the regulation and maintenance of pluripotency. Expression analyses revealed that Lrrc34 is predominantly expressed in pluripotent stem cells and has an impact on the expression of known pluripotency genes, such as Oct4. Methylation studies of the Lrrc34 promoter showed a hypomethylation in undifferentiated stem cells and chromatin immunoprecipitation-quantitative polymerase chain reaction analyses of histone modifications revealed an enrichment of activating histone modifications on the Lrrc34 promoter region. Further, we could verify the nucleolus-the place of ribosome biogenesis-as the major subcellular localization of the LRRC34 protein. We have verified the interaction of LRRC34 with two major nucleolar proteins, Nucleophosmin and Nucleolin, by two independent methods, suggesting a role for Lrrc34 in ribosome biogenesis of pluripotent stem cells. In conclusion, LRRC34 is a novel nucleolar protein that is predominantly expressed in pluripotent stem cells.

Ma, R., S. Bonnefond, et al. "Stemness is Derived from Thyroid Cancer Cells." Front Endocrinol (Lausanne). 2014 Jul 15;5:114. doi: 10.3389/fendo.2014.00114. eCollection 2014.

BACKGROUND: One hypothesis for thyroid cancer development is its derivation from thyroid cancer stem cells (CSCs). Such cells could arise via different paths including from mutated resident stem cells within the thyroid gland or via epithelial to mesenchymal transition (EMT) from malignant cells since EMT is known to confer stem-like characteristics. Furthermore, EMT is a critical process for epithelial tumor progression, local invasion, and metastasis formation. In addition, stemness provides cells with therapeutic resistance and is the likely cause of tumor recurrence. However, the relevance of EMT and stemness in thyroid cancer progression has not been extensively studied. METHODS: To examine the status of stemness in thyroid papillary cancer, we employed a murine model of thyroid papillary carcinoma and examined the expression of stemness and EMT using qPCR and histochemistry in mice with a thyroid-specific knock-in of oncogenic Braf (LSL-Braf((V600E))/TPO-Cre). This construct is only activated at the time of thyroid peroxidase (TPO) expression in differentiating thyroid cells and cannot be activated by undifferentiated stem cells, which do not express TPO. RESULTS: There was decreased expression of thyroid-specific genes such as Tg and NIS and increased expression of stemness markers, such as Oct4, Rex1, CD15, and Sox2 in the thyroid carcinoma tissue from 6-week-old BRAF(V600E) mice indicating the dedifferentiated status of the cells and the fact that stemness was derived in this model from differentiated thyroid cells. The decreased expression of the epithelial marker E-cadherin and increased EMT regulators including Snail, Slug, and TGF-beta1 and TGF-beta3, and the mesenchymal marker vimentin demonstrated the simultaneous progression of EMT and the CSC-like phenotype. Stemness was also found in a cancer thyroid cell line (named Marca cells) derived from one of the murine tumors. In this cell line, we also found that overexpression of Snail caused up-regulation of vimentin expression and up-regulation of stemness markers Oct4, Rex1, and CD15, with enhanced migration ability of the cells. We also showed that TGF-beta1 was able to induce Snail and vimentin expression in the Marca cell thyroid cancer line, indicating the induction of EMT in these cells, and this induction of EMT and stemness was significantly inhibited by celastro a natural inhibitor of neoplastic cells. CONCLUSION: Our findings support our earlier hypothesis that stemness in thyroid cancer is derived via EMT rather than from resident thyroid stem cells. In mice with a thyroid-specific knock-in of oncogenic Braf (LSL-Braf((V600E))/TPO-Cre), the neoplastic changes were dependent on thyroid cell differentiation and the onset of stemness must have been derived from differentiated thyroid epithelial cells. Furthermore, celastrol suppressed TGF-beta1 induced EMT in thyroid cancer cells and may have therapeutic potential.

Ma, R., N. Minsky, et al. "Stemness in human thyroid cancers and derived cell lines: the role of asymmetrically dividing cancer stem cells resistant to chemotherapy." J Clin Endocrinol Metab. 2014 Mar;99(3):E400-9. doi: 10.1210/jc.2013-3545. Epub 2014 Feb 25.

CONTEXT: Cancer stem cells (CSCs) have the ability to self-renew through symmetric and asymmetric cell division. CSCs may arise from mutations within an embryonic stem cell/progenitor cell population or via epithelial-mesenchymal transition (EMT), and recent advances in the study of thyroid stem cells have led to a growing recognition of the likely central importance of CSCs in thyroid tumorigenesis. OBJECTIVE: The objectives of this study were to establish the presence of a stem cell population in human thyroid tumors and to identify, isolate, and characterize CSCs in thyroid cancer cell lines. RESULTS: 1) Human thyroid cancers (n = 10) and thyroid cancer cell lines (n = 6) contained a stem cell population as evidenced by pluripotent stem cell gene expression. 2) Pulse-chase experiments with thyroid cancer cells identified a label-retaining cell population, a primary characteristic of CSCs, which at mitosis divided their DNA both symmetrically and asymmetrically and included a population of cells expressing the progenitor marker, stage-specific embryonic antigen 1 (SSEA-1). 3) Cells positive for SSEA-1 expressed additional stem cell markers including Oct4, Sox2, and Nanog were confirmed as CSCs by their tumor-initiating properties in vivo, their resistance to chemotherapy, and their multipotent capability. 4) SSEA-1-positive cells showed enhanced vimentin expression and decreased E-cadherin expression, indicating their likely derivation via EMT.

Madonna, R., Y. J. Geng, et al. "High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1." Biochim Biophys Acta. 2014 Aug 7. pii: S0925-4439(14)00251-8. doi: 10.1016/j.bbadis.2014.07.030.

Background and objective: Hyperglycemia leads to adaptive cell responses in part due to hyperosmolarity. In endothelial and epithelial cells, hyperosmolarity induces aquaporin-1 (AQP1) which plays a role in cytoskeletal remodeling, cell proliferation and migration. Whether such impairments also occur in human induced pluripotent stem cells (iPS) is not known. We therefore investigated whether high glucose-induced hyperosmolarity impacts proliferation, migration, expression of pluripotency markers and actin skeleton remodeling in iPS cells in an AQP1-dependent manner. Methods and results: Human iPS cells were generated from skin fibroblasts by lentiviral transduction of four reprogramming factors (Oct4, Sox2, Klf4, c-Myc). After reprogramming, iPS cells were characterized by their adaptive responses to high glucose-induced hyperosmolarity by incubation with 5.5mmol/L glucose, high glucose (HG) at 30.5mM, or with the hyperosmolar control mannitol (HM). Exposure to either HG or HM increased the expression of AQP1. AQP1 co-immunoprecipitated with beta-catenin. HG and HM induced the expression of beta-catenin. Under these conditions, iPS cells showed increased ratios

of F-actin to G-actin and formed increased tubing networks. Inhibition of AQP1 with small interfering RNA (siRNA) reverted the inducing effects of HG and HM. Conclusions: High glucose enhances human iPS cell proliferation and cytoskeletal remodeling due to hyperosmolarity-induced upregulation of AQP1.

Mallol, A., J. Santalo, et al. "Psammaplin A Improves Development and Quality of Somatic Cell Nuclear Transfer Mouse Embryos." Cell Reprogram. 2014 Jul 28.

Abstract Faulty reprogramming of the donor somatic nucleus to a totipotent embryonic state by the recipient oocyte is a major obstacle for cloning success. Accordingly, treatment of cloned embryos with epigenetic modifiers, such as histone deacetylase inhibitors (HDACi), enhances cloning efficiency. The purpose of our study was to further explore the potential effect of valproic acid (VPA), used in previous studies, and to investigate the effect of psammaplin A (PsA), a novel HDACi, on the development and quality of cloned mouse embryos. To this aim, cloned embryos were treated with 5, 10, and 20 muM PsA or 2 and 4 mM VPA for 8-9 h (before and during activation) or 16 h or 24 h (during and after activation), and their in vitro developmental potential and blastocyst quality were evaluated. Treatments with 10 muM PsA and 2 mM VPA for 16 h were selected as the most optimal, showing higher blastocyst rates and quality. These treatments had no significant effects on the expression of Nanog, Oct4, and Cdx2 or on global histone and DNA methylation levels at the blastocyst stage, but both increased global levels of histone acetylation at early developmental stages.

Mojsin, M., J. M. Vicentic, et al. "Quercetin reduces pluripotency, migration and adhesion of human teratocarcinoma cell line NT2/D1 by inhibiting Wnt/beta-catenin signaling." Food Funct. 2014 Aug 20.

Quercetin, a bioflavonoid found in plant foods, has a wide range of therapeutic effects. In order to examine the therapeutic potential of quercetin in teratocarcinoma, we used the human teratocarcinoma cell line NT2/D1 as an in vitro model. We have shown that quercetin inhibits the proliferation, adhesion and migration of NT2/D1 cells and downregulates the expression of pluripotency factors SOX2, Oct4 and Nanog. Our results further suggest that the anticancer effect of quercetin against human teratocarcinoma cells is mediated by targeting the canonical Wnt signaling pathway. Quercetin antagonized the Wnt/beta-catenin signaling pathway in NT2/D1 cells by inhibiting beta-catenin nuclear translocation and the consequent downregulation of beta-catenin-dependent transcription. These data suggest that quercetin as a potent inhibitor of Wnt signaling may be an effective therapeutic agent in cancers with aberrant activation of the Wnt pathway.

Mosbech, C. H., T. Svingen, et al. "Expression pattern of clinically relevant markers in paediatric germ cell- and sex-cord stromal tumours is similar to adult testicular tumours." Virchows Arch. 2014 Jul 30.

Paediatric germ cell tumours (GCTs) are rare and account for less than 3 % of childhood cancers. Like adult GCTs, they probably originate from primordial germ cells, but the pattern of histopathological types is different, and they occur predominantly in extragonadal sites along the body midline. Because they are rare, histology of paediatric GCTs is poorly documented, and it remains unclear to what extent they differ from adult GCTs. We have analysed 35 paediatric germ cell tumours and 5 gonadal sex-cord stromal tumours from prepubertal patients aged 0-15 years, to gain further knowledge, elaborate on clinical-pathological associations and better understand their developmental divergence. The tumours were screened for expression of stemness-related factors (OCT4, AP-2gamma, SOX2), classical yolk sac tumours (YSTs; AFP, SALL4), GCTs (HCG, PLAP, PDPN/D2-40), as well as markers for sex-cord stromal tumour (PDPN, GATA4). All YSTs expressed AFP and SALL4, with GATA4 present in 13/14. The majority of teratomas expressed SOX2 and PDPN, whereas SALL4 was found in 8/13 immature teratomas. Adult seminoma markers AP-2gamma, OCT4, SALL4 and PDPN were all expressed in dysgerminoma. We further report a previously unrecognised pathogenetic relationship between AFP and SALL4 in YST in that different populations of YST cells express either SALL4 or AFP, which suggests variable differentiation status. We also show that AP-2gamma is expressed in the granulosa layer of ovarian follicles and weakly expressed in immature but not in mature granulosa cell tumours.

Muller, U., C. Bauer, et al. "TET-mediated oxidation of methylcytosine causes TDG or NEIL glycosylase dependent gene reactivation." Nucleic Acids Res. 2014 Sep 1;42(13):8592-604. doi: 10.1093/nar/gku552. Epub 2014 Jun 19.

The discovery of hydroxymethyl-, formyl- and carboxylcytosine, generated through oxidation of methylcytosine by TET dioxygenases, raised the question how these modifications contribute to epigenetic regulation. As they are subjected to complex regulation in vivo, we dissected links to gene expression with in vitro modified reporter constructs. We used an Oct4 promoter-driven reporter gene and demonstrated that in vitro methylation causes gene silencing while subsequent oxidation with purified catalytic domain of TET1 leads to gene reactivation. To identify proteins involved in this pathway we screened for TET interacting factors and identified TDG, PARP1, XRCC1 and LIG3 that are involved in base-excision repair. Knockout and rescue experiments demonstrated that gene reactivation depended on the glycosylase TDG, but not MBD4, while NEIL1, 2 and 3 could partially rescue the loss of TDG. These results clearly show that oxidation of methylcytosine by TET dioxygenases and subsequent removal by TDG or NEIL glycosylases and the BER pathway results in reactivation of epigenetically silenced genes.

Muthusamy, T., O. Mukherjee, et al. "A method to identify and isolate pluripotent human stem cells and mouse epiblast stem cells using lipid body-associated retinyl ester fluorescence." Stem Cell Reports. 2014 Jun 12;3(1):169-84. doi: 10.1016/j.stemcr.2014.05.004. eCollection 2014 Jul 8.

We describe the use of a characteristic blue fluorescence to identify and isolate pluripotent human embryonic stem cells and human-induced pluripotent stem cells. The blue fluorescence emission (450-500 nm) is readily observed by fluorescence microscopy and correlates with the expression of pluripotency markers (OCT4, SOX2, and NANOG). It allows easy identification and isolation of undifferentiated human pluripotent stem cells, high-throughput fluorescence sorting and subsequent propagation. The fluorescence appears early during somatic reprogramming. We show that the blue fluorescence arises from the sequestration of retinyl esters in cytoplasmic lipid bodies. The retinoid-sequestering lipid bodies are specific to human and mouse pluripotent stem cells of the primed or epiblast-like state and absent in naive mouse embryonic stem cells. Retinol, present in widely used stem cell culture media, is sequestered as retinyl ester specifically by primed pluripotent cells and also can induce the formation of these lipid bodies.

Nawata, J., Y. Kuramitsu, et al. "Active Hexose-correlated Compound Down-regulates Sex-determining Region Y-box 2 of Pancreatic Cancer Cells." Anticancer Res. 2014 Sep;34(9):4807-11.

BACKGROUND/AIM: Active hexose-correlated compound (AHCC) is an extract of basidiomycete mushroom. It has been used as health food due to its efficacy of enhancing antitumor effects and reducing adverse effects of chemotherapy. Our previous research showed that AHCC down-regulated heat-shock protein (HSP)-27 and exhibited cytotoxic effects against gemcitabine-resistant pancreatic cancer cells. Sex-determining region Y-box 2 (SOX2) is reported to be up-regulated in other kinds of cancer cells and involved in carcinogenesis and malignancy. The aim of this study was to investigate the effects of AHCC on protein expression of SOX2 in the gemcitabine-resistant pancreatic cancer cell line

KLM1-R. MATERIALS AND METHODS: AHCC was applied to KLM1-R cells and expression of SOX2 was analyzed by western blotting. RESULTS: AHCC down-regulated SOX2 in KLM1-R cells. Nanog and Oct4, co-workers of SOX2 in maintaining pluripotency, did not exhibit any significant change in protein expression. CONCLUSION: We showed the potential of AHCC to be a candidate for combinatorial therapy in anticancer drug regimens. This result suggests that the target of AHCC in expressing therapeutic efficacy was not the pluripotent cells such as cancer stem cells (CSCs) but SOX2-specific.

Oktem, G., A. Bilir, et al. "Expression profiling of stem cell signaling alters with spheroid formation in CD133/CD44 prostate cancer stem cells." Oncol Lett. 2014 Jun;7(6):2103-2109. Epub 2014 Mar 24.

Cancer stem cells (CSC) isolated from multiple tumor types differentiate in vivo and in vitro when cultured in serum; however, the factors responsible for their differentiation have not yet been identified. The first aim of the present study was to identify CD133high/CD44high DU145 prostate CSCs and compare their profiles with non-CSCs as bulk counterparts of the population. Subsequently, the two populations continued to be three-dimensional multicellular spheroids. Differentiation was then investigated with stem cell-related genomic characteristics. Polymerase chain reaction array analyses of cell cycle regulation, embryonic and mesenchymal cell lineage-related markers, and telomerase reverse transcriptase (TERT) and Notch signaling were performed. Immunohistochemistry of CD117, Notch1, Jagged1, Delta1, Sox2, c-Myc, Oct4, KLF4, CD90 and SSEA1 were determined in CSC and non-CSC monolayer and spheroid subcultures. Significant gene alterations were observed in the CD133high/CD44high population when cultured as a monolayer and continued as spheroid. In this group, marked gene upregulation was determined in collagen type 9 alpha1, Islet1 and cyclin D2. Jagged1, Delta-like 3 and Notch1 were respectively upregulated genes in the Notch signaling pathway. According to immunoreactivity, the staining density of Jagged1, Sox2, Oct4 and Klf-4 increased significantly in CSC spheroids. Isolated CSCs alter their cellular characterization over the course of time and exhibit a differentiation profile while maintaining their former surface antigens at a level of transcription or translation. The current study suggested that this differentiation process may be a mechanism responsible for the malignant process and tumor growth.

Ovchinnikov, D. A., D. M. Titmarsh, et al. "Transgenic human ES and iPS reporter cell lines for identification and selection of pluripotent stem cells in vitro." Stem Cell Res. 2014 Jun 8;13(2):251-261. doi: 10.1016/j.scr.2014.05.006.

Optimization of pluripotent stem cell expansion and differentiation is facilitated by biological tools that permit non-invasive and dynamic monitoring of pluripotency, and the ability to select for an undifferentiated input cell population. Here we report on the generation and characterisation of clonal human embryonic stem (HES3, H9) and human induced pluripotent stem cell lines (UQEW01i-epifibC11) that have been stably modified with an artificial EOS(C3+) promoter driving expression of EGFP and puromycin resistance-conferring proteins. We show that EGFP expression faithfully reports on the pluripotency status of the cells in these lines and that antibiotic selection allows for an efficient elimination of differentiated cells from the cultures. We demonstrate that the extinction of the expression of the pluripotency reporter during differentiation closely correlates with the decrease in expression of conventional pluripotency markers, such as OCT4 (POU5F1), TRA-1-60 and SSEA4 when screening across conditions with various levels of pluripotency-maintaining or differentiation-inducing signals. We further illustrate the utility of these lines for real-time monitoring of pluripotency in embryoid bodies and microfluidic bioreactors.

Papanayotou, C., A. Benhaddou, et al. "A novel nodal enhancer dependent on pluripotency factors and smad2/3 signaling conditions a regulatory switch during epiblast maturation." PLoS Biol. 2014 Jun 24;12(6):e1001890. doi: 10.1371/journal.pbio.1001890. eCollection 2014 Jun.

During early development, modulations in the expression of Nodal, a TGFbeta family member, determine the specification of embryonic and extra-embryonic cell identities. Nodal has been extensively studied in the mouse, but aspects of its early expression remain unaccounted for. We identified a conserved hotspot for the binding of pluripotency factors at the Nodal locus and called this sequence "highly bound element" (HBE). Luciferase-based assays, the analysis of fluorescent HBE reporter transgenes, and a conditional mutation of HBE allowed us to establish that HBE behaves as an enhancer, is activated ahead of other Nodal enhancers in the epiblast, and is essential to Nodal expression in embryonic stem cells (ESCs) and in the mouse embryo. We also showed that HBE enhancer activity is critically dependent on its interaction with the pluripotency factor Oct4 and on Activin/Nodal signaling. Use of an in vitro model of epiblast maturation, relying on the differentiation of ESCs into epiblast stem cells (EpiSCs), revealed that this process entails a shift in the regulation of Nodal expression from an HBE-driven phase to an ASE-driven phase, ASE being another autoregulatory Nodal enhancer. Deletion of HBE in ESCs or in EpiSCs allowed us to show that HBE, although not necessary for Nodal expression in EpiSCs, is required in differentiating ESCs to activate the differentiation-promoting ASE and therefore controls this regulatory shift. Our findings clarify how early Nodal expression is regulated and suggest how this regulation can promote the specification of extra-embryonic precusors without inducing premature differentiation of epiblast cells. More generally, they open new perspectives on how pluripotency factors achieve their function.

Park, B. W., S. J. Jang, et al. "Cryopreservation of human dental follicle tissue for use as a resource of autologous mesenchymal stem cells." J Tissue Eng Regen Med. 2014 Jul 23. doi: 10.1002/term.1945.

The main purpose of this study was to develop a cryopreservation method for human dental follicle tissue to maintain autologous stem cells as a resource. A modified cryoprotectant, consisting of 0.05 m glucose, 0.05 m sucrose and 1.5 m ethylene glycol in phosphate-buffered saline (PBS) was employed, with a slow-ramp freezing rate. We observed > 70% of cell survival rate after 3 months of tissue storage. Isolated and cultured human dental stem cells (hDSCs) from cryopreserved dental follicles expressed mesenchymal stem cell markers at a level similar to that of hDSCs from fresh tissue. They also successfully differentiated in vitro into the mesenchymal lineage, osteocytes, adipocytes and chondrocytes under specific inductions. Using immunohistochemistry, the early transcription factors OCT4, NANOG and SOX2 were moderately or weakly detected in the nucleus of both fresh and cryopreserved dental follicles. In addition, p63, CCND1, BCL2 and BAX protein expression levels were the same in both fresh and cryopreserved tissues. However, the positive-cell ratio and intensity of p53 protein was higher in cryopreserved tissues than in fresh tissues, indicating direct damage of the freeze-thawing process. Real-time PCR analysis of hDSCs at passage 2 from both fresh and cryopreserved dental follicles showed similar levels of mRNA for apoptosis- and transcription-related genes. Based on these results, a newly developed cryoprotectant, along with a slow ramp rate freezing procedure allows for long-term dental tissue preservation for later use as an autologous stem cell resource in regenerative cell therapy. Copyright (c) 2014 John Wiley & Sons, Ltd.

Pipino, C., S. Mukherjee, et al. "Trisomy 21 Mid-Trimester Amniotic Fluid Induced Pluripotent Stem Cells Maintain Genetic Signatures During Reprogramming: Implications for Disease Modeling and Cryobanking." Cell Reprogram. 2014 Aug 27.

Abstract Trisomy 21 is the most common chromosomal abnormality and is associated primarily with cardiovascular, hematological, and neurological complications. A robust patient-derived cellular model is necessary to investigate the pathophysiology of the syndrome because current animal models are limited and access to tissues from affected individuals is ethically challenging. We aimed to derive induced pluripotent stem cells (iPSCs) from trisomy 21 human mid-trimester amniotic fluid stem cells (AFSCs) and describe their hematopoietic and neurological characteristics. Human AFSCs collected from women undergoing prenatal diagnosis were selected for c-KIT+ and transduced with a Cre-lox-inducible polycistronic lentiviral vector encoding SOX2, OCT4, KLF-4, and c-MYC (50,000 cells at a multiplicity of infection (MOI) 1-5 for 72 h). The embryonic stem cell (ESC)-like properties of the AFSC-derived iPSCs were established in vitro by embryoid body formation and in vivo by teratoma formation in RAG2-/-, gamma-chain-/-, C2-/- immunodeficient mice. Reprogrammed cells retained their cytogenetic signatures and differentiated into specialized hematopoietic and neural precursors detected by morphological assessment, immunostaining, and RT-PCR. Additionally, the iPSCs expressed all pluripotency markers upon multiple rounds of freeze-thawing. These findings are important in establishing a patient-specific cellular platform of trisomy 21 to study the pathophysiology of the aneuploidy and for future drug discovery.

Saenz-de-Juano, M. D., D. S. Penaranda, et al. "Does vitrification alter the methylation pattern of OCT4 promoter in rabbit late blastocyst?" Cryobiology. 2014 Aug;69(1):178-80. doi: 10.1016/j.cryobiol.2014.06.002. Epub 2014 Jun 12.

Vitrification is replacing slow freezing as the most popular method for oocyte and embryo cryopreservation. However, very little information is available on alterations in epigenetic regulation. Previous studies reported post-implantation effects of vitrification on fetal development and gene expression. This study was conducted to determine if vitrification procedure induce alterations in OCT4 promoter methylation profile which could determine the set point of fetal losses and transcriptomic alterations observed after implantation. Rabbit morulae were recovered at Day 3 of development and vitrified and transferred, or directly transfer, to recipient till Day 6. A conserved regulation region of OCT4 promoter was examined in control and vitrified embryos by bisulfite sequencing and quantitative PCR was used to measure the gene expression. No significant differences were observed in methylation levels or gene expression of OCT4. This work was the first approach in rabbit to the study of possible epigenetic alterations associated with vitrification procedure.

Scheerlinck, E., K. Van Steendam, et al. "Detailed method description for noninvasive monitoring of differentiation status of human embryonic stem cells." Anal Biochem. 2014 Sep 15;461:60-6. doi: 10.1016/j.ab.2014.05.026. Epub 2014 Jun 5.

The (non)differentiation status of human embryonic stem cells (hESCs) is usually analyzed by determination of key pluripotency defining markers (e.g., OCT4, Nanog, SOX2) by means of reverse transcription quantitative polymerase chain reaction (RT-qPCR), flow cytometry (FC), and immunostaining. Despite proven usefulness of these techniques, their destructive nature makes it impossible to follow up on the same hESC colonies for several days, leading to a loss of information. In 2003, an OCT4-eGFP knock-in hESC line to monitor OCT4 expression was developed and commercialized. However, to the best of our knowledge, the use of fluorescence microscopy (FM) for monitoring the OCT4-eGFP expression of these cells without sacrificing them has not been described to date.

Schonitzer, V., R. Wirtz, et al. "Sox2 Is a Potent Inhibitor of Osteogenic and Adipogenic Differentiation in Human Mesenchymal Stem Cells." Cell Reprogram. 2014 Aug 15.

Abstract Human mesenchymal stem cells (hMSCs) are a promising target for cell-based bone regeneration. However, their application for clinical use is limited because hMSCs lose their ability for cell division and differentiation during longer in vitro cultivation. The osteogenic differentiation is regulated through a complex network of molecular signal transduction pathways where the canonical Wnt pathway plays an important role. Sox2, a known key factor for maintenance of cellular pluripotency in stem cells, is supposed to influence the Wnt pathway in osteoblasts. In this study, we overexpressed Sox2 in immortalized hMSCs by lentiviral gene transfer. Sox2 overexpression significantly reduced the osteogenic and adipogenic differentiation potentials. This effect was abolished by knockdown of Sox2 overexpression. In addition, Oct4 and Nanog, other key transcription factors for pluripotency, are strongly upregulated when Sox2 is overexpressed. Furthermore, Dkk1, a target gene of the Sox2-Oct4 heterodimer and a Wnt antagonist, is downregulated. Sox2 overexpression causes higher expression levels of beta-catenin, the central transcription factor of the canonical Wnt pathway. These results suggest that Sox2 keeps hMSCs in an undifferentiated state by influencing the canonical Wnt pathway. Regulated expression of Sox2 may be a promising tool to cultivate hMSCs in sufficient quantities for cell and gene therapy applications.

Sureban, S. M., R. May, et al. "XMD8-92 inhibits pancreatic tumor xenograft growth via a DCLK1-dependent mechanism." Cancer Lett. 2014 Aug 28;351(1):151-61. doi: 10.1016/j.canlet.2014.05.011. Epub 2014 May 28.

XMD8-92 is a kinase inhibitor with anti-cancer activity against lung and cervical cancers, but its effect on pancreatic ductal adenocarcinoma (PDAC) remains unknown. Doublecortin-like kinase1 (DCLK1) is upregulated in various cancers including PDAC. In this study, we showed that XMD8-92 inhibits AsPC-1 cancer cell proliferation and tumor xenograft growth. XMD8-92 treated tumors demonstrated significant downregulation of DCLK1 and several of its downstream targets (including c-MYC, KRAS, NOTCH1, ZEB1, ZEB2, SNAIL, SLUG, OCT4, SOX2, NANOG, KLF4, LIN28, VEGFR1, and VEGFR2) via upregulation of tumor suppressor miRNAs let-7a, miR-144, miR-200a-c, and miR-143/145; it did not however affect BMK1 downstream genes p21 and p53. These data taken together suggest that XMD8-92 treatment results in inhibition of DCLK1 and downstream oncogenic pathways (EMT, pluripotency, angiogenesis and anti-apoptotic), and is a promising chemotherapeutic agent against PDAC.

Sutiwisesak, R., N. Kitiyanant, et al. "Induced pluripotency enables differentiation of human nullipotent embryonal carcinoma cells N2102Ep." Biochim Biophys Acta. 2014 Nov;1843(11):2611-9. doi: 10.1016/j.bbamcr.2014.07.013. Epub 2014 Jul 30.

Embryonal carcinoma (EC) cells, which are considered to be malignant counterparts of embryonic stem cells, comprise the pluripotent stem cell component of teratocarcinomas, a form of testicular germ cell tumors (GCTs). Nevertheless, many established human EC cell lines are nullipotent with limited or no capacity to differentiate under normal circumstances. In this study, we tested whether an over-expression of Yamanaka's reprogramming factors OCT4, SOX2, c-MYC and KLF4 might enable differentiation of the human nullipotent EC cells N2102Ep. Using OCT4 knockdown differentiated N2102Ep cells, we are able to derive reprogrammed N2102Ep cell lines. The induced pluripotency of N2102Ep allows the cells to differentiate toward neural lineage by retinoic acid; the expression of SSEA3 and SSEA4 is down-regulated, whereas that of neural surface markers is up-regulated. Consistent with the up-regulation of neural surface markers, the expression of the master neuroectodermal transcription factor PAX6 is also induced in reprogrammed N2102Ep. We next investigated whether PAX6 might induce spontaneous differentiation of nullipotent stem cells N2102Ep. However, while an ectopic expression of PAX6 promotes differentiation of NTERA2, it induces cell death in N2102Ep. We nevertheless find that upon induction of retinoic acid, the

reprogrammed N2102Ep cells form mature neuronal morphology similar to differentiated pluripotent stem cells NTERA2 as determined by TUJ1 expression, which is absent in N2102Ep parental cells. Altogether, we conclude that the nullipotent state of human EC cells can be reprogrammed to acquire a more relaxed state of differentiation potential by Yamanaka's factors.

Taha, M. F., A. Javeri, et al. "Upregulation of Pluripotency Markers in Adipose Tissue-Derived Stem Cells by miR-302 and Leukemia Inhibitory Factor." Biomed Res Int. 2014;2014:941486. doi: 10.1155/2014/941486. Epub 2014 Jul 23.

The expression pattern of pluripotency markers in adipose tissue-derived stem cells (ADSCs) is a subject of controversy. Moreover, there is no data about the signaling molecules that regulate these markers in ADSCs. In the present study, we studied the roles of leukemia inhibitory factor (LIF) and miR-302 in this regard. Freshly isolated mouse ADSCs expressed hematopoietic, mesenchymal, and pluripotency markers. One day after plating, ADSCs expressed OCT4 and Sox2 proteins. After three passages, the expression of hematopoietic and pluripotency markers decreased, while the expression of mesenchymal stem cell markers exhibited a striking rise. Both supplementation of culture media with LIF and transfection of the ADSCs with miR-302 family upregulated the expression levels of OCT4, Nanog, and Sox2 mRNAs. These findings showed that mouse adipose tissue contains a population of cells with molecular resemblance to embryonic stem cells, and LIF and miR-302 family positively affect the expression of pluripotency markers.

Takenaka-Ninagawa, N., Y. Kawabata, et al. "Generation of rat-induced pluripotent stem cells from a new model of metabolic syndrome." PLoS One. 2014 Aug 11;9(8):e104462. doi: 10.1371/journal.pone.0104462. eCollection 2014.

We recently characterized DahlS.Z-Leprfa/Leprfa (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS in vitro. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty of comparing to other rodents such as mouse. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. We aimed to establish riPSCs from MSCs in adipose tissues of both DS/obese rats and their lean littermates, DahlS.Z-Lepr+/Lepr+ (DS/lean) rats using lentivirus vectors with only three factors Oct4, Klf4, and Sox2 without c-Myc. The morphology, gene expression profiles, and protein expression of established colonies showed embryonic stem cell (ESCs)-like properties, and the differentiation potential into cells from all three germ layers both in vitro and in vivo (teratomas).

Talaei-Khozani, T., N. Zarei Fard, et al. "Delayed BMP4 exposure increases germ cell differentiation in mouse embryonic stem cells." Rom J Morphol Embryol. 2014;55(2):297-303.

Fate mapping studies have revealed that bone morphogenetic protein 4 (BMP4) signaling has a key role in segregation of primordial germ cells from proximal epiblast. Adding BMP4 to the culture media of embryonic stem (ES) cells could induce expression of germ cell markers; however, to provide a desired number of germ cells has remained a challenge. In the current study, we intended to establish an in vitro system to obtain reliable germ cells derived from ES cells. Differentiation was induced in ES cells via embryoid body (EB) and monolayer culture system. Cells were cultured with BMP4 from the beginning (++BMP4) or after 48 hours (+BMP4) of culturing for five days. The cultures were assessed for alkaline phosphatase (ALP) activity, expression of Oct4, Mvh and c-kit. In EB culture protocol, the expression of Mvh, Oct4 and ALP activity significantly increased in +BMP4 culture condition, but a significant down-regulation in the expression of germ cell markers was shown in ++BMP4 condition compared with the control group. Parallel differentiation experiments using monolayer culture system indicated the number of putative germ cells did not change. In the current study, we compared two differentiation methods (EB and monolayer) to achieve an optimal germ cell production.

Talluri, T. R., D. Kumar, et al. "Non-viral reprogramming of fibroblasts into induced pluripotent stem cells by Sleeping Beauty and piggyBac transposons." Biochem Biophys Res Commun. 2014 Jul 18;450(1):581-7. doi: 10.1016/j.bbrc.2014.06.014. Epub 2014 Jun 10.

The generation of induced pluripotent stem (iPS) cells represents a promising approach for innovative cell therapies. The original method requires viral transduction of several reprogramming factors, which may be associated with an increased risk of tumorigenicity. Transposition of reprogramming cassettes represents a recent alternative to viral approaches. Since binary transposons can be produced as common plasmids they provide a safe and cost-efficient alternative to viral delivery methods. Here, we compared the efficiency of two different transposon systems, Sleeping Beauty (SB) and piggyBac (PB), for the generation of murine iPS. Murine fibroblasts derived from an inbred BL/6 mouse line carrying a pluripotency reporter, Oct4-EGFP, and fibroblasts derived from outbred NMRI mice were employed for reprogramming. Both transposon systems resulted in the successful isolation of murine iPS cell lines. The reduction of the core reprogramming factors to omit the proto-oncogene c-Myc was compatible with iPS cell line derivation, albeit with reduced reprogramming efficiencies. The transposon-derived iPS cells featured typical hallmarks of pluripotency, including teratoma growth in immunodeficient mice. Thus SB and PB transposons represent a promising non-viral approach for iPS cell derivation.

Tang, M. K., L. M. Lo, et al. "Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells." F1000Res. 2014 May 8;3:102. doi: 10.12688/f1000research.4092.1. eCollection 2014.

Currently, there are genetic- and chemical-based methods for producing pluripotent stem cells from somatic cells, but all of them are extremely inefficient. However, a simple and efficient technique has recently been reported by Obokata et al (2014a, b) that creates pluripotent stem cells through acid-based treatment of somatic cells. These cells were named stimulus-triggered acquisition of pluripotency (STAP) stem cells. This would be a major game changer in regenerative medicine if the results could be independently replicated. Hence, we isolated CD45 (+) splenocytes from five-day-old Oct4-GFP mice and treated the cells with acidified (pH 5.7) Hank's Balanced Salt Solution (HBSS) for 25 min, using the methods described by Obokata et al 2014c. However, we found that this method did not induce the splenocytes to express the stem cell marker Oct4-GFP when observed under a confocal microscope three to six days after acid treatment. qPCR analysis also confirmed that acid treatment did not induce the splenocytes to express the stemness markers Oct4, Sox2 and Nanog. In addition, we obtained similar results from acid-treated Oct4-GFP lung fibroblasts. In summary, we have not been able to produce STAP stem cells from neonatal splenocytes or lung fibroblasts using the acid-based treatment reported by Obokata et al (2014a, b, c).

Taylor, U., W. Garrels, et al. "Injection of ligand-free gold and silver nanoparticles into murine embryos does not impact pre-implantation development." Beilstein J Nanotechnol. 2014 May 21;5:677-88. doi: 10.3762/bjnano.5.80. eCollection 2014.

Intended exposure to gold and silver nanoparticles has increased exponentially over the last decade and will continue to rise due to their use in biomedical applications. In particular, reprotoxicological aspects of these particles still need to be

addressed so that the potential impacts of this development on human health can be reliably estimated. Therefore, in this study the toxicity of gold and silver nanoparticles on mammalian preimplantation development was assessed by injecting nanoparticles into one blastomere of murine 2 cell-embryos, while the sister blastomere served as an internal control. After treatment, embryos were cultured and embryo development up to the blastocyst stage was assessed. Development rates did not differ between microinjected and control groups (gold nanoparticles: 67.3%, silver nanoparticles: 61.5%, sham: 66.2%, handling control: 79.4%). Real-time PCR analysis of six developmentally important genes (BAX, BCL2L2, TP53, OCT4, NANOG, DNMT3A) did not reveal an influence on gene expression in blastocysts. Contrary to silver nanoparticles, exposure to comparable Ag(+)-ion concentrations resulted in an immediate arrest of embryo development. In conclusion, the results do not indicate any detrimental effect of colloidal gold or silver nanoparticles on the development of murine embryos.

Theunissen, T. W., B. E. Powell, et al. "Systematic Identification of Culture Conditions for Induction and Maintenance of Naive Human Pluripotency." Cell Stem Cell. 2014 Jul 24. pii: S1934-5909(14)00298-7. doi: 10.1016/j.stem.2014.07.002.

Embryonic stem cells (ESCs) of mice and humans have distinct molecular and biological characteristics, raising the question of whether an earlier, "naive" state of pluripotency may exist in humans. Here we took a systematic approach to identify small molecules that support self-renewal of naive human ESCs based on maintenance of endogenous OCT4 distal enhancer activity, a molecular signature of ground state pluripotency. Iterative chemical screening identified a combination of five kinase inhibitors that induces and maintains OCT4 distal enhancer activity when applied directly to conventional human ESCs.

Torres, A. C., D. Boruszewska, et al. "Lysophosphatidic acid signaling in late cleavage and blastocyst stage bovine embryos." Mediators Inflamm. 2014;2014:678968. doi: 10.1155/2014/678968. Epub 2014 Apr 15.

Lysophosphatidic acid (LPA) is a known cell signaling lipid mediator in reproductive tissues. In the cow, LPA is involved in luteal and early pregnancy maintenance. Here, we evaluated the presence and role of LPA in bovine early embryonic development. In relevant aspects, bovine embryos reflect more closely the scenario occurring in human embryos than the mouse model. Transcription of mRNA and protein expression of enzymes involved in LPA synthesis (ATX and cPLA2) and of LPA receptors (LPAR1-4) were detected in Days 5 and 8 in vitro produced embryos. Embryonic LPA production into culture medium was also detected at both stages of development. Supplementation of culture medium with LPA (10(-5) M) between Days 2 and 8 had no effect on embryo yield and quality and on blastocyst relative mRNA abundance of genes involved in prostaglandin synthesis (PTGS2, PGES, and PGFS) and steroidogenesis (3betaHSD).

Toth, A., K. Fodor, et al. "Generation of induced pluripotent stem cells by using a mammalian artificial chromosome expression system." Acta Biol Hung. 2014 Sep;65(3):331-45. doi: 10.1556/ABiol.65.2014.3.9.

Direct reprogramming of mouse fibroblasts into induced pluripotent stem cells (iPS) was achieved recently by overexpression of four transcription factors encoded by retroviral vectors. Most of the virus vectors, however, may cause insertional mutagenesis in the host genome and may also induce tumor formation. Therefore, it is very important to discover novel and safer, non-viral reprogramming methods. Here we describe the reprogramming of somatic cells into iPS cells by a novel protein-based technique. Engineered Oct4, Sox2 and Klf4 transcription factors carrying an N-terminal Flag-tag and a C-terminal polyarginine tail were synthesized by a recently described mammalian artificial chromosome expression system (ACEs). This system is suitable for the high-level production of recombinant proteins in mammalian tissue culture cells. Recombinant proteins produced in this system contain all the post-translational modifications essential for the stability and the authentic function of the proteins. The engineered Oct4, Sox2 and Klf4 proteins efficiently induced the reprogramming of mouse embryonic fibroblasts by means of protein transduction. This novel method allows for the generation of iPS cells, which may be suitable for therapeutic applications in the future.

Tucker, B. A., F. Solivan-Timpe, et al. "Duplication of TBK1 Stimulates Autophagy in iPSC-derived Retinal Cells from a Patient with Normal Tension Glaucoma." J Stem Cell Res Ther. 2014 Jan 25;3(5):161.

Duplication of theTBK1 gene causes normal tension glaucoma (NTG); however the mechanism by which this copy number variation leads to retinal ganglion cell death is poorly understood. The ability to use skin-derived induced pluripotent stem cells (iPSCs) to investigate the function or dysfunction of a mutant gene product in inaccessible tissues such as the retina now provides us with the ability to interrogate disease pathophysiology in vitro. iPSCs were generated from dermal fibroblasts obtained from a patient with TBK1-associated NTG, via viral transduction of the transcription factors OCT4, SOX2, KLF4, and c-MYC. Retinal progenitor cells and subsequent retinal ganglion cell-like neurons were derived using our previously developed stepwise differentiation protocol. Differentiation to retinal ganglion-like cells was demonstrated via rt-PCR targeted against TUJ1, MAP2, THY1, NF200, ATOH7 and BRN3B and immunohistochemistry targeted against NF200 and ATOH7. Western blot analysis demonstrated that both fibroblasts and retinal ganglion cell-like neurons derived from NTG patients with TBK1 gene duplication have increased levels of LC3-II protein (a key marker of autophagy).

Ulbright, T. M., S. K. Tickoo, et al. "Best practices recommendations in the application of immunohistochemistry in testicular tumors: report from the International Society of Urological Pathology consensus conference." Am J Surg Pathol. 2014 Aug;38(8):e50-9. doi: 10.1097/PAS.0000000000000233.

The judicious use of immunostains can be of significant diagnostic assistance in the interpretation of testicular neoplasms when the light microscopic features are ambiguous. A limited differential diagnosis by traditional morphology is required for the effective use of immunohistochemistry (IHC); otherwise, the inevitable occurrence of exceptions to anticipated patterns will lead to "immunoconfusion." The diagnosis of tumors in the germ cell lineage, the great majority of primary tumors of the testis, has been considerably facilitated over the past decade by IHC directed at developmentally important nuclear transcription factors, including OCT4, SALL4, SOX2, and SOX17, that are mostly restricted to certain tumor histotypes. In conjunction with other markers, a specific diagnosis can be achieved in most instances through a panel of 3 or 4 immunostains and often fewer. IHC among tumors in the sex cord-stromal group may produce a significant proportion of false-negative cases until more sensitive and equally specific markers are validated.

Vaiphei, K., S. K. Sinha, et al. "Comparative analysis of Oct4 in different histological subtypes of esophageal squamous cell carcinomas in different clinical conditions." Asian Pac J Cancer Prev. 2014;15(8):3519-24.

BACKGROUND: Esophageal squamous cell carcinoma (ESCC) is a common cancer with poor prognosis. It has been hypothesized that Oct4 positive radioresistant stem cells may be responsible for tumor recurrence. Hence, we evaluated Oct4 expression in ESCC in pre-treatment, post neo-adjuvant residual and post-surgical recurrent tumours. MATERIALS AND METHODS: Endoscopic mucosal biopsies were used to study Oct4 expression and the observations were correlated with histological tumor grades, patient data and clinical background. RESULTS: All

patients presented with dysphagia with male predominance and a wide age range. Majority of the patients had intake of mixed diet, history of alcohol and tobacco intake was documented in less than half of the patients. Oct 4 expression was significantly higher in poorly differentiated (PDSCC) and basaloid (BSCC) subtypes than the other better differentiated tumor morphology. Oct4 was also expressed by adjoining esophageal mucosa showing low grade dysplasia and basal cell hyperplasia (BCH). Biopsies in PDSCC and BSCC groups were more likely to show a positive band for Oct4 by polymerase chain reaction (PCR). Dysplasia and BCH mucosa also showed Oct4 positivity by PCR. All mucosal biopsies with normal morphology were negative for Oct4. Number of tissue samples showing Oct4 positivity by PCR was higher than that by the conventional immunohistochemistry ($p>0.05$). Oct4 expression pattern correlated only with tumor grading, not with other parameters including the clinical background or patient data. CONCLUSIONS: Our observations highlighted a possible role of Oct4 in identifying putative cancer stem cells in ESCC pathobiology and response to treatment. The implications are either in vivo existence of Oct4 positive putative cancer stem cells in ESCC or acquisition of cancer stem cell properties by tumor cells as a response to treatment given, resulting ultimately an uncontrolled cell proliferation and treatment failure.

Vossaert, L., P. Meert, et al. "Identification of histone H3 clipping activity in human embryonic stem cells." Stem Cell Res. 2014 Jul;13(1):123-34. doi: 10.1016/j.scr.2014.05.002. Epub 2014 May 12.

Posttranslational histone modifications are essential features in epigenetic regulatory networks. One of these modifications has remained largely understudied: regulated histone proteolysis. In analogy to the histone H3 clipping during early mouse embryonic stem cell differentiation, we report for the first time that also in human embryonic stem cells this phenomenon takes place in the two different analyzed cell lines. Employing complementary techniques, different cleavage sites could be identified, namely A21, R26 and residue 31. The enzyme responsible for this cleavage is found to be a serine protease. The formation of cleaved H3 follows a considerably variable pattern, depending on the timeframe, culture conditions and culture media applied. Contrary to earlier findings on H3 clipping, our results disconnect the link between declining Oct4 expression and H3 cleavage.

West, J. A., A. Cook, et al. "Nucleosomal occupancy changes locally over key regulatory regions during cell differentiation and reprogramming." Nat Commun. 2014 Aug 27;5:4719. doi: 10.1038/ncomms5719.

Chromatin structure determines DNA accessibility. We compare nucleosome occupancy in mouse and human embryonic stem cells (ESCs), induced-pluripotent stem cells (iPSCs) and differentiated cell types using MNase-seq. To address variability inherent in this technique, we developed a bioinformatic approach to identify regions of difference (RoD) in nucleosome occupancy between pluripotent and somatic cells. Surprisingly, most chromatin remains unchanged; a majority of rearrangements appear to affect a single nucleosome. RoDs are enriched at genes and regulatory elements, including enhancers associated with pluripotency and differentiation. RoDs co-localize with binding sites of key developmental regulators, including the reprogramming factors Klf4, Oct4/Sox2 and c-Myc. Nucleosomal landscapes in ESC enhancers are extensively altered, exhibiting lower nucleosome occupancy in pluripotent cells than in somatic cells. Most changes are reset during reprogramming. We conclude that changes in nucleosome occupancy are a hallmark of cell differentiation and reprogramming and likely identify regulatory regions essential for these processes.

Whitworth, D. J., J. E. Frith, et al. "Derivation of Mesenchymal Stromal Cells from Canine Induced Pluripotent Stem Cells by Inhibition of the TGFbeta/Activin Signaling Pathway." Stem Cells Dev. 2014 Sep 4.

In this study we have generated canine mesenchymal stromal cells (MSCs), also known as mesenchymal stem cells, from canine induced pluripotent stem cells (ciPSCs) by small-molecule inhibition of the transforming growth factor beta (TGFbeta)/activin signaling pathway. These ciPSC-derived MSCs (ciPSC-MSCs) express the MSC markers CD73, CD90, CD105, STRO1, cPDGFRbeta and cKDR, in addition to the pluripotency factors OCT4, NANOG and REX1. ciPSC-MSCs lack immunostaining for H3K27me3, suggesting that they possess two active X chromosomes. ciPSC-MSCs are highly proliferative and undergo robust differentiation along the osteo-, chondro- and adipogenic pathways, but do not form teratoma-like tissues in vitro. Of further significance for the translational potential of ciPSC-MSCs, we show that these cells can be encapsulated and maintained within injectable hydrogel matrices that, when functionalized with bound pentosan polysulfate, dramatically enhance chondrogenesis and inhibit osteogenesis. The ability to efficiently derive large numbers of highly proliferative canine MSCs from ciPSCs that can be incorporated into injectable, functionalized hydrogels that enhance their differentiation along a desired lineage constitutes an important milestone towards developing an effective MSC-based therapy for osteoarthritis in dogs, but equally provides a model system for assessing the efficacy and safety of analogous approaches for treating human degenerative joint diseases.

Wyles, S. P., S. Yamada, et al. "Inhibition of DNA Topoisomerase II Selectively Reduces the Threat of Tumorigenicity Following Induced Pluripotent Stem Cell-Based Myocardial Therapy." Stem Cells Dev. 2014 Aug 21.

The advent of induced pluripotent stem cell (iPSC) technology creates new opportunities for transplant-based therapeutic strategies. The potential for clinical translation is currently hindered by the risk of dysregulated cell growth. Pluripotent stem cells reprogrammed by three-factor (Sox2, Klf, and Oct4) and four-factor (Sox2, Klf, Oct4, and c-Myc) strategies result in the capacity for teratogenic growth from residual pluripotent progeny upon in vivo transplantation. However, these pluripotent stem cells also have a stage-specific hypersensitivity to DNA-damaging agents that may allow separation of lineage-specific therapeutic subpopulation of cells. We aimed to demonstrate the selective effect of DNA topoisomerase II inhibitor, etoposide, in eliminating pluripotent cells in the early cardiac progenitor population thus decreasing the effect of teratoma formation. Immunodeficient murine hearts were infarcted and received implantation of a therapeutic dose of cardiac progenitors derived from partially differentiated iPSCs. Etoposide-treated cell implantation reduced mass formation in the intracardiac and extracardiac chest cavity compared with the same dose of iPSC-derived cardiac progenitors in the control untreated group. In vivo bioluminescence imaging confirmed the localization and engraftment of transplanted cells in the myocardium postinjection in both groups. Comparatively, the equivalent cell population without etoposide treatment demonstrated a greater incidence and size of teratoma formation. Hence, pretreatment with genotoxic etoposide significantly lowered the threat of teratogenicity by purging the contaminating pluripotent cells, establishing an adjunctive therapy to further harness the clinical value of iPSC-derived cardiac regeneration.

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