Induced Pluripotent Stem Cell (ips) Literatures

Mark H Smith

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

Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on induced pluripotent stem cell (ips).
[Smith MH. **Induced Pluripotent Stem Cell (ips) Literatures**. *Stem Cell* 2011;2(4):82-90] (ISSN 1545-4570). http://www.sciencepub.net/stem. 7

Key words: stem cell; life; gene; DNA; protein; induced pluripotent stem cell (ips)

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into many different types of cells in life bodies, that are exciting to scientists because of their potential to develop into many different cells, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situuition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

1. Literatures:

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

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

Brown, M. T. (2009). "Moral complicity in induced pluripotent stem cell research." Kennedy Inst Ethics J **19**(1): 1-22.

Direct reprogramming of human skin cells makes available a source of pluripotent stem cells without the perceived evil of embryo destruction, but the advent of such a powerful biotechnology entangles stem cell research in other forms of moral complicity. Induced pluripotent stem cell (iPSC) research had its origins in human embryonic stem cell research and the projected biomedical applications of iPS cells almost certainly will require more embryonic stem cell research. Policies that inhibit iPSC research in order to avoid moral complicity are themselves complicit in preventable harms to patients. Moral complicity may be unavoidable, but a Blue Ribbon Panel charged with assessing the need for additional embryonic stem cell lines may ease a transition from embryonic stem cell research to clinical applications of iPS cells.

Esteban, M. A., J. Xu, et al. (2009). "Generation of induced pluripotent stem cell lines from Tibetan miniature pig." J Biol Chem **284**(26): 17634-40.

Induced pluripotent stem cell (iPS) technology appears to be a general strategy to generate pluripotent stem cells from any given mammalian species. So far, iPS cells have been reported for mouse, human, rat, and monkey. These

four species have also established embryonic stem cell (ESC) lines that serve as the gold standard for pluripotency comparisons. Attempts have been made to generate porcine ESC by various means without success. Here we report the successful generation of pluripotent stem cells from fibroblasts isolated from the Tibetan miniature pig using a modified iPS protocol. The resulting iPS cell lines more closely resemble human ESC than cells from other species, have normal karyotype, stain positive for alkaline phosphatase, express high levels of ESC-like markers (Nanog, Rex1, Lin28, and SSEA4), and can differentiate into teratomas composed of the three germ layers. Because porcine physiology closely resembles human, the iPS cells reported here provide an attractive model to study certain human diseases or assess therapeutic applications of iPS in a large animal model.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" Curr Stem Cell Res Ther **4**(3): 168-77.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

Hong, H., K. Takahashi, et al. (2009). "Suppression of induced pluripotent stem cell generation by the p53-p21 pathway." Nature **460**(7259): 1132-5.

Induced pluripotent stem (iPS) cells can be generated from somatic cells by the introduction of Oct3/4 (also known as Pou5f1), Sox2, Klf4 and c-Myc, in mouse and in human. The efficiency of this process, however, is low. Pluripotency can be induced without c-Myc, but with even lower efficiency. A p53 (also known as TP53 in humans and Trp53 in mice) short-interfering RNA (siRNA) was recently shown to promote human iPS cell generation, but the specificity and mechanisms remain to be determined. Here we report that up to 10% of transduced mouse embryonic fibroblasts lacking p53 became iPS cells, even without the Myc retrovirus. The p53 deletion also promoted the induction of integration-free mouse iPS cells with plasmid transfection. Furthermore, in the p53-null background, iPS cells were generated from terminally differentiated T lymphocytes. The suppression of p53 also increased the efficiency of human iPS cell

generation. DNA microarray analyses identified 34 p53-regulated genes that are common in mouse and human fibroblasts. Functional analyses of these genes demonstrate that the p53-p21 pathway serves as a barrier not only in tumorigenicity, but also in iPS cell generation.

Li, C., H. Yu, et al. (2009). "Germline-competent mouse-induced pluripotent stem cell lines generated on human fibroblasts without exogenous leukemia inhibitory factor." PLoS One **4**(8): e6724.

Induced pluripotent stem (iPS) cells have attracted enormous attention due to their vast potential in regenerative medicine, pharmaceutical screening and basic research. Most prior established iPS cell lines were derived and maintained on mouse embryonic fibroblast (MEF) cells supplemented with exogenous leukemia inhibitory factor (LIF). Drawbacks of MEF cells impede optimization as well as dissection of reprogramming events and limit the usage of iPS cell derivatives in therapeutic applications. In this study, we develop a reproducible protocol for efficient reprogramming mouse neural progenitor cells (NPCs) on human foreskin fibroblast (HFF) cells via retroviral transfer of human transcriptional factors OCT4/SOX2/KLF4/C-MYC. Two independent iPS cell lines are derived without exogenous LIF. They display typical undifferentiated morphology and express pluripotency markers Oct4 and Sox2. Transgenes are inactivated and the endogenous Oct4 promoter is completely demethylated in the established iPS cell lines, indicating a fully reprogrammed state. Moreover, the iPS cells can spontaneously differentiate or be induced into various cell types of three embryonic germ layers in vitro and in vivo when they are injected into immunodeficient mice for teratoma formation. Importantly, iPS cells extensively integrate with various host tissues and contribute to the germline when injected into the blastocysts. Interestingly, these two iPS cell lines, while both pluripotent, exhibit distinctive differentiation tendencies towards different lineages. Taken together, the data describe the first genuine mouse iPS cell lines generated on human feeder cells without exogenous LIF, providing a reliable tool for understanding the molecular mechanisms of nuclear reprogramming.

Lu, M., M. D. Kardel, et al. (2009). "Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells." Exp Hematol **37**(8): 924-36.

OBJECTIVE: Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) constitute unique sources of pluripotent cells, although the molecular mechanisms involved in their differentiation into specific lineages are just beginning to be defined. Here we evaluated the ability of MEDII (medium conditioned by HepG2 cells, a human hepatocarcinoma cell line) to selectively enhance generation of mesodermal derivatives, including hematopoietic cells, from hESCs and hiPSCs. MATERIALS AND METHODS: Test cells were exposed to MEDII prior to being placed in conditions that promote embryoid body (EB) formation. Hematopoietic activity was measured by clonogenic assays, flow cytometry, quantitative real-time polymerase chain reaction of specific transcript complementary DNAs and the ability of cells to repopulate sublethally irradiated nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice for almost 1 year. RESULTS: Exposure of both hESCs and hiPSCs to MEDII induced a rapid and preferential differentiation of hESCs into mesodermal elements. Subsequently produced EBs showed a further enhanced expression of transcripts characteristic of multiple mesodermal lineages, and a concurrent decrease in endodermal and ectodermal cell transcripts. Frequency of all types of clonogenic hematopoietic progenitors in subsequently derived EBs was also increased. In vivo assays of MEDII-treated hESC-derived EBs also showed they contained cells able to undertake low-level but longterm multilineage repopulation of primary and secondary nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice. CONCLUSIONS: MEDII treatment of hESCs and hiPSCs alike selectively enhances their differentiation into mesodermal cells and allows subsequent generation of detectable levels of hematopoietic progenitors with in vitro and in vivo differentiating activity.

Marion, R. M., K. Strati, et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-54.

Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and

H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Miura, K., Y. Okada, et al. (2009). "Variation in the safety of induced pluripotent stem cell lines." Nat Biotechnol **27**(8): 743-5.

We evaluated the teratoma-forming propensity of secondary neurospheres (SNS) generated from 36 mouse induced pluripotent stem (iPS) cell lines derived in 11 different ways. Teratoma-formation of SNS from embryonic fibroblast-derived iPS cells was similar to that of SNS from embryonic stem (ES) cells. In contrast, SNS from iPS cells derived from different adult tissues varied substantially in their teratoma-forming propensity, which correlated with the persistence of undifferentiated cells.

Nakayama, M. (2009). "Cell Therapy Using Induced Pluripotent Stem (iPS) Cells Meets Next-Next Generation DNA Sequencing Technology." Curr Genomics **10**(5): 303-5.

The recent development of induced pluripotent stem (iPS) cell technology brings cell and gene therapies to patients one large step closer to reality. Technical improvements in various research fields sometimes come together fortuitously, leading to approaches to treating disease. If iPS cell technology continues to progress smoothly as expected and is actually applied to patients, the next logical step to ensuring the success of iPS cell therapy is to make use of next-next generation DNA sequencing technology and bioinformatics of recipient genomes. Before a patient-derived iPS cell colony is used for clinical therapy in a patient, the colony should undergo whole-genome DNA sequencing, thus avoiding risks associated with spontaneously mutagenized iPS cells. Researchers participating in the Human Genome Project need to take full advantage of both technologies-iPS cell technology and DNA sequencing-as doing so will help us achieve the original long-term goal of the project: developing therapies that will benefit human health.

Shi, Y. (2009). "Induced pluripotent stem cells, new tools for drug discovery and new hope for stem cell therapies." Curr Mol Pharmacol **2**(1): 15-8.

Somatic cell nuclear transfer or therapeutic cloning has provided great hope for stem cell-based therapies. However, therapeutic cloning has been experiencing both ethical and technical difficulties. Recent breakthrough studies using a combination of four factors to reprogram human somatic cells into pluripotent stem cells without using embryos or eggs have led to an important revolution in stem cell research. Comparative analysis of human induced pluripotent stem cells and human embryonic stem cells using assays for morphology, cell surface marker expression, gene expression profiling, epigenetic status, and differentiation potential have revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. These advances in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The cellular models created will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to design customized patient-specific stem cell therapies with economic feasibility.

Sommer, C. A., M. Stadtfeld, et al. (2009). "Induced pluripotent stem cell generation using a single lentiviral stem cell cassette." Stem Cells **27**(3): 543-9.

Induced pluripotent stem (iPS) cells can be generated using retroviral vectors expressing Oct4, Klf4, Sox2, and cMyc. Most prior studies have required multiple retroviral vectors for reprogramming, resulting in high numbers of genomic integrations in iPS cells and limiting their use for therapeutic applications. Here we describe the use of a single lentiviral vector expressing a "stem cell cassette" composed of the four transcription factors and a combination of 2A peptide and internal ribosome entry site technology, generating iPS cells from postnatal fibroblasts. iPS cells generated in this manner display embryonic stem cell-like morphology, express stem cell markers, and exhibit in vivo pluripotency, as evidenced by their ability to differentiate in teratoma assays and their robust contribution to mouse chimeras. Combining all factors into a single transcript achieves the most efficient reprogramming system to date and allows derivation of iPS cells with a single viral integration. The use of a single lentiviral vector for reprogramming represents a powerful laboratory tool and a significant step toward the application of iPS technology for clinical purposes.

Tanaka, T., S. Tohyama, et al. (2009). "In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes." Biochem Biophys Res Commun **385**(4): 497-502.

The lethal ventricular arrhythmia Torsade de pointes (TdP) is the most common reason for the withdrawal or restricted use of many cardiovascular

and non-cardiovascular drugs. The lack of an in vitro model to detect pro-arrhythmic effects on human heart cells hinders the development of new drugs. We hypothesized that recently established human induced pluripotent stem (hiPS) cells could be used in an in vitro drug screening model. In this study, hiPS cells were driven to differentiate into functional cardiomyocytes, which expressed cardiac markers including Nkx2.5, GATA4, and atrial natriuretic peptide. The hiPS-derived cardiomyocytes (hiPS-CMs) were analyzed using a multi electrode assay. The application of ion channel inhibitors resulted in dose-dependent changes to the field potential waveform, and these changes were identical to those induced in the native cardiomyocytes. This study shows that hiPS-CMs represent a promising in vitro model for cardiac electrophysiologic studies and drug screening.

Vallier, L., T. Touboul, et al. (2009). "Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells." Stem Cells **27**(11): 2655-66.

Human pluripotent stem cells from embryonic origins and those generated from reprogrammed somatic cells share many characteristics, including indefinite proliferation and a sustained capacity to differentiate into a wide variety of cell types. However, it remains to be demonstrated whether both cell types rely on similar mechanisms to maintain their pluripotent status and to control their differentiation. Any differences in such mechanisms would suggest that reprogramming of fibroblasts to generate induced pluripotent stem cells (iPSCs) results in novel states of pluripotency. In that event, current methods for expanding and differentiating human embryonic stem cells (ESCs) might not be directly applicable to human iPSCs. However, we show here that human iPSCs rely on activin/nodal signaling to control Nanog expression and thereby maintain pluripotency, thus revealing their mechanistic similarity to human ESCs. We also show that growth factors necessary and sufficient for achieving specification of human ESCs into extraembryonic tissues, neuroectoderm, and mesendoderm also drive differentiation of human iPSCs into the same tissues. Importantly, these experiments were performed in fully chemically defined medium devoid of factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications. Together these data reveal that human iPSCs rely on mechanisms similar to human ESCs to maintain their pluripotency and to control their differentiation, showing that these pluripotent cell types are functionally equivalent.

Ye, L., J. C. Chang, et al. (2009). "Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases." Proc Natl Acad Sci U S A **106**(24): 9826-30.

The innovation of reprogramming somatic cells to induced pluripotent stem cells provides a possible new approach to treat beta-thalassemia and other genetic diseases such as sickle cell anemia. Induced pluripotent stem (iPS) cells can be made from these patients' somatic cells and the mutation in the beta-globin gene corrected by gene targeting, and the cells differentiated into hematopoietic cells to be returned to the patient. In this study, we reprogrammed the skin fibroblasts of a patient with homozygous beta(0) thalassemia into iPS cells, and showed that the iPS cells could be differentiated into hematopoietic cells that synthesized hemoglobin. Prenatal diagnosis and selective abortion have been effective in decreasing the number of beta-thalassemia births in some countries that have instituted carrier screening and genetic counseling. To make use of the cells from the amniotic fluid or chorionic villus sampling that are used for prenatal diagnosis, we also showed that these cells could be reprogrammed into iPS cells. This raises the possibility of providing a new option following prenatal diagnosis of a fetus affected by a severe illness. Currently, the parents would choose either to terminate the pregnancy or continue it and take care of the sick child after birth. The cells for prenatal diagnosis can be converted into iPS cells for treatment in the perinatal periods. Early treatment has the advantage of requiring much fewer cells than adult treatment, and can also prevent organ damage in those diseases in which damage can begin in utero or at an early age.

Yu, J., M. A. Vodyanik, et al. (2007). "Induced pluripotent stem cell lines derived from human somatic cells." Science **318**(5858): 1917-20.

Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undifferentiated state. We show that four factors (OCT4, SOX2, NANOG, and LIN28) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem (ES) cells. These induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such induced pluripotent human cell lines should be useful in the production of new disease models and in drug

development, as well as for applications in transplantation medicine, once technical limitations (for example, mutation through viral integration) are eliminated.

Yuan, T. F. and O. Arias-Carrion (2008). "Locally induced neural stem cells/pluripotent stem cells for in vivo cell replacement therapy." Int Arch Med **1**(1): 17.

Neural stem cells hold the key to innovative new treatments for age-associated degeneration and traumatic injury to the brain and spinal cord. We hypothesized that the in vivo induced pluripotent stem cells or neural stem cells through "forced gene expression" can be used to repair damaged brain areas or treat degenerative diseases. Hopefully, these in vivo patient-specific stem cells can bring a new avenue for cell replacement therapies.

Yuasa, S. and K. Fukuda (2008). "Recent advances in cardiovascular regenerative medicine: the induced pluripotent stem cell era." Expert Rev Cardiovasc Ther **6**(6): 803-10.

Induced pluripotent stem (iPS) cells have recently been established by transfecting mouse and human fibroblasts with the transcription factors Oct3/4, Sox2, Klf4 and c-Myc, known to be expressed at high levels in embryonic stem (ES) cells. These cells have great potential in regenerative medicine as they have the capacity to differentiate into all three germ layer-derived cells and are syngeneic. The differentiation of ES cells into cardiomyocytes mimics the early processes involved in heart development. Recent studies describe the contribution of various growth factors and corresponding inhibitors to heart development during embryogenesis. Bone morphogenetic proteins, Wnt protein and Notch signals play critical roles in heart development in a context- and time-dependent manner. Consistent with ES cells, the exposure of iPS cells to such growth factors is hypothesized to augment differentiation into cardiomyocytes. The combination of iPS cells and appropriate developmental signal information has the potential for providing the foundations for future regenerative medicine.

2. Definition of Stem Cells

The definition of stem cell is “an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell” *(*Stedman's Medical Dictionary, 2002).

3. Characterization of Stem Cell

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

4. Sources of Stem Cells

Aristotle (384-322 BC) deduced that the embryo was derived from mother’s menstrual blood, which was based on the concept that living animals arose from slime or decaying matter. This concept was accepted in western world for over 2000 years, and it controlled western philosophy for over 2000 years either. In 1855, Virchow supposed that all cells in an organism are derived from preexisting cells. Now we know that all the human cells arise from a preexisting stem cell – the fertilized egg, that come from the mating of a man and a woman naturally but now can be produced in the laboratory tube. The counter hypothesis of spontaneous generation was accepted until 1864, when the French scientist Louis Pasteur demonstrated that there would be no microorganisms’ growing after sterilizing and sealing.

The animal body has an unlimited source of stem cells, almost. However, the problem is not in locating these stem cells, but in isolating them from their tissue source.

Five key stem cells have been isolated from human: (1) Blastocysts; (2) Early embryos; (3) Fetal tissue; (4) Mature tissue; (5) Mature cells that can be grown into stem cells.

Up to today, only stem cells taken from adults or children (known generically as "adult stem cells") have been used extensively and effectively in the treatment of degenerative diseases.

5. Embryonic Stem Cell

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are

pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

6. Somatic Stem Cell

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

7. Isolation and Characterisation of Stem Cells

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterisation of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

7.1 Prostatic tissue collection and culture

When using human tissue, formal consent by the donator must be obtained before tissue collection. Tissue sections are obtained under sterile conditions. Each individual tissue section is bisected with half being sent for histological analysis for diagnostic evaluation and the remainder used for tissue culture. After then, tissue sections are chopped and placed in collagenase type I at 200 U/ml in RPMI 1640 medium with 2% v/v FCS overnight on a shaking platform at 37°C. The digest is then broken down further by shaking in 0.1% trypsin in PBS with 1% BSA and 1 mM ethylenediaminetetraacetic acid (EDTA) for 15-20 min. The cell suspension is then washed three times in PBS with 1% BSA and 1 mM EDTA before resuspending in RPMI 10% v/v FCS. Prostate epithelial cells are separated from fibroblasts by differential centrifugation (360 g, 1 min without braking). This process produced a supernatant enriched for fibroblasts and a pellet enriched for epithelia. The epithelial cell suspension is then spun on a metrizamide gradient (1.079 g/ml), and the cells are isolated from the interface (Bhatt, 2003).

7.2 Ber-EP4/α_2/CD45 labelling of cells

Isolated epithelial cells are labeled at ambient temperature with either anti-human integrin α_2 monoclonal antibody or Ber-EP4 antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Samples are then dual labelled with CD45-FITC (1 μg/ml in 1% BSA/PBS) for 30 min (Bhatt, 2003).

7.3 Ber-EP4/α_2 and Hoechst labelling for flow cytometry

Isolated epithelial cells are labelled at ambient temperature with anti-human integrin α_2 monoclonal antibody (8 μg/ml in 1% BSA/PBS) for 30 min before the addition of the secondary antibody, RAMBO (2.6 μg/ml in 1% BSA/PBS) for 30 min. After washing with PBS, the cells are incubated for 20 min in the dark with streptavidin PE-Cy7 (20 μg/ml). Hoechst staining could be performed by using the protocol for HSC as described by Rupesh, et al (Bhatt, 2003). Briefly, epithelial cells are resuspended in Hoechst buffer (Hanks' balanced salts solution, 10% FCS, 1% D-glucose, and 20 mM HEPES) and warmed to 37°C. Hoechst 33342 is then added to give a final concentration of 2 μM and the cells incubated at 37°C for 2 h. Fifteen min before the end of incubation, the cells are labelled with monoclonal anti-human Ber-EP4 directly conjugated to FITC (8 μg/ml). The cells are then washed in ice-cold Hoechst buffer before resuspending in ice-cold Hoechst buffer containing propidium iodide (PI) at 20 ng/ml (Bhatt, 2003).

7.4 Flow cytometry isolation of the SP fraction

Flow cytometry is carried out using a Becton Dickinson FACS Vantage SE flow cytometer. Hoechst 33342 is excited with an argon ion, ultraviolet-enhanced laser at 350 nm, and its fluorescence is measured with a 424/44 BP filter (Hoechst BLUE) and a 675DF20 BP optical filter (Hoechst RED; Omega Optical, Brattleboro VT). A 640 LP dichroic mirror is used to separate the emission wavelengths. PI fluorescence is also measured through the 675DF20 BP (having been excited at 350 nm). A second argon ion laser is used to excite the additional fluorochrome PE-Cy7 at 488 nM. PE-Cy7 is measured using a 787RDF40 (Omega Optical) filter (Bhatt, 2003).

7.5 Cell cycle characterisation of SP fraction

Epithelial cells are isolated and all fractions are resuspended in Hoechst buffer and warmed to 37°C. Hoechst 33342 is then added to give a concentration of 2 μM and incubated at 37°C for 45 min. Pyronin Y (250 ng/μl) is added to each tube, and the samples are incubated for 45 min. Monoclonal anti-human Ber-EP4 FITC (8 μg/ml) is added as appropriate 15 min

before the end. After this, ice-cold Hoechst buffer is added immediately and the samples are washed then resuspended in ice-cold Hoechst buffer. The samples are analysed immediately by flow cytometry. Flow cytometry is performed using a modification of the method described above. Cells under study are selected by positive labelling for Ber-EP4 FITC before being analysed for Hoechst and Pyronin Y staining. These cells are then analysed by plotting the Hoechst profile on the x-axis and Pyronin Y along the y-axis in a linear scale (Bhatt, 2003).

7.6 Cytokeratin phenotype studies

Samples are processed as above, divided into two fractions, and labelled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labelled with Ber-EP4 FITC and integrin α_2 PE-CY7. Flow cytometry is performed as described and analysed on forward (FSC) and side (SSC) scatter (Bhatt, 2003).

8 Application of Stem Cells in Clinical Medicine

There are over four thousand registered diseases specifically linked to genetic abnormalities. Although stem cells are unlikely to provide powerful treatment for these diseases, they are unique in their potential application to these diseases.

Indeed, in many research projects, scientists have demonstrated that stem cells can be used to replenish or rejuvenate damaged cells within the immune system of the human body and that damaged stem cells can repair themselves and their neighbors. For example, in what is regarded as the first documented case of successful gene-therapy "surgery", scientists at the Necker Hospital for Sick Children in Paris of French succeeded in treating two infants diagnosed with Severe Combined Immunodeficiency Disease, a life-threatening degenerative disease caused by defects on the male (X) chromosome. With the identification of stem cell plasticity several years ago, multiple reports raised hopes that tissue repair by stem cell transplantation could be within reach in the near future (Kashofer, 2005). In cardiovascular medicine, the possibility to cure heart failure with newly generated cardiomyocytes has created the interest of many researchers (Condorelli, 2005). Gene clone techniques can be widely used in the stem cell researches and applications (Ma, 2004).

9 Debates on Stem Cell Research

There are a lot of debates on the stem cell research. Stem cell research is a high-tech question and the people involved in this rebates should have certain scientific knowledge on the stem cell. It is OK for the politicians or religionists to show their opinions on any topic they are interested in, but not suitable for them to make decisions (or make laws) that will significantly influence the scientific research as this field the politicians or religionists are not specialized. Such as, it is not suitable for the American President George W. Bush to show the power in the stem cell research. It is scientists' job. When politics and science collide, science should do scientific way, rather political way. Major ethical and scientific debates surround the potential of stem cells to radically alter therapies in health care (Williams, 2005).

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8/9/2011