Stem Cell Description

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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.
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1. Introduction

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. In many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

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.

In 2003, scientists in Edinburgh have identified the gene that gives foetal stem cells their ability to multiply without limit and never grow old (Hawkes, 2003).

The discovery may make it possible to create foetal stem cells from adult cells, and use them to treat diseases. At present the only way to get such cells is to create embryos. This is controversial, especially in the United States where federal research money cannot be used for embryonic research of this kind.

The gene, which the team has named Nanog after the mythical Celtic land where nobody grows old, is a regulator that controls the operation of many other genes. It operates only in embryonic stem cells, which are pluripotent (able to develop into any of the body's specialised cells).

Nanog's role, according to papers published in the journal *Cell* by the team from Edinburgh University and Nara Institute of Science and Technology in Japan, is to maintain stem cells and to make them grow.

Ian Chambers, of the Institute for Stem Cell Research at Edinburgh, said that nanog was a master gene, which "makes stem cells immortal".

Unlike specialised cells, that can only divide a limited number of times before they die, embryonic stem cells can go on dividing for ever. This means that a culture of stem cells can be kept alive for transplantation into patients where they will diversify into necessary cells — brain, muscle, liver or skin, for example.

For this to be possible, scientists need to understand how it is that stem cells can either divide without limit, or choose instead to differentiate into specialised cells. Nanog appears to be the key.

Nanog does`not disappear in adult cells, but it lies dormant. This means that if a way could be found to reactivate it, adult cells could be persuaded to become embryonic cells again.

James Thompson, of the University of Wisconsin, told the *Washington Post*: "As we know more and more about pluripotency, it will probably be possible to reprogramme cells to make stem cells out of any cell in the body. This is an important step in that direction."

The Edinburgh paper is published alongside a study from Shinya Yamanaka, from the Nara Institute. The two groups realised that they had discovered the same gene last year and have since collaborated in completing the research.

The next step is to work out how Nanog is switched on and off. To achieve that it may be necessary to continue working on embryonic stem cells and watching the process as it happens. British scientists have long argued that while work on adult stem cells is important, understanding how they work still requires the use of embryos.

Most of the research so far has been conducted in mice, but humans have an almost identical gene. In one experiment the Edinburgh team inserted the human Nanog gene into embryonic mouse cells, and subjected those cells to conditions that would normally make them turn into specialist cells. The human Nanog gene stopped that process.

The long-term implications of stem cell therapy could be a revolution in medicine. Many diseases are caused by the death of cells vital to the proper functioning of the organs.

Heart failure, for example, is often caused by damage to the muscles caused by a blood clot. Stem cells injected into the heart could recreate the heart muscle.

Type 1 diabetes is caused by the destruction of the pancreatic cells that make insulin. These cells might be reintroduced as stem cells.

Parkinson's disease is caused by a loss of cells. In animal experiments stem cells have been shown to reduce symptoms of the disease.

Some of the most notable recent findings are as follows: (1) the 'stemness' profile may be determined by approximately 250 genes; (2) organ-specific stem-cell growth and differentiation are stimulated during the reparative phase following transient injury; (3) two bone marrow stem-cell types show a remarkable degree of differentiation potential; (4) some organs contain resident marrow-derived stem cells, and their differentiation potential may only be expressed during repair; (5) the metanephric mesenchyme contains pluripotent and self-renewing stem cells; (6) marrow-derived cells invade the kidney and differentiate into mesangial and tubular epithelial cells, and these processes are increased following renal injury; and (7) epithelial-to-mesenchymal transition generates renal fibroblasts (Oliver, 2004).

After the claims of the first cloning of patient-specific stem cells using somatic nuclear transfer as published in Science in 2004 and 2005, former Seoul National University Professor Woo Suk Hwang became a national hero of Korea and an international celebrity. His academic reputation was down after January 11, 2006 when a nine-member investigation panel at Seoul National University reported that his data were intentionally fabricated. To many Koreans who once firmly believed in the future promise of this new therapeutic regimen, this heart-breaking event cast a dark shadow implying that stem cell therapy is hype and its future is uncertain. More importantly, for many Koreans this led to pessimism about the future of Korean stem cell science, as well as life science in general. In spite of these negative effectives from both scientific and non-scientific communities, stem cell research in Korea is bouncing back with better focus and balance as evidenced below. First, the Korean government has been reassuring by vowing to continue to support stem cell research in Korea with a long-term spending plan of $454 million over the next 10 years. This represents an even higher level of funding than before the Hwang scandal. Some Korean scientists even anticipate that the Korean government's support for stem cell research could be furthermore expanded. Second, non-government sectors are also continuing to push their plans to support stem cell science. Third, despite concerns and unfounded worries, Korean biologists are performing exceedingly well and continue to publish their results in prestigious international journals. Given the youth of stem cell science and largely unmet promises of stem cell therapy to treat intractable human diseases, the necessity of intimate and effective international collaborations is applicable to stem cell researchers not only in Korea but worldwide (Kwang-Soo Kim, 2007).

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.

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 labeled 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 labeled 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 labeled 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 analyzed 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 analyzed for Hoechst and Pyronin Y staining. These cells are then analyzed 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 labeled with either cytokeratin 8 or 14 indirectly conjugated to PE-Cy5. Samples are then dual labeled with Ber-EP4 FITC and integrin α_2 PE-CY7. Flow cytometry is performed as described and analyzed 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. Renal Stem Cells

Functional recovery in acute renal failure is well known, and the adult kidney is generally recognized to have the capacity to regenerate and repair. The adult stem cells exist in the kidney, including slow-cycling cells, side population cells, CD133+ cells and rKS56 cells. However, in vivo differentiation of bone marrow-derived cells into renal tubular cells may not occur at all, or is at most a minor component of the repair process. Moreover, it is generally accepted that stem cells and multipotent cells contribute to the regenerative process by producing protective and regenerative factors rather than by directly differentiating to replace damaged cells. Therefore, for clinical regenerative medicine in kidney disease, the focus of stem cell biology will shift from multiple

differentiation of cells or cell-therapy to multiple functions of the cells, such as the production of bone morphologic protein-7 and other regenerative factors (Hishikawa and Fujita, 2006).

Adult stem cells have been characterized in several tissues as a subpopulation of cells able to maintain generate, and replace terminally differentiated cells in response to physiological cell turnover or tissue injury. Little is known regarding the presence of stem cells in the adult kidney but it is documented that under certain conditions, such as the recovery from acute injury, the kidney can regenerate itself by increasing the proliferation of some resident cells. The origin of these cells is largely undefined; they are often considered to derive from resident renal stem or progenitor cells. Whether these immature cells are a subpopulation preserved from the early stage of nephrogenesis is still a matter of investigation and represents an attractive possibility. Moreover, the contribution of bone marrow-derived stem cells to renal cell turnover and regeneration has been suggested. In mice and humans, there is evidence that extrarenal cells of bone marrow origin take part in tubular epithelium regeneration. Injury to a target organ can be sensed by bone marrow stem cells that migrate to the site of damage, undergo differentiation, and promote structural and functional repair. Hematopoietic stem cells are mobilized following ischemia/reperfusion and engrafted the kidney to differentiate into tubular epithelium in the areas of damage. The evidence that mesenchymal stem cells, by virtue of their renoprotective property, restore renal tubular structure and also ameliorate renal function during experimental acute renal failure provides opportunities for therapeutic intervention (Morigi, 2006).

Acute renal failure has 50-80% mortality and treatment options for this life-threatening disease are limited. Stem cells offer an exciting potential for kidney regeneration. This review discusses pathogenesis of acute renal failure resulting from ischemia-reperfusion injury and the role of stem cells in reversing or mitigating this disorder. Specifically, the issues of differentiation of kidney cells from embryonic stem cells and bone marrow stem cells, and whether adult kidney stem/progenitor cells exist in the postnatal kidney are discussed. Evidence to support the conclusion that intra-renal cells, including surviving tubular epithelial cells and potential renal stem/progenitor cells, are the main source for renal regeneration is provided. Future research in selecting the type(s) of stem cells and optimizing the dose, frequency and route of administration of the cells will be fundamental in successful cell replacement therapy in acute renal failure. (Lin, 2006).

Repair of inflammatory and/or ischemic renal injury involves endothelial, mesangial and epithelial regeneration. These structures may be rebuilt by resident progenitor cells and bone marrow-derived stem cells. Resident progenitor cells in adult kidney have not yet been conclusively identified. They are likely to be slowly cycling cells located mainly in the outer medulla and renal papilla. In glomerulonephritis with mesangiolysis, mesangial regeneration involves progenitor cells migrating from the juxtaglomerular apparatus and also bone marrow-derived cells. In acute ischemic renal failure, epithelial regeneration of proximal tubules results from the migration, proliferation and differentiation of resident progenitor cells; bone marrow-derived cells may play an accessory role. Molecular mechanisms underlying these repair processes could be targets for new therapeutic approaches (Baud, 2005).

Ischemia causes kidney tubular cell damage and abnormal renal function. The kidney is capable of morphological restoration of tubules and recovery of function. Recently, it has been suggested that cells repopulating the ischemically injured tubule derive from bone marrow stem cells. In GFP chimeras, some interstitial cells but not tubular cells express GFP after ischemic injury. More than 99% of those GFP interstitial cells are leukocytes. In female mice with male bone marrow, occasional tubular cells (0.06%) appeared to be positive for the Y chromosome, but deconvolution microscopy revealed these to be artifactual. In beta-gal chimeras, some tubular cells also appear to express beta-gal as assessed by X-gal staining, but following suppression of endogenous (mammalian) beta-gal, no tubular cells could be found that stain with X-gal after ischemic injury. Whereas there is an absence of bone marrow-derived tubular cells, many tubular cells expressed proliferating cell nuclear antigen, which is reflective of a high proliferative rate of endogenous surviving tubular cells. Upon i.v. injection of bone marrow mesenchymal stromal cells, postischemic functional renal impairment was reduced, but there was no evidence of differentiation of these cells into tubular cells of the kidney. Bone marrow-derived cells do not make a significant contribution to the restoration of epithelial integrity after an ischemic insult. It is likely that intrinsic tubular cell proliferation accounts for functionally significant replenishment of the tubular epithelium after ischemia (Duffield, 2005).

Acute renal failure (ARF) is a common disease with high morbidity and mortality. Recovery from ARF is dependent on the replacement of necrotic tubular cells with functional tubular epithelium. Recent advancement in developmental biology led to the discovery of immature mesenchymal stem cells (MSCs) in bone marrow and several established

organs and to the definition of their potential in the recovery from tissue injury (Herrera, 2004).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is an area of intense investigation. Many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. Extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but and vast majority of tubular cells derive from an intrarenal source. Interstitial cells may represent another extratubular stem cell niche. It is not clear whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells (Humphreys, 2006).

The kidney has a dramatic capacity to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is currently an area of intense investigation. Studies from our laboratory and others have supported a model whereby many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated. A third hypothesis is that extratubular cells contribute to repair of damaged epithelium. Bone marrow-derived stem cells have been proposed to contribute to this process but our work and work of others indicates that the vast majority of tubular cells derive from an intrarenal source. Recent evidence suggests that interstitial cells may represent another extratubular stem cell niche. The fundamental unanswered questions in this field include whether renal stem cells exist in the adult, and if they do where are they located (interstitium, tubule, cortex, medulla) and what markers can be relied upon for the isolation and purification of these putative renal stem cells. In this review we focus on our current understanding of the potential role of renal and extrarenal stem cells in repair of the adult kidney and highlight some of the controversies in this field (Humphreys, 2006).

The capacity of the kidney to regenerate functional tubules following episodes of acute injury is an important determinant of patient morbidity and mortality in the hospital setting. After severe injury or repeated episodes of injury, kidney recovery can be significantly impaired or even fail completely. Although significant advances have been made in the clinical management of such cases, there is no specific therapy that can improve the rate or effectiveness of the repair process. Recent studies have indicated that adult stem cells, either in the kidney itself or derived from the bone marrow, could participate in this repair process and might therefore be utilized clinically to treat acute renal failure. This review will focus on our current understanding of these stem cells, the controversies surrounding their in vivo capacity to repopulate the renal tubule, and further investigations that will be required before stem cell therapy can be considered for use in the clinical setting (Cantley, 2005).

While it remains unknown whether there is a stem cell in the adult kidney, characterization of the cell populations involved in renal repair and misrepair is allowing a new understanding of the mechanisms that are responsible for renal homeostasis (Oliver, 2004).

Ischemia-reperfusion injury (I/R injury) is a common cause of acute renal failure. Recovery from I/R injury requires renal tubular regeneration. Hematopoietic stem cells (HSC) have been shown to be capable of differentiating into hepatocytes, cardiac myocytes, gastrointestinal epithelial cells, and vascular endothelial cells during tissue repair. The current study tested the hypothesis that murine HSC can contribute to the regeneration of renal tubular epithelial cells after I/R injury (Lin, 2003).

The kidney has the ability to restore the structural and functional integrity of the proximal tubule, which undergoes extensive epithelial cell death after prolonged exposure to ischemia. Small numbers of peritubular endothelial cells to be derived from bone marrow cells that may serve in the repair process (Duffield, 2005).

Renal progenitor tubular cells [label-retaining cells (LRC)] are identified in normal kidneys by in vivo bromodeoxyuridine (BrdU) labelling. In normal and contralateral kidneys, LRC are observed scattering among tubular epithelial cells. After unilateral ureteral obstruction (UUO), the number of the LRC significantly increase, and most of them are positive for proliferating cell nuclear antigen (PCNA). In contrast, PCNA+ cells lacking BrdU label are

rarely observed. LRC are not only in tubules but also in the interstitium after UUO. Laminin staining showed that a number of the LRC are adjacent to the destroyed tubular basement membrane. Some tubules, including LRC, lose the expression of E-cadherin after UUO. A large number of cell populations expressed vimentin, heat shock protein 47, or alpha-smooth muscle actin in the UUO kidneys, and each population contained LRC. None of the LRC is positive for these fibroblastic markers in contralateral kidneys. When renal tubules from BrdU-treated rats are cultured in the gel, some cells protruded from the periphery of the tubules and migrated into the gel. Most of these cells are BrdU+. Neither the total content of BrdU in the kidneys nor the number of LRC in bone marrow significantly is changed after UUO. LRC is a cell population that proliferates, migrates, and transdifferentiates into fibroblast-like cells during renal fibrosis (Yamashita, 2005).

10. Human embryonic stem cell (hESC)

Scientific progress in human embryonic stem cell (hESC) research and increased funding make it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Several issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. To minimize the risk of hESC transplantation, donors of materials used to derive hESC lines will need to be recontacted to update their medical history and screening. Because of privacy concerns, such recontact needs to be discussed and agreed to at the time of donation, before new hESC lines are derived. Informed consent for Phase I clinical trials of hESC transplantation also raises ethical concerns. In previous Phase I trials of highly innovative interventions, allegations that trial participants had not really understood the risk and benefits caused delays in subsequent trials. Thus researchers should consider what information needs to be discussed during the consent process for hESC clinical trials and how to verify that participants have a realistic understanding of the study. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies.

Increased funding and continued scientific progress have opened a new era in the ethics of human embryonic stem cell (hESC) research. These developments will reframe the ethical debate, which to date has focused on the moral status of the embryo and the acceptability of using embryos for research purposes. Although such philosophical questions have not been resolved, the issue is no longer *if* hESC research should proceed, but rather *how* it should proceed. The rapid pace of research makes it imperative to look ahead to the ethical issues generated by the expected use of hESC for transplantation. Some of these issues should be addressed now, even though Phase I clinical trials of hESC transplantation are still in the future. Crucial issues concerning safety of hESC transplantation and the need to recontact donors of materials used to derive new hESC lines are best resolved when these materials are donated. In addition, informed consent for hESC transplantation Phase I clinical trials will present particular challenges, which will require modification of the usual consent process for clinical trials. Failure to address these ethical issues may delay or preclude clinical trials that will test whether interventions based on hESC are safe and effective.

To the extent that disagreements over these questions might delay clinical trials, these issues also need to be addressed in advance. In conclusion, for hESC to fulfill its promise as therapy, a chain of activities needs to be established, including funding, basic science, and clinical trials. Recent events have increased funding and shown that the science may proceed rapidly. But a chain is only as strong as its weakest link. Attention to ethical issues raised by clinical trials is an essential part of the chain. The issues we have discussed are based on lessons from previous experience with related but not identical fields; invariably other unforeseeable issues will arise. Lack of attention to the special ethical concerns raised by clinical trials of hESC transplantation and their implications for the derivation of new hESC lines may undermine or delay progress towards stem cell therapies (Bernard, 2005).

11. Selected Protocols for Stem Cell Researches

11.1 INFT2 Protocol

Hematologic malignancies (blood cell cancers) in very young children are hard to treat with standard doses of chemotherapy (anti-cancer drugs). Stem cell transplantation (infusion of healthy blood forming cells) has been used but has not always been successful. The best donor of stem cells is a sibling (brother or sister) who is a match (the sibling's cells match the subject's immune type, or HLA type). But few very young children with leukemia have a matched sibling donor. This research study is for those children who do not have a matched sibling donor. In this study, a parent will be the stem cell donor. Using a parent donor (a parent donor is a partial match for the subject's HLA type) increases the risk of graft-versus-host disease (GVHD). GVHD occurs when the donor cells (the graft) recognize that the body tissues of the child (the host) are different. Because severe GVHD can be life-threatening, the

parent's stem cells will be filtered using a machine called the CliniMACS system, which removes the cells that cause GVHD. This system has not been approved by the Food and Drug Administration (FDA) and is considered experimental. In addition to the stem cell transplant, parent donor natural killer (NK) cells will be given. NK cells are special cells in the immune system (the body organs and cells that defend the body against infection and disease) that target cancer cells. NK cells may help donor cells to grow and may reduce the chance of GVHD. In this experimental treatment, chemotherapy will be used in addition to the stem cell and NK cell transplants. It is unknown if these treatments will work better than the treatments now being used to treat very young children with hematologic malignancies. **(**Leung, 2007**)**

11.2 OPBMT2 Protocol

Malignant osteopetrosis **is a** genetic disease in which cells in the bone tissue (osteoclasts) do not function properly. These cells are unable to perform their biological job of breaking down old bone tissue as new bone tissue is being made. This causes the bone tissue to build up, producing thick bones that do not work properly and causing the child to lose his/her bone marrow space, where red cells, platelets, and white cells are made.

This study is designed to use a haploidentical parental donor in the event that a matched sibling donor is unavailable. Using a parental donor would enable transplantation earlier in the disease process than waiting for a matched unrelated donor. This might reduce the chance of the disease getting worse before the transplant is done. With a parental donor, the risk of graft rejection (the patient's body will not accept and allow donated cells to grow) may be greater than the risk of rejection using a matched sibling donor.

The purpose of this study is to learn more about the cause and treatment of malignant osteopetrosis. It is designed to determine if children with malignant osteopetrosis can properly accept a parental donor transplant and to study the genetic (characteristics carried by genes) factors which cause the disease (Kasow, 2007).

A stem cell transplant has been shown to help, and possibly cure, patients with sickle cell disease. Stem cells taken from a brother or sister may provide bone marrow that is a perfect match (same tissue type) for the recipient. Unfortunately, only about 10-20% of sickle cell patients have a matched sibling donor. Stem cells from partially matched (partial tissue match) family members have been tried with a few children with sickle cell disease. The risk and benefits of these types of transplants are not as well known as transplants using a matched donor. When children with sickle cell disease have no matched brother or sister donor, allogeneic transplants are a possible treatment available for these patients (Paul Woodard, 2007)

11.3 SCT521 (COG # ASCT0521) Protocol

Idiopathic pneumonia syndrome is a complication that may occur in children who have had a stem cell transplant. Often patients with pneumonia have a cough and chest pain, are short of breath, or require oxygen to help them breathe. In some transplant patients, pneumonia is caused by a bacteria or virus. However, with idiopathic pneumonia syndrome, pneumonia occurs in the absence of infection. Despite corticosteroids and supportive care, this condition may be fatal. This research study will use a drug named etanercept. The drug has been approved by the Food and Drug Administration (FDA) for the treatment of certain joint or skin conditions in children over 4 years of age. Etanercept works by blocking the effects of a protein known as Tumor Necrosis Factor (TNF). TNF has been found in lung fluid from patients with idiopathic pneumonia syndrome. TNF may be involved in the development of lung injury in idiopathic pneumonia syndrome. An earlier study has determined the largest amount of etanercept that can be given without causing bad effects. A small research study has been done with adults and children with idiopathic pneumonia syndrome. Etanercept was found to be safe, and several patients had improvement in their breathing (Madden, 2007).

12. Stem Cell Facts

Mouse embryonic stem cells were first discovered in 1981. Since then, they have been an invaluable tool of modern biology and medical research. They have provided models to study diseases, they have brought about the discovery of many genes associated with diseases and they have been used to cure certain human disorders in animal models. After 20 years of exciting research, the mouse embryonic stem cell has helped to establish the value of these cells in *regenerative medicine*, which is the creation of cells or organs to replace tissues lost to disease or injury. The discovery of *human* embryonic stem cells in 1998 triggered important ethical controversy and debate, yet scientists are convinced that they hold enormous potential for clinical applications. Many diseases plaguing the modern world may be improved, or even cured, with therapies using human stem cells. Whether human embryonic stem cells or adult stem cells are used in future therapies will depend on the type of disease or injury. There are specific advantages for each stem cell type. Thanks to the ease of growing them in the laboratory,

human embryonic stem cells may one day become the source of artificial organs. Or scientists might one day be able to mobilize one's own adult stem cells to repair tissue damage caused by trauma, disease, and even aging. To reach such goals, both human embryonic and adult stem cells will have to be extensively studied. The complementary information acquired from studying both stem cell types is the key to unlocking their full potential.

13. Techniques of Human Embryonic Stem Cells

Immune rejection of transplanted stem cells could be avoided if the therapeutic cells derived from the human embryonic stem cells express a patient's own genes and proteins. A method to generate these types of stem cells is by *nuclear transfer*. The nuclear transfer technique is similar to the process of generating a blastocyst from the fertilization of an egg by a sperm cell; however, in this process the DNA in an egg is exchanged for the DNA from a cell of the patient. The egg is then coaxed to divide in a culture dish into a blastocyst. The human embryonic stem cells derived from this blastocyst will be an identical genetic match to the patient and can provide "customized" replacement cells for any disorder.

14. 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).

15. Stem Cell Glossary

(1) **Adult stem cells:** Stem cells found in different tissues of the developed, adult organism that remain in an undifferentiated, or unspecialized, state. These stem cells can give rise to specialized cell types of the tissue from which they came, i.e., a heart stem cell can give rise to a functional heart muscle cell, but it is still unclear whether they can give rise to all different cell types of the body.

(2) **Blastocyst:** A very early embryo consisting of approximately 150 cells. It contains the inner cell mass, from which embryonic stem cells are derived, and an outer layer of cells called the trophoblast that forms the placenta.

(3) **Cell line:** Cells that can be maintained and grown in culture and display an immortal or indefinite life span.

(4) **Differentiation:** The process of development with an increase in the level of organization or complexity of a cell or tissue, accompanied with a more specialized function.

(5) **Embryo:** The product of a fertilized egg, from the zygote until the fetal stage.

(6) **Embryonic stem cell:** Also called ES cells, embryonic stem cells are cells derived from the inner cell mass of developing blastocysts. An ES cell is self-renewing (can replicate itself), pluripotent (can form all cell types found in the body) and theoretically is immortal.

(7) **In vitro fertilization:** A procedure where an egg cell and sperm cells are brought together in a dish so that a sperm cell can fertilize the egg. The resulting fertilized egg, called a zygote, will start dividing and after a several divisions, forms the embryo that can be implanted into the womb of a woman and give rise to pregnancy.

(8) **Mesenchymal stem cell:** Also known as bone marrow stromal cells, mesenchymal stem cells are rare cells, mainly found in the bone marrow, which can give rise to a large number of tissue types such as bone, cartilage, fat tissue, and connective tissue.

(9) **Multipotent stem cells:** Stem cells whose progeny are of multiple differentiated cell types, but all within a particular tissue, organ, or physiological system. For example, blood-forming (hematopoietic) stem cells are single multipotent cells that can produce all cell types that are normal components of the blood.

(10) **Nucleus:** A part of the cell, situated more or less in the middle of the cell, which is surrounded by a specialized membrane and contains the DNA of the cell, which is the genetic, inherited material of cells.

(11) **Plasticity:** A phenomenon used to describe a cell that is capable of becoming a specialized cell type of different tissue.

(12) **Pluripotent stem cells:** Stem cells that can become all the cell types that are found in an implanted embryo, fetus, or developed organism.

(13) **Progenitor cell:** An early descendant of a stem cell that can differentiate, but cannot renew itself. By contrast, a stem cell can renew itself (make more stem cells by cell division) or differentiate (divide and with each cell division evolve more and more into different types of cells).

Regenerative medicine: Medical interventions that aim to repair damaged organs, most often by using stem cells to replace cells and tissues damaged by aging and by disease.

(15) **Reproductive cloning:** Somatic cell nuclear transfer used for the production of a fetus and delivery of a live offspring that is genetically identical the donor of the somatic cell DNA.

(16) **Somatic cells:** All the cells within the developing or developed organism with the exception of germline (egg and sperm) cells.

(17) **Stem cells:** Cells that have both the capacity to self-renew (make more stem cells by cell division) and to differentiate into mature, specialized cells.

(18) **Therapeutic cloning:** Somatic cell nuclear transfer for the isolation of embryonic stem cells. The embryonic stem cells are derived from the blastocyst (before it becomes a fetus) and can be instructed to form particular cell types (e.g. heart muscle) to be implanted into damaged tissue (e.g. heart) to restore its function. If the stem cells are placed back into the individual who gave the DNA for the somatic cell nuclear transfer, the embryonic stem cells and their derivatives are genetically identical and thus immunocompatible (they will not be rejected).

(19) **Transdifferentiation:** The ability of a particular cell of one tissue, organ or system, including stem or progenitor cells, to differentiate into a cell type characteristic of another tissue, organ, or system; e.g., blood stem cells changing to liver cells.

(20) **Transplantation biology:** The science that studies the transplantation of organs and cells. Transplantation biologists investigate scientific questions to understand why foreign tissues and organs are rejected, the way transplanted organs function in the recipient, how this function can be maintained or improved, and how the organ to be transplanted should be handled to obtain optimal results.

(21) **Umbilical cord stem cells:** Hematopoietic stem cells are present in the blood of the umbilical cord during and shortly after delivery. These stem cells are in the blood at the time of delivery, because they move from the liver, where blood-formation takes place during fetal life, to the bone marrow, where blood is made after birth. Umbilical cord stem cells are similar to stem cells that reside in bone marrow, and can be used for the treatment of leukemia, and other diseases of the blood. Efforts are now being undertaken to collect these cells and store them in freezers for later use.

(22) Zygote: The cell that results from the union of sperm and egg during fertilization. Cell division begins after the zygote forms.

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