Eternal Life and Stem Cell

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Abstract: As the nature will, to live eternally is an extracting dream in all the human history. Stem cell is the original of life and all cells come from stem cells. Germline stem cell (GSC) is the cell in the earliest of the cell stage. It is possible to inject the GSC into adult human body to get the eternal life. This article is to try to describe the stem cell and to explore the possibility of the eternal life with the stem cell strategy [Nature and Science. 2007;5(1):81-96].

Key words: DNA; eternal; life; stem cell; universe

1. Introduction

For a person, the most attracting will is to live longer, and the extreme dream is to live eternally. The number two important will for a person is to live happily. Humankind has a history longer than millions of years, and people never stopped the efforts to find a way to live eternally, no matter he/she was a beggar or an emper. There were many ways people considered as the way to keep life longer, even eternal, but people never got the eternal goal.

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into all different types of cells in life bodies, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003), and also gives a hope to let us get the eternal life. 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 situition 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.

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).

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).

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.

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.

2. Germline Stem Cell (GSC)

Testis is the organ for animal to reproduce the generation. As the new generation always has a young feature for the life, no matter how old the parents are, it is possible for the mature life to use the stem cell coming out from the reproduce organ (germline stem cells) to replace the old cells, to keep the mature body always young. The recently developed testis cell transplantation method provides a powerful approach to studying the biology of the male germline stem cell and its microenvironment, the stem cell niche.

In the insect *Drosophila* germline stem cells of the testis, one centrosome remains anchored to the region of the cortex at the interface between germ cells and somatic hub cells, while the other centrosome migrates to the opposite side to establish mitotic spindle orientation. This is important for the germline cells of the testis of *Drosophila.*

Human male germline stem cells, called spermatogonial stem cells (SSCs) in postnatal mammals, are the foundation of spermatogenesis (the process for spermatozoa production) and, together with oocytes from females, are essential for species continuity. SSCs and eggs are the original of the life individual and these kinds of cells are always keep young, no matter how old the parents are. SSCs reside on the basement membrane of the seminiferous tubule in the testis and are almost completely surrounded by somatic Sertoli cells, which form a microenvironment or niche. Within the niche, growth factors and extracellular signals regulate the fate decisions of SSCs either to self-renew or to form daughter cells that will begin the complex differentiation process of spermatogenesis.

The first step in spermatogenesis is the fate decision of an SSC to produce daughter cells committed to differentiation. The availability of a functional transplantation assay and a culture system that allows long-term replication of SSCs made it possible to examine intracellular signals that influence self-renewal and differentiation invitro in a rigorous manner that is not available for most adult stem cells. Stem cell recovery and cryopreservation may be applicable to all mammalian species and could be used to preserve the male germ line of valuable livestock animals, companion animals, and endangered species.

There are three particularly important areas include should be mentioned: (1) Further definition of factors and signals that support self-renewal of SSCs, relative to those that initiate differentiation in order to provide a better understanding of this fate decision; (2) Extension of the serum-free culture system to other species, including domestic animals, endangered species, and humans to confirm that self-renewal signals are conserved among mammals and for relevant applications; (3) Development of methods to allow in vitro differentiation of stem cells to provide mature spermatozoa, which would be enormously valuable in understanding the complex process of spermatogenesis and would have great practical use.

Stem cells are unique cell populations that are able to undergo both self-renewal and differentiation and are found in the embryo, as well as in the adult animal. In the early mammalian embryo, pluripotent embryonic stem cells are derived from the blastocyst stage and have the ability to form any fully differentiated cell of the body. As the embryo develops, stem cells become restricted in their ability to form different lineages (multipotent stem cells). Multipotent stem cells are also found in a wide variety of adult tissues such as bone marrow and brain. However, in the adult animal, the ability of certain stem cells to differentiate can be restricted to only one cell lineage (unipotent stem cells). Examples of mammalian unipotent stem cells include the stem cells residing in the gut epithelium, the skin, and the seminiferous epithelium of the testis.

In the mammalian testis, the germ line stem cells are a small subpopulation of type A spermatogonia that proliferate and ultimately differentiate into sperm under the control of both endocrine and paracrine factors. The ability to isolate, culture, and manipulate the germ line stem cell in vitro would allow us to unravel the molecular mechanisms that drive the first steps of spermatogenesis and to characterize the signaling pathways that induce spermatogonial differentiation versus self-renewal. It is important to know the biochemical and biophysical reasons for the ways how the germline stem cells keep young.

3. 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.

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. Embryonic stem (ES) cells can be cultured in conditions that either maintain pluripotency or allow differentiation to the three embryonic germ layers. Heparan sulfate (HS).

4. 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.

5. Isolation and Characterization 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 characterization of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

5.1 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).

5.2 Ber-EP4/α_2/CD45 labeling 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).

5.3 Ber-EP4/α_2 and Hoechst labeling 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).

5.4 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).

5.5 Cell cycle characterization 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 labeling 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).

5.6 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).

6. 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).

7. 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) labeling. 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).

8. The current scientific, ethical, and policy context of human embryonic stem cell research

New human embryonic stem cell lines are needed if human embryonic stem cells or their products are to be used for transplantation into humans. The twenty or so human embryonic stem cell lines approved for federally funded studies in 2001 by President Bush were derived using nonhuman feeder cells and serum and express the nonhuman antigen Neu5Gc. Thus, they would probably be immunologically rejected by the recipients unless this problem was remedied. Derivation of new human embryonic stem cell lines will be stimulated by the $3 billion in funding for stem cell research authorized by California voters in 2004. This measure will give priority to funding research that cannot be funded by NIH, which is currently the case for derivation of new human embryonic stem cell lines. Other states and private funders have followed suit in providing nonfederal support for human embryonic stem cell research. Outside of the U.S., human embryonic stem cell research is advancing vigorously. In May 2005, researchers from South Korea reported the derivation of 11 human embryonic stem cell lines using somatic cell nuclear transfer, demonstrating that technical obstacles to developing such stem cell lines can be overcome more readily than expected. In turn, such findings will stimulate further research.

Current ethical and policy guidelines for human embryonic stem cell research focus on the derivation of new human embryonic stem cell lines. In May 2005, a National Academy of Sciences (NAS) panel called for voluntary adoption of ethical guidelines in human embryonic stem cell research. Their recommendations included institutional oversight of human embryonic stem cell research

protocols through Embryonic Stem Cell Research Oversight Committees (ESCROs), informed consent from donors of materials for new human embryonic stem cell lines, restrictions on payment to gamete donors, and guidelines for banking stem cells and documentation. The twenty-three NRC recommendations have been endorsed by academic and scientific organizations and adopted as interim regulations for research funded by the state of California. That same month, the FDA issued regulations on screening and testing donors of human cells, tissues, and cellular and tissue-based products (HCT/P). While valuable, these initial efforts do not address crucial ethical issues in clinical trials of human embryonic stem cell transplantation, which have important upstream implications for how human embryonic stem cell lines should be derived, as well as for the conduct of the trials themselves. Our analysis begins with the need both to protect participants in Phase I trials of human embryonic stem cell transplantation and to respect the confidentiality of donors of materials used for derivation of human embryonic stem cell lines. These ethical responsibilities need to be addressed during the initial process of donating materials for new human embryonic stem cell lines. Next we consider challenges confronting informed consent for Phase I trials of human embryonic stem cell transplantation. We present specific recommendations for resolving these ethical issues.

9. Balancing the need to protect participants in Phase I clinical trials against the need to respect donors

The goal of Phase I clinical trials is to assess the safety and feasibility of the investigational intervention and to determine dosages for subsequent clinical trials. Direct therapeutic benefit, although hoped for, is unlikely in early trials, particularly if the first participants receive low doses. The guiding ethical principle of Phase I studies should be “Do no harm.” This ethical responsibility to protect the subjects in Phase I trials has important implications for the derivation of human embryonic stem cell lines. A major safety concern is transmission of infectious agents or serious genetic conditions through transplanted human embryonic stem cell cells or products. The public will expect strong protections against diseases transmitted through human embryonic stem cell transplantation, just as it demands that blood transfusions and solid organ transplants be tested for very rare but serious communicable diseases.

A broader perspective on protecting recipients of transplanted human embryonic stem cell materials is needed because of several clinical features of human embryonic stem cell transplantation. First, there is likely to be a considerable time period between donation of biological materials used to derive human embryonic stem cell lines and clinical trials involving transplantation of human embryonic stem cells or products from them. Polymorphisms and biomarkers associated with risk for specific diseases are being defined at a rapid pace. Second, in human embryonic stem cell transplantation, serious genetic conditions might also be transmitted, some of which may not have been apparent at the time the materials were donated. For instance, after donating, donors may develop cancer or a strong family history of cancer. Third, immunosuppressive drugs, which may be essential after cell transplantation to reduce rejection, will increase the risk of communicable diseases and cancer in recipients. Fourth, if human embryonic stem cell transplantation proves clinically effective, many patients may receive transplantation from a single human embryonic stem cell line over time. Hence many recipients may be at risk for diseases transmitted from donors. In order to safeguard recipients of human embryonic stem cell transplantation, researchers need to recontact persons whose gametes were used to derive the human embryonic stem cell lines at the time of clinical human embryonic stem cell transplantation trials to update information and perhaps do additional testing. Furthermore, if human

embryonic stem cell transplantation becomes a proven clinical treatment, periodic updating of the clinical status of donors would be prudent.

How can screening and testing of donors of materials for human embryonic stem cell lines be updated in an ethically acceptable manner? The responsibility to protect human embryonic stem cell transplant recipients from harm must be balanced against a responsibility to respect donors and protect their confidentiality. To resolve these countervailing mandates, researchers will need to obtain permission to recontact donors if human embryonic stem cell cells or materials derived from their gametes or embryos will be used for transplantation. Researchers need to tell donors about the kinds of information or testing that might be requested later and the reasons the information is needed. Such permission for recontact needs to be obtained when materials are donated for research. Without this permission, it would be a serious invasion of privacy to later recontact the donors. Also, donors who had not agreed to be recontacted might object strongly to a subsequent contact, refuse to provide information about their interim medical history, or undergo additional testing. Previous reports on the consent process for donating gametes and embryos for human embryonic stem cell research have not discussed the issue of recontact in depth. Obtaining permission to recontact will undoubtedly complicate the consent process for donating embryos for human embryonic stem cell research. However, permission for recontact will likely minimize the disqualification of human embryonic stem cell lines late in the development process for use in transplantation studies because of inadequate follow-up with donors. Recontacting donors presents logistical challenges because donors may move and contact may be lost. It would be desirable to ask donors to provide contact information for a relative or friends who will know their new address should they move. Confidentiality must be carefully protected because breaches might subject donors to unwanted publicity or even harassment. Concerns that their identities will not be kept confidential may deter some individuals from agreeing to be recontacted. Because of the intense public interest in and contentiousness over human embryonic stem cell research, it would be prudent for researchers and research institutions to develop stringent mechanisms, extending beyond those employed in routine clinical care, in order to assure donors that their identity and contact information remain protected.

10. Human embryonic stem cell transplantation in Phase I clinical trials

Current procedures for obtaining informed consent are likely to be inadequate to address particular issues faced by recipients of human embryonic stem cell transplantation in Phase I clinical trials. Because the matter is complex and any changes in policy will need careful consideration, discussions of the consent process need to begin now. Problems with informed consent commonly occur in clinical trials. Participants in cancer clinical trials commonly expect that they will benefit personally from the trial, even though the primary purpose of Phase I trials is to test safety rather than efficacy. This tendency to view clinical research as providing a personal benefit has been termed the “therapeutic misconception”. Analyses of consent forms suggest that such misunderstandings in cancer clinical trials do not reflect information in the consent forms. Indeed, cancer patients seeking therapeutic benefit may decide to enroll in a clinical trial before they meet the research staff, before they learn about the risks and benefits of the study or read a consent form.

Several measures may reduce the therapeutic misconception in recipients of human embryonic stem cell transplantation in Phase I clinical trials. First, researchers should frame their discussions with participants in the context of publicity about the potential for human embryonic stem cell to treat serious diseases. Second, investigators in human embryonic stem cell clinical trials must discuss a

broader range of information with potential participants than in other clinical trials. Informed consent requires researchers to discuss with potential participants information that is pertinent to their decision to volunteer for the clinical trial. Third, and most importantly, researchers should verify that participants have a realistic understanding of the study. The crucial ethical issue about informed consent is not what researchers disclose in consent forms or discussions, but rather what the participants in clinical trials understand. Lack of attention to the special ethical concerns raised by clinical trials of human embryonic stem cell transplantation and their implications for the derivation of new human embryonic stem cell lines may undermine or delay progress towards stem cell therapies (Bernard, 2005).

11 Why Are Embryonic Stem Cells So Valuable?

While grown in a dish, human embryonic stem cells can maintain their *"stem-cellness"* and provide an unlimited supply of more stem cells, as well as specialized cells that can be used for experiments and for the development of therapies. Apart from their potential to treat or cure diseases, human embryonic stem cells also provide a model to study very early human development and some of the disorders that lead to birth defects and childhood cancers. Many of these disorders develop in early pregnancy and are impossible to study in humans. Also, human embryonic stem cells also can be used to examine the genes that are turned "on" or "off" as stem cells generate more specialized cell types, permitting a unique understanding of the genetics of human development. The specialized cells derived from human embryonic stem cells also can be used to study the effectiveness of potential new drugs to treat diseases. This provides a human cellular model and can reduce animal experimentation and drug development costs. Additionally, embryonic stem cells can be derived from human blastocysts with specific genetic abnormalities. These types of blastocysts are identified through genetic diagnosis during IVF treatment, to screen out genetically abnormal blastocysts, and are usually discarded. The stem cells from them can provide a unique resource to understand genetic diseases and to develop cures. Human embryonic stem cells also could be used to understand the origin or causes of various diseases such as Alzheimer's disease or Parkinson's disease, which are currently unknown. Stem cells derived through *nuclear transfer* (more info below) from patients with such afflictions would provide special tools to study these diseases and possibly develop drugs for treatments.

12. Embryonic Stem Cells in the Clinic

Embryonic stem cells have not yet been used in treating humans. But numerous animal studies have shown that many of the specialized cells derived from them can indeed integrate into damaged tissues and function properly. Thus, diseases such as myocardial infarction, severe immune deficiency, diabetes, Parkinson's disease, spinal cord injury, and demyelination have been successfully treated in animal models. But the pathway from animal models to the clinic is still complex and burdened with obstacles to be overcome. First, not all specialized cells derived from human embryonic stem cells have been shown to integrate into animal tissue and function properly. This can be due to the poor quality of the specialized cells derived in culture, or to a lack of adequate communication between the human cells and the animal environment in which they are placed. Then there is the problem of scaling up to yield enough of the specialized cells to treat a human, since this requires many more cells than to treat a tiny mouse. Such cells will have to be produced under specific conditions to ensure safety for use in patients. Most human embryonic stem cells are still grown on a layer of mouse feeder cells, a potential source of contamination. Last, there's the problem of immune rejection by the patient. While the drugs

used in the organ transplantation field to suppress immune rejection have been improved over the years, rejection is still a major problem.

13. 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).

14. Eternal Life

The production of functional male gametes is dependent on the continuous activity of germline stem cells. The availability of a transplantation assay system to unequivocally identify male germline stem cells has allowed their in vitro culture, cryopreservation, and genetic modification. Moreover, the system has enabled the identification of conditions and factors involved in stem cell self-renewal, the foundation of spermatogenesis, and the production of spermatozoa. The increased knowledge about these cells is also of great potential practical value, for example, for the possible cryopreservation of stem cells from boys undergoing treatment for cancer to safeguard their germ line

According to Greek mythology, the hapless mortal Tithonus mistakenly asked the goddess Eos to confer eternal life rather than eternal youth, and he thus found himself condemned to immortal decrepitude. A new report suggests that if Tithonus had cut a side deal with Dionysus, the god of wine, he might have fared much better.

The study knits together threads of recent molecular research on aging, the venerable antiaging strategy of calorie restriction, and, surprisingly, the health benefits of moderate tippling. David Sinclair of Harvard Medical School in Boston and colleagues identify several naturally occurring small molecules that extend the life of yeast cells by approximately 70% and offer some protection to cultured human cells exposed to radiation. The molecules activate genes known to extend life span in laboratory animals. They belong to a family of chemicals known as polyphenols, some of which are prominent components of grapes, red wine, olive oil, and other foods.

The work by Sinclair and collaborators at the biotech firm BIOMOL Research Laboratories in Plymouth Meeting, Pennsylvania, including Konrad Howitz, is the latest in an increasingly hot field exploring the molecular biology of calorie restriction, a phenomenon first demonstrated in the 1930s. Laboratory rats fed a limited diet live about 40% longer than normal and are resistant to many chronic illnesses typical of aging. The observations have been replicated in yeast, fruit flies, nematodes, fish, spiders, and mice, with hints from ongoing experiments that they hold true for primates. These findings have fueled interest in understanding how calorie restriction works--and an increasingly spirited search for molecules that might mimic the process without requiring a draconian diet.

Research in the Massachusetts Institute of Technology laboratory of Leonard Guarente, for example, has shown that increasing the activity of a single gene, called *SIR2*, can extend the life span

of yeast. And without the gene, calorie restriction doesn't prolong life. The new research shows that certain molecules activate *SIR2* in yeast, as well as an analogous gene, *SIRT1*, in human cells. Sinclair says that preliminary data from experiments in nematodes and fruit flies are "encouraging," in terms of whether similar activation of *SIR*-like genes, known collectively as sirtuins, can occur in those organisms, too. The study "establishes that you can get activation of *SIR2*," says Guarente, who has co-founded a company called Elixir Pharmaceuticals, which is searching for drugs that target the Sir pathway.

Working with colleagues at Harvard, BIOMOL researchers began screening a library of compounds about 2 years ago for molecules that trigger *SIRT1* activity. The initial screen yielded two polyphenols, quercetin (found in apples and tea) and piceatannol. The team then searched for other molecules with similar structures. That canvass yielded another 15 compounds, the most potent of which turned out to be resveratrol, found in grapes and red wine. It increased *SIRT1* activity 13-fold, the team reports online 24 August in *Nature*.

Resveratrol's *SIRT1*-activating power adds another dimension to the work, because it suggests a link to the so-called French paradox, the observation that despite a high-fat diet, people in France suffer about 40% less cardiovascular disease than expected; epidemiologists have linked this effect to the moderate consumption of red wine. Sinclair and colleagues speculate that these benefits may derive from activation of *SIR*-like genes. Increased *SIRT1* activity in human cells seems to blunt the activity of the tumor-suppressor gene *p53*, blocking programmed cell death. Sinclair suggests that the *SIR*-activating compounds buy time for cells to heal themselves rather than commit suicide.

In addition to its immediate implications for aging and life extension, the new work bolsters the notion that there is an evolutionarily conserved mechanism to stall the aging process during times of stress, such as when food is scarce. It also raises the possibility that the sirtuin-activating compounds reflect an interaction between plant and animal species. According to this hypothesis, which Sinclair calls "xenohormesis," plants increase their own production of polyphenols in response to environmental stresses such as drought, and that message of impending crisis may be passed on to animals that eat the plants. "Other unrelated, nonplant species can get chemical clues from the plant world," Sinclair says, "which causes them to mount their own defense response." Alternatively, he adds, the plant compounds may simply be similar to analogous, unidentified molecules in human biology.

Richard Weindruch of the University of Wisconsin, Madison, who is conducting calorie-restriction experiments in monkeys and other animals, applauds the new report but adds, "I think one needs to be very cautious about making dramatic leaps from the yeast model into mammals." He notes that it was unclear, for example, whether resveratrol affected the aging process in the kind of cells in the heart and brain that are particularly susceptible to degeneration with age.

"It's kind of romantic that red wine contains something that could extend your longevity, don't you think?" says Cynthia Kenyon, who researches aging at the University of California, San Francisco, after seeing the data presented at a meeting in Switzerland last week. But the results have not caused Sinclair to renegotiate his relationship with Dionysus. "I'd already increased my red wine consumption prior to this discovery," he confesses with a laugh (Hall, 2003).

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