

Patenting Human Genes and Stem Cells

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Received: November 10, 2006; Accepted: November 22, 2006; Revised: November 29, 2006

Abstract: Cell lines and genetically modified single cell organisms have been considered patentable subjects for the last two decades. However, despite the technical patentability of genes and stem cell lines, social and legal controversy concerning their 'ownership' has surrounded stem cell research in recent years. Some granted patents on stem cells with extremely broad claims are casting a shadow over the commercialization of these cells as therapeutics. However, in spite of those early patents, the number of patent applications related to stem cells is growing exponentially. Both embryonic and adult stem cells have the ability to differentiate into several cell lineages in an organism as a result of specific genetic programs that direct their commitment and cell fate. Genes that control the pluripotency of stem cells have been recently identified and the genetic manipulation of these cells is becoming more efficient with the advance of new technologies. This review summarizes some of the recent published patents on pluripotency genes, gene transfer into stem cells and genetic reprogramming and takes the hematopoietic and embryonic stem cell as model systems.

Keywords: Human genes, human stem cells, hematopoietic stem cells, embryonic stem cells, gene transfer, nuclear transfer, patents.

INTRODUCTION

It is currently accepted world-wide that any patent should be justified on the bases of its novelty, originality, industrial application (e.g. medicine is included as industrial application) and sufficient disclosure. However, over a century ago, clarification of the concept of invention needed to be made. It was in 1903 when an English judge made the statement of clarifying the difference between *discovery* and *invention* [1].

In the 1980s, as recombinant DNA technology gets established and widely used, some of the first biotech patent applications involving cells and cell lines with altered genetic material were filed. Since then, patenting human genes has become acceptable to patent authorities and policy makers in many countries, but it has also created much social and ethical controversy [2-4], bringing us back to the statement made at the beginning of the twentieth century. Some consider that the commercialization of humans and their genetic material infringes human dignity [3] and argue that: (i) genes are 'naturally occurring' subjects and therefore should not be owned by any individual or group, (ii) genes are, therefore, discovered and not invented, (iii) genes are not 'new' or novel, they already exist in nature and (iv) in this post-genomic era, gene isolation and cloning is not an inventive technique (we have been doing it for more than 25 years). Just as the authorities struggle with the debate associated with human gene patents, scientific breakthroughs in stem cell research are clouding the issue even further. Those opposing the patentability of human genes would agree that the same first three claims would also apply to

patenting human stem cells. Stem cells also exist in nature and they are therefore 'discoveries' and not 'inventions'. Conversely, for those supporting the patentability of human genetic material and human cells it seems that the first objection is based on the misleading notion of what 'ownership' a patent grants. A patent represents an agreement to limit the commercial exploitation of an invention to an inventor for a period of time, and not a permission to exploit the invention. The second claim may appear to have some weight, since it is difficult to think of genes and stem cells as inventions. However, pioneers in the field of genetic and stem cell research would have had to establish new experimental methods and protocols in order to discover human genes and stem cells. Hence, patent applications may involve methods for isolating or purifying these so-called products and processes used in their isolation, culturing or modification (e.g. genetic modification of stem cells that may involve gene or nuclear transfer). Thus after a century of 'inventions' and two decades of patents in Life Sciences, the debate about patenting human genetic material and human cells is a current issue whilst the number of patents applications on human stem cells is rapidly increasing. As an example, the number of filed patent applications where inventions involve human and non-human stem cells is estimated to exceed two thousand applications worldwide. Over 500 of these applications claim inventions on human ES cells [5]. Forty four of these patents have been published in the first eight months of 2006.

This review is not intended to list all the published patents in the field, but will focus on discussing recent patents related to pluripotent stem cells and pluripotency genes, and technology developed to manipulate the genetic material of those stem cells. Recently, some of the molecular mediators of pluripotency and genetic (nuclear) reprogramming have been identified in embryonic stem cells (e.g., Oct4, Nanog and Sox2 genes) [6]. By contrast, the

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development of vectors to carry out genetic manipulation of stem cells has evolved in parallel to gene therapy studies related to adult hematopoietic stem cells (HSC). In addition to gene transfer, nuclear transfer techniques have given a whole new dimension to the stem cell research field, expanding their therapeutic potential in regenerative medicine. Therefore, this review takes breakthroughs in embryonic stem (ES) cells and HSC research as examples of patents in these two model systems. Details of each specific patent can be found using the search engine at <http://es/espacenet.com/>.

THE HEMATOPOIETIC STEM CELL (HSC)

There are cells found in both adult and fetal tissues that show characteristics of stem cells. In the adult, the bone marrow has been traditionally considered the most significant source of pluripotent stem cells and the HSC is by far the best characterized adult stem cell (for review, see [7-9]). Thus, the criteria that identify HSC have also been used to define other tissue-specific stem cells. First, HSC are rare, occurring with the frequency of 1 in 10^4 to 10^5 total bone marrow mononuclear cells. Second, they can produce identical daughter cells after a relatively large number of cell divisions. They have the ability to balance self-renewal and differentiation, maintaining a highly clonogenic population [10]. Third, they are pluripotent cells, giving rise to all blood cell lineages during the life span of an individual [11,12]. Although, HSC can be purified using cell surface markers (e.g. CD34), they are considered a heterogeneous population. Therefore, it is not surprising that early patents in the field claim methods for isolating a human cell population enriched in HSC to use in bone marrow transplantation (see Table 1 [13-28], EPO662512 and US5840580). The above cell population is identified as having a CD34⁺/CD38⁻/HLA-DR⁺ phenotype and preferably expressing markers such as CD13, CD33 or CD71. Human HSC are represented in the CD34⁺ cell population of blood and bone marrow. Within this population, the CD34⁺/CD38⁻ HSC subset is thought to be more primitive than the CD34⁺/CD38⁺ subset [29] containing long-term repopulating hematopoietic cells. HSC have been used successfully in bone marrow transplantation for the last 40 years [30], turning them into the first regenerative pharmaceuticals [11,12,31,32]. Both autologous and allogeneic (human leucocyte antigen (HLA)-matched related or unrelated donor) bone marrow transplants are performed, hence the importance of carrying out HLA typing and matching. Since HSC are rare, scientists have attempted to culture them *ex vivo*, and where possible, to expand primitive HSC by a variety of methods. These include the use of leptin to maintain and expand HSC (Table 1, WO9818486) and the treatment of these cells with specific polypeptides and stem cell survival factors such as stem cell factor (SCF) and fms-related tyrosine kinase 3 (Flt-3) ligand (Table 1, WO 03076613). Furthermore, bone marrow-derived HSC and mesenchymal stem cells (MSC) have been reported to give rise to non-hematopoietic cell lineages, such as endothelial, neural and muscle cells [33], further extending their therapeutic potential. Hence, patents have been filed using HSC for a therapeutic application other than bone marrow transplantation. It may be of interest that a number of them claim an HSC population that contains CD31⁺ endothelial progenitor cells (EPC) capable of forming blood vessels and

the use of these EPC to treat ocular vascular diseases (Table 1, US2005063961, MXPA05000972 and MXPA05011752). The same patents extend their claims to genetically modified EPC that may express anti-angiogenic peptides or trophic factors.

GENETIC MANIPULATION OF STEM CELLS

Genetic manipulation of cells can be achieved by gene or nuclear transfer, as shown in Fig. (1). In the first instance, the gene of interest or transgene is introduced into the cell that is the subject of genetic manipulation where its expression is vector-driven. As a result, the modified cell acquires the characteristic of the added transgene. In the second paradigm, the nucleus of an undifferentiated or differentiated somatic cell acting as donor replaces the nucleus of the recipient oocyte. The donor cell genetic material can be 'manipulated' or reprogrammed by the enucleated oocyte cytoplasm.

In the gene therapy field, HSC have proven to be quite recalcitrant to gene transfer techniques compared to other cell types [34]. Therefore the development of gene transfer vectors capable of expressing the transgene of choice and genetically modifying HSC have been the subject of numerous patents for commercial and medical applications. HSC have been identified as potential therapy for the permanent correction of hematological genetic defects, in particular some immunodeficiencies for which there is no other treatment (e.g. severe combined immunodeficiency (SCID) caused by defects in adenosine deaminase (ADA). Retroviral vectors (RV) for HSC have been traditionally based on the Molony Leukaemia virus (MLV) backbone. These vectors can be produced at high titer, they are replication-deficient and they ensure a sustained transgene expression since they integrate into the host genome. In order to improve safety or to expand their range of target cells, RV can be pseudotyped with a variety of envelope proteins such as the Vesicular Stomatitis Virus Glycoprotein (VSV-G) [35]. However, MLV-based RV can only transduce cells undergoing mitosis. HSC are generally quiescent *in vivo* and *in vitro*. *Ex vivo* treatment of HSC can promote the progression through the cell cycle that is required for MLV transduction, with the result of reducing self-renewal and long-term engraftment capacity. An early example of patents in this field claims a method for expressing a transgene in HSC and for optimizing gene transfer into HSC using RV (see Table 2, DE19704979). In addition, the exposure of HSC to chemicals such as hydroxyurea to increase their transduction efficiency *ex vivo* and their engraftment *in vivo* has also been a patentable subject (Table 2 [36-54], WO9748815).

Transgene expression from MLV vectors may be silenced by DNA methylation [55]. In order to avoid gene silencing and improve gene expression, researchers have engineered RV by adding sequences such as scaffold attachment regions (SARs). SARs are AT-rich DNA sequences, found upstream of, downstream of, or within genes, that have high affinity binding sites for nuclear matrix proteins defining boundaries of independent chromatin domains. Some SARs can enhance transgene expression in heterologous transfections *in vitro* and in transgenic mice [56]. Incorporation of the SAR from the human interferon

Table 1. Patents Related to Human Stem Cells and Pluripotency Genes

Publication number	Ref. No. and Title	Inventors	Publication Date
US5843780	[13] Primate embryonic stem cells	Thomson, J.A.	1998/12/01
WO9730151	[14] Cytokine expressed by DIA/LIF-deficient embryonic stem cells for the inhibition of differentiation	Dani, C., Chambers, I.P., Beuhr, M.L., Smith, A.G.	1997/08/21
US6875608	[15] Pluripotency determining factors and use thereof	Chambers, I.P., Smith, A.G.	2005/04/05
US2005255573	[16] Propagation and/or derivation of embryonic stem cells	Smith, A.G., Burdon, T.G.	2005/11/17
US2005196859	[17] Propagation and/or derivation of embryonic stem cells	Smith, A.G., Burdon, T.G.	2005/09/08
EP0662512	[18] Human hematopoietic stem cells	Terstappen, L.W., Loken, M. R., Huang, S., Olweus, J., Lund-Johnsson, F.	1995/07/12
US5840580	[19] Phenotypic characterization of hematopoietic stem cells	Terstappen, L. W., Loken, M. R., Huang, S., Olweus, J., Lund-Johansen, F.	1998/11/24
WO9818486	[20] Use of leptin to stimulate hematopoiesis	Rao, D.D., Sitnicka, E., Bartelmez, S.H., Hagen, F.S.	1998/05/07
WO03076613	[21] Protein sustaining undifferentiated stem cells as such	Ema, H., Nakauchi, H., Osawa, K.	2003/09/18
US2005063961	[22] Hematopoietic stem cells and methods of treatment of neovascular eye diseases therewith	Ema, H., Nakauchi, H., Osawa, K.	2005/03/24
MXPA05000972	[23] Hematopoietic stem cells and methods of treatment of neovascular eye diseases therewith	Dasilva, K.	2005/09/02
MXPA05011752	[24] Hematopoietic stem cells and methods of treatment of neovascular eye diseases therewith	Otani, A.	2006/06/06
JP06042663	[25] Discrimination marker of ES cell	Naatsuji, N., Tada, T., Tada, M.	2006/02/16
WO04072226	[26] Marker for the undifferentiated state of cell and composition and method for separation and preparation of stem cells	Naatsuji, N., Tada, T., Tada, M.	2004/08/26
JP05110565	[27] Agent for keeping differentiation/pluripotency	Yamanaka, N.	2005/04/28
WO06025802	[28] Method for maintaining pluripotency of stem/progenitor cells	Robson, P., Rodda, D., Ng Huck, H.	2006/03/09

gene into an MLV-based RV has resulted in increased transgene expression in quiescent cells [56]. The design of such vectors for commercial use has therefore, resulted in the filing of several patent applications (e.g. WO0140488 and US20020668362).

By contrast to MLV-based vectors, lentiviral vectors (LV) based on the human immunodeficiency (HIV) and the simian immunodeficiency (SIV) viruses or the human foamy virus (HFV) are types of retroviruses that can transduce non-dividing or slow-dividing cells. The transduction of quiescent cells, such as HSC, by these vectors is more efficient than by RV, since their pre-integration complex is more stable and able to cross the intact nuclear membrane of non-dividing cells [57]. Early patents filed by researchers at the Salk Institute for Biological Studies claimed a recombinant LV able to infect non-dividing cells (Table 2, CA2233867, ZA9608382, AU758600B and WO9812314). The term non-dividing cells include HSC. Similar to RV, LV can be produced at high titers, they are replication-defective, and

can be pseudotyped with different envelope glycoproteins. The design of LV has been further developed for gene transfer as follows: improved safety and transgene expression. To improve transgene expression researchers have engineered LV by adding a cis-acting post-transcriptional regulatory element originally derived from the woodchuck hepatitis virus (WPRE) (Table 2, AU751326B, WO9914310, US6287814, US6312912 and US6284469). WPRE is a useful RNA export sequence that ensures the accumulation of the desired mRNA in the cytoplasm of the transduced cell. In order to improve the safety of these vectors, more recent HIV vector systems include self-inactivating (SIN) long-terminal repeats (LTR) in their design [58]. SIN vectors have the majority of the 3' LTR deleted, resulting in a similar deletion of the 5' LTR upon reverse transcription and integration into the host genome. With this design, the expression of the transgene depends solely on an internal heterologous promoter, reducing the potential activation of adjacent genes [58]. In particular, specific promoters that

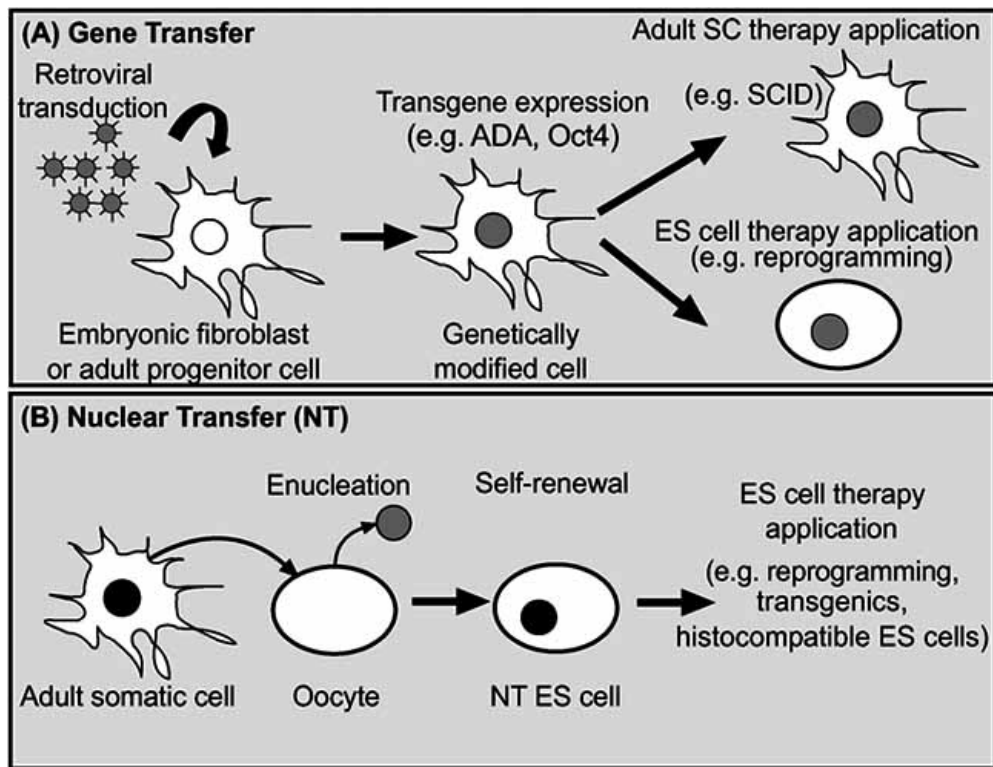


Fig. (1). Genetic manipulation of stem cells. (A) Gene transfer techniques. Embryonic fibroblasts or adult progenitor cells, such as HSC, are transduced with retroviral vectors (RV) encoding either a gene whose defect causes a disease (e.g. ADA) or a pluripotency gene (e.g. Oct4). The transduced cells acquire the phenotype associated to the transgene and can be used in cell therapy applications or to generate pluripotent stem cells. (B) Nuclear transfer techniques. Embryonic or adult somatic cells serve as nuclear donors. The nucleus is introduced into an enucleated oocyte and used to produce embryonic stem (ES) cells. Nuclear transfer ES cells have the capacity of self-renewal and can give rise to many cell lineages. These ES cells can be used in cell therapy applications such as cloning, the generation of transgenic animals or the production of histocompatible ES cells for transplantation (Figure adapted from Rodolfa and Eggan [99]).

show a maximum activity in HSC have been tested and included in the design of the latest LV (e.g. EF1-alpha promoter). Additional elements such as an inactive central polypurine tract and tissue-specific promoters have been also engineered into these vectors (Table 2, US2003008374, US2003082789, US2003138954, WO03091442 and AU 2002327412). In summary, the new generations of LV with a potential to be used in gene therapy are complex, safe, highly efficient and potent. They are capable of transducing very specific cell types such as human CD34⁺ HSC (e.g. patent NZ532060).

Although the most up-to-date gene transfer vectors are safer to use, there are aspects of their biology that are still not fully understood. The first successful gene therapy clinical trials employing RV to modify the patient's own HSC was controversially terminated because three of the X-SCID patients treated developed clonal T-cell leukemias [59-61]. Molecular analyses revealed clonal vector integration at the 5' end of the LIM domain only 2 (LMO2) gene in the leukemic cells of all three patients, suggesting that insertional mutagenesis at this specific site increased the expression of the LMO2 gene that has been linked to T-cell leukaemias [62]. This characteristic of RV is not shared by other vectors. LV do not preferentially integrate their genomes upstream of transcription start sites, thereby acting

as transcriptional activators [63]. However, there is no experimental evidence to suggest that the use of these vectors will reduce the risk of insertional mutagenesis [9].

THE EMBRYONIC STEM (ES) CELL

Stem cells are normally classified according to their differentiation potential. Totipotent stem cells, embryonic stem cells or ES cells possess a vast developmental potential, giving rise to all cell types in the body as shown in Fig. (2) [64, 65]. ES cells become more committed to specific cell lineages as the embryo develops, turning into pluripotent and multipotent tissue-specific stem cells in the adult. In this review, we have already described the HSC as a model of adult pluripotent stem cell. Historically, differential gene expression between the embryo and the adult has been thought to involve silencing of specific sets of genes, so that in most adult tissues a heterogeneous population of multipotent stem cells, progenitor cells and differentiated cells is found. ES cells can be isolated from the inner cell mass of a blastocyst at early stages of the mammalian embryo. They can be cultured *in vitro* over a long period of time and can be subjected to genetic manipulation. Since the mid 1990s, patents have been filed that claim the isolation and propagation of pluripotent mouse ES cells (e.g. WO9730151) and their use to generate transgenic animals. Recently, the isolation and culturing of human ES cells has

Table 2. Patents Related to Gene Transfer into Hematopoietic Stem Cells (HSC)

Publication number	Ref. No. and Title	Inventors	Publication Date
DE19704979	[36] Retroviral vector for expressing interleukin- antagonist in human hematopoietic stem cells	Bargou, R., Baum, C., Hoenemann, D.	1997/08/14
WO9748815	[37] Method for enhancing gene transfer	Uchida, N., Tsukamoto, A., Weissman, I.	1997/12/24
WO0140488	[38] Increased transgene expression in retroviral vectors having a scaffold attachment region	Murray, L.J., Plavec, I.	2001/06/07
US2002068362	[39] Increased transgene expression in retroviral vectors having a scaffold attachment region	Murray, L.J., Plavec, I.	2002/06/06
CA2233867	[40] Vector and method for use for nucleic acid delivery to non-dividing cells	Gallay, P.A., Naldini, L., Verma, I., Trono, D.	1997/04/10
ZA9608382	[41] Vector and method for use for nucleic acid delivery to non-dividing cells	Verma, I., Trono, D., Naldini, L., Gallay, P.	1997/06/27
AU758600B	[42] Vector and method for use for nucleic acid delivery to non-dividing cells	Verma, I., Trono, D., Naldini, L., Gallay, P.	2003/03/27
WO9812314	[43] Retroviral vectors capable of transducing non-dividing cells	Trono, D., Gallay, P.A.	1998/03/26
AU751326B	[44] Vector and method of use for nucleic acid delivery to non-dividing cells	Verma, I., Trono, D., Donello, J.E.	2002/08/15
WO9914310	[45] RNA export element and methods of use	Hope, T., Zufferey, R., Trono, D., Donello, J.E.	1999/03/25
US6287814	[46] Method for increasing cytoplasmic concentration of a nucleic acid transcribed within a cell	Hope, T., Zufferey, R., Trono, D., Donello, J.E.	2001/09/11
US6312912	[47] Method for identifying a cis-acting RNA export element	Hope, T., Zufferey, R., Trono, D., Donello, J.E.	2001/11/06
US6284469	[48] Method for isolating proteins that bind to the woodchuck hepatitis virus RNA export element	Hope, T., Zufferey, R., Trono, D., Donello, J.E.	2001/09/04
US2003008374	[49] Methods and composition relating to improved lentiviral vectors and their applications	Trono, D., Salmon, P.	2003/01/09
US2003082789	[50] Methods and composition relating to improved lentiviral vector production systems	Trono, D., Zufferey, R.	2003/05/01
US2003138954	[51] Methods and composition relating to restricted expression of lentiviral vectors and their applications	Trono, D., Wiznerowicz, M.	2003/07/24
WO03091442	[52] Improved chimeric glycoproteins and pseudotyped lentiviral vectors	Trono, D., Cosset, F.-L., Sandrin, V., Boson, B., Negro, D.	2003/11/06
AU2002327412	[53] Methods and composition relating to improved lentiviral vector production systems	Trono, D., Zufferey, R.	2003/02/17
NZ532060	[54] Methods and composition relating to restricted expression of lentiviral vectors and their applications	Trono, D., Wiznerowicz, M.	2005/11/25

given the opportunity to use them in regenerative medicine, but ES cell research has been surrounded by a myriad of ethical and technical challenges [66]. The first and better known granted patent on human ES cells is held by the Wisconsin Alumni Research Fund (WARF), with Dr. James A. Thomson named as inventor (see Table 1). Patent US5843780, published on 12 January 1998 and granted by the US Patent Trademark Office (USPTO) three years later,

claims a method of isolation and a preparation of totipotent primate ES cells with a normal karyotype and able to proliferate in an undifferentiated state after one year in culture. The claims of this patent are very broad and include human ES cells. Since the isolation of ES cells, there has been an attempt to understand which factors are required for maintaining the pluripotency of stem cells.

PLURIPOTENCY GENES

The development of new research tools such as global gene expression analyses, have made it possible to define the molecular mechanisms that maintain self-renewal and to identify pluripotency genes in stem cells. Gene profiling experiments have revealed that totipotent ES cells express a large number of so-called 'pluripotency genes' and lineage specific genes that are found to be expressed in neuronal and hematopoietic stem/progenitor cells [67,68]. Hence, the concept of 'stemness' is thought to reflect the transcriptional 'open mind' state of a stem cell [69]. Factors that are required for maintaining the pluripotency of ES cells are both extracellular and intracellular mediators. Extracellular factors include general signaling molecules such as WNT and bone morphogenic protein (BMP) [70]. In addition to the extracellular factors, intracellular mediators such as Oct4, Sox2 and Nanog have been implicated in the transcriptional control of self-renewal and pluripotency of both mouse and human ES cells [71]. Examples of patents related to pluripotency genes are shown in Table 1. Thus it is assumed that the expression of these genes and their products will maintain stem cells in their pluripotent state. Genetic manipulation, as described previously in this review, is not the only solution to express the product of a transgene in stem cells culture. The supply of recombinant proteins to stem cells in avoids long-term changes that will alter their genetic material. Designer Tat-fusion proteins are examples of these recombinant proteins. They consist of soluble forms of the desired recombinant protein fused to a short peptide sequence of the HIV-1 Tat protein that confers cellular delivery [72]. Tat-fusion proteins have the ability to cross the cell membrane and therefore their effect can be propagated to all cells in the culture. In 2003, Krosi *et al.*, published their work on HOXB4, a transcription factor that contain homeo-box (HOX) DNA binding domains and is important for HSC development. In an elegant study, the authors produced recombinant TAT-HOXB4 protein that, supplied to HSC, increased their expansion *in vitro* and *in vivo* [73]. Following the same experimental design, patent JP2005

110565 claims a method for maintaining the pluripotency of ES cells and somatic stem cells by delivering a polypeptide consisting of the fusion protein TAT-Nanog (Table 1). However, the key to control pluripotency and self-renewal is to understand how the expression of genes such as Oct4, Sox 2 and Nanog is regulated. There are examples of patents that claim methods to regulate the transcription of these genes by expressing other pluripotency genes (JP2006042663, WO 2004072226 and WO2006025802). Some go even further designing either a kit for determining the pluripotency or differentiation state of a stem cell (WO2004072226) or a reporter gene assay that uses the Nanog gene promoter and a transgene to eliminate teratomas and other cancers (WO2006025802).

Current research in the field is focusing on the delineation of the downstream targets of pluripotency genes [71]. Genome-wide analysis has revealed that Oct4, Sox2 and Nanog target genes that generally encode other developmentally regulated transcriptional repressors that are silent in pluripotent ES cells [71,74,75]. These results are in agreement with the idea that inhibition of differentiation pathways is essential for the maintenance of pluripotency. Polycomb group (PcG) proteins are transcriptional repressors found to control the expression of developmental genes in ES cells [74,75]. Many Oct4, Sox2 and Nanog target genes have been previously identified as PcG targets, thus indicating that pluripotency gene products may function in concert with PcG proteins to silence key regulators of cell differentiation. A strong possibility exists that new patents related to Nanog, Sox 2 and Oct 4 target genes will be filed and published in the near future.

NUCLEAR TRANSFER, TRANSGENIC ANIMALS AND CLONING

ES cells have been used extensively to generate transgenic animals, especially mice. ES cells are derived from a blastocyst cultured *in vitro* and can be subjected to DNA manipulation by inserting the desire transgenes. Genetically manipulated ES cells injected into the mouse

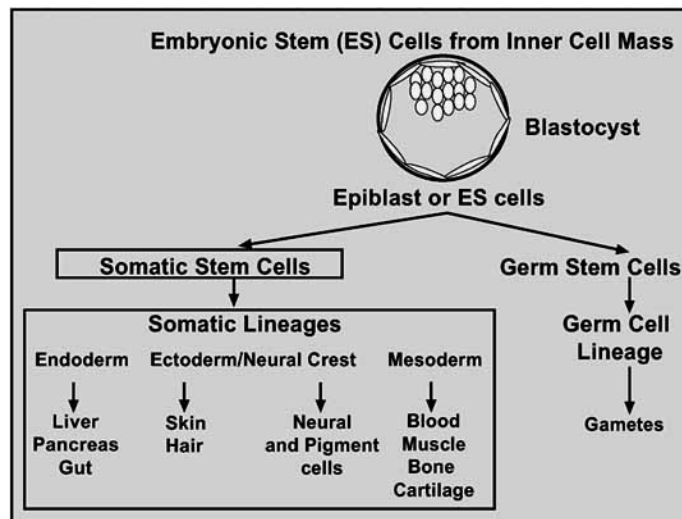


Fig. (2). Totipotent embryonic stem cells. Embryonic stem (ES) cells are isolated from the inner cell mass of a blastocyst at early stages of the mammalian embryo. ES cells have the potential to generate all cell types in the organism, including somatic cell lineages that form endoderm, ectoderm and mesoderm, and germ lineages that form the gametes.

Table 3. Patents Related to Nuclear Transfer

Publication number	Ref. No. and Title	Inventors	Publication Date
WO9424274	[76] Isolation, selection and propagation of animal transgenic stem cells	Smith, A.G., Mountford, P.S.	1994/10/27
WO9517500	[77] Embryonic stem cells as nuclear donors and nuclear transfer techniques to produce chimeric and transgenic animals	Stice S.L., Strelchencko N., Bethhauser, J., Scott, B., Jurgella, G.	1995/06/29
WO9516770	[78] Ungulate preblastocyst derived embryonic stem cells and use thereof to produce cloned transgenic and chimeric ungulates	Strelchencko, N., Stice, S.L.	1995/06/22
WO9508625	[79] Mammalian cloning by embryonic stem cell nuclear transplantation and embryonic stem cells	Reed, M.A., Wagner, T.E., Edward, M.J.A.	1995/03/30
WO9607732	[80] Totipotent cells for nuclear transfer	Mcwhir, J., Campbell, K.H.S.	1996/03/14
WO0234890	[81] Pluripotent stem cells	Smith, A.G., Ying, Q.L.	2002/05/02
MXPA00010473	[82] Process for obtaining stem cells	Smith, A.G.	2002/04/24
JP2005323609	[83] Isolation, selection and propagation of animal transgenic stem cells	Smith, A.G., Mountford, P.S.	2005/11/24/
US2005196858	[84] Isolation, selection and propagation of animal transgenic stem cells	Smith, A.G., Mountford, P.S.	2005/09/08
GB2318578	[85] Quiescent cell populations for nuclear transfer	Campbell, K.H.S., Wilmut, I.	1998/04/29
GB2331751	[86] Quiescent cell populations for nuclear transfer	Wilmut, I., Campbell, K.H.S.	1999/06/02
WO0229000	[87] Methods for minimizing immunological rejection of nuclear transfer fetus	Davies, C.J., Schlafer, D.H., Hill, J.R.	2002/04/11
US2004029825	[88] Methods for minimizing immunological rejection of nuclear transfer fetus	Davies, C.J., Schlafer, D.H., Hill, J.R.	2004/02/12
EP1621610	[89] A method of producing nuclear transfer embryonic stem cells by somatic cell nuclear transplantation technique	Mao, X., Mo, Y., Huang, J.	2006/02/01
US2003232430	[90] Method for making and using reprogrammed human somatic cell nuclei and autologous and isogenic human stem cells	Cibelli, J., West, M., Campbell, K.	2003/12/18
MXPA04005010	[91] Methods for making and using reprogrammed human somatic cell nuclei and autologous and isogenic human stem cells	Campbell, K.	2005/04/08
WO03008554	[92] Methods and composition for cell therapy	Cibelli, J., Dominko, T.	2003/01/30
US2006083722	[93] Methods and composition for cell therapy	Cibelli, J., Dominko, T.	2006/04/20
WO03100018	[94] A bank of stem cells for producing cells for transplantation having HLA antigens matching those of transplant recipients, and methods for making an using such a stem cell bank	West, M.	2003/12/04
US2004091936	[95] Bank of stem cells for producing cell for transplantation having HLA antigens matching those of transplant recipients, and methods for making an using such a stem cell bank	West, M.	2004/05/13

embryo grow giving rise to all the cell types in the adult, including germ cells (Fig. (2)). Chimeric mice are tested for germline transmission of the transgenes to create transgenic colonies. Examples of patents in this field are shown in Table 3 [76-95]. An early patent on mouse ES cells claim the use of totipotent cells of embryonic origin as donors for nuclear transfer experiments (WO9424274, WO9517500, WO9516770, WO9508625 and WO9607732). Nuclear transfer-derived embryos are used to produce transgenic animals (Fig. (2) (e.g., ungulates and placental mammals). This technology has great potential for use in agriculture and human and veterinary medicine. More recently, the same technology has been used to obtain pluripotent stem cells

transferring the nucleus of progenitor cells such as neural and hematopoietic progenitor cells (WO0234890 and MXPA00010473). The pluripotent cell is then selected by the expression of a genetic marker under the control of the Oct4 promoter. The differential expression of selectable markers, under the control of this promoter, gives survival advantages to pluripotent ES cells compared to non-ES cells. The use of this method of isolation, selection and propagation of ES cells has also been extended to animal transgenic stem cells (JP2005323609 and US2005196858).

The first patents for somatic cell nuclear transfer and cloning methods (GB2318578 and GB2331751) were granted in the year 2000 to researchers at the Roslin

Institute, Edinburgh, Scotland. These patents claim the use of quiescent cells in nuclear transfer and any cloned animal produced by this method. As expected, the patents covered critical issues and techniques used to create 'Dolly' the sheep. They also include claims that cover the use of this technology in human therapeutic cloning. Somatic cell nuclear transfer is also important for the production of stem cells that are identical to those of the potential recipient, therefore minimizing the immunological rejection when transplanted into the recipient. This is of particular interest in agriculture where early bovine pregnancy losses are much more common in nuclear transfer embryos than in normal pregnancies. Recent patents in the field are directed to improve the rate of nuclear transfer pregnancies (WO0229000 and US2004029825). Their claims include a method for minimizing immunological rejection by transferring a nuclear transfer embryo into a recipient with compatible MHC-Class I antigen. Others go even further and try to resolve the problem of rejection whilst developing a therapy to treat neurological injuries and diseases of the nervous system (EP1621610). In this instance, the patent claims include a method for transferring the nucleus of an adult neural cell into an enucleated stem cell obtained from the germ line. Although the patent refers to animal stem cells, the invention may also be of great benefit to humans.

Matching the MHC molecules of a donor to those of the recipient significantly increases the success rate of clinical transplantation. At present, there are more patients needing a transplant than donors. Therefore, new sources of histocompatible cells and tissues for transplantation hold a great potential. Two recent patents address these immunological issues by claiming the production of human embryos by somatic nuclear transfer (US2003232430 and MXPA 04005010). Banking of human stem cells has recently attracted great attention in the private sector. Therefore it is not surprising to see published patents related to collections of histocompatible stem cells generated by parthenogenesis, *in vitro* fertilization (IVF), same-species or cross-species nuclear transfer or by genetic targeting (WO03100018 and US2004091936). The collections or banks consist of human totipotent and/or pluripotent stem cell lines that are homozygous for one or more histocompatibility antigens. The homozygous genotypes are representative of the most prevalent MHC alleles in the population. Such collections of cell lines with homozygous MHC alleles will increase the chance of a match for those patients in need of a transplant.

CURRENT & FUTURE DEVELOPMENTS

The first bone marrow transplant carried out in mice took place in 1961 [96]. Since then, bone marrow transplantation in humans using adult pluripotent HSC has been performed successfully, converting HSC in the first cell-based therapeutics with industrial/medical application. Therefore, it is not surprising that patents have been filed relating to the isolation, characterization and genetic modification of such cells. In an attempt to extend the wealth of knowledge acquired in the field of hematology and bone marrow transplantation to other fields, scientists and clinicians are looking to use stem cells to repair damaged tissues such as cardiac muscle and vascular tissue after myocardial infarction and neurons to treat neurodegenerative diseases.

But adult HSC are limited in numbers and they cannot multiply as ES cells. The use and patentability of human ES cells and their genetic material is seen by some as an infringement of human dignity and has provoked serious ethical and social concerns to say the least [3].

This controversy surrounding the patenting of human genes and ES cells has erupted in recent years, and it is not only the ethical and social arguments in favor or against patenting human genetic material and cells, but also the decisions made by patent offices world-wide that are fuelling this debate. For example, the patent held by Amgen on using recombinant DNA technology to produce the hormone erythropoietin has been invalidated recently in the UK [97]. Both, the Court of Appeal and the House of Lords considered that gene sequences were to be assessed as 'discoveries' and not 'inventions'. In 2002, the Nuffield Council on Bioethics, also in the UK, published a report recommending patent offices around the world to refrain from awarding patents on DNA sequences. The report discriminates between the different uses of a DNA sequence; this being either a gene therapy application or a specific genetic test or the production of a recombinant polypeptide with therapeutic application. Statements such as the one made by the Nuffield Council of Bioethics may help clarify the ethical debate over patents on human genes. However, implementing their recommendations will not only prove difficult, but it will require a drastic change in thinking for other patent offices. The same could be said for patents related to stem cells. Also in 2002, the European Patent Office (EPO) restricted the controversial patent on animal transgenic stem cells, granted on 1999 to Edinburgh University (inventors: Prof. A. Smith and Dr. P. Mountford). According to EPO the claims of the original patent (WO9424274) were too vague and lead to the reading that the patent may have covered the creation of human beings. As a result, the patent holders agreed to remove all references to human or animal ES cells, leaving only the claims related to adult stem cells [98]. However, a year before EPO limited the claims of the Edinburgh patent, the USPTO granted a few patents with very broad claims on human ES cells, including the WARF patent (US5843780) relating to the techniques used to derive ES cell lines. WARF claims that its patent covers all import and use of human ES cells in the United States. Such broad claims seem to have put a halt to stem cell research in the country. Those experts in the field consider that decisions like the ones faced by the Amgen, Edinburgh and WARF patents may have wider implications in the future. Others consider that decisions like this will not have such a global impact. On the contrary, they think that patents will be looked at on an individual case basis. Thus, although the future of stem cell research and commercialization may seem contentious at present, especially with examples such as the erythropoietin patent, there are other examples that have yielded encouraging results. For example, when 'Dolly' the sheep was born a decade ago, it was difficult to predict the legal and social impact that this publication was going to have on cloning. Patents GB2318578 and GB2331751 describe important techniques involved in the creation of Dolly. Therapeutic cloning has been legal in the UK since 2001. In 2005, Professor Ian Wilmut (who cloned 'Dolly' the sheep)

was granted a license to carry out human therapeutic cloning for the treatment of diseases such as motor neuron disease. The work involves extracting the DNA from a cell of a patient that suffers motor neuron disease and inserting this DNA into a stem/progenitor cell taken from an embryo. The embryonic cell will develop into motor neurons that will degenerate in motor neuron disease. Therapeutic cloning has raised important moral, ethical and religious concerns, but in the case of this life-threatening disease, medical associations and policy makers have made the decision of supporting research that have the potential to develop a future treatment for this condition.

In conclusion, future directions on patenting human genes and stem cells are following the new and tighter guidelines established by patent offices such as USPTO. Nevertheless, ethical debate about the patentability of human genes and stem cells and policy makers are still lagging behind the fast developments in stem cell research. The great therapeutic potential of stem cells is driving advances in basic and medical research and promoting new clinical trials, even without the support of the private sector.

ACKNOWLEDGEMENTS

D.J.B. is a Wellcome Trust Senior Fellow.

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