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Genetically modified stem cells for cellular therapy

Review Article

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Summary

Stem cells carry the promise to cure a broad range of diseases and injuries, from diabetes, to neurological diseases and injuries. Over the past decade, significant progresses have been made in stem cell research; the derivation of embryonic stem cells (ESCs) from human tissues, the development of somatic cell nuclear transfer (SCNT) technology, and the confirmation that neurogenesis occurs in the adult mammalian brain, including in human. Despite these advances, there may be decades before stem cell research translates into therapy. Beside the scientific and technical challenges, there are ethical and political constraints and debates over stem cell research, particularly on ESCs and SCNT. In this manuscript, I will discuss how gene therapy is applied to stem cell research, in an attempt to unlock some of the technical, ethical and political hurdles associated with stem cell research.

I. Introduction

ESCs are self-renewing pluripotent cells that generate cells from the three germ layers of embryos; neurectoderm, mesoderm and endoderm. ESCs carry the hope to cure a broad range of diseases and injuries, like diabetes, heart diseases, Alzheimer's disease, Parkinson's disease and spinal cord injuries (Wobus and Boheler, 2005). ESCs are derived from the inner cell mass (ICM) of blastocysts, and have been derived from human donated embryos produced by *in vitro* fertilization (Thomson et al, 1998). The generation of hESCs provides an unlimited source of tissues for cellular therapy. Because their derivation involves the destruction of blastocysts, there are technical, political and ethical debates and constraints over the use of human ESCs (hESCs) for clinical research and therapy (Wobus and Boheler, 2005). To overcome these issues, investigators are devising strategies and protocols to derive ESCs that genetically matched the patients and without the destruction of embryos.

Neural stem cells (NSCs) are self-renewing multipotent cells that generate the main cell types of the nervous system; neurons astrocytes and oligodendrocytes. Contrary to a long-held belief, neurogenesis occurs in the brain and NSCs reside in the adult central nervous system (CNS) in mammals, including in human (Gage, 2000; Taupin and Gage, 2002; Ming and Song, 2005). Hence, the CNS may be amenable to repair. Neural progenitor and stem cells have been isolated from adult tissues (Reynolds and Weiss, 1992; Gage et al, 1995), including human post-mortem (Palmer et al 2001), providing a source of tissues for the treatment of diseases and injuries of the nervous system. The origin, identity and potential of adult-derived neural progenitor and stem cells remain to be fully and unequivocally characterized before adult NSCs could be brought to therapy (Taupin, 2006a).

Genetically modifying cells has been determinant for the study of gene function, and as a therapeutic tool to restore gene function and produce biologically active substances, like neurotransmitter synthesizing enzymes and trophic factors (Verma and Weitzman, 2005). In this manuscript, I will review and discuss recent studies involving genetically modifying stem cells in aim to circumvent some of the technical, political and ethical hurdles of ESC research, and to bring NSC research to therapy.

II. Genetic engineering to derive ESCs without the destruction of blastocysts

Transplantations of ESCs derived from human embryos would require to genetically matching the grafts with the patients and/or use immune-suppressive drug, to avoid the rejection of the grafts by the patients. With the recent advance in SCNT, there is the potential to generate stem cell lines, tissues and organs that would have the patient own genetic make up, and thus not be rejected. SCNT is a cloning strategy in which nuclei are isolated from a donor's somatic cells, like fibroblasts, and are transferred into enucleated oocytes from female donors (Campbell et al, 1996). By mechanisms yet to be unraveled, the cytoplasm of the oocytes reprograms the chromosomes of the somatic cell nuclei and the cloned cells develop into blastocysts, from which ESCs can be derived (Wakayama, 2006). Thereby, by isolating nuclei from the patients' somatic cells, there is the potential to generate isogenic ESCs, carrying a set of chromosomes identical to that of the patients. The potential of SCNT for therapy is further highlighted by the study of Rideout et al. (2002). In this study the authors combined SCNT and gene therapy to develop strategies for the treatment of genetic diseases. The authors derived ESCs by SCNT from immune-deficient Rag2(-/-) mice, as a model of genetic disease. After correction of the ESCs' gene defect by homologous recombination, transgenic mice were generated by tetraploid embryo complementation and hematopoietic precursor cells differentiated in vitro were grafted in mutant mice, from the ESCs. An immuno-competent phenotype was restored after tetraploid embryo complementation, whereas grafting of genetically engineered ESCs leaded to immuno-competent leaded immunoglobulins detetectable in the host (Rideout et al, 2002). This show that SCNT combined with gene therapy has the potential to treat genetic and gene deficient diseases. There are however, ethical and political debates over the use SCNT and ESCs for therapy (Trounson and Pera, 1998; Jaenisch and Wilmut, 2001). Particularly, the generation of ESCs by SCNT, is subject to the same limitations as for their derivation from donated eggs, as it also involves the destruction of embryos.

Altered nuclear transfer (ANT) is a variation of SCNT proposed by Hurlbut in 2005. In ANT, the gene CDX2, a gene crucial for trophectoderm development, is inactivated *in vitro* in the donor cells. CDX2 encodes the earliest-known trophectoderm-specific transcription factor and is essential for establishment and function of the trophectoderm. Inactivating the gene CDX2 eliminates formation of the fetal-maternal interface, but spares the ICM from which ESCs could be derived. The nuclei deficient for CDX2 are then transferred into enucleated oocytes from female donors, and submitted to the same protocols as for SCNT. Because the eggs created from nuclei deficient for CDX2 produce embryos that are unable to implant into the uterus and do not pursue their developments, ANT has been proposed as a variation of nuclear transfer to derive ESCs, without the destruction of embryos (Hurlbut, 2005).

In 2005, Meissner and Jaenisch reported the use of ANT, to derive ESCs in mice. Meissner and Jaenisch, 2005 genetically modified the donor cells, mouse fibroblasts, by inserting in their genome a cassette coding for RNAi cdx2 and the green fluorescent protein (GFP), flanked by two LoxP sequences. The nuclei of genetically engineered fibroblasts, selected by means of GFP fluorescence, were transferred into enucleated oocytes, to produce eggs by ANT. The eggs divided, produced cloned blastocysts that were morphologically abnormal and lacked functional trophoblasts. The cloned blastocysts did not implant into the uterus, but ESCs could be derived from their ICMs. To maintain the developmental potential of the generated ESCs, the expression of Cdx2 was reestablished by deleting the cassette RNAi cdx2, using a lenti virus (Meissner and Jaenisch, 2005).

ANT is a source of controversies and debates; it is argued that ANT is "a flawed proposal", as there is no basis for concluding that the action of CDX2 or any other gene, represents a transition point at which a human embryo acquires moral status (Melton et al, 2004). So, ANT does not resolve the ethical and political issue over the derivation of ESCs without the destruction of embryos. In addition, though the expression of Cdx2 is reestablished in the cloned cells, it remains to further evaluate whether cloned ESCs with a temporarily inactivated gene CDX2 have the same developmental potential as ESCs derived from donated eggs. Studies have also reported that SCNT may alter the developmental potential of ESCs (Wakayama et al, 2006). All of which may affect the developmental and therapeutic potential of ESCs generated by ANT. Nonetheless, this study highlights the potential of genetically modifying cells for the advancement of research in stem cell biology.

In all, the therapeutic potential of SCNT combined with gene therapy is enormous. It has not only the potential to treat genetic and gene deficient diseases, but also to circumvent the ethical and political issues currently limiting ESC research. However, developmental issues and acceptance of these techniques remain the main concerns over their applications for the treatment of human diseases. Resolving the issues over the potential of ESC generated by SCNT will involve a deep understanding of the cells' developmental mechanisms. The acceptance of SCNT and ESCs for therapy will require further proofs of their potential to treat human diseases and strong legislation supporting and defining the research practice.

III. Genetically modifying adult-derived NSCs

Contrary to a long-held belief, neurogenesis occurs in the adult mammalian brain, including in human (Gage, 2000; Ming and Song, 2005). Neurogenesis occurs primarily in two areas of the adult brain, the dentate gyrus of the hippocampus and the subventricular zone. It is hypothesized that newly generated neuronal cells originate from stem cells in the adult brain (Gage, 2000). Neural stem and progenitor cells have been isolated and characterized *in vitro* from various regions of the adult CNS, including the spinal cord, supporting the existence of NSCs in the CNS (Taupin and Gage, 2002). The generation of new neuronal cells in the adult brain and the isolation and characterization of neural stem and progenitor cells from the adult CNS suggest that the adult

brain may be amenable to repair. Cell therapy in the adult CNS could involve the stimulation of endogenous neural progenitor or stem cells, or the transplantation of adult-derived neural progenitor and stem cells (Taupin, 2006b). Adult-derived neural progenitor and stem cells have been transplanted in animal models, and shown functional engraftment, supporting their potential use for therapy (Shihabuddin et al, 2000).

Adult neural progenitor and stem cells can be genetically modified by retroviral-mediated infection, rendering them a vehicle for gene therapy (Gage et al, 1995). Adult-derived stem cells can be genetically engineered to boost or force their differentiation into a specific pathway. To this aim neural progenitor and stem cells can be genetically engineered to express gene synthesizing enzyme or key transcription factors involved in stem cell differentiation. Adult-derived neural progenitor and stem cells genetically engineered to express the transcription factor Nurr1, a nuclear receptor involved in the differentiation of dopaminergic neurons, have been successfully grafted in animal model of Parkinson's disease and shown to improve functional deficits (Shim et al, 2007). Adult-derived neural progenitor and stem cells genetically modified to express acid sphingomyelinase reverse lysosomal storage pathology when transplanted into animal models of Niemann-Pick's disease (Shihabuddin et al, 2004). This highlights the potential of genetically modified NSCs for the treatment of neurodegenerative diseases, lysosomal storage diseases and other genetic diseases of the CNS.

Fetal-derived neural progenitor and stem cells have been grafted in various models of neurological diseases and injuries, like Parkinson's disease and spinal cord injury, and shown to improve their neurological deficits (Ourednik et al, 2002; Yan et al, 2004). In these studies, the most likely mechanism of functional recovery is through the synthesis and release of neuroprotective substances by the grafted cells. Genetically modifying neural progenitor and stem cells could therefore also be applied for delivering trophic factors for the treatment for neurodegenerative diseases.

These data highlight the potential therapeutic of genetically modifying neural progenitor and stem cells for the treatment of CNS diseases and disorders. The potential of genetically modified NSCs is further highlighted by their potential for the treatment of brain tumors. Neural progenitor and stem cells migrate to tumors, injured, diseased sites when transplanted in the CNS, either by systemic injection, or through the cerebrospinal fluid (Brown et al 2003; Fujiwara et al 2004). The injected cells migrate to the diseased or degenerated areas where they integrate the host tissue. The properties of NSCs to be genetically modified and to migrate to tumor sites have been proposed for the treatment of brain tumors. It is proposed to genetically modified NSCs with "suicide genes", like genes coding for cytolytic activities or anti-tumor cytokines, to attack and destroy brain tumor cells (Yip et al, 2003). This further extends the use of cell engineering of NSCs for cancer therapy in the CNS.

In all, adult neural stem cells have the potential to treat a vast array of neurological diseases, without the ethical and political and ethical issues surrounding ESC research. However, NSC remains an elusive cell. Further studies will aim at identifying and characterizing neural progenitor versus stem cells, at generating homogenous populations of neural progenitor or stem cells, and devising protocols to further enhance the differentiation potential of neural progenitor and stem cells.

IV. Conclusion

Stem cell therapy holds the promise to treat a broad range of diseases and injuries. The promise of stem cell research and therapy is to regenerate and reconstruct the original pathway to promote functional recovery, but it may be years away before it emerges as a viable therapy. Genetically modifying cells has proven valuable to understand gene function, and to deliver trophic factors or neurotransmitter synthesizing enzymes in the CNS. The studies reported show that genetically modifying stem cells may therefore offer an opportunity to bolster stem cell research and therapy. Further studies involving stem cell research and gene therapy will aim particularly at devising strategies to derive pluripotent stem cells without the destruction of embryos that are suitable for therapy, at understanding the role of trophic factors in the in mediating recovery in stem cell transplant and developing vectors allowing sustained expression of the transgene of interest.

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