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Review Article

Gene therapy and stem cells in the treatment of congenital diseases

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ABSTRACT

Several congenital diseases are particularly attractive candidates for intervention using gene therapy since the underlying molecular bases for most of the monogenic disorders are well-understood. Transplantation of *ex vivo* genetically modified stem cells has also shown promise. Although all of these systems are meritorious and worthy of continued investigation, this mini-review article focused on the platforms that have received the most attention and that are maturing in the clinical setting; in particular, the potential of *in vivo* gene therapy and human-induced pluripotent stem cells. Studies of apparently disparate diseases that are presumably linked through shared metabolic pathways are likely to provide greater insights into the biology of the diseases. This and other opportunities for exchange will hopefully foster acceleration in the development of new and innovative therapies for these devastating diseases.

KEY WORDS: Congenital diseases, frozen embryos, gene therapy, pluripotency, stem cells

INTRODUCTION

Congenital diseases are responsible for over a third of all pediatric hospital admissions. Advances in prenatal screening and molecular diagnosis have allowed the detection of many life-threatening genetic diseases early in gestation. The options of preemptive treatment of congenital diseases in utero by gene therapy or stem cell changes the perspective of congenital diseases since it may avoid the need for postnatal treatment and reduce future costs.

GENE THERAPY

Gene therapy is promising in the treatment of many congenital diseases. With all the hoopla surrounding the Human Genome Project, it is understandable that people would entertain high hopes for the advancement of gene therapy. It falls into three groups: (1) replacing a defective or bad adaptive gene that's responsible for some monogenic disease, (2) altering or killing an aberrant cell (e.g. infected by HIV or cancerous) and (3) inducing production of a therapeutic protein. Initially, gene therapy focused on the first group, but most current research focuses on the other two. Whatever the application, numerous hurdles stand in the way of developing a successful gene therapy. These obstacles include identifying an appropriate target for gene therapy such as getting a therapeutic transgene into the right cells (and only those cells) in the right amount; delivering the transgene with a vector that does not trigger an immune response or, in the case of certain viral vectors, revert to a pathogenic form; providing the appropriate regulatory elements for turning the gene on and off at the correct time; keeping the transgene in the target cell long enough for it to do its job; and keeping the transgene from causing damage elsewhere [1-6]. The advances in gene therapy hold significant promise for the treatment of ophthalmic conditions such as heritable diseases of the retina [7-9], endocrinology [10], rheumatic diseases [11-13], Alzheimer's disease [14], diseases of the gastrointestinal tract [15-22], therapy of cancer [23-27] and neurological disorders [28,29]. In addition, diseases like inborn errors of metabolism (include the diseases resulting from enzyme defects in biochemical reactions due to genetic mutations), and human peroxisomal disorders (caused by peroxisomal ABC half-transporters mutation which is localized in the peroxisomal membrane) could be candidate to gene therapy [30-33].

In addition, artificial chromosomes (ACs) are highly promising vectors for use in gene therapy applications. They are able to maintain expression of genomic-sized exogenous transgenes within target cells, without integrating into the host genome. The recent developments in AC technology present improved methods for the production, purification, delivery, and natural transgene expression of genomic sized loci. These technologies are all steps forward in alleviating problems associated with synthetically produced cDNA. In addition, AC technology is proving to be highly compatible with stem-cell research. With further development, ACs could be used to improve the

efficacy of gene therapy by providing physiologically appropriate expression of transgenes *in vivo* in target tissues [34]. Moreover, carbon nanotubes have been proposed and are actively being explored as innovative multipurpose carriers for biomolecules and diagnostic applications. They are used in the controlled release of drugs as well as delivery of genetic material such as DNA, genes, and antibodies [35-37].

Ethical and political issues need to be addressed, but, over the long-term, the future of drug therapy could be gene therapy. Then, this article focused on the potential of *in vivo* gene therapy and alternative sources of pluripotency as perspectives in the treatment of congenital diseases.

ALTERNATIVE SOURCES OF PLURIPOTENCY

It has recently been shown that the first cleavage plane of the mouse zygote defines the border between the embryonic and abembryonic parts of the blastocyst and that this border correlates with the sperm entry position (SEP). Plusa *et al.* [38] developed a means of fluorescently labeling sperm that can record the exact site of its penetration when the label transfers to the egg surface. This approach indicates that the SEP marks the first cleavage in the great majority (88%) of embryos, e.g. life.

In the same way, Takahashi *et al.* [39] and Yu *et al.* [40] suggested alternative sources of pluripotency. They demonstrated that expression of four specific transcription factors (Oct4, Sox2, Klf4, and c-Myc) gives adult human fibroblasts many of the characteristics of human embryonic stem cells (hESCs). Refinements of this procedure will make it possible to produce pluripotent human cell lines without the use of an embryo [41-46]. In addition, human induced pluripotent stem (iPS) cells obtained by reprogramming technology are a source of great hope, not in terms of applications in regenerative medicine, such as cell transplantation therapy, but also for modeling human diseases and new drug development. In particular, the production of iPS cells from the somatic cells of patients with intractable diseases and their subsequent differentiation into cells at affected sites (e.g., neurons, cardiomyocytes, hepatocytes, and myocytes) has permitted the *in vitro* construction of disease models that contain patient-specific genetic information [47-95]. For example, disease-specific iPS cells have been established from patients with neuropsychiatric disorders, including schizophrenia and autism, as well as from those with neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease [96-102].

Finally, stem cells have the capability to proliferate and differentiate into various cells of the body. Few stem cell sources have been approved for transplantation; among them are the hematopoietic progenitor cells which are progenitors of the myeloid and erythroid lineage in the hematopoietic system that continually provides mature blood cells during the lifespan of the individual. These well-characterized stem cells are clinically relevant in the treatment of diseases such as breast cancer, leukemias, and congenital immunodeficiencies. In addition, the investigation of mesenchymal stem cells secretome is accumulating continuously increasing interest given the potential use of these cells in regenerative medicine [103-107].

De Coppi *et al.* [108] reported the isolation of human and rodent amniotic fluid-derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. Differentiated cell derived from human AFS cells are displaying specialized functions which include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue-engineered bone [109-116].

Despite many advances in hESC technology; the ethical dilemma involving the destruction of a human embryo is an important factor limiting the development of hESC based clinical therapies [117]. The application of embryo freezing to human *in-vitro* fertilization (IVF) has revolutionized its clinical practice and helped to convert IVF from an experimental procedure to the widespread practice. In Australia, more than 7000 babies have been born following the transfer of frozen-thawed embryos [118,119].

It is worth considering how couples can be encouraged to donate rather than discard their surplus frozen embryos. At present, there are numerous frozen embryos ready for donation. Moreover, it has been speculated that the primary means by which reactive oxygen species reduce the fertility of semen subjected to refrigeration or long-term liquid storage is its impact on sperm DNA integrity [120,121]. An educational program around the world on relevant legal, social, and clinical issues may facilitate this, and therefore, give an ethic destination to the human embryo frozen.

Author contribution

Paula RS Câmara wrote the paper and reviewed it.

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