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Fetal gene therapy: Opportunities and risks☆,☆☆

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ABSTRACT

Advances in human prenatal medicine and molecular genetics have allowed the diagnosis of many genetic diseases early in gestation. In-utero transplantation of allogeneic hematopoietic stem cells (HSC) has been successfully used as a therapy in different animal models and recently also in human fetuses. Unfortunately, clinical success of this novel treatment is limited by the lack of donor cell engraftment in non-immunocompromised hosts and is thus restricted to diseases where the fetus is affected by severe immunodeficiency. Gene therapy using genetically modified autologous HSC circumvents allogeneic HLA barriers and constitutes one of the most promising new approaches to correct genetic deficits in the fetus. Recent developments of strategies to overcome failure of efficient transduction of quiescent hematopoietic cells include the use of new vector constructs and transduction protocols. These improvements open new perspectives for gene therapy in general and for prenatal gene transfer in particular. The fetus may be especially susceptible for successful gene therapy due to the immunologic naiveté of the immature hematopoietic system during gestation, precluding an immune reaction towards the transgene. Ethical issues, in particular those regarding treatment safety, must be taken into account before clinical trials with fetal gene therapy in human pregnancies can be initiated.

Contents

1. Introduction

Over the last two decades extensive efforts have been made to develop techniques for early prenatal diagnosis of certain genetic diseases. Today, fetal material can be obtained invasively for genetic analysis as early as in the first trimester by chorionic villus sampling

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(CVS) [1]. Prenatal diagnosis for chromosomal defects or single gene disorders is based on molecular biology techniques such as PCR using either fetal material from an invasive test or, alternatively, uses fetal cells or fetal cell-free DNA from maternal blood [2]. In particular, non-invasive techniques have been shown to be feasible in prenatal diagnosis of hemoglobinopathies [3,4].

Using these modern techniques, many genetic diseases of the fetus including immunodeficiencies and diseases of the hematopoietic, connective tissue and skeletal systems can now be diagnosed. Examples include thalassemias, sickle cell anemia, severe combined immunodeficiency syndrome (SCID), osteogenesis imperfecta and storage diseases like Hurler's syndrome or globoid-cell-leukodystrophy (Krabbe's Disease). With the advent of new molecular methods including DNA-chip technology, it is conceivable that non-invasive screening of populations at risk might rapidly increase the number of diseases diagnosed prenatally. Termination of pregnancy when the fetus is diagnosed by a potentially fatal disease should, however, not be the consequence of prenatal diagnosis. Rather, advancements in treatment options must be the ultimate goal to improve long-term prospects for the future child.

2. Current issues of postnatal gene therapy

2.1. Allogeneic stem cell transplantation

Postnatal treatment of genetic diseases is often limited. For some of them, transplantation of allogeneic HSC from bone marrow, mobilized peripheral blood or umbilical cord blood has the potential for definitive cure. However, although promising results have been achieved with stem cell transplantation (SCT) [5–8], several obstacles with postnatal SCT therapy remain. Only for about one third of the patients a MHC-compatible donor can be found. The need for immunosuppression and bone marrow ablation in the recipient (except for SCID patients) leads to potentially life-long treatment-associated morbidity. Graft versus host disease (GVHD) and graft failure are common complications of non-related donor stem cell transplantation. Additionally, underlying genetic disease may lead to irreversible damage to the fetus already before birth (e.g., storage diseases or severe alpha-thalassemia). It is because of these limitations that many parents choose the option to terminate pregnancy when facing the diagnosis of a severe genetic defect in a previable fetus.

2.2. Gene therapy

Many postnatal gene therapy approaches are targeting a range of non-hematopoietic diseases such as hemophilic disorders (i.e., factor IX deficiency) or cystic fibrosis (CF). For some of these disorders, initial clinical experience has already been obtained, with variable success [9]. HSC are particularly attractive targets for somatic cell-based gene therapy, because they have the potential to produce permanently progeny containing a therapeutic gene lifelong. Current clinical protocols of postnatal gene therapy in paediatric patients with genetic diseases are based on ex vivo retroviral transduction of lymphocytes [10] or hematopoietic stem/progenitor cells from cord blood or bone marrow, followed by autologous transplantation of these engineered cells back into the patient. Initial trials showed the feasibility and safety of gene therapy using cord blood cells in patients with ADA-deficiency, although only very limited clinical efficacy has been achieved as reported in a follow-up study [11,12]. Recently, however, clinical success has been reported ten month after gene therapy in X-SCID disease using autologous retrovirally transduced $CD34^+$ bone marrow cells [13,14].

In spite of its positive aspects, several issues must be solved before gene therapy can gain broad clinical application [15,16]. In particular, the problem of insertional mutagenesis [17,18] must be addressed as a primary safety issue. The development of T-cell leukaemia following the otherwise successful treatment of patient with X-SCID in gene therapy trials using haematopoetic stem cells reported in 2003 has led to a re-evaluation of this approach [19–21] Also, the variables that need to be addressed include transduction efficiency, random integration of vector-gene-construct into host genome, duration of expression of the therapeutic gene ("gene silencing"), host immune response against vector, gene or gene product, and reproducible production of safe replication-free high-titer vectors [22]. Gene expression can be severely impaired by spontaneous cessation of regulatory sequence activity that control gene expression, by inactivation of promoters (e.g. by methylation) in the transduced host cell, by specific host defense mechanisms or by elimination of the transduced cells by the host immune system recognizing the foreign gene product [23]. Inflammatory cytokines such as TNF-α or IFN-γ have been shown to be involved in the host immune response towards the "foreign" gene or gene product by direct inhibition of the expression of transgenes [24]. Although some success has been achieved in mouse models [25], recent results in large animal models reveal that gene expression in vivo can still be severely impaired despite successful engraftment of genetically modified autologous HSC [26]. A further difficulty is the identification of the target for gene transfer, i.e. HSC, because definitive markers for undifferentiated, quiescent stem cells are still lacking. Recent reports suggest that there is no single HSC marker, leading to the assumption that the HSC compartment is heterogeneous [27–29].

Gene delivery systems to HSC include retroviral and adenoviral vectors, adeno-associated vectors (AAV), lentiviral (HIV-based) vectors and non-viral (liposome) vectors [22]. New generation AAV vectors have just recently been reported as leading to successful long-term gene expression and correction of hemophilia B in canine and mouse models after intrahepatic [30] or intramuscular [31] injection. Furthermore two new studies report improvements in the vision of patients with Leber's congenital amaurosis, a genetic degenerative disease of vision subsequent to injection of adeno-associated virus (AAV) into the subretinal space [32]. Because retroviruses — as opposed to adenoviruses — have the property to stably integrate into the host genome, they are preferentially used to deliver genes into HSC in experimental animal models and clinical settings. However, retroviral gene transfer into non-dividing cells is inefficient, in addition to the lack of control of long-term transgene regulation and expression in transduced cells [33]. Recent advancements in gene transfer techniques aim an enhancing transduction efficiency [34]. Host DNA replication can, however, be achieved only after stem cells are released from quiescence by prestimulation with growth factors in culture, which in turn leads to cell differentiation and loss of self-renewal and multilineage differentiation capacity [15]. New strategies for efficient and stable retroviral transduction of hematopoietic stem/progenitor cells by new generation of retroviral vectors and optimized transduction conditions, including prestimulation with novel growth factors such as thrombopoietin and flt3-ligand, centrifugation ("spinoculation") or transduction on fibronectin fragments, are evolving and yield promising results [35,36]. Nevertheless, because non-cycling cells cannot be transduced efficiently, release from quiescence in vitro is still necessary. The development in the field of non-viral vectors such as human artificial microchromosomes as vectors for gene delivery into mammalian cells [37] is rapidly evolving, though these vectors are still far away from application.

New viral vector generations include replication-deficient lentiviral HIV-based vector systems, which can integrate stably into the host genome in dividing and non-dividing cells [38]. Different replication-defective lentiviral vectors based on HIV have been constructed, including some in which one or more of accessory genes are deleted because of their association with intracellular pathological effects [39]. Requirements for HIV regulatory and accessory genes depends on the nature of target cells and can have a major impact on transduction efficiency, as has been shown by in