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Placenta-directed gene therapy for fetal growth restriction

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A B S T R A C T

Fetal growth restriction (FGR) is a serious pregnancy complication affecting ~8% of all pregnancies. There is no treatment to increase fetal growth in the uterus. Gene therapy presents a promising treatment strategy for FGR, with the use of adenoviral vectors encoding for proteins such as vascular endothelial growth factor (VEGF) and insulin-like growth factor demonstrating improvements in fetal growth, placental function, and neonatal outcome in preclinical studies. Safety assessments suggest no adverse risk to the mother or fetus for VEGF maternal gene therapy; a clinical trial is in development. This review assesses research into placenta-directed gene therapy for FGR, investigating the use of transgenes and vectors, their route of administration in obstetrics, and the steps that will be needed to take this treatment modality into the clinic.

1. Introduction

Gene therapy allows for the transfer of genetic material into a target cell with the aim of achieving therapeutic benefit. Since the first gene therapy trials in the 1990s, there has been hope that gene therapy could improve the management and outcomes of genetic diseases, particularly single-gene disorders. There are currently >1800 completed or ongoing gene therapy clinical trials, of which more than two-thirds are on cancer. Gene therapies are now becoming licensed for use in the clinic, the first being Gendicine™ in 2003 in China, for the treatment of head and neck squamous cell carcinoma, closely followed by Oncorine™, a p53 gene therapy for late-stage nasopharyngeal cancer in 2005 [1]. In 2012 Glybera™, a treatment for familial lipoprotein lipase deficiency, became the first gene therapy product to be approved for licensing in Europe [2].

Preclinical studies show that adeno-associated viral vector gene therapy to the fetus may cure single-gene disorders such as haemophilia. Concerns about germ-line gene transfer and off-target effects in the fetus, however, are holding back translation into the clinic of fetal-directed gene therapy [3]. Serious maternal obstetric diseases such as pre-eclampsia and fetal growth restriction (FGR) also affect the fetus and neonate long term. Examination of the molecular basis of these untreatable obstetric diseases has led to an understanding of the potential role that placenta-directed gene therapy might play [4]. This review considers the application of gene therapy to the placenta to treat fetal growth restriction. Results of preclinical studies are compelling and clinical trials are being planned. The limitations and risks of gene therapy in the maternal setting are evaluated, and the current ethical and regulatory issues are presented.

2. Fetal growth restriction

Optimal fetal growth depends on functioning maternal, placental and fetal factors, the external environment, in conjunction with a genetically predetermined growth potential. FGR may occur due to a malfunction of a single or number of these factors. It is potentially life-threatening and affects 8% of all pregnancies, contributing to 50% of stillbirths [5]. Of those diagnosed with FGR, about one in 500 cases is classified as both severe and early onset, occurring before 28 weeks of gestation. Some severe FGR is caused by structural abnormalities of the fetus, maternal medical disorders, and congenital infections. Most usually, impaired uteroplacental function restricts delivery of nutrients to the fetus, resulting in slowing or even in cessation of fetal growth, termed placental insufficiency.

In normal pregnancies, effective first-trimester infiltration of the trophoblast in the maternal spiral arteries leads to the creation of a high-flow, low-resistance maternal circulation. Angiogenesis and vasodilation in the placenta are enhanced by the production of factors such as placental growth factor (PlGF), vascular endothelial

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growth factor (VEGF), and insulin-growth factor (IGF) [6,7], which facilitates a reduction in placental resistance. The obstetric syndromes of pre-eclampsia and FGR appear to be interrelated through VEGF biology. An increase in soluble fms-like tyrosine kinase 1 (sFlt1), which acts as a soluble receptor for VEGF in the maternal circulation, is observed in both conditions [8,9]. Treatments based on the manipulation of VEGF and related angiogenic factors are therefore likely to be effective for FGR and pre-eclampsia.

When FGR is severe and early in onset, management involves prompt delivery of the fetus before death or irreversible organ damage occurs, especially to the brain. However, delivering the fetus in severe early-onset FGR adds additional risks to the baby from extreme prematurity [10]. In this situation the question of viability also arises, and decision-making with parents is challenging [3]. Substantial improvements in morbidity and mortality may be seen if delivery of such pregnancies is delayed by even one week (e.g. from 26 to 27 weeks) and if there are modest increases in birth weight (e.g. 100 g) [11]. It is in these severe early-onset cases of FGR that maternal gene therapy is initially being considered, where the benefit of gaining gestation or improved fetal weight might outweigh the potential risks of a novel therapy. If it is found to be safe and efficacious there is potential to use maternal gene therapy in more moderate FGR, which affects a larger number of pregnancies.

3. What is gene therapy?

Gene therapy is the transfer of genetic material to targeted cells in order to modify or treat a disease. To produce a therapeutic result, genetic information, otherwise known as a transgene, is introduced using a vector into the target cells. The transgenic protein that is produced by transcription of the transgene generates a therapeutic effect. More recently gene editing has emerged as a potentially more targeted form of gene therapy whereby a nuclease cuts the DNA helix to create a specific double-stranded break, which is then repaired using template DNA to enable the production of a therapeutic protein [12].

Somatic or stem-cell gene therapy is applied directly to target organs or cells, but does not cause multi-generational effects. Germ-line therapy, however, would be passed on to future generations, which current legislation precludes. Transgenes consist of several elements: a promoter, which regulates when and how the transgene is activated; an exon, the part of the gene that encodes mature RNA to produce a coding sequence; and finally, an intron, which acts as a transcriptional stop sequence. Gene therapy has proved to be successful in single gene disorders, whereby a missing or defective gene can be replaced, as in the case of haemophilia, β-thalassaemia, and X-linked severe combined immunodeficiency. Achieving safe, long-term expression continues to be a challenge in monogenic disorders [2]. Within cardiovascular disease, VEGF gene therapy is being applied to coronary artery disease [13].

3.1. Types of vector

The choice of vector is critical in gene therapy. Manufacture of the vector would ideally be simple and cost-effective, it should be capable of being targeted to the specific tissue or organ, and it should generate a transgenic protein for the required length of time to have a therapeutic effect without causing side-effects [14]. For obstetric conditions such as FGR and placental insufficiency, the therapeutic timeframe would be short, limited by the length of gestation. When targeting specific organs such as the placenta, the method of delivery may have considerable impact on the level and site of gene expression.

The most widely tested vectors in fetal gene therapy preclinical studies have been adenovirus and adeno-associated virus, lentivirus, and retrovirus vectors. The characteristics of these and other less widely used vector systems are listed in Table 1. Manipulating the vector structure and the transgene may alter vector properties. Pseudotyping, for example, involves changing the virus capsid (outer covering) for one of a different serotype or of a completely different virus, thus altering its ability to infect particular cell types or organs [15]. Using alternative enhancer-promoters may improve gene transfer to specific organs or tissues, and may even be manipulated to allow regulatable gene expression if required [16]. Many replication-deficient lentiviruses are based on the immunodeficiency virus, with a theoretical possibility of reversion to the wild type. In third- and fourth-generation lentivirus vectors, however, the risk of in-vivo generation of replication competent viruses is reduced by removal of the *tat* gene. Modification of virus elements, such as mutating the integrase in lentiviral vectors, renders it incapable of integrating and greatly reduces the risk of insertional mutagenesis [17]. Clinical grade production of vectors is tested rigorously for replication-competent viruses.

Gene editing is an attractive alternative approach to correcting gene defects, which avoids the use of vectors to introduce therapeutic transgenes [12]. Reports of successful applications to genomic targets are appearing at an accelerating rate. RNA-guided engineered nucleases (RGENs) derived from the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)-Cas (CRISPR-associated) system are now available that give high-precision genome editing.

4. Maternal gene therapy

Maternal gene therapy aims to facilitate expression of proteins in the mother that have translational benefits to the fetus, given the antenatal interdependence between a mother and fetus. Only short-term gene expression would be required to manage obstetric disease, and the concerns surrounding monogenetic and oncologic conditions requiring long-term gene therapies would not apply.

4.1. Vascular endothelial growth factor

Vascular endothelial growth factor stimulates angiogenesis and vasculogenesis, and plays an important role in hypoxic conditions where circulating blood flow is insufficient. Overexpression of VEGF may also cause diseases such as cancers, where improvements in blood circulation promote tumour growth. In mammals, five of seven known proteins within the VEGF family occur naturally: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). Exon splicing of the gene that encodes VEGF-A creates five molecular variants, differentiated by their amino acid count, of which the predominant form is VEGF-165. Angiogenic effects have been observed in VEGF-A and VEGF-D; VEGF-D may be pre-processed to a shorter more highly active VEGF-$D^{\Delta N \Delta C}$ isoform [18]. Three known VEGF receptors – VEGFR-1, VEGFR-2, and VEGFR-3 – bind proteins in order to generate angiogenic and lymphangiogenic effects [19]. Vasculogenesis is reliant on VEGF-A, with inactivation of even a single allele resulting in dysfunctional vascular development and even in embryonic death [20,21].

Experiments in pregnant sheep and guinea-pigs have demonstrated that adenovirus vectors containing VEGF isoforms A_{165} (Ad.VEGF-A_{165}) or preprocessed D isoform (Ad.VEGF-$D^{\Delta N \Delta C}$) increase uterine blood flow and fetal growth in normal and FGR pregnancy. In sheep, mid-gestation injection of the Ad.VEGF-A_{165} vector into the uterine artery via laparotomy was compared with the effect of contralateral uterine artery injection of a control β-galactosidase (Ad.LacZ) vector containing a non-vasoactive transgene LacZ. Uterine artery blood flow was significantly increased in

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