In Utero Gene Therapy

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Gene therapy holds the promise of therapies for genetic diseases, cancer, heart disease, and more. However, the promise and associated expectations, so far, greatly exceed reality. As we try to move theoretical and laboratory bench technologies to the bedside, Mother Nature confronts us with ever-greater hurdles. Great advances have been made in the identification and construction of genes, but attempts at inserting them and coaxing them to function normally within the human body have been fraught with frustration. The immune system seeks out and destroys cells expressing our newly inserted genes and, recognizing them as foreign, silences the man-made constructs before they are productive. Our messengers for better health lose their way within the vastness of the human body.

Solving these and other barriers to successful gene replacement and therapeutic efficacy are the main goals of the gene therapist. In the search for safe, reliable, and effective gene therapy, two different but complementary general approaches have been fostered. The first involves extensive modification of the vectors (usually viral in origin) that carry the genes. The vectors are designed in an attempt to reduce their antigenicity and thus minimize their recognition by the immune system. The second approach involves actual suppression of the immune system. Certainly, these approaches will be successful over time, but there are many hurdles, and new obstacles appear with each successful leap forward.

Our laboratory has pioneered a different approach to gene insertion that works within the natural development of cell function. During human fetal development, a window of opportunity exists for the therapeutic insertion of genes without incurring the wrath of the immune system. The fetus is an immune tolerant site that is full of progenitor cells designed to last a lifetime. The insertion of genes into this receptive environment could provide the opportunity to correct genetic deficiencies and prevent diseases rather than just treat their destructive effects.

<table>
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<th>Cell Type</th>
<th>Definition</th>
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<tr>
<td>Somatic</td>
<td>Mature cell that has a defined life span and a specific function. An example is the skin epithelial cell.</td>
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<tr>
<td>Stem</td>
<td>Immature cell, also called a multipotential cell, that can mature through differentiation into a variety of related cell types. An example is the hematopoietic stem cell.</td>
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<td>Germ line</td>
<td>Egg or sperm cell (haploid in chromosome number) having the potential to develop into every cell in the body and, thus, can pass any genetic material on to subsequent generations.</td>
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In some ways, this is comparable to our vaccinations of the newborn to prevent infectious diseases much later in life. In this special case, the developing treatment of genetic disorders, the injections are performed at an earlier stage of life.

In utero gene therapy offers several advantages in the treatment of genetic disorders. The large amount of somatic stem cells readily available for gene transfer in the fetus has made this a promising technology (Table 1). The permanent replacement of a gene into a somatic stem cell would ensure that daughter cells would also carry the gene, obviating the need of repeated therapy during the lifetime of the affected individual. However, in searching for somatic stem cells as targets for gene therapy, researchers have discovered that they are difficult to find in the adult. Even the adult bone marrow has only a small percentage of stem cells. Not only are the hematopoietic stem cells present in greater quantities in the fetus, but, at the appropriate gestational age, stem cells of epithelial origin are present in organs such as the intestine and the lung. Stem cells in developing organs express high levels of integrins, cell surface proteins that promote the attachment and uptake of the viral vectors. Therefore, the availability of target cells and the efficiency of gene transfer into them are improved in the fetus.

The second approach to in utero gene therapy makes use of the greatly reduced inflammatory response of the fetus. Because the fetus is an immune-privileged site, the immune reactions against the vector and the transgene protein are diminished. This also improves the efficacy of the viral infection. In utero transfer simplifies gene therapy with the vector technology currently available.

Both the amniotic fluid and the fetal circulation are readily available for fetal gene delivery. The umbilical vein is used to reach the fetal circulation and may be useful for systemic replacement strategies. This method of delivery, however, increases the risk of germ line transmission. Although the subject of much controversy, many researchers feel that, given our current level of knowledge, the avoidance of gene transfer to the germ line is most responsible at this time. In some countries, such as France, germ line transfer is illegal. Germ line transfection is unlikely when the gene is delivered via the amniotic fluid, especially after the primordial germ cells have migrated to the gonads (at 7 weeks’ gestation in humans).

Our group at the Alton Ochsner Medical Foundation and Louisiana State University Health Sciences Center has made the high-efficiency adenoviral-mediated transfer to the fetus via the amniotic fluid a reality (1–4). Genes injected into the uterine sac and the amniotic fluid with the aid of ultrasound can reach the epithelium of the fetal lungs and gut via normal fetal swallowing and breathing movements.

Because the epithelial cells lining the intestine and lung start differentiating towards the end of gestation, a working knowledge of lung and gut development is crucial to the success of this therapy and the application of the research. When a fetus is targeted too late, an insertion will reach only the trachea and an inflammatory response will occur. Researchers must also take into consideration the variations in developmental timing even between species when planning injections. For example, when mice pups are born, their lungs are still very immature compared to the rodent, rhesus primate, and human. At birth the rodent lung is immature compared to the human and rhesus lung. The shaded bar represents the window of opportunity, early in the second trimester of primate pregnancy, most conducive to vector infection.
with a 28-week (196-day) human fetus. Alveoli do not develop in this rodent species until almost a week following birth. In contrast, the lungs of primates (Rhesus and humans) are more mature at birth and contain developed alveoli. Therefore, it is necessary to target humans and rhesus early in the second trimester (Figure 1) to achieve successful infection.

A good motto for in utero gene therapists is “as little as possible, as early as possible.” Infection with high concentrations of virus in utero is a self-defeating strategy since a large inoculum of virus fails to take advantage of the unique environment of the fetus and, if challenged sufficiently, an inflammatory response occurs (though blunted) in the fetus.

Candidates for in utero gene therapy include diseases corrected by replacement of an inactive or absent protein. Many autosomal recessive disorders function in this manner. Autosomal dominant disorders are more difficult to correct because the symptoms of the diseases often result from the expression of structurally abnormal proteins.

One of the main candidates considered for in utero gene therapy is cystic fibrosis (CF), which is thought to be caused by mutations to the cystic fibrosis transmembrane conductance regulator gene (cfr) resulting in the absence of the protein’s chloride channel (3). Recent in utero gene therapy experiments targeting the cfr gene in mice yielded encouraging results (2,3). The S489X cystic fibrosis knockout mouse strain (cfrtm1Unc) mimics the abnormalities of the gastrointestinal tract seen in human CF patients (5). Approximately 5%-10% of newborns with CF present with meconium ileus, a form of intestinal obstruction. Without intervention, the S489X cfr-mutant (cfr/-) mice develop intestinal obstruction, resulting in less than 5% survival to adulthood. The complete reversal of the lethal intestinal blockage phenotype of the knockout mouse occurs following a single in utero treatment with adenovirus carrying the normal gene (2).

Following in utero transfer with an adenovirus vector carrying the cfr gene, low levels of adenoviral DNA were detectable in the fetal gut up to 72 hours after infection but not after birth. Although the transgene is gone by birth and no traces of the cfr protein are found, knockout mice survive over a year of age while their untreated littermates die before reaching adulthood.

These results show the viability of the in utero gene therapy approach and the possibility for extraordinary results. These data confirmed what many suspected: symptoms of CF cannot be wholly explained by the absence of cfr function in adulthood; cfr must play a role during development. In fact, cfr is highly expressed in the developing lung and gut. More cfr protein is expressed in the lung of a 25-week fetus than in an adult lung, suggesting that cfr is required for normal development but not for normal function in the adult organ (3). Therefore, it is possible that the clinical symptoms of CF can, at the very least, be improved if not prevented with a single intrauterine vaccination via the amniotic fluid.

The correction of the lung defect in CF is an obvious candidate for this technology. In addition, the highly vascular nature of the lung might also make possible the replacement of enzyme deficiencies. The release of enzymes into the bloodstream from permanently transfected stem cells in the lung might replace a function missing in the liver. Retrovirus and adeno-associated viral systems can integrate into somatic stem cells, and thus those individual cells could express protein.

Retroviruses require dividing cells for integration. In the developing undifferentiated airway epithelium, approximately one-third of the cells are dividing at any given time. Unfortunately, adenovirus is more stable in amniotic fluid than retrovirus, which is partially inactivated by the fluid. In addition, there is the remote chance that insertional mutagenesis (the insertion of the transgene into a functioning gene and subsequent mutation of that host gene) can occur using retroviruses. In this regard, careful monitoring will be required to assure that the germ line remains unaffected before any human trials occur.

Based on this knowledge of lung development, our laboratory recently started trials in the fetal rhesus primate, targeting the fetuses at the same time as the successful rodent research. Experimental success with fetal gene therapy in the mouse and rat via amniotic fluid injection targets the equivalent of a human fetus of 10–20 weeks’ gestation. Because amniocentesis is performed routinely at this gestational age, in utero gene therapy can be considered feasible for human use.

Rhesus macaque (Macaca mulatta) fetuses were injected with adenovirus vectors encoding reporter genes at different gestational ages to evaluate feasibility and timing in primates. The fetuses developed normally following gene transfer and no maternal adverse effects were noted. Highly efficient viral uptake and transgene protein expression occurred in the target organs of the lung and intestine. The lungs exhibited no immune response, and transgenic protein was observed up to 30 days post-infection. Polymerase chain reaction detection for adenovirus DNA was consistently negative in tissues not in contact with the amniotic fluid, such as kidneys, liver, gonads, and eyes. These studies demonstrate the safety and efficacy of in utero gene therapy in primates.
The fetus offers the gene therapist a new venue for exploring the promise of this technology. As shown by the results with CF in utero gene transfer, however, there may be surprising results when targeting stem cells. The practice of in utero gene therapy requires an open mind that is not anchored to concepts based on previous experience with adult somatic cell gene transfer.

References


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