Gene Therapy

In Utero Gene Therapy

Ideally, gene therapy strategies aimed at correcting genetic defects should target somatic stem cells. The permanent placement of a therapeutic gene into somatic stem cells could prevent the need for repeated therapy later in life. Avoiding the germline should ensure that the gene is not transferred to subsequent generations. The greatest availability of somatic stem cells occurs before birth, and the strategies that target these cells are known as fetal or in utero gene therapy.

Fetal somatic cell gene therapy avoids many problems currently faced by other gene therapy protocols. First, there is a large somatic stem cell population in the fetus, whereas in the adult it is difficult to find sufficient stem cells to adequately replace gene activity. Even the bone marrow has only a small percentage of stem cells in the adult. In the fetus, hematopoietic stem cells are present in higher proportions and, at the appropriate gestational age, stem cells of epithelial origin in the developing lung and intestine are accessible via the amniotic fluid. The simplistic structures of the early lung and gut contribute to this accessibility.

A second factor is that, at least with adenoviral vectors, efficiency of transfer may be improved. Integrins, the cell surface proteins that promote attachment and uptake of adenoviruses, are expressed at high levels in the developing lung. Thus, lower doses of virus are required for adequate transfer. Host response to adenovirus is dose-related, and the use of less virus results in a diminished immune reaction.

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The third condition favoring in utero gene therapy is the greatly reduced inflammatory response of the fetus. Immune reactions against the vector and the transgene protein are diminished because the fetus is an immune-privileged site. The lessened immune response also improves the efficacy of the viral infection. Basically, in utero transfer simplifies gene therapy with the currently available vector technology.

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 germline transfection, which is unlikely when the gene is delivered via the amniotic fluid, especially after the primordial germ cells have migrated to the gonads (seven weeks' gestation in the human). Genes introduced transabdominally into the amniotic fluid with ultrasound guidance reach the epithelium of the lungs and gut via fetal breathing and swallowing movements.

Perhaps the most critical factor in gene therapy in utero is gestational age. The advantages of fetal gene therapy are lost as the gestation approaches term. As an example, differentiation of epithelial cells follows a cephalocaudad pattern down the airways. As term approaches, the airways become complex and differentiated, integrin expression decreases, and the inflammatory response of the fetus improves.

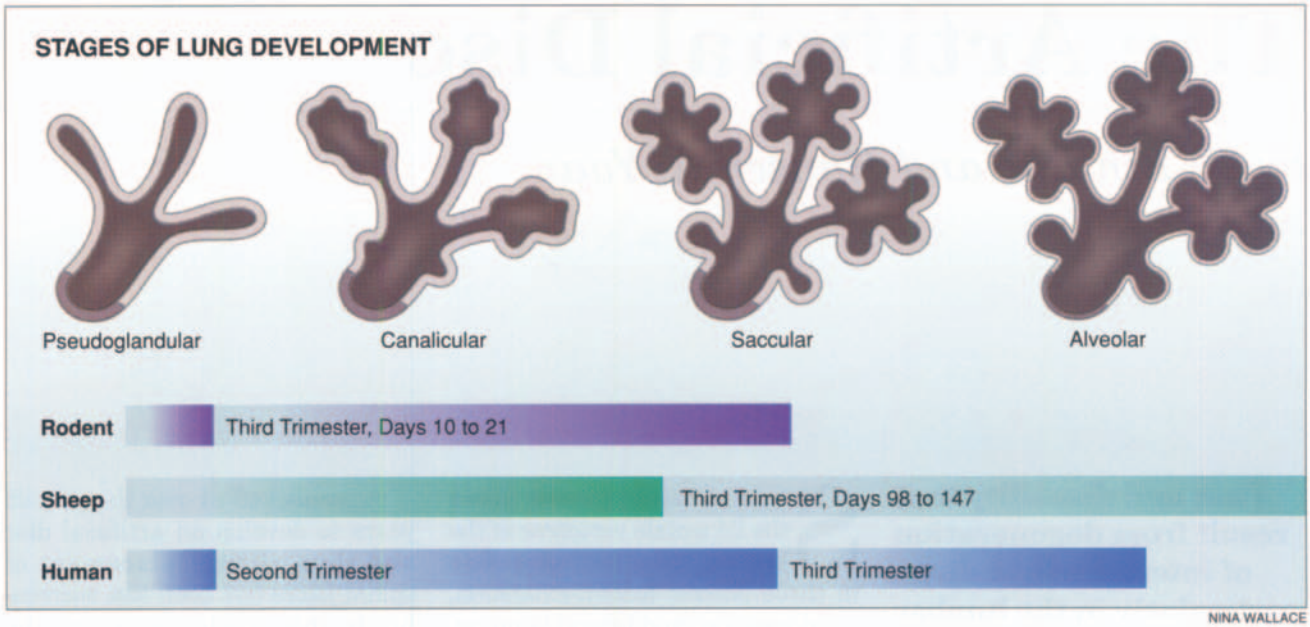
Differences in the timing of transfection can explain the mixed results of attempts at in utero gene therapy. A working knowledge of lung and gut development is crucial to success, and interpreting results obtained in other species is difficult. When rats and mice are born, their lungs are comparable in maturation to those of a 28-week human fetus. Alveoli do not develop in these species until almost a week after birth. In contrast, the lungs of a sheep at birth are more mature than those of a human.

If the rat and the sheep are targeted at the equivalent point during gestation, the lung of the rat is more likely to be successfully transfected because it is less mature. If transfection is attempted too late, only the trachea is infected and an inflammatory response ensues.

Experimental success in the mouse and rat with fetal gene therapy via amniotic fluid injection targets the equivalent of a human fetus at 10 to 20 weeks' gestation. Because amniocentesis is performed routinely at this gestational age, in utero gene therapy can be considered feasible for human use.

Candidates for in utero gene therapy include diseases corrected by replacement of an inactive or absent protein. Many autosomal recessive disorders can be treated in this manner. Autosomal dominant disorders are difficult to correct because the symptoms of the disease often result from expression of structurally abnormal proteins. In addition, tissue-specific expression in organs other than lung and gut is impossible to achieve via the amniotic fluid.

One of the main candidates for in utero gene therapy is the autosomal recessive disease cystic fibrosis, which results from mutations in a single gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The regulator is a cAMP-activated chloride channel that can also regulate a variety of other cationic and anionic channels. Although the under-



lying biological mechanisms are not completely understood, cystic fibrosis is thought to result from the failure of these channels to function correctly. Therefore, gene therapy strategies for cystic fibrosis aim to restore the normal phenotype by giving the patient the normal CFTR gene.

Approximately 5 to 10% of newborn humans with cystic fibrosis present with meconium ileus, a form of intestinal obstruction. A knockout mouse strain lacking the CFTR gene mimics the abnormalities of the gastrointestinal tract seen in patients with cystic fibrosis. Without intervention, the mutant mice develop intestinal obstruction, resulting in less than 5% survival to adulthood.

Recent experiments conducted in our laboratory with in utero gene therapy to replace the CFTR gene in these mice yielded surprising and encouraging results. Complete reversal of the lethal phenotype of the knockout mouse followed a single in utero transfer by injection into the amniotic fluid of a first generation adenoviral vector carrying the CFTR gene.

Low levels of adenoviral DNA were detectable in the fetal gut for up to 72 hours, but not after birth. Although no traces of the cAMP-activated chloride channel were found, 19 knockout mice were rescued, the oldest of which have now survived over a year. In contrast, all control mice with cystic fibrosis died within 40 days of birth.

These results show the viability of in utero gene therapy and the possibility for extraordinary results. In addition, these data confirm what many suspected, that symptoms of cystic fibrosis cannot be wholly explained by the absence of a cAMP-regulated chloride channel. CFTR must play a role in development of the intestines as well. Consistent with this conclusion is the previous finding that CFTR is highly expressed in the developing lung and gut. In fact, higher levels of CFTR occur in the lung of a 25-week fetus than in an adult lung. Therefore, it is possible that the clinical symptoms of cystic fibrosis could be at least improved if not prevented with a single intrauterine vaccination via the amniotic fluid.

Correction of the lung defect in cystic fibrosis is an obvious candidate for in utero gene therapy. In addition, the highly vascular nature of the lung might make replacement of enzyme deficiencies possible. Retroviral and adeno-associated viral vector systems can integrate into somatic stem cells, so that release of enzymes into the bloodstream from permanently transfected stem cells in the lung might replace a function missing in the liver.

Retroviral vectors transfer genes more efficiently than adenoviruses but require dividing cells for integration. In the developing undifferentiated airway epithelium, approximately one third of the cells are dividing at any given time. Unfortunately, retroviruses are less stable in amniotic fluid than adenoviruses, and there is also a remote chance that insertional mutagenesis will occur using retroviruses. Careful monitoring will be required to assure that the germline remains unaffected before human trials can begin.

The fetus offers the gene therapist a new venue for exploring the promise of this technology. As shown by the results with cystic fibrosis, however, be prepared for surprising results when targeting stem cells and do not be anchored to concepts based on somatic cell gene transfer.

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