CFTR gene therapy, a method to rescue lung hypoplasia in congenital diaphragmatic hernia?

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Despite progress in neonatal care and despite changing concepts in treatment in the recent years, the mortality rate of infants with congenital diaphragmatic hernia (CDH) remains high (20). Pulmonary hypoplasia and vascular alterations associated with CDH have tremendous postnatal functional impact on respiratory function, including ventilatory insufficiency and pulmonary hypertension. In the current article in focus, Larson and Cohen (Ref. 14, see p. L4 in this issue), using an experimental model of CDH in the rat, propose a very innovative new approach to treat lung hypoplasia that consists of inducing transient overexpression of cystic fibrosis transmembrane conductance regulator (CFTR) in fetal lung.

The etiology of CDH has remained elusive. CDH sporadically occurs with an incidence of about 1 of 2,500 living births and may be isolated (>50% of instances) or associated with other abnormalities. The diaphragmatic lesion presents as a hole that is left-sided in 85% of the cases, right-sided in 13%, and bilateral in 2%. Whether lung hypoplasia results only from compression of lung tissue by herniated viscera in the rib cage or from a primary lung defect remains a matter of debate. The possibility to surgically reproduce most of the features of the disease argues in favor of the first assumption, whereas the effects of the herbicide nitrofen, which induces in rodents other isolated lung hypoplasia or lung hypoplasia associated with CDH, support the second one. However, normal diaphragm development in mice lacking fibroblast growth factor 10, whose lungs fail to develop, argues against a primary lung hit (4). The leading causes of diaphragmatic defect are unknown, but gene-validation approaches in the mouse have recently allowed several genes involved in diaphragm development to be identified. This suggests the probable multifactorial origin and multiplicity of possible causes of the disease. Whatsoever, CDH lungs display immature appearance with fewer alveoli, thickened alveolar walls, increased interstitial tissue, and markedly diminished alveolar air spaces and gas-exchange surface area (8). Although these features are most prominent in the lung ipsilateral to the hernia, abnormalities are present also to some extent in the contralateral lung. Lung hypoplasia of CDH appears to result, at least in part, from impaired branching morphogenesis in early lung development (3), but abnormal development at later stages throughout intrauterine life is likely.

The anatomical defect of CDH is correctable surgically. Nevertheless, postnatal correction of the diaphragm defect does not solve the problem (18), which is not surprising if one takes into account its lack of immediate consequence for pulmonary hypoplasia and vascular abnormalities. Prenatal diaphragm closure by in utero surgery, although technically feasible, raised considerable problems, including tocolytic failure and maternal-fetal morbidity. A randomized trial in 1999 put an end to this technique because there was no benefit in terms of survival and cost (10). Trying to correct lung hypoplasia in utero by a less aggressive method and waiting for delivery to correct the diaphragm defect finally appeared as the best way to treat the disease. Most of current experimental and clinical trials explore the possibility of enhancing lung growth through an increase of lung tissue stretch obtained by fluid retention consecutive to tracheal occlusion, the so-called PLUG technique (12). This can be designated a physical method. The novel approach suggested by Larson and Cohen (14) through CFTR gene therapy, which represents a molecular method, though apparently radically different, may in fact relate to the same principle.

PLUG stands for “plug the lung until it grows.” The method is based on the principle that the degree to which the fetal lungs are expanded is a critical determinant of lung growth and maturation (9). First clinical attempts in human fetuses with CDH (11) were undertaken following the demonstration that lung hypoplasia can be reversed by temporary obstruction of the trachea in fetal sheep with lung hypoplasia of various origins, including surgically created diaphragmatic hernia. It has indeed been long recognized that the epithelium of the developing fetal lung secretes fluid that fills the future air spaces. Tension exerted by this fluid on lung tissue plays an essential role in lung development. Whereas tracheal ligation, which prevents lung fluid from escaping in amniotic fluid thereby inducing its accumulation, enhances lung growth and maturation, fluid drainage leads to lung growth impairment (2). Upper airways control fluid efflux, which determines the volume of lung fluid and thus maintains a constant pressure. Consistently, intrauterine respiratory movements that allow the future air spaces to communicate with the amniotic space, thus releasing lung fluid through the trachea and the pharynx and regulating its volume, are also crucial for lung growth and development (1). Underlying mechanisms of stretch-induced lung growth and maturation have only begun to be identified. In brief, lung growth response results from stimulated DNA synthesis and cell proliferation, but structural development is affected also, with thinning of septa, increased alveolar surface area, and accelerated elastin deposition. It is likely that mechanical stretch transfers information to cell nuclei to regulate gene expression, but signal transduction pathways are poorly defined. Recently, a systematic gene-profiling study allowed a variety of genes up- or downregulated by increased lung expansion to be identified in fetal sheep, thus opening new perspective in the field (19). Deleterious effects of occlusion...
are also observed with excessive differentiation of type II into type I alveolar epithelial cells (5), which points out the necessity to define an optimal duration of occlusion and to proceed to its prenatal release (16). Nevertheless, investigations in the lamb fetus have clearly demonstrated the benefit for lung function (6). At the moment, endoscopic occlusion of the trachea with an extractable latex balloon appears as the best method. A multicentric clinical trial is presently in progress with promising preliminary results (13).

The leading hypothesis of the study by Larson and Cohen (14) is the following: because CFTR overexpression during fetal lung development resulted in enhanced epithelial proliferation and accelerated differentiation in different species including primates, they assumed that it would improve the pulmonary hypoplasia associated with CDH. To test this hypothesis, they used the nitrofen model in rats and intra-amniotic administration of an adenoviral CFTR expression vector. The treatment improved internal surface area, saccular density, saccular number, and amount of saccular air space in the lungs of fetuses with CDH compared with control vector. Importantly, the efficiency of in utero gene transfer to the lung was affected by the gestational age at which nitrofen exposure occurred, indicating that it is affected by the developmental maturity of the lung at the time of gene therapy. They little speculated about the possible underlying mechanisms. However, it should be emphasized that active chloride-ion transport creates the driving force of fetal lung liquid secretion (17). Because CFTR gene encodes a chloride channel protein, its transfer in fetal lung may thus enhance the rate of lung fluid secretion and, subsequently, the tension of lung tissue. Moreover, the authors mention in the Discussion of their article (14) that recent unpublished experiments on CFTR-dependent gene expression in fetal lung indicate that CFTR is involved in regulation of stretch-induced differentiation of the lung by controlling airway smooth muscle contraction. Thus stretch-induced growth and differentiation are likely to represent the common denominator between tracheal occlusion and CFTR gene therapy in the correction of lung hypoplasia.

It is indeed too early to forecast the place that CFTR gene therapy might finally take in the management of CDH-linked pulmonary hypoplasia. It is clear that the least invasive approach of the disease will be the most preferable. Endoscopy may be used to deliver CFTR expression vector into the fetal airways. This would limit the amount of vector to deliver compared with intra-amniotic delivery. However, it should be determined first whether CFTR gene therapy would be efficient in correcting lung disorders in other animal models of CDH, including surgical models in lambs or rabbits, before considering a possible use in humans. Another major question is the safety of inducing overexpression of CFTR in the fetal and infant lung. Overexpression of CFTR in the normal lung of developing cfrt heterozygous mice can be lethal (15). The presence of CFTR protein in amounts exceeding normal levels is therefore not harmful. The fact that CFTR overexpression was not lethal in rat fetuses with CDH may relate to differences between species, but most likely, it results from the fact that nitrofen-treated rat fetuses with CDH were deficient in CFTR (14). Transfer of a CFTR expression vector into their lungs thus led to correction of this deficit, not simply to overexpression. It therefore appears crucial to determine whether the human fetus with CDH is deficient in CFTR to some extent. Temporal changes of CFTR expression have been studied during ovine lung development (7), but similar data are not available for the human fetus, all the more so in the presence of CDH. It would also be of particular interest to determine whether tracheal occlusion changes lung CFTR expression. If clinical use of CFTR gene transfer in the human fetus with CDH were to be considered, numerous preliminary studies in relevant animal models would be necessary to determine optimal and safe conditions of delivery to achieve just sufficient and transient expression of the transgene.

REFERENCES

