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Regenerative medicine solutions in congenital diaphragmatic hernia

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ABSTRACT

Congenital diaphragmatic hernia (CDH) remains a major challenge and associated mortality is still significant. Patients have benefited from current therapeutic options, but most severe cases are still associated to poor outcome. Regenerative medicine is emerging as a valid option in many diseases and clinical trials are currently happening for various conditions in children and adults. We report here the advancement in the field which will help both in the understanding of further CDH development and in offering new treatment options for the difficult situations such as repair of large diaphragmatic defects and lung hypoplasia. The authors believe that advancements in regenerative medicine may lead to increase of CDH patients' survival.

Introduction

In the EU-27, approximately 2100 babies with congenital diaphragmatic hernia (CDH) are born annually. Mortality is still up to 30% due to insufficient lung growth and persistent pulmonary hypertension. In the Western world, >60% of cases are diagnosed at the latest by the second trimester screening ultrasound (US). In *isolated* cases, one can make an individualized prognosis and counsel parents about prenatal options. The ability to identify a future non-survivor *prenatally* warrants the search for a prenatal intervention that can avoid that outcome. Today, lung development can already be stimulated prenatally by fetal endoscopic tracheal occlusion (FETO). FETO prevents egress of lung liquid, causing increased pulmonary stretch, which accelerates lung growth.[1] This has been clinically translated into a percutaneous procedure under local anesthesia, without serious maternal morbidity. FETO is associated with an apparent increase in survival from <15% to 50% (for left-sided cases) and reduction of early neonatal respiratory morbidity compared to historical controls.[2,3] This potential benefit is now being investigated in an ongoing global randomized clinical trial (RCT).[4] Unfortunately, FETO is invasive and has an up to 25% risk for preterm delivery, partly offsetting the beneficial effects. Also, the maximum post-FETO survival reported is 50–60%, which in part is caused by insufficient airway growth and/or limited vascular development. Therefore, alternative strategies are required.[5,6]

Regenerative solutions

Regenerative medicine combines tissue engineering and cell transplantation, with an aim of replacing damaged tissues and organs, using living cells.[7] While degenerative diseases have represented the main focus of the field, new interesting approaches could have a major influence in the outcome of children affected by congenital malformations such as CDH. In particular, there are at least 3 levels on which translational research in this field could have a direct impact: (1) understanding the mechanism of CDH using modeling; (2) engineering tissue to substitute the missing diaphragm; and (3) regenerating or substituting the affected lungs.[8–10]

Understanding CDH

Developmental biology has explored for decades the possible genesis of CDH with very little understanding of the actual mechanism or how to possibly prevent it from occurring. However, thanks to knowledge derived from the stem cell technologies,

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more hypotheses have been formulated in the past few years. For example, embryology textbooks normally describe the development of the phrenic nerve and diaphragm based on anatomical dissections of embryonic tissue. More recent work however showed that myogenic cells and axons that ultimately form the neuromuscular component of the diaphragm coalesce within the pleuroperitoneal fold (PPF). Indeed, PPFs are ultimately the source of the diaphragm's muscle connective tissue and regulate muscle development.[11] This had direct evidence in the nitrofen rat model of CDH where malformation of the PPF mesenchymal substratum was found to be correlated to the defect characteristic of CDH, yet also pulmonary hypoplasia.[11,12] Similarly, mutations in cells derived from PPF can have a major impact on the genesis of the diaphragm. Specifically, it has been shown that Gata4 mosaic mutations in PPF-derived fibroblasts resulted in the development of localized amuscular regions ultimately leading to the formation of CDH.[13] Thus, the PPFs and muscle connective tissue are critical for the development of the diaphragm, and mutations in PPF-derived fibroblasts can lead to CDH.[13] Indeed, mutagenesis of FOG2, a transcriptional co-regulator known to functionally interact with GATA4, can be linked to CDH and pulmonary hypoplasia in humans and mice.[14] Additionally, analysis of wild-type mouse embryos demonstrated co-expression of Gata4 and Fog2 in mesenchymal cells of the developing diaphragm and lungs, further indicating the possible correlation.[14] Gata4 may also act in conjunction with Wt1. Firstly; WT1-null mouse embryos develop CDH.[14] Moreover, if there is a conditional deletion of Wt1 present in the lateral plate mesoderm, 80% of G2-Gata4(Cre);WT1(fl/fl) embryos developed a typical Bochdalek-type CDH.[15] Interestingly, dietary supplement with RA to pregnant females, which was earlier shown to rescue pulmonary hypoplasia in the nitrofen model of CDH[16,17] was also partially effective in the mouse model of conditional deletion of WT1.[15] Based on that recent information there might possibly be 2 types of CDH, which would originate in different phases during development. CDH due to defective generation of the PPF mesenchyme appears in models of RA deficiency and also in models with loss of function of WT1. In later stages, CDH can develop by defective muscularization of the pleuroperitoneal septa. This type of CDH occurs in models of GATA4 conditional deletion[13] and also in c-Met deficiency.[11] While interesting, those preliminary findings should be validated by human data. However, preliminary studies in human specimens revealed that in the thick muscular border of CDH diaphragm, there is a high number of mature muscle cells and a significant increase of quiescent muscle stem cells (defined as satellite cells; PAX7+, Mib1−).[18] The abnormal architecture may affect the normal myogenic process and thus signaling and cell–cell interactions of myocytes. However, the expression of Fog2 in mesothelial and mesenchymal cells in CDH seems to indicate the absence of a genetic defect involving Fog2 in most clinical cases. Being that Fog2 is expressed in muscle cells at an early stage supports the hypothesis that the altered diaphragmatic genesis may undermine also the muscular component instead of only the mesenchymal one.[18] Similarly to what is happening for the diaphragm, lung development in normal and pathological situations using stem cell tools has revealed new knowledge on CDH, which could lead to innovative treatments. This is the case, for example, for persistent pulmonary hypertension (PPH), which is one of the prominent causes of death and severe morbidity in congenital diaphragmatic hernia (CDH). Next to a reduction of the number of pulmonary vessels hence cross-sectional diameter, PPH is also caused by increased vascular cell proliferation and endothelial cell (EC) dysfunction.[19] It has been shown that levels of C-Kit and its ligand, stem cell factor (SCF), are significantly increased in ECs of CDH fetuses during development, compared to controls that may ultimately contribute to vascular remodeling and thus to PPH.[20] Not only could the pathological development of EC lead to a malformed lung, but also malformed innervation. In particular, defective parasympathetic innervation may also contribute to airway branching, and lung hypoplasia abnormalities in CDH. In the murine nitrofen model of CDH, airway branching and airway contractions were significantly decreased compared with controls. However, lungs of nitrofen-exposed dams receiving carbachol displayed increased airway contractions and branching. Nitrofen-exposed lungs exhibited an increased number of proliferating Sox9-positive distal epithelial progenitor cells, which were decreased and normalized by treatment with carbachol.[21] In vivo treatment of nitrofen-treated embryos via amniotic injection of carbachol at E10.5 resulted in modest increases in lung size and branching at E17.5 (Figures 1 and 2).

Finally, specific problems related to CDH, such as skeletal muscle development or lung hypoplasia, could lately be explored in vitro using newly developed models. The latter take advantage of tissue engineering and microfluidic techniques using the patient's own cells giving the possibility of verifying in a human system what has been previously reported in animal models. Skeletal muscle can be easily engineered in vitro using bioreactors capable of inducing mechanical or electrical stimuli even at the level of single muscle fibers.[22,23] On the other hand, the so-called lung-on-a-chip has the advantage of offering personalized medicine solutions, e.g., by giving the possibility for evaluation of new drugs or interventions with systems that can more closely mimic the human development.[24] One of the most elegant studies looking at providing a method for generating patient-specific airway epithelial cells for disease modeling and in vitro drug testing has been reported for cystic fibrosis (CF), a lethal genetic disease caused by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. A directed differentiation protocol for generating functional CFTR-expressing airway epithelia from human pluripotent stem cells has been described using exogenous growth factors that mimic endoderm developmental pathways in vivo followed by air–liquid interface culture. At terminal maturation of the induced cells, patches of tight junction–coupled differentiated airway epithelial cells were seen demonstrating active CFTR transport function.[25] Similar systems could be eventually developed to test developmental cues or innovative drugs that could interfere with pulmonary hypoplasia typical for CDH.

Patch repair

CDH patients who survived the first few weeks of life undergo anatomical repair, but large diaphragmatic defects are closed using a patch. At present, the materials used in our surgical practice are less than ideal, mainly because of recurrence and chest

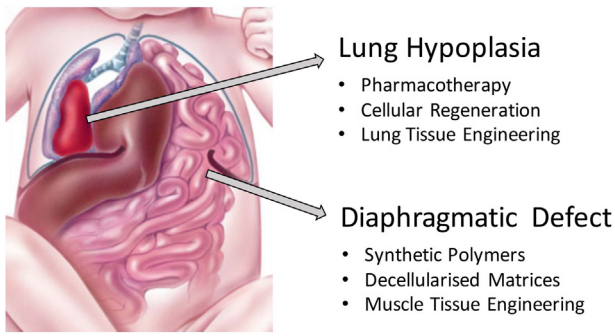

Fig. 1. Schematic representation of the future therapeutic options offered by regenerative medicine. (Reproduced with permission of the University Hospitals Leuven; artist: Dream-team, Loonbeek, Belgium.)