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Premature differentiation of vascular smooth muscle cells in human congenital diaphragmatic hernia

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ABSTRACT

Background: Congenital diaphragmatic hernia (CDH) is a rare congenital anomaly characterized by the herniation of abdominal organs into the chest cavity. The high mortality and morbidity of CDH patients are primarily caused by the associated pulmonary hypertension (PH), characterized by the thickening of the vascular media and adventitia. The media consist of heterogeneous populations of vascular smooth muscle cells (VSMC), ranging from synthetic to the characteristic contractile cells. VSMCs are influenced by developmental and environmental cues and may play a role in the development of the structural changes observed in CDH patients. Therefore, we hypothesized that the distribution of the VSMC populations may already be different at the origin of CDH development.
Methodology: We analyzed the protein expression of specific markers associated with synthetic and contractile VSMC phenotypes in human lungs at different developmental stages. Next, we compared lungs of premature and term CDH patients, as well as patients with lung hypoplasia due to renal agenesis or PROM, with age-matched controls.
Results: Synthetic and contractile VSMCs are distributed in a temporal and spatial specific pattern along the proximodistal axis of the lung. CDH patients have more abundant contractile VSMCs which are also more distally distributed. This different distribution pattern is already observed from 19 weeks of gestation onwards.
Conclusion: Our data suggest that the more extensive distribution of contractile VSMCs is associated with an early maturation of the pulmonary vasculature, contrasting the concept that CDH might be the result of delayed maturation of the epithelium.

Introduction

Pulmonary hypertension is a severe cardio-respiratory disorder that is associated with a variety of diseases (Simonneau et al., 2009). An increased pulmonary vascular resistance caused by structural and functional changes in the pulmonary vasculature results in a restricted pulmonary flow and ultimately in right ventricle failure leading to death (Morrell et al., 2009).

In congenital diaphragmatic hernia (CDH) pulmonary hypertension, in combination with lung hypoplasia, is the major determinant of the significant mortality and morbidity in these patients (Thebaud and Tibboel, 2009). Morphologically, the structural abnormalities in the pulmonary vasculature of CDH patients are well described and characterized by a reduced vascular bed, increased thickness of arterial media and adventitia and an excessive muscularization of arterioles (Rottier and Tibboel, 2005; Shehata et al., 2000). Modern treatments aim to reach a diminished pulmonary vascular tone and restrict iatrogenic damage of the epithelial components of the lung by gentle ventilation (Sluiter et al., 2011; Wung et al., 1995). However, the poor outcome of

Abbreviations: α-SMA, alpha smooth muscle actin; CDH, congenital diaphragmatic hernia; CRBP1, cellular retinol binding protein; ECMO, extra corporeal membrane oxygenation; MYL-9, myosin regulatory light chain 9; PH, pulmonary hypertension; PROM, premature rupture of membranes; RA, retinoic acid; SM-MHC, smooth muscle myosin heavy chain; VSMC, vascular smooth muscle cell.

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CDH, even with ECMO, is related to the disease process, as we recently reviewed (Sluiter et al., 2011).

The vascular wall consists of three layers, the intima, media and adventitia and each layer contains specific cells. The endothelial cells of the intima are surrounded by medial vascular smooth muscle cells (VSMCs) in arteries and veins, which are involved in the (pulmonary) vascular function (regulation of vascular tone) and structure (remodeling). The VSMCs are heterogeneous and their phenotypes associate with their function, ranging from synthetic (immature) to contractile (mature). The different phenotypes can be distinguished based on morphology, expression of cytoskeleton and contractile markers (Fig. 1) and responsiveness to several growth factors (Archer, 1996; Owens et al., 2004). The VSMC phenotype is developmentally and environmentally regulated and may play a role in vascular remodeling as seen in pulmonary hypertension (Stenmark and Frid, 1998). A case-report of two patients with primary pulmonary hypertension showed that VSMCs with an immature phenotype were present in the intima, whereas the media only had mature, contractile VSMCs (Mitani et al., 2001).

Morphological analysis performed more than 40 years ago of the lung hypoplasia in CDH revealed an early arrest in branching of both airway and vasculature (Areechon and Eid, 1963; Kitagawa et al., 1971), with a histological immature lung (George et al., 1987), suggesting that lung hypoplasia in CDH results from a delay in development. However, a more recent study showed that in human the maturation of alveolar type II cells and surfactant production was not delayed (Boucherat et al., 2007).

Since VSMC plays an important role in abnormal vascular remodeling and the structural abnormalities in CDH already develop in utero (Shehata et al., 1999; Taira et al., 1998), we hypothesized that the VSMC population in CDH is already intrinsically different early in development.

Therefore, we performed immunohistochemical stainings with specific markers associated with distinct VSMC phenotypes to analyze the VSMCs in pulmonary hypertension in CDH infants. We show clear differences in the expression of VSMC markers in CDH compared to age-matched controls, which suggests that VSMCs in CDH infants do not have a delayed development, but rather display an early differentiation into a mature, contractile phenotype.

Material and methods

Human lung samples

Human lung samples were retrieved from the archives of the Department of Pathology of the Erasmus MC, Rotterdam, following approval by the Erasmus MC Medical Ethical Committee. Fifty control lungs were used for the tissue microarray for normal lung development, subdivided in pseudoglandular, canalicular, saccular and alveolar stage (Table 1).

For the CDH versus control experiments, 20 CDH patients were selected (median lung/body weight ratio of term CDH patients 0.0054, normal ratio 0.012), which did not show severe hemorrhage and/or necrosis. The control group consisted of age-matched patients (15–40 weeks) that either died in utero or the duration of postnatal survival and spontaneous breathing and/or ventilation was comparable with the CDH patients (Table 2). We divided the CDH patients and age-matched controls in two groups; immature/premature (gestational age < 37 weeks) and term (gestational age > 37 weeks). Five patients with lung hypoplasia due to renal agenesis (median lung/body weight ratio 0.0112) and 5 patients with lung hypoplasia due to premature rupture of membranes (PROM) (median lung/body weight ratio 0.0115) were selected to determine if possible differences in expression between CDH and control were caused by CDH or by lung hypoplasia in general (Table 3).

Tissue microarray

Tissue microarrays were constructed as described by Kononen et al. (1998). For each sample, three tissue core biopsies of 1.5 mm in diameter and 3.2 mm in depth were taken from preselected regions to ensure adequate representation of all lung structures. These biopsies were placed in linear arrays into empty recipient paraffin blocks, two for the normal development stages, one for CDH, and one for CDH control. Tissue cores of adult multi-slides were used as controls.

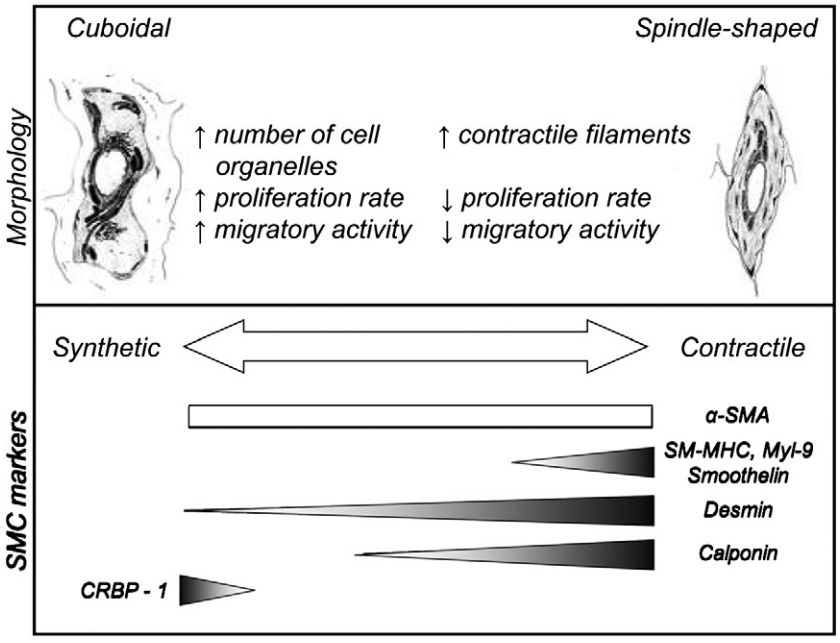

Fig. 1. Functional and molecular characteristics of smooth muscle cell phenotypes. Smooth muscle cells display different phenotypes ranging from synthetic to contractile, which can be characterized by cell morphology (cuboidal versus spindle shaped), function (proliferation, migration or regulation of vascular tone) and the expression of specific SMC markers.
Figure adapted from S.S.M. Rensen et al. Neth Heart J 2007;15:100–108.