

Embryological Origin of Airway Smooth Muscle

Kameswara Rao Badri¹, Yuanxiang Zhou¹, and Lucia Schuger¹

¹Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan

Airway smooth muscle (SM) develops from local mesenchymal cells located around the tips of growing epithelial buds. These cells gradually displace from distal to proximal position alongside the bronchial tree, elongate, and begin to synthesize SM-specific proteins. Mechanical tension (either generated by cell spreading/elongation or stretch), as well as epithelial paracrine factors, regulates the process of bronchial myogenesis. The specific roles of many of these paracrine factors during normal lung development are currently unknown. It is also unknown how and if mechanical and paracrine signals integrate into a common myogenic pathway. Furthermore, as with vascular SM and other types of visceral SM, we are just beginning to elucidate the intracellular signaling pathways and the genetic program that controls lung myogenesis. Here we present what we have learned so far about the embryogenesis of bronchial muscle.

Keywords: bronchial muscle; stretch; tension-induced myogenesis; cell shape; epithelial cell-derived morphogens

Airway smooth muscle (SM) develops from local mesenchymal cells that, at the initiation of the pseudoglandular period (gestation day 11 in mouse, 13 in rat, and week 5 in human), begin to elongate and express SM-specific proteins (1). Bronchial SM cells are first detected in the trachea/proximal main bronchi and follow the developing bronchial tree in a proximal to distal fashion (2–7). This pattern is the result of myogenesis proceeding in the opposite direction from mesenchymal cell precursors located at the tips of distal airways (8, 9). The appearance of bronchial SM precedes the appearance of vascular musculature by several days (3, 5, 6).

SM is found at sites that sustain mechanical tension in the form of hydrostatic pressure or shear stress, such as blood vessels and hollowed viscera, which during embryogenesis are filled with fluids. Furthermore, SM cells develop attached to basement membranes, which allow the former to spread and elongate, a process that generates additional cytoskeletal forces. Finally visceral SM, including that of the bronchial tree, originates around epithelia, which produce a variety of soluble factors with paracrine effect on the mesenchyme (10, 11). A growing body of evidence indicates that mechanical forces and epithelial-derived morphogenic factors are both involved in the development of airway SM.

EMBRYONIC MESENCHYMAL CELL SPREADING/ELONGATION AND AIRWAY SM MYOGENESIS

Evidence for the myogenic role of mechanical tension, in the form of cell spreading/elongation and stretching, comes mainly from our laboratory. Studies on the role of laminin (LN)-1,

a main component of epithelial–mesenchymal basement membranes (12–14), demonstrate that during lung development LN-1 expression is induced in epithelial and mesenchymal cells upon contact with each other and further accumulates at the epithelial–mesenchymal interface as a polymer (15) essential for basement membrane formation (12–14). Either blocking LN-1 polymerization and thereby basement membrane formation, or attachment of mesenchymal cells to the LN-1 polymer with epitope-specific antibodies or antisense probes, prevents the normal elongation of mesenchymal cells surrounding the epithelium (15–17). When prevented from elongating, the embryonic mesenchymal cells do not differentiate into bronchial SM cells, regardless of epithelial cell presence (15–17). These observations led us to study the role of the cell shape *per se* on SM myogenesis. Undifferentiated embryonic mesenchymal cells from different organs were plated on microspheres of diameters that either maintained the original round cell shape or facilitated cell elongation (18). Irrespective of their fate *in vivo*, after 18 hours in culture all the elongated cells differentiated into SM myoblasts, as indicated by the expression of SM-specific proteins and development of membrane potentials of -60 mV and voltage-dependent Ca^{2+} currents, characteristic of SM cells. After 72 to 96 hours in culture, the cells attained the same level of SM-specific proteins as found in mature SM cells. SM differentiation is equally observed in proliferating and quiescent cells, suggesting that the process is not significantly affected by cell proliferation. In contrast, round cells remain undifferentiated as indicated by the expression of high levels of α -fetoprotein, an embryonic marker (18). Known modulators of SM differentiation like transforming growth factor (TGF)- β 1 (19, 20), retinoic acid (21), platelet-derived growth factor (20, 22), sonic hedgehog (Shh) (23), and bone morphogenetic protein-4 (BMP-4) (9) neither stimulated round cells to differentiate nor prevented elongated cells from differentiating (18; Y. Zhou and L. Schuger, unpublished observations). Therefore, regardless of the organ of origin and normal fate of the cell *in vivo*, embryonic mesenchymal cells follow a myogenic pathway upon spreading/elongation. More recently it has been shown that human mesenchymal stem cells can be induced to differentiate either into SM or osteocytes by controlling the cell shape and tension with artificial matrices of different elasticity (24).

Undifferentiated mesenchymal cells do not express LN-2, the main LN produced by muscle (25), whereas when these cells are allowed to elongate, LN-2 expression and synthesis are induced (8). Similarly, LN-2 is synthesized by peribronchial mesenchymal cells when these elongate upon the onset of bronchial myogenesis (8). Blocking LN-2 with a specific antibody inhibits mesenchymal cell spreading/elongation and SM myogenic differentiation (8). Supporting these *in vitro* findings, decreases in the size and quantity of bronchial SM cells (8), as well as severe skeletal muscle abnormalities (26, 27), are seen in mice lacking LN-2 (26). In conclusion, these studies suggest that newly synthesized LN-1 and LN-2 accumulate around the developing bronchial tree, and by inducing spread/elongation of mesenchymal cell precursors, stimulate SM myogenesis (8).

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Correspondence and requests for reprints should be addressed to Lucia Schuger, M.D., Department of Pathology, Wayne State University, School of Medicine, 540 E. Canfield Street, Detroit, MI 48201. E-mail: lschuger@med.wayne.edu

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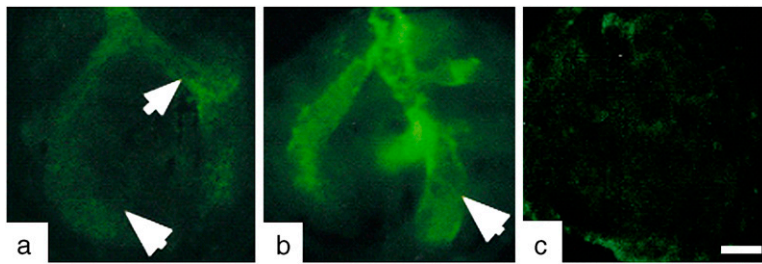


Figure 1. Embryonic whole lung explants cultured in the presence of extra- and intra-luminal dextran (volume expander) and immunostained for smooth muscle (SM) α -actin. (a) SM α -actin in control explants cultured without dextran (unmodified pressure). Some noticeable bronchial SM development is present (arrows). (b) SM α -actin in lung explants cultured with 5% dextran inside airways (increased pressure) shows hyperplastic bronchial muscle (arrow). (c) No bronchial myogenesis in lung explants cultured with 1% dextran in the culture medium (eliminated pressure). Bar = 100 μ m. Reprinted by permission from Reference 28.

EMBRYONIC MESENCHYMAL CELL STRETCHING AND BRONCHIAL SM DIFFERENTIATION

SM develops exclusively at sites that sustain mechanical tension such as hollow viscera and vasculature. In addition, cell spreading/elongation generate cytoskeletal tension forces. We therefore sought to determine whether stretch, by itself (in the absence of cell spreading) was sufficient to initiate SM myogenesis. Lung undifferentiated mesenchymal cells plated on silastic membranes were subjected to uniaxial continuous stretching. Five to ten percent stretching induced the expression of SM myosin, SM α -actin, desmin, and SM22 (28). Cells subjected to 0 or 1% stretch remain undifferentiated and synthesize high levels of α -fetoprotein (28). Undifferentiated mesenchymal cells from kidney and intestine do not respond to uniaxial stretch, suggesting a higher sensitivity of lung mesenchyme to mechanical tension. Embryonic lung explants from fetal mouse were further used to determine the effect of mechanical stretch upon SM myogenesis. The explants were cultured embedded in a thin collagen gel layer and their airway intraluminal pressure was increased, decreased, or eliminated.

Increase in intrabronchial hydrostatic pressure enhances SM myogenesis as visualized by increase in SM α -actin surrounding the bronchial tree, whereas in absence of hydrostatic pressure, there is no bronchial muscle development (28) (Figure 1). To determine whether abnormal mechanical tension may cause abnormal SM development, *in vivo* studies were focused on two types of human hypoplastic lungs: those related to oligohydramnion and those related to diaphragmatic hernia, since in both there is decreased lung distension (29–35). Supporting the *in vitro* findings, these hypoplastic lungs show severe decrease to near absence of airway SM cells and proportional decrease in elastin, which is mainly produced by SM cells (28) (Figure 2). Significant bronchial SM paucity was recently documented in mutant embryos lacking skeletal muscle and therefore unable to generate fetal breathing-like movements, decreasing thereby the intraluminal hydrostatic pressure (36). It has been recently shown that the intracardiac fluid forces generated from blood flow are essential for cardiogenesis (37) and that stretch of vascular wall in adult mice significantly increases SM gene expression (38). Therefore it is plausible that vascular myogenesis is also modulated by mechanical forces.

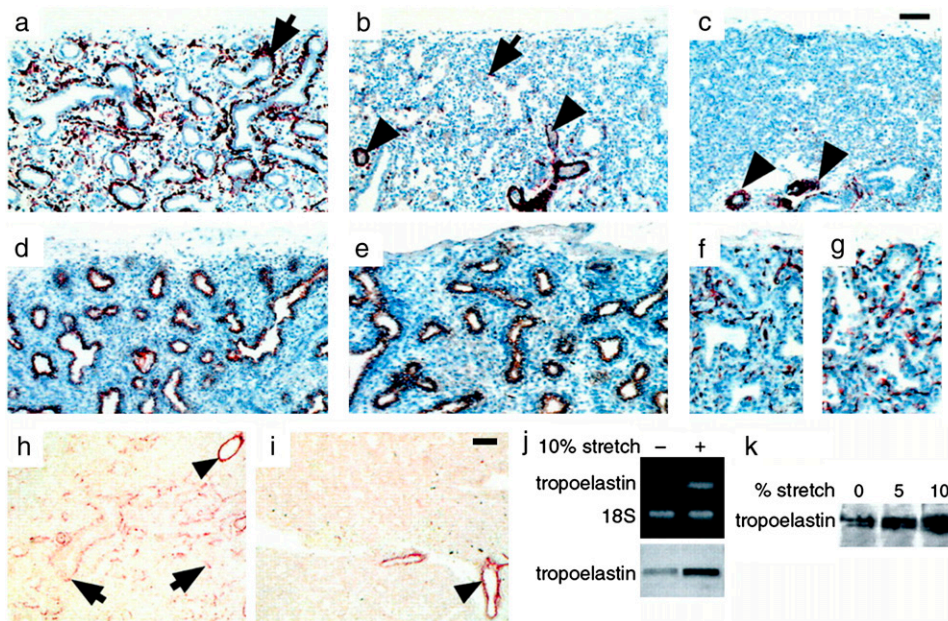


Figure 2. Immunohistochemistry showing paucity of bronchial SM cells in human fetal hypoplastic lungs. Shown are histologic sections from (a) normal lung, (b) hypoplastic lung caused by oligohydramnion, and (c) hypoplastic lung caused by diaphragmatic hernia, all at 22 weeks of gestation, immunostained for SM α -actin. There is significant decrease in bronchial SM cells (arrows) in the hypoplastic lungs (b and c), particularly in those compressed by intrathoracic herniation of abdominal viscera due to diaphragmatic hernia (c). The vascular musculature is unaffected (arrowheads). In the same hypoplastic lung shown in b, the epithelial cells, immunostained for cytokeratins (e), and the endothelial cells, immunostained for PECAM-1 (g), show no changes compared with controls (d and f). h and i demonstrate immunohistochemistry showing decrease in tropoelastin deposition in human hypoplastic lungs. (h) Histologic sections from normal lung at 20 weeks of gestation demon-

strate tropoelastin deposition around bronchi and bronchioli (arrows). (i) Histologic sections from same age hypoplastic lung reveal essentially no tropoelastin deposition, with the exception of vascular SM that shows no changes in tropoelastin when compared with controls (arrowheads). Bar = 60 μ m in a–e, h, and i, and 100 μ m in f and g. RT-PCR and immunoblot show stretch-induced upregulation of tropoelastin expression in mouse lung embryonic mesenchymal cells undergoing myogenic differentiation. (k) Immunoblot shows stretch-induced upregulation of tropoelastin synthesis in human lung embryonic mesenchymal cells undergoing myogenic differentiation. Reprinted by permission from Reference 28.

MOLECULAR MECHANISMS INVOLVED IN TENSION-INDUCED BRONCHIAL SM MYOGENESIS

The molecular pathways that lead an undifferentiated lung mesenchymal cell to differentiate into a bronchial SM cell are largely unknown. Serum response factor (SRF) and its dominant-negative isoform SRF Δ 5 play an important role in tension-induced myogenesis (28). SRF is one of the best-studied transcription factors regulating SM gene expression (39–41). SRF is a member of the MADS box family of transcription factors. SRF binds to the CARG box-like motif found in muscle-specific proteins and stimulates their transcription (39–41). SRF Δ 5 serves as naturally occurring dominant-negative isoform that blocks SRF-dependent muscle gene expression (42, 43). Undifferentiated mesenchymal cells synthesize SRF and SRF Δ 5. When these cells are subjected to mechanical tension, whether in the form of cell spreading/elongation or stretching, SRF Δ 5 production is suppressed while SRF production is concomitantly increased. This change in isoform pattern induces or at least facilitates the onset of myogenesis (28). The role of SRF and SRF Δ 5 is further confirmed by the fact that hypoplastic lungs associated with decreased intraluminal pressure still express both isoforms, whereas normal lungs of the same fetal age synthesized only full SRF (28). Indicating the importance of SRF for vascular SM development, recent studies demonstrated that conditional deletion of *Srf* in the vascular system results in a decrease in vascular SM cells proportional to the decrease in SRF-positive cells (44).

A search for genes differentially expressed during SM myogenesis indicated that *Rho-a* is one of the genes highly expressed in undifferentiated embryonic mesenchymal cells but expressed at low levels in their differentiated SM counterparts (45). RhoA is a small guanosine triphosphatase (GTPase) signaling protein that plays a critical role in the organization of the cytoskeleton (46). Additional studies confirmed that RhoA message, protein, and activity decrease during spread- and stretch-induced myogenesis (45; S. Jakkuraju and L. Schuger, unpublished observations) and *in vivo*, upon bronchial myogenesis (45). Functional studies in which constitutively active RhoA was expressed in undifferentiated embryonic mesenchymal cells show that high RhoA activity maintains the round, undifferentiated phenotype. However, if the cells begin to elongate and synthesize SM-specific proteins, the effect of RhoA is reversed from inhibitory to stimulatory (45). In round, undifferentiated mesenchymal cells both SRF and SRF Δ 5 are enriched in the cytoplasm. Upon cell elongation, SRF translocates to the nucleus, whereas SRF Δ 5 disappears. Transfection with dominant positive RhoA maintains the cells' round shape and prevents the SRF changes occurring upon cell elongation (45). Since LN-1 and mainly LN-2 were found to inhibit RhoA expression and activity (45), we proposed an integrative mechanism to explain how tension, in the form of cell spreading/elongation and stretch, may contribute to bronchial myogenesis (Figure 3) (47). More recently we identified a set of tension-responsive factors in lung embryonic mesenchymal cells, which we referred to as TIPs (tension-induced/inhibited proteins) (48). TIPs display signature motifs characteristic of nuclear receptor coregulators and chromatin remodeling enzymes. Functional studies revealed that TIP-1 and TIP-3 are involved in the cell's selection between the myogenic or the adipogenic pathway. TIP-1, induced by tension in the form of cell spreading and/or stretching, promotes myogenesis, while TIP-3, present in undifferentiated lung mesenchymal cells and inhibited by tension, stimulates adipogenesis. TIP-1 induces myogenesis by interacting with the SRF promoter and acetylating histones 3 and 4 at this promoter site, thereby activating its transcription. The

process is dependent on TIP-1 nuclear receptor binding box (48). At the present it is unknown whether TIPs participate in the mechanism proposed in Figure 3.

EMBRYONIC EPITHELIAL CELL-DERIVED MORPHOGENS AND BRONCHIAL SM DIFFERENTIATION

Evidence coming mainly from transgenic animal studies demonstrates that epithelial-derived morphogens and their up- and down-stream effectors modulate bronchial myogenesis. Sonic hedgehog (Shh) is the vertebrate homolog of drosophila hedgehog (Hh) and is the best characterized epithelial-derived morphogen with a role in bronchial myogenesis. Shh is critical for the development of multiple organs (49, 50). Transduction of the Shh signal occurs by activation of its receptor Patched-1 (Ptc-1) (51). Binding of Shh to Ptc-1 relieves Ptc-1-mediated inhibition of a signaling protein referred to as Smoothed, which results in the transduction of the signal to the Gli transcription factors (Gli1, Gli2, and Gli3). These translocate into the nucleus and bind to Shh-responsive elements (52) (Figure 5). Shh activity is restricted by its interaction with Ptc-1 and with Hedgehog interacting protein-1 (HIP-1) (52). Starting at E10.5, Shh is expressed in the embryonic lung epithelium at the tips of the distal buds (53–55). Concomitantly, Ptc-1 (23, 56) and HIP-1 (57) are expressed at high levels in the mesenchyme surrounding the epithelial tips.

Mutant mice lacking Shh show esophageal atresia/stenosis, tracheo-esophageal fistula, and tracheal and lung abnormalities (58–60). In these animals a rudimentary lung is represented by a dilated sac lined by bronchial-type epithelium and surrounded by one or two layers of mesenchymal cells, some of them undergoing apoptosis (59, 60). Airway SM is absent in these highly abnormal lungs (59, 60). In mice with conditional deletion of *Shh*, bronchial cystic dilation, scant mesenchyme, and lack of bronchial and vascular SM are seen in the areas of the lung in which *Shh* is deleted (60) (Figure 4). The effect of *Shh* deletion seems to affect particularly bronchial and vascular SM, since *Shh* null mice develop other visceral muscle, albeit in slightly smaller amount than normal (59–61). Exposure of lung embryonic mesenchymal explants to Shh in culture results in induction of SM α -actin/SM myosin-positive cells (23). However, *Shh* overexpression in the developing airway epithelium does not alter bronchial myogenesis (9).

Gli transcription factors act downstream of Shh. All three *Gli* genes are expressed in distinct but overlapping domains in lung mesenchyme, with expression being highest in the distal tips (62, 63). Mice in which the DNA-binding domain has been removed from *Gli1* are viable and appear normal (64). However mutant mice lacking *Gli2* function exhibit lung hypoplasia, which is more severe upon a 50% reduction in the gene dosage of *Gli3* (65). In these animals the lung phenotype is very similar to that of *Shh*-null mice, and although airway SM has not been studied, it is probably affected.

Forkhead box (Fox)a1 and Foxa2 act upstream of Shh (66). Foxa (previously termed hepatocyte nuclear factor [HNF]-3) transcription factors are a family that includes Foxa1, Foxa2, and Foxa3 (previously termed HNF3a, HNF3b, and HNF3d, respectively). These transcription factors are expressed primarily in endodermally derived tissues, where they influence embryonic patterning, cell differentiation, and function (67). The embryonic lung epithelium expresses only Foxa1 and 2 in a diffuse manner (68), and their combined conditional deletion results in significant airway dilatation with production of large cysts in the areas in which both genes are deleted. These areas show decreased *Shh* expression and absence of airway SM development (66).

In the developing lung, fibroblast growth factor (FGF) 9 also acts upstream of Shh (69). At embryonic day (E)9.5 and E10.5

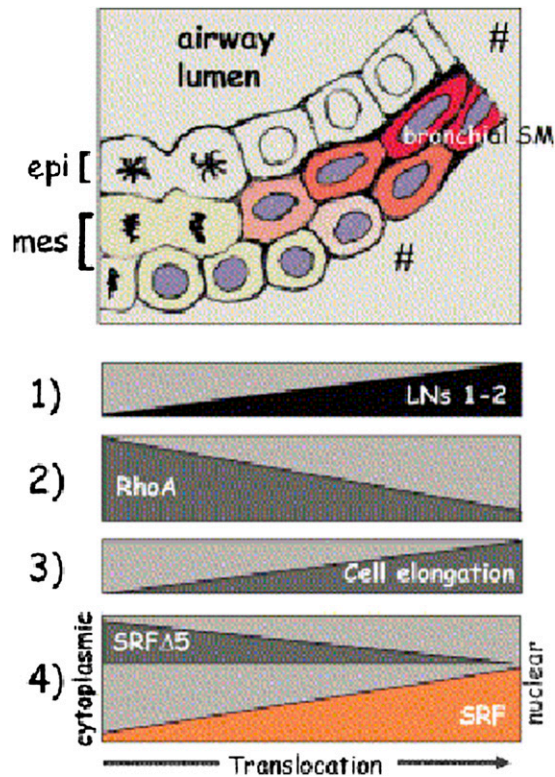


Figure 3. New epithelial–mesenchymal contacts at the tips of growing airway buds induce laminin (LN)-1 expression and deposition at the epithelial–mesenchymal interface (1). LN-1 is a powerful inhibitor of RhoA activity (2). Because high RhoA activity maintains the undifferentiated mesenchymal cells' round shape, its decrease allows for initial cell elongation (3). Cell elongation then switches the synthesis of LN-2 (1), which is an even more powerful inhibitor of RhoA. A positive feedback loop is created between LN-2, RhoA inhibition, and cell elongation (1–3). In the round, undifferentiated mesenchymal cells serum response factor (SRF) and SRFΔ5 are present in the nucleus and cytoplasm (4). Upon cell elongation, the cytoplasmic SRF isoform translocates gradually to the nucleus, whereas SRFΔ5 disappears (4). The increment of SRF plus the disappearance of SRFΔ5 contribute to the initiation of bronchial myogenesis. Reprinted by permission from Reference 47.

Fgf9 is expressed in both the mesothelial lining of the lung (future pleura) and the epithelium of the developing airways, but is restricted to the pleura by E12.5 and on (70). Lack of epithelial *Fgf9* expression results in decreased *Shh* expression (69); however, SM development has not been studied in these lungs. Overexpression of *Fgf9* in the airway epithelium increases *Shh* and *Ptc-1* expression (69). Lungs overexpressing *Fgf9* show bronchial dilation and increased mesenchymal cell proliferation, but notably lack bronchial SM (69) despite the increase in *Shh* and *Ptc-1* expression. These findings are in agreement with a previous study that showed that Shh induction of SM α -actin in mesenchymal lung explants in culture is inhibited by FGF9-soaked beads (23). Therefore either absence of Shh or combined increase in Shh and FGF9 levels both lead to lack of bronchial SM. A similar dual response seems to occur with FGF9 and FGF10. *Fgf9* overexpression up-regulates FGF10 along with the suppression of bronchial SM (69); however, FGF10 down-regulation decreases rather than increases bronchial myogenesis (9). Therefore either a decrease in FGF10 or a combined increase in FGF9 and FGF10 levels inhibit airway SM development (Figure 5).

BMP-4 is another epithelial-derived morphogen with a role in bronchial SM development, although it is currently unclear

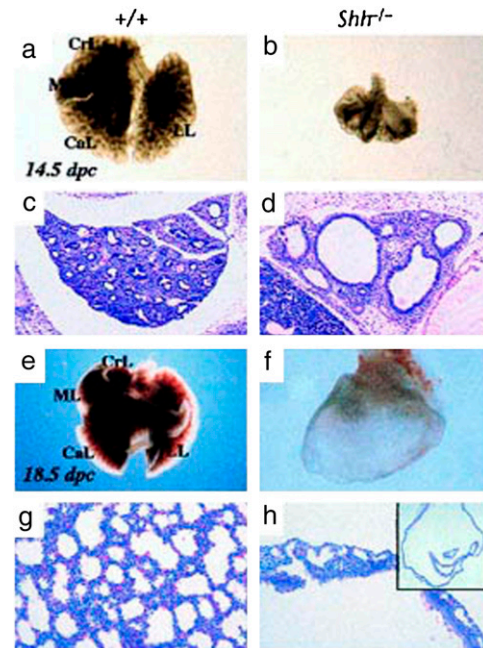


Figure 4. *Shh* is essential in lung development. Whole mount view of (a, e) wild-type and (b, f) *Shh*^{-/-} mutant lungs at (a, b) 14.5 and (e, f) 18.5 days after conception. Wild type shows characteristic lobulation (CrL, cranial lobe; ML, middle lobe; CaL, caudal lobe; LL, left lobe), which is not present in the mutant. Note that the mutant lung at 18.5 days after conception is reduced to a dilated sac (h, inset). (c, d, g, and h) Hematoxylin and eosin–stained sections of (c, g) wild type and (d, h) mutant lungs at (c, d) 14.5 and (g, h) 18.5 days after conception. Reprinted by permission from Reference 58.

whether under physiologic conditions BMP-4 stimulates or inhibits myogenesis. BMP-4 is a member of the TGF- β superfamily of proteins and has been shown to play a role in diverse developmental processes (71). Similar to *Shh*, during lung development, BMP-4 transcripts are localized in high levels at the distal tips of the terminal epithelial buds (53), while BMP receptors are expressed throughout the lung epithelium as well as in the distal mesenchyme (53). Epithelial overexpression of BMP-4 seems to induce extensive mesenchymal SM differentiation (9) along with mesenchymal cell death (53). On the other hand, *Fgf9*-overexpressing lungs, which lack airway SM, have increased BMP-4 level (69). Similarly, BMP-4 is upregulated in *Shh* null mice, which lack bronchial muscle (59). Furthermore, a study in which the BMP antagonist noggin was overexpressed in the bronchial epithelium showed a significant increase, rather than a decrease, in SM α -actin–positive mesenchymal cells (72). Finally, *noggin*-null embryos show no abnormalities in bronchial SM (23), and no lung abnormalities are described in mice with BMP-4 haploinsufficiency (73). Therefore, the physiologic role of BMP-4 in the process of bronchial myogenesis is yet unclear (Figure 5).

Wnt signaling seems to play a role in bronchial morphogenesis, but as it happens with other morphogenic factors, it is currently unknown whether Wnt signaling stimulates or inhibits myogenesis during normal lung development. The vertebrate Wnt growth factor family contains at least 21 secreted cysteine-rich glycoproteins that regulate cell–cell interaction in many embryonic tissues (74). Wnts interact with at least 10 known Frizzled (Fzd) receptors and signals through a canonical and a noncanonical pathways. The canonical pathway involves Fzd receptor binding and nuclear translocation of hypophosphorylated β -catenin, which then activates lymphoid enhancer-bind-

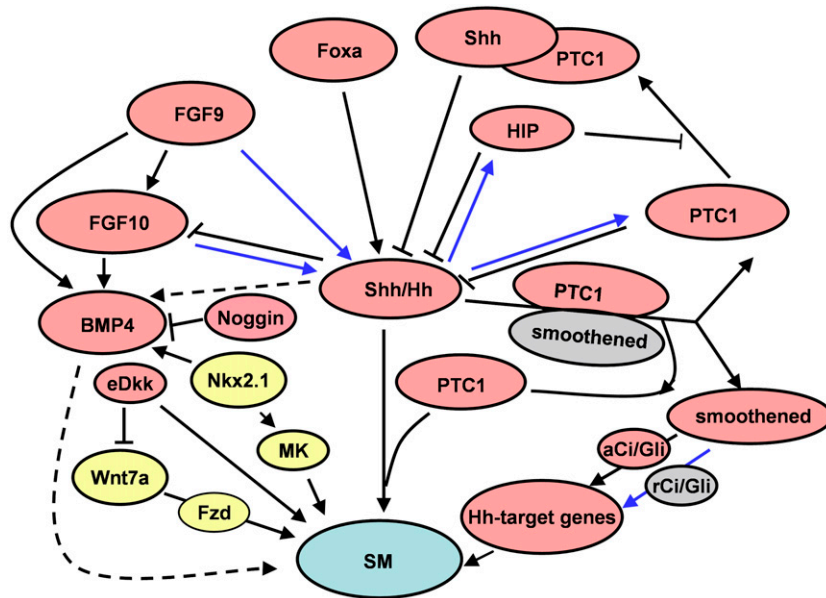


Figure 5. A schematic representation of signaling pathways in lung smooth muscle myogenesis. *Blue lines* indicate a negative regulation on Shh-mediated smooth muscle myogenesis. *Dotted lines* represent relationships that are not yet clear. *Yellow* represents involvement in vascular smooth muscle myogenesis only. *Gray* represents repressed protein or complex. Shh, sonic hedgehog; Hh, hedgehog; PTC1, patched-1; HIP, hedgehog-interacting protein; aCi/Gli, Ci/Gli activator; rCi/Gli, Ci/Gli repressor; Foxa, Forkhead box-a; FGF, fibroblast growth factor; BMP4, bone morphogenetic protein-4; eDkk, epithelial Dickkopf-1; Fzd, Frizzled; MK, midkine, SM, smooth muscle myogenesis.

ing factor/T cell factor (LEF/TCF)-mediated gene transcription. The noncanonical pathway involves either activation of c-Jun kinase or regulation of calcium flux (75, 76). Several Wnts are expressed in the lung and the biological function of Wnt5a and Wnt7a has been determined through the generation of mutant mice (77, 78). None of these mice seems to have abnormal bronchial SM, although Wnt7a-null mice present abnormalities in the differentiation and integrity of lung vascular SM (78). This finding points to a different regulation for vascular and bronchial myogenesis. The role of the Wnt signaling system on bronchial myogenesis is suggested by studies focusing on Dickkopf-1 (Dkk-1). Dkk-1 is a secreted Wnt antagonist that acts by inhibiting the Wnt/ β -catenin signaling (79). Dkk-1 is produced in the embryonic lung by the distal epithelium (80). When Dkk-1 is overexpressed in the developing distal lung epithelium, lung bronchial myogenesis increases (78). However, and contrary to this observation, treatment of embryonic lung explants with Dkk-1 results in an almost complete absence of bronchial SM (80). Interestingly, *Dkk1*-null mice have no lung phenotype (81) and mice with conditional lung deletion of β -catenin have normal airway SM development (82).

Altogether these studies indicate that specific levels of morphogens originating from specific lung compartments and acting in specific combinations at specific times are required for normal bronchial myogenesis. Experimental alteration in any of these conditions results in either increased or decreased/absent bronchial SM.

LUNG VASCULAR SM DIFFERENTIATION

Early development of pulmonary vasculature include three processes: formation of central vessels (angiogenesis), peripheral vessels (vasculogenesis), and a lytic process to establish luminal connection between the two (83, 84). Pulmonary arteries develop close to the bronchi whereas veins form at sites away from bronchi. Arterial vascular SM originate partly from migration of adjacent airway SM cells and from surrounding undifferentiated mesenchymal cells (vasculogenesis), but venous vascular SM arises completely by vasculogenesis (85–87). In humans arteries acquired SM α -actin at 38 days, whereas veins did at 56 days (85). Besides mechanical forces/stretch, SRF, Shh, and Wnt7b (discussed in previous paragraphs), other

factors affecting vascular smooth muscle myogenesis are Nkx2.1 (Thyroid transcription factor1, TTF1) and midkine (MK) (Figure 5). Suppression or targeted disruption of Nkx2.1, a homeodomain transcriptional regulator, resulted in defects in pulmonary vascular SM development, leading to the death of animals due to abnormal lung induced respiratory failure. In these animals bronchial airway SM is well formed with no changes in the expression of Shh mRNA (88, 89). It is noteworthy that Nkx2.1 is expressed in Shh(-/-) lungs (59). Midkine is a retinoic acid-responsive, heparin-binding growth factor regulated by Nkx2.1 (90, 91). MK transgenic mice showed increase in SM-specific genes (SM α -actin, calponin, and SM-22) in the vascular SM cells and vascular SM cell precursors, resulting in the increase in muscularization of small pulmonary arteries. The presence of normal bronchial SM in these animals indicate that MK has a selective effect on vascular SM (90, 91). MK expression was not detected in lungs of Nkx2.1 (TTF1)-null mice (90, 91).

CONCLUSIONS

The data currently available indicate that mesenchymal cell tension and epithelial-derived paracrine morphogenic factors control airway SM development. Whether these signals interrelate is a question that will require additional study. Several possibilities, however, may be proposed to link mechanical and biochemical myogenic signals. One possibility is that epithelial-derived morphogenic factors such as Shh may be required to render the embryonic mesenchymal cells responsive to mechanical forces. Another possibility is that morphogens act in part by promoting changes in mesenchymal cell shape. For example, Shh may induce cells to elongate when added to embryonic mesenchymal cell explants, and this in turn may induce SM α -actin and SM myosin expression, as seen in studies by Weaver and coworkers (23). This possibility may explain why embryonic mesenchymal cells do not respond to the myogenic effect of Shh when prevented from elongating (Y. Zhou and L. Schuger, unpublished observation). Alternatively, soluble factors and mechanical signals may both activate a common myogenic signaling pathway. Finally, there is a mechanical element that may be potentially contributing to the decrease or lack of bronchial SM observed in several transgenic lungs. In most of these lungs the airways are significantly dilated and therefore

withstand increased hydrostatic pressures that may be high enough to inhibit rather than stimulate SM differentiation, as has been previously shown (28). In conclusion, during the last decade we learned most of what is known about bronchial myogenesis. Further research is needed to integrate mechanical and biochemical signaling pathways and to unveil intracellular myogenic mechanisms.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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