Histol Histopathol (2005) 20: 1261-1266

**http://www.hh.um.es**

**Histology and Histopathology**

*Cellular and Molecular Biology*

## *Review*

# The role of fetal breathing-like movements in lung organogenesis

**M.R. Inanlou, M. Baguma-Nibasheka and B. Kablar**

Dalhousie University, Faculty of Medicine, Department of Anatomy and Neurobiology Halifax, NS, Canada

**Summary.** In this review the recent findings concerning the role of fetal breathing-like movements (FBMs) on lung organogenesis are discussed. We first review the consequences that the lack of FBMs has on lung organogenesis and then we discuss the possible pathways that may be employed in this process. Specifically, we review the data in support of the notion that FBMs are required for the cell cycle kinetics regulation (i.e., cell proliferation and cell death) via the expression of growth factors, such as platelet derived growth factors (PDGFs) and insulin growth factors (IGFs), and thyroid transcription factor 1 (TTF-1). Moreover, the role of FBMs on biochemical differentiation of Clara cells, type I and type II pneumocytes is reviewed. Interestingly, even though type II pneumocytes are able to synthesize surfactant-associated proteins (SPs), in the complete absence of FBMs, they are unable to compile, store and release the surfactant. Similarly, in spite of the expression of some early differentiation markers, in the absence of FBMs, type I pneumocytes are unable to flatten in order to allow the gas exchange in the lung. In fact, we are currently employing the cDNA microarray analysis in search for the molecules that might be specific for the lacking functions in pneumocytes.

**Key words:** Lung hypoplasia, Mouse embryo, Myf5, Myod, Lung cell differentiation, Microarray lesions

### Introduction

Organogenesis is one of the most critical processes during development that leads to creation of a fully functional organ. In addition to well established genetic regulatory programs that are required for organogenesis, mechanical forces generated in the embryo also play a role in how differentiating tissues respond to gene instructions. Lack or impairment of the physical forces has been shown to change the state of the organs (e.g., immobilization of the limbs causes muscle atrophy and bone demineralization) (Skinner, 1989).

Apparently, the lung is enormously subjected to the mechanical forces both intrauterine and after birth (Skinner, 1989). A great number of *in vitro* and *in vivo* studies show that mechanical forces influence fetal lung development through pulmonary distension (Kitterman, 1996; Harding, 1997; Liu and Post, 2000). The mechanical forces can be categorized in four groups: 1) adequate intrathoracic space, 2) adequate amount of amniotic fluid, 3) normal fetal breathing-like movements (FBMs), 4) normal balance of fluid volumes and pressures in prospective pulmonary conductive and respiratory systems (Harding, 1997). Among these factors, FBMs are produced by rhythmic contractions of the respiratory muscles with varying frequency and amplitude. These movements are responsible for intermittently reducing intrathoracic pressure and expanding the fetal lung during intrauterine life and are created by neuronal activities of the respiratory center in the brainstem that are transferred into the respiratory muscles (Harding, 1997). FBMs are detected at embryonic day (E) 14.5 in the mouse (Abadie et al., 2000) and at 10 weeks gestation in human embryos (de Vries et al., 1986). FBMs cause lung cells to operate in a biochemical as well as mechanical environment. Clinical reports (e.g., fetal akinesia) and laboratory experiments have revealed that the absence of FBMs in the embryo leads to pulmonary hypoplasia, indicating that these mechanical forces are necessary for normal lung development. However, the mechanochemical signal transduction pathways that translate mechanical stimuli to meaningful gene instructions for the pulmonary cells have yet to be identified. In this review we summarize the recently found mechanisms that attempt to explain how mechanical forces produced by FBMs influence lung growth and pulmonary cell differentiation required for functional maturation of the lung.

*Offprint requests to:* Dr. Boris Kablar, Inanlou, Dalhousie University, Faculty of Medicine, Department of Anatomy and Neurobiology, 5850 College Street, Halifax, NS, Canada B3H 1X5. e-mail: bkablar@dal.ca

### FBMs and the lung growth

Lung primordia evaginate as endodermally derived ventral buds into the surrounding splanchnic mesenchyme (Cardoso, 2000). After budding, the lung primordium undergoes four main stages of development: pseudoglandular, canalicular, saccular and alveolar (Ten Have-Opbroek, 1981). As the name implies, at the psuedoglandular stage, the primitive lung appears like a gland and is made of tubular structures called acinar tubules (Ten Have-Opbroek, 1981). Pulmonary blood supply and conductive airways develop at the canalicular stage (Laudy and Wladimiroff, 2000). Saccular stage is defined by the accelerated increase in the formation of smooth walled large airspaces named saccules (Laudy and Wladimiroff, 2000). Eventually, saccules give rise to the formation of the alveoli after birth (Ten Have-Opbroek, 1981).

Lack of FBMs has been reported to cause pulmonary hypoplasia (Liggins et al., 1981; Kitterman, 1996; Harding, 1997; Tseng et al., 2000; Inanlou and Kablar, 2003, 2005a,b). Lung hypoplasia is a common congenital problem in lung development among neonates (Porter, 1998). It is reported to be found in 14% of perinatal autopsies (Wigglesworth and Desai, 1982) and is considered to be an important cause of death within minutes or hours after birth. Hypoplastic lungs are detected as small lungs in a small or normal thoracic cavity with the ratio of wet lung weight to body weight to be less than normal. Compared to a normal lung, fewer and smaller peripheral airspaces are recognizable in hypoplastic lungs and they appear to be arrested at earlier stages of lung development and are histologically immature for the gestational age (Porter, 1998).

Normal growth of any organ relies on regulated cell cycle kinetics (i.e., cell proliferation and cell death). Because of the reduced size of hypoplastic lungs, it has been hypothesized that the lack of FBMs may contribute to disturbed cell cycle kinetics. Indeed, insufficiency or absence of FBMs has been associated with decreased proliferation and increased apoptosis of pulmonary cells in the hypoplastic lungs (Tseng at al., 2000; Inanlou and Kablar, 2003, 2005a,b). Recent data show that the pathological finding of attenuated lung cell proliferation in the hypoplastic lungs becomes more prominent with the advancing gestational age in the absence of FBMs and this may imply that the role of pulmonary distension on lung cell proliferation becomes more critical at later stages of lung development (Tseng et al., 2000; Inanlou and Kablar, 2003, 2005a,b).

In addition to cell proliferation, programmed cell death or apoptosis is also vital for normal development of many organs including lung by balancing cell proliferation (Vaux and Korsmeyer, 1999) and therefore an increased rate of pulmonary apoptosis can cause lung hypoplasia (Tseng et al., 2000; Inanlou and Kablar, 2003, 2005a,b). For instance, according to our recent findings, mdx:MyoD9th embryos (Inanlou and Kablar, 2003), *Myf5-/-* embryos (Inanlou and Kablar, 2005a) and *Myf5-/-:MyoD-/-* embryos (Inanlou and Kablar, 2005b) all have a different muscle phenotype and quite similar, but not identical, lung phenotype. mdx:MyoD9th embryos have significantly reduced diaphragm, Myf5-/- embryos have no rib cage and nonfunctional intercostal muscles and *Myf5-/-:MyoD-/-* embryos have no muscles at all. While mdx:MyoD9th embryos have the least severe lung phenotype (i.e., the elevated apoptosis is never an issue), *Myf5-/-* embryos have a more severe lung phenotype (i.e., apoptosis starts to be elevated at E16.5) and, finally, *Myf5-/-:MyoD-/-* embryos have even more severe phenotype, as indicated by the elevated lung apoptotic index as early as E14.5. However, the embryos of all three genotypes die shortly after birth due to the lung hypoplasia. Therefore, we think that even though the end result is the same (i.e., pulmonary hypoplasia and death), different mechanisms may be involved leading to that end result. In fact, the double-mutant embryos' lung phenotype suggests that to be the case (Inanlou and Kablar, 2005b). Therefore, even though the insufficient FBMs lead to decreased proliferation index, a parallel increase of the apoptosis index is observed only in the complete absence of FBMs, indicating that pulmonary cell proliferation pathways are more susceptible to mechanical factors than apoptosis pathways (Tseng et al., 2000; Inanlou and Kablar, 2003, 2005a,b).

Lung organogenesis is controlled by mediators including thyroid transcription factor-1 (TTF-1) and growth factors, such as platelet derived growth factors (PDGFs) and insulin-like growth factors (IGFs). TTF-1 is a member of NKX2 family of homeodomain transcription factors and is found in the thyroid, forebrain and lung (Lazzaro et al., 1991). Expression of TTF-1 in the lung is restricted to the epithelium at all stages of development and no expression can be identified in the surrounding mesenchyme (Hackett and Gitlin, 1997). During lung development, TTF-1 expression is suppressed in the epithelium of the proximal conductive ducts. However, its expression persists in the epithelium of lung periphery, leading to the formation of a proximal-to-distal increasing gradient in the expression of TTF-1 (Zhou et al., 2001). This gradient has been shown to be disturbed in pulmonary hypoplasia (Zhou et al., 2001; Inanlou and Kablar, 2003; 2005a,b) (Fig. 1). The exact mechanism(s) through which TTF-1 plays a role in normal lung organogenesis is unknown. However, TTF-1 has been recently shown to regulate the expression of midkine, a 13 kDa growth factor which mediates various developmental processes including cell proliferation (Reynolds et al., 2003). The described difference in the expression of TTF-1 along the proximal-to-distal axis may influence lung growth by its effect on the expression of midkine, although the precise mechanisms of that action are still unknown.

PDGFs serve as mitogenic competence factors that are composed of two polypeptide chains termed A and B. Competence growth factors have the ability to initiate the transition of quiescent (G0), non replicating cells

into the G1 phase of the cell cycle (Pledger et al., 1977) and to control cell cycle kinetics. Even though PDGF-AA is required for pulmonary branch morphogenesis, PDGF-BB regulates lung growth. Mechanical stretch has been reported to up-regulate PDGF-BB and its receptor (PDGF-ßR) in lung cells (Liu et al., 1995). Indeed, lack of FBMs is associated with down-regulation of PDGF-BB and its receptor in vivo (Inanlou and Kablar, 2005a,b), explaining a possible mechanism for decreased cell proliferation in the hypoplastic lungs. In addition to its role in cell proliferation, other studies show that PDGFs also prevent cell apoptosis (Desai and Gruber, 1999). Consistently, a consequent increase in cell apoptosis of pulmonary cells in the absence of FBMs can be attributed to the down-regulation of PDGF-BB and its receptor (Inanlou and Kablar, 2005a,b).

The other factors that control cell cycle kinetics are the IGFs. They are two single chain polypeptides that are named IGF-I and IGF-II. These factors are expressed in a great variety of tissues, indicating their autocrine or paracrine regulatory roles of cellular function. IGF-I is a progression growth factor and advances cells through the remaining hours of G1 phase (Fabisiak and Kelley, 1992). Interestingly, similarly to PDGF-BB, IGF-I promotes cell proliferation and prevents cell apoptosis (Baxter, 1988; Barres et al., 1992). Pulmonary expansion increases the expression of IGF-I in the lung (Joe et al., 1997). Previous studies have shown that the lack of mechanical forces in vivo contributes to the down-regulation of IGFs (Harding et al., 1993; Hooper et al., 1993; Inanlou and Kablar, 2005a,b), providing an explanation for the lack of FBMs resulting in decreased cell proliferation and increased cell apoptosis in the hypoplastic lungs.

It has been shown that in the absence of FBMs changes in cell cycle kinetics of pulmonary cells (i.e., decreased cell proliferation and increased cell death) start at earlier stages of lung development (i.e., psuedoglandular and canalicular). However, down-regulation of PDGFs and IGFs only occurs at the later stage (i.e., saccular) (Inanlou and Kablar, 2005a,b). These findings may imply that in addition to PDGFs and IGFs, other mediators are also involved in the regulation of cell cycle at earlier stages of lung development. The identity of these early factors has yet to be revealed.

In conclusion, FBMs seem to be important for lung growth because of their effects on lung cell proliferation and death, possibly by establishing the gradient of TTF-1 expression and regulating the expression of PDGFs and IGFs.

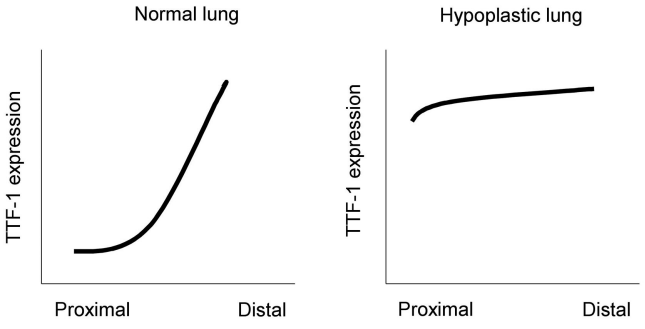


**Fig. 1.** TTF-1 expression is disturbed in the absence of FBMs. In the normal lung, the expression of TTF-1 is suppressed in the epithelium of proximal conductive ducts and it persists in the epithelium of the lung periphery, leading to the formation of the proximal-to-distal increasing gradient of TTF-1 expression. In the absence of FBMs this gradient is disturbed in the hypoplastic lungs and the epithelium of proximal conductive ducts continues to express TTF-1.

### FBMs and the lung maturation

In addition to lung growth, FBMs are also required for lung maturation. Maturation of all organs in the body relies on cell differentiation. Lung is composed of different cell types including Clara cells, type I and type II pneumocytes. Biochemical differentiation of lung cells is defined by the expression of their specific markers. Expression of a 10 kDa protein termed CC10 is believed to characterize biochemical differentiation of Clara cells, the non-ciliated epithelial cells of the pulmonary airways (Singh et al., 1988). Lack of FBMs does not reveal any difference in the expression of CC10 between normal and hypoplastic lungs (Inanlou and Kablar, 2005a,b), indicating that biochemical differentiation of Clara cells remains intact in the absence of mechanical stimuli *in vivo*.

Surfactant-associated proteins (SPs), that are synthesized and secreted by pneumocytes type II (Whitsett and Sever, 1997), are considered to be specific markers for the biochemical differentiation of type II cells (Phelps and Floros, 1991). Distribution of SPs expression has been found normal in pulmonary hypoplasia caused by the lack of FBMs (Tseng et al., 2000; Inanlou and Kablar, 2005a,b), suggesting that mechanical forces are not vital for the biochemical differentiation of type II cells in vivo. During pulmonary development, type II cells give birth to type I cells (Mallampalli et al., 1997). One of the specific markers of type I cells is T1a (or Gp38) (Williams, 2003), an apical membrane protein of unknown function. Even though in different stages of development this protein is found in several organs, in the lung it is exclusively expressed by type I cells. Previous in vitro studies report that mechanical distension up-regulates RTI40, a rat homologue of T1α (Dobbs and Gutierrez; 2001). By contrast, in our mouse in vivo models of pulmonary hypoplasia it appears that T1a is not influenced by FBMs (Inanlou and Kablar, 2005b), suggesting that the early differentiation of type I pneumocytes is also independent on FBMs.

In addition to the early biochemical differentiation, in some studies the later morphological differentiation of type I and II pneumocytes is reported to be intact in the absence of FBMs (Alcorn et al., 1980; Tseng et al.,

2000). However, others believe that final differentiation of these cell types is dependent on mechanical stimuli in vivo (Nagai et al., 1988; Benachi et al., 1999; Inanlou and Kablar, 2005a,b). Maturation of type II pneumocytes is reported to be associated with a decrease in the cytoplasmic glycogen and a simultaneous increase in the number of intracellular organelles called lamellar bodies. Lamellar bodies are composed of layers of membranes surrounding a non-lamellar proteinous central core and they are required for assemblage and storage of surfactant (Chi, 1985; Ten Have-Opbroek et al., 1990). Cytoplasmic glycogen is used by type II cells as a substrate for surfactant assemblage (i.e., compiling of surfactant-associated proteins and phospholipids) (Bantenburg, 1992). Periodic acid Schiff (PAS) staining for detection of cytoplasmic glycogen has revealed that type II cells are unable to utilize glycogen for the synthesis of the surfactant. Consistently, transmission electron microscopy (TEM) shows that the number of cytoplasmic lamellar bodies per cell is significantly reduced in the hypoplastic lungs (Nagai et al., 1988; Brandsma et al., 1993; Inanlou and Kablar, 2005b), while the intraalveolar lamellar bodies are scarce, loose and disorganised (Inanlou and Kablar, 2005b). To form a phospholipid monolayer upon alveolar surfaces, lamellar bodies are transformed to intermediate structures called tubular myelins (Williams, 1977). Compared to the normal lungs, tubular myelins are hard to find and their structure seems to be loose and disorganized in the hypoplastic lungs (Inanlou and Kablar, 2005b). These findings suggest that type II cells are unable to complete their morphological differentiation and consequently synthesis, assemblage and secretion of the surfactant are disturbed.

Type I cells are the other group of pulmonary cells that are morphologically characterized by a flattened nucleus and an extended cytoplasm containing numerous, well defined small cytoplasmic vesicles (Williams, 1990). In the absence of FBMs no cells with the characteristics of differentiated type I cells is found in the hypoplastic lungs and instead of squamous type I cells, cuboidal cells are found without well-defined cytoplasmic vesicles. These findings indicate that type I cells are unable to complete their morphological differentiation and consequently are non functional (Inanlou and Kablar, 2005b).

In summary, FBMs appear to affect pulmonary development by influencing both lung growth and lung cell differentiation. Lung growth appears to be regulated by the FBMs via growth factors (e.g., PDGFs and IGFs) and TTF-1 that act upon cell cycle kinetics. Lung cell differentiation apparently also requires FBMs, since the accomplishment of morphological differentiation of type I and type II cells is not possible in the complete absence of respiratory muscles (Fig. 2).

Currently, we are comparing the lung of embryos that contain type I and II pneumocytes arrested as described at different stages of differentiation (i.e., the lung of *Myf5-/-:MyoD-/-* embryos that develop pulmonary hypoplasia) to the lung of normal control embryos, using cDNA microarray analysis (Pastorian et al., 2000). By way of this approach, it is possible to perform molecular comparisons (e.g., the type of the gene and the amount of the gene expressed) between the mutant and the control tissues. Molecules that are not present in the mutant lung are assumed to be specific for the lacking differentiation steps of type I and II pneumocytes. For example, we have identified two molecules that are very likely important for type I and II pneumocyte differentiation. One gene is found to be more than 12 times down-regulated in the mutant lung tissue and is probably relevant to type II differentiation steps. Previous studies have identified this gene as important in lung vasculogenesis during development (Wani et al., 1999). The second gene is also found down-regulated in the mutant lung tissue and is probably relevant to type I differentiation steps. Previous studies have revealed that this gene plays a role in cell locomotion and cytokinesis (De Hostos, 1999).

In conclusion, we believe that the identification of molecules responsible for pneumocyte I and II differentiation will allow us to perform numerous follow-up experiments, which in turn will increase our understanding of processes that lead to pulmonary hypoplasia, an important cause of neonatal morbidity and mortality.

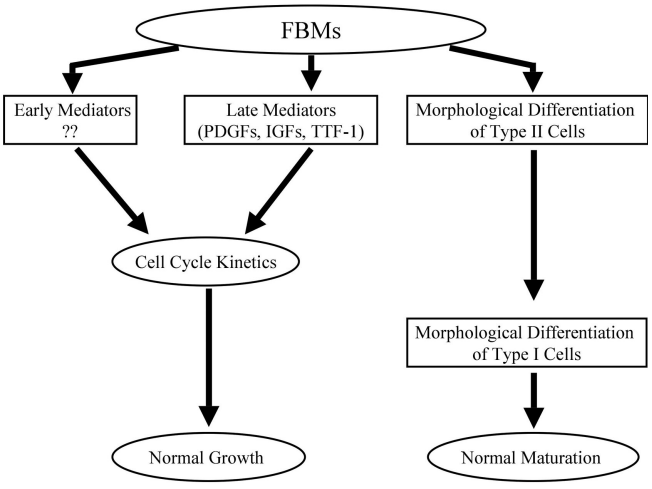


**Fig. 2.** FBMs, lung growth and functional maturation. FBMs seem to play a role in lung growth through their effects on lung cell cycle kinetics by regulating the expression of growth factors, such as PDGFs and IGFs, and establishing the gradient of TTF-1 expression at the last stage of lung organogenesis (i.e., "late" mediators). It is also suggested that FBMs influence the expression of other unknown growth factors (i.e., "early" mediators), that are responsible for changes in cell cycle kinetics at earlier stages of lung development. FBMs appear to also be required for the accomplishment of the morphological differentiation of type I and type II pneumocytes.

*Acknowledgements.* This work was supported by an operating grant from Natural Sciences and Engineering Research Council of Canada (NSERC), Lung Association of Nova Scotia (LANS) and infrastructure grants from Canada Foundation for Innovation (CFI) and Dalhousie Medical Research Foundation (DMRF) to B.K. M.R.I. is recipient of the Nova Scotia Health Research Foundation (NSHRF) fellowship for this project.

## References

Abadie V., Champagnat J. and Fortin G. (2000). Branchiomotor activities in mouse embryo. Neuroreport 11, 141-145.

Alcorn D., Adamson T.M., Maloney J.E. and Robinson P.M. (1980). Morphological effects of chronic bilateral phrenectomy or vagotomy in the fetal lamb lung. J. Anat. 130, 683-695.

Batenburg J.J. (1992). Surfactant phospholipids: synthesis and storage. Am. J. Physiol. 262, L367-L385.

Barres B.A., Hart I.K., Coles H.S., Burne J.F., Voyvodic J.T., Richardson W.D. and Raff M.C. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. Cell 70, 31-46.

Baxter R.C. (1988). The insulin-like growth factors and their binding proteins. Comp. Biochem. Physiol. B 91, 229-235.

Benachi A., Delezoide A.L., Chailley-Heu B., Preece M., Bourbon J.R. and Ryder T. (1999). Ultrastructural evaluation of lung maturation in a sheep model of diaphragmatic hernia and tracheal occlusion. Am. J. Respir. Cell Mol. Biol. 20, 805-812.

Brandsma A.E., Tibboel D., Vulto I.M., Egberts J. and Ten Have-Opbroek A.A. (1993). Ultrastructural features of alveolar epithelial cells in the late fetal pulmonary acinus: a comparison between normal and hypoplastic lungs using a rat model of pulmonary hypoplasia and congenital diaphragmatic hernia. Microsc. Res. Tech. 26, 389-399.

Cardoso W.V. (2000). Lung morphogenesis revisited: old facts, current ideas. Dev. Dyn. 219, 121-130.

Chi E.Y. (1985). The ultrastructural study of glycogen and lamellar bodies in the development of fetal monkey lung. Exp. Lung Res. 8, 275-289.

De Hostos E.L. (1999). The coronin family of actin-associated proteins. Trends Cell Biol. 9, 345-350.

Desai B.J. and Gruber H.E. (1999). Anti-apoptotic actions of cytokines in mammalian cells. Proc. Soc. Exp. Biol. Med. 221, 1-13.

de Vries J.I., Visser G.H. and Prechtl H.F. (1986). Fetal behaviour in early pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 21, 271-276.

Dobbs L.G. and Gutierrez J.A. (2001). Mechanical forces modulate alveolar epithelial phenotypic expression. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 129, 261-266.

Fabisiak J.P. and Kelley J. (1992). Platelet-derived growth factor. In: Cytokines of the lung. Kelley J. (eds). Marcel Dekker Inc. New York. pp 3-39.

Hackett B.P. and Gitlin J.D. (1997). Role of transcription factors in the development of the pulmonary epithelium. In: Lung growth and development. McDonald J.A. (ed). Marcel Dekker Inc. New York. pp 55-80.

Harding R., Hooper S.B. and Han V.K. (1993). Abolition of fetal breathing movements by spinal cord transection leads to reductions in fetal lung liquid volume, lung growth, and IGF-II gene expression. Pediatr. Res. 34, 148-153.

Harding R. (1997). Fetal pulmonary development: the role of respiratory movements. Equine Vet. J. Suppl. 24, 32-39.

Hooper S.B., Han V.K. and Harding R. (1993). Changes in lung expansion alter pulmonary DNA synthesis and IGF-II gene expression in fetal sheep. Am. J. Physiol. 265, L403-L409.

Inanlou M.R. and Kablar B. (2003). Abnormal development of the diaphragm in mdx:MyoD-/- 9th embryos leads to pulmonary hypoplasia. Int. J. Dev. Biol. 47, 363-371.

Inanlou M.R. and Kablar B. (2005a). Abnormal development of the intercostal muscles and the rib cage in *Myf5-/-* embryos leads to pulmonary hypoplasia. Dev. Dyn. 232, 43-54.

Inanlou M.R. and Kablar B. (2005b). Contractile activity of skeletal musculature involved in breathing is essential for normal lung cell differentiation, as revealed in *Myf5-/-:MyoD-/-* embryos. Dev. Dyn. (in press).

Joe P., Wallen L.D., Chapin C.J., Lee C.H., Allen L., Han V.K., Dobbs L.G., Hawgood S. and Kitterman J.A. (1997). Effects of mechanical factors on growth and maturation of the lung in fetal sheep. Am. J. Physiol. 272, L95-L105.

Kitterman J.A. (1996). The effects of mechanical forces on fetal lung growth. Clin. Perinatol. 23, 727-740.

Laudy J.A. and Wladimiroff J.W. (2000). The fetal lung. 1: Developmental aspects. Ultrasound Obstet. Gynecol. 16, 284-290.

Lazzaro D., Price M., de Felice M. and Di Lauro R. (1991). The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. Development 113, 1093-1104.

Liggins G.C., Vilos G.A., Campos G.A., Kitterman J.A. and Lee C.H. (1981). The effect of spinal cord transection on lung development in fetal sheep. J. Dev. Physiol. 3, 267-274.

Liu M. and Post M. (2000). Mechanochemical signal transduction in the fetal lung. J. Appl. Physiol. 89, 2078-2084.

Liu M., Liu J., Buch S., Tanswell A.K. and Post M. (1995). Antisense oligonucleotides for PDGF-B and its receptor inhibit mechanical strain-induced fetal lung cell growth. Am. J. Physiol. 269, L178-L184.

Mallampalli R.K., Acarregui M.J. and Snyder J.M. (1997). Differentiation of the alveolar epithelium in the fetal lung. In Lung growth and development. McDonald J.A. (eds). Marcel Dekker Inc. New York. pp 119-162.

Nagai A., Thurlbeck W.M., Jansen A.H., Ioffe S. and Chernick V. (1988). The effect of chronic biphrenectomy on lung growth and maturation in fetal lambs. Morphologic and morphometric studies. Am. Rev. Respir. Dis. 137, 167-172.

Pastorian K., Hawel L. 3rd and Byus C.V. (2000). Optimization of cDNA representational difference analysis for the identification of differentially expressed mRNAs. Anal. Biochem. 283, 89-98.

Phelps D.S. and Floros J. (1991). Localization of pulmonary surfactant proteins using immunohistochemistry and tissue in situ hybridization. Exp. Lung Res. 17, 985-995

Pledger W.J., Stiles C.D., Antoniades H.N. and Scher C.D. (1977). Induction of DNA synthesis in BALB/c 3T3 cells by serum components: reevaluation of the commitment process. Proc. Natl. Acad. Sci. USA 74, 4481-4485.

Porter H.J. (1998). Pulmonary hypoplasia--size is not everything. Virchows. Arch. 432, 3-6.

Reynolds P.R., Mucenski M.L. and Whitsett J.A. (2003). Thyroid transcription factor (TTF) -1 regulates the expression of midkine (MK) during lung morphogenesis. Dev. Dyn. 227, 227-237.

Singh G., Singh J., Katyal S.L., Brown W.E., Kramps J.A., Paradis I.L., Dauber J.H., Macpherson T.A. and Squeglia N. (1988). Identification, cellular localization, isolation, and characterization of human Clara cell-specific 10 KD protein. J. Histochem. Cytochem. 36, 73-80.

Skinner S.J.M. (1989). Fetal breathing movements: a mechanical stimulus for fetal lung cell growth and differentiation. In: Advances in fetal physiology. Gluckman B.D., Johnston B.M. and Nathanielsz P.W. (eds). Perinatology Press. New York. pp 133-151.

Ten Have-Opbroek A.A. (1981). The development of the lung in mammals: an analysis of concepts and findings. Am. J. Anat. 162, 201-219.

Ten Have-Opbroek A.A., Otto-Verberne C.J. and Dubbeldam J.A. (1990). Ultrastructural characteristics of inclusion bodies of type II cells in late embryonic mouse lung. Anat. Embryol. (Berl). 181, 317-323.

Tseng B.S., Cavin S.T., Booth F.W., Olson E.N., Marin M.C., McDonnell T.J. and Butler I.J. (2000). Pulmonary hypoplasia in the myogenin null mouse embryo. Am. J. Respir. Cell Mol. Biol. 22, 304-315.

Vaux D.L. and Korsmeyer S.J. (1999). Cell death in development. Cell 96, 245-254.

Wani M.A., Wert S.E. and Lingrel J.B. (1999). Lung Kruppel-like factor, a zinc finger transcription factor, is essential for normal lung development. J. Biol. Chem. 274, 21180-21185.

Whitsett J.A. and Sever Z. (1997). Respiratory epithelial cell gene transcription. In: Lung growth and development. McDonald J.A. (eds). Marcel Dekker Inc. New York. pp 37-54.

Wigglesworth J.S. and Desai R. (1982). Is fetal respiratory function a major determinant of perinatal survival? Lancet 1, 264-267.

Williams M.C. (1977). Conversion of lamellar body membranes into tubular myelin in alveoli of fetal rat lungs. J. Cell Biol. 72, 260-277.

Williams M.C. (1990). The alveolar epithelium, structure and study by immunocytochemistry. In: Electron microscopy of the lung. Schraufnagel D.E. (eds). Marcel Dekker Inc. New York. pp 121-147

Williams M.C. (2003). Alveolar type I cells: molecular phenotype and development. Annu. Rev. Physiol. 65, 669-695.

Zhou H., Morotti R.A., Profitt S.A., Langston C., Wert S.E., Whitsett J.A. and Greco M.A. (2001). Expression of thyroid transcription factor-1, surfactant proteins, type I cell-associated antigen, and Clara cell secretory protein in pulmonary hypoplasia. Pediatr. Dev. Pathol. 4, 364-371.

Accepted May 3, 2005