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Review

Role of skeletal muscle in lung development

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Summary. Skeletal (striated) muscle is one of the four basic tissue types, together with the epithelium, connective and nervous tissues. Lungs, on the other hand, develop from the foregut and among various cell types contain smooth, but not skeletal muscle. Therefore, during earlier stages of development, it is unlikely that skeletal muscle and lung depend on each other. However, during the later stages of development, respiratory muscle, primarily the diaphragm and the intercostal muscles, execute so called fetal breathing-like movements (FBMs), that are essential for lung growth and cell differentiation. In fact, the absence of FBMs results in pulmonary hypoplasia, the most common cause of death in the first week of human neonatal life. Most knowledge on this topic arises from *in vivo* experiments on larger animals and from various i*n vitro* experiments. In the current era of mouse mutagenesis and functional genomics, it was our goal to develop a mouse model for pulmonary hypoplasia. We employed various genetically engineered mice lacking different groups of respiratory muscles or lacking all the skeletal muscle and established the criteria for pulmonary hypoplasia in mice, and therefore established a mouse model for this disease. We followed up this discovery with systematic subtractive microarray analysis approach and revealed novel functions in lung development and disease for several molecules. We believe that our approach combines elements of both *in vivo* and *in vitro* approaches and allows us to study the function of a series of molecules in the context of lung development and disease and, simultaneously, in the context of lung's dependence on skeletal muscle-executed FBMs.

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Introduction

During the last third of gestation, in humans and in mice, intermittent fetal breathing-like movements (FBMs) generate pressure changes in the developing lung. These prenatal pressure changes are believed to be similar to the pressure changes caused by postnatal breathing (N.B., unlike regular breathing, the FBM bursts are separated by a resting period). Mounting evidence suggests that FBMs are important for normal lung growth and functional maturation, because absence of FBMs results in pulmonary hypoplasia (Kitterman, 1996; Inanlou et al., 2005), which is the leading cause of death, in the neonate (Liggins, 1984). FBMs seem to produce strain-induced fine tuning of lung growth and alveolar epithelial differentiation because an increased volume and pressure of the fluid in the developing lung is shifted back and forth between different parts of the lung as a consequence of the intermittent distension caused by the FBMs (Wirtz and Dobbs, 2000; Inanlou et al., 2005).

Physical forces sometimes referred to as stretch or distension are in fact more precisely describable as a variety of different mechanical forces, such as: stress (force per unit surface area), strain (lengthening of a structure), shear stress (force of fluid flow on cell surface), spring force (returns the spring to its original length), surface tension (differences in intracellular adhesion and cytoskeletal contractility), and pre-stress (isometric tension that balances intracellular and extracellular tensional pulling), etc. (Wirtz and Dobbs, 2000; Mammoto and Ingber, 2010). These different types of mechanical forces generated inside and between cells, tissues and organs are as essential as genes and chemical signals for the control of embryonic

development, neoplastic transformations, and stem cell potency. This realization calls for synergistic action of biologists, physicists, engineers and other scientists to define an interdisciplinary approach leading to the discovery of links between mechanical, chemical and other factors that operate to form a functional living body.

Various methods have been developed to study the role of physical forces in lung growth and alveolar epithelial differentiation. For example, surgically-induced tracheal ligation, diaphragmatic hernia, spinal cord or phrenic nerve transection were performed to cause the lack of different types of physical stimulations to the developing lung (Wirtz and Dobbs, 2000; Inanlou et al., 2005). In summary, these different surgically-induced approaches resulted in at least somewhat different and sometimes even opposing effects on the lung. Generally speaking, under-distension (as it occurs in diaphragmatic hernia, lung liquid drainage, abolition of FBMs) caused the lung to be too small and it favored type II cell phenotype (at the expense of the type I cell phenotype), while over-distension (as it occurs in tracheal ligation) favored type I cell phenotype (at the expense of the type II cell phenotype) (Wirtz and Dobbs, 2000; Inanlou et al., 2005). This is relevant information, considering that during lung development undifferentiated cuboidal alveolar epithelial cells further differentiate into type II cells, and subsequently some of type II cells differentiate into type I cells (Liu and Post, 2000).

Lung tissue consists of a number of cell types and shows a great degree of cellular and special complexity. It is therefore difficult to ascribe the effects of mechanical cues to individual components *in vivo*. For that reason, various *in vitro* approaches have also been developed to investigate the role of mechanical forces in lung development (e.g., primary cultures, cell lines, 2D and 3D cultures with single or mixed cell type), resulting in diversity and discrepancy, but also essentially agreeing that mechanical cues affect lung cell cycle and differentiation (Liu and Post, 2000). It seems however, that the major issue is in finding a way to differentiate between the intermittent versus continuous static and cyclic stretch. It has been found that intermittent stretch stimulates cell proliferation and extra-cellular matrix production, without causing cell injury, while continuous stretch increases cytokine production and cell injury (Liu and Post, 2000). Therefore, the type of physical force will likely employ different transduction pathways and molecules to translate the mechanical stimuli to the meaningful information at the cellular level.

FBMs and lung volume are interdependent and have so far been studied within the same *in vivo* experimental approach, mainly by employing the spinal cord transection procedure. This approach eliminates the FBMs, but it also reduces the amount of the fetal lung liquid (Harding et al., 1993), making it difficult to discriminate between the effects of the static (and continuous) stretch of the liquid and the cyclic (and intermittent) stretch from FBMs.

In an attempt to approach *in vivo* (but non-surgically) at least some aspects of the complex relationship that operates to connect the mechanical, molecular and other factors to form a body, we developed a two-step process based on mouse mutagenesis, anatomical sciences and microarray analysis. We consider this a genetically-induced approach, as opposed to the previously performed surgically-induced approaches, mentioned before. More specifically, our interest was to understand the nature of interactions between the skeletal (or striated) muscle, the executor of FBMs, and therefore the provider of a particular kind of mechanical force, and the developing mouse lung (N.B., as mentioned before, there are various kinds of physical forces that act upon lung *in vivo* and therefore various types of mechanical forces, and their combinations, were previously studied in different *in vivo* approaches). In other words, the elimination of FBMs without eliminating the innervation by spinal cord transaction may provide a "cleaner" model for studying the role of cyclic (intermittent) stretch from FBMs in lung development, because this approach affects less the static (and continuous) stretch by the liquid, as it leaves the innervation intact (Inanlou and Kablar, 2005b). We were particularly interested in the lung growth and its functional maturation, as viewed by studying the two types of alveolar epithelial cells, the one that produces the surfactant (type II cells) and the one that is the part of the blood-air barrier for gas exchange (type I cells). The result of interactions between the muscle and the lung is not predictable from the intrinsic development of either the muscle or the lung, but could be understood only by studying the connection (i.e., relationship) between the two (Kablar, 2011). The involvement of skeletal muscle in the shaping of developing cells, tissues and organs is an important example of Waddingtonian epigenetics, i.e., the study of the sum of genetic and non-genetic factors that control gene expression and produce phenotypic complexity.

The current review will explain the steps we undertook to date and represents the continuation of our work previously reviewed (Inanlou et al., 2005). We believe that the approach currently reviewed combines some interesting abilities of both *in vivo* and *in vitro* approaches, making it possible to suggest particular molecular players that bridge the gap between the mechanical cues produced by the muscle and the effect that they have on lung growth and cell differentiation. The intention of this review is to provide principal points of our discoveries, so that the readers can easily follow the steps we undertook, in case they would like to apply our approach to their own research topic.

Step I: mechanical relationship between the skeletal muscle and the developing lung

The first attempt that we undertook towards elucidation of the complex relationship between the

skeletal muscle and the lung was to examine lung development in the complete absence of all skeletal musculature (in *Myf5:MyoD* null embryos and fetuses), as well as in the absence of particular groups of respiratory muscles, such as the diaphragm and the intercostal muscles (Inanlou and Kablar, 2003, 2005a,b). We established that the lung in the absence of the muscle (and therefore mechanical forces from the FBMs) was hypoplastic, and that type I and II pneumocytes failed to fully differentiate (Inanlou and Kablar, 2005b). Indeed, even though it is possible that the muscle also has a paracrine role in this process (e.g., as the source of certain circulatory proteins), it is unlikely that a muscle-specific protein or a group of proteins, as opposed to just mechanical forces, had an impact on the lung phenotype found in muscleless fetuses (Inanlou et al., 2006). Furthermore, Myf5 and MyoD are not expressed or contained in the lung (Inanlou and Kablar, 2003, 2005a). Details of our findings can be found in individual papers cited in the current review, and also in the review article and book chapter previously published (Inanlou et al., 2005; Kablar, 2011). Here, we can summarize that in the analyzed Myf5:MyoD null embryos and fetuses, the lung weight was significantly decreased due to reduced cell proliferation and increased cell death in the lung tissue. Histopathologically, the hypoplastic lung was arrested at the canalicular stage. Thyroid transcription factor (TTF)-1 lost its normal proximal-to-distal distribution gradient. In addition to the failure in growth, the lungs also exhibited failures in cell differentiation. Specifically, type II pneumocytes, responsible for the synthesis of surfactant, failed to assemble (i.e., did not utilize glycogen adequately), store (i.e., had irregular lamellar bodies) and secrete (i.e., had irregular myelin figures) the surfactant. At the same time, type I pneumocytes, responsible for gas exchange, failed to differentiate from a cuboidal cell type into the squamous cell type (i.e., failed to flatten) in order to become a part of the blood-air barrier (Inanlou and Kablar, 2005b). Taken together, we concluded that the growth of the lung, and the differentiation of the alveolar epithelium (type I and II pneumocytes), depended on the mechanical stimuli from the respiratory musculature. Indeed, recent *in vitro* study also concludes that mechanical stretch promotes, via growth factors, fetal type II cell differentiation (Wang et al., 2009). Together, we are getting closer to an understanding of how lung cells sense and convert mechanical signals into biochemical responses essential for lung development.

Step II: molecular relationship between the skeletal muscle and the developing lung

The next step of our analysis was to identify molecular players that may be involved in this mechanical relationship between the muscle and the lung. The specific differentiation failure of type I and II pneumocytes, found in *Myf5:MyoD* null fetuses, prompted us to perform the so called systematic subtractive microarray analysis approach (SSMAA) to reveal a profile of genes involved in type I and II pneumocyte differentiation. In other words, we hypothesized that the difference in gene expression patterns between the control and the mutant lung would be related to the described differentiation failures of the alveolar epithelium. Indeed, our Affymetrix Gene Chip cDNA microarray analysis revealed 9 up-regulated and 54 down-regulated genes (Baguma-Nibasheka et al., 2007). Out of the 54 down-regulated genes, the literature and databases search detected 24 viable and fertile knockout mice that did not show an abnormal lung phenotype as single knockouts (N.B., generation of double-knockouts would be useful for further elucidation of the role of these 24 molecules in lung development and disease). Furthermore, two knockout mice died too early during development to be useful for studies relevant to lung organogenesis (N.B., generation of conditional mutants would be useful for further elucidation of the role of these two molecules in lung development and disease). Finally, our analysis revealed four molecules whose knockouts die at birth due to respiratory failure: *T-cell receptor ß, variable 13 (Tcrb-V13), connective tissue growth factor (Ctgf), special AT-rich sequence binding protein 1 (Satb1), and myeloblastosis oncogene (Myb).*

Tcrb-V13 (also known as *LKLF*) nulls had been analyzed before our microarrays were performed, by another group. Consistent with our microarray data, pulmonary hypoplasia was revealed in the mouse chimeras, with apparently normal pneumocytes (Kuo et al., 1997).

Ctgf null lungs revealed all the criteria for mouse pulmonary hypoplasia and specifically a failure of type II pneumocytes to properly assemble and store the surfactant (Baguma-Nibasheka and Kablar, 2008).

Satb1 null lungs (Alvarez et al., 2000; kindly provided by Dr. Terumi Kohwi-Shigematsu and Dr. Masaru Miyano, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, at postnatal day, P1) revealed several criteria for mouse pulmonary hypoplasia (e.g., 67% reduction of lung weight, disturbance of the TTF-1 gradient) and, specifically, the ratio between type II and I pneumocytes was strikingly affected. While controls contained 66±11% of type II cells and 34±10% of type I cells within the pneumocyte population, the number of type II cells was significantly increased (85±10%) and the number of type I cells was significantly decreased (15±9%) in the *Satb1*-/- lungs ($p<0.05$) (Fig. 1).

Myb null lungs (Sumner et al., 2000; kindly provided by Dr. Jonathan Frampton, Birmingham University Medical School, Birmingham, UK, at embryonic day, E15.5) revealed several criteria for mouse pulmonary hypoplasia (e.g., 63% reduction of lung weight, decrease in the cell proliferation index, PI, as revealed by PCNA). The average PI in *Myb*-/- lungs was decreased to 43±5% in the epithelium and 34±4% in the mesenchyme as compared to 75±5% and 65±4%,

respectively, in the controls ($p<0.05$) (Fig. 2).

In addition, we examined the fetal lungs of mice lacking some genes which microarray analysis has shown to be down-regulated in our amyogenic fetuses, but whose knockout does not appear to affect viability (Baguma-Nibasheka et al., 2007), such as: *Rag1* (*recombination activating gene 1*) null lungs (Mombaerts et al., 1992; kindly provided by Dr. Claus Nerlov, EMBL, Monterotondo, Rome, IT, at E18.5) and *Rock2* (*Rho-associated kinase 2*) null lungs (Pelosi et al., 2007; kindly provided by Dr. Nadia Rosenthal and Dr. Michele Pelosi, EMBL, Monterotondo, Rome, IT, at E18.5), that showed a normal lung phenotype (Fig. 2).

In conclusion, we provided here several examples of SSMAA that accomplished its purpose by revealing several molecular players involved in the mechanical relationship between the muscle and the lung. A number of molecules from our microarray list are in the pipeline

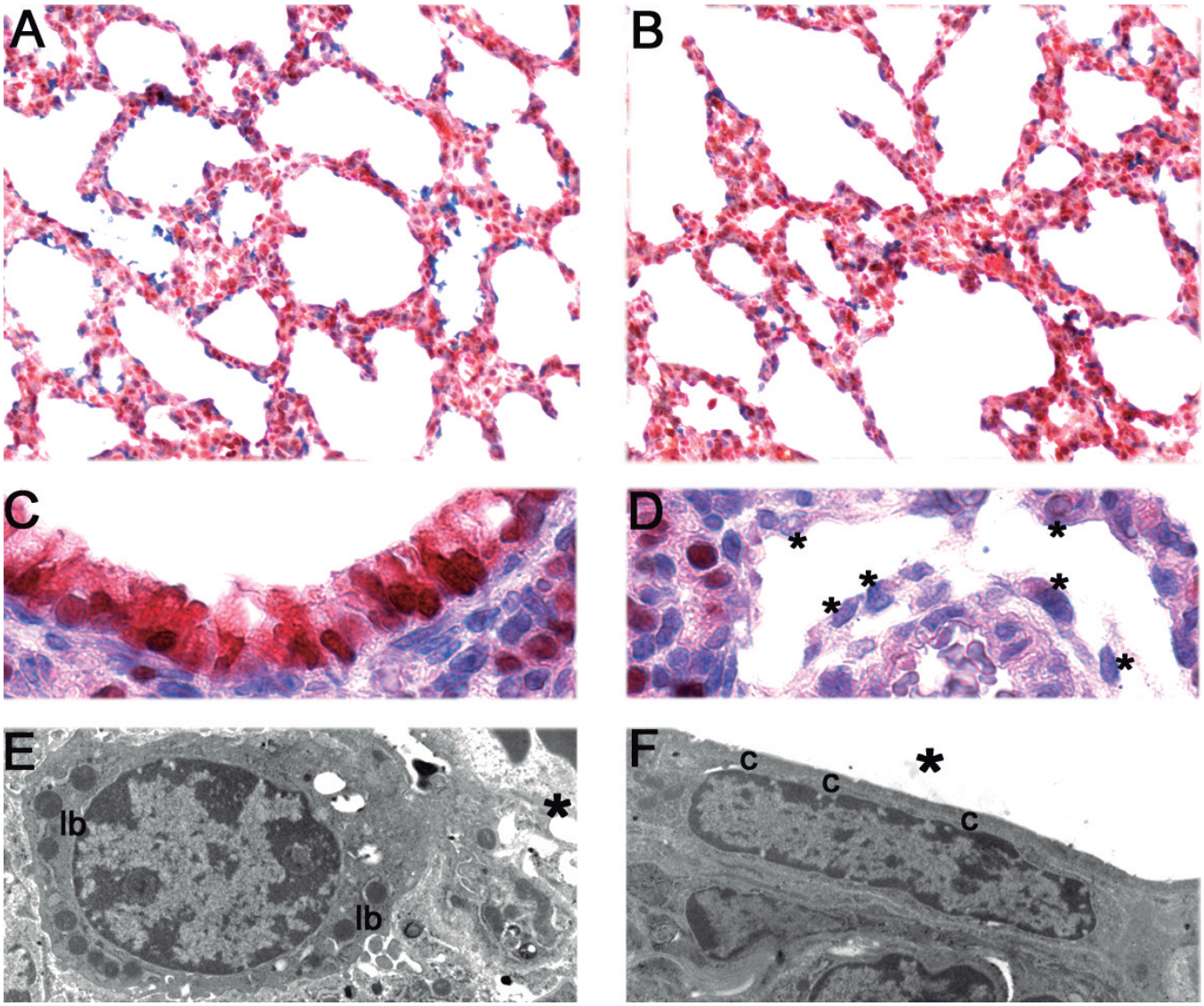

Fig. 1. *Satb1-/-* lung has disturbed TTF-1 distribution pattern and decreased number of type I pneumocytes at P1. **A, B.** *Satb1-/-* **(B)** neonatal lung is 67% reduced in weight, but histologically appears similar to the control (**A). C, D.** The TTF-1 (Santa Cruz rabbit polyclonal against TTF-1, 4 μg/ml) gradient is reversed in *Satb1-/-* lungs, with almost all the columnar epithelial cells of the proximal conductive ducts **(C)** still staining strongly for TTF-1 (as in pulmonary hypoplasia). Unlike pulmonary hypoplasia (and unlike the controls), TTF-1 is absent in the distal ducts **(D)**. The asterisks in **D** are placed inside the alveolar space and near the nucleus of unstained (TTF-1-negative) alveolar epithelial cells. **E, F.** (TEM, Transmission Electron Micrograph). Type II cell **(E)** is cuboidal, containing normal lamellar bodies (lb) in its cytoplasm. Type I cell **(F)** contains a few organelles and has an exquisitely thin cytoplasm (c) and a flat nucleus. Morphometry, performed on these cells in four randomly selected grid squares covering an area of 0.03 mm^2 for each embryo, as previously described (Baguma-Nibasheka and Kablar, 2008), revealed a misbalance in type II vs. type I cells. The asterisk in **E and F** indicates the alveolar space. A, B, x 400; C, D, x 630; E, F, x 12,000

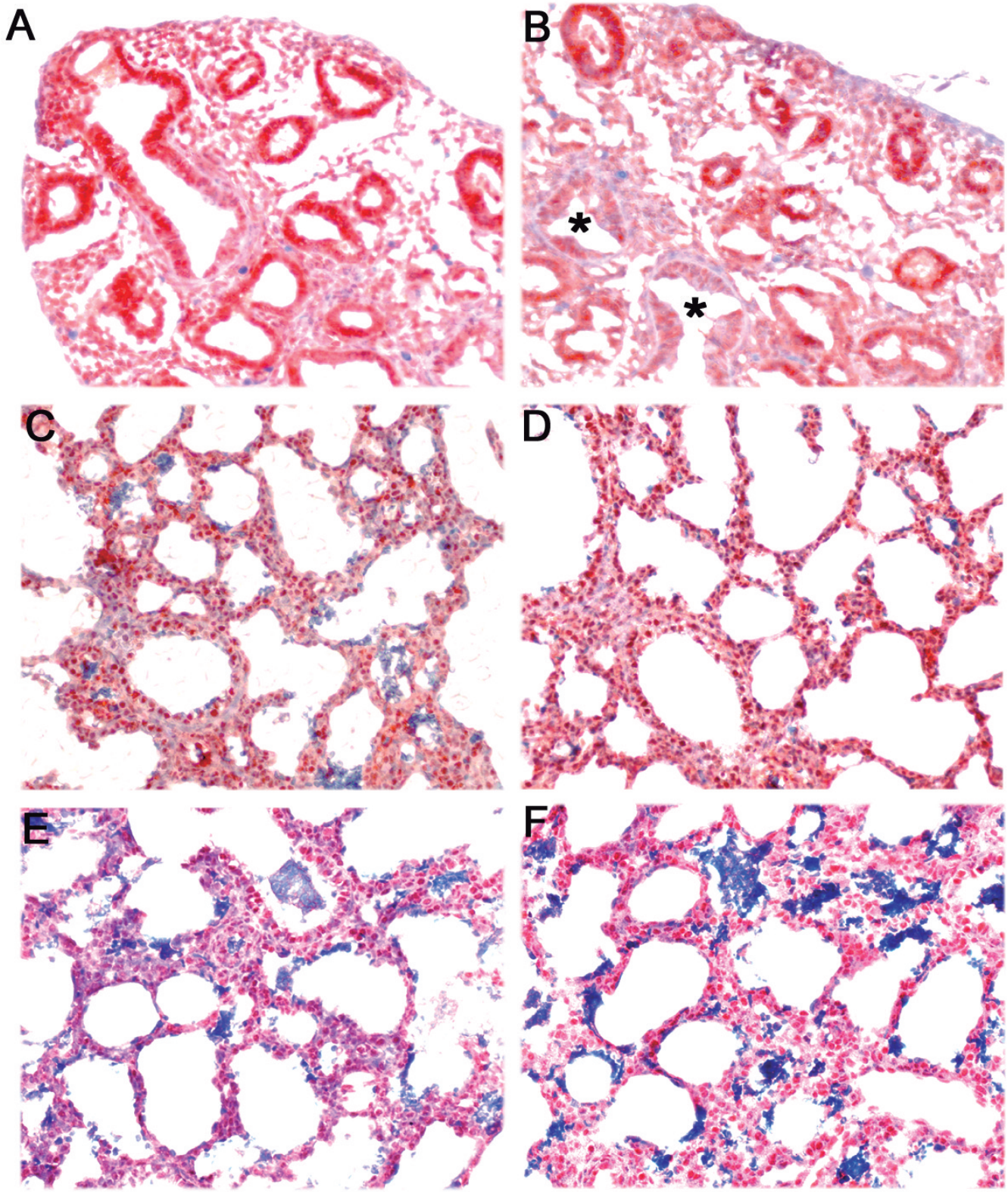

Fig. 2. *Myb-/-* lung is hypoplastic, while the lung of *Rag1-/-* and *Rock2-/-* fetuses is normal. **A, B.** *Myb-/-* E15.5 lung **(B)** is 63% reduced in weight, with proliferation index (PI) decreased in both the epithelial and the mesenchymal cell compartments, in comparison to the control **(A)**. In fact, it is clearly visible that some regions of the *Myb-/-* lung epithelium (asterisks in the ducts, in **B)** are completely PCNA-negative, as revealed by immunohistochemistry (Dako mouse monoclonal against PCNA, 1 μg/ml) on paraffin sections at E15.5. **C, D.** *Rag1-/-* lungs **(D)** show normal histopathological features in comparison to the control littermates **(C)** and have no alterations in the PI, as revealed by PCNA immunohistochemistry on paraffin sections at E18.5. **E, F.** *Rock2-/-* lungs **(F)** show normal histological features like the control littermates **(E)** and have no alterations in the PI, as revealed by PCNA immunohistochemistry on paraffin sections at E18.5. x 400

of the International Knockout Mouse Consortium (IKMC) (consisting of EUCOMM-KOMP-NorCOMM-TIGM) for conditional mouse mutagenesis. Our ultimate goal is to identify new molecular functions of the players from our list, and to specifically attribute functions in lung development and disease to these players, while defining which features of the lung phenotype are the result of the absence of the gene alone and which are due to the absence of all mechanical forces from the muscle via the execution of FBMs.

Mouse model for FBM-dependent pulmonary hypoplasia

The mechanochemical signal transduction pathways that translate mechanical stimuli from the muscle to meaningful gene instructions for final pulmonary cell differentiation are still unclear. In a recent experiment therefore, we used oligonucleotide microarrays to identify genes possibly involved in pneumocyte differentiation in amyogenic mouse embryos (Baguma-Nibasheka et al., 2007). From the muscle developmental biology perspective (which would be our point of view), we accomplished our goal of providing an example of how to study *in vivo* the complex relationship between the muscle (the executor of FBMs) and the developing lung (the recipient of mechanical stretch). So far, we have provided evidence to suggest that SATB1, MYB and CTGF are potential mechano-chemical transduction pathway players which translate the mechanical

Table 1. Summary of the lung phenotypes in the complete absence of muscle and FBMs (*Myf5:MyoD* nulls) as compared to the lung phenotypes described in: *Ctgf, Satb1, Myb, Tcrb-V13* null mice, as part of the systematic subtractive microarray analysis approach (SSMAA).

Genotype/ phenotype	Pulmonary Hypoplasia	Type II pneumocyte (surfactant production)	Type I pneumocyte (gas exchange)	
Myf5:MyoD	+ (7 criteria)	assembly, storage, secretion affected	absent	Inanlou and Kablar, 2005a,b
Ctgf	+ (7 criteria)	assembly and storage affected	normal	Baguma-Nibasheka and Kablar, 2008
Satb1	+ (2 criteria)	increased number	decreased number	Figure 1, current review
Myb	+ (2 criteria)	N/A (conditional mutagenesis necessary)	N/A (conditional mutagenesis necessary)	Figure 2, current review
Tcrb-V13 (or LKLF)	+ (3 criteria)	normal *	normal*	Kuo et al., 1997

*TEM, Transmission Electron Micrograph is needed to properly verify type II and I pneumocyte differentiation in *Tcrb-V13-/-* mice, which, in turn, would also require conditional mutagenesis approach to recreate the mice.

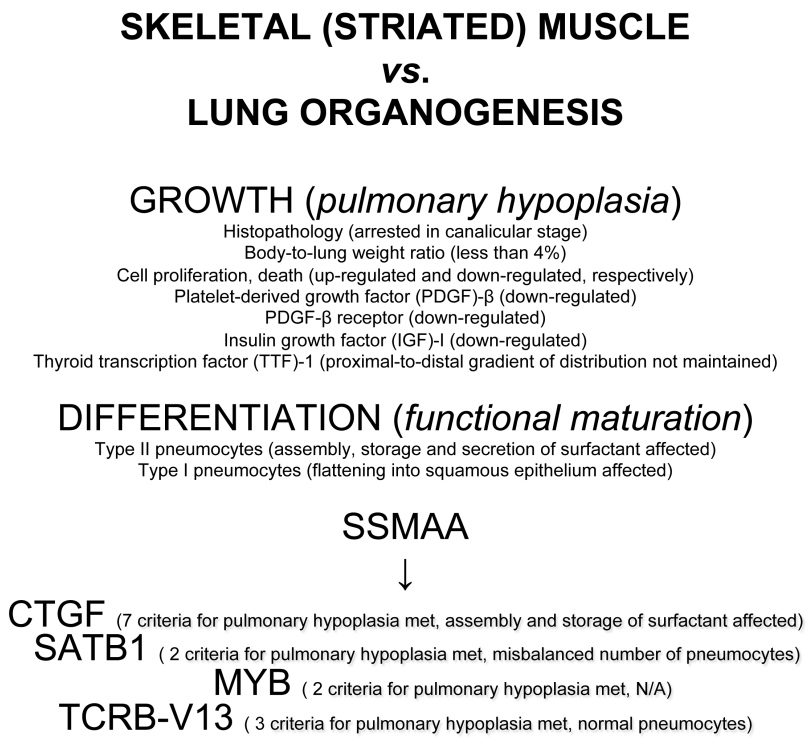

Fig. 3. Lung model for the Systematic Subtractive Microarray Analysis Approach (SSMAA). Step I: In the absence of the musculature and FBMs, the growth of the lung is severely affected and seven criteria for pulmonary hypoplasia in mice can be met. The cuboidal type II pneumocytes, currently thought to be the source of squamous type I pneumocytes, had failures in assembly, storage and secretion of surfactant. The type I pneumocytes could not flatten to become squamous epithelial cells and to function in the blood-air barrier for gas exchange. Step II: To discover new molecular players with precisely attributed functions in lung development and disease, we performed Affymetrix Gene Chip cDNA microarray analysis, followed by the analysis of mouse mutants. There are so far four examples of the SSMAA that worked, because out of approximately 25000 genes we did identify four genes whose knockout mice had some specific differentiation failures as "predicted" by the original phenotype (i.e., the phenotype described in *Myf5:MyoD* nulls). Therefore, this unique *in vivo* (whole-animal) approach allows us to suggest that the mechanical cues from the skeletal muscle, executed upon the developing lung as a consequence of the FBMs, are instrumental for the lung growth and alveolar epithelial differentiation, and that these mechanical cues act via a number of molecular players, and in particular via *Ctgf, Satb1, Myb and Tcrb-V13*, who appear to have very precise and somewhat different roles in this process. This figure is an updated version from Kablar, 2011.

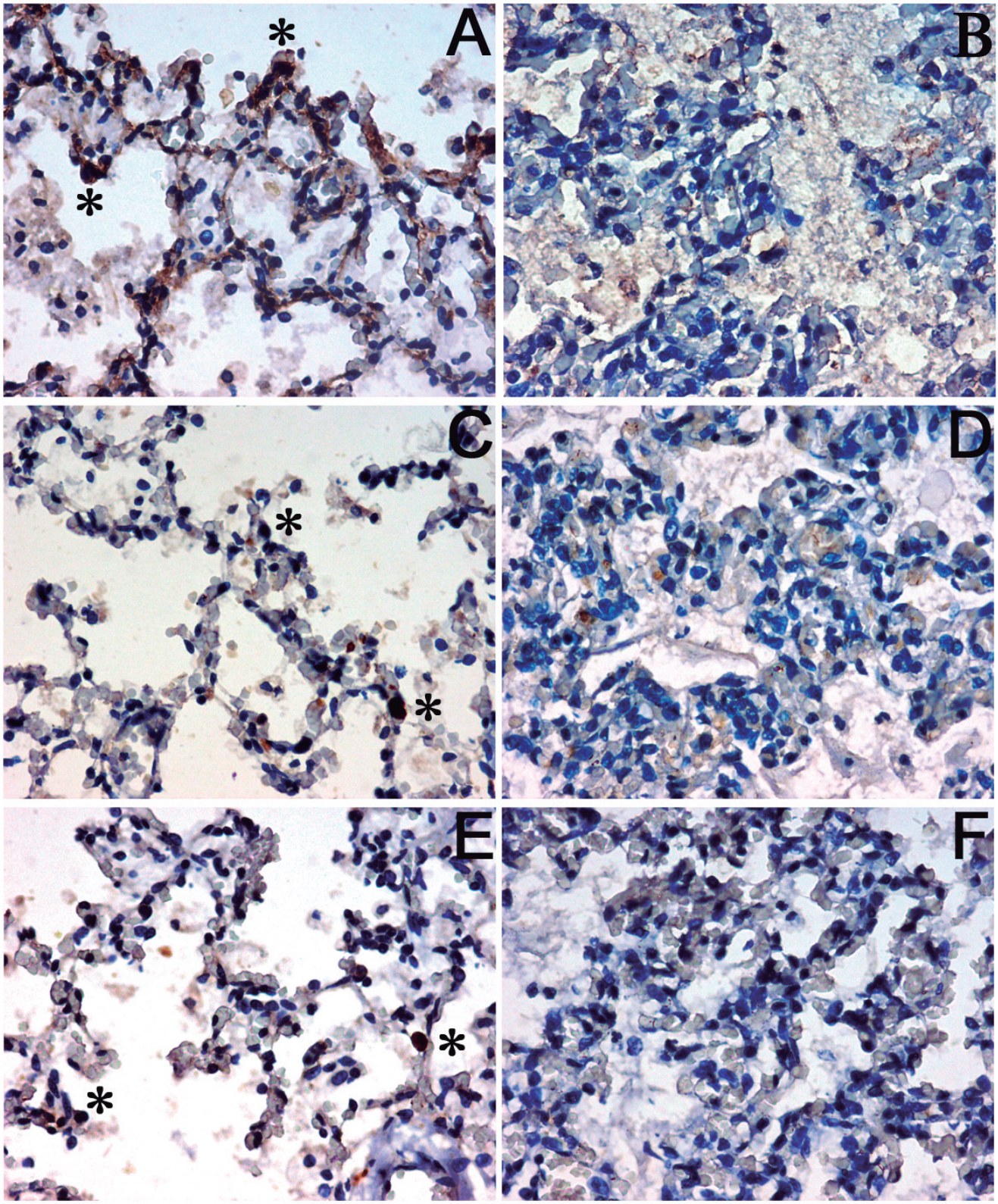

Fig. 4. Distribution of SATB1, MYB and CTGF in human lung development corresponds to their function in mice. **A, B.** During the 40th week, SATB1 (Santa Cruz goat polyclonal against SATB1, 40 μg/ml) is contained in the alveolar epithelial cells of the normal lung (A, asterisks) and in a very low number of only mesenchymal cells of the hypoplastic lung (**B). C, D.** At the same age, MYB (Santa Cruz rabbit polyclonal against MYB, 40 μg/ml) is visible in both the epithelial and the mesenchymal cells of the normal lung **(C)**, while in the hypoplastic lung **(D)** MYB is present in only a small number of mesenchymal cells. **E, F.** Similarly, CTGF (Santa Cruz goat polyclonal against CTGF, 40 μg/ml) is present in both the epithelial and the mesenchymal cells of the normal lung **(E)** and essentially abolished in the hypoplastic lung **(F)**. Controls for immunostaining were the negative results when SATB1, MYB and CTGF antibodies were used on the appropriate mouse mutants. x 400.

information from the muscle into the meaningful cell signal in the alveolar epithelium. Additional evidence for this statement could be provided by an *in vitro* experiment in which stress is applied to either a single fetal alveolar epithelial cell or a sheet of cells, while various molecules, and in particular SATB1, are monitored to see in what way type II-to-I pneumocyte transition depends on this particular mechanical cue and its transducing molecules.

Meanwhile, we propose here a mouse model for FBM-dependent lung hypoplasia employing SSMAA (Fig. 3). In the absence of the respiratory musculature and FBMs, the growth of the lung is severely affected, showing seven criteria for pulmonary hypoplasia in mice (Inanlou and Kablar, 2005a,b). Additionally, very specific differentiation defects of the alveolar epithelium were found in the hypoplastic lung. For example, the cuboidal type II pneumocytes, currently thought to be the source of type I pneumocytes, had failures in assembly, storage and secretion of surfactant, while the type I pneumocytes could not flatten to function in the blood-air barrier for gas exchange. To discover new molecular players with precisely attributed functions in lung development (i.e., differentiation of the type I and II alveolar epithelial cells) and disease (i.e., pulmonary hypoplasia), we performed Affymetrix Gene Chip cDNA microarray analysis, followed by the analysis of mouse mutants. In fact, *Ctgf, Satb1, Myb* and *Tcrb-V13* are four examples of successful SSMAA, because, out of approximately 25000 genes in the Affymetrix Gene Chip, we (and others, for *Tcrb-V13*) identified four genes whose knockout mice had specific differentiation failures "predicted" by the original mouse phenotype (i.e., the phenotype described in *Myf5:MyoD* nulls). Moreover, defining which features of the phenotype were the result of the absence of the individual genes (e.g., *Ctgf, Satb1, Myb, Tcrb-V13*) and which were due to the general absence of mechanical forces, as seen in the muscleless *Myf5:MyoD* nulls, was another advantage of this approach. An illustration of this approach's advantage is shown in Table 1.

Further, we believe that an approach analogous to this one would be successful for the analysis of the molecular basis of the interface between various other tissues and the skeletal muscle. In fact, by employing this approach, we have continued to analyze the development of various tissues and organs of the fetal or embryonic body that were affected by the absence of the skeletal musculature, such as the retina (Baguma-Nibasheka and Kablar, 2009a,b), crista ampullaris of the inner ear (Rot and Kablar, 2010), the palate (Rot-Nikcevic et al., 2006), temporomandibular joint (Rot-Nikcevic et al., 2007) and motor neurons innervating limb and back musculature (reviewed by Kablar, 2011).

Preliminary human studies and future directions

The data discovered in mice using mouse mutagenesis and the battery of phenotypic analysis approaches will eventually deliver a precise map of functions of various molecules. However, the function of various molecules discovered in mice as models of human diseases will have to be examined employing human material, adult and embryonic, normal and diseased. So far, there are several large scale projects that are trying to provide information on the distribution pattern of various molecules employing human material. For example, *The Human Protein Atlas*, http://www.proteinatlas.org, Uppsala University, contains information about the distribution pattern of a number of molecules in normal adult human tissues and also in neoplastic tissues. Unfortunately, this *Atlas* does not yet contain information on protein distribution pattern in embryonic, fetal, neonatal normal and diseased material. As a collaborative effort of our two laboratories (in Canada and Croatia), our intention is to increase the amount of information on molecules' distribution patterns, employing human embryonic, fetal and neonatal normal and diseased archival materials.

For the purposes of this theme, on the role of FBMs in lung development, we would like to stress that FBMs in normal human fetuses are an ultra-sound measure of the fetal wellbeing. There are several human conditions that may impair FBMs, such as: various causes of fetal hypoxemia and hypoglycemia, maternal alcohol, narcotics, sedatives consumption and smoking, *in utero* infections, etc (Harding, 1997). It is impossible to obtain a "clean" experimental system in humans, since an impairment or absence of FBMs often comes with other factors associated with pulmonary hypoplasia, such as premature rupture of fetal membranes and oligohydramnios (Harding, 1997). However, various deformations of the human rib cage may be comparable to the mouse studies on the role of FBMs in our model.

For example, as a further follow up to our microarrays (Baguma-Nibasheka et al., 2007), the distribution pattern of the three molecules (SATB1, MYB and CTGF) with the newly discovered functions in lung development and disease in mice (Figs. 1 and 2 in this review; Baguma-Nibasheka and Kablar, 2008), was studied employing human neonatal normal and diseased archival material (i.e., pulmonary hypoplasia caused by mechanical impairments of lung development). We intended to verify if any of the three players have a distribution pattern in humans consistent with their role in mice. In the human adult lung, MYB (strong presence) and CTGF (moderate levels) are present in the alveolar epithelium, while SATB1 is not (*The Human Protein Atlas*, http://www.proteinatlas.org, Uppsala University). Here, in the human neonatal lungs (gestational age 40 weeks; archival material from the University of Split Medical School, Croatia), we show that during normal lung development, all three molecules were present in the epithelial lining, while MYB and CTGF were also visible in the mesenchyme (approximately 6% of the cells contained MYB or

CTGF, and many epithelial cells contained SATB1) (Fig. 4). The hypoplastic lung was considerably smaller than the control, with a weight of 8g (left lung; lung-to-body weight ratio was 0.0081) before fixation, versus the normal 25g. Histopathologically, the hypoplastic lung appeared to be arrested in the saccular to early alveolar stage. Alveoli were of a very small diameter and partly atelectatic (unexpanded). There was evidence of interstitial hemorrhage and tissue edema, with a single inflammatory focus (possibly aspiration pneumonia). In the hypoplastic lung, none of the three molecules were detectable in the epithelium, and the presence of the three molecules was restricted to the mesenchyme, where the number of cells containing the proteins was extremely reduced (less than 0.3% of the mesenchymal cells contained any of the three molecules) (Fig. 4). Together, these data indicate that SATB1, MYB and CTGF, as in mice, may also have a role in human lung development and in the pathogenesis of pulmonary hypoplasia. Similar preliminary data are available for fetal human lung (Gugic, Saraga-Babic and Kablar, unpublished data).

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