Basement Membrane Distortions Impair Lung Lobation and Capillary Organization in the Mouse Model for Fraser Syndrome*

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Fras1 is a putative extracellular matrix protein that has been implicated in the structural adhesion of embryonic epidermis to dermis. Moreover, mutations in Fras1/FRAS1 have been associated with the mouse blebed phenotype and the human rare genetic disorder Fraser syndrome, respectively. Here we report the mapping of Fras1 within the extracellular space and evaluate the effects of Fras1 deficiency on lung development in the mouse. Expression of Fras1 was detected in the mesothelial cells of the visceral pleura and in the conducting airway epithelia. Immunogold histochemistry identified Fras1 as a component of the extracellular matrix localized below the lamina densa of epithelial basement membranes in the embryonic lung. Embryos homozygous for a targeted mutation of Fras1 exhibited fused pulmonary lobes resulting from incomplete separation during development as well as a profound disarrangement of blood capillaries in the terminal air sacs. We demonstrate that loss of Fras1 causes alterations in the molecular composition of basement membranes, concomitant with local disruptions of epithelial-endothelial contacts and extravasation of erythrocytes into the embryonic respiratory lumen. Thus, our findings identify Fras1 as an important structural component of the sub-lamina densa of basement membranes required for lobar septation and the organization of blood capillaries in the peripheral lung.

Basement membranes are thin, sheet-like arrangements of extracellular matrix proteins that serve a variety of functions including the physical separation of cell layers and tissues, molecular ultrafiltration, and tissue morphogenesis (1–3). Electron microscopically, typical basement membranes display two distinct zones, the lamina lucida and lamina densa. The lamina lucida contains anchoring filaments that extend to the hemidesmosomes and mediate the attachment of the epithelial cells to the basement membrane. The anchoring of the basement membrane to the mesenchyme is ensured by anchoring fibrils that are associated with the lamina densa and transverse the underlying zone termed sub-lamina densa.

Recently, we reported the cloning of the Fras1 gene, which encodes a putative extracellular matrix multidomain protein (4010 amino acids) expressed from various epithelia of the developing embryo (4). Fras1 contains several distinct domains similar to those found in different proteins such as the C-domain of von Willebrand factor, the cysteine-rich motifs of furin proteases, the chondroitin sulfate proteoglycan core protein NG2, and the calcium-binding motif of Na\(^{+}\)-Ca\(^{2+}\) exchange proteins. Targeted inactivation of Fras1 reproduces the phenotype of the blebed (bl) mice that together with eye blebs (eb), myelencephalic blebs (my), head blebs (heb), and fetal hematoma (fh) make up the family of the mouse bleb mutants. These mice are characterized by the formation of sub-epidermal hemorrhagic blisters during embryonic development, unilateral or bilateral renal agenesis or dysgenesis, cutaneous syndactyly, and fused eyelids (5). Based on the similarity of the phenotypic characteristics, the bleb mutant mice have been considered as models for the human hereditary disease Fraser syndrome (6). This rare genetic disorder presents with cryptophthalmos (fused eyelids), syndactyly, renal agenesis, and a range of other developmental defects including genital malformations, heart anomalies, and abnormalities of the airway and lungs (7). The correlation between the bleb phenotype and Fraser syndrome has been confirmed by the identification of mutations in Fras1 and its human counterpart FRAS1 in blebed mice and Fraser syndrome patients, respectively (8). Four out of the five bleb mutants (bl, eb, my, and heb) have been mapped on different chromosomes, whereas the fh mutation remains to be mapped (5). This along with the very similar phenotypic features of the bleb mice suggest that at least four of the respective bleb gene products either act synergistically or function independently in distinct but related pathways. Besides the genetic correlation of Fras1 with the blebed mutation, two additional genes have been recently associated with bleb mice. The gene, Grip1, encoding the glutamate receptor-interacting protein, was found to be mutated in the eye blebs (9), whereas a new gene termed Frem1 was affected in the head blebs mutants (10). Most interestingly, both gene products are either functionally or structurally related to Fras1. Grip1, a PDZ-containing protein, was shown to interact physically with the short C-terminal cytoplasmic tail of Fras1 and to be involved in the correct targeting of Fras1 to the basal side of polarized epithelia. Finally, Frem1 shares similarities with Fras1 in the domain organization and is therefore considered as structurally related to Fras1. The above data suggest that the genes affected in the bleb mutants encode proteins structurally and/or functionally related to Fras1, the founding member of the family and the prototype model for Fraser syndrome upon functional deficiency.

In Fras1−/− mutants as well as in Fraser syndrome patients, blisters appear exclusively during intra-uterine life in contrast to other known blistering disorders (11). The blisters form between the dermis and the skin basement membrane that...
detaches from the dermis together with the epidermal epithelium. This indicates that Fras1 exerts its function at the interface between the epithelial basement membrane and the underlying mesenchyme. However, so far no data exist on the ultrastructural localization of Fras1 within the extracellular matrix. Because respiratory malformations belong to the most common causes of neonatal death of Fraser syndrome patients (7), here we focused on the developing lung and addressed the issues of Fras1 localization within the extracellular space and the phenotypic abnormalities resulting from Fras1 inactivation. We demonstrate that Fras1 is localized at the basal side of the conducting airway epithelia and of the mesothelial layer of the visceral pleura. Immunogold labeling localized the protein at the ultrastructural level below the lamina densa of the basement membrane zone. Targeted ablation of Fras1 resulted in defective lung lobation and in the disarrangement of blood capillaries in the terminal air sacs. We show that the above malformations are secondary to perturbations in the molecular and structural integrity of basement membranes. Our results thus suggest a role for Fras1 in the functional contact between basement membranes and the underlying mesenchyme. Moreover, these findings assign a function to Fras1 containing basement membranes as physical barriers of blood vessel sprouting.

MATERIALS AND METHODS

Mice—Fras1+/− mice (4) were maintained on the NMRi genetic background in the F5 generation.

Antibodies and Immunohistochemistry—Rabbit antibodies to β-galactosidase were purchased from Cappel; rat monoclonal anti-Pecam-1/MEC13.3 was from Pharmingen, and mouse monoclonal anti-T1 α was from the Developmental Studies Hybridoma Bank, University of Iowa, hybridoma number 8.1.1 (www.uiowa.edu/~dsbboxx), courtesy of Dr. Andrew Farr (12). Rabbit anti-TFF-1 was a gift from R. Di Lauro, and rabbit anti-Nidogen-1 and anti-Agrin were kindly provided by R. Timpl. Rabbit anti-Fras1 was produced as described previously (4). Embryos and tissue samples incubated with anti-Pecam-1 antibody were fixed with Zn2+. In all other cases, fixation was performed overnight in 4% PFA/phosphate-buffered saline. Fixed specimens were either embedded in paraffin or immersed in O.C.T. embedding medium (BDH) and sectioned at 5 μm. For detection of Fras1, Nidogen-1, Agrin, β-galactosidase, and T1 α unmasking of the epitopes was required prior to the application of the primary antibodies. Immunofluorescence staining was achieved by using an appropriate Alexa-488-, -568, or Cy5 conjugated secondary antibody (Molecular Probes), and nuclei were counterstained with propidium iodide. For peroxidase staining a biotinylated anti-rabbit (Vectastain Elite ABC Kit) secondary antibody was applied and visualized with diaminobenzidine (Sigma). Sections were counterstained with methyl green. Hematoxylin/eosin staining was performed using standard procedures. Specimens were observed and photographed either on a Nikon Eclipse E800 microscope or on a Leica SP confocal microscope.

Preparation of Postnatal Lungs for Immunohistochemistry—Mice were anesthetized and allowed to bleed by sectioning the posterior vena cava. After piercing the diaphragm, lungs were inflated through the trachea with 4% PFA/phosphate-buffered saline at a pressure of 25 cm of H2O until the lungs completely filled the chest cavity. To prevent fixative backflow, the trachea lumen was kept closed, and lungs were dissected out of the rib cage and allowed to fix overnight. After fixation lungs were processed for cryosectioning using standard procedures.

Immunofluorescent Lungs were fixed overnight in 4% PFA, 0.05% glutaraldehyde, and 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.2, embedded in gelatin, and sectioned at 70 μm with a VT1000S vibrating microtome (Leica). Antibody staining was performed on free floating sections that were preincubated in 20% normal goat serum diluted in TBS buffer (50 mM Tris, 0.9% NaCl, pH 7.4), for 1 h, and then incubated for 48 h at 4 °C with the primary antibody, diluted in 1% normal goat serum. After several washes in TBS, sections were incubated with goat-anti-rabbit affinity-purified Fab’ fragment coupled to 1.4 nm gold particles (Nanoprobes Inc.). Gold particles were enhanced by silver amplification for 8–12 min using the HQ silver kit (Nanoprobes Inc.). Sections were treated with 1% OsO4 and contrasted in 1% uranyl acetate before embedding in epoxy resin (Durcupan ACM, Fluka). Serial electron microscope sections (70–80 nm) were collected on pioloform-coated copper slot grids.

Transmission Electron Microscopy—Lungs from E18.5 embryos were fixed in 2% glutaraldehyde, 2% PFA, post-fixed in 2% aqueous OsO4, stained with 2% tannic acid for 60 min, dehydrated in alcohol, and embedded in a modified Mollenhauer’s resin. Ultra-thin sections were post-stained with uranyl acetate and lead citrate. Images were obtained using a JEOL 100C transmission electron microscope operating at 100 kV.

Scanning Electron Microscopy—For scanning electron microscopy, isolated lungs from E18.5 Fras1+/− and control embryos were fixed in 2% glutaraldehyde, 2% PFA in 0.08 M sodium cacodylate buffer, pH 7.4, for 24 h at 4 °C, washed in buffer, post-fixed in 2% aqueous OsO4 for 60 min at 4 °C, and dehydrated through a graded series of acetone. Dehydrated samples were critical point dried (Baltec CPD 030) and mounted on copper stubs prior to sputter coating with 20 nm thick gold/palladium (Baltec SCD 050). Samples were examined using a JEOL JSM-840 scanning electron microscope, operating at 20 kV. Images were obtained on Agfa APX professional 120 roll film.

RESULTS

Expression and Localization of Fras1 during Lung Development—Fras1 inactivation in mouse was achieved by homologous recombination in which the gene for β-galactosidase was inserted in-frame to Fras1 coding sequences, resulting in a chimeric protein containing the N-terminal signal peptide of Fras1 fused to β-galactosidase (4). Although the resulting β-galactosidase chimeric protein was properly expressed and reflected endogenous Fras1 expression, it lacked enzymatic activity, most likely because of its improper oligomerization within the secretory pathway (13). We therefore assayed for Fras1 expression in the developing lung by using antibodies against β-galactosidase on histological sections from Fras1+/− embryos that appear phenotypically normal. β-Galactosidase immunostaining was evident in the mesothelial layer of the visceral pleura and in the epithelia of the conducting tubules but not in the intervening mesenchyme (Fig. 1A), in accordance with our previous findings demonstrating the epithelial origin of Fras1 (4, 8). To localize Fras1 during the course of lung development and maturation, we applied Fras1-specific antibodies to sections from the embryonic through the postnatal stages. During the embryonic stages of lung development, Fras1 immunoreactivity delineated the basal side of the mesothelial layer and of the conducting airway epithelia (Fig. 1, B and C). After birth, Fras1 can still be detected underlining the pleura mesothelium, whereas immunoreactivity at the basal side of the respiratory epithelia gradually declined and was completely absent by P12 (Fig. 1D). In the lungs of mice older than 1 month of age, Fras1 was no longer detectable (data not shown), suggesting that it is predominantly required for organ formation during embryonic development rather than for organ function in adult life. Similar to the lung, expression of Fras1 in the skin epithelium was shown to be down-regulated in neonates (4), suggesting that the restriction of Fras1 expression to embryonic and early postnatal stages could reflect a general mode of Fras1 function.

Previous reports demonstrated that dermal-epidermal separation upon blister formation in Fras1+/− mutants occurs below the lamina densa, implying a role for Fras1 at the level of basement membrane-mesenchymal adherence (4, 8). So far, however, no data exist on the ultrastructural localization of Fras1 within the extracellular space. Therefore, we performed pre-embedding immunogold labeling experiments using Fras1-specific antibodies (4) that recognize a central domain of the protein homologous to the NG2 chondroitin sulfate proteoglycan (14). The Fras1 protein distribution was evident as silver intensified gold particles located below the lamina densa of the basement membrane zone of the airway epithelia (Fig. 1E) and of the mesothelial monolayer (data not shown). These results

1 The abbreviation used is: PFA, paraformaldehyde.
indicate that a central portion of Fras1 is located within the sub-lamina densa of epithelial basement membranes consistent with the mode of blister formation and dermal-epidermal detachment in Fras1−/− mutants.

Fras1−/− Mice Exhibit Pulmonary Lobation Defects—Lung development in the mouse begins around embryonic day 9.5 (E9.5) with the formation of the laryngotracheal groove, a ventral protrusion of the foregut endoderm that subsequently separates from the primitive esophagus and bifurcates laterally to generate two symmetrical primary lung buds (15–17). Under the influence of the surrounding mesenchyme, the epithelial buds begin a process of continuous branching that gives rise to the bilaterally asymmetric bronchial tree. The left lung develops as a single lobe, whereas the right lung consists of four different lobes that are well established by E12.

Morphological examination of Fras1−/− lungs at E11.5 revealed that lung bud formation occurred properly (data not shown). However, ~95% of the analyzed mutants exhibited complete or partial fusion of the right lung lobes (Fig. 2B) compared with wild type controls of the corresponding stage (Fig. 2A). Cross-sections of embryonic lungs demonstrated the presence of five distinct lobes in wild type embryos (Fig. 2C), whereas in the mutants, the medial and caudal lobes appeared completely fused, and the cranial and accessory lobes exhibited some degree of septation (Fig. 2D). The defects in lobar septation of Fras1−/− embryos that are established during the early stages of lung development are maintained throughout embryogenesis and adulthood, suggesting that they do not significantly affect the development of the lung to a functional organ capable of sustaining respiration. Most importantly, the lungs of Fras1−/− embryos displayed the same degree of airway branching as wild type embryos (C). The abbreviations used are as follows: al, accessory lobe; cal, caudal lobe; crl, cranial lobe; e, airway epithelia; le, left lung; ml, medial lobe. Scale bar in A–D, 20 μm; in E, 200 nm.

FIG. 1. Fras1 expression and protein localization in the developing mouse lung. A, immunofluorescence pattern of β-galactosidase in the lung of an E14.5 Fras1−/− embryo. Staining reflects endogenous Fras1 expression in mesothelial cells (arrows) and in the epithelia of the conducting airways but not in the mesenchyme (*). B–D, detection of the Fras1 protein on lung paraffin (B and C) or cryosections (D) with specific antibodies. B and C, Fras1 underlines the mesothelial layer of the visceral pleura and the airway epithelia (arrowheads) at E11.5 (B) and E16.5 (C). D, after birth, Fras1 activity in the lung gradually declines. By P12, Fras1 is restricted to the visceral pleura and is no longer detectable in the alveoli. E, ultrastructural immunolocalization of Fras1 on lung sections of E14.5 embryos. Clusters of gold particles (arrows), corresponding to Fras1 molecules, are distributed underneath the lamina densa (arrowheads) of the basement membrane zone of a terminal airway. The abbreviations used are as follows: al, alveoli; aw, conducting airways; e, bronchial epithelial cell; m, mesothelial cells; vpl, visceral pleura. Scale bar in A–D, 20 μm; in E, 200 nm.

FIG. 2. Defective septation of right lung lobes in Fras1−/− embryos. A and B, scanning electron micrographs of E15.5 wild type and Fras1−/− lungs. A, whole lung of a wild type embryo displaying complete septation of right lung lobes (arrowheads). B, fused medial and caudal lobes in the right lung of Fras1−/− embryos (arrowhead). C and D, hematoxylin-eosin staining of lung sections from E16.5 wild type and Fras1−/− lungs. C, a wild type control displaying the normal four-lobed pattern of the right lung. D, Fras1 homozygous mutants usually exhibit complete fusion of the medial and caudal lobes of the right lung, whereas the cranial lobe is partially separated (arrowheads). E and F, whole lungs of wild type and Fras1−/− embryos at E12.5. Lungs of Fras1−/− embryos (F) exhibit the same extent of epithelial branching as wild type controls (C). The abbreviations used are as follows: cal, caudal lobe; crl, cranial lobe; e, airway epithelia; le, left lung; ml, medial lobe. Scale bar in A and B, 400 μm; in C and D, 10 μm.
basement membrane was compared with wild type controls (Fig. 5, A–H), and at E18.5, TTF-1 was no longer detectable in differentiated squamous epithelial cells (Fig. 4C). Staining for TTF-1 in the lungs of Fras1−/− age-matched embryos produced a pattern similar to wild type (Fig. 4, B and D), suggesting that respiratory epithelial cell differentiation in homozygous mutants occurred normally. We further applied antibodies for the protein markers T1α and pro-SP-C to distinguish between differentiated type I and II pneumocytes, respectively. At E18.5, staining for T1α (Fig. 4, E and F) and pro-SP-C (data not shown) revealed that respiratory epithelial cell differentiation in the terminal air sacs of Fras1−/− embryonic lungs was comparable with wild type, indicating that Fras1 ablation does not affect lung maturation.

**Basement Membrane Distortions Result in Disarrangement of Blood Capillaries in the Terminal Air Sacs of Fras1−/− Embryonic Lungs**—A pivotal process during fetal lung maturation is the establishment of the air-blood barrier to facilitate efficient gas exchange during postnatal life. This occurs during the late gestational stages when dramatic anatomic changes remodel the distal air sacs and result in the close apposition of respiratory epithelia and capillary endothelial cells (23). The fusion of the basement membranes of capillary endothelial and type I epithelial cells results in the formation of a thin air-blood interface (24).

By using antibodies for the endothelial specific protein Pecam-1 and Fras1, we detected Fras1 in the interface between endothelial and respiratory epithelial cells (Fig. 4G) at E18.5, the period of distal air sac remodeling. The above localization of Fras1 prompted us to examine the development of the vasculature in the lungs of Fras1−/− embryos. To visualize blood capillaries and the basement membrane in the distal airways, we used antibodies against Pecam-1 and the basement membrane marker Agrin in double immunostaining experiments. At E16.5, mutant and control lungs displayed no significant differences in the extent of vascular development and in the organization of blood capillaries around the distal airways (data not shown). In E18.5 wild type lungs, blood capillaries, labeled by Pecam-1, appeared well arranged in the terminal air sacs, with regard to a readily conspicuous basement membrane demarcated by Agrin (Fig. 4, H and J). However, in Fras1−/− lungs of the same stage, blood vessels in the terminal sacs appeared strikingly disorganized (Fig. 4I) compared with wild type. Furthermore, blood capillary disarrangement in the peripheral lung of Fras1−/− embryos correlated well with the absence of a distinguishable basement membrane outline (Fig. 4, I and K).

Ultrastructural examination of the interface between endothelial and epithelial type I cells showed that basement membranes in Fras1−/− lungs appeared wider and less dense compared with wild type controls (Fig. 5, A and B). Moreover, the mature alveoli are lined by differentiated alveolar type I and II epithelial cells that sustain gas exchange and surfactant production, respectively, and develop from a multipotential primitive epithelium. The differentiation of the epithelial cells in the distal air sacs to type I and II pneumocytes occurs around E17 parallel to the thinning of the mesenchyme and the gradual establishment of the air-blood barrier.

To evaluate epithelial cell differentiation in the peripheral lung of Fras1−/− embryos, we employed specific antibody markers in immunohistochemical experiments. TTF-1 is a homeodomain-containing transcription factor expressed in undifferentiated cuboidal cells in the peripheral respiratory epithelia (21, 22). The onset of cuboidal cell differentiation to squamous type I pneumocytes is accompanied by the down-regulation of TTF-1. At E16.5, TTF-1-specific antibodies labeled the nuclei of virtually all epithelial cells in the terminal bronchi of wild type embryos (Fig. 4A), and at E18.5, TTF-1 was no longer detectable in differentiated squamous epithelial cells (Fig. 4C). Staining for TTF-1 in the lungs of Fras1−/− age-matched embryos produced a pattern similar to wild type (Fig. 4, B and D), suggesting that respiratory epithelial cell differentiation in homozygous mutants occurred normally. We further applied antibodies for the protein markers T1α and pro-SP-C to distinguish between differentiated type I and II pneumocytes, respectively. At E18.5, staining for T1α (Fig. 4, E and F) and pro-SP-C (data not shown) revealed that respiratory epithelial cell differentiation in the terminal air sacs of Fras1−/− embryonic lungs was comparable with wild type, indicating that Fras1 ablation does not affect lung maturation.

**Normal Differentiation of Respiratory Epithelial Cells in Fras1−/− Mutants**—Accumulating evidence indicates that basement membranes, beside their structural function in epithelial-mesenchymal adhesion, play a pivotal role in mediating signals that regulate cell differentiation (19, 20). In the lung,
endothelial cells were locally detached from the basement membrane in Fras1−/− mutants (Fig. 5C). The loss of adherence of endothelial cells to the basement membrane in the terminal sacs could account for the extravasated erythrocytes that were systematically observed in the respiratory lumen of Fras1−/− lungs (Fig. 5D). The results show that loss of Fras1 affects the basement membrane-endothelial cell attachment and suggest that Fras1-containing basement membranes play an important role in the proper arrangement of blood capillaries around the respiratory epithelia.

**DISCUSSION**

In the present study we examined the expression and function of Fras1 in the developing mouse lung. Fras1 originates from epithelial cells and was detected in the epithelial-mesenchymal interfaces during lung development. Ultrastructural immunogold labeling using antibodies raised against the central NG2-like domain of Fras1 detected the protein underneath the lamina densa of embryonic lung epithelia. This finding is in accordance with our reported indications that Fras1 exerts its function below the lamina densa (4). These include the detection of the lamina densa at the roof of the detached epidermis and the loss of collagen VI deposition in the skin basement membrane. Most notably, Fras1 staining displayed a clustered colloidal gold deposition resembling that of collagen VII, which is a component of the anchoring plaques of the sub-lamina densa (25, 26), and that of collagen XVIII, which colocalizes with fibrillar structures underneath the lamina densa (27). The above imply that Fras1 could be a component of an anchoring complex that serves the adhesion of the basement membrane to the underlying matrix. Whether there is a potential colocalization between Fras1 and collagen VII and/or collagen XVIII remains to be determined. Nevertheless, given the length of Fras1 (4010 amino acids), we cannot exclude the possibility that the remaining domains of the protein coincide with other structures of the basement membrane within or below the lamina densa.

The phenotype of Fras1−/− lungs includes the incomplete septation of the right lung lobes, disorganization of blood capillaries in the peripheral lung, and extravasation of erythrocytes in the respiratory lumen. Most importantly, fused pulmonary lobes and extravasated erythrocytes have also been reported in Fraser syndrome fetuses (28, 29). Thus, Fras1−/− mice are a useful model for the study of lung abnormalities in Fraser syndrome patients. It is not clear whether the lung malformations described in this study contribute to neonatal lethality observed both in Fras1−/− mice as well as in Fraser syndrome patients. The lobar septation defects are evident in homozygous animals that survive, indicating that the above malformation does not interfere with viability. It is, however, possible that more severe cases of erythrocyte extravasation could lead to lung hemorrhage and neonatal death due to respiratory insufficiency.

A recent study (30) reported that mice deficient for laminin-α5, a basic component of basement membranes, exhibit incomplete separation of lung lobes, although branching morphogenesis was not affected. This demonstrated that pulmonary lobation and branching morphogenesis can be independent processes contrary to previous assumptions (31–33). Moreover, the absence of the visceral pleura basement membrane in laminin-α5-deficient embryos was considered as the primary cause for
the loblar septation defects. In contrast to laminin-α5 knock-out embryos, the visceral pleura basement membrane in Fras1–/– mutants remains detectable both at the ultrastructural level (not shown) as well as with antibody fluorescent markers. Loss of Fras1 rather seems to cause perturbations in the molecular composition of the visceral pleura basement membrane, suggesting that not only the complete absence but even distortions in the integrity of the visceral pleura basement membrane could impair normal lobation in the mouse lung without disturbing epithelial branching morphogenesis.

Fras1 deficiency further affected the structural integrity of the respiratory epithelia basement membrane. Our results identified a correlation between the proper organization of blood capillaries around the distal air sacs and the structural integrity of the basement membrane. The disarrangement of blood capillaries observed in the lungs of Fras1–/– mutants implicates Fras1 in the maintenance of a barrier function of basement membranes on blood vessel protrusion. This could be accomplished either by the contribution of Fras1 to basement membrane structural integrity or, alternatively, by a direct interaction between Fras1 and endothelial cells. Indeed, both the localization of Fras1 in the sub-lamina densa and the presence of endothelial basement membrane detachment sites in the lungs of Fras1–/– embryos argue for a role of Fras1 in preserving basement membrane-mesenchymal contact. The disruption of endothelial cell basement membrane attachment could be the primary cause for the extravasation of erythrocytes observed in the respiratory lumen of Fras1–/– embryos. These findings lead to the intriguing hypothesis that Fras1 exerts a similar function also in the skin basement membrane. The loss of the barrier properties of the skin basement membrane on blood vessel protrusion resulting from Fras1 dysfunction could provide the basis to explain the hemorrhagic nature of the blisters that occur in utero in Fras1–/– embryos, in the absence of mechanical friction or trauma.

Previous studies (33, 34) reported that mouse embryos heterozygous for the Foxf1-targeted allele display fused lung lobes and defects in pulmonary vasculature resulting in perinatal lung hemorrhage. Furthermore, it could be demonstrated that the Foxf1–/– phenotype was accompanied by reduced levels of BMP4 and vascular endothelial growth factor (34). On the other hand, elevated levels of BMP4 inhibited expression of Foxf1 (35). These indicate that defects in the regulation of vascular endothelial growth factor and/or growth factors of the transforming growth factor-β superfamily could account for the lung malformations of the Foxf1–/– embryos. Given the apparent similarity of the lung phenotypes between Fras1–/– and Foxf1–/– embryos, it is conceivable that Fras1, besides its structural role, could have been involved in the modulation of the activity of vascular endothelial growth factor, BMP4, or other transforming growth factor-β-related proteins. This could be accomplished through the N-terminal cysteine-rich repeats of Fras1 that share similar structure to chordin- and furin-type cysteine motifs that have been implicated in the regulation of the activity of members of the transforming growth factor-β family (36–38).

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