Original Article

# Expression of Fibroblast Growth Factor-10 during Histogenesis of Human Lung in Prenatal Period

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## Abstract

**Introduction:** The developing human lung has five stages. Many important signaling molecules have been identified at each stage of the developing lung. Fibroblast growth factor (FGF) signaling, mainly FGF10, is a key molecule in airway branching. Failure of the expression of FGF10 may lead to hypoplastic lung and blunted tracheal end. In this view, the expression of FGF10 was studied in each stage of human lung development. **Material and Methods:** In the present study, ten aborted fetuses of gestational age 14–26 weeks were procured from the Department of Obstetrics and Gynaecology, LNH, New Delhi, after ethical clearance. After fixation in 10% formalin, serial sections of each lung tissue were stained with hematoxylin and eosin, and immunostaining was done to see the expression of FGF10. **Results:** The amount of mesenchymal tissue decreased and condensed with advancing gestational age. At 14-week gestation, the first expression of FGF10 was seen in mesenchyme by immunostaining. **Discussion and Conclusion:** As the gestational age advances, the amount of mesenchymal tissue decreases and gradual diminution of the height of epithelium was seen. By the 26$^{th}$ week of intrauterine life, fetal lung attains morphological maturity so as to support the extrauterine life of a growing fetus.

**Keywords:** *Apert syndrome, diverticulum, mesenchyme, parenchyma, pneumocytes*

## Introduction

The development of lung is a very complex process, designed to provide a large internal surface area in which inspired air and capillary blood come in intimate contact with each other. The developing respiratory system has been described histologically as progressing through embryonic stage (26 days to 6 weeks), pseudoglandular stage (6–16 weeks) canalicular stage (16–28 weeks), saccular stage (28–32 weeks), and alveolar stage (36 weeks to term).[1-3]

In embryonic stage, ventral respiratory diverticulum begins from foregut endoderm.[3] At 4 weeks, the tip of lung bud elongates, divides, and dilates into surrounding splanchnic mesenchyme and forms the right and left bronchi.[4] The expression of thyroid transcription factor-1 (TTF-1), NKx 2.1, appears in the ventral endoderm of foregut before the appearance of definitive lung buds and lung cell lineage.[5,6] In pseudoglandular stage, rapid expansion and branching morphogenesis of respiratory tubules are seen up to terminal bronchioles.[6,7] After formation of bronchial bud, fibroblast growth factor-10 (FGF10) is required for further branching and growth.[6] Mesenchyme expresses FGF10 in localized fashion in close proximity with distal epithelial tubules.[8] FGF10 is a chemotactic and proliferation factor for endoderm.[9] The core signaling molecules that control branching morphogenesis in the lung include FGF10 and their receptors and sonic hedgehog (SHH). In mammals, signaling by FGF10 and FGF receptor 2b (FGFR2b) is crucial for bronchial bud formation.[1,9,10] SHH is expressed only in epithelium and FGF10 in mesenchyme. SHH signaling represses FGF10 expression, as stronger the inhibitory effect of SHH on FGF10 expression, the thinner is the mesenchyme. Locally thinner mesenchyme would lead to local repression of FGF10 expression leading to the accumulation of FGF10 on the sides, thus triggering bifurcation and outgrowth.[11] At this stage, due to the growth and branching of all conductive airways, tubular tree is formed up to future terminal bronchioles.[12] Along with branching of airway, vascular network develops and



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progressive differentiation of epithelial cells occurs to form the adult structure of cartilage, gland, and bronchial smooth muscles.[6] In canalicular stage, air–blood barrier becomes thin enough to support gas exchange. The saccular stage is characterized by further development of primitive alveoli with type I and type II pneumocytes. Finally, the formation of definitive cup-shaped alveoli marks the last alveolar stage.

There are a number of studies on development of the lung in human, but there are no studies regarding histogenesis of the human lung along with observing the FGF10 expression. FGF10 is an essential factor for directional outgrowth and induction of epithelial bud. Failure of the expression of FGF10 leads to lung hypoplasia and blunted tracheal end that affects normal birth and survival of fetuses. A detailed knowledge of normal development of the lung with expression of FGF10 will also have a role in promoting lung stem cell injury repair.[13,14] It aids the development of targeted therapeutic strategies to combat lung disease.

## Material and Methods

The study was conducted on ten aborted fetuses [Table 1] from mid-gestation age onward (14–26 weeks). The fetuses were procured from the Department of Obstetrics and Gynaecology from our hospital after obtaining written informed consent of the parents and institutional ethical clearance. Fetuses of more than 20 weeks were obtained from spontaneous abortion while 14–20 weeks were collected from cases aborted by medical termination of pregnancy). All normal fetuses (without any gross congenital anomaly and maceration) were the part of this study. Determination of gestational age was done by measurement of crown-rump length (CRL), crown-heel length (CHL), biparietal diameter (BPD), foot length (FL), and weight of fetuses. The fetuses were immersion fixed in 10% formalin after a midline longitudinal incision on the anterior thoracic wall for better fixation of the thoracic viscera. After fixation, small parts of the lung close to hilum were dissected out and preserved in fresh formalin for 1–2 weeks. The specimens were processed in paraffin. Thin section (5 μm) were generated by rotatory microtome and stained.

### Staining

Tissue sections were stained with hematoxylin and eosin (H and E) stain to see the normal morphology. Immunostaining of every tenth section of each fetal lung was done to see the expression of FGF10 using polyclonal FGF10 antibody. All stained sections were examined under the BX 61 motorized microscope, and images were captured with the Olympus DP71 camera with Image-Pro Plus MC 6 software by (Media Cybernetics, Inc. 401 N. Washington Street, Suite 350 Rockville, MD 20850 USA).

## Results

### 14-week gestation

The developing lung had differentiating mesenchyme in which cut sections of various sizes of tubules (tubulogenesis) were seen. Between the lobules, connective tissue septa with few small blood vessels were clearly seen. Few tubules showed further branching into smaller tubules. At this stage, the developing fetal lung resembled an exocrine gland [Figure 1a]. At higher magnification, most of the tubules were lined by columnar epithelium, and in some places, lining epithelium showed cilia. Very few developing tubules had smooth muscles in their wall. Faint expression of FGF10 was seen in entire tissue with an intense expression in mesenchyme close to the branching point [Figure 1b].

### 18-week gestation

The fetal lung mesenchyme became more differentiated. The blood vessels in mesenchyme showed a moderate increase in number and size. Tubules were also enlarged in size with more successive branching. Primitive cartilage plates with perichondrium were seen around developing intrapulmonary bronchi. Few of large developing tubules had infolding and lined by pseudostratified epithelium [Figure 2a]. Smooth muscles were seen around some enlarging tubules. Immunostaining revealed intense staining in connective tissue close to developing tubules. Few lining columnar cells and some intraluminal cells of tubules showed the expression of FGF10.

### 20-week gestation

By this time, the amount of parenchymal tissue had relatively decreased but cellularity increased as compared

**Table 1: Fetal measurements and collection data**

| Age group (weeks) | CRL (cm) | BPD (cm) | CHL (cm) | FL (cm) | Number collected |
|---|---|---|---|---|---|
| 14 | 10.2-12 | 2.6-2.8 | 13.9-14.5 | 0.9-1.7 | 3 |
| 18 | 11-11.3 | 2.8-3.1 | 14.1-15.3 | 1.7-2.1 | 2 |
| 20 | 18.8-19.6 | 4.3-5.3 | 28.9 | 3.2-3.4 | 3 |
| 26 | 25-25.8 | 5.8-6.1 | 38.3-39.1 | 5-5.3 | 2 |

CRL: Crown-rump length, BPD: Biparietal diameter, FL: Foot length, CHL: Crown-heel length

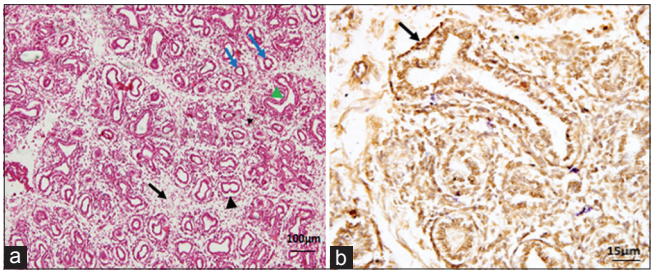


**Figure 1: 14 weeks: (a) Hematoxylin and eosin staining showing parenchyma of the developing lung containing differentiated mesenchyme (black arrow). The tubular structures (blue arrows) with few branching tubules (green arrowhead) are embedded in it. The connective tissue septa are seen. The smooth muscles cells (black arrowhead) are seen around most of the tubules (scale bar: 1 cm = 100 μm) (×10). (b) Immunostaining with anti-fibroblast growth factor-10 antibody demonstrates diffuse light brown stained in the mesenchyme. At branching point of tubules, dark brown (arrow) stained mesenchyme is seen (scale bar: 1 cm = 15 μm) (×40)**

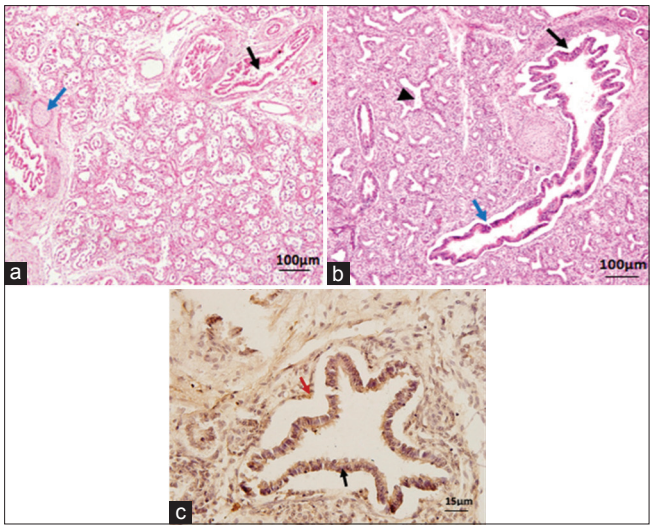


**Figure 2: 18–20 weeks: (a) Hematoxylin and eosin staining showing larger tubules (black arrow) with more branching and epithelial infolding. The bigger tubules show pseudostratified epithelium. Few bigger tubules (intrapulmonary bronchus) were surrounded by the developing cartilage plates (blue arrow) (scale bar: 1 cm = 100 µm) (×10). (b) Hematoxylin and eosin staining showing large number of branching tubules (arrowhead). The tubules without any cartilage plate (blue arrow) are seen arising from intrapulmonary bronchus (black arrow) (scale bar: 1 cm = 100 µm) (×10). (c) Immunostaining with anti-fibroblast growth factor-10 antibody showing dark brown immunopositive cells in entire lining epithelium (black arrow) and basement membrane, mainly near branching point (red arrow) (scale bar: 1 cm = 15 µm) (×40)**

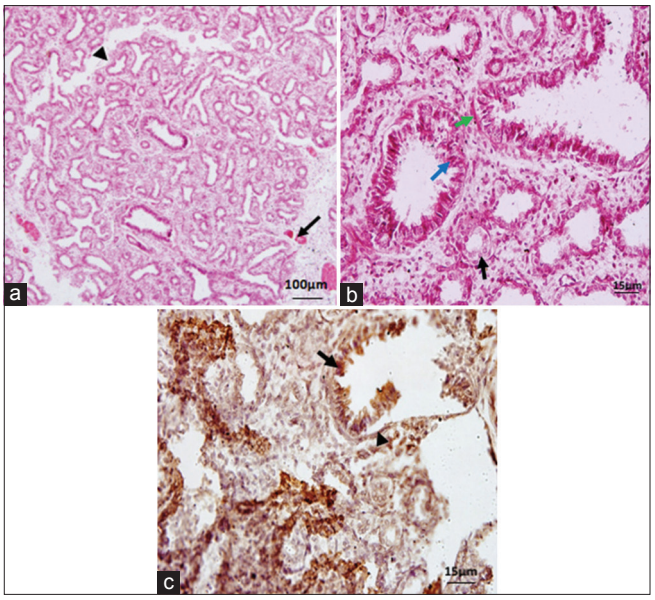


**Figure 3: 26 weeks: (a) Hematoxylin and eosin staining showing distinct lobular structure surrounded by connective tissue septa. The mesenchymal tissue is well differentiated. The blood vessels (arrow) apposed to the developing tubules. Alveolar duct with alveoli is seen as elongated tubule with lateral outpouching (arrowhead) (scale bar: 1 cm = 100 µm) (×10). (b) Hematoxylin and eosin staining depicting tubules lined by well-developed pseudostratified ciliated columnar epithelium (blue arrow). Few tubules show cuboidal epithelium (black arrow). The smooth muscle cells (green arrow) are well-developed around tubules (scale bar: 1cm = 15 µm) (×40). (c) Immunostaining showing darkly stained immunopositive cells in the entire lining epithelium (arrow) and basement membrane (arrowhead) of tubules (scale bar: 1 cm = 15 µm) (×40)**

to lower gestational age sections. The parenchyma had many branching tubules with irregular lumen. Well-developed, moderately larger vessels were also observed in connective tissue septa. Cartilage plates were well developed around larger tubules. Most of the larger tubules were lined by pseudostratified ciliated epithelium. Most of the pseudostratified lined tubules had cilia [Figure 2b]. Medium-to-small lumen tubules were lined by columnar-to-cuboidal epithelium. Smooth muscles were seen in the wall of tubules. Immunostaining showed more intense staining than a younger fetus in entire epithelial lining and their basement membrane at branching site [Figure 2c]. The rest of lung tissue showed less intense expression.

### 26-week gestation

The amount of mesenchymal tissue became more mature and well differentiated. Connective tissue had very well-developed large blood vessels. Pleura was seen as a layer of simple squamous cells with underlined loose connective tissue. Many septa were arising from loose connective tissue of pleura. The lobular architecture became more pronounced. A lot of successive branched expanding tubules were seen in the mesenchyme. Expanding tubules showed an irregular wavy pattern. In medium magnification (×40), most of the tubules were lined by cuboidal epithelium whereas larger tubules had well-developed pseudostratified ciliated columnar epithelium [Figure 3b]. Alveolar duct with alveoli was recognized as elongated tubules with lateral outpouching [Figure 3a]. Squamous epithelium-lined lateral outpouching is from cuboidal-lined alveolar duct. Thicker and more developed smooth muscles were found along pseudostratified lined bigger tubules but were thinner in other tubules. The interlobular connective tissue septa appeared less cellular in comparison to intralobular connective tissues around developing tubules. Immunohistochemistry sections showed intense stained immunopositive cells in entire epithelial lining of tubules and basement membrane [Figure 3c].

## Discussion

The study of normal development of the human lung is very important to know the pathogenesis of defective lung developments such as congenital diaphragmatic hernia, respiratory distress syndrome, and Apert syndrome and also in the treatment of these diseases. Most of the previous studies were on lower animals. The present study was done on human fetuses ranging from 14 to 26 weeks of gestation. In this study, we correlate the normal morphology of various stages of developing human lung along with immunostaining by FGF10. FGF10 is a mesenchymal marker for branching morphogenesis. Fetal age was estimated using different fetal parameters, i.e., CRL, CHL, BPD, FL, and the ultrasonographic data of age provided by

an obstetrician along with last menstrual period of mother. Hence, the error in the fetal age estimation was minimized.

The lung develops as an endodermal-lined outgrowth (respiratory diverticulum) from the ventral wall of foregut at 3–4 weeks of gestation into surrounding mesenchyme. The respiratory diverticulum forms trachea and two bronchial buds.[6] Each of the buds enlarges to form the right and left main bronchi. Then, the right bronchus forms three and left bronchus forms two secondary bronchi. During further development, tertiary bronchi and further 17 generations of subdivisions have formed. The cartilaginous, muscular, and connective tissue components of trachea and lungs are developed from surrounding mesoderm.[1,2,4,15] The developing lung requires integration of many important signaling molecules and transcription factors at various stages of development. The signaling molecules are FGF, SHH, TTF, Wnt, bone morphogenetic protein, GATA-6, Foxa2, Foxj, Foxf, RARα/β, Hox-b5, and Gli family.[16] In FGF signaling, particular FGF10 signal to FGFR2 is vital for bronchial bud formation and branching morphogenesis. The FGF10 acts as a potent mitogen and its expression in adjacent mesenchyme is important for initiating the outgrowth of new branches.[9,12] FGF10 is one of the early and important signaling molecules required for normal development of the lung.

In this study, the youngest fetus was of 14 weeks of gestation. The developing lung had abundant primitive mesenchymal tissue, in which developing tubules were embedded. Most of the developing tubules were lined by columnar epithelium, but some tubules had cuboidal epithelium. Previous studies on human fetus showed that the terminal buds had many mesenchymal cells without features of condensation. The lining epithelium consisted of low columnar cells with round nuclei and clear cytoplasm. One literature shows that distal airways lined by columnar epithelium have prominent subnuclear vacuolation. This finding is in consonance with our study. The developing lung had an appearance similar to the exocrine gland, which has also been manifested by previous workers.[2,3] Many others have described very prominent hyaline cartilage plates in bronchus and bronchioles lined by cuboidal epithelium. At 14 weeks of gestation, we did not see very prominent cartilage plates around the developing tubules.[17] This difference in the size of cartilage plates may be due to the difference in site of section taken from the fetal lung. At 14 week, the expression of FGF10 was present in mesenchyme of developing tubules with a stronger expression close to the distal part of tubules. Literature shows that epithelial–mesenchymal interaction is very important for lung morphogenesis.[8,18,19]

At 18 weeks of gestation, a number of developing tubules were lined by simple columnar epithelium, and a few were lined by cuboidal epithelium. The lumen of bigger tubules appeared irregular and lined by pseudostratified epithelium. The cartilage plates surrounded the bigger tubules. The previous studies done are in accordance with our finding at 18-week gestation. According to few studies, at this age of gestation, the blood vessels get apposed between the developing air spaces for the first time.[20] Similar results were observed in our study.

We found that the parenchyma became more evident by 20-week gestation. The lumen of most of the tubules became irregular. Previous studies on human fetuses showed more evolved lobular structure.[21]

At the age of 26 weeks, respiratory bronchioles, alveolar duct, and alveolar sac were prominent. The primitive alveoli lined by cuboidal-to-squamous epithelium were seen. The air–blood barrier becomes thin that implies that lung is viable enough to support the life of a fetus in preterm delivery.

Immunostaining at 20 weeks and 26 weeks revealed that FGF10 shows its expression with its receptor at the entire endoderm lining of branching tubules. This interaction of FGF10 and its receptor is mainly responsible for directional outgrowth and induction of epithelial bud.[3] The disruption of FGF10-FGFR2b is lethal at birth. The FGF10 regulates the activation, expansion, and differentiation of mesenchymal stem cells.

## Conclusion

The developing respiratory tree showed a decrease in the amount of mesenchymal tissue and gradual diminution of the height of epithelium. It is evident that by 26 weeks, lungs attain morphological maturity, thus indicating the viability of extrauterine life of a baby born after this period. The initial FGF10 expression was found at as early as 14 weeks of gestation, i.e. in pseudoglandular stage. The FGF10 expression was shifted from mesenchyme to epithelial lining of tubules. Thus, we conclude that FGF10 has a significant role in airway branching, division, and differentiation. This knowledge can be utilized in the development of targeted therapeutic strategies to combat lung diseases.

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### Conflicts of interest

There are no conflicts of interest.

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