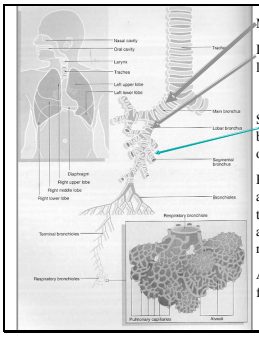
Slide 1

Lung histology

- Cast of Characters
  - Airways
    - Conducting
    - Respiratory
  - Vessels
    - Arteries, arterioles - pulmonary and bronchial
    - Capillaries
    - Veins/Venules and Lymphatics
  - Pleura - visceral and parietal

Understanding embryology of the lung requires basic understanding of the histologic components, both microanatomically and types of cells. The function of the lung requires air to enter a zone of particle filtration and air warming (conducting airway). The second zone requires development of a thin membrane for gas exchange. As the goal of gas exchange is an interface between air and blood, the vasculature also follows progressive branching to the capillary bed.

Slide 2



Main stem bronchus

Lobar bronchus (5 lung lobes)

Segmental bronchus (10 bronchopulmonary segments on right, 9 on left

Branching continues as airways become bronchioles, then at terminal bronchioles airways transition into respiratory bronchioles

About 20 branch generations from beginning to end

Starting from the larynx, the airway continues as the trachea, which branches into 2 bronchi. His is followed by lobar bronchi, then segmental bronchi (10 right, 9 left). Conducting airways continue as bronchioles then into respiratory bronchioles, the beginning of the gas exchange zone.

Slide 3

Lung Histology: Conducting zone

- Airways Conducting Zone
  - Trachea
  - Bronchi - ciliated and goblet cells, elastic tissue, smooth muscle, glands, cartilage
  - Bronchioles - (1 mm) - No cartilage or bronchial glands, ciliated lining,no goblet cells, smooth muscle
- Cell types
  - CILIATED CELL - beating of cilia contribute to mucociliary elevator
  - GOBLET CELL - Mucus secretion
  - BASAL CELL - reserve cell
  - KULCHITSKY CELL - neuroendocrine cells.

The cells of the conducting zone (proximal) include epithelial cells which are ciliated (slender surface projections that beat to move mucus and particles). Cells are required that make mucous, and regenerative cells are present. In addition, fibroblasts, smooth muscle and cartilage are cellular components in this zone.

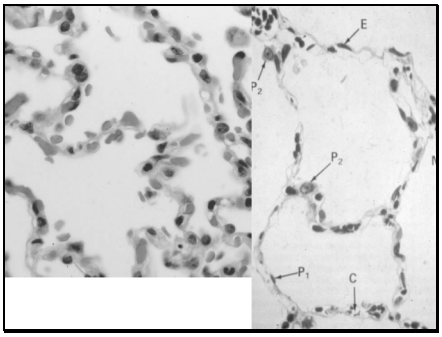
Slide 4

Pulmonary Histology

- Airways Respiratory Zone
  - Terminal bronchiole to Respiratory bronchiole - lined by ciliated cells and **CLARA CELLS; by transitional zone to RB, all Clara cells.**
  - Alveolar ducts/sacs
    - **Type I cells** 90% of alveolar surface
    - **Type II cells**
- Cell types
  - CLARA CELLS - produce a component of surfactant and are the bronchiolar reserve cell
  - TYPE I CELLS - Thin lining cell for gas exchange
  - TYPE II CELLS - surfactant and alveolar reserve cell

In the respiratory (distal) zone, the cells are geared towards surfactant production (which is needed to keep alveoli open by reducing surface tension) and generating a thin layer for gas exchange. The alveoli have a thin type I epithelial cell closely apposed to a thin capillary endothelial cell. In addition, type II cells make surfactant and are the reserve cell.

Slide 5



The histology of mature alveoli reveals the thin gas exchange membrane with “E” as the thin type I epithelial cells and “C” as the closely apposed capillary endothelial cells. Type II cells are seen. The histology is background to the embryology, trying to help you visualize the endpoint and complexity of the lung. Epithelial and mesenchymal (endothelial) populations must grow and expand in a coordinated fashion, and at the same time, signals must also foster differentiation towards type I and type II cells

Slide 7

Laryngeal development

- Week 4
  - Respiratory primordium arises from distal/caudal pharynx
    - Laryngo -tracheal groove
  - Endodermal derivative of epithelium of larynx trachea and bronchi
  - Connective tissue, smooth muscle and cartilage from splanchnic mesenchyme surrounding the foregut

By week 4, the distal/caudal pharynx develops a groove on its ventral surface. The endodermal derivative of this groove gives rise to epithelium and glands, while the splanchnic mesoderm/mesenchyme gives rise to vessels, muscle and cartilage.

Slide 8

Laryngeal development

- LT groove evaginates and forms LT diverticulum
- This becomes invested with splanchnic mesoderm to form lung bud
- This maintains a laryngeal inlet

The septum that forms by folds and fusion keeps a septate inlet that becomes trachea and esophagus

The LT groove evaginates to form the LT diverticulum. This evagination maintains an opening to the larynx. Meanwhile as it grows forward a septum forms that eventually becomes circumferentially invested with splanchnic mesenchyme to have a separate larynx/trachea and esophagus.

Slide 9

Epithelium of the larynx

- Endoderm of proximal/cranial end of LT tube and cartilage from neural crest origin
- Formation of proximal larynx – cranial tube
  - Arytenoid swellings grow towards tongue
  - Airway gets closed off, eventually recanalizes
- Laryngeal webs – Incomplete recanalization
- Laryngeal atresia – ascites, hydrops and lungs do not properly form.

At the proximal end of this developing structure forms the larynx. The epithelium of the larynx is endodermal derived and the cartilage is from neural cartilage origin. This growth and proliferation closes off the larynx which is eventually recanalized. If the recanalization is incomplete, a congenital anomaly called a web occurs. Complete atresia (blockage) of the larynx leads to abnormal lung development.

Slide 10

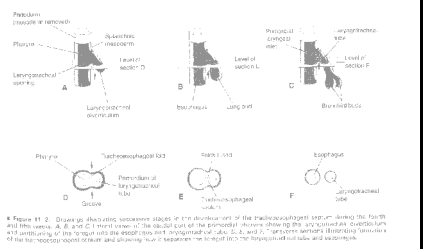
Trachea

- Endoderm of distal LT tube
  - Epithelium of trachea and lung
- Splanchnic mesenchyme
  - Connective tissue
- 4th week
  - If esopahgeal separation from LT tube is incomplete, develops into TE fistula

The distal portion of the LT tube develops into the trachea and lungs.

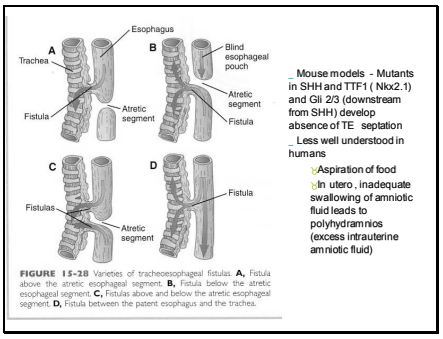
The septation of the trachea from the esophagus and the eventual separation is critical. In the absence of complete septation, a tracheo-esophageal fistula can develop – an abnormal connection between the trachea and esophagus

Slide 11



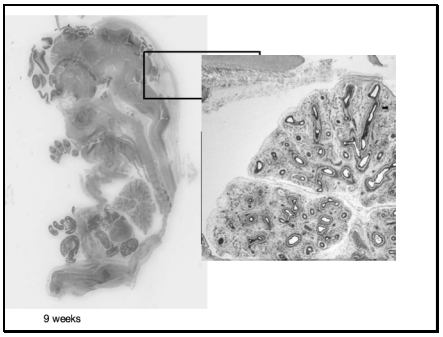
This diagram is from Moore's Developing Human. It demonstrates the formation of the larynx, LT tube and septation/separation between the respiratory and digestive systems.

Slide 12



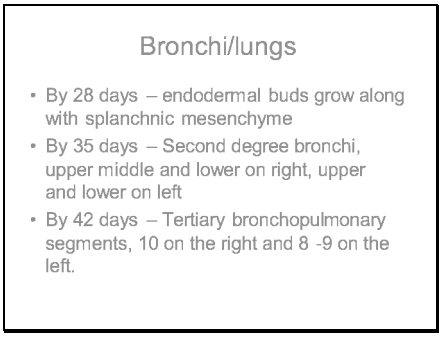
There are variations of tracheoesophageal fistula. Polyhydramnios (excess amniotic fluid) can occur when insufficient fluid is ingested and absorbed due to esophageal blockage. The most common type is shown in B with esophageal pouch and connection of trachea to stomach. Infants with TE fistulas aspirate food and secretions that accumulate in the blind esophageal pouch. Interestingly, mouse models with TTF1 (thyroid transcription factor), SHH (sonic hedgehog) and Gli gene mutations develop absence of TE septation.

Slide 13



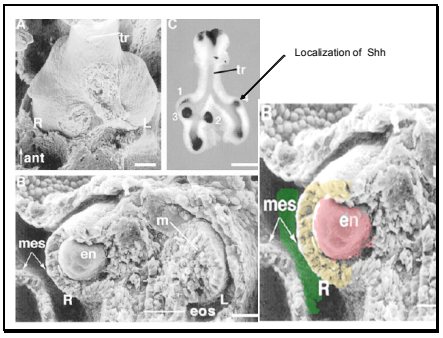
This slide shows a whole mount section of an approx 9 week fetus. The lecture slide will show a higher view with pharynx to larynx transition, trachea and lungs in the pseudoglandular phase (see below). By 9 weeks much of the structural development of the upper conducting airways is apparent.

Slide 14



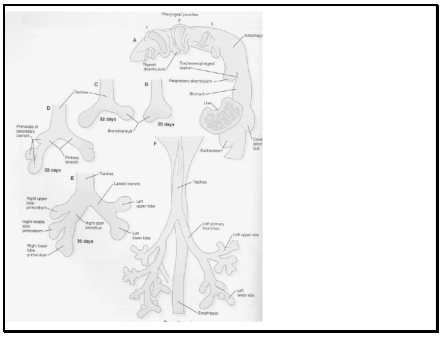
The budding of the endoderm invested with splanchnic mesenchyme continues. By 28 days, bronchial buds are identified. By 35 days, second degree bronchi, and by 42 days bronchopulmonary segments are defined by conducting airway branching.

Slide 15



These scanning electron microscopic images show the right and left bronchial buds, and the lower images show endoderm (pink) surrounded by mesechyme in yellow. What also occurs is an outer layer of splanchnic mesenchyme which becomes visceral pleura, a thin membranous layer that surrounds the lung.

Slide 16



This is a diagram illustrating the budding process that leads to the branching pattern described in slide 14.

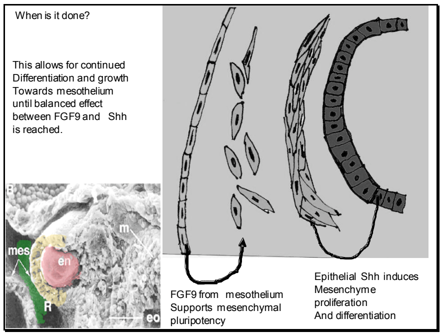
Slide 17

Branching morphogenesis

- By 24 weeks, 17 orders of bronchi and respiratory bronchioles (7 more after birth)
- Lungs grow to pleura – visceral pleura from splanchnic mesenchyme and parietal pleura from somatic mesoderm.

Branching continues so that by 24 weeks 17 orders of bronchi and bronchioles have formed. The lungs grow to the pleura.

Slide 19



What we observe microscopically during this branching period illustrates the balance between growth, differentiation and growth arrest that must occur simultaneous between epithelium, mesenchyme and pleura. As in the step in the electron microscopic image, a balance must occur between growth and differentiation signals derived from the different layers. More discussion to follow.

Slide 20

Table 1. Lung development (human) and regulatory factors

Stage	Duration	Characteristic events	Major molecular mediators
Embryonic	4–7 weeks	Outgrowth of trachea, right and left main bronchi and major airways	HNF-3β, TTF-1, RA, RAR, Shh, Ptch, Gli2, Gli3, FGF-8, FGF-10, HNF-4, N-cadherin, activin-β, activin-β-R IIA, lefty-1/2, nodal, Pitx-2
Pseudoglandular	5–17 weeks	Formation of bronchial tree up to a preacinar level	GATA-6, N-myc, PDGF, PDGF-R, EGF, EGF-R, FGF, TGF-β, Shh, Ptch, VEGF, BMP-4, RA, RAR
Canalicular	16–26 weeks	Formation of the pulmonary acinus and of the future air-blood barrier; increase of capillary bed; epithelial differentiation; first appearance of surfactant	GATA-6, TTF-1, HNF-3β, Mash-1, VEGF
Saccular	24–38 weeks	Formation of transitory air spaces	HNF-3β, TTF-1, NF1, VEGF, VEGF-R
Alveolar	36 weeks to 2 postnatal years	Alveolarization by forming of secondary septa	PDGF, PDGF-R, FGF, FGF-R, VEGF, VEGF-R, angiopoietins, ephrins, RA, RAR
Microvascular maturation	Birth to 3 postnatal years	Thinning of interalveolar walls; fusion of the capillary bilayer to a single layered network	VEGF, VEGF-R, PDGF, PDGF-R, angiopoietins, ephrins

Kleiner Roth and Post Biol Neonate, 2003

The maturation of the lung is defined by stages which are approximate and differ by 1 week or so depending on texts. After the embryonic stage during which left right structure and major airways form, the pseudoglandular stage is characterized by completion of the conducting zones/proximal airways. This is followed by the canalicular stage during which the future alveoli are formed with an intervening capillary bed. During the saccular stage the mature configuration of alveoli develops, which continue to develop in the alveolar phases after birth.

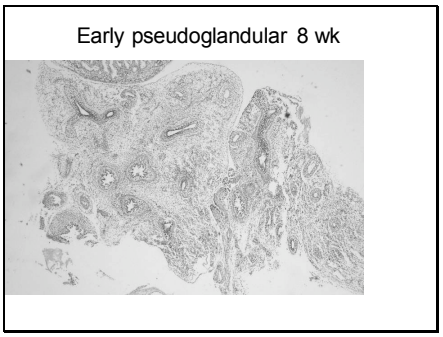
Slide 21

Lung Maturation

- Pseudoglandular (5-17 weeks)
  - No gas exchange zones
  - Lung resembles an exocrine gland
- Canalicular (17-25 weeks)
  - Terminal bronchioles enlarge and branch 2 -3 respiratory bronchioles then 3 -6 alveolar ducts. Terminal sacs begin to form
    - VAscularized – caudal slower than cranial
- Terminal sac (25 weeks to 34 weeks) – blood flow and surfactant
  - Epithelium thins to become type I like
  - Capillaries grow in
  - Blood air barrier forms
    - Type I and type II cells
      - Surfactant reduces surface tension allowing expansion.
- Alveolar period (late fetal to childhood)
  - Surfactant
  - Gas exchange
  - Pulmonary vs systemic circulation
  - Alveoli mature from age 3 -8. Numbers increase from 50 million at birth and 300 million at age 8 (adult number)

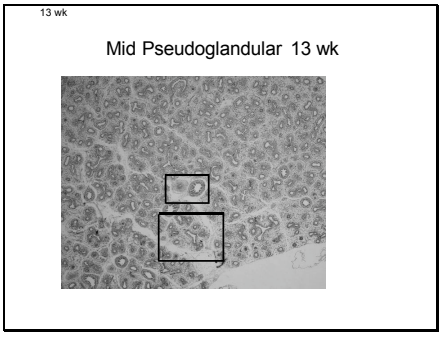
If we correlate the stages to morphologic/histologic appearance, the pseudoglandular stage looks like an exocrine gland- with ducts and immature "lobules". In the canalicular phase, the airway branching finishes, but these "lobules" begin to develop as sacs with ingrowth of capillary vessels. Epithelial differentiation can be seen, proximal versus distal, with the appearance of surfactant. By the terminal sac phase, distal epithelial differentiation completes into type I and II cells, with recognizable air-blood barrier and surfactant production

Slide 22



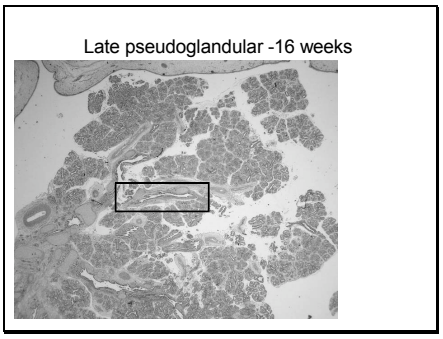
At 8 weeks – pseudoglandular duct structures and mesenchyme

Slide 23



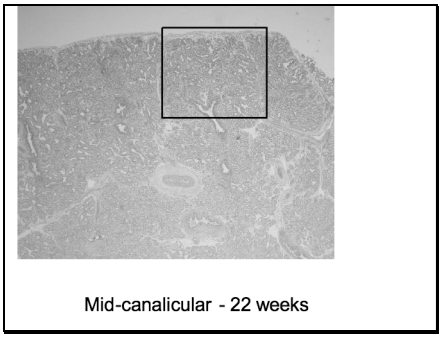
By 13 weeks, much more airway branching has formed, with recognizable broncho-vascular bundle and a “lobular” appearance.

Slide 24



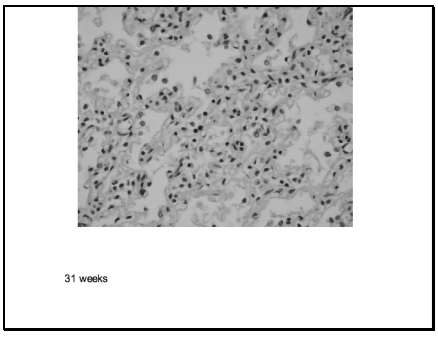
By late pseudoglandular, the proximal airways are well developed and differentiated. Epithelium is ciliated and cartilage is seen in airways. Bronchioles are formed, and conducting airways developed. Distal sacs are still immature, but their precursors are identified with vessels in the interstitium. Interlobular septa are seen. Vasculature is clearly identified but not to the capillary level.

Slide 25



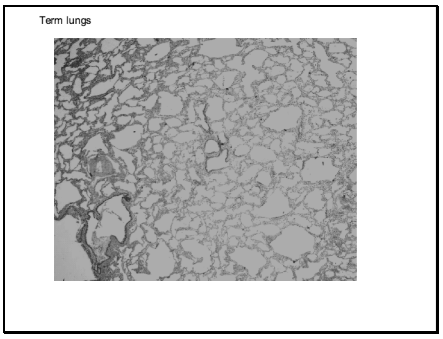
In the canalicular phase, bronchioles are differentiated and alveoli begin to form. They are cellular and immature, and not yet thin walled for gas exchange. Cells do not yet resemble flat type I cells.

Slide 26



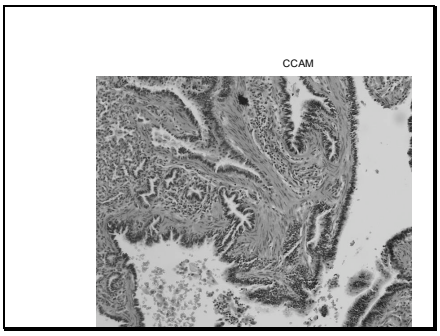
By 31 weeks, alveolar spaces are easily identified and the alveolar wall is thin with a flat type I cell and capillary

Slide 27



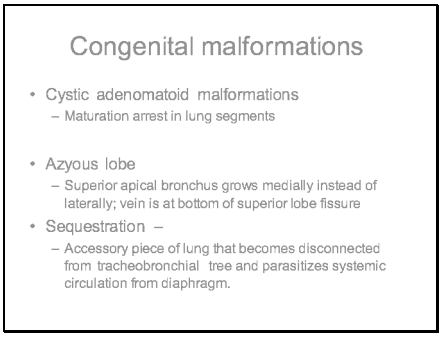
By term, alveoli are well formed and numerous. Alveoli are thin walled although they are slightly more cellular than adult alveoli. At birth, the alveolar lining cells are both type I and II cells. Alveoli continue to form until about age 8.

Slide 28



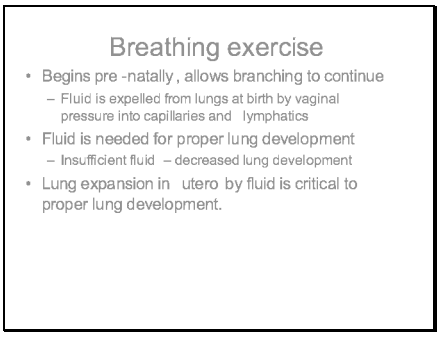
There are congenital malformations that recapitulate phases of lung maturation. These congenital cystic adenomatoid malformations likely represent maturation arrest at different points in the maturation of a lung segment or lobule. These are often detected early in life, although uncommonly they are first detected in adulthood.

Slide 29



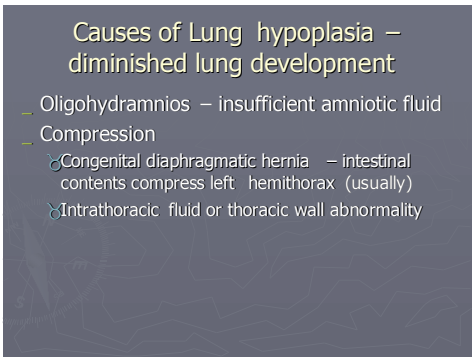
In addition, some defects represent abnormal development of lung and vascular development, such as an azygous lobe.
A pulmonary sequestration represents a segment of lung that becomes separated from the normal bronchial tree, and parasitizes its arterial blood supply from the diaphragm.

Slide 30



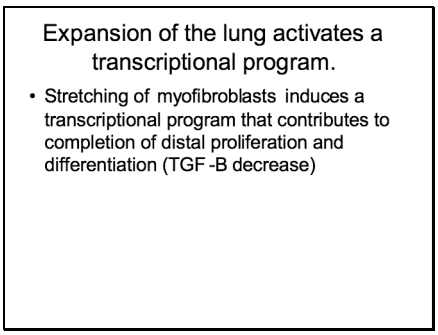
Lung development is linked to breathing movements, possibly to maintain intrapulmonary fluid and expansion.

Slide 30A

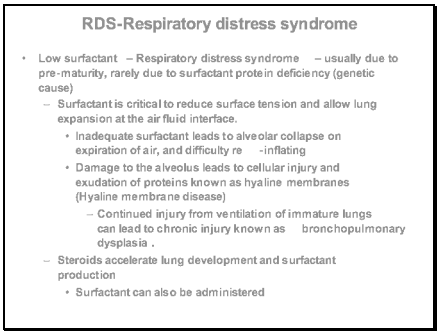


Amniotic fluid originates in the kidney. Lack of fetal urine results in oligohydramnios and results in lung hypoplasia. Lung can also be compressed during development causing hypoplasia. Loss of amniotic fluid can impact lung development.

Slide 31

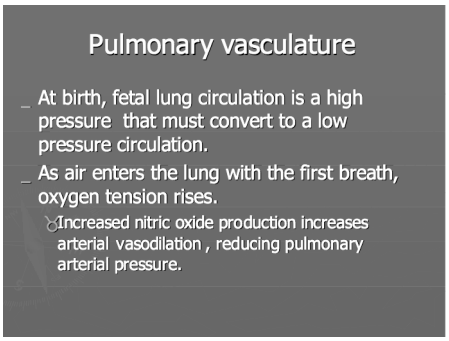


Slide 32



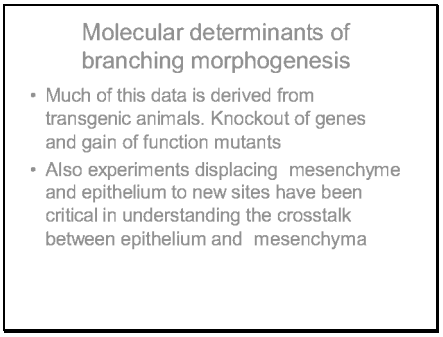
Low surfactant is usually the result of preterm labor and immaturity of the fetal lungs. Without surfactant, lungs cannot properly expand, and mechanical ventilation is needed to inflate the lungs. Necrosis of epithelium and protein exudation from vessels lead to intra-alveolar material known as hyaline membranes. Steroids can accelerate lung maturity/surfactant production and surfactant can be administered. Mechnical ventilation can cause chronic injury (bronchopulmonary dysplasia).

Slide 32A



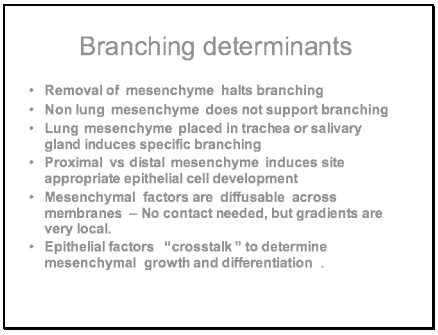
The fetal lung circulation is a high pressure circulation that must rapidly convert to a low pressure circulation with the first breath. With the first breath, oxygen tension rises and pulmonary vasodilation is induced by nitric oxide. This reduces pulmonary arterial pressure. Some newborns fail to fully convert from fetal circulation to post natal configuration, a condition known as persistent pulmonary hypertension of the newborn. This condition is treatable with oxygen, NO and more aggressive interventions, but is life threatening.

Slide 33



Now that we have tracked the morphologic changes of lung development and some of their pathologic consequences, we will discuss some molecular information regarding lung development. The basic growth pattern of lungs from the initial bronchial bud is progressive branching, followed by differentiation.

Slide 34



Mesenchyme drives the branching process and also is determinant of epithelial differentiation. Proximal versus distal mesenchyme induces differentiation of epithelium into proximal and distal type epithelial cells. This requires no contact and these are diffusible factors. While no contact is needed, the effect can be very local. While mesenchyme has an important effect, the epithelium also releases factors that impact on mesenchyme growth and differentiation.

Slide 35

Factors in branching morphogenesis

- Proliferation
  - Growth factors, factors that promote pluripotency
- Differentiation
  - Transcription factors and downstream effectors leading to differ ent cell types
  - Proximal and distal differences (e.g cartilage, type II cells)
- Inhibition of proliferation
  - Growth eventually stops, also a key to branch points
- Apoptosis
  - Structures must be remodeled in second wave of growth.
  - May deal with local proliferation excess
- Coordinated growth of different cell populations – "crosstalk "
  - Elements may be intracellular pathways, cellular receptor specif icity, as well as diffusable factors with very local gradients
- Differential adhesion.
  - Cell to cell, cell to matrix.
  - Adhesion molecules may be expressed reducing cellular fluidity, fostering attachment of epithelium to mesenchyme via common extracellular matrix proteins

Slide 36

FGF-fibroblast growth factor

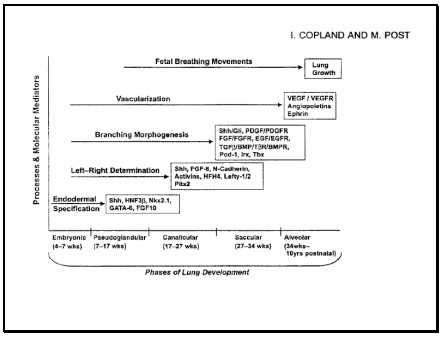
- Loss of epithelial FGF receptor leads to loss of branching
- FGF10 in mice from mesenchyme binds to FGFR2 on epithelial cells.
- Epithelial Shh (sonic hedgehog) shuts off FGF10 from mesenchyme , stopping growth. New buds form in Shh negative areas that have persistent FGF10 production
- Other FGF (e.g. FGF7) and other FGFR (e.g. FGFR3 and 4) may play a similar role in later stages of distal/alveolar lung development.

Slide 37

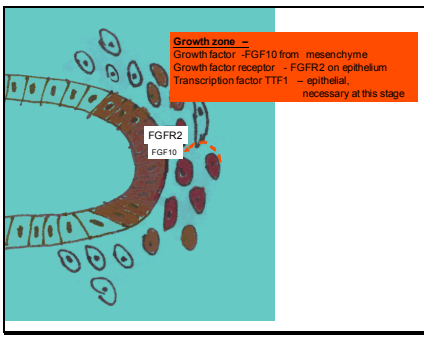
Branching parameters

- BMP4 may prevent proximal type differentiation, allowing cells to accept signals for distal development.
- TTF1 and HNF3B may promote differentiation towards surfactant producing cells.
- Shh may induce proliferation and differentiation in mesenchyme and inhibit epithelial proliferation signals (FGF10).
- TGF-B may induce extracellular matrix production, which in turn may further anchor and stabilize epithelial cells.

Slide 38



Slide 39



The process requires several different signals to occur. Cellular growth is driven by growth factors, but growth cannot go on uncontrolled to development an organized structure. A key to branching is a zone of growth arrest. These processes must occur in a coordinated manner requiring epithelial and mesenchymal cross talk. Once structures are formed, cellular differentiation needs to occur so that structures can perform their specialized function. In addition, certain types of differentiation including cell-cell adhesion and cell to matrix adhesion makes mature structures permanent.

Loss of FGF10 in mouse models results in absence of lung development. FGF10 from mesenchyme binds to FGFR2 on epithelial cells, inducing their growth. Epithelial Shh shuts off FGF10 production, halting growth. New buds form in zones beyond the inhibitory effect of Shh that have FGF10 production. Other FGFs may play a similar role is distal lung branching.

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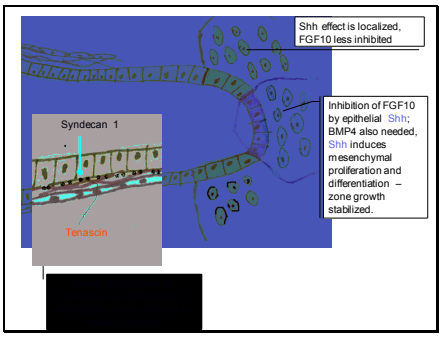

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