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.

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.

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 mucus, and regenerative cells are present. In addition, fibroblasts, smooth muscle and cartilage are cellular components in this zone.

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.

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.
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.

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.

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.

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.

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.
HD11- Lung Development

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.

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 13

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 14

- By 28 days – endodermal buds grow along with splanchnic mesenchyme
- By 35 days – Second degree bronchi, upper middle and lower on right, upper and lower on left
- By 42 days – Tertiary bronchopulmonary segments, 10 on the right and 8-9 on the left.

These scanning electron microscopic images show the right and left bronchial buds, and the lower images show endoderm (pink) surrounded by mesenchyme 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 15

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.

**Slide 19**

What we observe microscopically during this branching period illustrates the balance between growth, differentiation and growth arrest that must occur simultaneously 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**

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.

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 21**

**Lung Maturation**
- Pseudoglandular (5-17 weeks)
  - No gas exchange yet
  - Lung resembles an exocrine gland
  - Early airways are not airways
  - Terminal sacs (stage 0) to 3rd generation bronchioles (1-4) airways form
  - Tissues appear dense but vascularity present
  - Terminal sacs (stage 0) - blood flow and surfactant
  - Epithelium: squamous to columnar
  - Type II cell proliferation
  - Surface: cuboidal
  - Type I cell formation
  - Basilar/myofibroblastic differentiation

- Canalicular (17-32 weeks)
  - Terminal sacs to 3rd generation bronchioles (1-4) airways open
  - Terminal sacs (stage 2-8) - blood flow and surfactant
  - Epithelium: columnar
  - Type II cell formation
  - Surface: squamous
  - Type I cell formation
  - Basilar/myofibroblastic differentiation

- Saccular (32-40 weeks)
  - Terminal sacs to 3rd generation bronchioles (1-4) airways open
  - Terminal sacs (stage 2-8) - blood flow and surfactant
  - Epithelium: columnar
  - Type II cell formation
  - Surface: squamous
  - Type I cell formation
  - Basilar/myofibroblastic differentiation

- Fetal (40 weeks)
  - Terminal sacs to 3rd generation bronchioles (1-4) airways open
  - Terminal sacs (stage 2-8) - blood flow and surfactant
  - Epithelium: columnar
  - Type II cell formation
  - Surface: squamous
  - Type I cell formation
  - Basilar/myofibroblastic differentiation
At 8 weeks – pseudoglandular duct structures and mesenchyme

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

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.

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.

By 31 weeks, alveolar spaces are easily identified and the alveolar wall is thin with a flat type I cell and capillary
HD11- Lung Development

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.
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. Mechanical ventilation can cause chronic injury (bronchopulmonary dysplasia).

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 postnatal 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. 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.

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.
Factors in branching morphogenesis

- Proliferation
  - Growth factors, factors that promote
  - Differentiation
  - TGF-β may induce extracellular matrix production, which in turn may further anchor and stabilize epithelial cells.
- Homoebox proteins
  - TTF1 – epithelial, necessary at this stage
- Growth factor – FGF10 from mesenchyme
- Growth factor receptor – FGFR2 on epithelium

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 in later stages of distal/alveolar lung development.

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.

Other FGFs may play a similar role in distal lung branching.