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# Fractal Analysis of the Human Fetal Lung Development

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**Abstract.** In order to characterize quantitatively the development of the fetal lung, we applied the method of fractal geometry to 3D images of human fetal airways reconstructed from serial histologic sections. Four human fetal right lungs were subjected, whose cranio-rump lengths were 103 mm, 132 mm, 145 mm, and 190 mm, respectively. The former and the latter two corresponded to the pseudogladular stages and the canalicular stages, respectively. Fractal analysis with 3D box-counting method was made for four cubes with side 0.432 mm. The means of fractal dimensions of the former two were 1.7, that of the third was 2.1, and the last was non-fractal. These results show that a self-similar branching growth remains in the pseudoglandular stage and a surface-increasing growth occurs in the canalicular stage. This transition of growth modes may correspond to the functional difference between the airway completed in the pseudoglandular stage and the air space developing in the canalicular stage.

## 1. Introduction

The morphogenesis of the human fetal lung begins at the third gestation week as the formation of a ventral diverticulum of the foregut (BOYDEN, 1975). This diverticulum quickly divides into right and left branches and repeats a numerous number of divisions. The developmental process is classified into three stages according to microscopic findings, i.e., the pseudoglandular, the canalicular, and the saccular stages (BOYDEN, 1975; ADAMSON, 1991). During the pseudoglandular stage, the fetal airway down to future terminal bronchioles (TBs) are formed (BUCHER and REID, 1961a), while the further development occurs in the canalicular stage. However, our recent work estimating the number of endtips of the fetal airway (KITAOKA *et al*., 1996) has revealed that the branching process reaches already to the level of future alveolar ducts in the late pseudoglanduar stage, which is consistent with the recent cyto-immunochemical and ultrastructural morphometric studies (JOYCE-BRADY and BRODY, 1988; OTTO-VERBERNE *et al*., 1988; MOSCHOPULOS and BURRI, 1993). The canalicular stage is then considered as that of deformation of parts distal to future respiratory bronchioles (RBs). Thus, the airway attains a complicated system at relatively early stage.

Fractal geometry, proposed by Mandelbrot is useful for describing such complicated configurations (MANDELBROT, 1982; WEIBEL, 1991). In this paper, we characterize

quantitatively the lung morphognesis in terms of the fractal geometry and discuss its mechanism compared to a viscous finger phenomenon showing fractal growth.

## 2. Materials and Methods

### *2.1. Three-dimensional reconstruction of airways*

Four human fetal right lungs with crown-rump lengths (CRLs), 103 mm (Case 1), 132 mm (Case 2), 132 mm (Case 3), and 190 mm (Case 4), respectively, were treated. The exact gestation ages were unknown. Each lung was completely serially sectioned at a section thickness of 12 $\mu$m, and stained with Azan (Fig. 1). Three-dimensional reconstruction were performed in each lung in the anterior segment of the upper lobe and the anterior basal segment of the lower lobe. In these segments an area to be reconstructed was chosen arbitrarily at about one-fourth or one-fifth of the distance from the pleura to the pulmonary hilus, so as not to contain large airways. For each area, 36 serial sections were subjected for reconstruction and two cubes with side 0.432 mm were obtained.

One each section, light micrographs were recorded at a magnification of 100 ×, and the cross sectional contours of internal surfaces lined by the epithelial cells of the airways were traced on a sheet of semi-translucent paper. All the branches contained in a sample under investigation were followed in consecutive sections until they reached a bifurcation or a blind end. The drawing were input into a microcomputer (PC-9801; NEC, Tokyo) by tracing with a cursor and 3D reconstruction of airways were performed (Fig. 2).

### *2.2. Measurement of fractal dimension and the airway volume*

A 3D box-counting method linked with 3D reconstruction system was used (SHIMIZU *et al*., 1989; KITAOKA and ITO, 1991). This method is briefly explained as below.

Enclose part of the space into a cube with side $P$ and divide the cube into $(P/a)^3$ small cubes with side $a$. Let $N(a)$ be the number of small cubes which overlap the object in question. Count $N(a)$ and plot $\log N(a)$ against $\log a$. If plotted data lie almost on a line, then the object can be regarded as self-similar and the absolute value of the slope gives the fractal dimension $D$, i.e.

$$N(a) = Ka^{-D},$$

where $K$ is a constant. The 3D box-counting program consists of the parts of cubic grid indication and counting overlapping cubes.

Volumes of the internal space of the airway contained in the observed cubes were measured by a software for 3D volumetry by the Cavalieri method. The volume density of the airway (the ratio of the volume of the airway to that of the observed cubes) were then calculated.

## 3. Results

Table 1 shows $N(a)$ counted in four regions of four cases. Here $a$ is a multiple of the section thickness, 12 $\mu$m. Figure 3 shows two log-log plots of $a$ and $N(a)$ in Cases 1 and 4,

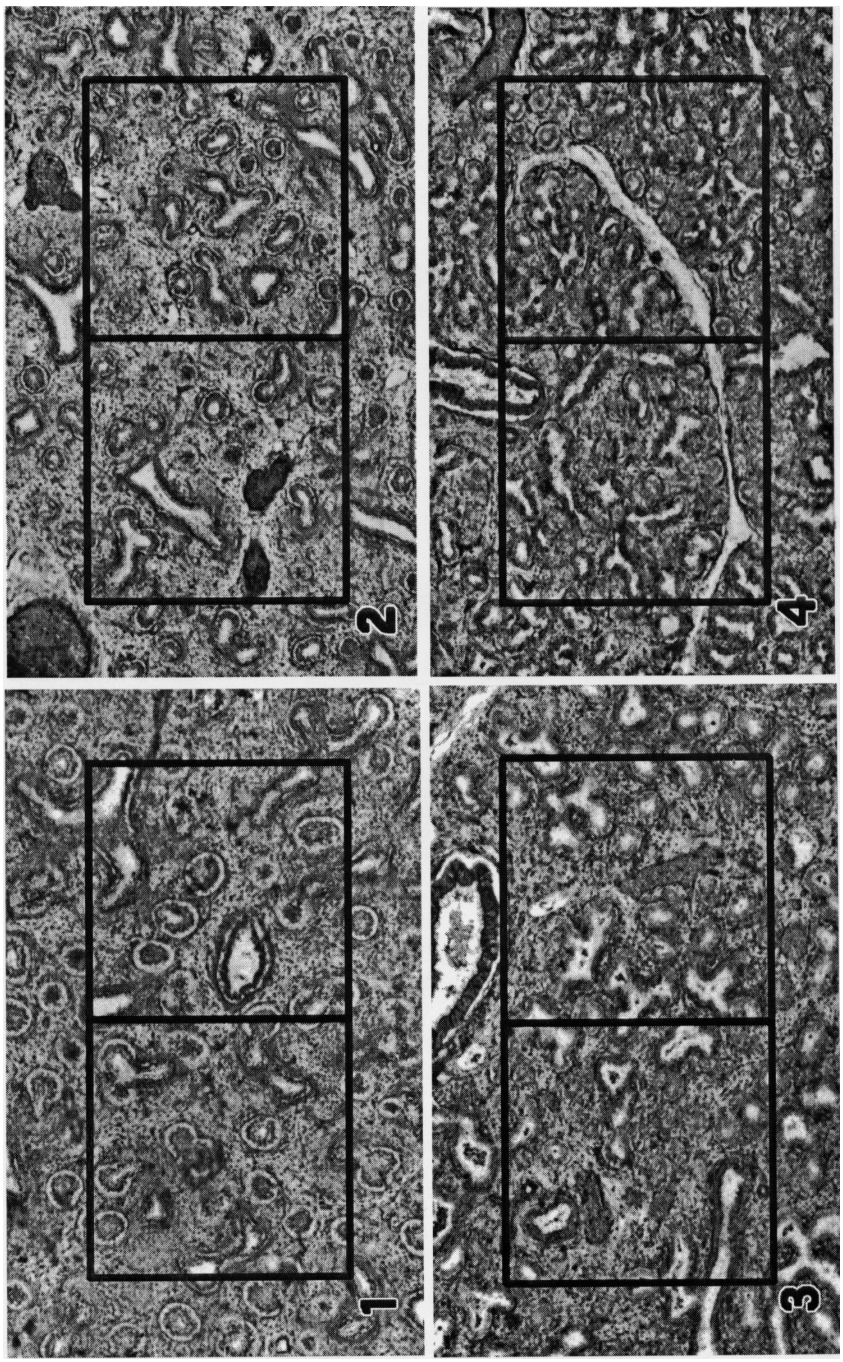


Fig. 1. Photomicrographs of reconstructed areas. Characters correspond to the Cases. Area enclosed by the two squares, 0.432 mm × 0.432 mm, were reconstructed. While patterns of the airway in Cases 1 and 2 are quite similar, the difference among in Cases 2, 3, and 4 is obvious. Note that the micrograph of Case 2 appesrs as if its magnification is slightly smaller than that of Case 1.

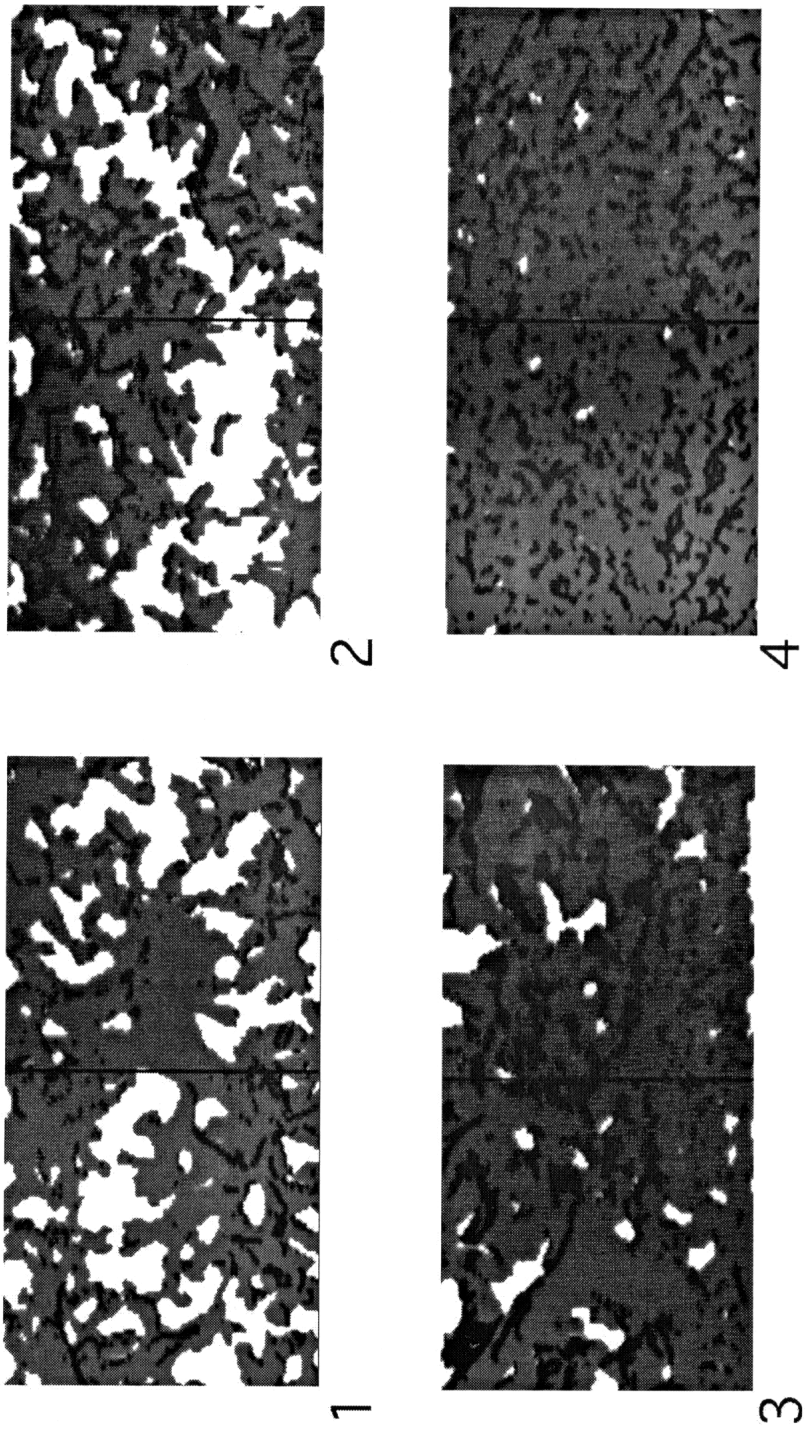


Fig. 2. Three-dimensional images of the reconstructed airways from serial 36 slices. Fractal dimensions were measured for each cube.

Table 1. Counted $N(a)$ in the researched regions of four cases.

| | Researched regions | $N(12)$ | $N(24)$ | $N(36)$ | $N(48)$ | $N(72)$ |
|---|---|---|---|---|---|---|
| Case 1 | Upper lobe | 4,011 | 1,201 | 624 | 401 | 184 |
| | | 2,694 | 805 | 400 | 272 | 123 |
| | Lower lobe | 4,017 | 1,182 | 605 | 393 | 176 |
| | | 4,871 | 1,371 | 666 | 422 | 196 |
| Case 2 | Upper lobe | 4,961 | 1,591 | 797 | 466 | 199 |
| | | 4,448 | 1,382 | 732 | 451 | 194 |
| | Lower lobe | 4,608 | 1,397 | 729 | 455 | 198 |
| | | 3,800 | 1,219 | 636 | 410 | 179 |
| Case 3 | Upper lobe | 9,995 | 2,560 | 1,217 | 636 | 215 |
| | | 10,009 | 2,480 | 1,130 | 603 | 214 |
| | Lower lobe | 10,241 | 2,568 | 1,185 | 683 | 211 |
| | | 10,675 | 2,521 | 1,102 | 607 | 213 |
| Case 4 | Upper lobe | 12,429 | 3,629 | 1,526 | 705 | 216 |
| | | 12,925 | 3,659 | 1,570 | 714 | 216 |
| | Lower lobe | 12,371 | 3,550 | 1,520 | 705 | 215 |
| | | 13,521 | 3,818 | 1,571 | 719 | 216 |

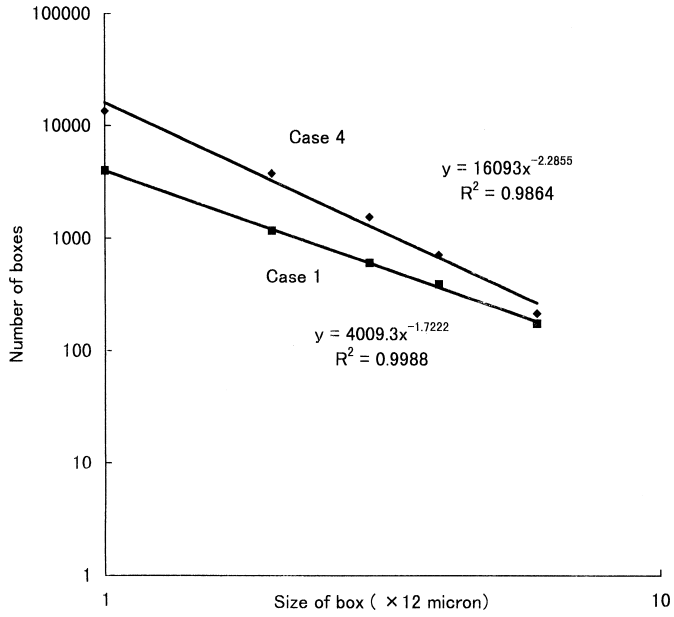


Fig. 3. Log-log plots of the size of box ($x$-axis) and the number of boxes containing internal space of the airway ($y$-axis) from Cases 1 and 4. The regression is very high in Case 1, however, plots are not accordant with a line in Case 4.

respectively. In Case 1, the regression line is highly accordant with all five plots with correlation coefficient of more than 0.998. In Case 4, correlation coefficient of the regression line was larger than in other cases. We regarded here a case with correlation coefficient larger than 0.99 as "fractal".

Table 2 shows the summary of all four cases. There is no significant difference in the slopes and the correlation coefficients of the regression lines between Cases 1 and 2, while certain amount of differences exist among Cases 2, 3, and 4 ($p < 0.001$). It is remarkable that the fractal dimensions of Cases 1 and 2 are equal in spite of significant differences in the volume densities of the airways ($p < 0.01$) and the numerical densities of endtips ($p < 0.001$).

## 4. Discussion

Our present study shows that the histologic difference between pseudoglandular stage and the canalicular stage, which has been described qualitatively, are characterized quantitatively with fractal analysis. During the pseudoglandular stage, the fractal dimension of the airway distribution does not change in spite of increasing the number of endtips. It suggests a self-similar growth during these stages. The value of fractal dimension 1.7 is the same as that obtained from the human adult airways down to TBs (KITAOKA and ITOH, 1991). It is worth noting that the configuration of conductive airway in the fetal lung is similar to that of adult stage in spite that it does not function as an air duct in the fetal stage. This fact means that morphogenetic principles of the branching system is consistent with teleonomical argument.

On the other hand, in the early canalicular stage, the fractal dimension exceeds 2, while the coefficient of correlation becomes smaller than those in the pseudoglandular stage. Such configuration change is observed in future alveolar ducts and sacs. These results suggest that in the canalicular stage the mode of growth changes from the self-similar branching to the surface increasing. The latter assures the function of air space, i.e. gas

Table 2. 3D Morphometry of four human fetal lungs.

| | CRL (mm) | Histologic classification | Fractal dimension (Correlation coefficient) | Volume density of airway (%) |
|---|---|---|---|---|
| Case1 | 103 | Late Pseudoglandular | $1.73 \pm 0.05$ $(0.999 \pm 0.000)$ | $8.3 \pm 0.8$ |
| Case 2 | 132 | Late Pseudoglandular | $1.73 \pm 0.07$ $(0.998 \pm 0.000)$ | $10.1 \pm 1.6$ |
| Case 3 | 145 | Early Canalicular | $2.12 \pm 0.03$ $(0.995 \pm 0.002)$ | $21.9 \pm 0.2$ |
| Case 4 | 190 | Middle Canalicular | $(2.27 \pm 0.02)$ $(0.986 \pm 0.000)$ | $27.5 \pm 0.5$ |

exchange, which needs a lot of surface.

It has been generally agreed that the intraluminal pressure of the airway affects the lung development in a late gestation state (ALCORN *et al*., 1977; FEWELL *et al*., 1983). A similar mechanism is considered to work even in the earlier stage, though it is nearly impossible to undertake the same experiments in this stage.

As is well known, branching patterns are observed not only in the living organ but also as physical phenomena. It is plausible that there will be a common mechanism ammong pattern formations both in biological and non-biological branching systems. Although the morphogenesis is performed through genetic expression in the biological system, the gene expression itself is under the control of physicochemical conditions.

The viscous fingering phenomenon between two fluids with different viscosities is known as an example of fractal growths of surfaces. LUBKIN and MURRAY (1995) proposed a mathematical model of branching in lung morphogenesis at very early stage based on a viscous finger model in Hele-Shaw cell. They assume that the low-viscosity fluid is the amnion filling the lumen of airway and the extremely high viscosity one is the lung parenchyma surrounding airway. The epithelium of airway is regarded as a surface of the two fluids. We consider that this model is applicable not only to the first several branching but also to later stages of the lung development, though the governing equations may be much more complicated.

In human fetal development, there is a hydrostatic pressure difference between the amniotic cavity and the lumen of the foregut until about 14 week, when the circulation of amnion between the two space is completed (LANGMAN, 1975). It may be possible to image that this hydrostatic pressure can be a driving force which generates a self-similar branching growth. At the end of pseudoglandular stage, this hydrostatic pressure may disappear because of the beginning of the amniotic circulation, however, at the same time, excretion of bronchial glands begins (BUCHER and REID, 1961b), which may make a hydrostatic pressure gradient between proximal and distal parts of the airway tree. This new pressure gradient may change the growth mode from self-similar branching into surface increasing as shown in viscous fingering experiments (VIZECK, 1990).

It is not easy to prove experimentally the above hypothesis. However, we believe that not only molecular biological approach, but also this kind of physical approach is necessary for understanding the structures of living organ.

## 5. Conclusion

We characterized quantitatively the fetal development of the airway tree by the use of fractal analysis. The result shows that self-similar branching growth remains during the pseudoglandular stage and surface-increasing growth occurs in the canalicular stage. This change of growth mode corresponds to the functional difference between the airway which is completed in the pseudoglandular stage and the air space which develops in the canalicular stage. Fractal analysis is useful for clarifying changes of growth modes.

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