学 位 論 文 の 要 旨

氏名　Shigeru Moriyama

学 位 論 文 名　Mathematical Representation and Analysis of the Number of Epithelial Cells in the Intestine of the Mouse Embryo

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著 者 名　Shigeru Moriyama, Junko Kuramoto, Hiroki Otani

論 文 内 容 の 要 旨

INTRODUCTION

Epithelial tubular tissues throughout the body including the intestinal epithelium develop regulating common basic events such as cell proliferation and cell death. Tissue-specific cell differentiation and differential regulation of cell proliferation/death as well as movement/positional rearrangement of the cells lead the formation of various organs. For understanding these complex developmental phenomena, the most basic information is the exact cell number in each location. Due to lack of information of the cell number, the wrong development-related theories tend to retain for many years in the field and repeatedly mentioned in the world-wide standard textbooks. One of these examples is the temporary occlusion of the duodenal lumen during the organogenetic period. This interesting finding has been interpreted as due to the overproliferation of the epithelium to occlude the lumen. However, by simply counting the cell number in the transverse sections of the midgut/duodenum during the period of the temporal occlusion of the lumen, we previously elucidated that the occlusion occurs due to the convergent extension but not due to the overproduction of the epithelial cells. It is thus indispensable to measure the total number of cells over the entire length of the intestinal tract in order to accurately grasp the phenomenon in the developing intestine. Further, upon the acquisition of the cell number as a basic information, the mathematical analyses are necessary to elucidate the principle of the developmental events such as how cell proliferation is regulated to reproducibly form the tissues and organs of normal size and shape. Therefore, in the present study, we measured the total number of cells in the developing intestine, and constructed the mathematical model.

MATERIALS AND METHODS

Animals. All experiments with animals in this study were approved by the Animal Care

and Use Committee of Shimane University (IZ26-20, IZ30-54). C57BL/6J mice (CLEA Japan, Tokyo) between 8 and 20 weeks of age were used. The mice were housed in the Department of Experimental Animals, Interdisciplinary Center for Science Research, Organization for Research and Academic Information, Shimane University. Noon of the day when a vaginal plug was observed was defined as embryonic day 0.5 (E0.5). One embryo for each E11.5, E13.5, E15.5 was obtained under the deep anesthesia (total number of embryos, n = 3, one from each dam). The crown-rump length (CRL) was measured. The intestine was fixed in 4% formaldehyde/70% ethanol at 4°C overnight. The fixed samples were embedded in paraffin and 5 μm-thick serial transverse sections were made from the junction between the stomach and duodenum to the cecum. **Caudal-type homeobox protein2 (Cdx2) immunostaining.** The sections were immune-stained with anti-Cdx2 antibody for cell counting. Cdx2 is a transcription factor which is expressed in the intestinal epithelial cells. After deparaffinization, endogenous peroxidase was deactivated by 0.03% hydrogen peroxide/99% methanol. The primary antibody reaction was performed with monoclonal mouse anti-human clone DAK-CDX2 (1:50, Dako, Glostrup, Denmark), followed by the secondary reaction using M.O.M. biotinylated anti-mouse Ig G reagent and Avidin-Biotin Complex reagent (Vector). Sections were then visualized with 0.05% 3, 3'-diaminobenzidine-tetrahydrochloride (Dako). **Cell number counting by stereology.** Using the Stereo Investigator®, the measurement of the total cell number of the intestinal epithelium was performed at E11.5 with 240 μm interval (11 measured / total 249 sections), at E13.5 with 600 μm interval (22 measured / total 2545 sections), and at E15.5 with 1 mm intervals (36 measured / total 7100 sections). **Analysis software.** Image J (National Institutes of Health, Bethesda, Maryland, USA) and Excel built-in software (exponential function analysis, multinomial analysis, linear regression analysis) were used.

RESULTS AND DISCUSSION

Morphology of the intestine and cell counting. At E11.5, E13.5, E15.5, the CRL of the mouse embryo was 7.25 mm, 10.75 mm,16.46 mm and the length of the intestine was 1.25 mm, 12.73 mm, 35.50 mm, respectively. During this period, CRL increased 2.27-fold, whereas intestinal length increased 28.4-fold. The intestinal tract elongated and showed a physiological hernia at E13.5 and E15.5. The epithelium was pseudostratified, and at E11.5, the pseudostratified cell layer number is 1 to 2 in the rostral and caudal parts, and 2 to 3 in the middle part. At E13.5, the pseudostratified cell layer number is 3 throughout the entire length. At E15.5, the epithelium at the rostral part was simple columnar and villi formation was observed, whereas in the more caudal parts, the epithelium was pseudostratified with the more layer number and villi were the less developed. **Number of cells counted by stereology.** At E11.5, E13.5, E15.5, the number of cells were 26992, 301770, 2746667, respectively. The number of cells increased 11.2-fold from E11.5 to E13.5 and 9.1-fold from E13.5 to E15.5. **Mathematical**

expression of epithelial cell proliferation. A mathematical model of the proliferation pattern of epithelial cells was created from the total number of cells obtained on each date. We used Excel built-in software and obtain the formula. $Y = 0.0472e^{1.1557X}$, Y is the cell number, e is the napier constant (2.718), and X is the day number. The reliability of this approximation software is $R^2 = 0.9993$, which indicates that it is almost suitable as an approximation curve for measurement data. Therefore, it was suggested that the increase in the total cell number of the intestinal epithelium is an exponential growth of mouse embryos from E11.5 to E15.5. **The biological importance of the exponential growth of the intestinal epithelium from E11.5 to E15.5.** The current finding that cells grow exponentially in *in vivo* tissues, as in the log phase *in vitro* cultures was unexpected for the authors, because there are many complex conditions / interactions *in vivo*, such as those from the mesenchyme and muscle layers that limit the surrounding space. Current findings do not support the exponential growth of cells in the intestine at other developmental dates or stages, or in other organs. What is the biological importance of the exponential cell growth in the intestine during this period? As observed in this study, villus formation occurs during this and the next developmental stage, in addition to intestinal elongation, and there is an urgent need for a very rapid increase in the surface area surrounding the newly formed villi. At least one of the significances to increase the epithelial cell numbers is to cover the rapidly increasing surface area. **The mechanism that enables exponential growth of the intestinal epithelium from E11.5 to E15.5.** We previously reported the existence of the interkinetic nuclear migration (INM) in the intestinal epithelium throughout the entire length of the intestine during this period. INM is a regulatory mechanism of the epithelial cell proliferation / differentiation of the epithelial tubular tissues and organs. In INM, the epithelial cell nuclei show oscillatory movement in synchrony with the progression of the cell cycle, generating the pseudostratified structure of the epithelium. INM has been suggested to maximize the numbers of mitosis at the limited apical surface by piling up the cell nuclei toward the basal side while maintaining a mono-layer structure (pseudostratified structure) and thus allowing the efficient stem cell expansion. In the present study, at E11.5, E13.5, E15.5, the epithelium was pseudostratified in different modes depending on the different dates and different parts of the intestine. These findings suggest that INM play an important role to enable the exponential growth of the epithelial cells in the intestine during this period.

CONCLUSION

The number of epithelial cell layers in the intestinal tract of mouse embryos from E11.5 to E15.5 was measured by stereology. Based on the data, the intestinal epithelial cell proliferation during this period can be expressed as an exponential growth model.