学 位 論 文 の 要 旨

氏名　新田　哲哉

学　位　論　文　名　Spatiotemporal Difference in the Mode of Interkinetic Nuclear Migration in the Mouse Embryonic Intestinal Epithelium

発　表　雑　誌　名　Shimane Journal of Medical Science
（巻，初頁～終頁，年）　(in press)

著　　者　　名　Tetsuya Nitta, Noriko Ogawa, Dereje Getachew, Akihiro Matsumoto, Jun Udagawa, Hiroki Otani

論 文 内 容 の 要 旨

INTRODUCTION

Interkinetic nuclear migration (INM) is a phenomenon in which stem cell nuclei move along the apical-basal (AB) axis of the pseudostratified epithelium in synchrony with the cell cycle. INM has been extensively analyzed in the neural epithelia and suggested to play important roles in regulation of stem/progenitor cell proliferation/differentiation. While the panel of INM distribution are extending, variation in the modes of INM has been noticed among the different organs and tissues as well as between the different dates of gestation in the same epithelium. Since INM has been implicated in the regulation of stem cell proliferation and differentiation in the epithelium, it is speculated that the INM mode is differentially regulated corresponding to the different needs in the different tissues and organs. We previously reported the difference in the INM mode in the mouse embryonic midgut between embryonic day (E) 11.5 and E12.5 using immunohistological observation of distribution change of nuclei marked with 5-bromo-2’-deoxyuridine (BrdU) along the AB axis and the statistical analysis of the distribution patterns by multidimensional scaling (MDS). However, there is no report whether INM exists in the epithelia throughout the intestine including the hindgut and whether there are spatiotemporal differences in the INM modes in the developing intestinal epithelia. In the preset study, we first proved the existence of INM using 5-ethynyl-2'-deoxyuridine (EdU) for marking the nuclei and MDS analysis in the entire length of the intestinal epithelium, and further examined spatiotemporal differences in the INM modes.

MATERIALS AND METHODS

C57BL/6J mice (CLEA Japan, Tokyo, Japan) between 8 and 20 weeks of age were used. Single male and female mice were mated overnight in the same cage. Noon of the day when a vaginal plug was observed was defined as E0.5.

We observed the structure of intestinal epithelium histologically by hematoxylin and eosin (HE) staining as well as by γ-tubulin immunostaining, and by using scanning electron microscopy (SEM). At E11.5 and E13.5, mouse dams were injected with 5-ethynyl-2'-deoxyuridine (EdU) and embryos were obtained 1, 2, 4, 6, 8, 10 and 12 hours later. EdU was injected to label cells in S phase, and the distribution of EdU (+) nuclei in the epithelium was examined at these time points after injections. These embryos were processed into paraffin blocks, and serially cross-sectioned at 5 μm. Prepared sections were used both for histological analysis and for EdU assay using a Click iT EdU Alexa Fluor 488 Kit. The position of EdU (+) nuclei in the epithelia along the AB axis was measured at each time point. The numbers of EdU (+) nuclei counted were 98-336 for E11.5, and 80-1276 for E13.5 per each time point. EdU (+) nuclei were represented using a nuclear population histogram (%). We then analyzed the distribution patterns of EdU (+) nuclei in histograms using the multidimensional scaling (MDS). This makes it possible to detect differences in the INM mode genuine *in vivo* condition between the parts of organs or developmental stages. The analyses were performed on the intestine parts, 1) proximal: from the end of stomach to the cecum, and 2) distal: from the cecum to the anus, separately, to analyze the regional difference.

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

SEM images of cross-sectioned surfaces of the intestine showed single columnar morphology of the epithelial cells, whereas light microscopic observation showed that the nuclei were positioned at different levels along the AB axis of the epithelium, thus proving the pseudostratified structure of the intestinal epithelium. Most of the mitotic figures were observed at the apical surface, however, some were located at the intermediate position in the epithelium at E13.5. Immunostaining for γ-tubulin is localized at the apical surface and supports the single-layered structure.

INM has been extensively studied in the neural epithelium and other epithelia of ectoderm origin using various methods. On the other hand, INM-like nuclear localization shift as well as

apical-limited localization of mitotic figures have long been observed in many other epithelia, not only of endoderm and mesoderm origin in vertebrates, but also in epithelia of invertebrates.

Histograms of EdU (+) nuclei population (%) along the AB axis showed a tendency that EdU (+) nuclei are relatively more basally distributed at first and last of the observed period, whereas they are distributed more apically at 4, 6, and 8 h points in the E11.5 series, and 4 and 6 h points in the E13.5 series.

By MDS, EdU (+) nuclei distribution data at seven time points were visualized by plotting them in two dimensions based on the similarities between the histogram patterns. The present MDS analysis of the histograms showed the general finding that both in E11.5 and E13.5, as well as the intestine parts both proximal and distal to the cecum showed a cyclic change in the EdU (+) nuclei distribution pattern, indicating the existence of INM. On the other hand, there appeared regional and chronological differences in the INM modes of the intestinal epithelium. From the MDS analysis, the basal-to-apical nuclear movement that corresponds to the G2 phase of the cell cycle is for 4 h and 6 h in the proximal and distal parts, respectively. The apical –to-basal shift that corresponds to the M/G1 phase occurs for 8 h and 2 h in the proximal and distal parts, respectively.

The observed cyclic change in the nuclear localization in synchrony with the cell cycle strongly suggests the existence of INM in the entire length of the developing mouse intestinal epithelium. On the other hand, the present study suggests regional and chronological differences in the mode of INM. The present study revealed that G2 phase shift are within the reported time course between the proximal and distal parts of the intestine, however, the M/G1 phase shift apparently differs between the two parts. The previous and present findings strongly suggest that the molecular mechanism differs between the basal-to-apical and apical-to-basal movement of the nuclei in INM, and that the latter may work more actively in the differential manner correspondingly to the developmental needs specific to the organ, region, and developmental phase.

CONCLUSION

The present study elucidated the existence of INM through the entire intestine, and that there is spatiotemporal difference in the mode of INM between the proximal and distal parts of the intestine as well as between E11.5 and E13.5.