学 位 論 文 の 要 旨

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

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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.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

Interkinetic nuclear migration (INM) は、単層円柱上皮幹細胞の核が細胞周期と同期して頂底軸に沿って上下に移動することにより、偽重層上皮の形態をとり分裂像が頂表面に限局する現象である。INMは、神経上皮など外胚葉由来上皮の発生過程中に幹細胞の増殖分化の調節機能をもつ。申請者の研究室において、これまでにINMの存在を、中腸前半を含む内・中胚葉由来上皮組織に示してきたが、腸管全長においては不明であった。

本研究では、腸管全長におけるINMの存在と部位・時期による違いを検討した。Jcl:C57BL/6J成熟雌雄マウスを交配させ、膣栓確認日の正午をembryonic day 0.5 (E0.5) とした。E11.5、E13.5に5-ethynyl-2'-deoxyuridine (EdU) を母獣に腹腔内投与し、1、2、4、6、8、10、12 時間後に母獣 1 匹から各胎仔 1 匹を得て、腸管全長の5 μm 連続切片を作成した。盲腸を境として近位、遠位の2部に分けて、EdU染色した核の頂底軸に沿って6等分した層における分布を計測し、その割合をヒストグラムで表して各時点のヒストグラム間の類似性を多次元尺度構成法 (multidimensional scaling: MDS) にて解析した。HE染色、走査電顕にて上皮形態を、γ-tubulin 免疫染色にて一次線毛の局在を観察した。その結果、E11.5、E13.5、近・遠位部とも腸管上皮は偽重層の形態で分裂像はほとんど頂表面に限局していた。MDSにより、EdU陽性細胞核の分布は、E11.5、E13.5、近・遠位部とも、基底側から頂表面、さらに基底側へと周期的に変化し、腸管全体におけるINMの存在が示された。一方、周期はE11.5、E13.5とも近位部で12時間、遠位部で8時間と異なり、またE13.5では一部で周期的な分布変化に逆行するパターンが観察され、細胞分化などINM以外の現象が重複する可能性が示唆された。

本研究は、幹細胞の増殖調節機構と深く関係するINMが、発生過程において、時期によっては例外的なパターンも示すものの腸管全体に渡り上皮組織に存在することを初めて明らかにした重要な研究であり学位授与に値すると判断した。

最終試験又は学力の確認の結果の要旨

申請者は、幹細胞の増殖分化調節機構に関与するINMが腸管全体に渡り存在し、時期や部位により細胞周期や分布パターンに相違があることを初めて明らかにした。学位授与に値する充分な研究能力と関連知識を有していると判断した。（主査　松本健一）

申請者は組織学的手法と多変量解析を用い、胎性期のマウス中腸における腸管上皮細胞の偽重層上皮の形成が、細胞分裂およびその細胞周期と連動することにより形態形成に寄与することを示した。学術的に興味深く、学位授与に値する研究であると判断できる。（副査　松崎有未）

申請者は、細胞分裂を効率的に行うために起こると考えられるINMが、発生期マウスの全腸管の上皮細胞に認められることを示した。組織像を数理モデル解析し、近位と遠位の腸管では分裂速度や細胞停留等に差があることを示した。質疑を行い学位授与に値すると考えた。（副査　吉山裕規）