学 位 論 文 の 要 旨

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学　位　論　文　名　Interkinetic Nuclear Migration in the Mouse Embryonic Ureteric Epithelium: Possible Implication for Congenital Anomalies of the Kidney and Urinary Tract

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論 文 内 容 の 要 旨

INTRODUCTION

Interkinetic nuclear migration (INM) is a phenomenon in which progenitor cell nuclei migrate along the apico-basal axis (AB axis) of the pseudostratified epithelium, which is characterized by the presence of apical primary cilia, in synchrony with the cell cycle in a manner of apical mitosis. INM is suggested to regulate not only stem/progenitor cell proliferation/differentiation but also organ size and shape. INM has been reported in epithelia of both ectoderm and endoderm origin. We previously reported INM in the mouse embryonic endoderm-derived midgut epithelium, and described precise cyclic changes in the nuclear distribution based on the mathematical analysis of 5-bromo-2'-deoxyuridine (BrdU) immunostaining data. Although we previously observed in the ureter a change in epithelial nuclear distribution suggestive of INM, we could not confirm the existence of INM in the ureter. In this study, we examined the developmental process of the mesoderm-derived ureteric epithelium, and observed changes in the nuclear distribution consistent with INM as well as those in the mode of INM, i.e. the distribution/localization, speed and direction of nuclear kinetics, during the ureter development. We also discussed the significance of INM in the ureteric epithelium in comparison with other epithelia as well as its possible relation with CAKUT via primary cilia.

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 embryonic day (E) 0.5.

We observed the structure of ureter using histological analysis and scanning electron microscopy (SEM), and nucleus movement in the ureteric epithelium using BrdU-immunostaining and the multidimensional scaling (MDS) analysis (BrdU/MDS method). For SEM, pregnant dams were sacrificed at E11.5 and embryos were collected. Embryos were fixed paraformaldehyde and glutaraldehyde/phosphate buffer. Post-fixed in osmium tetroxide followed by tannic acid immersion and they were again treated in osmium tetroxide. After being washed with buffer and dehydration in a graded series of alcohol, the samples were placed into *t*-butyl alcohol and dried with a freeze-drying device. They were then coated with gold by an ion sputter coater and observed under a SEM. For histology and BrdU/MDS method, at E11.5, E12.5 and E13.5, mouse dams were injected with BrdU and embryos were sacrificed 1, 2, 4, 6, 8, 10 and 12 hours (h) later. BrdU was injected to label cells in S phase. These embryos were processed into paraffin blocks, and serially cross-sectioned at 5 μm. Prepared sections were used both for immunohistochemistry and histological analysis by hematoxylin-eosin staining. Transverse sections were stained with an anti-BrdU monoclonal antibody and 3,3'-diaminobenzidine (DAB) chromogen, and measured the position of BrdU (+) nuclei in the ureteric epithelia along AB axis at each time point. BrdU (+) nuclei were represented using a nuclear population histogram (%). MDS is a method for the statistical analysis of multi-dimensional information; it mathematically explores similarities or dissimilarities among data sets in a large matrix, and represents them on a 2-dimensional graph. MDS can thus visualize more easily similarities or dissimilarities among any phenomena. In this study, we interpreted changes in the distribution of BrdU (+) nuclei during the time course as reflecting the nuclear movement.

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 ureter showed columnar morphology of the epithelial cells. In the magnified views, primary cilia were observed on the apical surface of the cytoplasmic processes of epithelial cells. Immunohistochemistry for *γ*-tubulin confirmed the presence of apical primary cilia. Light microscopic observation of the cross sections of the ureteric epithelium revealed that the nuclei were positioned at different levels along the AB axis

of the epithelium, while all mitotic figures were observed at the apical surface. By BrdU-immunostaining, at E11.5, E12.5 and E13.5, at 1 h after injection, the labeled nuclei were located more prominently in the basal side of the epithelium. At the 4 h to 6 h time points, the percentages of labeled nuclei appeared to shift toward the more apical side. By 10 h to 12 h, the percentages of labeled nuclei in the apical-side layers decreased and those in the basal side again reached the initial levels. These findings supported the notion that the ureteric epithelium is a pseudostratified epithelium, and suggested that INM exists in the ureteric epithelium. The histogram patterns and MDS data revealed circular changes in the BrdU (+) nuclei distribution in the ureteric epithelia that suggest nucleus movement characteristic of INM. MDS data further showed that one cycle of nuclear movement from basal to apical to basal spanned 10 h to 12 h from E11.5 to E12.0, 8 h to 12 h from E12.5 to E13.0, and 6 h to 10 h from E13.5 to E14.0, indicating that the nuclear movement cycle tends to shorten during development. MDS data revealed that a shift from basal-to-apical nucleus movement during the G2 phase remained 4 h in duration in all stages. On the other hand, whereas in the E11.5 and E12.5 cycles nuclei stayed at the apical-most position from 4 h to 6 h during the S phase, in the E13.5 cycle the nuclei moved in the apical to basal direction from 4 h to 6 h in the G1 phase. These findings suggest both a similarity in the S/G2/M phase length and a dissimilarity in the G1 phase, suggesting a corresponding similarity and dissimilarity in the molecular mechanisms involved.

Primary cilia functions include spindle orientation, cytokinesis, cell proliferation, cell cycle progression, and checkpoint control, which are directly or indirectly related with INM and the dysfunction may contribute to the pathogenesis of ciliopathy. In recent years, loss/dysfunction of primary cilia has been linked to a number of diseases, the so-called nonmotile ciliopathy, including many CAKUT syndromes. In these syndromes, multiple epithelial tubular organs are affected, such as the kidney, ureter, liver, pancreas, and brain. Although precise mechanisms by which these multiple epithelial tubular tissues are affected remain to be clarified, the pathogenesis of at least some of the ciliopathies in CAKUT may be linked with INM observed in the present study via apical primary cilia and cytoskeleton-related regulatory mechanisms of the cell cycle and proliferation/differentiation.

CONCLUSION

Primary cilia were observed on the apical surface of the cytoplasmic processes of the ureteric epithelium of mesoderm origin. The circular change in the BrdU (+) nuclei distribution was observed in synchrony with the cell cycle, which is consistent with the existence of INM. We suggest that some of the ciliopathies in CAKUT may be linked with INM observed in the present study via apical primary cilia.