

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 (CAKUT)

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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 μ m. 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.

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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<p>学位論文名</p>	<p>Interkinetic Nuclear Migration in the Mouse Embryonic Ureteric Epithelium: Possible Implication for Congenital Anomalies of the Kidney and Urinary Tract</p>	
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<p>論文審査の結果の要旨</p>		
<p>外胚葉由来上皮の発生過程において、幹細胞の核が細胞周期と同期し頂底軸に沿って移動する現象は、interkinetic nuclear migration (INM)と呼ばれ幹細胞の重要な増殖・分化調節機構と考えられる。申請者の所属教室では、既に内胚葉由来の上皮管腔組織であるマウス胎仔の中腸上皮にINMの存在を確認し発生学的意義を報告している。本研究では、中胚葉由来の上皮管腔組織である尿管の発生過程におけるINMの存在と意義を証明し、先天性腎尿路奇形のうち一次線毛の機能障害により複数の上皮管腔臓器に異常を生じる病態とINMとの関連についても考察した。対象はC57BL/6Jマウスの母獣とし、embryonic day (E) 11.5では尿管上皮の形態と一次線毛の有無を走査型電子顕微鏡及およびγ-tubulin免疫染色により評価した。また、E11.5, E12.5, E13.5でbromodeoxyuridine (BrdU) を腹腔内投与し、頂底軸に沿った胎仔尿管上皮細胞の核移動をBrdU免疫染色により評価し、その局在パターンの変化を多次元尺度構成法により解析した。形態学的特徴として、走査型電子顕微鏡による観察では尿管上皮が偽重層であること、またγ-tubulin免疫染色では頂表面に一致した一次線毛の存在が確認された。加えて、いずれの日齢でも頂底軸に平行なBrdU陽性細胞核の分布に周期性がありINMが存在すること、さらに多次元尺度構成法によるパターン分析から各日齢に特異的な“INMに特徴的な核移動パターン”と細胞周期との間に関連性が認められた。上記より、外胚葉あるいは内胚葉由来の上皮発生過程以外にも、すなわち中胚葉由来組織である尿管上皮においてもINM特有の核移動と一次線毛が存在することが示された。この成果は一次線毛を介したINMの異常が複数の上皮管腔の形成異常を伴う先天性腎尿路奇形を呈する症候群の病態解明の一助となる可能性がある。</p>		
<p>最終試験又は学力の確認の結果の要旨</p>		
<p>申請者は中胚葉由来の尿管上皮の特徴を形態学的に評価すると同時に、発生学的観点から核移動のパターン分析を数理統計解析で評価した。一次線毛を介したINMの異常が線毛病という新しい概念を裏付ける極めて興味深い知見であり多くの発展性が期待される成果で学位に値するものと判断した。</p>		
<p style="text-align: right;">(主査 椎名浩昭)</p>		
<p>申請者は幹細胞の核が細胞周期と同期して頂底軸に沿って移動するという現象が中胚葉由来上皮管腔組織である尿管にも認められることを明らかにし、さらに、尿管上皮が偽重層であることおよび頂表面に一次線毛が確認されることも見出した。公開審査での質疑応答も的確であり、学位授与に値する高い学識と研究能力を示した。</p>		
<p style="text-align: right;">(副査 竹下治男)</p>		
<p>申請者は増殖細胞核染色法と多次元尺度構成法を用いて中胚葉上皮である胎児期尿管上皮にINMがみられることを明らかとした。関連領域の知識も豊富であり博士の学位に値すると判定した。</p>		
<p style="text-align: right;">(副査 木下芳一)</p>		

(備考) 要旨は、それぞれ400字程度とする。