学 位 論 文 の 要 旨

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学 位 論 文 名　Global Pattern of Interkinetic Nuclear Migration in Tracheoesophageal Epithelia of the Mouse Embryo: Inter-Organ and Intra-Organ Regional Differences.

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論 文 内 容 の 要 旨

INTRODUCTION

The trachea and esophagus start to develop from the common anterior foregut at embryonic day (E) 9.5 in mice, and the separation into the ventral trachea and dorsal esophagus is almost complete before E11.5. Epithelial expression of the transcription factors Nkx2.1 and Sox2 and the related signaling molecules has been shown to be essentially involved in the separation and further development of the trachea and esophagus. Experimental disruption of the expression of these transcription factors and the signaling pathways leads to the formation of esophageal atresia with/without fistula (EA/TEF). In spite of these findings on the final determination/perturbation of organ identities, the cellular events such as interkinetic nuclear migration (INM) in these normal and abnormal processes remain largely unknown. INM is an apicobasal (AB) polarity-based regulatory mechanism of proliferation/differentiation in epithelial stem/progenitor cells, i.e., the M-phase at the apical surface and S-phase at the basal side, and thus generates the pseudostratified structure of the epithelium. We previously documented INM in the endoderm-derived tracheal/esophageal epithelia at E11.5 and suggested that INM is involved in the development of both organs. However, detailed analysis of the INM mode on the inter-organ and intra-organ regional differences has not yet been done. The trachea has a clear structural difference between the ventral and dorsal sides, while in the esophagus there is no apparent structural difference between the ventral and dorsal sides. Thus, we hypothesized that the INM mode may be different between the two organs and between the ventral and dorsal sides in the trachea. Therefore, we here investigated inter-organ (trachea *vs.* esophagus) and

intra-organ regional (ventral *vs.* dorsal) differences in the INM mode in the tracheal and esophageal epithelia of the mouse embryo. We also analyzed convergent extension (CE), the epithelial tissue converges toward the central axis and extends along the perpendicular axis, and planar cell movement (PCM), i.e., cell movement along the long (L) axis.

MATERIALS AND METHODS

C57BL/6J mice (CLEA Japan, Tokyo) between 8 and 20 weeks of age were used. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University. The pregnant mice were intraperitoneally injected with 5-ethynyl-2'-deoxyuridine (EdU) at E11.5 and E12.5 and were sacrificed 1, 4, 6, 8 and 12 hr later to obtain the embryos. The distribution of labeled cell nuclei along the AB axis was chronologically analyzed in the total, ventral and dorsal sides of the epithelia. The percentage distribution of the nuclei population was represented by histogram and the chronological change was analyzed statistically using multi-dimensional scaling (MDS). The distance variation of each time point of the MDS results was further analyzed by using the MorphoJ software package. We ran a one-way ANOVA to perform a canonical variate analysis (CVA), which is used to maximize the variation among specified groups. The Procrustes distances among the groups were determined with the pairwise difference in means using permutation tests (10,000 rounds). A P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The trachea and esophagus sections were taken at equal distance in proximal-to-distal fashion, from the separation of the foregut tube to the bifurcation of the trachea into two main bronchi. To analyze intraorgan difference, we further divided the trachea and esophagus by using a ruler into the ventral and dorsal halves. We comparatively analyzed the INM mode to determine the inter-organ difference during E11.5–E12.0 and E12.5–E13.0, and then to determine the intra-organ regional difference in each trachea and esophagus. During E11.5–E12.0, the total trachea and total esophagus showed generally similar MDS patterns, with the 1 hr and 12 hr time points being located on the (+) side of the 1st dimension (basal side), while the 4 hr and 6 hr time points were on the (-) side of the 1st dimension (apical side) and the 8 hr time point was in the middle of the graph. The cell cycle was thus estimated to be 12 hr for both the total trachea and total esophagus. The basal-to-apical G2/M phase spanned 4 hr, and the return phase—i.e., the apical-to-basal G1 phase—took 8 hr for both organs. During E12.5-E13.0 the cell cycle was again estimated to be 12 hr for both the total trachea and total esophagus. The basal-to-apical G2/M phase spanned 4-6 hr, whereas the return apical-to-basal phase took 6 hr for both the trachea and esophagus. The inter-organ comparison of the INM mode during

E11.5–E12.0, but not E12.5–E13.0, showed a significant difference. During E11.5–E12.0 the trachea, but not the esophagus, showed a significant difference between ventral and dorsal sides in the INM mode. During E12.5–E13.0 neither organ showed regional differences.

Previous studies, including ours, revealed that the duration of the G1 phase nuclear shift varies significantly depending on the organs as well as on the developmental dates, whereas the G2/M phase shift is within a narrow time window and almost constant in each organ irrespective of dates. We also previously documented regionally different INM modes in the intestine. In the proximal intestine that later develops into the small intestine, the basal-to-apical and apical-to-basal movements of the nuclei take 4 hr and 8 hr, respectively, whereas in the distal intestine that corresponds to the future large intestine they require 6 hr and 2 hr, respectively. Thus, differential INM modes have been suggested to play an important role in the differential organogenesis and histogenesis of organs as well as in different regions of individual organs, and thereby in the differential structures and functions of organ regions. Further, the histograms of the EdU-positive nuclei distribution along the AB axis of the trachea and esophagus in the present study and those of the intestine and ureter in our previous studies all suggest that the nuclei are not all actively/continuously involved in the INM/cell cycle, but rather a significant part of the nuclei remain in the same position along the AB axis throughout the 12 hr interval. To elucidate the actual contribution of INM to the resultant total cell number in the organ/region, the ratios of the cells that are actively involved in INM must be elucidated. We examined the 12 hr interval difference in cell distribution along the L axis to investigate whether CE was involved in the elongation of the trachea and esophagus during E11.5 and E12.5. To analyze the PCM, we plotted the distribution of EdU-positive nuclei and EdU-negative nuclei on a graph and examined whether the ratio of the EdU-positive nuclei changes at the relative same position along the L axis after 12 hr. CE appeared to occur in both organs during E11.5–E12.0, while PCM was unclear in both organs.

CONCLUSION

The present findings support our hypothesis on the existence of different modes of INM between the two organs which derive from the common anterior foregut as well as between the dorsal and ventral sides of the trachea. We observed an inter-organ difference in the INM mode between the trachea and esophagus, and an intra-regional difference in the trachea, both during E11.5–E12.0. We further showed that CE occurs in both organs during E11.5–E12.0. Therefore, these cellular behavior differences in the INM mode, albeit during the limited time period immediately after the separation of the two organs, may be related with the differential normal and abnormal organogenesis and later histogenesis between the two organs as well as between the dorsal and ventral sides of the trachea.