別紙様式第４

学　位　論　文　要　旨

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[論文題名]

Formin homology 2 domain-containing 3 controls neural plate morphogenesis in mouse cranial neurulation by regulating multidirectional apical constriction

Formin homology 2 domain-containing 3 は多方向性の頂端収縮を調節することによってマウス頭部神経管形成における神経板の形態形成を制御する

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[要　旨]

Neurulation involves three-dimensional changes in the shape of the neural plate, where neuroepithelial cells tightly interconnected as a sheet undergo dynamic cell shape changes and reorganization of cell-cell junctions. These morphological processes of neuroepithelial cells are controlled spatially and temporally by rearrangement of the cytoskeleton, including the apical junctional complex lined with F-actin. Contraction of the F-actin network in the apical junctional complex causes apical constriction of individual neuroepithelial cells, leading to the localized bending and invagination of the neural plate.

Formins, another group of actin nucleators that promote the formation of linear unbranched F-actin, also seem to participate in junctional actin polymerization. in mammals, the roles of formins in epithelium have been investigated mainly using *in vitro* model systems but, the *in vivo* role of mammalian formins in epithelial morphogenesis during embryonic development remains unclear.

We have previously shown that Formin homology 2 domain-containing 3 (Fhod3), a formin family protein that is expressed abundantly in the heart and to a lesser extent in the brain and kidney, plays an essential role in cardiogenesis by organizing cardiac myofibrillogenesis. Fhod3-null embryos died around E11.5 due to defects in cardiac development but also showed defects in neural tube closure. The transgenic expression of Fhod3 in the heart sufficiently rescued the cardiac defects of Fhod3-null embryos but did not restore defects in neural tube closure, leading to exencephaly at the late embryonic stage. Fhod3 is therefore expected to play a crucial role also in neural tube closure, although the detailed mechanisms remain unclear.

To clarify the role of Fhod3 during neural development in mice, we examined the effect of Fhod3 deficiency on neurulation. In mice, neural tube closure is initiated at several different points. Around embryonic day (E) 8.0, the initial closure (closure I) starts at the hindbrain/spinal boundary and proceeds both rostrally towards the head and caudally towards the tail. Subsequently, closures II and III begin at the forebrain/midbrain boundary and the rostral end of the neural plate, respectively. By E9.5, the caudally-directed closure II/III meets the rostrally-directed closure I at the midbrain/hindbrain boundary region, thus completing cranial neural tube closure. In Fhod3-null embryos at E9.5, when cranial neural tube closure is normally completed, the caudally-directed closure II/III and the rostrally-directed closure I were aborted midway through.

We then examined the Fhod3 expression by *lacZ* staining of heterozygous *Fhod3*$^{+/-}$ and homozygous *Fhod3*$^{-/-}$ embryos. The Fhod3 expression in the neural tube was restricted to the hindbrain, specifically in rhombomeres 1 to 6 but not in the inter-rhombomere boundaries.

We next examined the Fhod3 expression in transverse sections of the hindbrain region and its relevance to the rostrally-directed closure I. Fhod3 expression was restricted to the lateral plate and completely absent from the floor and roof plates of the neural tube. In control embryos, the bilateral neural plates bent dorsomedially toward the midline; the ridges of the neural plates were then flipped and fused. In null embryos, the bilateral plates never bent toward the midline, leaving the dorsal ridges still separated from each other. The rostrally-directed closure occurred normally in rhombomeres 7–8 where *lacZ* staining is negative. The closure was delayed in rhombomeres 5–6 and never proceeded further.

To analyze morphological changes in the neural plates in Fhod3-null embryos, we examined the cellular organization of the neuroepithelium at the level of rhombomere 4 of E9.5 embryos. The neural plate of wild-type embryos formed a tightly packed pseudostratified columnar epithelium with a flat apical surface. In contrast, the neural plate of Fhod3-null embryos failed to form a columnar epithelium; the cells did not become compacted into a regular packed pattern.

To identify the effect of Fhod3 depletion on F-actin organization in neuroepithelial cells, we analyzed F-actin distribution in neuroepithelial cells at the level of rhombomere 4 of E9.5 embryos. In wild-type embryos, F-actin was accumulated at the apical surface of the lateral neural plates. By contrast, the apical accumulation of F-actin at the convex surface of bilateral neural plates of Fhod3-null embryos was significantly decreased. The tight junction protein ZO-1 at the convex surface was also decreased, although accumulation was detectable. On the other hand, the adherens junction protein cadherin

at the apical surface and lateral contacts was not substantially altered, suggesting that adherens junctions are better retained. We then estimated the force in cadherin-mediated junctions using the monoclonal antibody α18, which recognizes the force-induced active conformation of α-catenin, a linker molecule between cadherin and F-actin. In wild-type embryos, activated α-catenin was accumulated at the apical surface of the lateral plate, where myosin heavy chain (MHC) was also accumulated. In contrast, the accumulation of α18 signal at the apical surface was attenuated in null embryos. We further investigated the subcellular localization of Fhod3. The apical accumulation of endogenous Fhod3 was detected specifically in wild-type embryos.

To further examine the apical junctional complex, transmission electron microscopic analysis was performed. In Fhod3-null embryos, cell-cell contacts were sparsely distributed when compared to those in wild-type embryos, suggesting the loss of contraction of the apical plane. This loosely organized pattern is also observed in SEM images.

To clarify whether or not the loss of apical F-actin actually causes failure of apical constriction, we observed the apical surface of the lateral neural plate using sections tangential to the apical plane. The surface area of Fhod3-null embryos was more than twice as large as that of wild-type embryos, indicating reduced apical constriction.

We finally examined whether or not the apical constriction occurs not only towards the mediolateral axis but also the anteroposterior axis at the lateral plate of the mouse neural tube. To this end, we optically sectioned the apical surface of the lateral neural tube of the whole-mount embryos in the anteroposterior plane by confocal microscopy. Apical F-actin was accumulated in the center of rhombomeres. In contrast, F-actin was distributed evenly in Fhod3-null embryos, and no constriction pattern was obvious. Consistent with this idea, longitudinal sections of the neural tube in wild-type embryos showed uneven F-actin distribution along the anteroposterior axis; the intensity of apical F-actin in rhombomere regions was relatively high compared with that in boundary regions. Thus, contraction along the anteroposterior axis at the lateral plate, which is caused by the Fhod3-dependent accumulation of apical F-actin at the rhombomere centers, seems to be responsible for the morphological segmentation of rhombomeres.

We concluded that Fhod3 plays a crucial role in the morphological changes associated with neural tube closure at the hindbrain by mediating apical constriction not only in the mediolateral, but also in the anteroposterior direction, thereby contributing to tube closure and rhombomere segmentation, respectively.

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備考　論文要旨は、和文にあっては 2，000 字程度、英文にあっては 1，200 語程度