主論文の要旨

Radial glial cell-neuron interaction directs axon formation at the opposite side of the neuron from the contact site

（神経細胞は放射状グリア細胞と接触することで、
接触面と反対方向に軸索を形成する）

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[Background]

Neuronal polarization is integrated with neuronal migration through complex and distinct environment to ensure proper development of the laminar architecture of cortical regions of the mammalian brain and the following appropriate patterning of synaptic connectivity. During neurogenesis, newly born neocortical pyramidal neurons, mainly generated by asymmetric division of radial glia progenitors in the ventricular zone (VZ), exit the VZ and migrate through the subventricular zone (SVZ) and the lower intermediate zone (IZ) with multipolar morphology, dynamically extending and retracting multipolar neurites in random directions. Then they transit from multipolar shape to bipolar shape, with a thick leading process and a thin trailing process, and migrate along radial glial fiber to the developing cortical plate (CP). Leading process becomes dendrite and trailing process becomes axon, indicating that radial glia-guided migration and axon-dendrite polarity may share similar polarized signaling pathways. Therefore, MBT is a vital prerequisite for both radial glia-guided migration and axon-dendrite polarity, probably through establishing cell-polarity signaling cascades. It has been reported that the function of filamin A, Lis1 and DCX is required for the transition out of the multipolar stage. And mutations in the genes encoding these proteins in humans cause brain malformations accompanied by mental retardation and/or seizure, including periventricular nodular heterotopia, subcortical band heterotopia and lissencephaly. However, it is still unclear how MBT is spatiotemporally initiated and what is the underlying molecular and/or cellular mechanism orchestrating this process.

[Methods and results]

To evaluate whether interactions between radial glial cell and multipolar cells initiate axon-dendrite polarization, we performed in vitro neuron-radial glia interaction assay.

We found that the radial glial cell-cortical neuron interaction directs axon formation at the opposite side of the neuron from the contact site (Figure 1). Furthernore, we found that N-cadherin accumulated at the contact site between the Nestin-positive cell and stage 2 or stage 3 cortical neuron, and an N-cadherin-mediated Nestin-positive cell-cortical neuron interaction directs axon formation opposite of the contacting neurite. To extend these findings in vivo and to determine whether an N-cadherin-mediated RGC-pyramidal cell interaction is required for MBT, we first used immunohistochemistry to analyze the pattern of N-cadherin expression and its relationship to the localization of bipolar cells in the developing neocortex. We found that N-cadherin was prominently expressed in the MZ, the upper IZ and the luminal surface of the ventricle. MBT transition area localizes between the upper and the lower IZ, and MBT transition area coincides with the appearance of the predominant expression of N-cadherin in the upper IZ. Moreover, N-cadherin accumulated at the contact site between the leading-like process and the radial glial fiber, which is consistent with the notion that the N-cadherin-mediated RGC-pyramidal cell interaction may engage the MBT. To further

confirm that an N-cadherin-mediated RGC-pyramidal cell interaction is required for the MBT, we evaluated neuronal morphology following inhibition of N-cadherin in pyramidal cells using a dominant-negative approach. Three days after electroporation, most control neurons had transited from multipolar to bipolar morphology and migrated into the CP, whereas most N-cad-DN-expressing pyramidal cells failed to acquire bipolar morphology and enter the upper IZ. Moreover, in the IZ, the N-cad-DN-expressing cells exhibited an abnormal morphology with round soma and extensively elongated leading-like processes, most likely due to compromised N-cadherin function. To determine the mechanism by which the N-cadherin-mediated Nestin-positive cell-cortical neuron interaction directs the location of axon formation, we performed a screening assay for inhibitors that may interfere with signaling cascades involved in the regulation of neuronal polarity. We found that Rho-Rho-kinase signaling in cortical neurons is responsible for axon formation at the opposite side of the soma from the contacting neurite during the Nestin-positive cell-cortical neuron interaction in vitro. Furthermore, We found that N-cadherin-mediated Nestin-positive cell-cortical neuron interaction induces polarized distribution of activated RhoA and activated Rac1 in cortical neuron, thereby directing the location of axon formation to the side opposite of the contacting neurite. In vivo, we confirmed that Rho-Rho-kinase signaling in pyramidal cells is required for MBT and the following neuronal migration.

Collectively, these results suggest that 1) Radial glial cell-cortical neuron interaction directs axon formation at the opposite side of the neuron from the contacting site; 2) N-cadherin accumulates at the contact site between the radial glial cell and cortical neuron;

3) Inhibition of the N-cadherin-mediated adhesion decreases the oriented axon formation in vitro, and disrupts the axon-dendrite polarization in vivo; 4) Radial glial cell-cortical neuron interaction induces polarized distribution of active RhoA at the contact site and active Rac1 in the opposite neurite; 5) Inhibition of RhoA activity in neuron impairs the oriented axon formation in vitro, and prevents the axon-dendrite polarization in vivo.

[Discussion]

The establishment of axon-dendrite polarity is critical for neurons to integrate and transmit information in the nervous system. Here, we propose a novel model in which an N-cadherin-mediated RGC-pyramidal cell interaction directs the establishment of axon-dendrite polarity. In the IZ, multipolar cells interact with radial glial fibers through N-cadherin. This N-cadherin-mediated interaction subsequently induces a polarized distribution of active RhoA in the contacting neurite and active Rac1 on the opposite side of the cell and initiates the transition from multipolar to bipolar cell morphology with the axon forming at the opposite side (Figure 2). Our finding that N-cadherin-mediated RGC-neuron interaction directs axon formation from the opposite side of contacting neurite may provide a general mechanism for establishing axon-dendrite polarity in the developing nervous system.