外 国 語 要 約

学位論文題目　**“Role of vitronectin in mouse neurogenesis: division of neural stem cells, cell cycle exit, and neurite formation”**

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Vertebrates have a central nervous system that consists of a single tubular spinal cord and the brain. The central nervous system has a complicated structure in order to control behavioral and sensory functions, and its development begins during embryonic stage. In the neurogenesis of the early mammalian embryo, neural stem cells in the neuroepithelium replicate themselves by symmetric division and increase the cell number. After that, asymmetric division accompanied with self-reproduction generates neurons either directly or via neural progenitor cells that have neural differentiation potency. The neural progenitor cells exit the cell cycle after a certain number of divisions and become immature neurons. These neurons migrate to appropriate positions and grow into mature neurons that form neurites such as axons and dendrites. It has been reported that the extracellular matrix plays an important role in these processes of neurogenesis.

Vitronectin (Vtn), one of the extracellular matrix proteins, has been reported to be transiently expressed in the floor plate of neural tube and notochord during neurogenesis. Although the functions of Vtn in the process of neurogenesis remain unclear, it has recently been reported that Vtn promotes the differentiation of motor neurons in neural tube and is involved in the promotion of neurite outgrowth of retinal neuron. My laboratory reported that Vtn is involved in promoting the early differentiation stage and axonal determination in cerebellar granule cells via one of the receptors, αvβ5 integrin. However, the main role of Vtn in major events of neurogenesis has not been clarified. In addition, it is poorly understood which receptors bind to Vtn in order to regulate individual functions. This study focuses on symmetric / asymmetric division of neural stem cells, cell cycle exit and neurite formation of neural progenitor cells in order to clarify the role of Vtn and its receptors in each process of neurogenesis. The role of Vtn in the following two cases are examined: i) midbrain dopamine neuron development (*in vivo*), ii) retinoic acid (RA)-induced neuronal differentiation of mouse neuroblastoma cell line Neuro2a (*in vitro*).

In Chapter 1, the purpose of this study and recent findings on the relationship between neurogenesis and Vtn are summarized as an introduction.

In Chapter 2, the roles of Vtn in the symmetric / asymmetric division of neural stem cells in midbrain dopaminergic neurogenesis are examined using E12.5 mouse embryo. Neural stem cells are closely aligned in the ventricular zone of the ventral midbrain. While differentiation, the neural progenitor cells generated in this zone migrate to marginal layer along the processes of neural stem cells called radial glia. Immunofluorescent staining revealed that Vtn is expressed on radial glia. Next, comparative analysis

between Vtn knockout and wild type mice were performed. Vtn knockout increased the number of neural stem cells and neural progenitor cells immediately after division. However, no effect of Vtn knockout on the number of dopaminergic neuron and the efficiency of differentiation was observed. These results suggest that Vtn suppresses the asymmetric division of neural stem cells and promotes the symmetric division in the ventral region of midbrain.

In Chapter 3, the role of Vtn in the cell cycle exit and neurite morphology of neuronal progenitor cells were examined using RA-induced neuronal differentiation of Neuro2a cells as *in vitro* model. RA suppresses the cell proliferation and induces the cell cycle exit and morphological transition from multipolar to bipolar of Neuro2a cells. Since the expression levels of Vtn increased transiently after RA addition, the inhibition of Vtn was performed using Vtn antibody. As a result, Vtn inhibition significantly suppressed the efficiency of cell cycle exit and the morphological transition of neurites. In addition, immunofluorescent staining showed that the expression of Vtn was observed at the tips of neurites. Vtn was localized at both neurites in most cells displaying bipolar morphology, but in cells displaying multipolar morphology, not only Vtn-positive but also multiple Vtn-negative neurites were observed. Furthermore, Par6 mutant was transfected to Neuro2a cells in order to investigate the involvement of cell polarity regulator in morphological transition of neurites. Transfection of Par6 mutant caused disorder of morphological transition of neurites and disrupted the localization of Vtn at the tips of the neurites. Also, knockdown of the Vtn receptor candidates, β3 and β5 integrins, significantly reduced the efficiency of cell cycle exit. In contrast, knockdown of β5 integrin significantly suppressed the morphological transition, while knockdown of β3 integrin had no effect. These results indicate that Vtn promotes the cell cycle exit via αvβ3 and αvβ5 integrins, and that morphological transition from multipolar to bipolar requires the involvement of Vtn, αvβ5 integrin and Par6.

In Chapter 4, the roles of Vtn in neurite outgrowth were examined using Neuro2a cells. Vtn inhibition experiments in the same manner as in the previous chapter suppressed RA-induced neurite outgrowth in Neuro2a cells. In addition, knockdown of β3 integrin, but not β5 integrin, suppressed neurite outgrowth as well. These results suggest that Vtn promotes the neurite outgrowth via αvβ3 integrin.

In summary, this study revealed the following roles of Vtn: First, Vtn is involved in suppression of asymmetric division, promotion of symmetric division and self-reproduction of neural stem cells. Second, Vtn is also involved in cell cycle exit, morphological transition and neurite outgrowth in neural progenitor cells generated by asymmetric division. Finally, αvβ3 and αvβ5 integrins are involved in these roles, suggesting that Vtn can play multiple roles depending on these receptors.