学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on stiffness of culture substrate for astrocytic differentiation of mouse embryonic neural stem cells
（胎生期マウス神経幹細胞のアストロサイト分化に作用する培養基盤の硬さに関する研究）

Neurons and glia are differentiated from NSCs. Astrocyte, a type of glia is expected to be applicable in regenerative medicine for neurodegenerative diseases, such as spinal cord injury and amyotrophic lateral sclerosis. Astrocytes can be obtained from NSCs by passaging NSCs repeatedly or by adding serum and/or recombinant proteins in culture medium. On the other hand, these previously known methods are not ideal for regenerative medicine applications. For example, passaging NSCs or the repeated use of recombinant proteins is time-consuming and costly. Moreover, many undefined proteins, including unknown pathogens are contained in serum. Therefore, fast and low-cost methods for differentiating astrocytes from NSCs under pathogen-free conditions are in great demand.

We can obtain two types of NSCs from the mammalian brain. One is embryonic NSCs isolated from the ganglionic eminence of the embryonic brain, and the other is adult NSCs, isolated from the subventricular zone of the lateral ventricles and the subgranular layer of the hippocampus of the adult brain. Human NSCs are also existed in the brain. On the other hand, these human NSCs cannot be isolated from the brain by surgery for regenerative medicine. Alternatively, human NSCs can be obtained by differentiating human iPS cells into NSCs. However, NSCs induced from the human iPS cells have embryonic characteristics and astrocytic differentiation from human iPS cells is difficult. Over 10 passages/80 days of human NSCs are required to prepare for differentiation and 14 days of prepared NSCs to differentiate into human astrocytes in the serum contained culture medium. Therefore, faster and easier methods for differentiating embryonic and neurogenic NSCs into astrocytes are strongly required for regenerative medicine for neurodegenerative diseases, such as ALS and SCI.

Culture substrate stiffness influence differentiation of various types of cells strongly. It has been shown that the differentiation of adult NSCs is affected by stiffness of culture substrates. Adult NSCs tend to differentiate into astrocytes on stiff culture substrates, whereas they easily differentiate into neurons on soft culture substrates regardless of the presence or absence of serum. On stiff substrates, embryonic NSCs preferably differentiate into astrocytes in a culture medium containing serum, whereas in serum-free media, they easily differentiate to neuron. However, there are no reports that the differentiation of embryonic NSCs is regulated by soft substrate.

Actomyosin is a cytoskeletal complex that generates mechanical contractile force in cells. It has been reported that actomyosin is involved in the regulation of cell fate and differentiation. PP-MRLC upregulate actomyosin contractility and is increased in the cells on stiff substrates, and as a result, the cellular contractile force is enhanced. Meanwhile, MRLC dephosphorylation loosen cellular contractile force in cells on soft substrates. It is known that the activation of the RhoA signaling cascade enhance Di-phosphorylation of MRLC. ROCK is a key protein in the RhoA signaling cascade. It was shown that the ROCK inhibitor prevents the phosphorylation of MRLC in various cells and alters the morphology of the cells on stiff substrates. On the other hand, whether PP-MRLC in NSCs is regulated by substrate stiffness, and whether it directs the differentiation of NSCs is not well understood.

In this report, we examined whether astrocytic differentiation of embryonic NSCs was influenced by substrate stiffness in the absence of serum. The results revealed that astrocytic differentiation of embryonic NSCs was significantly increased on soft substrates compared with commonly used stiff plastic substrates. Moreover, the expression of PP-MRLC in embryonic NSCs cultured on soft substrates was lower than that in cells cultured on stiff substrates. Also, ROCK inhibitor reduced PP-MRLC in embryonic NSCs and promoted differentiation of embryonic NSCs into astrocyte on stiff surfaces. These results suggest that culturing embryonic NSCs on soft substrates or with ROCK inhibitor in serum-free conditions might be a suitable alternative to prepare astrocytes in regenerative medicine.