論　文　概　要 (Thesis Abstract)

○ 論 文 題 目
(Theme)

Effect of prolonged culture and hypoxic conditions
on in vitro neural differentiation potency
of embryonic stem cell-derived neural stem cells
（マウス胚性幹細胞由来神経幹細胞神経分化における低酸素および長期培養による影響の解析）

○ 指 導 教 員
(Supervisor)

人間総合科学研究科　　生命システム医学専攻　　大根田 修 教授
(Graduate School of Comprehensive Human Sciences
Doctoral Program in Biomedical Sciences
Professor Osamu Ohneda

（所　属）
(your position)

筑波大学大学院人間総合科学研究科　　生命システム医学専攻
(Graduate School of Comprehensive Human Sciences in University of Tsukuba
Doctoral Program in Biomedical Sciences)

（氏　名）
(your name)

LUKMANTO DONNY

Background and purposes [目的]

Pluripotent stem cells (PSC), such as: Embryonic stem (ES) cell, is expected to be source for neural cells through its neural differentiation capacity. In addition, ES cells have been utilized as an excellent model for the study of neural development. However, current method of neural induction does not generate neural cells exclusively. Moreover, the addition of developmental signals at specific time to mimic development led to complexity and higher cost of the protocols. Neural development is a dynamic process that regulated through time, as example: neural-glia switch was observed under the prolonged culture of brain slice-derived radial glia cells. On the other hand, microenvironment, such as low oxygen tension (hypoxia) is reported to enhance glial differentiation of neural stem cells. Despite these accumulating knowledges, the dynamic changes of ES cell-derived neural stem cells (ES-NSCs) under the effects of prolonged cell culture and hypoxic conditions are still obscured. Hence, to fulfil this gap and to ensure the characteristic of ES-derived neural stem cells, I aim for:

1. To simplify neural induction method of pluripotent stem cells,

2. to clarify the effects of prolonged culture and low oxygen tension on ES-derived neural stem cells.

Material and methods [対象と方法]

Sox1-GFP mouse ES cells were used as pluripotent stem cells in this study. Neural induction was performed with SFEB methods without addition of exogenous signals. ES-cells derived neural stem cells (ES-NSCs) is isolated through cell sorting by Sox-1 expression. Neural markers as analyzed with quantitative reverse transcription polymerase (qRT-PCR) and Immunostaining. In addition, the expression of Sox1 is measured with FACS. The expression of HIF-□□□were measured by immunostaining and western blot. Inhibition of HIF-1□ was performed by treatment with small interfering RNA target HIF-1□ (si-HIF-1□). Gene expression related to developmental signals was analyzed with qRT-PCR. Neural cells apoptosis was detected with TUNEL assay. Finally, statistical analyses were performed through unpaired Student's t-test or Turkey-Kramer test after a one-way analysis of variance. The software was used for the statistical analyses was GraphPad Prism 7.0 software program.

Results [結果]

In the present study, I effectively isolated the neuroectoderm cell population derived from ES cell aggregates by using a simple method combined of SFEB culture and cell-sorting with Sox1 as a marker. Next, I demonstrated that the isolated cells showed in vitro temporal neural specification which resulted in distinct cell fate after neural differentiation. In details, the early-passaged ES-NSCs gave rise to neurons, whereas late-passaged ES-NSCs gave rise to glial cells, similar to the in vivo dynamic changes during the neural development. Remarkably, hypoxic treatment induced the neural differentiation of ES-NSCs but did not affect the cell fate. Under hypoxic conditions, early-passaged ES-NSCs whether upregulation of neuronal markers, whereas late-passaged ES-NSCs showed the upregulation of a glial marker. In addition, the knockdown of the HIF-1α expression impaired the neuronal differentiation of early-passaged ES-NSCs under hypoxic conditions.

Discussion [考察]

My study demonstrated that cells sorting technology could be utilized to compensate the addition of exogenous signals during early neural induction. Of importance, I showed here the distinct effects of prolonged culture and hypoxic stimuli on the neural differentiation of ES-NSCs; prolonged culture was involved in the cell fate after neural differentiation, while hypoxic treatment efficiently promoted neural differentiation. In particular, my data suggested that loss of Sox1 expression might contribute to the loss of neurogenic capacity of neural stem cells due to prolonged culture whereas HIF-1 expression is responsible for upregulation of neural differentiation.

Conclusion [結論]

By the comparison of ESNSCs at different passaged numbers, which were equivalent to the in vivo developmental stage, we found that ES-NSCs recapitulate temporal neural development in which early stage ES-NSCs show characteristics of the neurogenic period, and late-stage ES-NSCs are predominantly gliogenic. Importantly, hypoxia treatment promotes neuronal differentiation in early passaged ES-NSCs, but then switches to promote glial differentiation in late-passaged ES-NSCs.Taken together, these results indicate that careful consideration about time and oxygen tension should be taken when developing neural cells from PSCs for clinical purposes.