Analysis of neuronal differentiation mechanism in iPS・EC cells

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(iPS･EC 細胞における神経細胞分化機構の解析)

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ABSTACT

Embryonic stem (ES) cells are undifferentiated cells derived from embryos. ES cells possess the ability to proliferate and self-renew indefinitely and are able to differentiate to cells of three germ layer. However, they are technically difficult to handle and are ethically controversial. Yamanaka and his group succeeded in reprogramming somatic cells into pluripotent state by forced expression of transcription factors establishing induced pluripotent stem (iPS) cells. iPS cells are similar to ES cells but are advantageous as they can be a source of patient/model specific cells. Embryonal carcinoma (EC) cells are also similar to ES cells but are malignant. Given their pluripotent nature, iPS and EC cells serve as valuable alternatives to ES cells in the study of pluripotency and differentiation mechanisms such as neurogenesis.

Neurogenesis is the process through which new nerve cells are generated from neural progenitor cells and has been reported to persist even in the adult nervous system. As is the case with other development processes, neurogenesis is a complex process intricately modulated by mechanisms yet to be fully understood. Alterations in neurogenesis interferes with brain development, function resulting to cognitive deficits and neurological conditions.

On the other hand, chromatin modulators such as histone deacetylase (Hdac) inhibitors have been used to improve reprogramming efficiency during iPS cell generation. Changes in histone acetylation status affect gene expression in turn controlling proliferation, differentiation and development. Hdacs consist of a number of isoforms that regulate cellular mechanisms differently.Hdac8 is an isoform expressed in the brain and in neuroblastoma and is associated with poor prognosis.

This research aimed to 1) generate rat iPS cells using a non-viral plasmid vector and establish a protocol to differentiate them to neural lineage and 2) investigate the role of selectively inhibiting Hdac8 on neurogenesis using retinoic acid treated P19 EC cells as a model.

We successfully generated rat iPS cells (riPSC) and applied a multi-step protocol to differentiate riPSC to a neuronal lineage comprising of glutamatergic and dopaminergic neurons. Glutamatergic neurons were responsive to agonist stimulation. We also found a glycophenotypic difference in expression of epitopes that bind R-10G antibody, which reacts with human ES/iPS cells but not EC cells. riPSC clone highly reactive to R-10G formed teratomas consisting of derivatives of all three germ cell layers. On the other hand, low reactive clones resulted in tumor masses made up of undifferentiated cells. Conventionally used tumor rejection antigen (TRA)-1-81 epitope expression was comparable.

In the second part of the study, we found HDAC8 inhibition suppressed proliferation, reducing size of P19 cell aggregates and 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) reducing activity without inducing cell death. Anti-proliferative effect was characterized by upregulation of the cell cycle inhibitor p21 and mRNA expression. HDAC8 inhibition also resulted in downregulation of Sox2 protein level as well as Musashi-1 and repressor type bHLH factor, Hes5, mRNA expressions.

The establishment of rat iPS cells and differentiation into neuronal lineage cells provides a model to help study neurogenesis processes as well as pharmacological and toxicological studies on neurons. Glygophenotypic difference with regard to R-10G found is potentially useful for rat iPS cell evaluation and to study the role of glycans in pluripotency and carcinogenesis in these cells. The findings of the second part of this thesis, uncover as well as suggest a role for HDAC8 inhibition in proliferation in retinoic acid induced P19 cells hence contributing to our understanding of epigenetic regulation by HDACs in neurogenesis and neuronal differentiation.

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学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

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2．論文提出者 (1) 所　　属　生命科学　　専攻

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3．審査結果の要旨（600～650 字）

本研究では、ウイルスベクターの代わりにプラスミドベクターを使用して作製した、ラット iPS 細胞の細胞特性について、in vitro 実験系および in vivo 実験系を用いて追究した。

6週令の雄性ラット腹部皮膚を牛胎児血清存在下に培養後、さらに継代培養した線維芽細胞に、c-Myc、Klf4、Oct3/4およびSox2の各発現プラスミドベクターを導入した。導入後の細胞は当該適切条件下に培養して、ラット由来iPS(riPS)細胞を作製した。同riPS細胞には、EC細胞マーカータンパク質、細胞表面タンパク質およびアルカリホスファターゼ活性とともに、各種多能性マーカー遺伝子群の発現が検出されたが、線維芽細胞には上記の各マーカー発現はいずれも観察されなかった。ケラタン硫酸抗原性の高いriPS細胞クローンを免疫不全マウスに皮下投与すると、種々組織で奇形腫発生が招来されたのに対して、同抗原性の低いクローン投与ではこのような強い奇形腫形成活性は確認されなかった。同抗原性の高いriPS細胞を神経細胞に分化誘導したところ、NMDA型受容体構成サブユニット遺伝子とタンパク質の発現だけでなく、アゴニスト刺激による細胞内Ca^{2+}濃度上昇がアンタゴニスト同時添加によって阻害されることが判明した。

以上の研究成績は、今後の iPS 研究分野において催奇性の低い遺伝子導入方法の開発への手掛かりを与えるとともに、同細胞の神経再生医療分野への応用の可能性を拡大することが期待される点で評価されるので、審査委員会は本論文が博士（薬学）に値すると判断した。

4．審査結果　(1) 判　　定（いずれかに○印）　(合　格)　・　不合格

(2) 授与学位　　博　士（　薬学　）