学位の種類	博士（医学）
報告番号	甲第１４８０号
学位記番号	第１０６６号
氏名	福田 誠
授与年月日	平成 27 年 3 月 25 日
学位論文の題名	Derivation of mesenchymal stromal cells from pluripotent stem cells through a neural crest lineage using small molecule compounds with defined media （化学合成培地を用いたヒト多能性幹細胞から神経堤細胞および間葉系間質細胞誘導法の確立） PLoS ONE 9(12): e112291. doi: 10.1371/journal.pone.0112291
論文審査担当者	主査： 澤本 和延 副査： 飛田 秀樹，大塚 隆信

Derivation of multipotent mesenchymal stromal cells from pluripotent stem cells through a neural crest lineage under chemically defined conditions

Makoto Fukuta[1,2,3†], Yoshinori Nakai[4†], Kosuke Kirino[5†], Masato Nakagawa[6], Yoshihisa Matsumoto[1,2,3], Katsutsugu Umeda[7], Toshio Heike[7], Naoki Okumura[8], Noriko Koizumi[8], Tatsutoshi Nakahata[5], Megumu Saito[5], Takanobu Otsuka[3], Shigeru Kinoshita[4], Morio Ueno[4*], Makoto Ikeya[2*], and Junya Toguchida[1,2,9*]

Background: Neural crest cells (NCCs) are an embryonic migratory cell population with the ability to differentiate into a wide variety of cell types that contribute to the craniofacial skeleton, cornea, peripheral nervous system, and skin pigmentation.
This ability suggests the promising role of NCCs as a source for cell-based therapy. Although several methods have been used to induce human NCCs (hNCCs) from human pluripotent stem cells (hPSCs), such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), further modifications are required to improve the robustness, efficacy, and simplicity of these methods, and also to use chemically defined culture conditions for clinical applications. **Methodology/Principal Findings**: Chemically defined medium (CDM) was used as the basal medium in the induction and maintenance steps. By optimizing the culture conditions, the combination of the GSK3β inhibitor and TGFβ inhibitor with a minimum growth factor (insulin) very efficiently induced hNCCs (70 - 80%) from hPSCs. The induced hNCCs expressed cranial NCC-related genes and stably proliferated in CDM supplemented with EGF and FGF2 up to at least 10 passages without changes being observed in the major gene expression profiles. Differentiation properties were

confirmed for peripheral neurons, glia, melanocytes, and corneal endothelial cells. In addition, cells with differentiation characteristics similar to multipotent mesenchymal stromal cells (MSCs) were induced from hNCCs using CDM specific for human MSCs.**Conclusions/Significance:** Our simple, robust, and chemically defined induction protocol enabled the generation of hNCCs as an intermediate material producing terminally differentiated cells for cell-based innovative medicine.

We believe that our current study will contribute significantly to this issue by following points.

1) Entire procedure can be done in chemically defined condition.
2) Requirement of growth factors is minimum.
3) The efficiency is high (70-80%)
4) Induced NCCs can be expanded with the original gene expression profile.
5) Induced NCCs can serve as a precursor for mesenchymal stromal cells, which also can be induced in a chemically defined condition.
6) Finally, using most up-dated materials, entire procedures from iPS cells to osteogenic cells can be performed in a xeno-free, chemically defined condition.