Original Article

Differentiation of Definitive Endoderm from Human Induced Pluripotent Stem Cells on hMSCs Feeder in a Defined Medium

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Abstract

Background: The Definitive Endoderm (DE) differentiation using the undefined media and non-human feeders can cause contaminations in the generated cells for therapeutic applications. Therefore, generating safer and more appropriate DE cells is needed. This study compared five different methods to establish an appropriate method for inducing an efficient DE differentiation from Human Induced Pluripotent Stem Cells (hiPSCs) on an appropriate feeder in a more defined medium.

Methods: Human Induced Pluripotent Stem Cells (hiPSCs) were cultured on inactivated feeders. Passaged hiPSCs, without feeder, were incubated for three days with Activin-A and different endodermal differentiation media including 1-FBS, 2-B27, 3-ITS and albumin fraction-V, 4-B27 and ITS and 5-like the third medium. The feeder cells in the first four methods were Mouse Embryonic Fibroblasts (MEFs) and in the fifth method were human adult bone marrow Mesenchymal Stem Cells (hMSCs). DE markers FOXA2, SOX17 and CXCR4 and also pluripotency marker OCT4 were evaluated using qRT-PCR, as well as FOXA2 by the immunocytochemistry.

Results: QRT-PCR analysis showed that after three days, the expression levels of DE and pluripotency markers in the differentiated hiPSCs among all five groups did not have any significant differences. Similarly, the immunocytochemistry analysis demonstrated that the differentiated hiPSCs expressed FOXA2, with no significant differences.

Conclusion: Despite this similarity in the results, the third differentiation medium has more defined and cost effective components. Furthermore, hMSC, a human feeder, is safer than MEF. Therefore, the fifth method is preferable among other DE differentiation methods and can serve as a fundamental method helping the development of regenerative medicine.

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Introduction

The application of cell culture-derived products is evolving in medical treatment [1]. If unlimited numbers of vital *in vitro* differentiated cells are obtained, the development of cell-based therapies and the study of the early stages of the drug discovery for the diseases will be improved [2]. The ability of hiPSCs for self-renewing and the potential to differentiate into three embryonic germ layers, *i.e.* ectoderm, mesoderm and endoderm, makes it possible to use them as an appropriate source of cells in regenerative medicine [3]. Unlike many other stem cells, Embryonic Stem Cells (ESCs) and iPSCs are able to unlimitedly proliferate without losing potency. The advantage of using iPSCs is the source of autologous cells they supply [4]. Thus, they are considered as an infinite source for the production of vital matured cells *in vitro* and might be used as a main component in the cell therapy [5].

Stem cells, such as iPSCs, are usually maintained on a layer of feeder cells. Feeder layers have many advantages for stem cells, such as maintaining their undifferentiated situation and pluripotency. hiPSCs are usually maintained on inactivated Mouse Embryonic Fibroblasts (MEFs) as a feeder, but applying non-human feeder cells can produce biological contaminants such