Stem Cells and Cloning: Advances and Applications

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LETTER

A Response to Article "Rho-Associated Protein Kinase Inhibitor and Hypoxia Synergistically Enhance the Self-Renewal, Survival Rate, and Proliferation of Human Stem Cells" [Letter]

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Dear editor

We read and review the article by Alsobaie et al[1] with great interest, which studied the synergistic effect of rho-associated protein kinase (ROCK) inhibitor Y-27632 and hypoxic condition to maintain propagation of human induced-pluripotent stem cells (IPSCs). We would like to give our insights particularly on the characterization method of IPSCs in this study.

First, we really appreciated the author's effort by using pluripotency markers as examined by flow cytometry such as Oct-4, SSEA-1, TRA-1-81 and so forth to get an overview of IPSC stemness in their cell culture. However, it is also vital to check whether their potential of trilineage differentiation (ectodermal, mesodermal and endodermal) is still retained or not. This can be done through teratoma formation by injecting IPSC into NOD/SCID mice although this approach may encounter issues as these animals are prone to developing thymus tumors besides ethical aspect of animal sacrifice itself.[2,3] Alternatively, one may carry out embryoid body (EB) formation, an aggregate of pluripotent stem cells with three germ layers. EB offers a sustainable approximation of trilineage development and serves as early prediction of their tendency to differentiate into one of three embryonic tissues.[4]

As shown in Supplementary Figure 3D, an IPSC colony appeared to undergo spontaneous differentiation. This suggested that the utilization of pluripotency markers measured via flow cytometry is not enough to assert that IPSC stemness is maintained throughout late passages and we should evaluate additional parameters. Gene expression related to three embryonic layers can be tested and compared both in control and test group. The researchers may pick one of those genes to be tested in PCR with each representation of three germ layers, that is, ectoderm (Nestin, PAX6); mesoderm (NCAM1, Brachyury) and endoderm (GATA4, FOXA2, CXCR4, SOX17).[5]

Second, as the authors referred to original paper by Siti-Ismail et al[6] with regard to immunohistochemistry method, we noticed that phosphate buffer saline (PBS) was seemingly used as primary diluent in alkaline phosphatase assay. The utilization of PBS may interfere with alkaline phosphatase staining as PBS provide phosphate ions as its substrate. Hence, other buffer such as Tris-HCl is recommended to get more optimized results.[7]

As the authors intend to conduct further in-depth studies on signaling pathways in hypoxia and their association with IPSC self-renewal, we truly hope that our inputs can be considered to improve validation of this research.

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All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

All authors have received no financial support and have no potential conflicts of interest in this communication.

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