BR OP 7

Excision Of Reprogramming Transgenes Improves The Differentiation Potential Of IPS Cells Into Hepatocyte-like Cells And Hepatic Organoid

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Background : Hepatocytes and hepatic organoids derived from human induced pluripotent stem cells (hiPSCs) have emerged as a very promising strategy for cell therapies in liver disease. The removal of reprogramming transgene affects the differentiation potential of hiPSCs at an early stage of three-germ layers, yet remains at hepatocytes and hepatic organoids as late-stage embryo development. So we Used an excisable polycistronic lentiviral system (cre-loxP system), generated both transgene-carrying iPSC and transgene-free iPSC from human fibroblasts, and demonstrated that excision of transgenes improves the differentiation potential of iPS cells into hepatocyte-like cells and generation of hepatic organoid exhibiting efficient hepatic differentiation.

Methods : we compared hepatic differentiation potential and characteristics of hepatic organoids from iPS cells following the removal of reprogramming transgenes using cre/loxP system.

Results : the pluripotent state in all hiPSCs was quite similar as shown by the expression of pluripotent markers, the embryonic body (EB) formation, and tri-lineage differentiation in vitro. However, after direct differentiation into hepatocytes, transgene-free hiPSCs were superior to those from transgene-residual hiPSCs. Interestingly, generation and hepatic differentiation of hepatic organoid (hHO) was significantly enhanced by the elimination of transgene in hiPSCs as observed by the upregulation of markers for fetal liver (CK19, SOX9, and ITGA6) and functional hepatocyte (Albumin, ASGR1, HNF4α, CYP1A2, CYP3A4, and AAT) under the culture of differentiation media, respectively.

Conclusions : the excision of reprogramming transgenes improves the differentiation potential of hiPS cells into hepatocyte-like cells and hepatic organoids with the property of liver progenitor cells. Thus, our findings provided important insights into the characteristics of hepatic organoids derived from iPSC.

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