

Significant induction of embryonic stem (ES) cells differentiating into functional hepatocytes

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ES cells are pluripotent stem cells that can become many differentiated cells both *in vivo* and *in vitro*. It has been indicated that mouse ES cells could differentiate into functional hepatocytes under several types of treatments in cultures and after transplantations. These previous publications encouraged us to use human ES cell to derive functional human hepatocytes for potential clinical therapy in future. For this long-term goal, it is necessary to improve the efficiency of hepatic differentiation from ES cells. Different from the natural process of differentiation for embryonic cells during embryogenesis, differentiation of ES cells *in vitro* is an artificial process, which provides us with the chance to derive hepatocytes efficiently by the optimal design of selecting medium contents and activating of signaling pathways. We have successfully divided the whole process of ES cells differentiating functional hepatocytes into multi-steps. Recently, our results indicated that ES cells cultured in monolayer could be directly induced to differentiate into definitive endodermal cells. The components required for maintaining the survival and differentiation of ES cells under monolayer culture have already been determined. Activation of Wnt signaling pathway is necessary for the differentiation of definitive endodermal cells from ES cells. Therefore, the agonist to activate Wnt signaling pathway was used to enhance the production of ES cell-derived definitive endodermal (ESDE) cells. We have found that a combined activation of both Wnt signaling pathway and Nodal signaling pathway resulted in a production of 80% to 90% ESDE cells in total ES cell-derived cells. For the next step, the self-renewal of ESDE cells maintaining in culture will be tested. ESDE cells are expected for further differentiation into hepatoblasts, which are bi-potential hepatic progenitor cells with expressions of both albumin and cytokeratin-19. The hepatoblasts could become functional hepatocytes after transplantation into liver of mouse with chronic liver injury. In conclusion, ES cells cultured in monolayer could be directly induced to differentiate into definitive endodermal cells. The activations of both Wnt pathway and Nodal pathway have been found to be better than single activation of Nodal pathway during the process of ES cells differentiating into ESDE cells. The newly established protocol in our study will significantly improve the efficiency of induced hepatic differentiation from ES cells.

Keywords: embryonic stem (ES) cells, hepatocytes, signaling pathway

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