Isolation and identification of hepatic progenitor cells from uninjured adult mouse liver

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Liver transplantation has become almost a routine procedure for the most severe cases of liver diseases, with one-year survival rates exceeding 80%. However, donor organ availability is severely limiting and the procedure is remaining risky. Hepatocyte transplantation has therapeutic potential for many liver disorders as one alternative to whole organ transplantation. Hepatic stem cells are an attractive potential source of hepatocytes for transplantation. For many reasons, the isolation of murine hepatic stem cells is comparatively difficult among all of the studied mammalian species. With the wealth of powerful genetics and extensive disease models, it is highly desirable to utilize adult mouse to study hepatic stem cells. This study is aimed to isolate and culture candidate cells of hepatic stem cells or progenitor cells from uninjured adult mouse liver, by investigating their ability to proliferate and potentials to differentiate *in vitro*. Hepatocytes were isolated by two-step collagenase perfusion and mechanical centrifugation, then cultured in modified DMEM and observed by phase contrast microscopy. It was found that a subpopulation of hepatocytes began to proliferate significantly and to form colonies on being activated after 2~3 days in culture. These proliferating cells are mainly mononucleate and their size is one-third to half that of mature hepatocytes. They showed definitely higher ability to proliferate and to expand under our experimental condition than reported hepatocytes and progenitor cells isolated from adult rat liver. The formed colonies could expand continually more than 2 months, with the occupied area of some reaching 0.64 mm² and cell dividing more than 10 rounds. Some larger cells, morphologically like mature hepatocytes, appeared at the edge of colony around day 30. At 24 h after attachment, all hepatocytes expressed Albumin, but were negative for AFP and CK19. After being activated, the proliferating cells expressed AFP and Albumin at day5, no CK19 expression. But at day55, the expanding colonies began to express CK19, and some cells within colonies manifested Albumin negative. Therefore, these cells are indicated as hepatic progenitor cells. In conclusion, these mouse-derived adult hepatic progenitor cells (AHPCs), are capable of proliferation, and are bipotential to differentiate into hepatocytes and duct cells. AHPCs may be useful tools as new *in vitro* cell models to investigate liver development and regeneration, and can facilitate the study of hepatic stem cells and progenitor cells that can be used in cell transplantation.

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