In vitro tissue expansion perspectives

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Besides orthotopic liver transplantation, therapies currently developed to treat liver diseases include transplantation of isolated mature adult hepatocytes by their infusion into blood stream, implantation of liver tissue substitutes and perfusion of blood through an extracorporeal bioartificial device containing liver cells.

With respect to liver cell transplantation (LCT), the success of the procedure closely depends on the viability and the stability of liver-specific functions of the infused liver cells. The number of needed hepatocytes is also another limiting factor because of donor organ shortage.

Hence, several attempts are investigating the optimal appropriate conditions for the improvement of both quantity and quality of the cells dedicated to LCT.

Primary hepatocytes represent the most common cell type in current liver-directed cell therapies. However, these cells showed a limited proliferation potential in vitro, even under the most optimal culture conditions, and a loss of differentiated functions over a period of days. The analysis of cell fractions obtained after liver isolation demonstrates that diploid hepatocytes are largely associated to the proliferation observed in primary cultures (1,2). Accordingly, the isolation and purification of this cell population may help to characterize their proliferation state in vitro whereas in vivo studies have to evaluate their engraftment potential. Stimulation of hepatocytes proliferation and differentiation can also occur if co-cultured with other cell types as for instance bone marrow stromal cells (3). These data obtained in adherent cell cultures need to be confirmed in cell suspension culture systems such as bioreactors. Indeed, these systems that allow stability of liver-specific functions thanks to a three-dimensional cultures will help to efficiently supply large amounts of hepatocytes.

At the molecular level, it has been clearly demonstrated that telomere length constitutes one of the parameters that regulate hepatocytes proliferation. Hence, several studies are currently focused on inhibiting its attrition and evaluating its effect on in vitro hepatocytes expansion. According to in vivo data showing that upon appropriate conditions, hepatocytes are able to proliferate with no alteration of differentiated functions, immortalization approaches have been developed. Oncogenic, viral, or chemical agents have been used for overcoming the growth limitations of primary hepatocytes. However, still additional data are mandatory especially regarding safeguards of their clinical use.

Mechanisms of aging have also been investigated in order to characterize the genes involved in conferring growth advantage and prolonging life span of hepatocytes in vitro.

Finally, isolation and characterization of liver stem and progenitor cells is another recent alternative thanks to the development of cell biology techniques. These self-renewing cell types of adult or embryonic origin may represent new therapeutic prospects if full characterization of the behaviour and the biology of these cells in vivo, has been performed.

In conclusion, advances and knowledge of hepatocytes and liver stem biology in vitro will be helpful to determine the optimal conditions for their in vitro expansion or even prolonged life span in order to counteract the limitation of cell supply and to improve the quality of cell suspension for LCT.

References

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