

Culture of omentum-induced regenerating liver yielded hepatocyte-committed stem cells

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Earlier we showed that when omentum, activated by inert particles, is allowed to fuse to a wedge cut in the liver, it induces stem cell proliferation in the liver resulting in massive liver regeneration. Here, we attempt to culture stem cells from the omentum-induced regenerating liver tissue. Cells from regenerating liver tissue were harvested and cultured. Cultured cells were characterized by immune staining, fluorescence activated cell sorting analysis, growth factor assay, *in vitro* differentiation, and their ability to engraft to injured sites *in vivo*. Culture yielded cells with a mesenchymal stem cell phenotype that could be maintained in culture indefinitely. These cells, called regenerating liver stem cells, expressed both adult and embryonic stem cell markers, secreted high levels of vascular endothelial growth factor, and expressed albumin. When grown on matrigel in the presence of hepatocyte growth factor, these cells differentiated into hepatocyte-like cells in culture, but they did not differentiate to adipogenic and osteogenic lineages when grown in specific differentiation medium. The differentiated cells expressed α -fetoprotein and secreted high levels of albumin and urea. After systemic injection, the undifferentiated cells engrafted only to the injured sites in the liver and not to the normal areas of the liver. In conclusion, omentum-induced regenerating liver yields hepatocyte-committed stem cells in culture. Such cells could prove to be useful in cell transplantation therapies. (Translational Research 2010; 156:358-368)

Abbreviations: AFP = α -fetoprotein; α -SMA = α -smooth muscle actin; BSA = bovine serum albumin; CD = cluster of differentiation; CXCR4 = chemokine receptor-4; DMEM = Dulbecco's modified Eagle medium; FACS = fluorescence-activated cell sorting; FBS = fetal bovine serum; FITC = fluorescein isothiocyanate; GEC = glomerular epithelial cells; HGF = hepatocyte growth factor; MSCGM = mesenchymal stem cell growth medium; PBS = phosphate buffered saline; PBS-T = PBS with 1% Tween-20; PH = partial hepatectomy; PMC = primary mesangial cells; RLSC = regenerating liver stem cells; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; VEGF = vascular endothelial growth factor; WT-1 = Wilm tumor suppressor gene

In a previous study, we showed that when inert foreign-body particles are placed in the abdominal cavity, the omentum mobilizes and expands to surround the particles. In this state of the omentum (called activated omentum), it becomes a reservoir of stem cells

and growth factors.^{1,2} After the activated omentum is allowed to fuse with an injured site in the liver, the liver grows and expands to 1.5 times the size of the original liver.³ We found that the growth of the liver depended not on the expansion of hepatocytes but on the

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