Human fetal liver cultures support multiple cell lineages that can engraft immunodeficient mice.

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**Public Summary:**
In this report, Fomin and colleagues have created cultures of human fetal liver cells supporting many of the different cell types found in the liver during prenatal development. These include fetal hepatoblasts, the precursors of hepatocytes and biliary cells, as well as specialized endothelial cells, known as liver sinusoidal endothelial cells, found only in the liver. The liver cultures also contained blood stem cells. Transplantation of the cultured liver cells into mice, modified to accept human cells, demonstrated engraftment of hepatocytes and endothelial cells in the liver and establishment of a human immune system. This work demonstrated for the first time a culture system that supports multiple cell lineages found in the developing liver. The work also showed that liver sinusoidal endothelial cells can be grown in culture and successfully transplanted. These endothelial cells are the main source of Factor VIII, the clotting factor that is missing or reduced in patients with hemophilia A. Transplantation of liver sinusoidal endothelial cells may offer a new method of treating patients with hemophilia A. The ability to grow these cells in culture is an important step in developing ex vivo methods of expanding these cells for transplantation. These endothelial cells survived and grew in cultures without the addition of any growth factors that support endothelial cell growth, indicating that the growth factors and cell signals required by these cells for survival were being provided by the other cells in the cultures. Thus, these cultures provide a means to further explore the complex interactions between liver sinusoidal endothelial cells and other liver cells. Robust engraftment of human liver sinusoidal endothelial cells was shown in two lines of immunodeficient mice, one with chronic liver damage and the other with acute liver damage, thereby demonstrating that engraftment and growth of liver sinusoidal endothelial cells does not require a chronic state of liver damage. This is an important finding with respect to envisioning a cell therapy for hemophilia A as such patients have otherwise normal liver function. The animal transplant studies suggest that transplanted liver sinusoidal endothelial cells may be able to expand in the liver of a transplant recipient. The newly described liver cultures offer evidence that liver sinusoidal endothelial cells can be expanded in culture and the transplants into humanized mice show that these cultured cells can still engraft and further grow in the liver. This work supports the idea that a cell therapy for hemophilia A based on transplantation of liver sinusoidal endothelial cells may be a future viable option for treating this bleeding disorder.

**Scientific Abstract:**
During prenatal development the liver is composed of multiple cell types with unique properties compared to their adult counterparts. We aimed to establish multilineage cultures of human fetal liver cells that could maintain stem cell and progenitor populations found in the developing liver. An aim of this study was to test if maturation of fetal hepatocytes in short-term cultures supported by epidermal growth factor and oncostatin M can improve their ability to engraft immunodeficient mice. Fetal liver cultures supported a mixture of albumin(+) cytokertin-19(+) hepatoblasts, hepatocytes, cholangiocytes, CD14(++)CD32(+) liver sinusoidal endothelial cells (LSECs) and CD34(+)CD133(+) haematopoietic stem cells. Transplantation of cultured cells into uPA-NOG or TK-NOG mice yielded long-term engraftment of hepatocytes, abundant LSEC engraftment and multilineage haematopoiesis. Haematopoietic engraftment included reconstitution of B-, T- and NK-lymphocytes. Colonies of polarized human hepatocytes were observed surrounded by human LSECs in contact with human CD45(+) blood cells in the liver sinusoids. Thus, fetal liver cultures support multiple cell lineages including LSECs and haematopoietic stem cells while also promoting the ability of fetal hepatocytes to engraft adult mouse livers. Fetal liver cultures and liver-humanized mice created from these cultures can provide useful model systems to study liver development, function and disease.