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Ultrapure cells for liver research

Worldwide, hepatic fibrosis, cirrhosis and hepatocellular carcinoma cause considerable human suffering and high economic burdens. Causally responsible for the accumulation of excess scar tissue within the diseased liver are hepatic stellate cells (HSCs) representing 5-8% of the total number of liver cells. In normal liver, these cells are quiescent and contain numerous fat vacuoles storing vitamin A. Upon hepatic injury, these cells lose their quiescent phenotype and transdifferentiate into highly proliferative myofibroblasts capable to synthesize and deposit large quantities of collagens. Due to their exceptional patho-physiological relevance, HSC biology has been the focus of many studies. First protocols for HSC isolation were already established in 1982. In respective protocols, the liver is first enzymatically digested by pronase and collagenase using a perfusion system, and HSCs are subsequently enriched by centrifugation of the crude liver cell suspension through a density gradient.

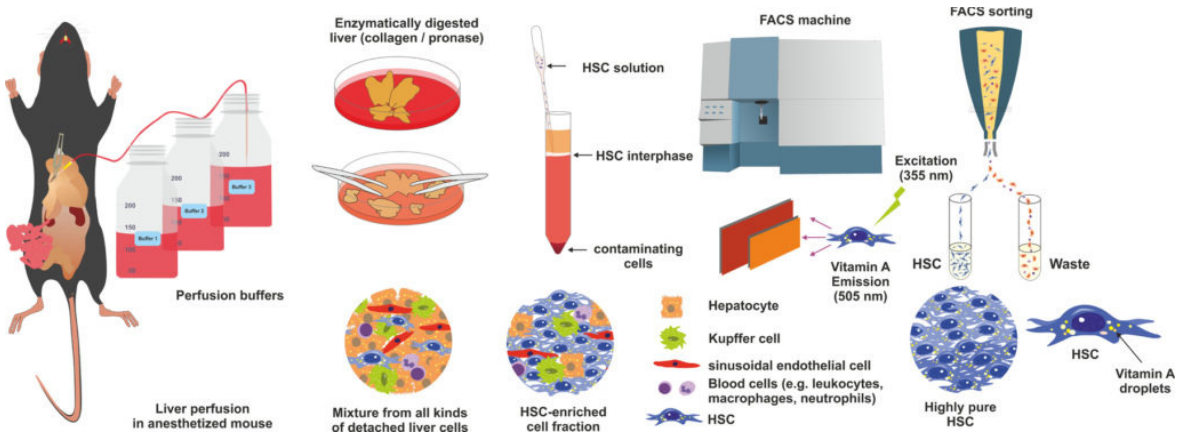

Fig. 1. Simplified scheme of the protocol used for isolation of murine HSC. In a first step, the liver of the anesthetized mouse is perfused with different enzymatic perfusion solutions. Subsequently, the liver is removed and the cells are dispersed in a sterile plastic culture dish. Thereafter, the cell solution is filtered and HSCs are enriched by density centrifugation. After centrifugation, HSCs are found in the white shining interlayer, while hepatocytes and other blood cells are predominately located at the bottom of the gradient. Final purification of HSCs is then performed in a FACS machine, in which they are identified by excitation with UV light from a 355 nm laser and measuring the emission at 505 nm resulting from the intracellular retinol (Vitamin A) content.

During centrifugation, HSCs separate from other cells (remaining hepatocytes, Kupffer cells, sinusoidal endothelial cells) because of their high lipid content and concomitant low density. At the end of centrifugation, the low-density fraction enriched in HSCs becomes visible as a thin white layer. Since sufficient cellular lipid droplets are an important precondition for successful HSC isolation, these protocols work best when relatively old mice are being used or when animals received diets enriched in vitamin A. Of course, these protocols had significantly contributed to the knowledge in HSC biology for many years. However, it is obvious that these conventional protocols, which are exclusively based on differences in cellular density, allow only the enrichment of cells (up to 50-70%) and are far beyond to yield virtually pure cell preparations. Therefore, considerable pitfalls may arise when analyzing these cell mixtures with the

infinitely large repertoire of novel, highly sensitive techniques nowadays available in molecular biology and biochemistry.

We have recently described an advanced method for high-purity isolation of murine HSCs (Fig. 1). Similarly to previous protocols, the liver is first enzymatically digested with pronase and collagenase to destroy parenchymal cells. Thereafter, HSCs are enriched by density centrifugation the crude cell mixture through a gradient made out of iohexol (a compound approved as a contrast agent). Thereafter, the HSC-enriched cell fraction is further purified by Fluorescence-activated cell sorting (FACS) using a FACS sorting instrument equipped with a UV laser suitable to detect HSC autofluorescence resulting from their intracellular vitamin A content. For HSC sorting, a gating strategy is used in which cell doublets are first discriminated, and single cells are then exposed to a UV laser and identified by their UV light excitation profile. Staining with sets of various antibodies directed against specific markers present exclusively on other contaminating cell populations, including leukocytes (CD45), neutrophils (Ly6G), and macrophages (CD11b and F4/80) can be further used to identify contaminating cells. In sum, this sorting strategy is highly suitable to identify and isolate HSCs at a very high purity and separate them reliably from all myeloid cells. Importantly, our protocol is also useful when different cell populations should be separated simultaneously from one liver, thus allowing a comprehensive comparison of different hepatic cell compartments from the same animal. Furthermore, the novel procedure, including FACS sorting is suitable for isolation of HSCs from injured and fibrotic livers, expanding the repertoire of experimental possibilities in investigating HSCs biology in diseased livers and murine models mimicking human liver disease. Although we must admit that the overall cell yield achieved by this method is much lower compared to conventional isolation procedures, the method is ideal for establishing pure HSC cultures required for analysis techniques aiming to uncover the transcriptomic or epigenomic landscape of HSCs without interfering factors.

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