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REVIEW

# Human hepatic stellate cell isolation and characterization

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**Abstract** The hepatic stellate cells (HSCs) localize at the space of Disse in the liver and have multiple functions. They are identified as the major contributor to hepatic fibrosis. Significant understanding of HSCs has been achieved using rodent models and isolated murine HSCs; as well as investigating human liver tissues and human HSCs. There is growing interest and need of translating rodent study findings to human HSCs and human liver diseases. However, species-related differences impose challenges on the translational research. In this review, we focus on the current information on human HSCs isolation methods, human HSCs markers, and established human HSC cell lines.

**Keywords** Human hepatic stellate cells · Human HSC isolation · Human HSC markers · Human hepatic stellate cell lines

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## Introduction

The hepatic stellate cells (also referred to as Ito cell, fat-storing cell, lipocyte, perisinusoidal cell, parasinusoidal cell) are one of the key nonparenchymal components in the sinusoid compartment with multiple functions in the liver. HSCs were first described and named "Sternzellen" in 1876 by Kupffer [1] using a gold-chloride impregnation technique. Later Toshio Ito [2] and Bronfenmajer [3] observed perisinusoidal cells containing lipid droplets in human livers. Wake [4] identified that "Sternzellen" were the same cells as the vitamin A-storing cells in the liver. In 1995, the international community of investigators recommended the nomenclature of hepatic stellate cell (HSC) [5]. The embryologic origin of HSCs remains unresolved. Based on expression markers, potential origins of HSCs include endoderm (cytokeratins) [6], or the septum transversum mesenchyme (Foxf1, vimentin) [7], or neural crest (GFAP, synaptophysin, N-CAM) [8] and P75 [9]). However, the neural crest origin has been challenged [10]. Recent studies utilizing cell-fate mapping in mice have suggested that HSCs may originate from septum transversum [11].

Under physiological conditions, HSCs reside in the space of Disse exhibiting a quiescent phenotype (qHSCs), and their main function is storing vitamin A in lipid droplets [12, 13]. In response to injury, qHSCs decrease vitamin A storage and peroxisome proliferator-activated receptor gamma (PPARϒ) expression, and activate into myofibroblasts (aHSCs), which are characterized by increased proliferation and high contractility with expression of pericellular matrix proteins (α-smooth muscle actin (α-SMA), vimentin), and secretion of abundant extracellular matrix proteins (fibronectin, collagen type I and III) [14–17]. HSCs release inflammatory, proliferative, and

fibrogenic cytokines such as IL-6, PDGF, and TGFβ, through direct contact with their neighboring cells [18]. It is reported that HSCs can also function as regulatory bystander and contribute to liver-induced tolerance [19]. HSCs also contribute to liver regeneration [20], and potentially mediate sinusoidal blood flow via contraction and regulate microvascular structure and function in liver [21].

The development of methodologies and techniques for isolating and culturing primary HSCs has provided a platform for great achievements in understanding this cell's unique and pleiotropic functions in liver pathophysiology. Knook et al., using density centrifugation and centrifugal elutriation, first established the isolation of HSCs from rat liver [22]. Subsequently, the first human HSC isolation and characterization was reported by Friedman from normal liver [23]. With the development of techniques for HSC isolation, cultivation and characterization, dramatic achievement has been made in exploring the physiological and pathological functions of HSCs. Mouse models have been a very valuable tool in characterizing cellular gene activation and protein-expression profiles as well as elucidating the signaling pathways involved. Especially, mouse models utilizing HSC specific markers has greatly advanced our understanding of the function of HSCs [24–27]. Meanwhile, significant advancement has been made in human liver pathology studies as well as in vitro studies using isolated primary human HSCs, for example, to study their reverting capacity and function in retinoid metabolism [28–33]. There is increasing interest in translating research from mouse models and mouse HSCs to human HSCs and human disease. In this review we will examine the current information on human HSCs, including the methodologies for HSC isolation, primary cultures of human HSCs, human liver tissues, as well as established human HSC cell lines.

## Human HSC isolation methods

An efficient method of HSC isolation and clear characterization of human HSCs is undoubtedly critical for a deep understanding of its role in human liver physiology and liver diseases. Two main methods for isolating HSCs from human liver have been described so far, one is to grow smooth muscle-like cells from liver tissue explants, and the other is using density gradient centrifugation similar to the isolation of HSCs in rodents [22, 34].

### Liver tissue explants

Culture and characterization of myofibroblasts grown from human liver explants of normal and fibrotic livers were reported about 40 years ago [35–37]. Tissue fragments were attached to plastic substratum. The outgrowth of cells with myofibroblast characteristics became detectable after 10–15 days, and the myofibroblastic cells were recovered and passaged by trypsinization after 3–4 weeks culturing from the explants of liver sections [38]. Generally, studies were carried out on homogeneous cultures of "smooth muscle cells" subcultured between three and ten passages, without noticeable phenotypic alterations and significant result variation from various passages [36, 38–41].

The HSCs/myofibroblasts grown from explants of human liver provide a useful research model to study human fibrogenesis. The cells are positive for desmin and smooth muscle α-actin, and demonstrate features typical of myofibroblasts, such as abundant rough endoplasmic reticulum (ER) and bundles of microfilaments under transmission electron microscopy. Typical lipid droplets were not detectable, since these cells were kept in culture long enough to be considered as fully 'activated' [38]. They express collagen types I, III, IV, and V; and also laminin B1 chain, fibronectin, matrix-metalloproteinase-2 (MMP-2) [37, 39], and fibroblast activation protein (FAP) [42].

Similar to the outgrowth from liver slice cultures, some studies have isolated human HSC-like myofibroblasts through culture purification from mixed crude liver cell populations, obtained from perfused normal human livers. Stellate-cell growth in mixed cultures revealed that more than 80% displayed desmin and α-SMA expression, and also express interstitial collagens type I and III. Using this cell culture system, researchers demonstrated that retinoic acid suppressed the response of myofibroblasts to PDGF, while this suppressive effect did not alter PDGFRα or β abundance or activation [43].

The limitations of this approach include the potential heterogeneity of the cells in culture. Under these culture conditions, two cell types, which resemble smooth muscle cells and vascular endothelium, grew from the liver tissue [37], while epithelial cells were no longer present in these subcultures [36, 37]. Moreover, using this culture method, early events of HSC activation cannot be traced and investigated, since it takes weeks for the cells to grow out of the liver tissue and onto plastic surface.

### Isolation of human HSCs using density gradient centrifugation

Friedman [23] first successfully isolated, cultured, and characterized human HSCs (lipocytes) from normal human livers. Isolation of human HSC (fat-storing cells) was also reported in other studies using density gradient centrifugation method [31, 44, 45]. In general, researchers isolated human HSCs from wedge sections of human liver

unsuitable for transplantation within 48 h. Sections of donor liver were isolated by catheter perfusion [23], or finely minced [44, 45], and digested using pronase and collagenase, followed by density gradient centrifugation using Larex (Stractan) or other gradient medium to remove other non-parenchymal cells. HSCs isolated with this method were reported to be highly viable and with purity of ~ 90% [23, 44]. In some studies, HSCs isolated from density gradient centrifugation were further enriched and purified by centrifugal elutriation [46].

The isolated hHSCs display vitamin A autofluorescence with numerous lipid droplets in the perinuclear zone [47]. Retinoid droplets were maintained for 9 days on uncoated plastic, with subsequent loss of vitamin A droplets and progression to a fibroblastic morphology with expression of matrix constituents including α-SMA on day 14 in culture on uncoated plastic. Conversely, cells cultured on basement membrane-like gel remained clustered and retained vitamin A fluorescence. Transmission EM demonstrated abundant retinoid droplets, prominent rough ER, and prominent microfilaments. Cytoskeletal and matrix protein expression investigation reported that desmin expression was negative in hHSCs within 2–3 day culture, and only seen in cells in longer-term culture (> 7 days) with polyclonal but not monoclonal primary antibody. Vimentin, collagens I, III, IV, FN, and laminin were identified [23, 47]. Researchers found that these hHSCs respond to vasoconstrictors such as thrombin, angiotensin-II, and endothelin-1, suggesting their role in regulating sinusoidal blood flow [44]. The cultured hHSCs produced monocyte chemotactic protein-1 (MCP-1) [45], and responded to TGFβ1 with increased PDGFRβ [48]. It has been demonstrated that density gradient isolated human HSCs can be reverted to a quiescent-like phenotypes through synergistic action of epidermal growth factor (EGF), fibroblasts growth factor 2 (FGF2), dietary fatty acids (oleic acid, palmitic acid) and retinol, as demonstrated by the abundant presence of retinyl ester-positive intra-cytoplasmic lipid droplets, and low expression levels of activation markers [31].

Density gradient separation remains the most widely used approach for HSC isolation, but this method targets the buoyancy of vitamin A-rich HSCs. This could result in inefficiency on isolating 'activated' HSCs. It has been demonstrated that upon liver injury, large number of HSCs were retrieved from higher density gradient layers in rat [34].

### NPC stepwise-separation method

Recently, a detailed protocol for human liver hepatocytes, NPC (non-parenchymal) fractions including Kupffer cells, liver endothelial cells, and HSCs was reported [49, 50]. In this method, NPCs were purified by Percoll density gradient centrifugation. Kupffer cells (KCs), liver endothelial cells (LECs) and hepatic stellate cells (HSCs) were separated using specific adherence properties and magnetic activated cell sorting (MACS). Specifically, KCs were isolated through adherence separation step since they adhere on cell culture plastics within a short period of time; then LECs were isolated from HSCs using MACS with CD31 micro beads; unlabeled HSCs were collected as the pass-through of the separation column during the MACS procedure. Using this method, HSCs with purity of ~ 93% can be achieved, which were GFAP positive.

### Fluorescent-activated cell sorting

Using the HSC characteristic of retinoid droplet storage, a pure HSC population was obtained by sorting of HSC based on endogenous vitamin A fluorescence with high side scatter (SSC) of incident light [51, 52]. The disadvantage of this method is the lower yield, higher cost, and requires a fluorescence-activated cell sorting (FACS). This method, however, remains valuable for obtaining pure hepatic stellate cells. Importantly, in human HSC isolation, a major concern is hepatocyte contamination, especially from a steatotic liver, in which contaminated hepatocytes also generate strong autofluorescence, making the sorting purification of HSCs challenging.

### Liver slice culture

Liver slice culture offers some unique advantages and has been used in various studies. The precision-cut liver slice model maintains cell–cell and cell–matrix interactions and therefore preserves the native physiologic milieu of resident liver cells [53] (Emilia Gore et al. 2017 keystone symposium) [54]. It was reported that slices of adult human [55] liver were cultured at the air–fluid interface for up to 28 days, with stellate cells positive for α-SMA and reticulin [53]. Using this method, the research demonstrated distinguished expression pattern of α-SMA, PDGFRβ and Thy-1 in normal, cirrhotic and cholestatic livers [56].

### Co-culture method

A recent study reported a 3D organotypic co-cultivation system for hepatocyte and non-parenchymal cells (NPCs). Using long-term cell co-culture, density gradient centrifugation and MACS, high purity and good separation of endothelial cells (ECs), Kupffer cells (KCs), dendritic cells (DCs), invariant natural killer T (iNKT) cells were obtained and then added back in a biogel into a 3D culture. In this system, HSCs were identified by desmin and GFAP

expression, and most of the HSCs were α-SMA positive [57].

## Human HSC markers (Table 1, Fig. 1)

Rodent studies have identified specific markers for HSCs. The most prominent proteins identified in rodent HSCs include desmin, GFAP, and α-SMA (when activated) [58, 59]. However, rodents and human HSCs not only show dramatic morphological discrepancy, but also the protein expression profile of human HSCs is quite different from mouse HSCs. In particular, immunostaining does not identify desmin or GFAP-positive cells in quiescent human HSCs [60, 61]. Therefore, it is important to identify specific markers for human HSCs.

### Morphologic identification

Transmission electron microscopy remains the gold standard for identification of HSCs based on location, cytoplasmic processes, lipid droplet content, rough endoplasmic reticulum, and bundles of microfilament [23, 62]. Morphologic features by light microscopy include presence of lipid droplets and stellate (star) shape of the cells. The most characteristic morphologic feature of HSCs in a normal liver is their storage of vitamin A in the form of cytoplasmic retinoid droplets [4]. These cytoplasmic lipid droplets are readily identifiable in live biopsies [63, 64]. Loss of retinoid is a prominent feature accompanying stellate cell activation. However, it was reported that in patients with normal liver histology, only 75% of the perisinusoidal cells contain lipids [65].

### Cytoskeletal proteins

*Desmin*

Yokoi et al. discovered desmin in rat HSCs [58]. Since then, desmin has been widely used as a 'gold standard' for identifying HSCs in rodent liver. However, data of desmin on HSC studies obtained in human subjects have been contradictory [38, 64, 66, 67]. Some studies have shown that perisinusoidal liver cells in normal adult liver tissue are devoid of desmin expression [66, 68], and desmin immunostain has been reported to be negative in fibrotic human livers [67, 69, 70]. While others have suggested positive desmin immunoreactivity in normal [38] or cirrhotic human livers [66]. As well, in isolated human HSCs, the results are not consistent. Positive immunostaining for desmin has been observed in primary isolated human HSCs in some studies [23, 31, 38], while negative immunoreactivity for desmin was reported in others [61, 71].

*Alpha-SMA (α-SMA)*

This is used as a reliable marker of activated and myofibroblastic HSCs. This cytoskeletal protein is absent from other resident liver cells except portal myofibroblasts and vascular smooth muscle cells [42, 66]. In normal and

**Table 1** Mouse HSC and human HSC markers

| Marker | Rodents | | Human liver tissue | | Isolated human HSCs | Isolated human HSCs Density gradient centrifugation | |
|---|---|---|---|---|---|---|---|
| | Quiescent | Activated | Normal | Diseased | Explants | Initial | Later passage |
| Desmin | + | + | + or − | + or − | + | − | + or weak or − |
| GFAP | + | + | − or + | + | − | + | + |
| P75 | + | + | + | + | | − | + or − |
| Trk-C | + | + | + | + | | | − |
| N-CAM | − | + | + | + | | | + or − |
| PDGFRβ | + | + | − | + | + | | + |
| CRBP-1 | + | + | + | + | | | |
| CYGB | + | + | + | + | | | |
| LRAT | + | − | + | + | | | |
| α-SMA | − | + | + or − | + | + | − | + |
| Vimentin | + | + | + | + | | | + |
| FAP | | | − | + | + | | |
| NT-3 | + | + | + | + | | | + |
| SYN | + | + | + | + | | | − |

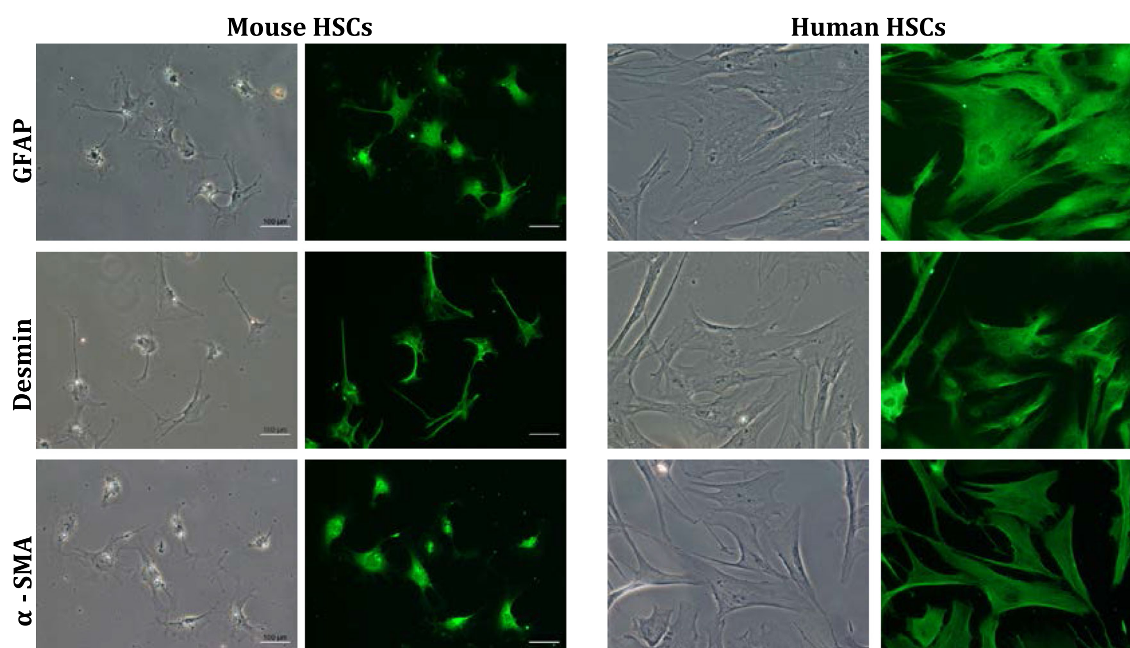


**Fig. 1** Immunofluorescence staining for GFAP, desmin, and α-SMA of primary mouse HSCs and primary human HSCs

pathological adult human livers, positive immunostaining of α-SMA was identified in fat-droplet-containing HSCs, and increased cell number and intensity of the staining signal was observed in the specimens with chronic liver disease [66, 68, 72]. Some studies reported no α-SMA immunoreactivity detected in normal human liver [42, 73]. Expression of vimentin, vinculin, procollagens I, III, collagen IV, V, laminin, and fibronectin are identified in human HSCs [23, 39, 64, 70].

## Neural markers

Stellate cells are directly adjacent to nerve endings [74], and studies have identified neurotrophin receptors [75] with functional studies confirming neurohumoral responsiveness of HSCs [18, 76, 77].

*GFAP (glial fibrillary acidic protein)*

Rodent HSCs express GFAP [59, 61]. In normal human liver tissue, GFAP immunoreactivity was absent [42]. However, a small subpopulation of periportal cells was reported as GFAP-positive in normal human liver in a different study [78]. In cirrhotic livers, GFAP was detected in focal clusters of cells in the periseptal regions of the regenerative nodules [42]. Positive GFAP staining was also identified in HSCs in fibrotic/cirrhotic livers [69]. Isolated human HSCs were reported as GFAP positive in some studies [49, 50, 61, 71], while no GFAP immunoreactivity was detected in isolated HSCs from cirrhotic liver explants [42].

*NGFRp75 (surface marker) (nerve growth factor receptor p75)*

Studies showed that human HSCs express the low-affinity nerve growth factor receptor p75, which was detected in perisinusoidal cells in normal donor liver sections. Also in fibrotic and cirrhotic livers, intense staining of p75 was observed immunolocalized with α-SMA-positive HSCs, and no p75 expression was observed in hepatocytes [69, 75, 79]. Quiescent (freshly isolated) HSCs did not express p75; its expression first became detectable in activated HSC after 7 days of culture in rat, and after 14 days of culture by Western-blot analysis in activated human HSCs [79]. P75 immunostain has shown inconsistent results in several human HSC cultures [61].

*Trk-C (surface marker) (NTRK3, neurotrophic receptor tyrosine kinase receptor 3)*

Notably, Trk-C is expressed in both rodent and human HSCs in normal and varying pathologic conditions [69, 75]. Vascular smooth muscle cells also express Trk-C [80]. However, lost expression of Trk-C was reported in cultured human HSCs [61].

*N-CAM (surface marker) (neural cell adhesion molecule)*

N-CAM was found to be present in periportal and intermediate-zonal human HSCs. Such intralobular heterogeneity of N-CAM expression might be related to the different maturational stages of the HSCs [81]. N-CAM-positive HSCs were also demonstrated in cirrhotic human livers co-localized with α-SMA [69]. N-CAM immunostain has shown inconsistent results in cultured human HSCs [31, 61].

*NT-3 (neurotrophin-3)*

NT-3 was detected in human HSCs in normal or various pathologic conditions; for example, positive immunostaining for NT-3 was observed in HSCs lining the sinusoids in human liver with alcoholic cirrhosis [69]. Weak immunoreactivity of NT-3 was also detected in hepatocytes in cryosections of human liver [75]. Positive NT-3 staining was observed in isolated human HSCs [61].

**Retinol processing proteins**

*CRBP-1 (cellular retinol binding protein-1)*

CRBP-1 is a carrier protein of intracellular retinol. Diffuse light staining by immunohistochemistry for CRBP-1 was seen in the cytoplasm of hepatocytes, while much more intense positive signal for HSCs were observed in rat [82]. In formalin-fixed paraffin embedded human live tissues, positive CRBP-1 staining was observed in lobular HSCs without reacting with smooth muscle cells and cholangiocyte positivity [70]. CRBP-1 was downregulated in human livers with advanced fibrosis, presumably due to a loss of vitamin A [64].

*CYGB/STAP (cytoglobin/stellate cell activation-associated protein)*

Cytoglobin was discovered by a proteome analysis of rat HSCs [83]. In the rat liver, Cygb is expressed in the quiescent HSCs and is increased when these cells were activated in fibrotic liver tissues. In normal human liver, CYGB-positive cells have similar distribution as in normal rat liver; however its expression is not increased around inflammatory lesions, which is not consistent with the observation in the inflammatory regions of rat liver [84]. Other hepatic constituent cells in liver lobules such as Kupffer cells, endothelial cells, hepatocytes, and bile-duct epithelial cells are negative for Cygb immunostaining [85]. Quiescent stellate cells, but not portal myofibroblasts, express both CYGB and CRBP-1 in normal human liver. In fibrotic and cirrhotic livers, it was shown that the distribution of CYGB was mutually exclusive with the distribution of Thy-1, and FBLN2 [64]. Thy-1$^{+}$ cells were located within the periportal tract in normal human liver [86], and its expression was observed in the fibrotic septa of cirrhotic liver [56]. Co-staining of Thy-1 and CD248 was detected in isolated human hepatic stellate cells [87]. It has been reported that cytoglobin expression is correlated with a more quiescent phenotype of HSCs and is regulated by extracellular matrix proteins dependent on FAK signaling in rat HSC-T6 cell line [88].

*LRAT (lecithin retinol acyltransferase)*

This enzyme is responsible for all retinyl ester synthesis within the liver and plays an indispensable role in the formation of HSC lipid droplets [63, 89], and was identified as a specific marker for HSCs [24]. LRAT-positive staining was demonstrated in the space of Disse of normal human liver, and was suggested to be recognized as a quiescent HSC marker in human tissue [73]. LRAT+/CRBP-1+ HSCs were demonstrated to contribute to portal fibrosis in human liver specimens in viral hepatitis [29].

**Other membrane proteins and markers**

*FAP: (fibroblast activation protein)*

FAP is a cell surface-bound protease of the prolyl oligopeptidase gene family expressed at sites of tissue remodeling. FAP mRNA and immunoreactivity were detected in cirrhotic, but not normal human livers. FAP colocalized with α-SMA in vivo and in isolated HSCs in vitro [42]. It was suggested that FAP expression was related to the severity of liver fibrosis [90].

*PDGFRβ (plate-derived growth factor receptor β)*

The PDGF receptor was the first membrane receptor identified on HSCs. Human stellate cells contain high levels of both PDGF α- and β-receptors, whereas rat cells contain predominantly the PDGF β-receptor [38, 43, 91–93]. PDGFRβ expression was identified in both quiescent and activated HSCs in rodents [91, 94, 95]. In cirrhotic human liver, PDGFRβ expression was markedly increased [28]. Using precision-cut liver slices, PDGFRβ expression was observed in fibrotic septa of cirrhotic liver before culture and was maintained after culture [56]. PDGFRβ mRNA was also detected in hHSCs grown from human liver tissue explants [38], and could be used as an activated HSC marker [84].

*B7-H1 (PDL1 or programmed death ligand-1)*

HSC expresses the co-stimulatory molecule on activated but not resting HSCs [96].

*SYN (synaptophysin)*

This neural marker was present in perisinusoidal HSCs in human normal liver biopsies, and increased in pathological conditions such as chronic biliary disease and chronic hepatitis C [69, 97, 98].

*ABCRYS (alpha B-crystallin)*

In normal and cirrhotic human livers, perisinusoidally located, stellate-shaped cells were stained positive for ABCRYS [61, 69, 71]. Cultured human HSCs, isolated from normal donor livers, were also shown positive ABCRYS immunoreactivity [71].

## Human hepatic stellate cell lines (Table 2)

There are obvious disadvantages in obtaining and usage of primary HSCs, particularly primary human HSCs, such as the heterogeneity of isolated cell populations and cellular characteristics, limited supply, considerable variations of cell preparation in different laboratories, as well as the isolation equipment and techniques requirements. Immortalized HSC lines were established and have been used in a wide range of research. These immortalized cell lines provide unlimited resource supply, homogeneity, and are suitable for genetic manipulation studies. They recapitulate many activated HSC features, and can serve as a useful tool for mechanistic investigation of HSC function in hepatic fibrosis and liver pathophysiological processes. The immortalized HSC lines currently in use have been generated from primary HSC through spontaneous immortalization during long-term culture, or by transformation with the simian virus 40 large T-antigen (SV40T), or ectopic expression of human telomerase reverse transcriptase (TERT). Notably, none of the published cell lines are reported to be tumorigenic. Considering these cells are 'genetically modified', careful evaluation of the reported studies is always warranted [18, 99].

### The LI90 line

The LI90 cell line is the first reported human HSC line derived from a human hepatic epithelioid hemangioendothelioma [100]. LI90 cells express α-SMA, vimentin, collagen types I, III, IV, V, and VI, fibronectin, laminin, and MMPs [101]. They are desmin negative and do not express endothelial or monocyte/macrophage-lineage markers. When exposed to medium supplemented with retinoids, LI90 cells accumulate vitamin A-containing lipid droplets [100].

**Table 2** Characteristics of human hepatic stellate cell lines

| Human HSC line name | Derivation resource | Derivation method | Expression markers | Transition to quiescent phenotype |
|---|---|---|---|---|
| LI90 | Human hepatic epithelioid hemangioendothelioma | Outgrowth from the diseased tissue | α-SMA, vimentin, collagen types I, III, IV, V, and VI, fibronectin, laminin and MMPs | Yes |
| TWNT-1<br>TWNT-4 | LI90 cell line | Retrovirally induced human telomerase reverse transcriptase | Col1α1, HGF; PDGFRβ, α-SMA, Col1α1 | Yes |
| hTERT | Normal human liver | Retroviral expression of the human telomerase reverse transcriptase | PDGFRα and β, GFAP, Col1α1 and α-SMA, etc. | Yes |
| LX-1 | Normal human liver | SV40 T antigen | α-SMA, vimentin, GFAP, PDGFR-β, Ob-$R_L$, DDR2, MMP-2, TIMP-1, MT1-MMP, neuronal genes | Yes |
| LX-2 | Normal human liver | Spontaneous immortalization in low serum condition | Same as LX-1 | Yes |
| GREF-X | Cirrhotic human liver | Polyoma virus large T antigen | α-SMA, vimentin, collagen I, IV, V and VI, fibronectin, laminin, MMP-2 | Yes |
| HSC-Li | Normal human liver | Retrovirus SV40LT | HGF, VEGF receptor 1, Col1α1, Col1α2, α-SMA, PDGFR-β, vimentin, TGF-β1 | Yes |

### TWNT-1 and TWNT-4 cell line

The TWNT-1 [102, 103] and TWNT-4 [104] cell lines were derived from retrovirally induced human telomerase reverse transcriptase into the LI90 cell line, since the parental LI90 cells were observed to enter replicative senescence. TWNT-1 cells synthesized Col1α1, HGF, and could uptake acetylated low-density lipoproteins, and TWNT-4 expressed PDGFRβ, α-SMA, Col1α1.

### hTERT HSC line

Schnabl et al. described a cell line immortalized by ectopic expression of the human telomerase reverse transcriptase (hTERT) gene in primary HSCs isolated from surgical specimens of normal liver. Extensive characterization of gene and protein expression revealed that this cell line has a similar gene expression profile as the activated human HSC including PDGFRα and β, GFAP, collagen1a1, and α-SMA [105, 106]. Importantly, this cell line undergoes transition to quiescent status when cultured in a basement membrane-like matrix.

### LX-1 and LX-2

LX-1 and LX-2 are the most utilized human HSC lines. LX-1 was generated by transformation with SV40 T antigen, and LX-2 was obtained through spontaneous immortalization in low serum culture [107]. Both cell lines express α-SMA, vimentin, GFAP, PDGFRβ, discoidin domain receptor 2 (DDR2), and leptin receptor OB-$R_L$. Both cell lines also express matrix remodeling factors, including MMP-2, TIMP-1, TIMP-2, MT1-MMP, and multiple neuronal genes. Both cell lines express mRNA for procollagen 1α1 and HSP47, and they retain key features of HSC such as accumulating retinol and converting it to retinyl ester [26, 108–110].

### GREF-X

This cell line was established by immortalizing human liver myofibroblasts, obtained from explants of human liver and transfected with a plasmid containing the coding sequencing of polyoma virus large T antigen expressed under the control of the early promoter of cytomegalovirus (CMV) [111]. GREX-X cells stain positive for α-SMA and vimentin, and express collagen types I, IV, V, and VI, fibronectin, and laminin, and secrete MMP-2. Importantly, GREF-X cells are able to take up and esterify retinol, and respond to TGFβ1 [111].

### HSC-Li

Recently, a human HSC line was established by immortalizing the primary human HSCs isolated from surgical specimen of adult liver donors using the simian virus 40 large T antigen (SV40LT) for application in a co-culture system with immortalized human hepatocytes in vitro [112]. HSC-Li cells were longitudinally spindle-like and contained fat droplets in their cytoplasm as observed under electron microscopy. It expresses mRNAs for hepatocyte growth factor (HGF), VEGF receptor 1 (Flt-1), Col1α1, and Col1α2, and positive for α-SMA, PDGFRβ, and vimentin proteins.

All of these cell lines should be categorized as myofibroblast-like cells since they contain few or no lipid droplets, express α-SMA, and synthesize collagen type I and fibronectin.

## Conclusions

In the normal liver, HSCs comprise 5–8% of total rat liver cells [113]. In mouse liver, HSCs comprise a population of 8–10% of total liver cells, but rapidly expand in response to chronic fibrogenic injury corresponding to ∼ 15% of total liver cells [114]. Pathogenic mechanisms responsible for development of hepatic fibrosis and liver failure are poorly understood. Newer studies are showing that there might be differences in pathways that are involved in hepatic fibrosis that are etiology dependent. For example, HSCs are identified as a major source of myofibroblasts in hepatotoxic-induced liver fibrosis, such as alcohol or CCl4. Following chronic injury, HSCs activate into myofibroblast-like cells, acquiring contractile, pro-inflammatory, and fibrogenic properties. They have also been shown to inactivate and acquire a quiescent-like phenotype, which might help with regression of liver fibrosis [114, 115]. As well, blockage of certain proteins in HSC activation pathways might prove to have therapeutic implication in human diseases [98, 116–118]. Mouse model studies and advanced HSC isolation techniques have contributed to the elucidation of this cell's functions. However, species-differences are critical to translational research. Protein expression patterns of normal and activated HSCs are not identical among species, which imposes challenges on the translational research, and therefore it is imperative to develop tools and techniques for investigating human HSCs to confirm and extend studies in rodent models.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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