

REVIEW

Pointing at Ito cell, from structure to function (... or Cinderella story in liver histology)

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Abstract

For more than 130 years, the hepatic stellate cells (also known as Ito cells) have been the object of numerous studies that aimed at their characterization both in normal and postlesional status, where they play a key role in fibrosis progression specific for chronic hepatic pathology. Converged studies on their histophysiology have revealed other functions, namely the involvement in liver embryological development and regeneration, metabolisms regulation, modulation of local inflammatory and immune reactions. Ito cells plasticity is surprising, as they are able to provide the connection between the complex sinusoidal and parenchymal microenvironment, influencing by autocrine and paracrine mechanisms the extracellular matrix content in tight correlation to growth and repair necessities. Last but not least, Ito cells take an active part in systemic homeostasis maintenance by their capacity to store and mobilize vitamin A, respectively. The evolving interest in their research will be undoubtedly followed by a better knowledge of the physiopathological sequences responsible for liver diseases, as new targets for the development of new therapeutic approaches directed toward improvement of prognosis and patients' quality of life.

Keywords: hepatic stellate cells, vitamin A, liver development, fibrosis, cytokines.

Introduction

Star-shape liver cells have been firstly described in 1876 by Kupffer, who was using gold chloride for morphological study of the liver, and identified in sinusoids a cellular type containing cytoplasmic vitamin A storage, which he named "sternzellen", according to their cellular shape [1].

However, the initial method of identification used by Kupffer did not achieve the accurate differentiation of star-shape cells from the macrophage-type cells residents in liver parenchyma, so microscopic studies have been further continued. Thus, in 1951, Ito published his own results regarding the liver sinusoidal cell population, describing it in association to macrophages (Kupffer cells) as a cellular line capable to store lipids in cytoplasm and correspondently naming it "fat-storing cells" [2]. Later, in order to reflect their role in lipids and vitamin A storage, Bronfenmajer *et al.* had proposed the name "lipocytes" [3, 4]. Therefore, since 1876, these cells are referred to by using a large variety of names, as follows: hepatic stellate cells (HSCs), perisinusoidal cells, Ito cells, lipocytes, parasinusoidal cells, or fat-storing cells [5–7].

The history of the HSC can be compared to Cinderella's story... Due to its morphology difficult to identify by usual light microscopy techniques, the Ito cell was, and, somehow, remains hidden among the other cells in the hepatic parenchyma – just like Cinderella, treated by her family as a maid. Nevertheless, this cell can transform itself by the capacity of developing supplementary functions, in normal status or depending on the associated pathological conditions (Figure 1). This metamorphosis

granting the HSC an important position in the pathogenesis of liver diseases is therefore similar to Cinderella's change to a princess.

Structure and ultrastructure of hepatic stellate cells

The embryological origin of HSCs is still a matter of interest. Despite the evidences of surface markers belonging to the three embryonic layers, current data have favored a double origin, mesodermal (from *septum transversum*) and endodermal, respectively [8–10].

Initially, the presence of glial markers on the surface of HSC has initially supported the hypothesis of ectodermal origin, which has been quickly infirmed by experimental studies [11]. Following continuous and thorough researches on human embryos, CD34 and cytokeratin 7/8 have been identified, which support the endodermal origin [12]. On the other hand, studies have demonstrated the expression of mesoderm transcriptional factor Foxf1, in an analogous manner to cells originating in the mesenchyme of *septum transversum* [13]. These first observations are also supported by recent experimental results, which have confirmed the *septum transversum* origin – specifically from a multipotent mesenchymal cell – not only of liver stellate cells, but also of perivascular mesenchymal cells, portal fibroblasts, smooth muscular fibers from central vein [9], and other cellular types like chondroblasts, osteoblasts, or adipoblasts [4].

In physiological status, HSCs are located in Disse subendothelial space (Figure 2), representing approximately 5–8% of the whole liver cell population and one

third of non-parenchymal cells [10, 14, 15]. A light concentration around the terminal vein has been ascertained,

without any known functional significance related to this location [3, 5].

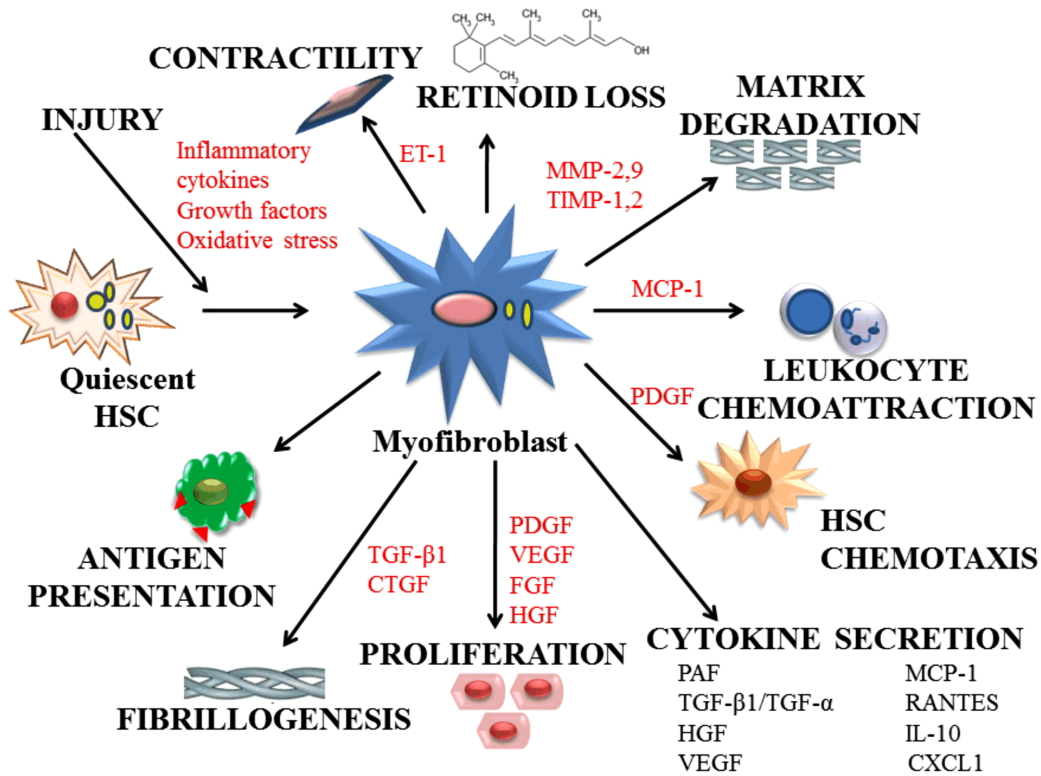


Figure 1 – Multifaceted role of the hepatic stellate cell.

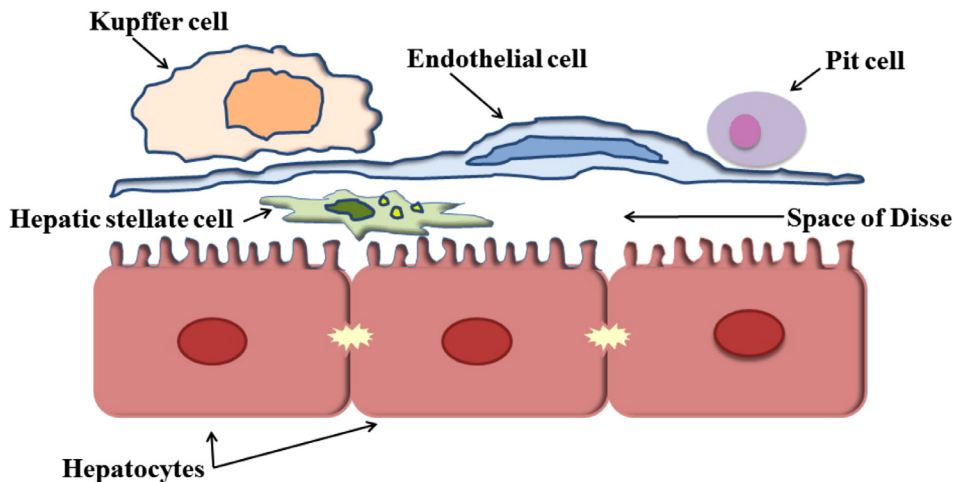


Figure 2 – Hepatic stellate cell location in liver parenchyma.

Ultrastructurally, HSC has a stellate cellular body due to extension of numerous cytoplasmic processes, an oval or ellipsoidal nucleus, moderately developed RER, reduced perinuclear Golgi complex, and variable cytoplasmic deposits of vitamin A [16]. The subendothelial cytoplasmic extensions are disposed in a perisinusoidal location, between endothelium and hepatocytes [17]. On the surface of these cellular prominences, there are numerous spine microprojections involved in reception of chemotactic signals able to induce the stimulation of HSC contractile system [18]. The close contact with sinusoids, on one hand, and hepatocytes, on the other hand, supports the theory of mediators or cytokines transport facilitation role of HSCs [5]. Moreover, the direct contact with free nerve endings sustains the expression of neurotrophin

receptors on their cellular surface and/or their neuro-endocrine activity [19–21].

Hepatic stellate cells functions

Developmental and liver regeneration function

The tight structural association between HSCs, endothelial cells and hepatocytes suggested the idea that they may intervene in modulation, growth, differentiation, or morphogenesis of all the other parenchymal cells. During angiogenesis, the direct interaction between pericytes and endothelial cells is an essential process which leads to the maturation of vascular morphological elements [22]. According to the most widespread data, based on thorough studies of liver embryology, HSCs are nowadays

considered as playing the role of the pericytes, with major impact on vasculature development [10, 23]. In support of this theory come also the results of an experimental study, which demonstrated that lack of β -catenin in mouse liver mesenchyme leads to highly activated HSCs and induction of dilated sinusoids development [24]. Nevertheless, recent evidences point out the role of HSCs in the differentiation of hepatocytes, by their capability to induce synthesis of extracellular matrix components [25], and in the development of intralobular biliary ducts [10].

Researches on liver histophysiology have demonstrated that HSCs may be also involved in normal liver hematopoiesis during intrauterine life, as they express on their SDF1 α (surface stromal cell-derived factor 1 α) or CXCL12, a powerful chemotactic factor for hematopoietic stem cells [26, 27].

All these data will certainly fundament future studies regarding the elaboration of more efficient specific protocols in hepatocytes cultivation (*in vitro* studies) based on stem cells, with potential major impact on liver transplant and successful therapies in acute and chronic liver diseases.

The liver regeneration capacity is well recognized, beginning with the first observations in experimental partial hepatectomy [28]. In these circumstances, liver regeneration is achieved by remnant hepatocytes and is taking place in the absence of necrosis or inflammation [28]. If hepatocyte division fails, it seems that liver regeneration is supported by the involvement of other types of cells, which mediate liver progenitor cells activation [28, 29]. The HSCs support the process of liver regeneration by direct secretion of a great variety of cytokines, or by remodeling extracellular matrix components [10, 30]. The hepatic regeneration process has finely regulated steps, which are naturally ending by the achievement of a specific cellular mass correspondent to the liver [31].

Unfortunately, the complete portrayal of HSCs involvement in liver regeneration might be achieved only in experimental models characterized by their total ablation – an ideal condition that is still unachievable. Moreover, the complexity of human liver microenvironment cannot be entirely replicated in experimental conditions.

Vitamin A storage

In normal functional status, 50–80% of vitamin A is stored in liver, 80–90% of it being in fact deposited as cytoplasmic droplets in HSCs [32, 33]. Electron microscopy distinguished two types of vitamin A lipid droplets in HSCs cytoplasm. Type I have various sizes, but not more than 2 μ m in diameter and are packaged by a peripheral membrane, while type II are much larger (up to 8 μ m) and devoid of peripheral membrane [10]. The relationship between the two types of vitamin A droplets is partially known, with opinions that support type II formation due to fusion of more type I droplets [34], or that type II might represent a precursor for type I vitamin droplets [35, 36].

Exogenous vitamin A is absorbed in the intestines and is transported to the liver by chylomicrons, where after uptake by hepatocytes is transferred to HSCs for storage, only small quantities being kept into hepatocytes [37].

Biochemically, HSCs cytoplasmic droplets contain as main components retinyl esters, and predominantly retinyl palmitate, but also small amounts of triglycerides, phospholipids, cholesterol, and free fatty acids [38].

Consequently to postlesional activation, HSCs achieve a series of ultrastructural changes consisting in loss of cytoplasmic vitamin A storage associated to significant rough endoplasmic reticulum (RER) and Golgi development, which would support an enhanced collagen synthesis characteristic for activated HSC or hepatic myofibroblast-like cells [39, 40].

Initial observations revealed that these ultrastructural modifications are the base of the cascaded events that result in fibrous tissue deposition in liver parenchyma. Therefore, the study on HSCs properties has been largely extended, with an increased focus on genes and mediators, which coordinate these events and, consequently, on new therapies for hepatic chronic diseases associated to variable degrees of fibrosis.

In this respect, a new hypothesis submits the idea of a common process of HSCs dedifferentiation and adipocytes and preadipocytes dedifferentiation into fibroblasts [41]. Thus, besides both adipocytes and HSCs being lipid storage cells, additionally, HSCs express collagen type IV and adipocytes specific genes, as adiponectin and adipisin [42, 43]. Based on these common features, a similar mechanism of liver lipocytes regulation and of adipocytes differentiation coordinated by PPAR γ (peroxisome proliferator-activated receptor gamma) acting as adipogenic transcription factor has been suggested [44]. PPAR γ promotes lipids intracellular storage, including that of retinyl esters in liver HSCs, concomitantly suppressing the activity of genes involved in collagen type I synthesis and, as a consequence, one of the factors whose therapeutic control might prevent liver fibrillogenesis [45, 46].

Recent data have shown that once activated, HSCs lose their cytoplasmic vitamin A and are capable of its conversion into retinoic acid, using alcohol dehydrogenases, specifically retinaldehyde dehydrogenases [47]. Retinoic acid mediates the interaction between HSCs and NK (natural killer) cells, both in alcoholic liver and in infections with liver tropism [47].

Inflammatory function: secretion of cytokines and fibrillogenesis

One of the most surprising functions is that of involvement in the modulation of liver inflammatory reactions, as HSCs are able to amplify the local response and the infiltration of parenchyma by mononuclear cells, and also by neutrophils, as in alcoholic hepatopathy [48].

So far, a large variety of cytokines secreted by HSCs has been identified, their list being most probably still incomplete [5].

Among these, PGF2 α (prostaglandin F2 α) and PGD2 (prostaglandin D2), or PGE2 (prostaglandin E2), are involved in hepatic metabolism and in local inflammatory processes, possibly supplemented by leukotrienes C4 and B4, whose complete activity is yet incompletely deciphered [5]. Local postlesional neutrophilic inflammation is amplified as result of HSCs action, as they are able to secrete PAF (platelet activating factor), which facilitates chemotaxis and stimulation of neutrophil granulocytes [49].

After their activation, HSCs are capable to synthesize a large spectrum of chemokines, as following: CCL21 (chemokine C-C motif ligand 21), MCP-1 (monocyte chemoattractant peptide-1), CCR5 (C-C chemokine receptor type 5), and RANTES (regulated on activation normal T-cell expressed and secreted) [5]. These substances are involved in variable immunomodulatory processes.

HSCs synthesize TGF- α (transforming growth factor α), TGF- β (transforming growth factor β) and EGF (epidermal growth factor), important growth factors involved in their own proliferation and that of the hepatocytes as well in an autocrine manner, a process finely regulated by PDGF (platelet-derived growth factor) [50, 51]. TGF- β , well known as the effective hepatic anti-proliferative factor produced by activated HSCs [52], has a major impact in liver fibrillogenic cascade. On the other hand, HGF (hepatocyte growth factor) is currently considered the most powerful hepatic mitogen factor delivered by HSCs [5]. HSCs may balance HGF stimulatory action toward liver regeneration, demonstrating the capacity to coordinate the type of cytokines secretion according to the requirements of this process.

Lately, CTGF (connective tissue growth factor) or CCN2, a cytokine with collagen synthesis promoter activity even in lung and kidney [53] has been added to the list of factors involved in liver fibrillogenesis – activated HSCs being one of its principal sources of secretion, beside hepatocytes. Apoptotic bodies formed after hepatocytes injury induce Kupffer and HSCs activation and the latter conversion to myofibroblasts [54]. Once stimulated, HSCs express on their surface a series of adhesion molecules and act as local APCs (antigen presenting cells), being able to stimulate T and NK cells activity [55, 56], achieving a pathological chain of hepatocytes necrosis/apoptosis perpetuation, and continuous extracellular matrix collagen deposition [57]. Myofibroblasts also possess contractile ability, as they express α -smooth muscle actin (α -SMA) [58].

Conversely, deactivation of HSCs induces collagen deposition arrest and facilitates extracellular matrix regression [39, 59, 60]. Procollagen type III deposition, followed by that of collagen type I and IV are early events in hepatic lesions, a process balanced by MMPs (matrix metalloproteinases) activation. MMPs are involved in collagen degradation, in order to maintain the extracellular matrix stability [61]. Simultaneously, MMPs inhibitors become expressed, as another reaction meant to maintain the hepatic extracellular matrix composition [62]. Matrix collagen degradation also depends on genic signals, which influence myofibroblasts apoptosis (by anti-apoptotic genes) and inactivation of HSCs (by PPAR γ gene) – effects balances by MMPs activity [63].

It is worth to mention that other mechanisms are contributing to local fibrillogenesis activation, such as the increase of antiapoptotic factors expression (particularly Bcl-2), supporting the lesional hepatocytes survival and, therefore, perpetuating the activation of HSCs and consequent collagen deposition [61].

Despite their important involvement in fibrillogenesis, HSCs are not the only structures responsible of collagen synthesis in liver parenchyma, as other types of cells are performing the same function – such as portal fibroblasts, mesenchymal cells, and local fibrocytes [64].

Relationship with the immune cells

HSCs spectrum of activities oriented toward maintaining the immune homeostasis in liver parenchyma is highly variable. Indisputable proofs have already certified HSCs capacity to modulate the activity of T and B cells and, furthermore, their capability to act as APCs has been also tested [65–67].

Complex experimental studies have demonstrated the involvement of activated HSCs in liver T-lymphocytes recruitment and apoptosis induction, a role mediated by PD-L1 (programmed death-ligand 1), a transmembrane protein with immunosuppressive function [68, 69]. The expression of this molecule is stimulated by IFN- γ (interferon γ) and, conversely, PD-L1 blocking is associated with the reduction of HSCs immunomodulatory activity, both in humans and mice [69–71].

Studies on liver immunopathology have demonstrated that CD8+ cells exert an enhanced fibrillogenic activity *via* HSCs when compared to CD4+ cells, partially supporting the high level of fibrosis noticed in patients presenting both hepatitis C virus (HCV) and human immunodeficiency virus (HIV), whose CD4+/CD8+ cells rate is reduced compared to patients only with HCV [51].

HSCs receive, in turn, numerous signals from immune cells [NK cells, CD56(+) T-cells, or $\gamma\delta$ T-cells] located in the sinusoid capillaries. These cells are able to produce a series of mediators, which stimulate HSCs activity. One of the most important HSCs activation pathways includes the intervention of nuclear factor κ B (NF- κ B), able to stimulate Toll-like receptor 4 (TRL4) or P2Y receptor activation [72]. Secondary to activation, HSCs produce pro-inflammatory cytokines, chemokines, or ROS (reactive oxygen species), or may act as APCs [73].

There are only few reports regarding the relationship between B-lymphocytes and HSCs in hepatic micro-environment. These results support the correlation between HSCs number, plasma cells number, and severity/degree of fibrosis in autoimmune hepatitis [74] and also the association between HSCs – plasma cells location and reduction of HSCs number in patients showing a favorable therapeutic response [75]. The intimate mechanism of interaction between these two types of cells has been recently demonstrated, also involving the direct intervention of PD-L1 [76].

The new perspective of HCS intervention as a non-professional APC in complex relationships with other types of cells of liver sinusoid capillary is still debated. Experimentally, numerous receptors have been identified on the plasma membrane of HSCs (members of the HLA class II, Fc (fragment crystallizable) of IgG, cathepsin S, and lipid-presenting molecules) – a feature which supports this theory [65–67]. As APCs, HSCs may induce the efficient stimulation of lymphocytes or apoptosis of virus-infected cells [48]. However, HSCs intervention as APCs is controversial. There are data to support that HSCs are not able to act alone as APCs but that they require the intervention of retinoic acid or of TGF- β 1, molecules which could stimulate T-lymphocytes activation and may induce differentiation of Foxp3+ subpopulation [77–79].

Hepatic stellate cells and hepatocellular carcinoma

Considering that HSCs promote and support secretion of cytokines which controls liver proliferation and regeneration, in specific clinical situations, their aberrant activation may result in severe diseases, such as hepatocellular carcinoma [10].

Frequently, hepatocellular carcinoma is developed on a preexistent liver pathology, as liver cirrhosis – the characteristic fibrosis present in the liver parenchyma being performed by HSCs [80]. Thus, more and more studies raise the question of the connection between the activity of HSCs and the occurrence of malignant tumors, as they intervene in liver carcinogenesis by autocrine secretion of numerous cytokines, such as TGF- β , PDGF, FGF 1 and 2 (fibroblast growth factors 1 and 2), IGF (insulin-like growth factors), and HGF [30, 53, 81–83]. This assessment is sustained by immunohistochemical studies results that revealed a high number of HSCs in tumoral sinusoids, capsule, and fibrous septae [84, 85].

In liver carcinogenesis initiation, these cells intervene not only by modulation of stromal growth, but also by inhibition of the local immune response or by stimulation of neoangiogenesis, features demonstrated in patients with chronic viral hepatitis [86–88]. All these findings lead to the conclusion that HSCs are “accomplices” which create this microenvironment required for tumor masses development, including secondary or metastatic types [89].

In achieving the goal of early detection of malignant transformation in chronic lesions associated to cirrhosis or viral hepatitis, a decisive role might be played by the activated HSCs. Thus, secretory products of these cells, as HGF, IGF, TGF- β 1, or osteopontin, whose level of secretion are much more amplified in patients with hepatocellular carcinoma, might be considered as novel diagnostic biomarkers with potential future applicability in current medical practice [90–92].

☞ Recent technical advances in HSCs research

Taking into account the technical requisites, HSCs isolation is a very difficult process. Historically, the first procedures were accomplished in rats [93, 94], but mouse models, including genetic ones, have been lately developed [5]. Nevertheless, the “gold standard” method applies the principle of density centrifugation using Iohexol, resulting in HSCs separation based on their physical characteristics in comparison to other cells of liver parenchyma. Following this method, viable HSCs, which are appropriate for cell culture research, are obtained [95]. More recently, other methods have become available for HSCs identification, such as: flow cytometry, quantitative real-time PCR, confocal microscopy, and molecular markers of cellular origin and of specific phenotype [5].

The FACS (fluorescence-activated cell sorting) procedure applied to obtain HSCs has been reported by several groups of researchers [41, 95–99]. Unfortunately, due to large variations of the protocols, a complete analysis of the isolated cells functional properties has not been yet achieved.

HSCs are major targets in anti-fibrotic therapies. Most

research in this direction has been mainly done using experimental models [100] designed to exploit the fibrosis mechanisms which involve HSCs. Consequently, different receptors for molecular therapies have been identified, such as: M6P/IGF-IIR (mannose-6-phosphate/insulin-like growth factor II receptor), PDGFR (platelet-derived growth factor receptor), RBPR (retinol binding protein receptor), α_2 -macroglobulin, ferritin, uroplasinogen, thrombin, matrix compounds (integrin, collagen type VI, and fibronectin) receptors [101, 102].

One of the most studied receptors is M6P/IGF-IIR, a regulatory transmembrane glycoprotein, acting as a clearance receptor able to provide protein degradation by endocytosis, or able to act as a signaling receptor involved in transduction of G-protein-linked signal [103, 104]. M6P/IGF-IIR has affinity for different ligand molecules, classified according to their binding modality in two categories: M6P-containing ligands (renin, latent TGF- β 1, thyroglobulin, proliferin, leukemia inhibitory factor, and granzyme B) and M6P-free ligands (IGF-II, retinoic acid, urokinase-type plasminogen activator receptor, and plasminogen) [104]. Furthermore, M6P binding to activated HSCs requires a carrier and the necessary abilities to perform this task are expressed by HSA (human serum albumin), as has been demonstrated by appropriate tests [105, 106].

M6P-HSA complex binding to HSCs results, by M6P internalization [106], in activation reduction – thus, representing the first proof of HSCs targeted therapy benefits [101]. A different mechanism used to obtain a selective carrier is based on albumin transformation by means of cyclic peptide moieties, which provide the binding to other molecules (*i.e.*, cytokines or growth factors) and these, in turn, adhere to HSCs. Thus, the precise orientation of some anti-fibrotic substances towards HSCs has been achieved, *in vitro* (*e.g.*, doxorubicin [107], pentoxifylline [108, 109] 18 β -glycyrrhetic acid (18 β -GA) [110], mycophenolic acid [106]), directly resulting in a reduction of the fibrosis process [102, 111].

Another target molecule is PDGFR β , which is over-expressed in PDGF-stimulated HSCs, with powerful mitogenic effect directly correlated with fibrosis [112]. An additional design tentative of a targeted drug delivery system dedicated to IFN- γ selective supply to HSCs has been using sterically stable liposomes adapted by specific cyclic peptides assuring PDGFR β binding [113–115]. This type of experiment is justified by the necessity to optimize the anti-fibrotic action of IFN- γ administered to human subjects [115].

A recent stage in specifically oriented cirrhosis therapy toward HSCs is represented by genetic approach. Antigenic therapy is aimed to provide HSCs genes and antisense material, *via* adenoviral or lipid based non-viral vectors [101]. The studies on this subject, using mainly adenoviral mediated transduction methods, are yet limited.

Although HSCs have a unique gene signature, this suffers major transformations during the transdifferentiation process into myofibroblasts [116–119]. However, HSCs isolation by FACS secures the preservation of the characteristic gene reservoir [99], including the major fibrogenic genes *Colla1* and *Acta2* [95].

The cluster of transdifferentiation-sensitive genes has

been continuously extending. This comprises transcription factors, extracellular matrix proteins, cellular adhesion molecules, cytokine receptors, and genes codifying proteins responsible for matrix transformation or cytoskeletal arrangement [102]. Some of them are considered as putative targets for genic therapies application [102, 104], with several experimental reports on *CSRP2* and *SM22 α* genes [120], *GFAP* gene [121–123], *vimentin* gene [61], *α -SMA* and *Collal* gene [124].

Targeted genic therapy may be also oriented toward several receptors, such as p75 neurotrophin [125], which bind to NGF (nerve growth factor) and results in activated HSCs apoptosis [125, 126]. Consequently, in case of a direct stimulation of p75NTR (p75 neurotrophin receptor), HSCs destruction could be achieved [125, 126].

Although the benefits of genic therapy are undoubtable, the pathway from experimental data to clinical application is far from being accomplished. The difficulties mainly derive from the inconsistency of the reported results, *in vitro* observations sometimes lacking *in vivo* correspondence – a feature understandable in relation to the complexity of regulation process responsible for HSCs genetic specificity [120].

Final remarks

Despite the amount of knowledge accumulating on HSCs, they still remain an enigma. Its pluripotential capacity and its major contribution in liver development and regeneration could represent challenging research targets in developing novel therapies. Moreover, extremely interesting data on HSCs involvement in regulation of immunotolerance in liver microenvironment – with significance for viral pathology – have been registered, suggesting subtle relationships between their activity and some other types of inflammatory cells. Last but not least, the use of HSCs for *in vitro* differentiation of hepatocytes followed by transplant in human liver could provide the base for new therapeutic approaches in liver diseases.

Conflict of interests

The authors declare that they have no conflict of interests.

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