Review

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Hepatic stellate cells: current state and open questions

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Abstract: This review article summarizes 20 years of our research on hepatic stellate cells within the framework of two collaborative research centers CRC575 and CRC974 at the Heinrich Heine University. Over this period, stellate cells were identified for the first time as mesenchymal stem cells of the liver, and important functions of these cells in the context of liver regeneration were discovered. Furthermore, it was determined that the space of Disse – bounded by the sinusoidal endothelium and hepatocytes – functions as a stem cell niche for stellate cells. Essential elements of this niche that control the maintenance of hepatic stellate cells have been identified alongside their impairment with age. This article aims to highlight previous studies on stellate cells and critically examine and identify open questions and future research directions.

Keywords: aging; CD133; mesenchymal stem cells; reelin; stellate cells; stem cell niche.

Introduction

Hepatic stellate cells contribute to fibrosis and cirrhosis in chronic liver diseases. In fibrotic livers, stellate cells produce extracellular matrix proteins that lead to scar formation and the ultimate loss of liver function. This process is preceded by the activation of stellate cells, which remain in a quiescent state in normal, healthy livers. In this quiescent state, stellate cells primarily store retinoids (vitamin A; Wake

1980) mainly as retinyl palmitate in membrane-coated vesicles and have long cellular extensions. During activation (e.g., by injury through a poisoning or infection of the liver), stellate cells release retinoids and increase the expression of extracellular matrix-associated genes, which leads to typical liver fibrosis in the case of persistent liver damage (Friedman 2008). This situation is of clinical relevance and explains the interest of various research groups in developing therapeutic strategies for treating patients with fibrosis. In this context, it is also important to determine whether stellate cells alone contribute to liver fibrosis. In addition to activated stellate cells, other fibroblastic cells in the liver (e.g., portal fibroblasts) can also contribute to fibrogenesis (Iwaisako et al. 2014). However, a recent study using single-cell RNA sequencing (scRNA-seq) to analyze stellate cells from mouse liver treated with a high-fat diet to induce non-alcoholic steatohepatitis (NASH) demonstrated that activated stellate cells can once again become quiescent after returning to a normal diet and fibrosis regression (Rosenthal et al. 2021). This finding suggests that elucidating the function and needs of stellate cells may open new ways to treat patients with liver fibrosis.

Although the function of stellate cells is predominantly observed in the formation of extracellular matrix proteins, many facets of this cell type remain enigmatic. The most obvious property of stellate cells is their retinoid content, which increases with age (Figure 1) and suggests that these cells remain in a quiescent state for a long time until the liver is injured. It has been proposed that stellate cells play an important role in regulating vitamin A homeostasis under physiological conditions. However, experimental evidence of this has not been provided and the molecular mechanism for the release of retinol by stellate cells remains unknown (Blaner et al. 2016). While it seems that retinoids help to preserve quiescence in stellate cells, their absence cannot trigger fibrosis (Kluwe et al. 2011; Yoneda et al. 2016). It has also been shown that retinoids promote hepatocyte survival after liver injury to facilitate appropriate tissue repair (Evarts et al. 1995; Shmarakov et al. 2013). In light of these findings, activating stellate cells could promote liver regeneration by releasing retinoids. This is in line with the observation of stellate cells

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unleashing a variety of growth factors and thus supports the reconstitution of liver mass after injury. Hepatocyte growth factor (HGF) is a factor released by stellate cells that promotes hepatocyte proliferation and maintenance (Rohn et al. 2020). Another noteworthy observation is that hepatic stellate cells express the molecular markers of all three germ layers (i.e., the mesoderm, endoderm, and ectoderm) (Geerts 2004). Additionally, factors that are normally found in cells with developmental potential (e.g., Hand2, embryonic stem cell-expressed RAS, GATA4, and nestin) have been identified in stellate cells, which discriminate them from other liver cells (Nakhaei-Rad et al. 2015, 2016; Niki et al. 1999; Reichert et al. 2021; Rosenthal et al. 2021; Yin et al. 2012). The expression of factors involved in cell development (i.e., nestin) and the discovery of the cell surface protein CD133 in stellate cells, as well as their resistance to apoptotic stimuli, prompted us to investigate whether stellate cells have stem cell characteristics (Kordes et al. 2007; Reinehr et al. 2008). At that time, it was shown that CD133 is expressed by stem cells and cancer stem cells (Corbeil et al. 2000; Miraglia et al. 1997; Singh et al. 2004).

CD133 in stellate cells

CD133 (also known as prominin-1) is a pentaspan transmembrane glycoprotein associated with cholesterol-containing membrane microdomains called lipid rafts, which are localized in filopodia, microvilli, and primary cilia (Dubreuil et al. 2007; Röper et al. 2000; Weigman et al. 1997). Several studies have shown that CD133 serves an important role in the organization and dynamics of these structures. For instance, CD133-deficient mice exhibit optic disc dysmorphogenesis and photoreceptor degeneration along with a complete loss of vision (Zacchigna et al. 2009). Furthermore, it was recently proposed that the interplay of CD133-ganglioside membrane complexes, phosphoinositide 3 kinase, and cytoskeleton components regulates microvillar architecture and that the silencing of CD133 in primary cultures of hematopoietic stem cells results in the loss of uropod-associated microvilli (Thamm et al. 2019). Moreover, CD133 serves an essential role in recruiting molecular compartments to primary cilia and can influence hedgehog signaling. Sonic hedgehog-mediated signaling triggers the translocation of the primary cilium-associated glioma-associated oncogene homolog (GLI) transcription factors and CD133 into the nucleus, which is a process that controls stem cell activation (Singer et al. 2019). Additionally, CD133 has been suggested as a target gene of β -catenin-dependent WNT signaling and seems to act as a protective factor for this pathway, which prevents

β -catenin degradation and has implications for cancer stem cell maintenance and growth (Brossa et al. 2018; Mak et al. 2012; Monoranjan et al. 2020). The downregulation of CD133 results in the upregulation of WNT inhibitory genes and reduction of nuclear β -catenin, which has been observed in melanoma cells (Rappa et al. 2008). In addition to these supportive effects on canonical WNT and hedgehog signaling, CD133 is released into various bodily fluids via small vesicles or exosomes that originate from the tips of microvilli and cilia (Dubreuil et al. 2007; Huttner et al. 2008; Karbanová et al. 2014; Marzesco et al. 2005) or the exocytosis of multivesicular bodies in non-epithelial cells (Bauer et al. 2011). This indicates that CD133 is also potentially involved in intercellular communication.

CD133 is expressed by a broad range of cells, particularly differentiated epithelial, glial, and photoreceptor cells as well as various somatic stem cells (e.g., neural and hematopoietic stem cells, cancer stem cells) (Corbeil et al. 2000, 2009; Karbanová 2008; Miraglia et al. 1997; Singh et al. 2004; Weigmann et al. 1997). In normal liver, CD133 is detectable on the apical membranes of cells present in the canals of Hering and bile ducts (Karbonová et al. 2008), hepatic progenitor cells (Suzuki et al. 2008), the microvilli of hepatocytes (Lee et al. 2020), and the cellular processes of hepatic stellate cells (Kordes et al. 2007; Rohn et al. 2018). CD133 shows a polarized appearance in stellate cells at specific cell membrane compartments that may be involved in intercellular communication (Figure 1). However, the function of this protein remains to be determined for stellate cells. Among cells that harbor developmental potential, CD133 has been found on hematopoietic stem cells (Miraglia et al. 1997) and mesenchymal stem cells of the bone marrow and adipose tissue (Kshitiz et al. 2019; Pal and Das 2017).

Stellate cells as mesenchymal stem cells

Before blood formation is established in the bone marrow, the liver represents an important site of hematopoiesis, which is performed by hematopoietic stem cells capable of forming all myeloid and lymphoid cell types of the blood (Spangrude et al. 1988). In the early 1900s, the fact that hematopoiesis is facilitated by progenitor cells in the bone marrow and that these cells are supported in this process by fibroblasts was discovered by Alexander Maximow, a Russian military physician (Maximow 1905, 1906). However, it became clear 100 years later that hematopoiesis could not occur without these supporting fibroblasts

Figure 1: Hepatic stellate cells and their perivascular niche.

In the normal liver, stellate cells reside in a quiescent state, as indicated by the presence of glial fibrillary acid protein (GFAP; red; left corners) and retinoids (blue; upper right corner). Moreover, unique proteins such as CD133/prominin-1 (green; upper left corner) and reelin (green; lower left corner) are expressed by quiescent hepatic stellate cells and have possible implications for cell-cell communication. Stellate cells are preserved in a quiescent state and maintained by their niche in the space of Disse, which is bounded by sinusoidal endothelial cells (SECs) and hepatocytes. This niche consists of basement membrane proteins such as laminin-521 (middle: proteome analysis of rat liver extracellular matrix) and collagen IV (COL IV; green; lower right corner) as well as signaling pathways such as the β -catenin-dependent WNT pathway. Stromal cell-derived factor 1 (SDF1/CXCL12) is released by endothelial cells and keeps stellate cells in their niche. Norepinephrine released by nerves of the sympathetic nervous system modulates prostaglandin synthesis in stellate cells. The fluid mechanics of the bloodstream sustain the expression of integrins and extracellular matrix proteins in hepatic stellate cells. The fluorescence images show normal rat liver tissue and were processed by 2D-deconvolution using the software cellSens Dimension (Olympus). The scale bars represent 20 μm . Cell nuclei were stained by 4',6-diamidino-2-phenylindole (DAPI; blue).

because the depletion of nestin-expressing fibroblasts in the bone marrow leads to impaired blood formation (Méndez-Ferrer et al. 2010). In the early 1990s, Arnold I. Caplan described these supporting fibroblasts as mesenchymal stem cells (MSCs), which seem to be derived from pericytes associated with blood vessels (Caplan 1991, 2008). Stellate cells are typical pericytes of sinusoids in the liver (Kostallari and Shah 2019). After the transplantation of hepatic stellate cells into lethally irradiated rats, we demonstrated for the first time that hepatic stellate cells themselves lack hematopoietic potential but assist hematopoietic stem cells in this process. Moreover, stellate cells are directly associated with hematopoietic GATA1-

positive cells in the fetal liver and home in the bone marrow after transplantation (Kordes et al. 2013a, 2014), which suggests that interaction with blood progenitor cells also seems conceivable *in vivo*. It took a further eight years for the supportive effect of stellate cells to be confirmed by another group, which found that stellate cells and endothelial cells maintain hematopoietic stem cells in the fetal liver by releasing stem cell factor (Lee et al. 2021).

Another typical property of MSCs is their differentiation into osteocytes (Friedenstein et al. 1968). Stellate cells also develop into osteocytes after treatment with differentiating culture media (Kordes et al. 2013a). However, MSCs not only contribute to bone formation via

differentiation into osteocytes but also develop into adipocytes and chondrocytes, among others. Notably, this differentiation capacity is widely used to identify MSCs. Although stellate cells can also transform into adipocytes and share the expression of many molecular markers with MSCs of the bone marrow (Kordes et al. 2013a), differences exist in the expression profiles of both cell populations. However, this can be explained by different functions, the status of their activation, and the influences of respective microenvironments. This can especially be seen in the fact that the MSCs of different organs and even of the same organ differ significantly based on scRNA-seq (Baryawno et al. 2019; Harman et al. 2020). Due to the well-known heterogeneity of MSCs, this cell type is mainly characterized by functional tests. As investigated by sqRNA-seq, stellate cells also exhibit distinct gene expression profiles depending on their localization in the liver and relative proximity to the central vein or portal field (Rosenthal et al. 2021). However, the striking similarities of stellate cells to MSCs known from other organs with respect to their differentiation potential and hematopoiesis support prompted our group to classify stellate cells as MSCs of the liver (Kordes et al. 2013a). Since stem cells are, by definition, self-renewing cells that harbor developmental potential (Till and McCulloch 1961), stellate cells were tested for their ability to differentiate (as previously outlined) and self-renew. If proliferating stellate cells are separated on a single-cell basis, cell clones can be generated that remain transplantable and retain developmental potential (Kordes et al. 2014).

Although the occurrence of MSCs has been postulated for all organs of the body (Crisan et al. 2008), stellate cells are difficult to identify as MSCs in normal liver. Only after their activation (e.g., by liver injury) do stellate cells show the upregulation of genes such as nestin (Niki et al. 1999), which are typically assigned to MSCs. Nestin expression is suppressed in quiescent stellate cells by epigenetic mechanisms such as histone modification (Reister et al. 2011). During activation, stellate cells show the strong cytosine demethylation of DNA (approximately 60%) (Götze et al. 2015; Schumacher et al. 2017). This demethylation of DNA mainly occurs in non-coding regions of the genome and accomplishes active enzyme-mediated processes that might involve a base excision repair mechanism (Schumacher et al. 2017). The mechanism and relevance of reduced DNA demethylation during stellate cell activation still require detailed investigation. The distinct epigenetic alterations involved in activating stellate cells are accompanied by profound changes in their gene expression that facilitate liver regeneration (Götze et al. 2015; Schumacher et al. 2017).

Stellate cells in liver regeneration

Stellate cells are activated after liver injury and release a variety of growth factors. This trophic effect has been described in the same manner for MSCs from other organs and likely represents a main function of stellate cells in the regenerating liver. Moreover, as per MSCs from bone marrow, hepatic stellate cells have immunomodulatory functions (Markov et al. 2021; Naji et al. 2019; Raicevic et al. 2015) that could also assist regenerative processes of the liver. Recent scRNA-seq analyses indicated that activated stellate cells in diseased liver (NASH) can be divided into four clusters. As expected, fibrogenic myofibroblasts were found, while intermediate activated, proliferating, and immune/inflammatory cell clusters were additionally identified (Rosenthal et al. 2021). Notably, the relevance of the immunomodulatory stellate cell cluster for diseased liver remains unknown. However, all clusters derived from the activation of stellate cells can regain a quiescent-like state during fibrosis regression (Rosenthal et al. 2021).

In addition to their trophic and immunomodulatory functions, stellate cells show a developmental potential that also includes the formation of hepatocytes and cholangiocytes, as demonstrated by our group and that of Anna Mae Diehl using transplantation and lineage tracing experiments (Kordes et al. 2012, 2014; Swiderska-Syn et al. 2014). The ability to differentiate into hepatocytes is a property of MSCs (Spitzhorn et al. 2018). However, other groups failed to detect a contribution of stellate cells to the reconstruction of liver mass when lineage tracing models were used (Lua et al. 2014; Mederake et al. 2013). Later studies showed that the outcome and involvement of facultative liver progenitor cells substantially depended on the injury model and its intensity (Raven et al. 2017). Moreover, hepatocytes and cholangiocytes exhibit high plasticity, making stem cell involvement in the regenerative process unnecessary in most cases. In this context, it is interesting to note that hepatoblasts (precursor cells of hepatocytes and cholangiocytes) with endodermal and mesenchymal markers were recently found in embryonic mouse liver using single-cell transcriptome analyses (Lotto et al. 2020). Notably, these hybrid cells were found approximately one day after stellate cells migrated into the developing liver. The origin of these hybrid cells remains to be elucidated. However, initiating stellate cell differentiation using a micromilieu in the embryonic liver that promotes developmental processes in migrating cells seems conceivable. The development of stellate cells into hepatocyte-like cells can be induced *in vitro* by using differentiating media containing growth factors or bile salts (Kordes et al. 2007, 2014; Sawitzka et al. 2015). Both

growth factors and bile salts can trigger differentiation and proliferation in stellate cells (Sawitza et al. 2015; Sommerfeld et al. 2009). However, it remains to be elucidated which signaling pathway networks control these competitive processes in stellate cells and under which conditions these cells form epithelial cells *in vivo*. Recent work has indicated that receptor tyrosine kinase-mediated signaling, adaptive stress response pathways, and β -catenin-dependent WNT signaling are involved in balancing cell proliferation, differentiation, and quiescence in hepatic stellate cells (Reichert et al. 2021). Notably, the micromilieu (or niche) of stellate cells ultimately controls their behavior.

Stellate cell niche

Stem cells maintain their potential to self-renew and differentiate in protected sites, which was first postulated by Raymond Schofield and termed “stem cell niche” (Schofield 1978). The interaction between hematopoietic stem cells and MSCs during hematopoiesis in the fetal liver suggests the existence of a stem cell niche in the liver (Kordes and Häussinger 2013b). Stellate cells are found as pericytes between sinusoidal endothelial cells (SECs) and hepatocytes in the space of Disse. Here, a basement membrane-like structure that contains collagen 4, fibronectin, and laminins can be found (Figure 1). According to proteomic analyses, laminin-521, which consists of $\alpha 5$, $\beta 2$, and $\gamma 1$ chains, is detectable in the liver matrix and maintains the quiescent state of stellate cells (Rohn et al. 2018). Thus, laminin-521 represents an important element of the stellate cell niche. Neighboring cells are also important to keep stem cells in their niche and preserve their characteristics. SECs can attract and retain stellate cells in the space of Disse via stromal cell-derived factor 1 (SDF1/CXCL12) release (Sawitza et al. 2009). Once activated, stellate cells also presumably begin to release SDF1 to attract other cells to sites of tissue injury or to retain hematopoietic stem cells in the fetal liver and ensure blood formation. SDF1 release by MSCs in bone marrow is controlled by the sympathetic nervous system through direct innervation (Katayama et al. 2006). Stellate cells are also innervated and release prostaglandins, among others, after stimulation by norepinephrine (Athari et al. 1994; Häussinger et al. 1987), which is the transmitter of sympathetic nerves (Figure 1). Additionally, SECs are capable of secreting ligands of the β -catenin-dependent WNT signaling pathway (Ding et al. 2010). This canonical WNT cascade favors the quiescent state of stellate cells and thus represents another important element of their niche (Kordes et al. 2008; Reichert et al. 2021). Additionally, there

is evidence of neighboring hepatocytes also releasing factors that control the target genes of this pathway and preserve the features of quiescent stellate cells (Sawitza et al. 2009). However, the ligands released by hepatocytes that trigger this effect in stellate cells remain unknown.

Although some elements of the stellate cell niche have been identified, the relevance of other potentially important factors on the maintenance of their function—such as cell-cell contacts (Notch and Hippo signaling pathways), low oxygen tension, the parasympathetic nervous system, and blood nutrient composition—is largely unknown and requires further analyses. Elevated oxygen levels, poor nutrition, and chronic inflammation can compromise the integrity of stem cell niches and trigger cell stress in stem cells, which impairs their ability to self-renew and initiates their differentiation (Hotamisligil and Davis 2016; Ito and Suda 2014; Jahandideh et al. 2020). Ultimately, the stem cell pool and regenerative capacity of an organ decline. The function of stellate cells is also impaired by aging processes, as demonstrated by our studies (Rohn et al. 2020). The aging liver shows an altered extracellular matrix and is significantly less perfused (Le Couteur and McLean 1998; Rohn et al. 2020). The latter is associated with the reduced mechanical stimulation of liver cells, which normally will stimulate cells to produce matrix and initiate the release of growth factors such as HGF from endothelial and stellate cells (Lorenz et al. 2018; Rohn et al. 2020). With age, stellate cells also exhibit the decreased expression of extracellular matrix proteins, integrins, and growth factors but an increase in inflammatory factors (Figure 2). This altered gene expression is partially regressed when stellate cells are exposed to mechanical forces (Rohn et al. 2020). These physical factors represent further elements of their niche (Figure 1).

Stellate cells express reelin

Several studies have reported the expression of reelin—a large, secreted glycoprotein that resembles extracellular matrix proteins—in the livers of different mammalian species (Botella-López et al. 2008; Carotti et al. 2017; Ikeda and Terashima 1997; Kobold et al. 2002; Samama and Boehm 2005; Smalheiser et al. 2000). Reelin is best known for its functions in the central nervous system, where it controls the positioning of postmitotic neurons and fulfills essential functions during neuronal development and in the adult brain (Wasser and Herz 2017). A major source of reelin outside the central nervous system is the liver, where its physiological functions are not well established. In the embryonic liver, reelin expression was detected as early as

Figure 2: Impaired stellate cell niche during aging.

Gene expression arrays suggest that stellate cells exhibit a senescence-associated secretory phenotype (SASP) and reduced glial fibrillary-acidic protein (GFAP) expression during aging. Additionally, the elevated expression of genes associated with cell migration (i.e., CXCR4/C-X-C motif chemokine receptor 4 and MMP13/matrix metalloproteinase 13) and the lower expression of integrins indicated that stellate cells are no longer retained in their niche when the liver ages. Furthermore, stellate cell functions are impaired by aging because the expression of extracellular matrix (ECM)-associated genes and growth factors (e.g., hepatocyte growth factor (HGF)) decline. An age-related decrease in the blood volume that enters the liver and regression of sinusoidal fenestrae is known. Thus, stellate cells are less exposed to mechanical stimuli that trigger the expression of genes such as integrin- $\alpha 5$, which decrease during aging. Moreover, stellate cells release HGF after exposure to mechanical stimuli in an integrin- $\alpha 5$ /integrin- $\beta 1$ -dependent manner and this effect significantly decreases in aged rat liver. Impaired stellate cell functions (e.g., HGF release) can contribute to the decreased regenerative capacity of aged livers.

E8.75 in hepatoblasts based on single-cell transcriptomics (Lotto et al. 2020). Early studies did not report gross morphological or developmental abnormalities in the livers of reelin-deficient reeler mice (Ikeda et al. 1997), where reelin expression is abrogated due to a spontaneous autosomal-recessive mutation in the *Reln* gene. Additional sources of extra-neuronal reelin include blood cells (i.e., lymphocytes and platelets), lymphatic endothelial cells, odontoblasts, and cells of the intestine (Khialeeva and Carpenter 2017; Liu et al. 2020; Smalheiser et al. 2000). Interestingly, reelin is also detected in the circulatory system. Plasma concentrations of reelin are altered under different pathophysiological conditions (e.g., it is

upregulated in multiple sclerosis, neurodegenerative diseases, and liver cirrhosis) (Botella-Lopez et al. 2006, 2008; Calvier et al. 2020), which suggests that circulating reelin concentrations might be useful as a diagnostic biomarker. Reelin has been described as a sensitive fibrosis marker in patients with hepatitis C virus infection (HCV)-associated liver fibrosis (Carotti et al. 2017; Dobie et al. 2019; Mansy et al. 2014) and as a prognostic factor in hepatocellular carcinoma (Okamura et al. 2011). On a functional level, several lines of evidence link reelin with liver fibrogenesis. In patients with liver fibrosis resulting from chronic HCV, levels of serum reelin are correlated with reelin immunoreactivity in the liver and the degree of liver fibrosis (Carotti

et al. 2017; Mansy et al. 2014). Thus, possible functions of hepatic reelin include the modulation of the inflammatory response mediated by liver-resident and invading immune cells and a contribution to regulating the activation state of hepatic stellate cells. This might involve auto-crine/paracrine signaling mechanisms, where reelin elicits changes in hepatic stellate cells and crosstalk with non-resident liver cells (e.g., macrophages and platelets). However, the underlying cellular and biochemical mechanisms have not been elucidated to date (Figure 3).

Furthermore, it remains unclear whether circulating reelin primarily originates from the liver or other sources. Somewhat surprisingly, hepatic stellate cells—not hepatocytes—were identified as the primary source of liver-derived reelin (Botella-Lopez et al. 2008; Carotti et al. 2017; Kobold et al. 2002; Lua et al. 2016; Magness et al. 2004). In a murine genetic liver injury/regeneration model where the hepatocyte-specific deletion of E3 ubiquitin ligase damaged DNA binding protein 1 blocks hepatocyte proliferation (Endo et al. 2012), the upregulation of reelin was

Figure 3: Reelin signaling in hepatic stellate cells.

Reelin is detectable in the blood, which suggests that stellate cell-derived reelin could exert paracrine effects on neighboring liver cells or be systemically relevant. Reelin may also exert autocrine effects as a component of the microenvironment of stellate cells (upper row). The potential functions of hepatic stellate cell-derived reelin involve modulation of the stem cell niche in the space of Disse and regulation of the quiescent/activation state of stellate cells. Stellate cell-derived reelin may also participate in regenerative processes. Possible target cells could include non-resident liver cells such as platelets or invading macrophages (bottom row, left). The binding of reelin oligomers to the extracellular domains of the lipoprotein receptors ApoER2/low-density lipoprotein receptor-related protein 8 (LRP8) or very-low-density lipoprotein receptor (VLDLR) induces phosphorylation of the intracellular adaptor protein disabled-1 (DAB1) via non-receptor tyrosine kinases of the Src family (SFK) (bottom row, middle) as known from experiments on responsive neurons. This leads to the activation of downstream signaling cascades, which eventually influence the differentiation, migration, and electrophysiological properties of responsive neurons. Thus far, it has not been demonstrated that this form of canonical reelin signaling also takes place in the context of the liver. Noncanonical reelin signaling via alternative receptors has also been described (bottom row, right). In addition to the classical high-affinity reelin receptors ApoER2 and VLDLR, other reelin-binding receptors (e.g., integrins) have been identified in different tissues and cell types, which may act independently of DAB1. Although it has not yet been shown, it is reasonable to assume that these noncanonical signaling pathways play an important role in mediating the effects of reelin also in the liver and other non-neuronal organs.

observed in bipotent transient-amplifying liver progenitor cells (oval cells), which give rise to hepatocytes and bile duct cholangiocytes following liver damage. This resembles the expression of reelin in bipotent migrating hepatoblasts during early liver organogenesis (Lotto et al. 2020). Thus, reelin seems to be expressed by cells that possess development potential. Notably, reelin is one of several neural and glial marker genes (e.g., glial fibrillary acid protein (GFAP), nestin, neurotrophins, neurotrophin receptors, and neural cell adhesion molecules) detected in hepatic stellate cells.

In the classical reelin signaling pathway first described in neurons, secreted reelin is bound by apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR), which belong to the LDL receptor gene family, whose members can perform classical signal-transducing functions in addition to transport and endocytosis functions (Herz and Bock 2002). The binding of oligomeric reelin complexes to the ectodomains of lipoprotein receptors induces the formation of receptor complexes that cause tyrosine phosphorylation of the cytoplasmic adapter protein disabled-1 (DAB1) (Hass et al. 2017), which interacts with the intracellular domains of ApoER2- and VLDLR (Figure 3). Src kinase-mediated DAB1 phosphorylation is essential for the intracellular propagation of the reelin signal. Phosphorylated DAB1 functions as an adaptor for the assembly of signaling complexes that modulate downstream signaling pathways including phosphoinositide 3 kinase, glycogen synthase kinase β , Rho GTPases, and cofilin; in this way, both the neuronal microtubule and actin cytoskeleton are regulated by reelin. A negative feedback loop via ubiquitination and the proteasomal degradation of phosphorylated DAB1 ensures the responsiveness of the signaling cascade (Bock and May 2016).

While liver-invading blood cells (monocytes) expressing components of the classical reelin signaling cascade (Baitsch et al. 2011) may interact with hepatic reelin via ApoER2 and VLDLR, communication with liver-resident target cells (e.g., hepatocytes, stellate cells, cholangiocytes) might involve alternative reelin signaling pathways (Figure 3). Alternative receptors that bind to reelin with lower affinity include β 1-integrin, the amyloid precursor protein, and Eph/ephrin proteins (Bouché et al. 2013; Hoe et al. 2009; Schmid et al. 2005). Interestingly, increased levels of DAB1 protein, which is barely expressed in non-fibrotic livers, were described in cells of the ductular reaction close to reelin-expressing cells (Carotti et al. 2017). This suggests a possible role of classical reelin signaling via DAB1 under pathological conditions. Hepatic reelin thus represents a diagnostically and pharmacologically relevant target, which has a

potentially causal influence on liver regeneration and fibrosis. However, the relevance of reelin for stellate cells and normal or regenerating liver requires investigation. Recent evidence suggests that reelin is expressed by human MSCs derived from induced pluripotent stem cells and prevents their proliferation (Zhang et al. 2019). While this finding may link the expression of reelin in stellate cells to their quiescent state, this relationship requires further investigation.

Conclusion

Over the last 20 years, our work on stellate cells has shown that the function of this cell cannot simply be reduced to extracellular matrix production. Instead, this is a response of stellate cells to the absence of proper reconstitution of liver mass in chronic liver diseases. Moreover, the activation of stellate cells is not negative per se. It is important for these cells to adopt altered gene expression and assist in the regeneration of the injured liver. This can occur through an increased release of trophic factors and, controlled by influences of the stellate cell niche, initiation of cell development. The properties and functions of activated stellate cells are similarly observed in the MSCs of other organs. The classification of stellate cells as MSCs initially seems to contradict findings indicating that MSCs are anti-fibrotic. However, this is not the case because MSC involvement in fibrogenesis has since been demonstrated by several studies (El Agha et al. 2017). New therapeutic approaches for patients with fibrosis/cirrhosis might lie in the further elucidation of stellate cell niche elements. Here, a comparison of the normal and chronically diseased situation may provide evidence of misdirected communication between stellate cells and their neighboring cells in the diseased liver. This presumably fails to provide appropriate feedback to stellate cells, which ultimately triggers fibrosis. Unraveling the demands of stellate cells will assist us in finding a suitable therapy to treat patients with chronic liver diseases.

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