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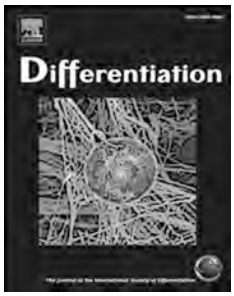

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The Role of Extracellular Matrix on Liver Stem Cell Fate: A Dynamic Relationship in Health and Disease

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Abstract

The liver stem cell niche is a specialized and dynamic microenvironment with biomechanical and biochemical characteristics that regulate stem cell behavior. This is feasible due to the coordination of a complex network of secreted factors, small molecules, neural, blood inputs and extracellular matrix (ECM) components involved in the regulation of stem cell fate (self-renewal, survival, and differentiation into more mature phenotypes like hepatocytes and cholangiocytes).

In this review, we describe and summarize all the major components that play essential roles in the liver stem cell niche, in particular, growth factor signaling and the biomechanical properties of the ECM.

Keywords: liver development, ECM, stem cell, progenitor cell, stem cell niche, growth factors, mechanobiology

1. Introduction

The stem cell niche is a specific and dynamic microenvironment in which many inputs such as signals departing from blood vessels, neural and supportive cells, as well as secreted factors, and extracellular matrix (ECM) components regulate stem cell behavior. These multiple signals with a physical, electrical, structural or biochemical nature are responsible for supporting stem cell properties.

In the last two decades, the role and importance of the ECM in cell biology has considerably increased, and currently, the ECM is recognized as an active entity composed of a variety of proteins and other molecules(Akhmanova et al., 2015). Besides, the structural, chemical and functional diversity of ECM components confers distinct biomechanical and biochemical properties to the ECM, providing a specific composition to the matrix, strictly correlated with the tissue, and sometimes species, of origin. In the liver, the extrahepatic stem cell niches are located in the peripheral or peribiliary glands inside the walls of the bile ducts, while the intrahepatic stem cell niches are found on the ductal plates of fetal livers and the Canals of Hering in the postnatal livers (Schmelzer et al., 2007). Although the major structural components of liver's ECM are collagens (COL) and fibronectin (FN), the matrix of bile ductules, where the progenitor cells reside, is mainly composed of COL IV and laminin (LN) (Terada and Nakanuma, 1994; Yasoshima et al., 2000).

The liver is known for its unique regenerative capability. The support of stem cell behavior provided by the stem cell niche helps maintain their quiescent state in homeostasis and regulates their self-renewal, expansion, and differentiation after activation. Following tissue injury, the surrounding microenvironment promotes self-renewal and differentiation of stem cells by activating and sending numerous signals. Moreover, the secretion of diverse growth factors (GF) such as TGF-α, EGF, FGF, and HGF play an essential role in liver development, health, and disease. The ECM is also a reservoir of GF and bioactive molecules by regulating their diffusion and availability (Hynes, 2009; Wilgus, 2012). It is constituted by adhesive molecules, notch signaling proteins, and proteoglycans which can bind and modulate GF activity (Lee et al., 2011).

Several authors have tried to determine which factors, signals or conditions control the fate choice of hepatic stem/progenitor cells to differentiate towards a specific cell type. This

review provides an in-depth summary of the most recent findings on the role of hepatic ECM in liver stem cell behavior.

2. Composition and function of stem cell niches in solid organs

In 1978, Raymond Schofield proposed that stem cells resided in specific locations called niches, which provided a complex multifactorial microenvironment (Schofield, 1978). Today, we can determine that a stem cell niche is an accurate and complex microenvironment within a specific anatomic location. It comprises cellular and acellular elements that interact with local and systemic signals for the regulation of the stem cell function (Kordes and Haussinger, 2013; Mohyeldin et al., 2010; Scadden, 2006), where the interactions of neighbors with stem cells via cadherins (Marthiens et al., 2010) and soluble paracrine signals play a critical role for stem cell maintenance and differentiation. Thus, the niche is not merely the place where stem cells reside, but it also compromises the microenvironment generated by the surrounding cells, involving the release of signals that induce stem cell quiescence. It also promotes stem cell asymmetric and symmetric divisions critical in tissue homeostasis, regeneration and repair, and ultimately the anchorage of stem cells and stromal cells to the ECM (Jones and Wagers, 2008). Although *in vivo* stem cell microenvironments are entirely inaccessible, it is usually regarded that the presence of a stem cell defines a genuine stem cell niche. Thus, despite the importance of all niche elements, the identification of a stem cell is the necessary evidence (Weissman, 2000).

Stem cells are frequently found on basement membranes (Fuchs et al., 2004), where specific adhesion molecules called integrins mediate the interactions. Alteration or loss of integrin expression gives rise to the recruitment of stem cells (Watt and Hogan, 2000). Additionally, the direct contact with peripheral nervous system elements helps to control stem cell recruitment.

A typical stem cell niche includes different components that are perfectly integrated and controlled. These components comprise the mentioned stem cells, stromal cells, ECM proteins, soluble factors, and innervation and neural inputs (Fig. 1). Other key niche factors are cell-cell and cell-ECM adhesions, as mentioned above. Although these are standard components, every particular tissue sustains different functional and specialized types of niches (Jones and Wagers, 2008). Mohyeldin *et al.* suggest that oxygen and other gaseous

messengers can also be considered as components of the stem cell niche, in the sense that they can also act as metabolic regulators of stem cell function (Mohyeldin et al., 2010).

3. Liver development

Hepatic development and organization begins by the third week of gestation and continues until the postnatal stage. The anterior portion of the hepatic diverticulum forms the intrahepatic biliary tree and the liver, whereas the posterior portion gives rise to the extrahepatic bile ducts and the gallbladder (Zorn, 2008).

From definitive endoderm, hepatocytes are originated during embryonic development after hepatic competence acquisition by ventral foregut endoderm and specification of those epithelial cells into hepatic endoderm (Zaret, 2008, 2016).

Primordial liver cells transition into a non-polarized cellular phenotype called hepatoblasts, which then generate the liver bud. These cells are bipotent progenitor cells that express fetal hepatic genes as well as cholangiocyte and hepatocyte lineage genes (Schmelzer et al., 2007). They can differentiate into both cell types depending on different signaling pathways. Notch and TGF-β promote biliary differentiation (Clotman et al., 2005; Decaens et al., 2008; Tanimizu and Miyajima, 2004). However, the specification of hepatoblasts toward hepatocyte fate is promoted when these pathways are down-regulated (Huch et al., 2013; Huch et al., 2015; Nantasanti et al., 2015).

4. Liver stem cell niches

The human hepatic stem cells are located in the ductal plates of the fetal and neonatal livers, and in the Canals of Hering in pediatric and adult livers (Kordes and Haussinger, 2013; Kuwahara et al., 2008; Saxena and Theise, 2004; Schmelzer et al., 2007; Stachelscheid et al., 2009; Zhang et al., 2008; Zhou et al., 2007). These cells constitute approximately 0.5-2% of the parenchyma and have a size that ranges between 7-10 µm in diameter with a high nuclear-cytoplasm ratio. They express EpCAM, NCAM, CD133, CXCR4, SOX9, SOX17, FOXA2, CK8/18/19, Hedgehog protein, Claudin 3, and ALB at deficient levels. They do not express AFP, ICAM-1 or endothelial, mesenchymal, and hematopoietic markers(Schmelzer et al., 2007).

The hepatoblasts reside mainly in the parenchyma of fetal and neonatal livers or as small aggregates in the Canals of Hering in adult livers (Zhang et al., 2008). These progenitor cells have a larger size than the hepatic stem cells (10-12 μm), as well as more substantial amount of cytoplasm; however, they have an overlapping antigenic profile (Schmelzer et al., 2006; Schmelzer et al., 2007; Theise et al., 1999). They share the expression of CXCR4, CD133, SOX17, CK8/18/19, Hedgehog proteins and the non-expression of hematopoietic, endothelial or mesenchymal markers. The differences are found in the reduction of the expression levels of EpCAM, in the presence of high levels of ALB with discrete cytoplasmic packing, the change of expression of NCAM by ICAM-1, and a strong expression of AFP, amongst others. The percentage of these cells in postnatal livers decreases to <0.01% of parenchymal cells (Schmelzer et al., 2007; Zhang et al., 2008). On the contrary, they undergo a significant expansion during specific regenerative processes associated with several diseases such as cirrhosis and toxic injury.

In the past decade, other niches of stem cells have been identified in the large intrahepatic bile ducts, which have a distinct histology, resembling the one found in the extrahepatic bile ducts (Cardinale et al., 2011; Carpino et al., 2012). This is a different stem cell niche located in the peribiliary glands, which contain a different stem cell population known as human biliary tree stem/progenitor cells (hBTSCs), which can be differentiated into cholangiocytes and hepatocytes (Reid, 2016; Semeraro et al., 2012). hBTSCs originated from the common bile duct are also amenable to be differentiated into pancreatic cell lineages (Cardinale et al., 2015; Wang et al., 2013), displaying the nature of a multipotent stem cell.

5. Role of liver ECM in the liver stem cell niche

5.1. The interaction between ECM and hepatic stem cells

The process of ECM renovation is a complex but remarkably synchronized procedure resulting from the equilibrium among production, secretion, degradation (Lu et al., 2011). Although ECM comprises less than 3% in a healthy liver section (Gressner, 1992), and minimal modification on the ECM has a direct consequence in hepatic functions (Bedossa and Paradis, 2003).

The principal structural components of the liver ECM are COL and FN. COL I and III are expressed in the portal stroma, space of Disse, liver capsule, and fibroid tissue (Aycock and Seyer, 1989; Martinez-Hernandez, 1984). COL IV and LN give rise to the basal lamina of the blood vessels and bile ducts (Martinez-Hernandez, 1984). COL V forms thin fibers located in the center of thick COL I and III fibrils. On the other hand, FN is a glycoprotein that can be found in the liver capsule, portal stroma, and space of Disse. FN levels increase during liver regeneration (Aziz-Seible and Casey, 2011), while the absence of that is correlated with cirrhosis, liver stiffness, and disorganized COL network (Iwasaki et al., 2016). Cell-cell interaction and also cell-matrix interplay are essential in the regulation of the stem cell behavior within niches (Spradling et al., 2001). In the liver, the ECM and basement membrane of bile ducts, where the progenitor cells reside, are mainly composed of COL IV and LN (Terada and Nakanuma, 1994; Yasoshima et al., 2000).

It is known that the liver has a significant regenerative ability. Although hepatocytes have a slow turn-over, hepatic injury promotes a rapid reconstitution of liver mass, where inflammatory cytokines such as TNF-α and IL-6 produced by Kupffer cells (Kwon et al., 2015) trigger hepatocytes to enter the cell cycle ($G_0 \rightarrow G_1$). As hepatocyte proliferation is overwhelmed by some drugs, toxins or in chronic liver diseases, liver stem/progenitor cells contribute to liver regeneration (Roskams et al., 2003; Vig et al., 2006). However, little is known about the surrounding environment of the hepatic progenitor cells. Different hepatic chronic diseases are characterized by excessive deposition of ECM because of continuous liver damage. Murata *et al*. demonstrated that fibrotic livers have more than five-fold increase in COL deposition compared to a healthy organ (Murata et al., 1984).

Stuart Forbes' research group demonstrated that during liver damage, both in rodents and humans, there are specialized niches around hepatic progenitors where the LN helps the maintenance of undifferentiated progenitor cells (Lorenzini et al., 2010). Furthermore, LN also promoted the expression of biliary/hepatoblast genes and significantly inhibited the expression of early hepatocyte genes, demonstrating the role of this protein in the control of liver progenitor cell fates (Lorenzini et al., 2010). Studying the expansion of liver progenitor cells in a choline-deficient ethionine-supplemented model, it was observed that the deposition of ECM precedes the expansion and migration of the progenitor cells (Van Hul et al., 2009). This accumulation of COL I and LN was found in front of these progenitor

cells along the portal-venous gradient of lobular invasion(Van Hul et al., 2009). Therefore, changes in the ECM composition alter the cell signaling in liver, facilitating either normal regeneration or paving the way for liver disease (Williams et al., 2014). Recently, Klaas *et al*. studied the role of the ECM in liver regeneration (Klaas et al., 2016). They observed considerable alterations (COL, FN, and elastin) as well as in non-structural proteins. These changes resulted in the rearrangement and increase of the stiffness in damaged liver ECM (Klaas et al., 2016). Takayama *et al*. succeeded to culture and expand pluripotent stem cell-derived hepatoblasts on dishes coated with human LN-111. These cells were maintained for more than three months and could differentiate into both hepatocyte-like cells and cholangiocyte-like cells (Takayama et al., 2013).

5.2. Growth factor presentation

GF are key intercellular signaling molecules (mostly proteins) that direct cells during development and adult organisms, controlling cell growth, migration, and differentiation (Lee et al., 2011).

Many GF and small molecules play critical roles in the hepatic specification and stem cell maturation (Chen et al., 2018). TGF-α and EGF are just a few examples of liver autocrine GF signaling. They are produced both by hepatocytes and non-parenchymal cells, and concentration gradients of these molecules induce different liver cell behaviors. In the case of HGF, it can cause hepatocyte loss, leading to an embryonic liver size reduction (Schmidt et al., 1995). FGF also plays an essential role in liver development, health and disease. Itoh *et al.* concluded that these molecules could work at the paracrine and endocrine level (Itoh et al., 2016). Examples of FGF as paracrine factors are FGF8 and FGF10, involved in embryonic liver development; FGF7 and FGF9, in tissue repair after liver injury, and FGF5, FGF8, FGF9 in the development and progression of hepatocellular carcinoma.

On the other hand, FGF15/19 and FGF21 are endocrine signals that play critical roles in the bile acid metabolism. Insulin is also known by preserving many hepatocyte-specific functions, like albumin secretion by hepatocytes, lipogenesis, and glycogenesis, amongst others. OSM is an interleukin family cytokine involved in hepatic maturation and the induction of hepatocyte-specific functions such as lipid synthesis, detoxification, and ammonia clearance. However, it has also been shown that progenitor cells that receive OSM

do not mature, despite stimulating hepatocyte differentiation. Interestingly, hepatocyte function is recovered when OSM is removed from the environment (Levy et al., 2015).

The ECM can additionally be considered as a reservoir of GF and bioactive molecules, regulating their diffusion (Hynes, 2009; Wilgus, 2012). It contains many components like adhesive proteins, notch signaling molecules, and proteoglycans which can bind and modulate GF activity (Lee et al., 2011). In other cases, GF can be released through ECM and proteoglycan degradation. Furthermore, the ECM can storage immature molecules until they are activated. For example, TGFβ, which stimulates biliary differentiation, is secreted in an inactivated way and remains retained in the ECM until its activation (Yue, 2014). Downregulation of this signaling pathway allows hepatoblasts to differentiate into hepatocytes (Huch et al., 2015) (Fig. 2). Additionally, some GF contain heparin-binding domains (HBD), which are critical for the modulation of biological activities like cell proliferation, differentiation, morphogenesis, and angiogenesis. The first growth factors isolated with this motif were bFGF (FGF2) (Bohlen et al., 1984) and aFGF (FGF1) (Bohlen et al., 1985). Since then, a vast family of GFs containing an HBD was identified (e.g., VEGF, PDGF, EGF, other FGFs, TGFbetc) (Rider and Mulloy, 2017).

Proteoglycans are proteins that carry glycosaminoglycans (GAGs), a vital carbohydrate that exists in four forms in the liver and in different quantities: heparan sulfate, chondroitin sulfate, dermatan sulfate, and hyaluronic acid (Baghy et al., 2016; Kjellen and Lindahl, 1991). Complexes formed between GAGs and GFs, like FGF-heparin or FGF-heparan sulfate complexes, protect the GFs from degradation. Furthermore, heparan sulfate plays key roles in binding and interacting with GF, plasma proteins, and other factors, allowing with this, the regulation of protein distribution, bio-availability, and action to target cells. Even though there are well-known signaling proteins that interact with heparan sulfate domains, there are still knowledge gaps that remain to be elucidated (Billings and Pacifici, 2015).

All these mechanisms act on stem cells and stem cell niches, modulating stem cell proliferation and differentiation.

5.3. Mechanobiology of ECM and liver Stem Cells.

In the last years, the field of biomedical engineering has continuously evolved. This progress has introduced new relevant knowledge, as the concept of mechanobiology.

Mechanobiology is a scientific area at the interface of the biology and engineering, focused on the study of the role that tissue biophysical stimulus and mechanical environment (force, geometry, topography, stiffness, matrix elasticity) play in cellular processes, including differentiation, injury response, physiology, and pathology (Engler et al., 2006; Ingber, 2018). Consequently, mechanobiology identifies that cells can recognize not only biochemical signals, but also physical factors that impact their normal functioning and maintenance (Lim et al., 2010).

Currently, it is well documented that all the cells in the body are mechanosensitive (Jaalouk and Lammerding, 2009), including liver cells. This organ was not considered to be associated with a mechanical load in the past. Hence, liver mechanobiology represents a field in progress that might be critical in areas such as development, pathology, and tissue engineering. In this section, we sought to provide a thorough description of hepatic mechanobiology and ECM contribution.

An essential component of cellular mechanosensitivity is the capability of the cell-cell and cell-matrix interactions to detect and respond to the mechanical stiffness of its surface. The focal adhesion complexes are the points where cell-matrix interactions take place, mediated by integrin molecules that link the ECM to the actin-myosin cytoskeleton of the cell. The contraction promoted by the complex actin-myosin cytoskeleton allows the cell to survey its mechanical environment through the movement of the integrin. In general, when the ECM is stiff, the contraction of the cellular cytoskeleton is more difficult, and it generates an accumulation of integrins, enlarged focal adhesions, and further development of the cytoskeleton (Janmey and Miller, 2011). The integrin movement is a downstream signaling pathway where the Rho/GTPases and the contraction of the actin-myosin cytoskeleton (Wells, 2008a) are the primary mediators. Cell-cell interactions are mediated via cadherins, creating a bridge between the cytoskeleton of two neighboring cells (Smutny and Yap, 2010). Taking into account this premise, the optimal stiffness for culturing and expanding any given cell type corresponds to the *in vivo* elastic modulus of its corresponding tissue (Pedro M.Baptista, 2014). Sometimes, the stiffness of a tissue or a substrate is defined regarding Elastic or Young's modulus, a constant that refers to the ability of a material to resist a determined deformation, or the ratio of strain when stress is applied. The liver, which is a soft organ, is described as viscoelastic with a non-linear stress-strain behavior

(Suh and DiSilvestro, 1999). The effects of these ECM properties on cell mechanosensitivity are mostly unknown (Pedro M.Baptista, 2014).

The chemical structure of a tissue and the organization of all its components, as COL, proteoglycans, and FN amongst others contribute to mechanical strength and behavior of the tissue. Consequently, modifications produced in any of these components, lead to alterations in the mechanical properties of the tissue (Wells, 2008c). The human liver is composed by a population of stem/progenitor cells located in the stem cell niche. The components of the ECM in the liver stem cell niche are exclusive when compared with the rest of the liver, and therefore, the mechanical properties or stiffness of these regions are different from the rest of the organ (Lozoya et al., 2011). An excellent example of mechanically controlled cell response is the human hepatic stem cells that can differentiate into hepatocytes on soft surfaces, while cholangiocytes are observed on more rigid surfaces (LeCluyse et al., 2012).

In the liver, the property of stiffness is not static, and it fluctuates with organ development, disease, and repair. During early liver development, human progenitor stem cells in their undifferentiated state are maintained in the endoderm due to a low stiffness environment. The creation and development of ECM structures, cytokines, and environmental stimuli usually initiate cell migration and differentiation of human progenitor stem cells into more mature parenchymal liver cells (Si-Tayeb et al., 2010a; Si-Tayeb et al., 2010b). These determinations have been observed in *vitro* experiments, where the use of low stiffness substrates promoted the maintenance and expression of stem cell markers in human progenitor stem cells (Schrader et al., 2011). Similarly, in work performed by Lozoya *et al*. (Lozoya et al., 2011), human hepatic stem cells were cultivated in 6 different hydrogel formulations during one week and then, the degree of differentiation was analyzed by using various markers. The generated results demonstrated a stiffness-dependent behavior of these cells (Smutny and Yap, 2010), suggesting that cells seeded on their desirable mechanical environment allow to organize themselves in the same way observed in the stem cell niche.

In mechanobiology, another significant stimulus is represented by the shear stress. Human liver stem, progenitor, and embryonic stem cells have been shown to differentiate into

mature liver cells when exposed to shear stress in perfusion bioreactor cultures (Miki et al., 2011; Schmelzer et al., 2010). The work developed by Toshio *et al.* demonstrated the potential of using a four-compartment 3D perfusion culture to induce hepatic differentiation in embryonic stem cells. In this study, the hepatic differentiation was carried out by using a combination of cytokines with the culture in a dynamic 3D perfusion system. This study showed that the 3D perfusion culture induced more functional maturation in embryonic stem-derived hepatic cells, compared with 2D cultures and consequently, the use of 3D perfusion bioreactor technologies may be useful for further studies on generating embryonic stem cell-derived hepatic cells (Miki et al., 2011; Schmelzer et al., 2010). Finally, another type of mechanical force to consider in the liver is the interstitial fluid pressure, which hepatocytes are sensitive and able to respond to (Hsu et al., 2010).

In the context of partial hepatectomy, the loss of tissue is completely regenerated *de novo* through hepatocyte proliferation and the activation of matrix-producing cells. Through regeneration, a provisional matrix is generated during the early stages, and it contains uncrosslinked COL I (T Kim, 2003). When regeneration is mediated by progenitor cells, the initial composition of the matrix maintains the hepatoblast phenotype until ECM structures are fully formed with the addition of COL IV and the crosslinking of COL I (Zhang et al., 2008). If proliferating hepatocytes initiate the regeneration, the production of the matrix takes place by human hepatic stem cells simultaneously. In this process, physical and chemical cues provided by the ECM control the initiation and cessation of cell propagation (Kordes et al., 2014). Along the same line, alterations in this process produce an increase in stiffness and consequently, liver dysfunction, being the last one an essential parameter in the prognosis of liver diseases and hepatocellular carcinoma (Jung et al., 2011; Tsukuma et al., 1993). During the fibrotic state, the human hepatic stem cells are activated and an excess amount of LN and COL, especially COL IV, are deposited (Wells, 2008b).

In summary, the correct interplay between the ECM biomechanical and biochemical environment is a crucial factor for liver stem/progenitor and more mature cells in homeostasis, development, regeneration, and disease.

5.4. Novel tools for ECM – Stem Cell interaction research

Ideally, the best method to study and determine the interactions of ECM components with stem cells is within *in vivo* studies. However, these studies are difficult to perform due to the complexity of the stem cell niche and the strict legislation that controls the human/animal experimentation. Focused on animal experimentation, apart from ethical considerations, the work with animals is time consuming, laborious and expensive (Andersen and Winter, 2017). These issues have forced researchers to find new alternatives to decrease the time and resources involved in these type of studies and, naturally, to decrease the number of animals used. The term 3R'S (reduction, refinement, and replacement), is an alternative strategy that was defined by Russell and Burch and that included a large variety of new techniques that intend to recreate the physiological environment found *in vivo* without the use of animals (Arora et al., 2011; Franco et al., 2018).

An excellent example of this strategy is the work developed by M. Huch *et al*. in 2013 when they turned to 3D cell culture systems to investigate the stem cell biology of the liver. The structures generated in these culture systems were called organoids (Huch and Koo, 2015) and defined as a 3D structure derived from either pluripotent stem cells, neonatal tissue stem cells or adult-derived stem/progenitor cells. In these, cells spontaneously self-organize into structures that resemble the *in vivo* tissue concerning the cellular composition and tissue function (Hindley et al., 2016). In the publication mentioned above, researchers showed that the Wnt-target Lgr5 labels actively proliferating cells in the adult mouse liver following toxic damage. When these Lgr5 positive cells were expanded and self-organized into 3D cystic structures by using Matrigel® and by adding a chemically defined growth medium containing specific GF, these cells had the potential of differentiating into both hepatocytes and cholangiocytes *in vivo* (Huch et al., 2013). In 2015, by employing a similar strategy, this research group was able to establish human hepatic organoids from both healthy liver biopsies and single EpCAM positive cells (Huch et al., 2015). Hence, organoid cultures represent an ideal tool for studying stem cell niche interactions in a 3D ECM microenvironment, since this method is amenable to ECM manipulations that might shed some light in knowledge gaps of certain liver diseases and help translational medicine, by providing a more physiologically relevant 3D *in vitro* model system.

Another tool that has attracted significant interest in the last decades is the use of tissue/organ decellularization. This process usually consists of the perfusion of a detergent

solution in order to remove cellular contents, preserving only the ECM of the organ. The decellularization method applied in human, porcine and rat liver ECM generated scaffolds containing the major proteins present in the liver ECM, like LN, COL, or FN, and also the ECM-bound GF. These properties improve the physiology of the *in vitro* microenvironment (Soto-Gutierrez et al., 2011; Wang et al., 2016). In a recent study, Vyas *et al.* showed that human fetal liver progenitor cells self-assembled inside acellular liver scaffold discs to then form 3D liver organoids, which were able to recapitulate several aspects of hepatobiliary organogenesis and resulted in the parallel formation of progressively more differentiated hepatocytes and bile duct structures. This model shows relevant information about mechanisms of hepatic and biliary development and could be an exciting model in the future of disease modeling and drug screening (Vyas et al., 2017).

The link between biomedical engineering and biology has allowed the creation of a new alternative tool: Bioprinting. It consists in the use of spatial patterning of living cells and other non-living biologic materials employing an additive manufacturing technique (Ozbolat et al., 2016). This technique allows for the precise control of the microarchitecture and macroarchitecture of tissues and organs, which is critical to the function of many biological structures (Leberfinger et al., 2017). A clear example of this technology was reported recently by the group of Alan Faulkner-Jones *et al.* where these researchers reported the first investigation into the bioprinting of human pluripotent stem cells, their response to a valve-based printing process, as well as their post-printing differentiation into hepatocyte-like cells. The hepatic-like cells were examined for the presence of hepatic markers to further validate the compatibility of the valve-based bioprinting process with fragile cell transfer (Faulkner-Jones et al., 2015).

Throughout this section, we have described technologically sophisticated engineering tools that allow more precise control over the liver stem cell microenvironment, amenable to test the relevant role played by the use of decellularized ECM or its multiple components, as well as the use of specific GFs. These new tools have the potential to promote further advances in diverse areas such as the study of liver development, homeostasis, and disease, by recreating much more accurately the micro/macroenvironment found *in vivo*.

6. Conclusion

It is well known that the liver is an organ with a remarkable regenerative capacity. Part of this regenerative ability is possible due to the synchronous proliferation of stem cells and ECM remodeling in the stem cell niche, which provides a complex multifactorial microenvironment where many inputs regulate stem/progenitor cell behavior.

Traditionally, decades ago, the liver ECM was considered to be an inert cell growth substrate. However, due to the developments made in the last decades, it is now recognized that the liver ECM is a dynamic structure, which is composed of a variety of proteins and other macromolecules that work as a supportive scaffold, regulating cell biological functions. Finally, the ECM mechanobiology is also vital in the regulation of stem cell behavior, where cells can detect and identify physical factors. Therefore, the correct performance and maintenance between the biophysical, biomechanical and biochemical microenvironment of the ECM and liver stem cells are essential to direct stem and progenitor cell quiescence, proliferation, and differentiation, profoundly impacting organ homeostasis, repair, regeneration, and disease.

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Competing interest

All the authors have read and understood the Journal of Differentiation policy on declaration of interests and declare that we have no competing interests.

List of Abbreviations

COL: Collagen

ECM: Extracellular Matrix

FN: Fibronectin

GF: Growth Factors

HBGF: Heparin-Binding Domains

LN: Laminin

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Figures and legends

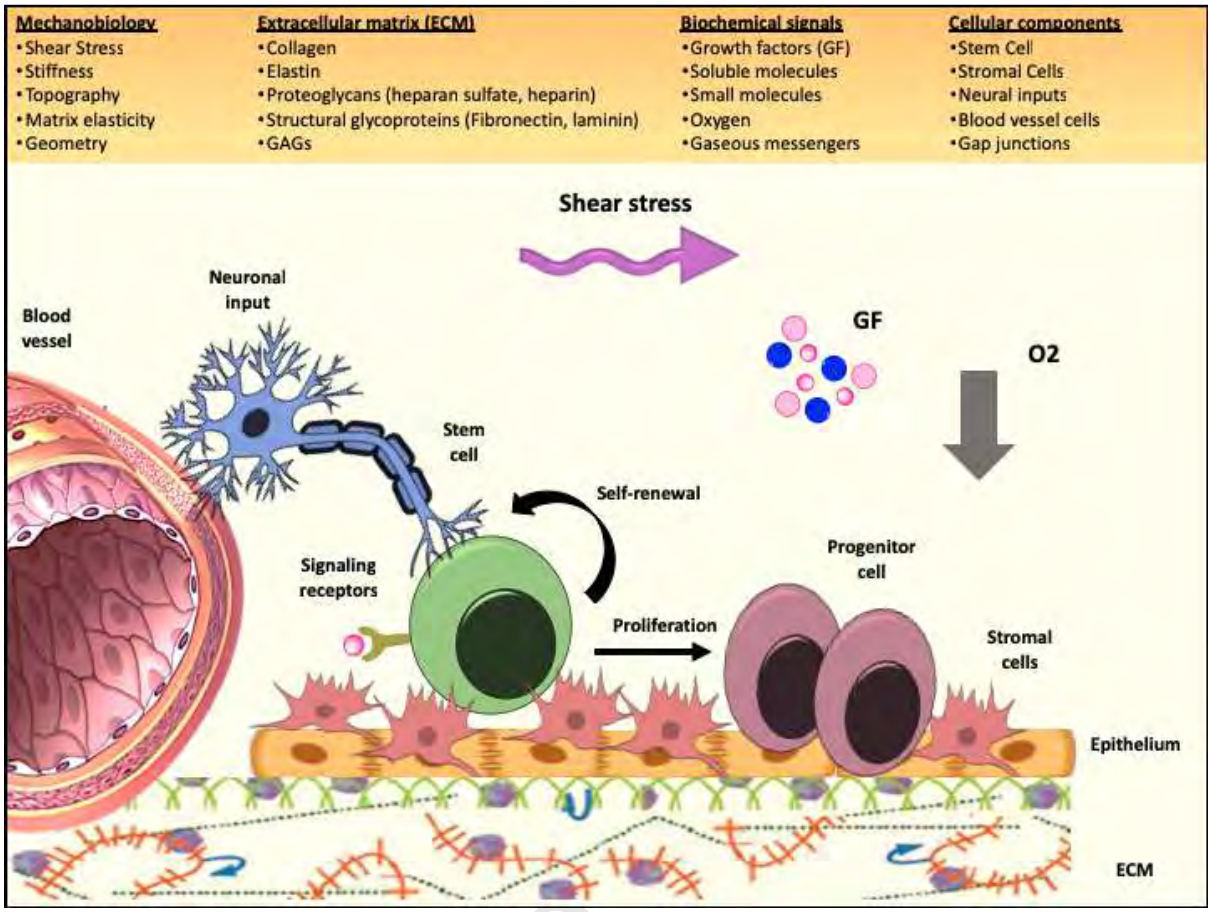

Figure 1: Stem cell niche components. Stem cell niches are specific microenvironments composed of multiple cells, such as neurons, stromal, epithelial, as well as blood vessels. Autocrine and paracrine signals mediate the interactions between all these componentssignals. Secreted molecules can also interact with cells which are not nearby, distributed via the bloodstream to different parts of the body. The ECM, which the main structural components are collagens and fibronectin, plays an essential role in the cellular niche functioning as a supportive scaffold that regulates the biological functions of the cells and it can be considered as a reservoir of growth factors and bioactive molecules, regulating their diffusion and availability. Here, it is also necessary to mention the role played by the mechanobiology, where physical signals are also critical for the correct ECM-stem cell interaction. The stem cell niche maintains and controls the fate of the stem cells, supporting self-renewal and maintaining the balance between quiescence, proliferation and differentiation.

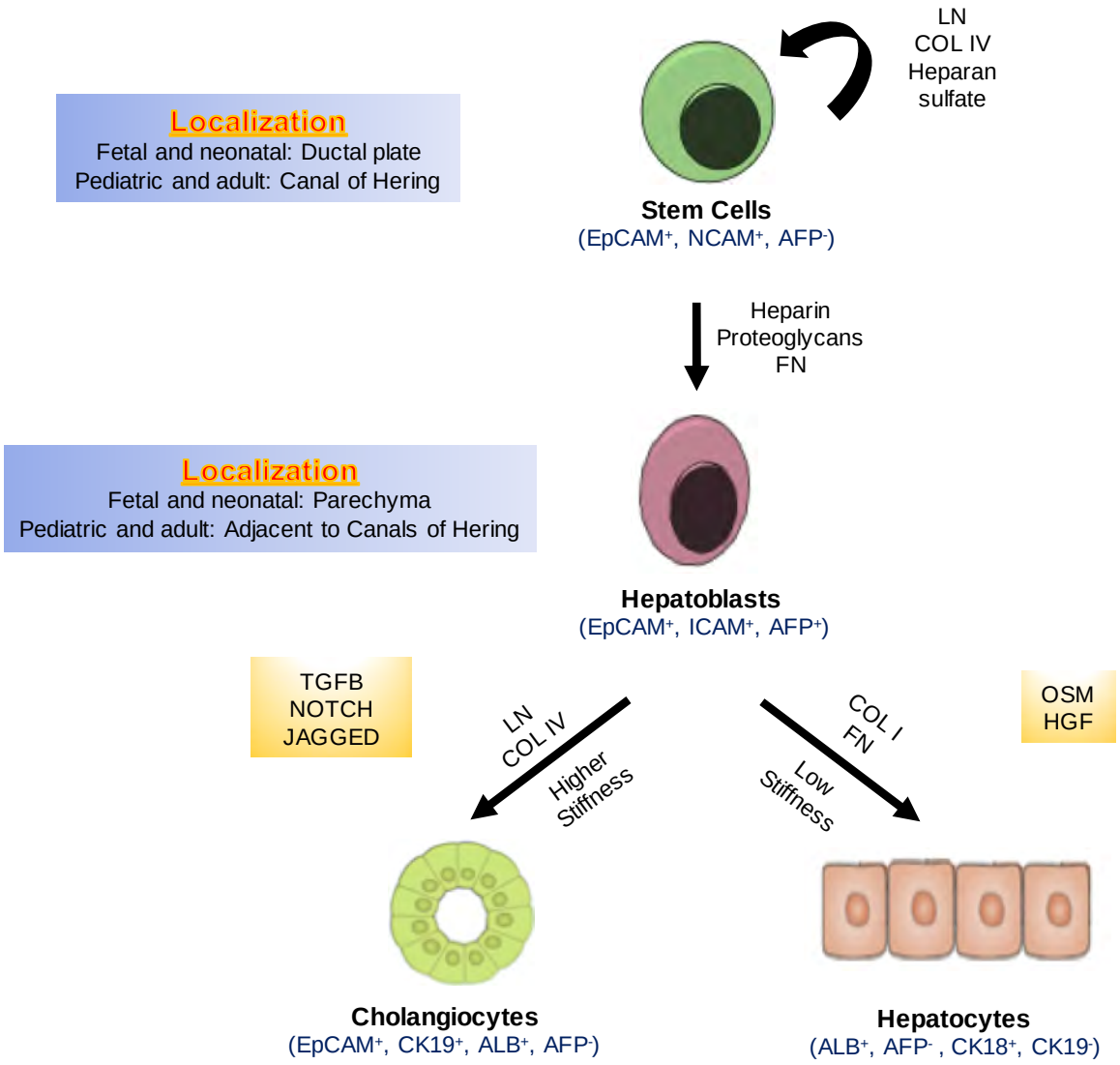

Figure 2: Hepatic lineage. Different ECM components, growth factors, and signaling pathways are involved in the hepatic cell fate choice, as well as the location of the stem cells and hepatoblasts during human development. Laminin, collagen IV and heparin sulfate are ECM components involved in the stem cell self-renewal. Heparin, proteoglycans, and fibronectin are necessary for stem cell differentiation into hepatoblasts. Laminin and collagen IV give rise to a cholangiocyte fate, whereas collagen I and fibronectin are responsible for hepatocyte differentiation. Hepatoblasts can differentiate into cholangiocytes and hepatocytes depending on different signaling pathways. Mainly, Notch, TGFB and Jagged promote biliary differentiation, meanwhile, Oncostatin M and HGF drive hepatocyte lineage. The primary markers that are specific of these cells are also described in brackets.