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Review

Tissue remodelling in liver diseases

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Summary. Tissue remodelling is a dynamic process that occurs during fetal or adult life and involves a modification of the original organization and function of a tissue. Tissue remodelling is observed in physiological and pathological conditions such as during wound healing or in the mammary gland during the course of pregnancy. In this review we will discuss the remodelling occurring in the liver as a consequence of chronic inflammation, as observed in chronic hepatitis, or as a consequence of hepatocellular carcinoma (HCC) progression in more detail. We will consider how altered deposition and turn-over of extracellular matrix (ECM) proteins could lead to development of liver fibrosis, and how the exacerbation of fibrosis could underlie the development of cirrhosis. The involvement of inflammatory and anti-inflammatory cytokines commonly used as therapeutic agents, such as Interferon-α, is then evaluated with a particular focus on modulation of ECM proteolysis. Finally, we analyze the role of alterations of the surrounding microenvironment including ECM, growth factors, cytokines and membrane receptors for ECM ligands in the development of HCC and in its invasive behaviour.

Key words: Tissue remodelling, Extra-cellular matrix proteins, Matrix metalloproteinases, Integrins, Chronic hepatitis, Liver fibrosis, Hepatocellular carcinoma

Introduction

The ability of the liver to regenerate was well known in ancient times, as illustrated by the legend of Prometheus, whose liver was eaten by an eagle every day and regenerated every night. Now we can say that regeneration of the liver and the consequent tissue remodelling that follows is not a never ending resource; on the contrary it may play a central role in the pathogenetic basis of chronic liver diseases regardless of their etiology.

In many physiological and pathological conditions, the liver, as well as other tissues, undergoes rearrangement of the original architecture, with consequent changes in the spatial organization and redefinition of the histological borders. This is well known to occur during embryonic life, when pluripotent stem cells differentiate to generate organs and tissues. There are also many examples in the adult life. The mammary gland is a typical example of an organ that completes its development during adult life with macroscopic modifications of the tissue. Another example of tissue remodelling is cancer development, which implies formation of new tissues in the context of a pre-existing organ structure. In this situation, break-down of tissue boundaries and rearrangement of tissue architecture seem to be regulated by matrix metalloproteinases (MMPs), a large family of zinc-endopeptidases with proteolytic activity toward extracellular matrix (ECM) components.

The family of MMPs includes at least 23 members, and the list seems likely to grow longer. According to current classifications, MMPs can be grouped on the basis of their biochemical characteristics or substrate specificity. Although some differences exist among the components of the MMP family, they also have major aspects in common, such as structural homology and overlapping activities toward ECM components. The reason for such biological redundancy is not well understood, but it probably points to the indispensable nature of MMP activities. One member of the MMP family is MMP2, widespread in the human body with a wide spectrum of action toward the ECM. It is secreted as a pro-enzyme, and activated at the cellular surface by a membrane-type MMP (MT1-MMP) with the aid of an MMP inhibitor, TIMP2 (Strongin et al., 1993; Itoh et al., 1995; Kinoshita et al., 1998; Nagase, 1998).

The proteolytic activity of MMP2 is balanced by the presence of TIMP2, that has a different role since it participates in MMP-2 activation, probably facilitating binding of MMP-2 to MT1-MMP, but it is also the natural inhibitor of MMP-2, so that a feedback system in

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the regulation of the proteolysis exists (Fridman et al., 1993; Itoh et al., 1998).

Tissue remodelling in physiological conditions

Tissue remodelling occurs in a number of physiological situations during adult life. For example, at sexual maturation, the mammary gland begins its development, which then continues during the ovarian cycles under hormonal regulation. The gland finally completes its growth during the first pregnancy, so that its main functions can be accomplished during the lactation phase. Breast epithelial cells proliferate following a pattern of branching morphogenesis. The cells located at the tip of the end buds penetrate through the fat pad, travelling for a minimum distance of 2 mm (Williams and Daniel, 1983; Werb et al., 1996; Giannelli et al., 1999). The development and morphogenesis of the mammary gland requires cross-talk between epithelial cells and the surrounding microenvironment, which includes myoepithelial and stromal cells, as well as extracellular matrix (ECM) components in the basement membrane (BM). Some of these interactions are ensured by a class of transmembrane receptors known as integrins, which bind to ECM macromolecules and mediate adhesion and migration. In addition, integrins can deliver intracellular signals that affect proliferation, apoptosis and differentiation. Changes in the ECM microenvironment sensed by integrins can therefore have dramatic effects on breast gland epithelial cells.

This scenario is complicated by the presence of hormones, cytokines, growth factors and proteases such as matrix metalloproteinases (MMPs), that rearrange and modify the surrounding microenvironment thus influencing epithelial cell behavior.

A dynamic model for studying tissue remodelling is offered by keratinocytes that in quiescent situation are almost immobile cells (Carter et al., 1991; Jones et al., 1991; Gil et al., 1994; Xia et al., 1996; Borradori and Sonnenberg, 1999). However, under certain circumstances such as wound healing, keratinocytes acquire a mobile phenotype until complete healing has been achieved and they revert to being static cells (Makela et al., 1999; Nguyen et al., 2000).

Tissue remodelling in chronic liver diseases

As reported for other tissues, the liver is also a site of tissue remodelling. This aspect is of clinical interest because chronic damage to the liver induces necrosis of the parenchyma with consequent increased deposition of ECM that underlies the occurrence of liver cirrhosis (fibrosis) (Moshage, 1997; Levy et al., 2000).

Mechanisms responsible for altered production and turnover of ECM proteins in the liver are not fully understood, although it is generally agreed upon that they play an important role in liver tissue remodelling (Bianchi et al., 1984; Knittel et al., 1996). Their role in the pathogenesis of liver fibrosis is also widely acknowledged (Yata et al., 2002), so that one goal of antiviral therapies, including alpha-Interferon, is to reduce viral replication and the consequent inflammation in order to reduce fibrosis and deterioration of the liver function (Poynard et al., 2002; Sanceau et al., 2002). While up to now evaluation of fibrosis has been reserved to histological analysis of liver biopsy samples, there is an urgent need for other markers capable of quantifying and staging the development of fibrosis.

Therefore, the use of some proteolytic fragments of ECM proteins expressed in the liver has been proposed as a prognostic indicator of the status of fibrosis (Guechot et al., 1994; Murawaki et al., 1995). However, the utility of these fragments would be bolstered by an understanding of the underlying mechanisms and of the proteolytic enzymes that are responsible for their production. Here we will discuss the role of proteolytic enzymes, in particular MMPs, in the remodelling of liver tissue.

MMP-2 is present in the liver as a product of Ito cells, although hepatocytes may interfere with the MMP-2 activation process (Arthur et al., 1989; Theret et al., 1997). In the course of liver fibrosis, increased levels of pro-MMP-2 have been detected, mainly in the serum, while in liver tissue extract the active form of MMPs has been shown to be increased with respect to normal liver (Ebata et al., 1997; Preaux et al., 1999; Murawaki et al., 2002). The increased concentration of MMP-2 in the liver tissue could be responsible for degradation of the ECM components which are physiologically expressed in hepatic tissue, and this might lead to activation of Ito cells toward a myofibroblastic phenotype with consequent secretion and deposition of new ECM proteins particularly enriched with Collagen type I and III, the main components of the fibrotic tissue in the cirrhotic liver (Theret et al., 1999; Yoshida et al., 1999; Neubauer et al., 2001; Yang et al., 2003). This hypothesis is further supported by an in vivo experimental model where acute lethal hepatitis in mice was blocked by MMP inhibitors such as BB-94, and by the better survival of MMP-2 knock-out animals (Wielockx et al., 2001). In addition, MMP-2 seems to play a role in allowing Ito cell migration within Disse spaces or toward the inflammation area in response to the degradation of the BM-like structure present in the Disse spaces (Yang et al., 2003). Loss of contact with a BM-like structure, either by remodelling of the surrounding microenvironment or disruption of the adhesive substrates, is also responsible for the activation of the Ito cells with consequent deposition of ECM proteins, migration, or MMP production (Friedman et al., 1989; Sohara et al., 2002; Issa et al., 2003). Proliferation of Ito cells is also important in the progression of liver fibrosis, and MMP-2 seems to work as a paracrine stimulator factor or by activating other growth factors such as transforming growth factor (TGF)-ß1, that directly stimulates cell proliferation (Yu and Stamenkovic., 2000; Giannelli et al., 2002b).

Another hypothesis suggests that MMP-2 could

degrade Collagen type I, showing an interstitial collagenase-like function, as observed in rabbits where specific MMP-2 inhibitors reduced Collagen type I degradation (Kerkvliet et al., 1999). Thus, it has been proposed that MMP-2 could have an anti-fibrotic activity although more data are needed to confirm this possibility. Similarly, it has been reported that MT1-MMP could also have a proteolytic activity toward Collagen type I with a cleavage site in the α-chain identical to that reported for MMP-1 (Ohuchi et al., 1997).

As previously described, TIMP-1 and TIMP-2 are involved in the progression of liver fibrosis because of the correlation with the inhibition of ECM degradation. Increased levels of TIMP-2 in serum and hepatic tissue (mRNA) correlated with progression of the disease and inflammatory status (Benyon et al., 1996; Ebata et al., 1997; Lichtinghagen et al., 2001). In particular, patients

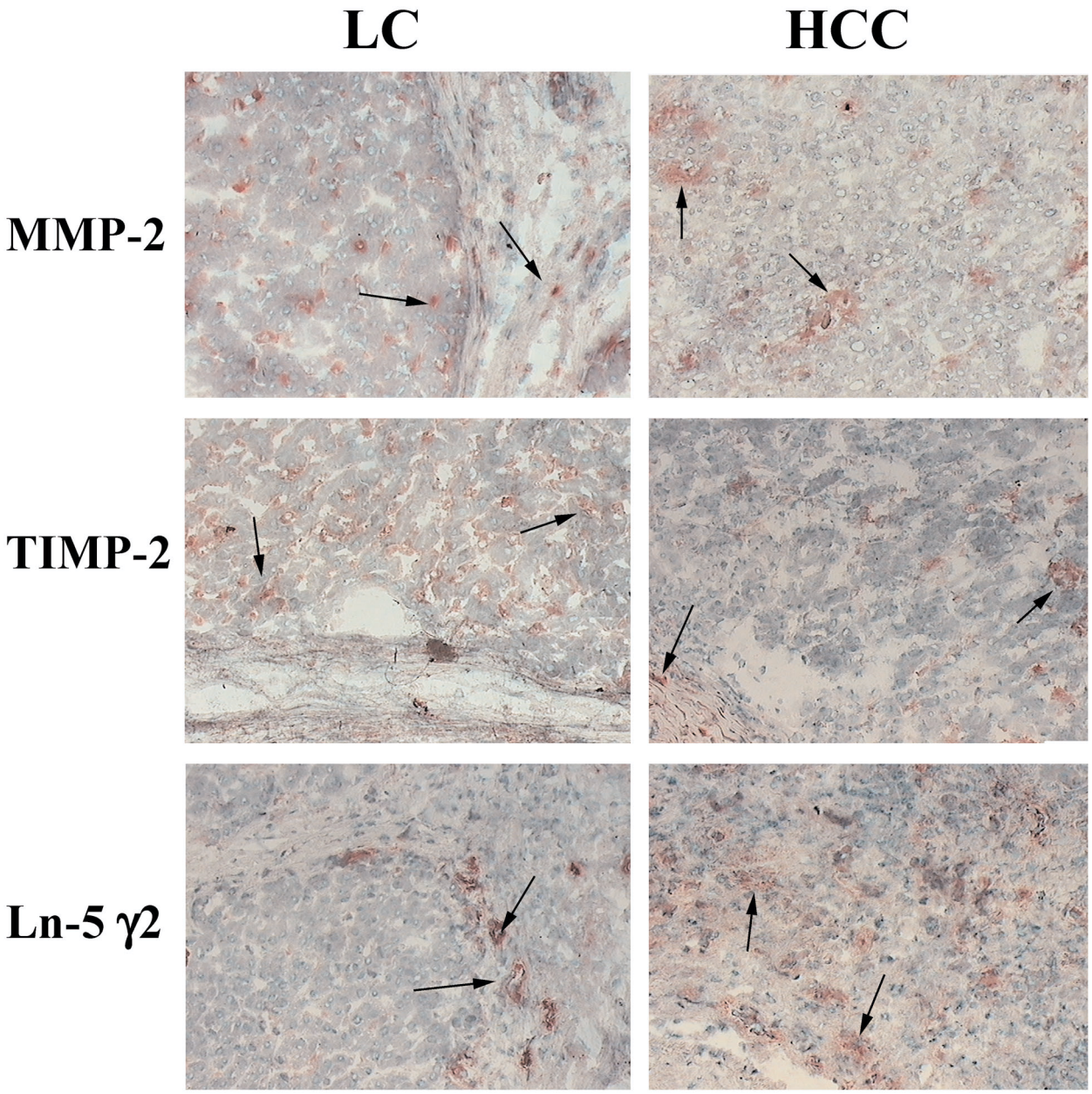

Fig. 1. MMP-2, TIMP-2 and Ln-5 γ2 chain expression in liver cirrhosis and HCC. MMP-2 expression is similar in liver cirrhosis (LC) and HCC, while TIMP-2 expression is stronger in LC than in HCC. Staining for the Ln-5 γ2 chain is restricted to the vessel BM in LC, whereas it is abundantly present in the HCC tissue. (x 20)

with liver cirrhosis had higher levels of TIMP-1 and TIMP-2 when compared to patients with chronic liver disease, and the latter had higher levels when compared to normal subjects (Benyon et al., 1996; Murawaki et al., 1999; Ninomiya et al., 2001; Giannelli et al., 2002a). An additional role for TIMP-1 in the course of chronic liver damage has been proposed, as it has recently been reported that TIMP-1 protects activated Ito cells from apoptosis both "in vitro" and "in vivo" because of the inhibition of MMPs (Iredale et al., 1998; Murphy et al., 2002). Furthermore, growth factors such as TGF-ß1, which are known to increase in the course of liver fibrosis, play a central role in the pathogenesis of liver fibrosis by means of several mechanisms including the regulation of TIMP-1 and TIMP-2 (Knittel et al., 1996; Moshage, 1997).

Tissue remodelling and cytokines

At this point we must consider the role of inflammation, and in particular of some mediators of inflammation, in the exacerbation of liver fibrosis. It has been reported that a number of different cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1ß, TGF-ß1 and platelet-derived growth factor (PDGF) are up-regulated in liver parenchyma during chronic hepatitis (Castilla et al., 1991; Neuman et al., 2002). The altered network of cytokines probably plays a role not only in mediating the inflammation, but also in modulating the expression of MMPs and TIMPs, thereby influencing the local proteolysis of ECM proteins (Knittel et al., 1999; Haruyama et al., 2000; Yang et al., 2003). This could trigger an altered turn-over of ECM proteins in the liver, activating Ito cells as previously mentioned.

On the other hand, anti-inflammatory cytokines such as Interferon (IFN)-α have largely been used in therapy for patients with chronic viral hepatitis. While IFN-α has so far been used mainly for its antiviral activity, it has recently been suggested to have a role in the modulation of the proteolytic activity mediated by MMPs and TIMPs, although no definitive conclusions can be drawn (Bauvois et al., 2002). It has also been shown that IFN-α inhibits cytokine-mediated proteolytic up-regulation, and therefore could play a role as an anti-fibrotic agent (Sanceau et al., 2002). Another possibility is that IFN-α

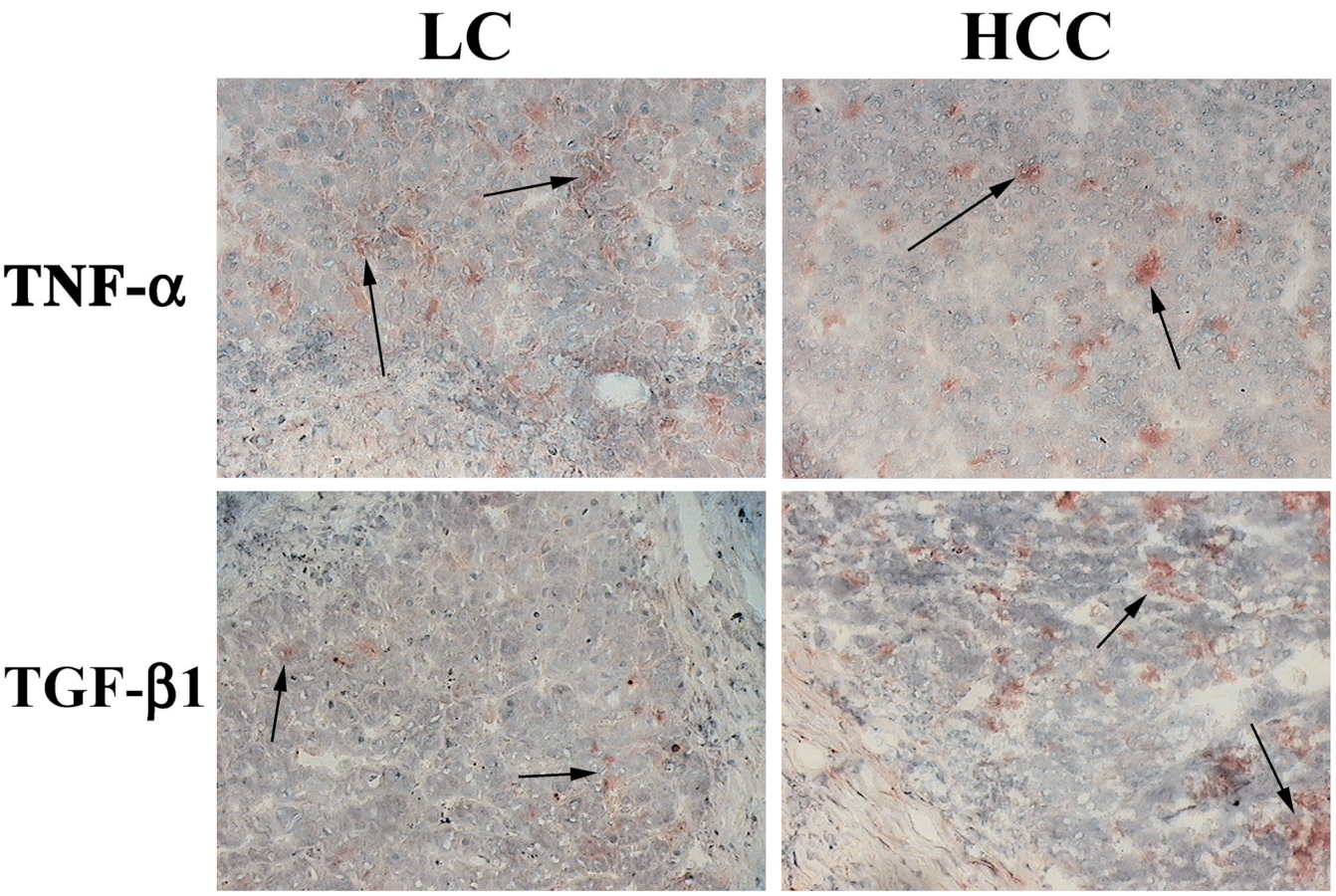

Fig. 2. TNF-α and TGF-ß1 in LC and HCC. TNF-α and TGF-ß1 are strongly expressed in LC and HCC tissue. (x 20)

down-regulates TIMP-1 expression, which could increase the MMP-1 activity, accelerating Collagen type I degradation and slowing fibrosis (Mitsuda et al., 2000; Ninomiya et al., 2001). These data are also supported by the indirect observation in a large number of patients that the status of fibrosis is a factor independent of viral load in the evaluation of sustained response to therapy (Myers et al., 2003). Finally, it has been shown that IL-10 inhibits prostaglandin-dependent MMP production (Mertz et al., 1994), and therefore it might play an important role in liver damage by reducing ECM degradation in the earlier steps of fibrosis (Koulentaki et al., 2002). In this regard, encouraging preliminary data have been reported with the use of IL-10 as a therapeutic agent (McHutchison et al., 1999), or by increasing the production of IL-10 using pirfenidone in "in vivo" animal models (Nakazato et al., 2002).

Tissue remodelling in hepatocellular carcinoma

Cancer development and metastasis occurrence are other examples of tissue remodelling. The most frequent epithelial malignancy in the liver is Hepatocellular carcinoma (HCC), which represents the fifth most frequent cancer in the world and the third most frequent cause of tumor-related death (Bruix and Llovet, 2002). In European countries and in North America HCC commonly develops in cirrhotic liver where the cancer cells grow embedded in fibrotic tissue enriched with ECM proteins. Therefore, MMPs may play a key role in the remodelling of the underlying tissue to allow cancer development and spread. MMPs, and in particular MMP-2, are thought to be involved in the spread and metastasis of several different malignancies (Liotta et al., 1980; Grigioni et al., 1994; Coussens and Werb, 1996; Deryugina et al., 1997; Nelson et al., 2000; Giannelli et al., 2001). In HCC up-regulation of MMP-2 and MT1-MMP as compared with the surrounding liver parenchyma has been reported (Terada et al., 1996; Yamamoto et al., 1997; Ogata et al., 1999), while in other studies an imbalance between MMP-2/TIMP-2 has been found, caused by a down-regulation of TIMP-2 (Giannelli et al., 2002a). Differences among these studies could be due to the absence or presence of cirrhosis in the surrounding peritumoral tissue, and this could perhaps mask the role of MMP-2 and/or MT1-MMP (Benyon et al., 1996; Colombo et al., 1989; Preaux et al., 1999). The bulk of data suggest increased proteolytic activity in HCC, regardless of MMP-2, MMP-9, MT1-MMP activity or decreased TIMP-1 and TIMP-2 expression. These biological findings may have clinical relevance since a more invasive and aggressive phenotype seems to be correlated with higher proteolytic activity, with the occurrence of metastasis and with worse prognosis and survival (Giannelli et al., 2002a). Therefore, modulation of the proteolytic activity seems to be an avenue to pursue to ameliorate clinical outcome in patients with HCC.

In "in vitro" models, it has been shown that MMP-2 activation can be reduced by the presence of a blocking functional antibody directed against integrin α3ß1 (Giannelli et al., 2001). Integrins are heterodimer transmembrane receptors involved in cell adhesion and communications that bind ECM proteins and are involved in a number of biological cellular functions including cell motility and differentiation (Hynes, 1987; Ruoslahti and Giancotti, 1989; Lora et al., 1998). Integrins are also a suitable target for modulation by soluble factors such as TGF-ß1, that up-regulates integrin expression on the surface of HCC cells, thus triggering a more invasive and aggressive phenotype that can be reversed by the addition of blocking functional antibodies against TGF-ß1 (Nejjari et al., 1999; Cai et al., 2000; Giannelli et al., 2002b).

In conclusion, remodelling of the surrounding microenvironment seems to play a key role in the spread of HCC cells. A possible scenario is that increased proteolytic activity could be responsible for processing ECM proteins, and for activation of TGF-ß1 stored in the ECM. Active TGF-ß1 could stimulate HCC cells with a paracrine effect and induce increased cell motility and invasion, up-regulating the expression of integrin receptors such as α3ß1. This integrin is one of the main receptors for Ln-5 (a Ln isoform), an ECM protein proposed to be associated with cancer growth and metastasis metastasization. Recently, expression of the Ln-5 chains has been detected in HCC in a differential fashion, which correlates with the metastatic phenotype (Giannelli et al., 2003), supporting the idea that modification of the microenvironment could have an important role in the metastatic process of HCC.

Conclusions

In the course of chronic liver damage, tissue remodelling with consequent deposition of ECM proteins takes place as a consequence of cellular damage. Altered ECM turnover may lead to liver fibrosis, and its progression is responsible for the development of cirrhosis and of HCC. Alterations in the surrounding microenvironment due to increased ECM protein production and/or increased proteolytic activity modulated by growth factors or cytokines could be responsible for a more or less invasive HCC phenotype. More studies are needed to gain a better understanding of the mechanisms regulating fibrosis and the modifications of the microenvironment responsible for excessive or faulty tissue remodelling which leads to liver failure and the onset of HCC.

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