7

Alcoholic Liver Disease and the Survival Response of the Hepatocyte Growth Factor

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1. Introduction

Hepatocyte growth factor (HGF), also known as a scatter factor plays a major role in liver repair and regeneration during injury. HGF displays a complex network of signaling pathways that activate key functions for liver repair, survival and cellular redox control (Gomez-Quiroz, et al. 2008; Nakamura and Mizuno 2010) all these functions are initiated by the binding of HGF to its receptor c-Met which, after autophosphorylation, recruits a wide variety of signal transduction proteins.

After injury, liver initiates repair process which could be leaded by proliferation of the mature, and normally quiescent, hepatocytes or, when damage is extensive and mature hepatocytes are enable to proliferate, by the activation of progenitor cells population or oval cells (Riehle, et al. 2011)

It is well recognized that the repair process begins with the activation of Kupffer cells, the liver-resident macrophagues, which respond to damage secreting tumor necrosis factor alpha (TNF-α) and interleukin (IL-) 6, these cytokines induce the activation of signaling pathways in hepatocytes such as nuclear factor κB (NF-κB) and signal transducer and activator of transcription 3 (Stat3), respectively. In addition, hepatic stellate cells (HSC) and inflammatory cells initiate the production of HGF which together with TNF-α and IL-6 lead to a cooperative signal to overcome cell-cycle checkpoint controls and shift cells from G0 through G1, to S phase, leading to hepatocytes proliferation and repair.(Riehle et al. 2011)

2. HGF/c-Met and its function in liver repair

HGF gene is encoded on chromosome 7 band 7q21.1 and its product is a precursor glycopolypeptide of 697 amino acids which is proteolytic processed in the extracellular environment by the serine endoprotease urokinase-type plasminogen activator (uPA) (Naldini, et al. 1992) generating the mature HGF consisting in a heterodimeric disulphide-linked two polypeptide chains (α and β). The α-chain consists of 463 amino acids residues and four kringle domains. The β-chain consists of 234 amino acid residues. It is thought that *in vivo* HGF acts in a paracrine fashion, being produced by mesenchymal or stromal cells (Stoker, et al. 1987), and targeting epithelial cells nearby (Sonnenberg, et al. 1993).

Among the multiple biological function triggered by HGF is important underline: cell proliferation, apoptosis protection, morphogenesis, scattering, cell motility, invasion and metastasis (Hanna, et al. 2009). The signal transduction that drives all these effects starts by the ligand-induced dimerization and activation of the HGF receptor, the proto-oncogen c-Met (cellular-mesenchymal epithelial transition factor) (Ma, et al. 2003).

Human c-Met gene is located on chromosome 7 band 7q21-q31; the product is a 150-kDa polypeptide that, after glycosylation and proteolytic processing, generates the mature α-β heterodimer receptor composed of one extracellular 50-kDa α-chain linked by disulfide bonds to a transmembrane 140-kDa β-chain. The β-subunit encloses multiple sites of regulatory phosphorylation. Ligand binding to the receptor leads to autophosphorylation of the tyrosines 1230, 1234 and 1235 located within the activation loop of the tyrosine kinase domain and activates the intrinsic kinase activity of the receptor. (Ma et al. 2003).

HGF also induces tyrosine 1313 phosphorylation which is preferred by phosphoinositide 3-kinase (PI3K) binding, while in the multi-substrate signal transducer docking site, located in the C-terminus domain, phosphorylation of tyrosines 1349, and 1356 lead to the activation of adapter and effectors proteins such as Shc, Src, Gab1, Grb2, PI3K, SHP2, phospholipase C-γ (PLC-γ) among others (Bertotti and Comoglio 2003), these pathways have been widely characterized both *in vivo* and *in vitro*, and it has been well defined the difference between HGF and other growth factors with tissue repair activities such as epidermal growth factor (EGF), TNF-α, or IL-6. Under this context it has been reported that HGF promotes tubulogenesis by the activation of GAB1, SHP-2, and Stat3; survival and antiapoptosis by NF-κB, Stat3 and Erk1/2; and proliferation and growth by Grb2 and PI3K (Boccaccio, et al. 1998; Fan, et al. 2005; Maroun, et al. 1999; Maroun, et al. 2000; Trusolino, et al. 2010)

In addition of these phosphorylations, there are other regulatory sites within the β-subunit; in the juxtamembrane domain the phosphorylation of serine 956 and tyrosine 1093 have negative regulatory functions (Ma et al. 2003) which are involved in the receptor internalization after activation (Kermorgant and Parker 2005) and posterior degradation by ubiquitin-proteosome pathway (Jeffers, et al. 1997).

Functions elicited by HGF are particularly important for liver repair and regeneration after liver injury by alcohol, drugs, viral infections or partial hepatectomy. In fact, HGF was originally discovered as a potent mitogen of adult rat hepatocytes (Nakamura, 1984). HGF is produced in the liver by non-parenchymal cells, such as HSC, sinusoidal endothelial, Kupffer cells and immune cells; but also it can be produced by other distant organs in the body, including lungs, and kidneys where it displays repair process as well. (Grenier, et al. 2002; Nakamura and Mizuno 2010; Taieb, et al. 2002b).

In healthy humans circulating levels of HGF are always present, and can be affected by age, gender and pregnancy. It can be established a normal range from 0.26 to 0.39 ng/ml, however it is well known the increase in serum HGF after both, acute and chronic liver damage (Funakoshi and Nakamura 2003), and this increment depends on the kind of damage or disease. For example, in alcoholic liver cirrhosis the HGF levels reported is 0.78 ng/ml (Antoljak, et al. 2001), in patients with hepatocarcinoma the average level is 1.06 ± 1.45 ng/ml and in patients with fulminant hepatitis it could reach values of 16.40 ± 14.67 ng/ml (Funakoshi and Nakamura 2003). The increment shows a positive correlation with markers of liver failure such as serum bilirubin or gamma-glutamyl transpeptidase, and

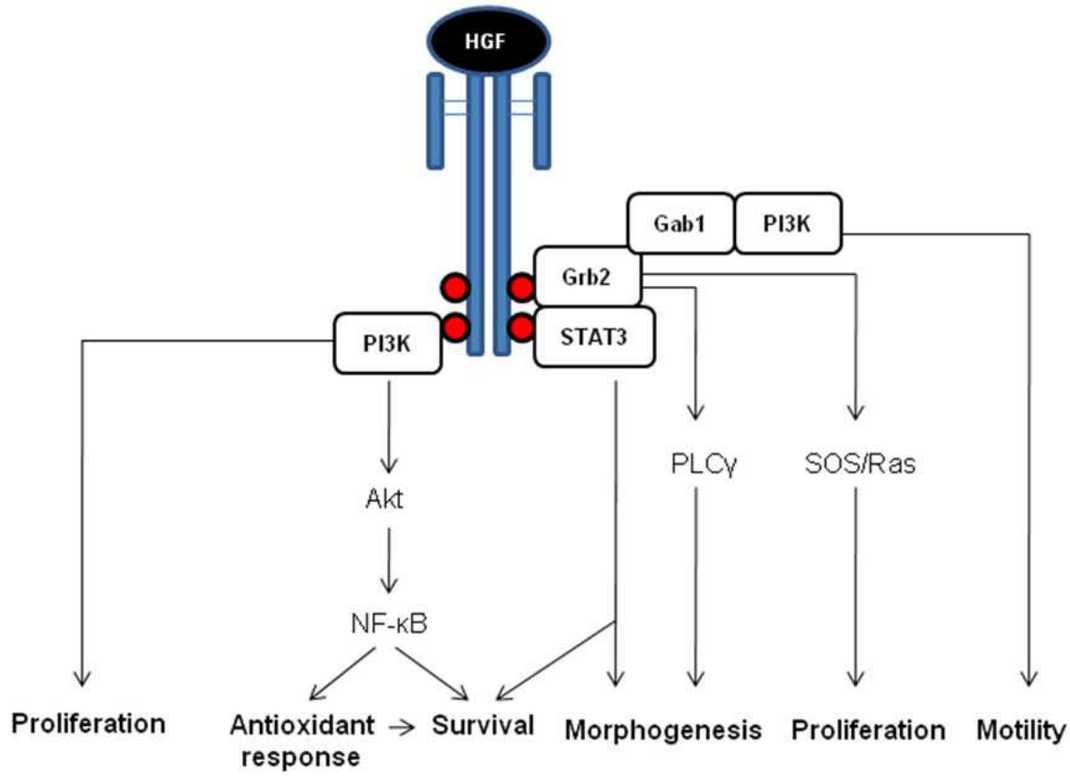

Fig. 1. Signaling pathways and effects elicited by HGF/c-Met. HGF, hepatocyte growth factor; Grb2, growth factor receptor-bound protein 2; Grb1 binding-associated binder1; PI3K, phosphoinositide-3-kinase; STAT3, signal transducer and activator of transcription 3; PLCγ, Phospholipase C gamma; SOS, son of sevenless, guanine nucleotide exchange factor; RAS, rat sarcoma, GTPase; Akt, Protein Kinase B; NF-κB, nuclear factor kappa B.

negative correlation with albumin content, suggesting that HGF levels can reflect the grade of illness and can be considered as a prognosis indicator.

The main evidence of the relevance of HGF on liver regeneration and repair came with the creation of the hepatocyte-specific c-Met signaling deletion mouse (MetKO) (Huh, et al. 2004). Loss of c-Met signaling appeared not to be detrimental to hepatocyte function under physiological conditions, but sublethal doses of Fas agonist (Jo2 antibody) induced a hypersensitization to damage generating massive liver apoptosis and necrosis, with no important effects in wild type mouse. Hepatocytes derived from MetKO mice exhibited sensitization to Jo2-induced apoptosis and impairment in motility and phagocytosis, activities which are required for a proper repair process (Gomez-Quiroz et al. 2008; Huh et al. 2004)

3. HGF and c-Met in alcoholic liver disease

Functions of HGF in ALD remains partially characterized. However it is clear that HGF and c-Met are involved in every stage of ALD, from inflammatory response to alcoholic steatohepatitis, fibrosis, cirrhosis and hepatocarcinoma (Cornella, et al. 2011; Tahara, et al. 1999; Taieb et al. 2002b).

3.1 Ethanol impairs HGF release from neutrophils

Ethanol-initiated damage lead to an inflammatory response characterized by neutrophilia and hepatic polymorphonuclear neutrophil (PMN) infiltration (Taieb et al. 2002b). We reported previously that acetaldehyde, the main metabolite of ethanol biotransformation, induces the production of IL-8, chemotactic factor for PMN, in HepG2 cells (Gomez-Quiroz, et al. 2003) and Taieb and coworkers (Taieb et al. 2002b) have shown that PMN are an important source of HGF specifically in patients with severe alcoholic hepatitis, finding that these patients presented higher levels of HGF (6.07 ± 0.738 ng/ml) in comparison with alcoholic hepatitis-free patients with cirrhosis (3.24 ± 0.438 ng/ml) and healthy controls (0.407 ± 0.027 ng/ml). Hepatic HGF levels correlated with the degree of hepatic PMN infiltration (P=0.0015, ρ=0.76). This study provides evidences that PMN cells are an important source of HGF, particularly in patients with large PMN infiltration, pointing out the PMN cells as coadjutants in liver repair, at least in acute processes.

Although PMN cells provide HGF to the injured liver, ethanol inhibits this effect. PMN cells treated with degranulation promoter agents (cytochalasin B and N-formyl-methionyl-leucyl-phenylalanine) induced the release of HGF, however the presence of different concentrations of ethanol significantly inhibited HGF degranulation in a concentration-dependent manner (Taieb, et al. 2002a), indicating that ethanol impairs HGF release from PMN cells obstructing liver repair process.

3.2 Free radicals, oxidative stress and ethanol metabolism

In terms of ethanol metabolism-induced oxidative stress, we can define two main levels of HGF-mediated protection. The first level is generating system regulation, particularly the microsomal ethanol oxidizing system (MEOS), and the second one is the control or neutralization of the free radicals formed as a consequence of ethanol metabolism.

A free radical is a molecule or atom that contains one or more unpaired electron, this characteristic makes them highly instable and reactive. In the cellular context, oxygen and nitrogen free radicals have a wide variety of actions ranging from modulation of gene expression to the induction of cell death.

Physiologically 1-2% of molecular oxygen is converted, by enzymatic and non-enzymatic mechanisms, to reactive oxygen species (ROS) which include hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), singlet molecular oxygen ($^1O_2$), hydroxyl (HO•), alkoxyl (RO•), and Peroxyl (ROO•) radicals (Cadenas 1989). In order to avoid the harmful effects of these molecules, cells enclose a battery of antioxidant defenses which include enzymatic and non-enzymatic components. Among the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione (GSH) peroxidase (GSHPx) and GSH reductase (GSHRd) sustain the major detoxifying activities. SOD1, 2 and 3 which are located in cytosol, mitochondria and plasma membrane respectively drive the transformation of $O_2^{\bullet-}$ in H_2O_2, although hydrogen peroxide is not a free radical, it is the precursor of the highest reactive and toxic specie among the ROS, the hydroxyl radical via either, Fenton reaction, which requires the presence of Fe^{3+} or Cu^{2+} for the process, or Haber-Weiss reaction. (Valko, et al. 2007)

To counteract this phenomenon H_2O_2 is transformed to H_2O by the action of catalase or GSHPx. GSHPx uses two molecules of GSH to reduce H_2O_2, generating the oxidized form of

glutathione (GSSG) which can be regenerated to GSH by the action of GSHRd using NADPH as electron donor.

GSH is the naturally occurring major thiol. It is produced in the cytosol by the action of two enzymes, the γ-glutamyl cysteine ligase (also known as γ-glutamyl cysteine synthetase, γ-GCS) that catalyses the formation of the dipetide L-γ-glutamyl-L-cysteine, and by glutathione synthase which converts this dipeptide in GSH (L-γ-glutamyl-L-cysteinyl-Glycine).

The ability of GSH to deal with ROS is not restricted as a cofactor of GSHPx, because GSH has, by itself, the capacity to scavenge free radicals, as it happens with others non-enzymatic antioxidants such as vitamin C and E, and β-carotene .

Ethanol metabolism is mainly driven by both, alcohol dehydrogenase and MEOS which main member is cytochrome P450 (Cyp) 2E1, both enzymes generate acetaldehyde as primary metabolite, but only MEOS produces, in addition, ROS such as $O_2^{\bullet}$, H_2O_2, and the highly toxic HO•, responsible of DNA, protein and lipid oxidation (Cadenas 1989). It has been reported that Cyp2E1 is induced by ethanol consumption principally in zone 3 hepatocytes. Biopsies from recently drinking subjects revealed an increment from 4- to 10-fold over normal values (Tsutsumi, et al. 1989).

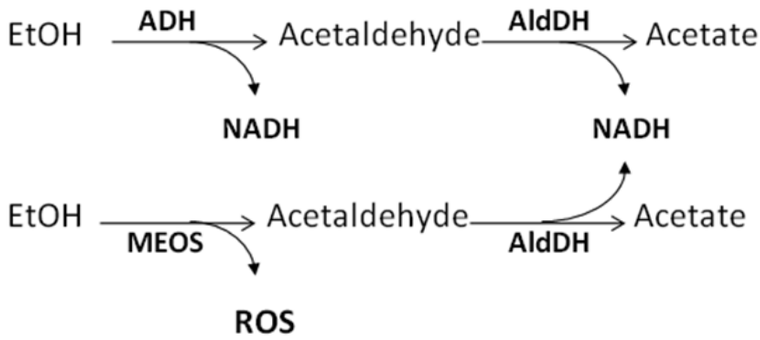

Fig. 2. Major alcohol metabolism processes. EtOH, ethanol; ADH, alcohol dehydrogenase; AldDH, aldeyde dehydrogenase; ROS, reactive oxygen species; MEOS, microsomal ethanol oxidizing system; NADH, reduced nicotine adenine dinucleotide.

Other Cyp isoforms have been implicated in ethanol metabolism, such as Cyp1A2 and Cyp3A4, thus the term MEOS characterizes the total microsomal ethanol oxidation, not only Cyp2E1 (Salmela, et al. 1998).

3.3 HGF regulates the ROS generating systems

Donato and coworkers reported in 1998 that HGF regulates the expression and activities of Cyp1A1/2, Cyp2A6, Cyp2E1 and, Cyp2B6 in human hepatocytes in primary culture (Donato, et al. 1998). Activities of Cyp1A2, Cyp3A4 and Cyp1A1/2 were significantly diminished at 72 h of HGF treatment (10 ng/ml). Particularly in five hepatocytes cultures from five different healthy donors, HGF treatment resulted in the reduction of Cyp2E1 activity ranging from 55 to 69% regarding not treated cultures. These effects were correlated with the decrease in the corresponding mRNA, suggesting that HGF transcriptionally and post-translationally downregulates some of the MEOS components. This regulation represents the first frontline of HGF-induced defense against the toxicity of ethanol.

In addition to the abrogation of MEOS components by HGF, it is reported that it can also downregulate another ROS generating systems such as NADPH oxidase (Gomez-Quiroz et al. 2008), which is involved in many liver diseases such as fibrosis(De Minicis, et al. 2010), hepatitis C virus infection (de Mochel, et al. 2010) and alcoholic liver disease where it is mainly activated in Kupffer cells leading to TNF-α production (Kono, et al. 2000).

3.4 HGF regulates the ROS produced by ethanol metabolism

The second HGF frontline against the oxidative effect of ethanol metabolism is leaded by the generation of antioxidants. It is well known that HGF/c-Met can regulate the activation of survival transcription factors such as NF-κB or Nrf2 (Gomez-Quiroz et al. 2008; Kaposi-Novak, et al. 2006; Valdes-Arzate, et al. 2009) which are responsible of the expression of a wide range of antioxidant, detoxifying and antiapoptotic proteins and it has been well documented elsewhere (Fan et al. 2005; Klaassen and Reisman 2010).

In vitro studies have revealed the prominent role of HGF in cellular survival against ethanol toxicity. One of the main problems to address the molecular mechanism of alcohol induced damage, at the cellular level, is that hepatocytes in primary culture loss the capacity to metabolize ethanol due to a downregulation in the MEOS and alcohol dehydrogenase. Cell lines expressing stable and constitutively Cyp2E1 and/or alcohol dehydrogenase have been established to sort the inconvenience in the absent of alcohol metabolism in normal hepatocytes in culture (Chen and Cederbaum 1998; Donohue, et al. 2006), particularly relevant is the cell line VL17A, which is a suitable *in vitro* model of ALD due to the expression of both Cyp2E1 and alcohol dehydrogenase (Donohue et al. 2006), which mimics much better conditions in normal hepatocytes than single gene transfected cells (Chen and Cederbaum 1998).

VL17A have been used to address the mechanism of HGF-induced protection in ALD (Valdez-Arzate 2009). HGF was capable to decrease ROS production, protein oxidation and lipid peroxidation damage due to ethanol metabolism, correlating with an increase in cell viability. Analysis of the mechanism involved in the HGF-induced protection against ethanol metabolism toxicity revealed that HGF induces the activation of NF-κB, with a concomitant expression of antioxidant enzymes such as catalase, SOD1, and γ-GCS, as well as an elevation in GSH synthesis as a consequence of the expression of γ-GCS. Inhibition of NF-κB, or the main upstream activators of this transcription factor (PI3K and Akt), significantly abrogated the protective role of HGF (Gomez-Quiroz et al. 2008; Valdes-Arzate et al. 2009)

HGF has demonstrated to increase the expression of catalse and GSHRd (Arends, et al. 2008). Catalase transforms H_2O_2 in H_2O, and GSHRd restores GSH from GSSG (Figure 2).

It has been widely characterized the relevance of GSH in ALD by Fernandez-Checa and coworkers (Fernandez-Checa, et al. 2002; Fernandez-Checa, et al. 1993; Fernandez-Checa, et al. 1996; Hirano, et al. 1992), and it is proposed as one of the key mechanism in the alcohol-induced hepatocellular damage, mainly by GSH depletion in mitochondria.

HGF has shown regulate strongly GSH homeostasis by inducing the expression of γ-GCS (Tsuboi 1999; Valdes-Arzate et al. 2009), and we are claiming that this is the main mechanism of protection against oxidative stress. In fact, c-Met KO mice are under oxidative

stress due to the increment in the NADPH oxidase activity, and depletion in GSH. MetKO mice subjected to the carcinogenic agent N-nitrosodiethylamine were more susceptible to the development of tumors in the liver, presenting an increment in multiplicity, size and incidence. This effect was abrogated by the oral administration of N-acetyl cysteine (NAC) which is precursor of GSH synthesis (Takami, et al. 2007). These findings were correlated in *in vitro* studies, MetKO hepatocytes are more susceptible to Jo2-induced damage, effect that was abrogated by NAC treatment, which induced GSH reservoirs restoration (Gomez-Quiroz et al. 2008).

The main consequence in ROS generation is the induction of hepatocytes apoptosis. ROS can induce the activation of jun kinease signaling pathway leading to the activation of AP-1 transcription factor that promotes the expression of proapoptotic proteins such as FasL, TNF-α and Bak. ROS also targets mitochondria inducing membrane permeabilization and the release of proapoptotic proteins (cytochorme c, Smac/diablo, EndoG, among others(Serviddio, et al. 2010), which participate in the cell death process.

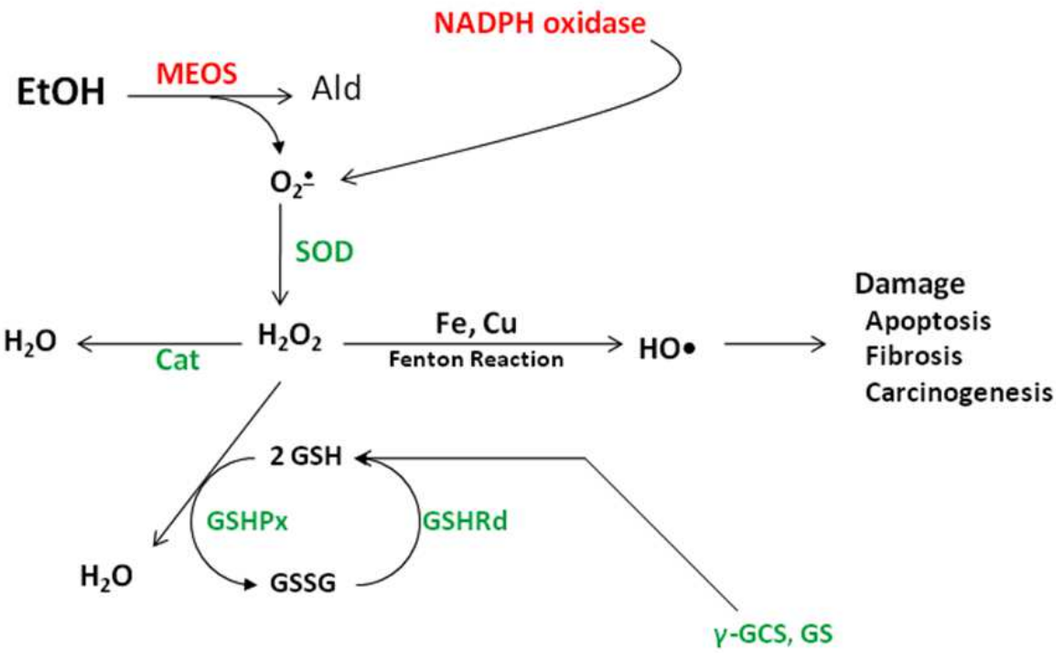

Fig. 3. Alcohol-mediated reactive oxygen species production and antioxidant response driven by HGF. Molecules downregulated (red) and upregulated (green) by HGF. EtOH, ethanol; MEOS, microsomal ethanol oxidizing system, Ald, Acetaldehyde; H_2O_2, hydrogen peroxide; $O_2^{\bullet-}$, superoxide anion; HO•,hydroxyl; SOD, Superoxide Dismutase; Cat, catalase; GSH Glutathione; GSHPX, Glutathione peroxidasa; Gluatathione –reductase; GSSG, Oxidized glutathione; γ-GCS, γ-glutamyl cysteine synthetase; GS, Glutathione Synthase.

It is well known the antiapoptotic effect of HGF, not only in hepatocytes, but in many cell types (Arends et al. 2008; Fan et al. 2005; Kitta, et al. 2001; Santangelo, et al. 2007). In addition to the control of ROS, HGF can induce the expression of antiapoptotic proteins by the activation of survival canonical pathways for example, Akt/NF-κB, Erk1/2 or Stat3, which induce Bcl2, Bcl-XL, and Mcl-1 expression (Gomez-Quiroz et al. 2008; Valdes-Arzate

et al. 2009), but also HGF-induction of GSH supports its antiapoptotic function, based in the fact that NAC treatment significantly reduced the Jo2-induced apoptosis in hepatocytes, and pretreatment with BSO, an inhibitor of γ-GCS, abrogated the protective effect of both NAC and HGF(Gomez-Quiroz et al. 2008).

The evidence gathered here clearly shows the central role of HGF in the control of ethanol-induced oxidative stress and GSH homeostasis as key issues in the hepatoprotective mechanism in ALD (Figure 2).

3.5 Regulation of lipid homeostasis and recovery from alcohol-induced fatty liver

The mechanism of alcohol-induced fatty liver disease is complex and remains not fully characterized. The main mechanism proposed is that alcohol impacts at three levels.

The first level is the induction of oxidative stress, starting by MEOS, and continuing with mitochondria dysfunction due to a decrease in mitochondrial GSH reservoirs. The second level is the increase in TNF-α, which augments mitochondria failure, and ROS production and deregulates adiponectin, which promotes fatty acids oxidation. The third level is the activation of transcription factors involved in lipid biosynthesis and export.

Liver packages triglycerides into a very low density lipoproteins (VLDL), forming a complex with the apolipoprotein B 100 in a process facilitated by the microsomal triglyceride transfer protein (MTTP), which is downregulated in rats fed with an ethanol-containing liquid diet (Sugimoto, et al. 2002). The first and second levels converge in the generation of endoplasmic reticulum (ER) stress and in the increment in the activation of sterol regulatory element binding protein 1c (SREBP1c), which is in the third level. SREBP1c is a transcription factor that remains inactivated in ER, and after stimulus, migrates to the Golgi for further process and finally arrives to the nucleus where it promotes the expression of genes involved in fatty acids synthesis (Ferre and Foufelle 2010). Furthermore, the AMP-activated protein kinase (AMPK), a serine-threonine kinase, which stimulates both, fatty acid oxidation by the activation of the peroxisome proliferator-activated receptors alpha (PPARα) and glycolisis, and inhibits fatty acid synthesis due to the inactivation of acetyl-CoA carboxylase (ACC), leads to the decrease of malonyl-CoA, a precursor in fatty acid synthesis and inhibitor of the carnitine palmitoyl transferase 1, the rate-limiting enzyme in fatty acid oxidation (Dobrzyn, et al. 2004; Hardie and Pan 2002). AMPK also inhibits the activation of SREBP1c, presenting AMPK as a multimodal regulator of lipid metabolism in the liver. Animals fed with an alcohol-containing diet exhibit a decrease in the activity of AMPK resulting in the development of hepatic steatosis (Garcia-Villafranca, et al. 2008).

In addition, it has been proposed that the increase in NADH/NAD^+ ratio due to alcohol metabolism drives the inhibition of the tricarboxylic acids cycle and fatty acid oxidation, but also it can induce ER stress (figure 3) (Lieber 2004)

HGF involvement in lipid homeostasis has been experimentally demonstrated, Kaibori and collaborators reported that HGF is capable to induce lipid synthesis under liver regeneration (Kaibori, et al. 1998), and the genetic elimination of c-Met signaling in hepatocytes induced changes in the expression of many proteins related to lipid metabolism such as Acox1, ASS1, Crot, Cyp4a10, Cyp 4a14, Fasn, Lipc, among others (Kaposi-Novak et al. 2006).

One of the firsts studies focused to address the regulation of alcohol-induced fatty liver by HGF was reported in 1999 by Tahara and collaborators (Tahara et al. 1999), they observed that rats fed with an ethanol containing diet for 30 days developed hepatic steatosis. The administration of HGF for 7 days (37 days on the diet) reduced lipid deposition in liver tissue. The major mechanism observed was the increment on lipid secretion via VLDL by the induction of ApoB100 expression.

The second HGF effect related to steatosis control is the restoration of the mRNA levels and activity of MTTP. Injection of HGF on animals subjected to an alcoholic diet restored MTTP levels, this observation was corroborated in HepG2 cells which were exposed to HGF resulting an enhanced expression of both MTTP and ApoB100 (Sugimoto et al. 2002).

The induction of VLDL and MTTP by HGF seems not to be exclusive for alcohol-induced steatosis, due to Kosone and collaborators (Kosone, et al. 2007) found the same results in a model of fatty liver generated by a high-fat diet and confirmed in HepG2 cells the decrease in lipid content.

Another evidence of HGF-induced alcoholic steatosis protection comes from the effect of pioglitazone, this drug is used in the treatment of diabetes and non-alcoholic steatoshepatitis, but it has been postulated that it can be also used in the treatment of alcoholic steatohepatitis (Tomita, et al. 2004). Ethanol reduced the mRNA and protein levels of c-Met, and pioglitazone restored the c-Met expression decreasing the lipid content in liver tissue. Both HGF and pioglitazone showed downregulate the expression of key genes involved in lipid homeostasis such as SREBP 1c and stearoyl-CoA desaturase, improving the deleterious effects in the alcoholic fatty liver.

Finally, recently has been published that HGF can promote the activation of AMPK signaling pathway (Chanda, et al. 2009) inducing the suppression on lipid synthesis and gluconeogenesis.

3.6 Fibrosis

The main consequence in the chronic alcohol ingestion is liver fibrosis/cirrhosis which is characterized by the hyper-accumulation of connective tissue components, mainly collagen, and a disorganization of the normal hepatic structure of regenerative nodules. Many reports have clarified the preventive and therapeutic effects of HGF on liver fibrosis or cirrhosis (Inagaki, et al. 2008; Jones, et al. 2010; Matsuda, et al. 1995; Matsuda, et al. 1997; Ueki, et al. 1999; Xia, et al. 2006).

HGF treatment in animals challenged with different inducers of liver fibrosis suppressed the connective tissue components expression and hydroxyproline levels, preventing the onset of scar formation (Matsuda et al. 1997). The mechanism of HGF-induced anti-fibrotic protection is focused to antagonize the transforming growth factor beta (TGF-β) signaling pathway. TGF-β is the main profibrogenic factor, inducing the expression of collagen, fibronectin and hepatocytes apoptosis. The effect of HGF on TGF-β has been observed at different levels. In the model of fibrosis induced by the bile duct ligation, HGF showed suppress the expression of TGF-β and the conversion bile duct epithelial to mesenchymal transition (Xia et al. 2006). TGF-β suppression by HGF was also observed in the model of DEN-induced fibrosis (Ueki et al. 1999).

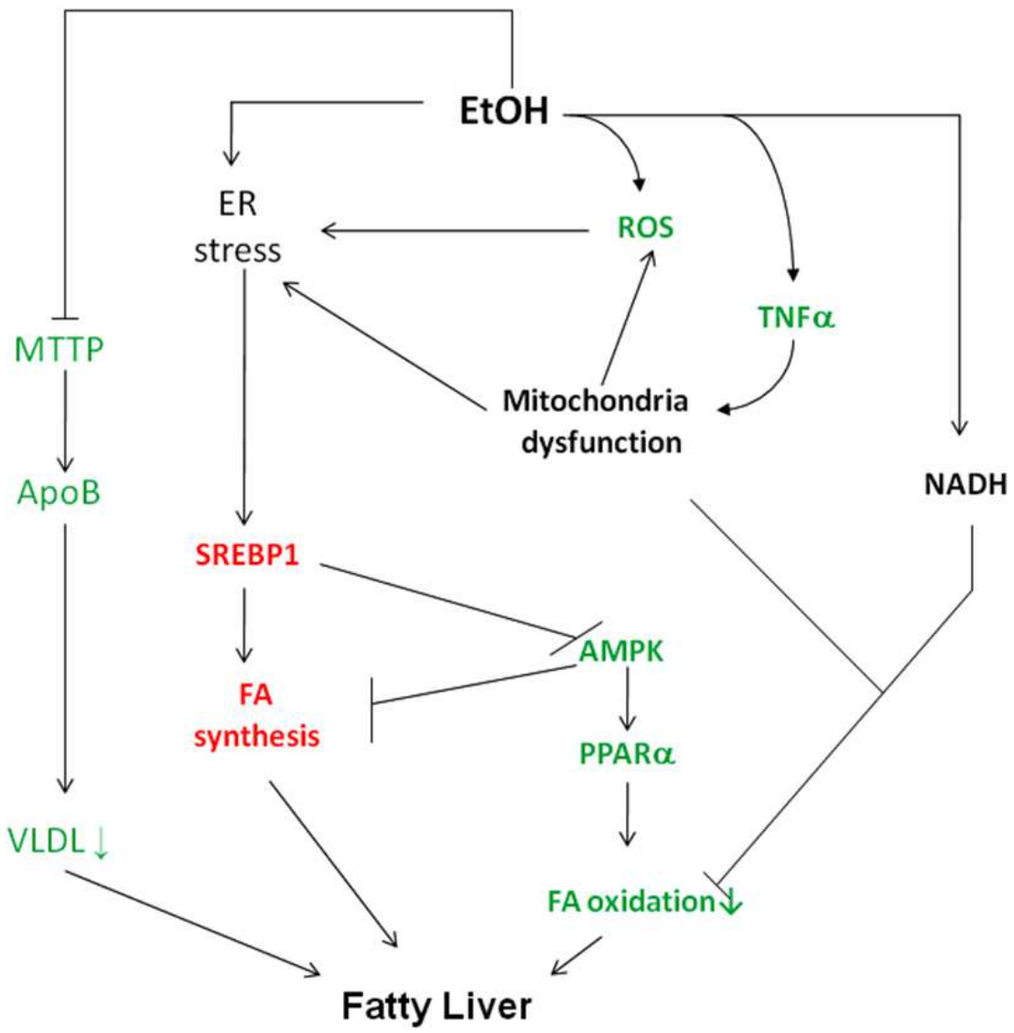

Fig. 4. Molecular mechanism of ethanol-mediated lipid deposition in the liver. Molecules or processes downregulated (red) and upregulated (green) by HGF. EtOH, ethanol; TNF-α, Tumor necrosis factor-alpha; ROS, reactive oxygen species; ER, Endoplasmic reticulum; NADH, Nicotine Adenine dinucleotide reduced; SREBP1c, sterol regulatory element binding protein 1c; AMPK, AMP-activated protein kinase; MTTP, microsomal triglyceride transfer protein; VLDL, very low density lipoproteins; PPAR-α; peroxisome proliferator-activated receptors alpha; FA, Fatty acid.

Another study showed that HGF decreased the expression of collagen 1A2 gene in a model of fibrosis induced by carbon tetrachloride by a mechanism dependent of the nuclear export of smad3 mediated by galectin-7 (Inagaki et al. 2008).

Recently an innovator system to study the anti-fibrotic and protective effect of HGF was developed (Jones et al. 2010). HGF was mixed with collagen and robotically printed onto standard glass slides to create arrays of 500 μm diameter spots, and then rat hepatocytes were seeded and challenged with 100 mM ethanol. Results showed that hepatocytes in the HGF-containing collagen spots presented less apoptosis comparing with those seeded in

HGF-free spots. To investigate impact on the fibrosis, a mix of hepatocytes and HSC were seeded and again challenged with alcohol. HSC were less activated (less fibrotic) in spots containing HGF compared to control. This *in vitro* study confirms the anti-fibrotic response of HGF

3.7 HGF, alcohol and cancer

Heavy long-term alcohol drinking (≥69.0 g alcohol/day in men, and ≥ 23.0 g alcohol/day in women) is a significant risk factor for the development of hepatocellular carcinoma (HCC) (Shimazu et al. 2011) . There is no efficient animal model to study the direct mechanism of alcohol-induced HCC, recently it has been reported that rats selectively bred for high alcohol preference with free access to water supplemented with 10% ethanol for 18 months developed hepatic neoplasia, ERK pathway activation, increased Cyp2E1 activity and oxidative stress (Yip-Schneider et al. 2011). As mentioned here, ethanol metabolism is the main harmful mechanism, due to ROS production; in addition ethanol increases the infiltration of inflammatory cells enhancing the oxidative damage, accelerating telomere shortening and favoring oncogenic mutation (Cornellá et al. 2011) in the carcinogenesis process.

The HGF functions, previously described inhere; strongly suggest the prominent role of this growth factor in the prevention of HCC. In fact, MetKO mice exhibited an accelerated DEN-induced hepatocarcinogenesis, demonstrating the protective and preventive roles of HGF in liver carcinogenesis (Takami et al. 2007), in this study, the accelerated tumor development was associated with increased rate of cell proliferation and prolonged activation of epidermal growth factor receptor (EGFR) signaling, MetKO mice also exhibited an elevated lipid peroxidation, decreased levels of GSH, and SOD expression increment, suggesting that oxidative stress could be a determinant factor for the acceleration of hepatocarcinogenesis. MetKO mice were more susceptible than WT mice to DEN-induced liver cancer increasing multiplicity, incidence and size of tumors. The negative effects of c-Met deletion were reversed by the chronic administration of NAC, blocking EGFR activation and reducing initiated hepatocarcinogenesis, confirming that c-Met deletion-induced oxidative stress is the main hallmark in the accelerated carcinogenesis and that c-Met integrity is required as a protective mechanism against chemical-induced carcinogenesis by the induction of cytoprotective enzymes such as antioxidant enzymes.

In contrast, a wide variety of human tumors exhibit sustained activation, overexpression or mutation of c-Met, including liver tumors. It is not hard to imagine that beneficial and protective roles of HGF in all stages of alcoholic liver disease are not longing in liver cancer. HGF-induced protection ends in the early stages of hepatocarcinogenesis. Once initiated the carcinogenic process, HGF and c-Met trigger signaling cascades resulting in proliferation, growth, survival, motogenesis and angiogenesis (Eder et al. 2009). Mouse and human cell lines that overexpress c-Met or/and HGF become tumorigenic and metastatic in athymic mice (Rong et al. 1994) and, in contrast, downregulation of c-Met or HGF expression decreases tumorigenic potential (Abounader et al., 2002), in fact, overexpression of c-Met or HFG are associated with poor prognosis and aggressive phenotype (Kaposi-Novak et al. 2006). In summary HGF/c-Met protects from carcinogenesis, but in established tumors induces the aggravation of the disease. Mutations in c-Met and HGF predispose to human cancer.

4. Conclusion

In conclusion HGF and c-Met are introduced as promissory therapeutic targets for the treatment of ALD, they can reverse or abrogate almost every stage of the disease, controlling the metabolism, the harmful molecules, the lipid homeostasis disturbing and the chronic effects of alcohol consumption. Although more research is needed to figure out the proper point of intervention in the signaling activated by HGF and c-Met, the experimental evidences gathered here support the promising success of HGF/c-Met.

5. Acknowledge

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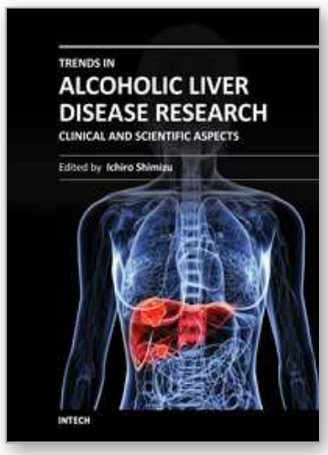

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Alcoholic liver disease occurs after prolonged heavy drinking. Not everyone who drinks alcohol in excess develops serious forms of alcoholic liver disease. It is likely that genetic factors determine this individual susceptibility, and a family history of chronic liver disease may indicate a higher risk. Other factors include being overweight and iron overload. This book presents state-of-the-art information summarizing the current understanding of a range of alcoholic liver diseases. It is hoped that the target readers - hepatologists, clinicians, researchers and academicians - will be afforded new ideas and exposed to subjects well beyond their own scientific disciplines. Additionally, students and those who wish to increase their knowledge will find this book a valuable source of information.

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