Addressing liver fibrosis by TRAIL targetied to hepatic stellate cells

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Chapter 1

Application of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for the treatment of liver fibrosis

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Abstract

Liver fibrosis is the result of an excessive production and over-accumulation of extracellular matrices in the liver. The development of liver fibrosis is associated with progressive chronic liver disease. The underlying pathophysiology of the disease presents hepatic stellate cells (HSCs) transitioning into an active form as the major event in the development of fibrosis. Therefore, a desirable anti-fibrotic therapy to eliminate activated HSC is considered the first step toward resolution of liver fibrosis. TRAIL has already been introduced as a selective agent that can induce apoptosis in activated HSC. However, the dual role of apoptosis in progression and resolution of liver fibrosis presents a real dilemma. Uncovering the specific effects of TRAIL on hepatic stellate cells, defining the interaction of TRAIL with different cell populations and characterizing the potential determinants in response to TRAIL during liver fibrosis will not only enable a more comprehensive insight into the role of TRAIL in liver fibrosis but also promote the discovery of safer and more effective therapeutic strategies in using TRAIL for resolving liver fibrosis. This review summarizes the significant findings that contribute to a better understanding of the therapeutic role of TRAIL with regard to liver fibrosis progression and resolution.

Introduction

Following chronic injury, the liver develops the pathology known as fibrosis. The key element in the development of liver fibrosis is a cell type called the Hepatic Stellate cell (HSC). Quiescent HSCs are dedicated to retinoid storage, yet through an activation process following injury they proliferate and transform into a fibroblastic phenotype. In this form, activated HSCs secrete extracellular matrix proteins, mainly collagen I and III, that accumulate over time and affect liver structure and function [1]. Liver fibrosis is considered a dynamic process. A range of growth factors and cytokines, including PDGF, TGF-β and IL-1b, mediate HSCs activation and their sustained proliferation. After activation, HSCs also express tissue inhibitors of metalloproteinase (TIMPs) that block membrane metalloproteinase 1 (MMP-1), a crucial enzyme that degrades and remodels deposited collagen in fibrotic tissue. Inhibition of the production of these cytokines could halt HSC proliferation and promote its phenotypic reversion to a quiescent form. Consequently, an upregulation of MMPs results in the degradation and removal of deposited collagens. However, the chronic nature of liver fibrosis, the underlying diseases and the complex and overlapping profibrotic signaling limit efficient removal of the involved profibrotic elements. Of several mechanisms that promote the resolution of liver fibrosis, the elimination of activated HSC, because of its central role in the fibrotic process, is considered a major step. Indeed, dissecting the mechanisms and pathophysiology underlying liver fibrosis provides a wealth of evidence that associates fibrosis resolution with the apoptotic elimination of activated HSCs. Activated HSCs are especially prone to the apoptotic agonist TRAIL as a result of higher expression of

its dedicated receptors on their surface [2,3]. In recent years, research has been conducted regarding the potential application of TRAIL as a tumoricidal agent. However, less attention has been paid to its use as an anti-fibrotic element. In this review, we discuss the benefits and deficiencies of TRAIL as an anti-fibrotic agent in liver fibrosis.

TNF-related ligands

TNF-α, CD59L (Fas L) and TRAIL are among the most studied factors in the TNF family that induce apoptosis and thus cell death. These ligands are naturally employed by a number of immune cells, especially NK and CTL cells, to induce controlled apoptosis in tumor cells or infected cells However, depending on the modulation and signaling pathway that they initiate and develop, their corresponding targeted cells undergo different and even contradictory consequences [4]. HSC cells have receptors for all three types of the mentioned ligands. CD59 induces cell death in activated HSC through JNK-assisted tyrosine phosphorylation of CD59, while it blocks the apoptotic pathway via the CD95 receptor tyrosine nitration and even has a thriving effect on quiescent HSC via the epidermal growth factor receptor (EGFR) phosphorylation [5]. TNF is secreted from mononuclear cells and damaged hepatic cells and binds to TNF-receptor-1 or 2 (TNFR1, 2). TNFR2 may induce cell death in active HSC via the Fas-Associated protein Death Domain (FADD), while TNFR1 signals proliferation and activation in HSCs [6]. TRAIL is mainly produced by activated natural killer cells or macrophages in a membrane-anchored or soluble form [7]. NK cells are particularly important in eliminating activated HSCs in liver

cells. For this reason, the liver accommodates NKCD56^{Bright} cells that have been specialized to produce substantial amounts of TRAIL ligands in comparison with peripheral NKCD56^{dim} cells [8]. NK cells from Hepatitis C Virus-infected patients efficiently eliminate primary activated HSCs *in vitro* in a TRAIL dependent manner [9]. Also, treatment with IFN-Y and IFN-α upregulates TRAIL expression of TRAIL- expressing NK cells in the liver and makes a significant contribution to viral clearance and the resolution of liver fibrosis [10–12]. On the other hand, cytokines such as TGF-β and IL-10 that are produced during chronic liver damage could impair hepatic NK cell function and subsequently its efficiency in resolving fibrosis [13–15]. Two types of apoptosis-inducing TRAIL receptors have been identified: TRAIL-R1 (also referred to as DR4) and TRAIL-R2 (also called DR5/killer/TRICK2). DR5 is expressed to a higher extent on the surface of activated HSC in comparison to DR4. TRAIL is associated with the induction of cell death through the intrinsic caspase pathway, caspase 9 activation and a distinct TRAIL mediated apoptosis pathway called paraptosis. Paraptosis is an osmotic dysregulation of HSCs induced by prolonged potassium channel activation [12, 16, 17]. In addition to efficient induction of apoptosis, TRAIL does not seem to have the extreme liver toxicity, including massive hemorrhagic necrosis, which is associated with other death-inducing ligands such as CD95 ligand and TNF-α. This makes TRAIL an attractive pro-apoptotic receptor ligand [18]. A direct relation has been established between fibrogenesis and the number of activated HSCs [19]. By removing activated HSCs as the source of extracellular matrix (ECM) production, TRAIL could indirectly down regulate proliferation of remaining HSCs via down

regulation of collagen and TGF-β production as an important activator of HSC [20]. TRAIL is capable of directly inhibiting the production of collagen by HSCs without killing them, because TRAIL does not interfere with the folding mechanism of collagen production. TRAIL regulates collagen production through interfering with nuclear translocation of heat shock factor 1(HsF1). Blocking HsF1 translocation leads to a decrease in Heat shock protein 47 (Hsp47) expression a of collagen-specific molecular chaperones in activated HSCs that are responsible for the correct folding and secretion of pre-collagen to form soluble collagen[21].

Selectivity of TRAIL variants in elimination of activated HSCs

TRAIL has a multivalent affinity for its decoy receptors DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), in addition to the DR4 and DR5 receptors that induce apoptosis. The dynamics of wild type and mutant TRAIL interaction with their receptors has been well characterized [22]. Receptor-specific agonistic TRAIL has been introduced both in the form of receptor-specific mutant TRAIL agonists or monoclonal antibodies against specific TRAIL receptors such as DR4 and DR5 [23–25]. Since the DR5 receptor is over expressed up to 105-times in activated HSC in comparison to quiescent HSCs [26], using DR5 agonists could be a highly efficient therapy for specific elimination of activated HSCs. DR5-specific agonists have been shown to reduce the decoy receptor-mediated antagonism more than 20-times. Thus, using DR5 receptor specific TRAIL should lower the required administered dose, possibly with fewer side effects [22, 27]. The application of receptor-specific agonists for DR5 could drastically reduce [24] the hepatotoxicity that is associated

with the use of wild type human TRAIL [23–25, 28]. This toxicity could be due to negligible amount of DR5 in comparison to DR4 on the surface of healthy hepatic cells [24, 28]. In some pathological cases, such as nonalcoholic steatohepatitis, steatotic hepatocytes release toxic saturated free fatty acids which induce the expression of DR5 and initiate receptor localization into cell surface lipid rafts with subsequent recruitment of the initiator caspase-8 upon binding to TRAIL [29].

Adverse effect of TRAIL on healthy liver hepatic parenchyma

A point of discussion in using TRAIL related agonists in clinical treatment has mainly centered on its unwanted effects on normal tissues, especially hepatic parenchyma. TRAIL is known to induce apoptosis in various organs: thymocytes, prostate epithelial cells and neural cells under certain conditions [28, 30–32]. In addition, some studies indicate TRAIL treatment is a source of potential damage to normal hepatic parenchyma [33]. However, hepatic cell death *in vivo* was substantially decreased in TRAIL-deficient mice or in the presence of TRAIL receptor inhibitors [34]. As stated above, apoptosis and the state of liver fibrosis are linked**.** Receptor-mediated apoptosis of liver cells initiates the release of chemotactic signaling mediators including macrophage inflammatory protein-2 (MIP2), monocyte chemotactic protein-1 (MCP-1) and CXC ligand-1 that recruit macrophages into the liver and promote hepatic inflammation. The engulfing of Apoptotic Bodies (ABs) by quiescent HSC facilitates the transformation to the fibroblastic phenotype of activated HSCs and the release of fibrogenic mediators including TGF-β. TGF-β increases apoptosis in hepatic cells as well as the

expression of ECM by HSCs (Figure 1) [17,35,36]**.** Linking TRAIL with other molecules could increase the formation of different TRAIL conformations and develop toxic responses toward hepatic cells [31, 37–40]. Moreover, the hepatic models that are used to evaluate the effects of TRAIL on normal hepatic parenchyma are inconsistent. Freshly isolated human but not nonhuman primate hepatocytes were found to be sensitive to TRAIL apoptosis [37–40]. Further studies, however, have shown that this was an *in vitro* artifact caused by the isolation procedure and the adaptation to culture conditions [38,39]**.**

TRAIL and increased hepatotoxicity in viral hepatitis

The state of viral hepatitis seems to be related to TRAIL-induced apoptosis. However, there is still a controversy regarding the exact role of TRAIL in viral induced hepatitis. Different viral components modulate the cellular response towards TRAIL. *In vivo* models for adenoviral hepatitis showed that apoptosis in infected hepatocytes was mediated by down regulation of the TRAIL decoy receptor [34]. Adenovirus E1A gene expression increases the cellular susceptibility towards TRAIL-induced apoptosis. On the other hand, E1B and to a greater extent E3 10.4K and 14.5K proteins (also known as E3-RID) neutralize the effect of TRAIL in affected cells[41]. The E1B 19K protein inhibits the activation of procaspase-8 (FLICE) through FLICE sequestration, thus rendering infected cells resistant to TRAIL apoptosis, while E3-RID facilitates the internalization and degradation of TRAIL receptor 1(DR1) [41]. However, most other types of viral hepatitis make hepatic cells vulnerable to the effects of TRAIL [42]. Hepatitis B Virus and its HBX

protein sensitize hepatocytes to TRAIL-induced apoptosis through up regulation of Bax protein [42], while the hepatitis C virus sensitizes the infected cells through up regulation of TRAIL receptors [43]. HIV glycoprotein gp120 binding to CXCR4 chemokine receptor selectively up-regulates TRAIL R2 expression on hepatocytes through JNK 2 kinase and confers an acquired sensitivity to TRAIL mediated apoptosis. Interestingly, co-infection of HCV and HIV increases hepatocyte apoptosis in comparison to HCV or HIV alone. It is supposed that this effect is mediated by the simultaneous TRAIL receptor and ligand up-regulation [44, 45].

Role of Cytokines in TRAIL-mediated resolution of liver fibrosis

TRAIL expression in the liver is highly inducible by a number of cytokines, including interleukin-2 (IL-2), interferon gamma (IFN-γ) and Interferon α and β (IFN α, β). IL-2 increases TRAIL expression in liver NK cells, whereas this effect is not present in peripheral NK cells [8]. Liver NK cells are important eliminators of liver fibrosis and, upon activation by IL-2, liver NK cells enhance TRAIL expression to induce apoptosis in their target cells [12]. IFN-γ is another cytokine that has a proven effect on reducing liver fibrosis. Several mechanisms have been proposed for the anti-fibrotic effect of IFN-γ in liver fibrosis, including interfering with TGF-β signaling, inhibiting HSC activation, slowing activated HSC proliferation and consequently reducing extracellular matrix secretion and deposition [46–49]. However, recent findings support the role of IFN-γ in enhancing bound and soluble TRAIL expression by NK-T cells and NK cells that further boost the killing efficiency of these effector cells against activated HSCs [7, 8, 12, 50].

IFN-γ also down regulates the TRAIL-Rs in healthy hepatic cells and therefore reduces the collateral damage to these cells [43, 44, 50, 51]. Both IFN- α and IFN-β antagonize the TGF-β signaling and SMAD3 stimulated collagen transcription in activated HSCs [52, 53]. IFN- α / β also induce TRAIL expression of NK cells through activation of the IFN stimulated gene factor-3 (ISGF3) transcription factor and play a critical role in limiting fibrosis during viral hepatitis[54]. In fact, clinical studies indicate that long term administration of IFN- α / β alleviates non-established liver fibrosis and reduces extra cellular matrix levels [53, 55].

HSCs sensitivity to TRAIL-mediated apoptosis and synergy with drugs

Activated HSCs are in constant need of supporting survival signals in order to sustain their proliferation and fibrotic state. Still, removal of fibrogenic cells is considered the first natural step toward resolving liver fibrosis. There is a delicate balance between apoptotic removal of activated HSCs and ABs in promoting fibrosis. Phagocytosis of the ABs by activated HSCs delivers survival signals to HSCs and promotes the progression of fibrosis. This process is mainly regulated through two pathways; sensing ABs reminiscence by the toll like receptor 9 on HSCs initiates a MYD88-dependent pathway of Nuclear factor kappa B (NF-κB) activation[35]; or, alternatively, of the phosphoinositide 3 kinase (PI3K)-dependent phosphorylation of NF-κB and subsequent translocation of NF-κB into the nucleus. Incorrect regulation of NF-κB has been linked to many inflammatory diseases, including liver fibrosis. NF-κB is responsible for inducing the expression of survival genes, including the anti-apoptotic Bcl-2 family proteins Bcl-XL. A direct

relation has been established between the amount of contra-apoptotic proto-oncogenes Bcl-XL in different stages of HSC activation and susceptibility toward death-inducing ligands [56]. The inhibition of NF-κB could therefore offer a potent mechanism for the direct induction of apoptosis in activated HSCs or for their sensitization to apoptosis by TRAIL agonists. Sulfasalazine and related compounds are commonly used as anti-inflammatory drugs that activate IκB kinase (IKK), an intermediate that activates the inhibitor of nuclear factor kappa-B subunit beta (I-Kβ) and that accelerates the recovery from liver fibrosis by eliminating activated HSCs [57]. Proteasome inhibition could prevent I-kB degradation, and thus inhibition of NF-κB. This effect is associated with the loss of survival proteins and consequent cell death. Inhibitors of the proteasome such as Bortezomib and MG132 induce apoptosis in activated and immortalized human HSCs. In addition, this inhibition elevates DR5 expression on the surface of HSCs and hence renders them more susceptible to TRAIL agonists [58]. Interestingly, TRAIL itself may induce NF-κB via TRAIL-induced activation of the JNK pathway in HSCs cells [59]. JNK pathway activation has been associated with a range of effects, including collagen expression and cell proliferation in HSCs. Leflunomide, a JNK inhibitory compound, prevents cell proliferation and subsequently enhances TRAIL- mediated apoptosis in culture-activated HSCs [59].

Role of growth factors in TRAIL mediated apoptosis

ECM producing cells such as HSC are highly responsive to a series of growth factors, such as EGF, Amphiregulin (AR), Beta Cellulin (BTC) and Platelet Derived

Growth Factor (PDGF), due to the increased expression of the corresponding receptors on their surfaces [60-62]. Upon attachment to their dedicated receptors, growth factors such as PDGF and EGF form homo or hetero dimmers and induce signal transduction through the cytoplasmic tail of the receptor. These factors play a pivotal role in the development of liver fibrosis because they increase HSC proliferation through a series of signaling kinases, including extracellular Kinas 1⁄2 (ERK1/2), Focal Adhesion Kinase (FAK), Phosphoinositide 3-kinase /Protein Kinase B (PI-3K/Akt) and c-Jun N-terminal kinase 26 (JNK 26). These growth factors may also block TRAIL-mediated apoptosis by interfering with the caspase-3 p17 phosphorylation [5, 60, 63]. The EGF family represents transmembrane anchored proteins on the surface of hepatocytes and HSCs. However, upon activation, HSCs increase the amount of free EGF in the environment through a process called ectodomain shedding. Ectodomain shedding occurs due to higher expression of membrane metalloproteinase, such as ADAM 12 in activated HSCs. ADAM 12 proteolytically cleaves and releases EGF-like ligands anchored on the surface of HSC in the liver environment [64]. Interestingly, TRAIL itself accelerates ectodomain shedding of EGF, hence antagonizing its apoptotic effect [63]. Targeted therapy of activated HSCs via an anti-EGF receptor scFv and a TRAIL fused protein was shown to be more efficient in eliminating activated HSC viability and ECM secretion [65].

TRAIL signaling: a balance between inducing apoptosis and killing fibrosis

Liver cirrhosis is considered an end stage liver disease and is the primary cause of the need for liver transplant. The development of liver fibrosis is associated with progressive chronic liver diseases. The best anti-fibrotic therapy is elimination of the underlying disease process. In situations where treating the underlying etiology is not possible or not sufficient for reversing the process, specific anti-fibrotic therapy would be highly desirable. HSCs play central role in liver fibrogenesis [2, 3]. Activation of HSCs is associated with the overexpression of death-inducing receptors such as TRAIL, which makes activated HSCs ideal targets for inducing apoptosis through TRAIL agonists. However, the safe and efficient application of TRAIL for the resolution of liver fibrosis requires addressing several issues. Due to the flow of survival- and growth factors that are released during the fibrotic process, efficient induction of apoptosis requires the circumvention or blocking of these signals. An even greater concern is TRAIL-induced hepatotoxicity and the notorious effect of TRAIL on parenchymal hepatic cells as innocent bystanders. Cytokines, inflammatory factors, viruses and fatty acids render hepatic cells susceptible to TRAIL, a consequence not observed in healthy conditions. In addition, the bifunctional nature of apoptosis as the nexus of liver injury and fibrosis casts doubt over the benefit of this approach. It is thus of crucial importance to direct the effect of TRAIL toward the activated HSCs while avoiding collateral damage to hepatic parenchyma. Such pinpoint accuracy might be achievable through a few customized strategies. Application of receptor-specific TRAIL or TRAIL fused with receptor-

specific ligands could circumvent binding to decoy receptors and induce more efficient apoptosis in activated HSCs. Simultaneous application of TRAIL and other inhibitors of inflammatory mediators of signaling and interferons could sensitize HSCs to TRAIL while protecting hepatocytes against the lethal effects of TRAIL. Finally, recent advances in gene delivery could enable the sustained local production of TRAIL in the liver via customized vectors, which would enable a limited distribution and a more efficient therapeutic administration of TRAIL in fibrosis-affected aerials while avoiding systematic effects of TRAIL [65].

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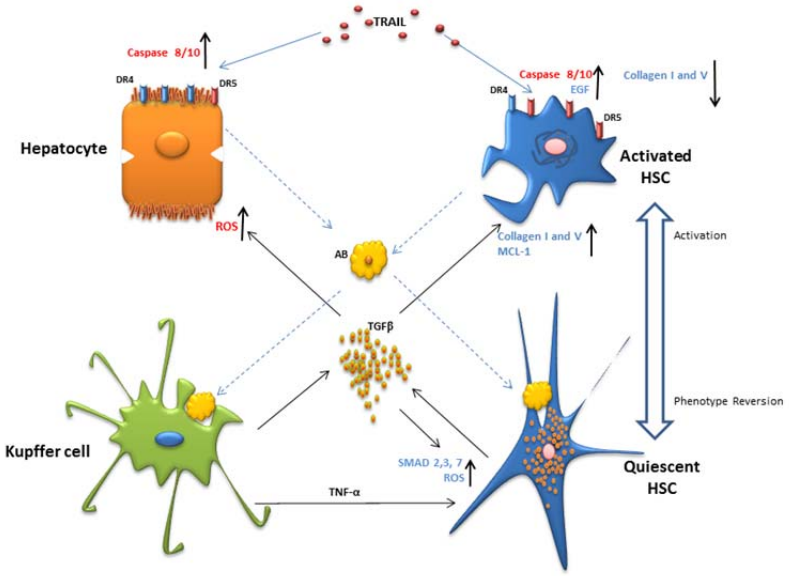

Figure 1: Relation between TRAIL-induced apoptosis and different cell types communication with emphasis on the central role of AB and TGF- β. The direct effect of TRAIL on activated HSCs is considered to be ameliorating liver fibrosis via elimination of these cells.. The indirect effect of TRAIL increases inflammation and accelerates fibrosis. MCL 1; *Myeloid Cell Leukemia 1 protein*, ROS; *Reactive oxygen species*, EGF; *Epidermal Growth Factor*