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The role of ADAM17 and other metalloproteases in liver pathological processes

Dissertation thesis

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Prohlášení:

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V Praze, 29.5.2020

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List of Abbreviations

ADAM(s)	A disintegrin and metalloproteinase(s)
APP	Amyloid precursor protein
AREG	Amphiregulin
BDL	Bile duct ligation
DDC	3,5-diethoxycarboncyl-1,4-dihydrocollidine
CANDIS	Conserved Adam seventeeN Dynamic Interaction Sequence
CCl_4	Carbon tetrachloride
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
HB-EGF	Heparin binding epidermal growth factor
HGF	Hepatocyte Growth Factor
IGFBP	Insulin-like growth factor-binding protein
IL	Interleukin
ILR	Interleukin receptor
MMP(s)	Matrix metalloproteinase(s)
MPD	Membrane proximal domain
PS	Phosphatidylserine
PTGS	Prostaglandin–endoperoxide synthase
TACE	TNF-α converting enzyme
TGF	Tumor growth factor
TIMP	Tissue inhibitor of metalloproteinases
TNFR	Tumor necrosis factor alpha receptor
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

1 Abstract

Liver fibrosis is a condition described by extensive accumulation of scar tissue in the liver. With further progression, it leads to cirrhosis or even to hepatocellular carcinoma. Liver fibrosis accompanies every chronic liver disease and its prevalence in adult European population is estimated to be around 4%. During my dissertation work, I studied the function of three members of Metzincin family of metalloproteinases - ADAM17, ADAM10 and MMP-19, in liver fibrosis and liver regeneration using mouse genetic models. ADAM17 and ADAM10 are important regulators of signalling pathways which are involved in immune response as well as differentiation. Both proteases are able to cleave ectodomains of their substrates from cell membrane, affecting bioavailability of ligands and functionality of receptors. Several of their substrates are involved in liver pathologies. MMP-19 on the other hand, is a metalloprotease mainly involved in extracellular matrix cleavage, important process in fibrosis development, as well as resolution of fibrosis.

Our results demonstrate that ablation of ADAM10 results in increased susceptibility to liver fibrosis in mice, both spontaneous and toxin induced. ADAM10 deficiency affected biliary epithelium, as we detected higher markers of biliary damage in serum of ADAM10 deficient mice. On the other hand, ADAM17 inhibition had protective effect in conditions involving biliary epithelial damage. ADAM17 deficiency did not influence development of fibrosis after CCl_4 intoxication, but it reverted exacerbating effect of ADAM10 deficiency on liver fibrosis. Interestingly, we showed that both studied ADAMs influenced levels of soluble TNF RI in serum, although in different manner. While ablation of ADAM17 inhibits cleavage of TNF RI into serum, deletion of ADAM10 leads to increased TNF RI release. Moreover, we show that ADAM17 and ADAM10 are involved in shedding of EGFR ligands and cMet receptor in partial hepatectomy, the model of liver regeneration. As a consequence, mice with combined deficiency of both proteases exhibited reduced EGFR signalling, but increased HGF/cMet signalling. Studies of MMP-19 whole body deficient mice revealed that ablation of MMP-19 is protective in CCl_4-induced fibrosis. Ablation of MMP-19 caused slower degradation of healthy extracellular matrix and reduced responsiveness to profibrotic TGF-β.

In conclusion, this work extends the knowledge of ADAM10/17-dependent release of EGFR ligands, cMet and TNF RI from liver cells in pathological states *in vivo.*

Furthermore, we described involvement of MMP-19 in liver fibrosis development. Our results demonstrate that inhibition of MMP-19 could be considered as potential treatment of liver fibrosis.

2 Abstrakt

Jaterní fibróza je patologický stav, při kterém dochází ke zmnožení vaziva v jaterní tkáni. V pokročilém stádiu vede k cirhóze nebo až k hepatocelulárnímu karcinomu. Tento jev se vyskytuje u všech chronických jaterních onemocnění a jeho prevalence u dospělé populace v Evropě se odhaduje okolo 4 %. V této práci jsem se zabývala rolí tří metaloproteáz z rodiny metzincinů - ADAM17, ADAM10 a MMP-19, v jaterní fibróze a regeneraci jater. Proteázy ADAM17 a ADAM10 jsou významné při regulaci signálních drah ovlivňujících imunitní odpověď a buněčnou diferenciaci. Obě proteázy odštěpují z buněčných membrán ektodomény svých substrátů, čímž mohou regulovat dostupnost ligandů nebo funkčnost receptorů. Mnoho z těchto substrátů hraje roli v jaterních patologiích. MMP19 je naproti tomu metaloproteázou, jež hraje důležitou úlohu při štěpení extracelulární matrix, což je proces významný jak při rozvoji, tak při hojení fibrózy.

Naše výsledky prokázaly, že inaktivace ADAM10 vede u myších modelů ke zvýšené náchylnosti k tvorbě spontánní i toxinem indukované jaterní fibrózy. Myší modely deficientní pro ADAM10 vykazovaly poškození epitelu žlučových kanálků, charakterizované zvýšením negativních biliárních markerů v séru těchto zvířat. Oproti tomu inhibice ADAM17 měla protektivní účinek v experimentálních modelech, jež vedou k poškození epitelu žlučových kanálků. Samotná inaktivace ADAM17 neměla vliv na rozvoj jaterní fibrózy po intoxikaci CCl_4, ale zvrátila negativní efekt na fibrózu vyvolanou deficiencí ADAM10. Překvapivým zjištěním byl protichůdný efekt proteáz ADAM na hladiny solubilního TNF RI v séru. Zatímco delece ADAM17 vede k nižšímu odštěpování TNF RI do séra, delece ADAM10 vede ke zvýšení TNF RI hladiny.

Kromě toho jsme ukázali, že ADAM10 a ADAM17 se podílí na uvolňování ligandů EGFR a receptoru cMet v rámci modelu pro regeneraci jater, parciální hepatektomie. Myši s kombinovanou deficiencí pro ADAM10 i ADAM17 tak vykazovaly sníženou signalizaci přes EGFR, ale zvýšenou signalizaci přes HGF/cMet. Analýza myší deficientních pro MMP-19 ukázala, že ablace této metaloproteázy má protektivní účinek při modelu fibrózy indukované CCl_4. Inaktivace MMP-19 vedla k pomalejší degradaci zdravé extracelulární matrix a minimalizovala profibrotické efekty způsobené TGF-β.

Celkově shrnuto, tato práce rozšiřuje naše znalosti o vlivu proteáz ADAM10/17 na uvolňování ligandů EGFR, cMet a TNF RI z jaterních buněk za patologických podmínek

in vivo. Dále jsme popsali roli MMP-19 při rozvoji jaterní fibrózy a naše výsledky ukazují, že inhibici MMP-19 lze využít v rámci léčby této patologie.

3 Introduction

3.1 ADAMs and MMPs

A disintegrin and metalloproteinases (ADAMs) and matrix metalloproteinases (MMPs) are two groups of a protein cleaving enzymes which belong to the superfamily named "metzincins". Metzincins (Bode et al., 1996) superfamily comprises distinct group of proteases, which differ greatly in their aminoacid sequence, however topology of their active site remained very similar among all members (Stocker et al., 1995), indicating all evolved from the common ancestral protein (Rawlings and Barrett, 1993). Typical feature shared by all members of metzincins is a presence of the zinc binding motif HExxHxxGxxH in their active site, where three histidines coordinates a zinc (II) atom. Zinc atom is necessary for polarization of water molecule followed by nucleophilic attack on peptide bond leading to its cleavage.

ADAMs and MMPs have a wide variety of substrates and therefor are involved in a numerous physiological processes, including embryonic development, tissue maintenance, wound healing. Protective roles in disease development was shown for several members of this versatile family (Chan et al., 2011; Endres and Deller, 2017; Lopez-Otin and Matrisian, 2007). On the other hand MMPs and ADAMs were as well linked to the development of many pathological states like cancer development, septic shock and many others (Shiomi et al., 2010; Zunke and Rose-John, 2017).

In this work I focus on three members of metzincin superfamily, namely ADAM17, ADAM10 an MMP19, and their role in pathological processes in liver.

3.1.1 ADAM family – structure and function

ADAMs are transmembrane proteases which cleaves their proteins extracellularly in close proximity of plasma membrane. This cleavage leads to release of whole ectodomain of their substrate from cell surface, which is also called "shedding". Besides proteolytic function, ADAMs are able to bind integrins, transmembrane receptors that facilitate cell adhesion (Hynes, 2002). ADAM family in humans is composed of 22 members (Weber and Saftig, 2012) with 12 being active proteases (ADAM8, 9, 10, 12, 15, 17, 19, 20, 21, 28, 30, and 33). Catalytically inactive ADAMs are mostly involved in egg-sperm fusion (Bigler et al., 1997; Weber and Saftig, 2012). These distinct functions, are

governed by different domains of ADAMs. Structural organization and function of individual domains in exerting and regulating ADAM activity is described in following chapter.

ADAMs have a typical domain structure, as seen on figure 1. C-terminal prodomain is followed by a metalloproteinase, disintegrin, cysteine-rich, EGF-like repeat-containing, transmembrane and a cytoplasmic tail domains.

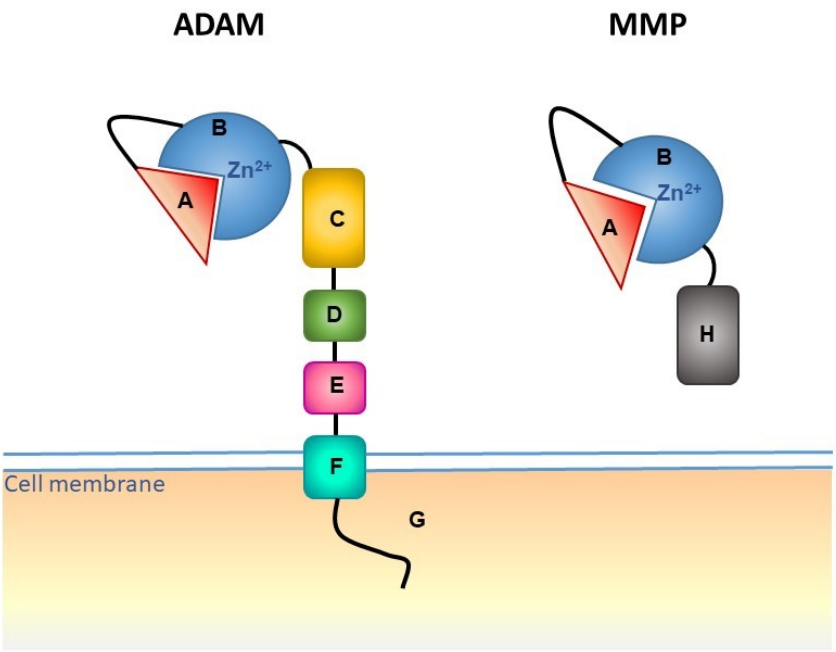

Figure 1. **Typical domain structure of ADAMs and MMPs.** A – a prodomain, B – a metalloproteinase domain, C – a disintegrin domain, D – a cystein rich domain (a membrane proximal domain in ADAM10 and ADAM17), E – an EGF-like domain (CANDIS domain in ADAM10 and ADAM17), F – transmembrane domain, G – cytoplasmic tail, H – a hemopexin-like domain. Adapted from (Klein and Bischoff, 2011).

ADAMs are expressed as a zymogens kept in an inactive state. One of the mechanisms implied in this inhibition is called “cystein switch” (Loechel et al., 1999), where a cysteine from prodomain ligates the zinc atom in the active site, thereby blocking proteolytic activity (Springman et al., 1990). This mechanism is common for prodomains of other metalloproteases including MMPs (Cao et al., 2000). Moreover, or maybe even

more importantly, the prodomain of ADAM proteins also functions as intramolecular chaperone (Anders et al., 2001; Suh et al., 2013) facilitating the proper folding of the other domains. ADAM protein is cleaved between prodomain and metalloproteinase domain by proteases furin (Peiretti et al., 2003) and protein convertase (Wong et al., 2015) in trans-golgi. Mature ADAM protease is then further trafficked to the plasmatic membrane. Most likely, cleaved prodomain is not degraded, but remains bound to rest of ADAM molecule, forming one of the structural components of mature ADAMs on the cell surface (Wewer et al., 2006). Interestingly, it has been shown that bound prodomain is not able to block proteolytic activity of mature ADAMs, but other regulatory mechanisms are involved instead (Le Gall et al., 2010).

Metalloproteinase domain of a catalytically active ADAMs contains the active site with a zinc atom. Substrate specificity for ADAM proteins is not given solely by amino acid sequence of the substrate (Tucher et al., 2014) but also substrate secondary structure (Stawikowska et al., 2013), interaction partners (Janes et al., 2005) and conformation of ADAM protease itself. Growing evidence show that activity of some ADAM proteases, e.g. ADAM17 is controlled by conformational changes which regulate access to its active site (Le Gall et al., 2010). Proposed mechanism involves whole molecule, where C-shaped closed structure is changed to accessible I-shaped structure (Guan et al., 2010), which is catalytically active. This allows very rapid activation of ADAM17 molecule on cell surface upon certain stimuli (Le Gall et al., 2010). In addition, tissue inhibitor of metalloproteinase-3 (TIMP3) binds to metalloproteinase domain of several ADAM proteases and acts as their natural inhibitor (Murphy, 2011).

Disintegrin, or disintegrin-like, domain was named due to the similarity with disintegrin proteins from viper venom (Giebeler and Zigrino, 2016), which inhibit function of integrins (Calvete, 2005) causing hemorrhage in victims of a snake bite. Unlike venom disintegrins, ADAM disintegrin domain does not contain RGD sequence, except for ADAM15, but binds integrins through consensus xCD sequence (Lu et al., 2007). Furthermore, disulfide bonds in disintegrin domain play role in C to I-shape conformational switch described in previous paragraph (Guan et al., 2010). ADAM17 was described to associate with integrin α5β1 (Bax et al., 2004). This interaction can occur between two neighbouring cells (trans) or on a membrane of one cell (cis) and leads to inhibition of ADAM17 proteolytic activity (Gooz et al., 2012). In addition, disintegrin

domains of proteolytically inactive ADAMs are involved in cell-cell as well as cell-ECM adhesion (White, 2003).

Function of a cystein rich and EGF-like domains is not fully understood, but this membrane proximal region was described to be important for substrate binding as well as for regulation of proteolytic activity of ADAMs (Smith et al., 2002). ADAM17 and ADAM10 differ from the rest of ADAM family by their unique structure which does not include a cystein rich and EGF-like domains, but instead contains a membrane proximal domain (MPD) followed by CANDIS (Conserved Adam seventeeN Dynamic Interaction Sequence) (Grotzinger et al., 2017). Similarly to disintegrin domain, MPD exists in two different conformations, opened and closed one (Dusterhoft et al., 2013). The switch from opened to closed conformations is governed by protein-disulfide isomerase (PDI) and serves as a negative regulator of ADAM catalytic activity. Upon activation stimuli, like PMA in case of ADAM17, negatively charged phosphatidylserine (PS) is exposed on outer leaflet of cell membrane. Created negatively charged hubs interacts with positively charged residues on MPD (Sommer et al., 2016). Only MPD present in open conformation is able to bind PS. It has been postulated that binding of MPD to cell membrane causes conformational change of ADAM ectodomain, bringing the metalloproteinase domain closer to the cell surface where it cuts its substrates (Sommer et al., 2016).

Similar mechanism applies for CANDIS domain, which is also able to bind a negatively charged PS, while higher presence of cholesterol blocks an interaction of CANDIS with the plasma membrane, leading to the inhibition of ADAM activity (Dusterhoft et al., 2015; Matthews et al., 2003). Moreover, CANDIS plays direct role in a substrate recognition as CANDIS is able to bind substrates on its own, which is not the case of MPD. However, this applies only to certain group of substrate, so far it has been shown that CANDIS of ADAM17 binds the interleukin 6 receptor (IL6R) and interleukin 1 receptor type II (IL-1R2), but is not able to bind tumor necrosis factor alpha (TNF-α), which is one of its the most emblematic substrates (Dusterhoft et al., 2014).

Cytoplasmic tail of ADAMs was originally proposed as important for transferring the intracellular signals to ADAM molecule leading to regulation of ADAM activity. A tyrosine phosphorylation of cytoplasmic tail by p38 was associated with ADAM17 activation (Killock and Ivetic, 2010; Xu and Derynck, 2010). Described mechanism links phosphorylation of a cytoplasmic tail to dimerization of ADAM17, where unphosphorylated ADAM17 forms homodimer and in this conformation binds its inhibitor

TIMP-3 (Xu et al., 2012). Upon phosphorylation, dimer dissociate, inhibitor is released and ADAM17 gains activity. However, different studies have shown that rapid stimulated activation of ADAM17 (Horiuchi et al., 2007b; Le Gall et al., 2010) and ADAM10 (Maretzky et al., 2015) on cell membrane does not require cytoplasmic tail. In addition, cytoplasmic tail of several ADAMs contains SH3 binding motive/motives (Ebsen et al., 2014) which can contribute to binding of intracellular interaction partners of ADAM that can influence their catalytic activity.

3.1.2 ADAM17 and ADAM10: the "shedding" enzymes

ADAM17, alternatively named tumor necrosis factor alpha (TNF-α) converting enzyme (TACE), and ADAM10 are considered as main proteases mediating ectodomain shedding of various substrates from cell surface and therefore are also called "sheddases". They are closest mutual relatives in ADAM family, evolutionary distinct from the other family members (Huxley-Jones et al., 2007), however their sequence similarity is only around 30% (Gooz, 2010). ADAM17 and ADAM10 are ubiquitously expressed in mammalian cells and have large number of substrates which partially overlaps between two proteases.

3.1.3 Physiologically important ADAM17 substrates

ADAM 17 is probably most characterized member of ADAM proteins. More than 70 substrates were identified to be cleaved by ADAM17 (Grotzinger et al., 2017). Cleavage of individual substrates leads to different, sometimes even antagonistic consequences as summarized in figure 2. Nevertheless involvement of ADAM17 in physiological and pathological processes is mostly connected with two signalling pathways: epidermal growth factor receptor (EGFR) and TNF-α pathways.

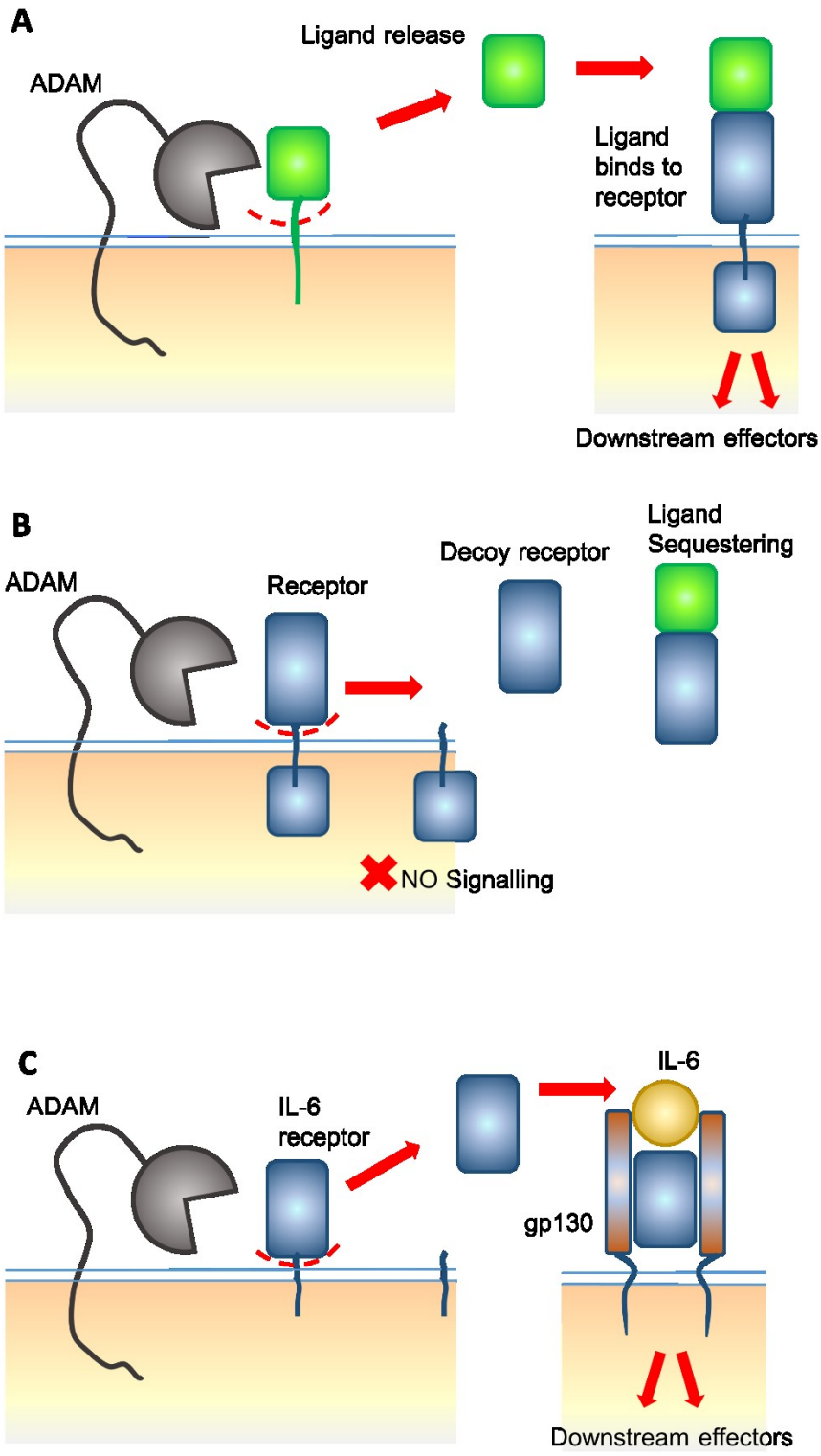

Figure 2. **Different consequences of ectodomain shedding by ADAMs.** A) Shedding of a membrane bound ligand molecule results in ligand bioavailability leading to triggering of signalling pathway. B) Shedding of a receptor leads to attenuation of signalling. Solubilized part of a receptor can exert a decoy function as it is able to by bind ligand without triggering signalling. This further influence bioavailability of ligand. C) Shedding of IL-6 receptor enables trans-signalling on neighbouring cells which do not express IL-6 receptor *per se*.

Several of EGFR ligands were identified as substrates of ADAM17: transforming growth factor alpha (TGF-α), heparin binding epidermal growth factor (HB-EGF), amphiregulin (AREG) and epiregulin. These ligands are expressed as transmembrane proteins. Upon activation of ADAM17, ectodomains of EGFR ligands are shed from cell membrane and subsequently bind to EGFR on same cells or on neighbouring cells, leading to activation of growth receptor cascade. Shedding process it therefore necessary for EGFR signalling which was confirmed *in vivo* in ADAM17-deficient mouse (Peschon et al., 1998). Complete ADAM17 deficiency caused phenotype resembling TGF-α (Luetteke et al., 1993) and EGFR-deficient mouse (Miettinen et al., 1995), manifested by skin defects and impaired development of multiple organs, leading to lethality between embryonic day 17.5 and postnatal day 1. Interestingly, few human patients with complete ADAM17 deficiency were described (Bandsma et al., 2015; Blaydon et al., 2011). Developmental defects were milder, without embryonal lethality, but of a similar nature as in mice. Importantly, patients were prone to the bacterial and viral infections, eventually leading to death in some cases. This underlines importance of ADAM17 in immune response.

ADAM17 protein was discovered in a search of protease responsible for release of TNF-α (Black et al., 1997), cytokine which is an important mediator of inflammation. Later on, TNF-α receptors TNFR I and TNFR II (Reddy et al., 2000; Solomon et al., 1999) were identified as ADAM17 substrate, pointing up role of ADAM17 in regulation of TNF-α pathway. In addition, several other molecules involved in immune response are shed by ADAM17, like interleukin 6 receptor, l-selectin, CX3CL1/Fractalkine of V-CAM. Thus, not surprisingly, high levels of ADAM17 were connected with several inflammatory diseases: rheumatoid arthritis (Charbonneau et al., 2007; Patel et al., 1998), osteoarthritis (Amin, 1999), inflammatory bowel disease (Cesaro et al., 2009), psoriasis (Kawaguchi et al., 2005) and others. Moreover, in experimental mouse model, ADAM17-defiency targeted to myeloid cells completely reverted endotoxin shock lethality (Horiuchi et al., 2007a). On the other side of spectra, complete deficiency in human patients described above was also connected with inflammatory bowel disease, skin inflammation and persistent sepsis (Bandsma et al., 2015; Blaydon et al., 2011).

3.1.4 Physiologically important ADAM10 substrates

ADAM10 is main alpha-secretase participating in Notch cleavage and whole body ADAM10 knockout mice die prenatally due to limited Notch signalling that results in improper development of vasculature and numerous other developmental problems (Hartmann et al., 2002).

Study of ADAM10 is to large extent concentrated on its role in Alzheimer disease development (Colciaghi et al., 2004; Kojro et al., 2001; Suh et al., 2013). ADAM10 cleaves amyloid precursor protein (APP) in a manner which prevents its accumulation and formation of β-amyloid plaques in brain (Kuhn et al., 2010; Lammich et al., 1999). Therefore, regulation of ADAM10 activity could potentially be interesting strategy for Alzheimer diseases therapy (reviewed in (Peron et al., 2018))(Lammich et al., 1999).

Though, EGFR signalling is mostly connected with ADAM17, ADAM10 participates in regulation of this pathway as well. Sahin et al. (2004) showed that EGFR ligands EGF and betacellulin are primarily cleaved by ADAM10. Moreover, even typical ADAM17 substrate AREG is in certain context cleaved by ADAM10 (Miller et al., 2013).

Among other identified substrates of ADAM10 belongs hepatocyte growth factor (HGF) receptor cMet (Schirrmeister et al., 2009), cell surface receptors for hyaluronic acid CD44 (Anderegg et al., 2009) and cell adhesion molecule E-cadherin (Maretzky et al., 2005).

3.1.5 MMP family - structure and function

Matrix metalloproteinases is a group of 23 proteins in human, named according their ability to cleave components of extracellular matrix. Originally, MMPs were connected only with mechanistic remodelling of tissues through extracellular matrix breakdown, but this view on their function proved to be misleading. Nowadays, we know that among their substrates belong important signalling molecules as cytokines, chemokines and angiogenic factors (Rodriguez et al., 2010). Moreover, MMP contributes to bioavailability of several signalling molecules which are not their direct proteolytic targets, e.g. vascular endothelial growth factor (VEGF) (Hawinkels et al., 2008) or transforming growth factor beta (TGF-β) (Imai et al., 1997; Maeda et al., 2002). These

growth factors resides in latency complexes bound to extracellular matrix through proteins which are cleaved by MMPs, leading to release and activation of growth factors.

MMP are either secreted enzymes or transmembrane proteins with active site facing extracellular space. Structure of secreted MMPs generally consist of a signalling peptide, an inhibitory prodomain, a catalytic domain, a hinge region and a hemopexin domain (Fig. 1). Function of a prodomain and catalytic domain was described in section about ADAM proteins. Hinge region is highly motile element (Osenkowski et al., 2005) allowing conformational arrangement of MMP (Fasciglione et al., 2012). Hinge region also influence stability of an active enzyme as this region is prone to autoproteolysis (Knauper et al., 1997). Hemopexin domain is important for substrate recognition (Patterson et al., 2001; Roeb et al., 2002). In addition, membrane type MMPs contain the transmembrane domain or glycosylphosphatidylinositol anchor.

According their substrate specificity and structural features, MMP are divided into several groups: collagenases, gelatinases, stromelysins, matrilysins and membrane type MMPs. Collagenases (MMP-1, MMP-8, MMP-13) cleave triple-helical collagen, e.g. collagen I. Gelatinases (MMP-2 and MMP-9) are able cleave denatured collagens and non-fibrillar collagens as collagen IV. Stromelysins cleave several components of extracellular matrix as laminin, aggrecan, fibronectin, but do not cleave fibrillar collagens. Matrilysins differ in structure from stromelysins, but share several of their substrates. Common feature of membrane type MMPs is that they are activable by furin protease.

3.1.6 MMP-19

MMP-19 is a secreted type MMP not classified in any of the above mentioned groups of MMPs (Vandenbroucke and Libert, 2014), though in the past it was sometimes included into stromelysins. Among its substrates belong components of basal membrane (e.g. laminin, nidogen-1 and collagen IV (Sadowski et al., 2005; Stracke et al., 2000; Titz et al., 2004)), extracellular matrix proteins (tenascin-C, fibronectin (Gueders et al., 2010; Stracke et al., 2000)), plasminogen (Brauer et al., 2011) and insulin-like growth factor-binding protein 3 (IGFBP-3) (Sadowski et al., 2003).

MMP-19 cleavage of nidogen-1 (Titz et al., 2004) and plasminogen (Brauer et al., 2011) leads to inhibition of capillary growth, which may be beneficial in certain types of tumors (Jost et al., 2006)((Impola et al., 2005). However, cleavage of extracellular matrix

proteins by MMP-19 results in increased invasivity of metastatic tumors and therefore increased expression of MMP-19 is associated with poor prognosis in several cancer types: ovarian (Wang et al., 2019), colorectal (Chen et al., 2019), pancreatic (Zhai et al., 2016) and lung cancer (Yu et al., 2014).

MMP-19 is also involved in immune reaction, it is expressed on myeloid cells and subtypes of T-cells (Mauch et al., 2002; Sedlacek et al., 1998). MMP-19 overexpression was described in rheumatoid arthritis pathology (Kolb et al., 1999; Sedlacek et al., 1998). On the other hand, MMP-19 plays role in normal T-cell development 1 (Wang et al., 1996).

MMP-19 plays protective role in lung fibrosis (Yu et al., 2012). Suggested mechanism involves increased expression of prostaglandin–endoperoxide synthase 2 (PTGS2). Moreover, MMP-19 deficient lung fibroblasts exhibit higher responsivity to profibrotic cytokine TGF-β, manifested by higher production of collagen I after treatment in comparison to wild type fibroblasts (Jara et al., 2015).

3.2 Liver- function, structure and pathology

Liver is the largest internal organ in human body. About 500 distinct functions of liver has been described including I) metabolism of carbohydrates, lipids and amino acids, II) detoxification of waste products and toxic substances, III) bile production, IV) synthesis of plasma proteins, like albumin and coagulation factors, V) vitamin storage. With this range of different functions, there is no other possibility for patients with irreversible liver damage then liver transplantation, as no device that could sufficiently substitute liver functions is recently available (Rozga, 2006; Sussman and Kelly, 2014).

3.2.1 Liver cell types

Liver parenchyma is constituted by hepatocytes and biliary epithelial cells (or cholangiocytes). Hepatocytes present around 50% of all liver cells in number, but forms about 80% of liver volume and are responsible for majority of liver functions. Biliary epithelial cells are cells lining bile ducts and are important for bile secretion. Major non parenchymal cell types present in normal liver are Kupffer cells (resident macrophages) (Naito et al., 1997), hepatic stellate cells (vitamin A storage cells, responsible for

extracellular matrix production) (Friedman, 2008) and liver sinusoidal endothelial cells (Poisson et al., 2017).

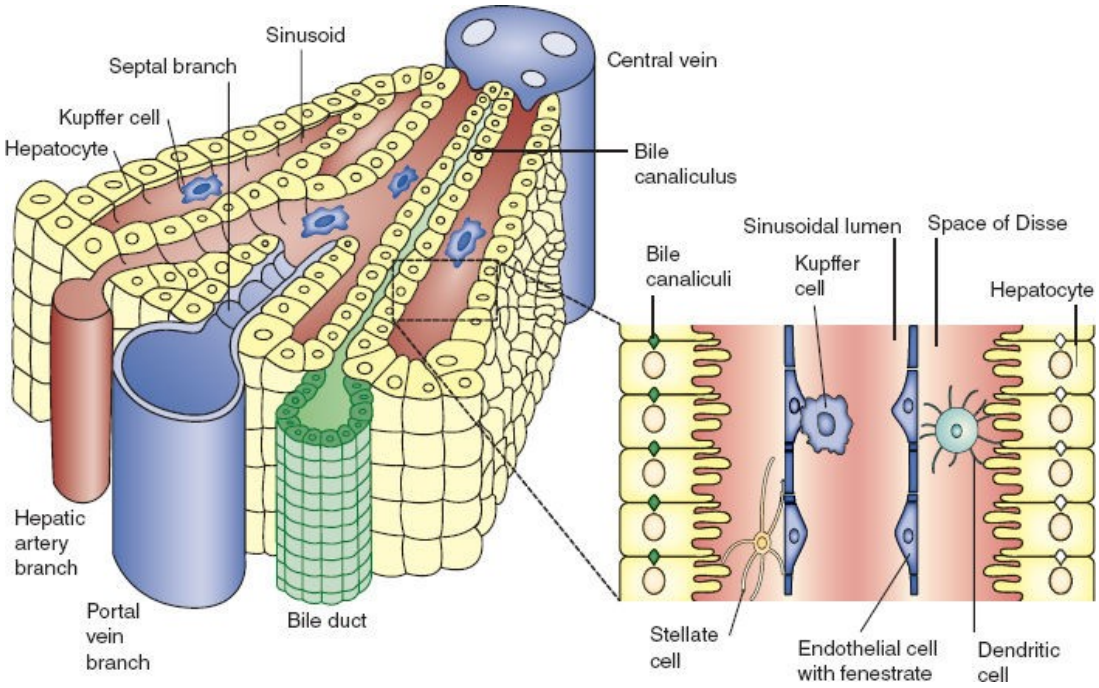

Figure 3. **Microanatomy of liver depicting all main cell types.** *Nature Rev Immunol*. 2006;6:244–251, with permission.

Organization of liver tissue is uniform through whole organ. Liver microarchitecture is depicted in figure 3. Liver sinusoid (Brunt et al., 2014) is a capillary connecting central vein and portal triad composed of portal vein, bile duct and liver artery. Sinusoid is discontinuously lined by endothelial cells without basement membrane. Other cells present in liver sinusoid are immune cells and hepatic stellate cell that reside in the space between endothelial cells and hepatocytes, called also space of Disse. Hepatocytes are arranged around sinusoid, interconnected with tight junctions (Gissen and Arias, 2015), forming capillary-like structure between each two adjacent hepatocytes called bile canaliculi. Hepatocytes secrete bile salts which are collected through bile canaliculi into bigger bile ducts which further join to form common bile duct carrying bile from liver into duodenum. Near portal triad resides small pool of liver progenitor cells (Itoh, 2016), also called oval cells according their shape, which are able to differentiate into hepatocytes or biliary epithelial cells in certain pathological conditions.

3.2.2 Molecular mechanism of liver injury

Liver is an organ with remarkable regenerative capacity. Differentiated hepatocytes are able to enter into cell cycle and start to proliferate as a part of normal organ renewal (Malato et al., 2011; Wang et al., 2015), but in more massive way during liver injury (Malato et al., 2011). Though some studies (Furuyama et al., 2011) suggested significant contribution of liver progenitor cells to this process, with more data collected, liver progenitor cells are now viewed as a backup for the states when differentiated hepatocytes are no more able to proliferate (Miyajima et al., 2014). Differentiation of liver progenitor cells into hepatocytes and biliary epithelial cells is thus present during chronic liver diseases and occurs proximal to the bile duct in portal triad.

Leading cause of chronic liver diseases in human patients is chronic hepatitis C infection (Shirazi et al., 2020). However, with current trends, it will be soon replaced by nonalcoholic steatohepatitis, condition connected with western lifestyle and metabolic syndrome. Chronic alcohol abuse is the third most common aetiology of chronic liver diseases (Shirazi et al., 2020).

No matter what is the original cause, all chronically injured livers proceed through stage of liver fibrosis (Friedman, 2003), which in its end stage results in liver cirrhosis and eventually hepatocellular carcinoma. Liver fibrosis occurs when damaged liver tissue is replaced by scar tissue, rich in fibrillary collagens, predominantly collagen I (Burt et al., 1990), but also collagen III or elastin (Yasui et al., 2016). It is a reversible state (Benyon and Iredale, 2000; Ramachandran and Iredale, 2012), but generally untreated in human patients with no clinical symptoms. If the scaring continues, fibrillary tissue starts to interfere with the normal liver functions, fibrosis comes to cirrhotic stage (Tsochatzis et al., 2014), characterized by liver architectural disruption.

Accumulation of fibrotic extracellular matrix has two aspects: i) production of fibrillary collagens on one side and ii) cleavage of fibrillary tissue and restoration of normal matrix on the other. Fibrillary collagens are produced by myofibroblasts, cells that appear in liver tissue after injury. Major source of myofibroblast in liver are activated hepatic stellate cells (Tsuchida and Friedman, 2017), though other sources contribute, including bone marrow fibroblasts or portal myofibroblasts (Forbes et al., 2004; Lemoinne et al., 2013). Hepatic stellate cells are in quiescent state in resting liver, with retinoid storage as their most distinguished function. After injury they became activated, loose lipid

droplets, start to express α-smooth muscle actin and secrete collagen I and other proteins of fibrotic extracellular matrix (Friedman et al., 1985; Iredale, 2007). TGF-β, produced by Kupffer cells (Gressner et al., 1993), is a key cytokine regulating transdifferentiation of hepatic stellate cells into myofibroblasts (Dooley et al., 2000; Tang et al., 2017).

Fibrosis reversal includes loss of myofibroblasts from liver, cleavage of fibrotic collagens and its replacement with normal extracellular matrix. Results from rodent models suggest, that part of activated hepatic stellate cell undergo apoptosis (Kim et al., 2005) and part revert to inactive phenotype, similar to quiescent state, but more prone to another potential activation (Kisseleva et al., 2012). Removal of fibrotic matrix will be described later in section: "MMPs in liver fibrosis".

3.2.3 Experimental models of liver injury

Most used rodent model of liver fibrosis are carbon tetrachloride (CCl_4) intoxication and bile duct ligation (BDL). Diet enriched in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC diet) is valuable tool to study liver progenitor cells in mice (Dorrell et al., 2011).

CCl_4 is a substance, which is bioactivated in hepatocytes, giving rise to toxic intermediates, which cause hepatocyte death (Slater et al., 1985). Subsequent necrosis of liver tissue leads to activation of Kupffer cells, activation of hepatic stellate cells and fibrosis within 4-6 weeks of regular application or even cirrhosis when applied over longer period of time (Liedtke et al., 2013). Once toxin is no longer applied, fibrosis start to resolve and complete regeneration is reached after 4-6 weeks. Therefore, CCl_4 is a valuable model, in which all parts of liver fibrosis development as well as reversal can be studied.

BDL is a model of liver fibrosis originating from damage of biliary epithelia. This type of injury in human patients is most commonly caused by autoimmune diseases or obstructions of biliary system like gallstones of carcinoma. BDL in rodents is laparoscopic operation, in which common bile duct is ligated and dissected (Tag et al., 2015). This blocks bile outflow from liver, causing pressure on small bile ducts in liver parenchyma as well as accumulation of toxic bile acids, leading to massive proliferation of biliary epithelia, activation of Kupffer cells and fibrosis within 2-4 weeks (Liedtke et al., 2013). Common bile duct can be surgically reconnected in rats to study fibrosis reversal (Popov et al., 2010), however this recovery model is not used in mice.

Feeding with DDC diet leads to formation of poorly soluble protoporphyrin crystals, which accumulates in bile canaliculi and bile ducts, forming plugs interfering with bile outflow (Fickert et al., 2007). Liver injury after 2-3 weeks feeding is similar to the one caused by BDL, mostly affecting the biliary system, with bile duct enlargement, formation of new bile ducts and accumulation of fibrotic tissue around them. Higher number of bile ducts leads to enrichment in liver progenitor cells pool (Dorrell et al., 2011) in mice. Advantage of this model is the possibility to study recovery process by diet switch to standard chow.

Two thirds (2/3) partial hepatectomy is a rodent model used to study proliferative capacity of liver cell (Martins et al., 2008). It is performed by surgical removal of two thirds of liver. Remaining portion of liver immediately starts recovery process in which differentiated hepatocytes re-enter cell cycle and start to proliferate to compensate lost liver mass (Michalopoulos, 2010). Within first two days hepatocytes undergo one to two cycles of cell duplication, followed by division of non-parenchymal cells. Complete restoration of original liver occurs after one to two weeks.

3.2.4 Regulation of TNF-α signalling by ADAM17 in liver injury

TNF-α was described to play a role in many pathological states of the liver and have distinct effects on hepatocytes depending on the strength of a signal and length of signal duration. ADAM17 is a key player in TNF-α signalling, indispensable for TNF-α release. Mice lacking ADAM17 specifically in myeloid linage almost did not release TNF-α into serum after LPS application (Horiuchi et al., 2007a). Interestingly, hepatocyte specific ADAM17 deficient mice had reduced shedding of TNF-α into serum (McMahan et al., 2013). Thus both, tissue macrophages and hepatocytes, are significant source of TNF-α. Once released from cell surface, TNF-α binds to TNF-α receptors on Kupffer cells and hepatocytes. Nevertheless, ADAM17 can also shut down TNF-α signalling by cleavage of TNF-α receptors. Cellular context is therefore important for the outcome of ADAM17 activity on TNF-α signalling.

In a model of 2/3 partial hepatectomy, serum levels of TNF-α dramatically rises one hour after removal of liver mass and drops again to normal within next hour. TNF-α starts regeneration process, in which differentiated hepatocytes enter cell cycle and divide to compensate for the lost liver mass. Even though several publications described TNF-α

as cytokine necessary for liver regeneration (Sudo et al., 2008; Yamada et al., 1997), its role is now questionable as TNF-α deficient mice did not show altered regeneration after partial hepatectomy (Hayashi et al., 2005) neither did mice with deficiency in ADAM17 (McMahan et al., 2013). On the other hand chronically enhanced shedding of TNF-α produce great obstacles to liver regeneration. Mice deficient in ADAM17 inhibitor, TIMP-3, have constantly enhanced ADAM17 activity and consequently higher TNF-α shedding (Mohammed et al., 2004). TNF-α levels are high during whole process of regeneration after partial hepatectomy in serum of these mice, unlikely in wild type mice where TNF-α peaks and drops. This chronic exposure to TNF-α leads to enhanced apoptosis of hepatocytes and at some point to death of animals which are unable to restore removed liver mass (Mohammed et al., 2004).

Even though hyperactivity of ADAM17 hampered liver regeneration after partial hepatectomy, it can be beneficial in other pathological condition of liver. Timp3 deficient mice exhibited delayed apoptosis in Fas-induced hepatitis (Murthy et al., 2010), which was result of excessive TNFR1 shedding. Moreover, adenoviral delivery of ADAM17 into wild type mice prevented from lethal dose of acetaminophene through same mechanism (Murthy et al., 2010).

Similarly, in model of liver intoxication, TNF-α plays both protective and damaging role. High levels of TNF-α produced in liver after CCl_4 injection contributes to apoptosis of hepatocytes (Sato et al., 2014) and neutralization with TNF-α antibody is beneficial in this type of injury. Interestingly, with too high doses of neutralizing antibody beneficial effect vanished, resulting in even higher damage than without antibody (Dong et al., 2016). This suggest that certain small amount of TNF-α has protective effect during liver intoxication. ADAM17 deficient mouse in myeloid linage shed less TNF-α after CCl_4 application compared to WT leading to lower release of hepatocyte damage markers into serum (McMahan et al., 2013). However, attenuation of TNF-α signal did not result in reduced apoptosis and necrosis of hepatocytes in this model. To complete the whole picture, CCl_4 intoxication leads to elevated serum levels of cleaved TNFR1 and TNFR2 (Ijiri et al., 2014).

3.2.5 ADAM17 and ADAM10 as regulator of growth factor signalling in liver

EGFR pathway is important signalling in the liver, it protects hepatocytes from apoptosis (Svinka et al., 2017) and it is needed for hepatocyte division (Natarajan et al., 2007). Bioavailability of EGFR ligands is provided by ADAM proteases as was described in previous chapter.

Individual EGFR ligands have different source, EGF coming mainly from duodenum, HB-EGF produced by Kupffer cells, amphiregulin and TGF-α hepatocytes and hepatic stellate cells, but generally all have similar effects when it comes to liver pathology, they promote proliferation of hepatocytes after liver challenge as reported in many rodent models (Berasain et al., 2005; Kiso et al., 1995; Mitchell et al., 2005; Skov Olsen et al., 1988). On the other hand, EGFR is expressed also on non-parenchymal cells of a liver and several studies reported that EGFR ligands enhanced liver fibrosis through their mitogenic effect on hepatic stellate cells (McKee et al., 2015; Perugorria et al., 2008).

To sum up, regulation of EGFR receptor signalling by ADAMs in liver is complex process, which is not fully understood so far. One problem is redundancy between EGFR ligands, some of which can be cleaved not only by ADAM17 but also other ADAMs-ADAM10 (Miller et al., 2013) and ADAM12 (Higashiyama and Nanba, 2005). Moreover, as mentioned above, EGFR ligands are produced and shed in different cell types of liver. Therefore, mouse models which targets specific cell type, e.g. hepatocytes, biliary epithelial cells, hepatic stellate cells, present useful model for EGFR research in liver.

HGF/cMet pathway is another important growth factor pathway involved in liver regeneration. Interestingly, also cMet is regulated by shedding by both ADAM10 and ADAM17 (Yang et al., 2012). This regulation is not through shedding of ligands but shedding receptor itself. After cleavage, cMet is no longer available on cell surface and signalling is attenuated.

Several studies shows that combined disruption of both EGFR and cMet pathways leads to dramatic effects, livers of experimental animals are not able to recover from partial hepatectomy, necrosis after intoxication is aggravated (Paranjpe et al., 2016) (Scheving et al., 2015; Seki et al., 2008) and most strikingly homeostasis of unchallenged liver is compromised with fatal outcome (Tsagianni et al., 2018). Even though both cMet and EGFR provide fungible mitogenic signal for hepatocytes, they have distinct role in differentiation of hepatic progenitor cells, also called oval cells. Kitade et al. (2013)

showed that cMet induced hepatic progenitor cells differentiation to hepatocytes while EGFR signalling led to Notch1-dependent differentiation towards biliary epithelial cells. Roles of ADAMs in differentiation process remains to be established.

3.2.6 MMPs in liver injury

MMP-2 and MMP-9 involved in tissue remodelling (Corbel et al., 2000; Salo et al., 1994), when extracellular matrix needs to be cleaved and re-established. In liver fibrosis MMP-2 and MMP-9 levels are elevated (Jiang et al., 2004), they cleave normal healthy extracellular matrix (Takahara et al., 1995), which is than replaced by fibrotic scar tissue. Moreover, MMP-2 and MMP-9 also directly participate in profibrotic signalling cascades, by release of TGF-β from extracellular matrix (Yu and Stamenkovic, 2000) leading to activation of hepatic stellate cells (Han et al., 2004). Also MMP-8 was shown to promote hepatic stellate cell activation (Baig et al., 2016).

Recovery from fibrosis also includes remodelling of tissue. Components of fibrotic scars, mainly collagen I and collagen III, need to be cleaved and removed, while new healthy extracellular matrix is synthesized. Main human collagenase MMP-1 is absent in rodents, where MMP-13 (Yata et al., 1999) and MMP-8 (Harty et al., 2005) are predominantly responsible for cleavage of fibrillary collagens in mice and rats. Consequently, MMP-13 deficient mice suffer from hampered recovery of CCl_4 induced fibrosis (Fallowfield et al., 2007). Therefore, adenoviral application of collagenases could be promising approach in treatment of established fibrosis. Beneficial effects were shown experimentally with all collagenases MMP-1 (Iimuro et al., 2003), MMP-13 (Endo et al., 2011) and MMP-8 (Siller-Lopez et al., 2004).

Interestingly, MMP-2 seems to have direct negative effect on collagen I production by hepatic stellate cells (Radbill et al., 2011), therefore it is needed for fibrosis resolution. Indeed, MMP-2 deficient mice exhibit enhanced fibrosis after CCl_4 treatment (Onozuka et al., 2011; Radbill et al., 2011), pointing out protective role of MMP-2 over destructive.

TIMP-1, major inhibitor of MMPs (Brew et al., 2000), is upregulated during liver fibrosis. Transgenic mice overexpressing TIMP-1 developed more prominent fibrosis after CCl_4 intoxication (Yoshiji et al., 2000). Conversely whole body deficiency of TIMP-1 also resulted in increased susceptibility to CCl_4-induced fibrosis (Wang et al., 2011). This may sound contradictory, but TIMP-1 inhibits large number of MMPs which have profibrotic

as well as antifibrotic functions (Hemmann et al., 2007). Nevertheless, experimental blockage of TIMP-1 by adenoviral delivery of siRNA (Cong et al., 2013) or TIMP-1 activity by MMP-9 mutants, inhibited development of liver fibrosis (Roderfeld et al., 2006).

4 Aims of the study

Primal aim of this study was to clarify the role of hepatocyte derived ADAM17 during the development of liver fibrosis and in the liver regeneration. However, first results from animal experiments led to the decision to involve ADAM10, closest relative of ADAM17, to the study. As a complementary project I studied the role MMP19, metalloprotease which is also inhibited by TIMP3, in development of liver fibrosis and fibrosis resolution.

Aims were set as follows:

- **Clarify the role of ADAM17 and ADAM10 in liver fibrosis *in vivo* using a murine model.** TNF-α signalling is regulated by ADAM17 on several levels. As important mediator of inflammation TNF-α contributes to the development of fibrosis. On the other hand, balance between necrosis and proliferation of hepatocytes dictates the outcome of fibrosis development in this model.

- **Identify the impact of hepatocyte derived ADAM17 and ADAM10 in liver regeneration.** Many substrates of ADAM17 and ADAM10 were described previously to be important in hepatocyte proliferation, most importantly several EGFR ligands. As shedding of EGFR ligands by ADAMs is necessary part of their activation, aim was to study the contribution of ADAM17 and ADAM10 activity to the hepatocyte proliferation after partial hepatectomy using mouse models with deficiency of proteases in liver parenchymal cells - hepatocytes and biliary epithelial cells.

- **Aim of my complementary project was to establish the role of MMP19 in the murine model of CCL4 induced liver fibrosis.** MMP19 is not much studied gene with abundant expression in liver. Therefore, it is important to clarify its role in liver pathology.

5 Results

Results are described in detail in individual publications, which are enclosed as supplements to this thesis. In following section are mentioned the most important results with indication where to find them.

5.1 ADAM10/17 mediated release of cMet correlates with fibrosis progression

To establish the role of ADAM17 and ADAM10 in liver fibrosis, we first looked on its expression in course of fibrosis development using mouse model of DDC diet. We showed that ADAM17 and ADAM10 expression on mRNA level is significantly increased in fibrotic state, reaching its peak in the early recovery state, and again decreasing to control levels with fibrosis resolution ((Chalupsky et al., 2013), Fig. 5). mRNA levels of ADAMs reflected histological findings, where inflammation and collagen deposition was evaluated ((Chalupsky et al., 2013), Fig. 6-7). Two ADAM17 substrates were determined in serum of treated mice, cMet and TNF-α. Both substrates were increased in peak fibrosis and declined in recovery phase. Especially cMet strongly correlated with state of fibrosis, during recovery phase even better than standardly used biochemical markers ((Chalupsky et al., 2013), Fig. 7). Therefore, we proposed its use as a biomarker of liver damage.

5.2 UDCA influences maturation of ADAM17

Ursodeoxycholic acid (UDCA, 3α,7β-dihydroxy-5 β-cholanic acid) is an approved drug for the treatment of primary biliary cirrhosis and is also used to treat several other cholestatic conditions. It has been described before that UDCA regulate the expression of TNF-α and other inflammatory cytokines. We showed that UDCA interfered with maturation of ADAM17 in human liver hepatocellular carcinoma cell line HepG2 ((Buryova et al., 2013), Fig. 3). This in consequence led to lower shedding of ADAM17 substrates TNF-α, TGF-α and cMet into culture medium ((Buryova et al., 2013), Fig. 1). Similar effect was recapitulated in context of whole organism using BDL model. Mice that underwent pretreatment with UDCA developed less severe liver injury, determined from biochemical markers ((Buryova et al., 2013), Fig. 6) and histological sections ((Buryova

et al., 2013), Fig. 7). Reduced injury was accompanied by lower shedding of cMet into serum. This lead to the conclusion, that hepatoprotective effect of UDCA in acute cholestasis is at least in part mediated through inhibition of ADAM17.

5.3 Spontaneous liver fibrosis in ADAM10 deficient strain with higher dose of Alb-Cre.

To further study the role of ADAM17 liver pathology we prepared liver specific knockout by crossing Alb-Cre mouse to ADAM17 flox strain. Same way were prepared ADAM10 liver deficient mice, which were added to the study. Double deficient mouse strain was prepared by crossing the ADAM17 deficient strain and ADAM10 strain together. This study was mainly performed with ADAM10 and ADAM17 deficient mice, which were homozygous for floxed alleles but carried Cre only on one chromosome. Those lines are marked as ADAM10$^{\Delta Alb}$, ADAM17$^{\Delta Alb}$ and ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$. Neither of two single deficient strains, nor double deficient strain displayed any spontaneous phenotype.

However, in Muller et al. (2016) we used the ADAM10 deficient strain which had Alb-Cre on both chromosomes to increase the Cre expression and thus its effect. Strikingly, this strain, marker B6.ADAM10$^{\Delta hep\Delta ch}$ developed spontaneous liver fibrosis at the age of 15 weeks ((Muller et al., 2016), supplementary Fig. 8). In literature it is typical to breed Alb-Cre to hemizygous state for generation of conditional deficient mouse strain using Cre-Lox system, e.g. (Das et al., 2011; Gazit et al., 2012). However, we found that our Alb-Cre generated ADAM10 deficient strain displayed spontaneous phenotype only when Cre was bred to homozygosity, suggesting Cre dosage is important to ensure Cre-Lox recombination in whole population of liver parenchymal cells.

5.4 ADAM17 deficiency ameliorates enhanced liver fibrosis caused by ADAM10 deficiency

ADAM10$^{\Delta Alb}$, ADAM17$^{\Delta Alb}$ and ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ and their control littermates were subjected to intoxication with CCl_4. After 4 weeks, all groups developed pronounced fibrosis. ADAM17$^{\Delta Alb}$ developed fibrosis to the same extent as their control littermates but ADAM10$^{\Delta Alb}$ developed more advanced bridging phase of fibrosis. This is a state when

fibrotic fibers interconnect central veins, forming bridges between them. Interestingly, double deficient ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ developed fibrosis to the same extent as their control littermates (Zbodakova et al., manuscript, Fig 4).

Together with higher hepatocellular damage, manifested by increased aminotransferase activities in serum, ADAM10$^{\Delta Alb}$ displayed enhanced ductal reaction with higher release of alkaline phosphatase into serum and enhanced expression of CD44 in comparison to control littermates, or ADAM17$^{\Delta Alb}$ and ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ (Zbodakova et al., manuscript, Fig. 5).

Levels of serum TNF RI increases with CCl4 induced damage. ADAM17$^{\Delta Alb}$ exhibited lower shedding of sTNF RI into serum with liver fibrosis development (Zbodakova et al., manuscript, Fig. 6). Unexpectedly, levels of sTNF RI in serum of ADAM10$^{\Delta Alb}$ were increased. Same effect was detected in primary hepatocytes. Observed increase did not result from increased expression of *TnfrI* in liver and was not caused by increased activity of ADAM17, as ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ also showed increased levels of sTNF RI in unchallenged state (Zbodakova et al., manuscript, Fig. 6). This points to two different pathways of sTNF RI release influenced by ADAM17 and ADAM10.

5.5 ADAM10 and ADAM17 together regulate release of EGFR ligands and cMet after partial hepatectomy.

To study the role of ADAM10 and ADAM17 in liver regeneration we applied model of ⅔ partial hepatectomy. As a first screen we looked on the time point six hours after partial hepatectomy and we found that phosphorylation of AKT kinase is significantly decreased in ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$, but not in single deficient group (Zbodakova et al., manuscript, Fig. 2). At the same time point, ADAM10/17 deficiency led to decreased release of EGFR activating factor into serum. This was determined by an experiment in which we applied sera from mice after partial hepatectomy on the control primary hepatocytes and we saw decreased phosphorylation of EGFR in hepatocytes treated by sera originating from hepatectomized ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ (Zbodakova et al., manuscript, Fig. 2). Data suggest that that decrease in AKT signalling was most likely caused by decreased shedding of EGFR ligands and that both ADAM17 and ADAM10 are important for this shedding in liver regeneration.

However, initial decrease in pro survival signals was compensated in the later time points as 40 hours after hepatectomy, ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ did not exhibit delayed hepatocyte proliferation in comparison with control littermates (Zbodakova et al., manuscript, Fig.3). We explain this compensation by shift from EGFR signalling towards HGF signalling, mediated through impaired cMet shedding. Shedding of cMet receptor was strongly increased after hepatectomy in controls, together with expression of HGF (Zbodakova et al., manuscript, Fig. 3). However, ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ had lower levels of shed cMet in serum during whole course of experiment (Zbodakova et al., manuscript, Fig. 3). Lower shedding of cMet should lead to higher availability of functional cMet on the cell surface. This was confirmed on primary hepatocytes, where hepatocytes from ADAM10$^{\Delta Alb}$ADAM17$^{\Delta Alb}$ exhibited higher phosphorylation of cMet upon HGF stimulation than hepatocytes from controls. As single deficient ADAM10$^{\Delta Alb}$ and ADAM17$^{\Delta Alb}$ did not have significant impairment of cMet shedding, we concluded that both proteases are important sheddases for cMet *in vivo*.

5.6 MMP-19 deficiency protects from liver fibrosis

MMP-19 whole body deficient mouse, marked as MMP19KO, were prepared previously by insertion of PGK-neomycin cassette (Beck et al., 2008). Unchallenged animals did not exhibit any apparent liver phenotype. To study involvement of MMP-19 in liver fibrosis, mice were exposed to CCl_4 for 4 or 6 weeks and some groups were let to recover for 10 or 15 days after initial 6 week CCl_4 treatment to look at fibrosis resolution. MM19KO developed less severe fibrosis, with lower collagen deposition and lower aminotransferase serum levels ((Jirouskova et al., 2012), Fig 1) and subsequently recovered faster ((Jirouskova et al., 2012), Fig 2). Slower fibrosis progression was accompanied with lower activation of MMP-2 and higher deposition of collagen IV ((Jirouskova et al., 2012), Fig 4). Thus MMP-19 deficiency seems to influence MMP network, reducing their potential to degrade components of basal membrane, which could play protective role in liver fibrosis development.

Furthermore, we screened components of profibrotic TGF-β pathway. MMP19KO mice exhibited lower phosphorylation of SMAD3, TGF-β signalling mediator, in both fibrosis development and recovery, which is consistent with lower collagen deposition in MMP19KO ((Jirouskova et al., 2012), Fig 5). Data were supported by experiment *ex vivo*,

where MMP19KO primary hepatocytes treated with TGF-β1 expressed lower levels of profibrotic genes *Snail1* and *vimentin* ((Jirouskova et al., 2012), Fig 6) than primary hepatocytes from wild type animals. All together this points towards impaired TGF-β signaling in MMP19KO.

6 Discussion

6.1 Role of ADAM17 and ADAM10 in liver fibrosis

Among the identified substrates of ADAM17 and ADAM10 belongs many molecules, which has been described previously as important signalling mediators, involved in liver diseases, or conversely, which may be protective in liver pathology. One of the aims of this study was to clarify role of ADAM10 and ADAM17 in liver, using several mouse models.

At the beginning of this study, there was little information about role of ADAM17 or ADAM10 to liver pathology *in vivo*. Data from whole body ADAM10 (Hartmann et al., 2002) or ADAM17 deficient mice clarified that ablation of this proteases does not lead to liver developmental defect), despite embryonic lethality of both deficient strains. To establish their role in liver pathology, we prepared conditional ADAM10 and ADAM17 deficient mice, with deficiency targeted to hepatocytes and biliary epithelial cells. We applied several models of liver disease, three different models of liver fibrosis (BDL, DDC diet and CCl4 induced fibrosis) and model of liver regeneration (⅔ partial hepatectomy).

ADAM10 and ADAM17 expression was increased in fibrosis induced by DDC (Chalupsky et al., 2013), but was not in CCl_4-induced fibrosis. However, expression levels are not the best measure when considering involvement of ADAM10/17 to certain biological process. It was shown, that ADAM17 can be readily activated on the membrane, without change in expression and disregarded of TIMP-3 levels or even prodomain cleavage (Le Gall et al., 2010). Therefore, release of ADAM's ligand gives much more valuable picture than mere expression. We always determined levels of several ADAM ligands in the serum when studying different mouse models. Especially HGF receptor, cMet, proved to be valuable one in the study of liver diseases.

Shedding of cMet was once attributed mainly to ADAM10. Using specific ADAM17 inhibitor, we have shown that ADAM17 contributes to shedding of cMet in HepG2 and LX2 cell lines (Chalupsky et al., 2013). More importantly, those findings were confirmed *in vivo*, as only simultaneous deficiency of both ADAM10 and ADAM17 lead to significant decrease of serum sMet (soluble cMet) in mice (Zbodakova et al., manuscript). Shedding of cMet was strongly induced in liver fibrotic conditions (Chalupsky et al., 2013) (Buryova et al., 2013) as well as after partial hepatectomy

(Zbodakova et al., manuscript). Serum sMet levels very well reflected histological findings of hepatocellular damage and fibrosis therefore we suggested its potential as marker of liver damage (Chalupsky et al., 2013).

To further study involvement of ADAM10 and ADAM17 in liver pathology we prepared conditional deficient strains using Cre recombinase expressed under human albumin promoter. This promoter is expressed in hepatocytes but also liver progenitor cells (Qin et al., 2004; Tian et al., 1997) starting in embryonic state (Weisend et al., 2009). In consequence, using Alb-Cre transgene leads in adult mouse to deficiency in the hepatocytes, as well as in the biliary epithelial cells, which do no express albumin *per se*, but have differentiated from liver progenitor cells. Our ADAM10 or ADAM17 deficient mice, which expressed Cre from one chromosome, did not display any gross liver phenotype (Zbodakova et al., manuscript). Nor did the double deficient strain which lacks both proteases.

However, our collaborators, who prepared ADAM10 deficient mice using the same ADAM10 floxed strain but Alfp-Cre transgene, reported spontaneous fibrosis in ADAM10 deficient mice, starting as early as 4 weeks after birth (Muller et al., 2016). Alfp-Cre regulates Cre by both albumin and α-fetoprotein elements (Kellendonk et al., 2000) and its expression pattern does not differ from our Alb-Cre, as was checked using reporter strain with floxed YFP gene. Nevertheless, its expression could be stronger as Alfp-Cre transgene contains additional enhancer elements. Therefore, we further bred our ADAM10 deficient strain to the state, when Alb-Cre was present on both chromosomes to enhance Alb-Cre expression. Alb-Cre transgene has Cre inserted in identified locus, which is safe for generation of homozygous Alb-Cre transgene on both chromosomes ((Postic et al., 1999), JAX (2020): https://www.jax.org/strain/003574).

Ablation of ADAM10 using Alfp-Cre resulted in several different pathological consequences (Muller et al., 2016). ADAM10 deficiency directly influenced expression of bile acid transporters in hepatocytes, which consequently resulted in hepatocyte necrosis and concomitant liver fibrosis. ADAM10 deficient animals exhibited ductular reaction with enhanced proliferation of biliary epithelial cells and liver progenitor cells. However, architecture of biliary tree and bile canaliculi was not compromised in ADAM10 deficient mice. In addition, differentiation of BMOL cell line was shifted toward hepatocyte fate in absence of ADAM10. Of note, fibrotic phenotype was not uniform in all ADAM10

deficient mice, approximately half of the animals showed no signs of liver damage. As mentioned before, our ADAM10 deficient strain with hemizygous Alb-Cre did not develop any spontaneous liver damage. In contrast, ADAM10 deficient strain with homozygous Alb-Cre exhibited liver damage of similar nature as Alfp-Cre generated strain, but milder. There were no signs of liver fibrosis at the age of 4 weeks in these mice, but deposition of fibrillary collagens was clearly detectable on histology from 15-weeks old animals. Even though fibrosis in homozygous Alb-Cre took longer time develop, phenotype was more consistent among animals than in Alfp-Cre transgenes. Differences between Alfp-Cre generated and homozygous Alb-Cre ADAM10 deficient strain could be caused by different background of mice (129/C57BL/6 or pure C57BL/6 respectively) and different housing conditions in two animal facilities.

Important conclusion from this part of our study was, that even though Alb-Cre transgene is estimated to contain 7 copies of Cre recombinase in heterozygous state ((Postic et al., 1999), JAX (2020): https://www.jax.org/strain/003574), it does not lead to recombination in 100% of hepatocytes. From semiquantitative PCR analysis of hepatocyte DNA, recombination was estimated to 90% in Alb-Cre hemizygots (Zbodakova et al., manuscript). Proportion of hepatocytes with not recombined Adam10 gene can revert spontaneous liver fibrosis phenotype caused by ADAM10 deficiency. Breeding Alb-Cre transgene to homozygosity improved recombination efficiency.

Though ADAM10 deficient mice with incomplete recombination in hepatocytes (Alb-Cre hemizygots) did not develop spontaneous fibrosis, they were more prone to CCl_4 induced fibrosis than control littermates (Zbodakova et al., manuscript). After 4 weeks of CCl_4 intoxication, ADAM10 deficient mice reached bridging fibrosis, stage in which fibrotic tissue interconnects central veins. This condition was not observed in control littermates at this point of CCl_4 intoxication. Extensive bridging of fibrotic scars is the cause of a disruption of liver architecture in cirrhosis.

ADAM17 deficient animals developed CCl_4 induced fibrosis to the comparable extent as control littermates, as determined from serum ALT/AST levels and quantitative analysis of staining of fibrillary collagens on histological sections. Interestingly, mice with the combined ablation of both ADAM10 and ADAM17 exhibited same level of fibrosis as controls of ADAM17 single deficient mice (Zbodakova et al., manuscript). Thus,

ADAM17 deficiency did not have protective effect in CCl_4 induced fibrosis, but eliminated exacerbating effect of ADAM10 deficiency.

How can ablation of ADAM17 be protective in ADAM10 deficient conditions? One hint comes from our experiment with hepatoprotective bile acid UDCA. As for liver fibrosis treatment, there are very few substances registered with proven hepatoprotective effect. UDCA is one of them, indicated for use in primary biliary cirrhosis (Beuers et al., 2015). As it was documented that UDCA treatment reduced levels of TNF-α, we were interested how UDCA influences ADAM17 activity. Indeed, we saw decreased release of several ADAM17 substrates upon UDCA treatment of HepG2 cells, as well as *in vivo* in model of BDL (Buryova et al., 2013). Moreover, UDCA interfered with the maturation of ADAM17, shifting the proportion of ADAM17 pool in the cell towards the form with prodomain.

Results from UDCA treatment suggest that ADAM17 inhibition plays part in the hepatoprotective effect of UDCA. However, ADAM17 deficiency in liver did not result in protection against acute CCl_4 induced damage, as was shown by McMhan et al. (2013), or chronic CCl_4 induced fibrosis, as shown by us (Zbodakova et al., manuscript). Reason, for this is most likely different nature of liver damage, where CCl_4 mostly affects hepatocytes, while BLD primarily affects biliary epithelial cells. Unfortunately, we do not have data from DDC or BDL model in ADAM17 deficient mice, which would be more suitable to confirm the conclusion from UDCA experiments. Yet, we had shown that ADAM17 deficiency can revert negative effect of ADAM10 deficiency on fibrosis development caused by CCl_4. One of the differences between ADAM10 deficient and ADAM10/17 double deficient mice in this model was that ADAM10 deficient mice had affected biliary epithelia. This ductular reaction in ADAM10 deficient mice was demonstrated by increased serum levels of alkaline phosphatase, marker of liver biliary damage, and increased expression of CD44, protein highly expressed on activated biliary epithelial cells. We cannot completely rule out that increased CD44 expression is not caused by overexpression in other cell type, e.g. activated hepatic stellate cells, or Kupffer cells, however activated biliary epithelial cells express CD44 in high levels (He et al., 2008) and ablation of ADAM10 in those cells could be directly linked to higher levels of CD44, as CD44 is known substrate of ADAM10 (Anderegg et al., 2009). Importantly, ADAM10/17 deficient animals had ALP and CD44 levels comparable to control

littermates (Zbodakova et al., manuscript). This further points to the conclusion that ADAM17 inhibition is protective in liver damage which affects biliary epithelia and in this respect it is worth additional examination.

CCl_4 induced liver damage is connected with elevated levels of soluble TNF RI (Ijiri et al., 2014). ADAM17 is its primary sheddase and we determined reduced levels of TNF RI in serum of ADAM17 deficient mice. As reduction was around 30%, significant portion of sTNF RI in serum comes from cell types other than liver parenchyma. Unexpectedly, we found that ADAM10 deficiency leads to increased levels of TNF RI in serum. It seems that this difference comes directly from liver cells, as this phenomenon was seen also in primary hepatocytes, indicating direct effect of ADAM10 on TNF RI release. We confirmed that observed differences of sTNF RI levels are not result of different expression of TNF RI between deficient strains and controls. Higher release of TNF RI could be caused by increased shedding of TNF RI, however most likely not through ADAM17. If ADAM10 deficiency would cause increased activity of ADAM17, double deficient strain should behave as ADAM17 deficient in the respect of TNF RI shedding, however, we detected increased TNF RI levels in serum of untreated ADAM10/17 double deficient mice. Apart from shedding, TNF RI could be released into serum via exosome-like vesicles 14745008 (Hawari et al., 2004). It is possible that in unchallenged state, ADAM10 deficiency increase exosomal release of TNF RI, while in pathological states, ADAM17 activity is induced, which causes further release of TNF RI by shedding. This would be in agreement with TNF RI release kinetics we measured in ADAM10/17 double deficient mice during partial hepatectomy (Zbodakova et al., manuscript), where untreated animals exhibit higher release of TNF RI, but after hepatectomy, serum levels of TNF RI increased in controls but not in ADAM10/17 deficient mice. In primary hepatocytes, situation resembles rather hepatectomy conditions than unchallenged state, which could be expected, as isolation itself causes activation of several pathways similar to injured liver (Elaut et al., 2006; Vinken et al., 2014). During toxic liver injury, TNF-α signalling plays dual role. It has damaging effect on hepatocytes, but complete inhibition of TNF-α (Dong et al., 2016), or ablation of TNF RI (Yamada and Fausto, 1998), aggravated liver damage after CCl_4 intoxication. Therefore, TNF-α signalling needs to be well balanced and shedding of TNF RI could serves to regulate TNF-α pathway on individual cell types, shutting down the signalling. In addition, soluble

TNF RI binds serum TNF-α, influencing its bioactivity (Aderka et al., 1992). Our results show that both, ADAM10 and ADAM17 influence levels of TNF RI in serum, but in different manner.

6.2 Role of ADAM17 and ADAM10 in liver regeneration

⅔ partial hepatectomy is well studied model of liver regeneration. Signalling pathways involved in liver regeneration process include growth factor receptor pathways: EGFR and cMet. As ADAM10 and ADAM17 are involved in shedding of several EGFR ligands but also cMet receptor, we were interested in role of ADAM10 and ADAM17 in liver regeneration. In our study we showed, that ADAM10 and ADAM17 both contribute to regulation of EGFR signalling, as well as cMet signalling, in regenerating liver.

It was reported before, that ADAM17 deficient mice phenocopy in some cases EGFR deficient mice. However, we showed, that not only ADAM17 but also ADAM10 is involved in shedding of EGFR ligands after partial hepatectomy. Only ADAM10/17 double deficient mice exhibited reduced pro survival AKT phosphorylation, not single deficient ADAM17 or ADAM10 stains (Zbodakova et al., manuscript). We also showed, that serum from ADAM10/17 deficient mice after hepatectomy had decreased potential to activate EGFR compared to serum from hepatectomized control littermates. As our mice had targeted deficiency to liver cells, this confirms that hepatocytes themselves are significant source of EGFR ligand in the early stage of liver regeneration.

Several EGFR ligands has been shown to promote proliferation of hepatocytes, though not all to the same extent. Physiological involvement of amphiregulin, TGF-α, and HB-EGF in liver regeneration after partial hepatectomy was affirmed using deficient mouse strains. However there are little data about other EGFR ligands, e.g. betacellulin, which is highly expressed by hepatocellular carcinoma cells (Moon et al., 2006). Unlike rest of the mentioned EGFR ligands, shedding of betacellulin is primarily mediated by ADAM10 (Sanderson et al., 2005). Therefore, betacellulin would be one potential explanation of decreased EGFR signal in ADAM10/17 double deficient mice.

Apart from EGFR, HGF/cMet pathway provides by itself fungible signal for hepatocyte proliferation (Patijn et al., 1998) and was shown to be involved in liver regeneration after partial hepatectomy (Zhang et al., 2020). After partial hepatectomy, levels of HGF in serum rises prominently. We observed, that this was accompanied with

significant increase of its receptor, cMet, in serum (Zbodakova et al., manuscript), indicating that cMet shedding is a part of the mechanism to fine-tune HGF signalling.

As already mentioned, levels ADAM10/17 double deficient mice had reduced levels of sMet in serum, while levels of HGF were the same as in control littermates (Zbodakova et al., manuscript). Decreased levels of shed cMet implies that there is higher amount of full-length functional receptor on the cell surface of hepatocytes, which would result in more robust HGF/cMet signalling. We confirmed this implication by showing that ADAM10/17 deficient primary hepatocytes exhibit stronger phosphorylation of cMet upon HGF treatment.

To sum up, ADAM10 and ADAM17 simultaneous deficiency decreased the release of EGFR ligands into serum after hepatectomy, leading to impaired EGFR signalling, but concurrently caused enhanced signalling through cMet receptor by inhibition of cMet shedding. This resulted in reduced AKT phosphorylation 6 hours after hepatectomy, but there was no significant delay in hepatocyte proliferation or liver mass gain in later time points (Zbodakova et al., manuscript). This indicates, that both ADAM10 and ADAM17 are important regulators of growth factor signalling in liver on several different levels.

6.3 Protective role of MMP-19 deficiency in liver fibrosis

Apart from ADAMs, which mainly regulate inflammation and growth factor pathways, we focused our research also on one enzyme from MMP family, which are more connected with extracellular matrix degradation, important process in liver fibrosis. MMP-19 is a well expressed in liver tissue (Mueller et al., 2000), but complete MMP-19 deficiency in mouse do not compromise liver development. Therefore we aimed to establish MMP-19 function in liver pathology.

In our study we showed that MMP-19 deficient mice develop less severe liver fibrosis after CCl_4 intoxication and recover faster than wild type controls after withdrawal of toxin (Jirouskova et al., 2012). Milder hepatocellular damage in MMP-19 deficient mice was demonstrated by reduced levels of aminotransferases in serum, reduced necrotic areas and lower deposition of fibrillary collagens as determined by hydroxyproline content in liver tissue or histological staining.

During fibrosis development, healthy extracellular matrix is cleaved and replaced by fibrotic tissue (Benyon and Arthur, 2001). Among MMP-19 substrates are several proteins of basal membrane and we indeed observed increased protein levels of non-fibrillary collagen IV in MMP-19 deficient mice compared to wild type controls in early stages of fibrosis development. Moreover, MMP-19 deficiency caused decreased MMP-2 activation at this time point, which most likely contributed to higher levels of collagen IV. In addition, as MMP-2 is one of the proteases that activate TGF-β, decreased amount of active MMP-2 could be to some extent responsible for hampered fibrogenesis in MMP-19 deficient mice. With further fibrosis progression, situation shifted and MMP-2 processing toward active form was pronouncedly increased in MMP-19 deficient mouse. MMP-2 protective role was reported in mouse models of liver fibrosis by inhibition of collagen I synthesis (Radbill et al., 2011). Therefore, MMP-2 increased activation at peak fibrosis in MMP-19 deficient mice could directly contribute to hepatoprotective effect observed in those animals.

TGF-β is the main profibrotic signalling pathway (Biernacka et al., 2011) and its blockage prevents liver fibrosis in experimental animals (Qi et al., 1999). Apart from activation of hepatic stellate cells (Dooley et al., 2000), it has direct effect on hepatocyte proliferation (Russell et al., 1988) and triggers hepatocyte apoptosis. Our results indicate that ablation of MMP-19 causes impaired responsiveness to TGF-β (Jirouskova et al., 2012). At peak fibrosis, MMP-19 deficient animals had the same expression of TGF-β mRNA, higher amount of TGF-β activating MMP-2, but TGF-β signalling was reduced as determined by phosphorylation of mediator of TGF-β signalling, SMAD3. This was in agreement with *ex vivo* experiment with primary hepatocytes, where MMP-19 deficient hepatocytes expressed lower levels of *vimentin* and *Snail1* in response to treatment with recombinant TGF-β1 than wild type hepatocytes. At the same time, AKT phosphorylation was slightly increased in MMP-19 deficient hepatocytes, which is in agreement with known function of AKT in inhibition of TGF-β (Godoy et al., 2009). Tendency towards higher phosphorylation of AKT was seen also in livers during recovery phase. This correlated with accumulation of binucleated tetraploid hepatocytes in MMP-19 deficient livers during recovery phase, process under control of AKT pathway (Celton-Morizur et al., 2009).

This all together indicates, that part of hepatoprotective effect of MMP-19 deficiency in liver fibrosis involves inhibition TGF-β in hepatocytes through enhanced

AKT signalling. It would be interesting to examine further whether MMP-19 deficient hepatic stellate cells are less responsive to TGF-β activation.

In summary, we showed that hepatocyte derived ADAM17 and ADAM10 modulate liver processes on several levels, through regulation of EGFR and cMet and TNF RI signalling. Inhibition of their activity have different consequences in liver pathology, ablation of ADAM10 in liver parenchyma enhance susceptibility to toxic damage of the liver, while ADAM17 inhibition seems to be protective in cholestatic liver damage. In addition, we established significant role of MMP-19 in liver fibrosis, showing MMP-19 deficiency have protective effect on fibrosis development. Ablation of MMP-19 influence activation of MMP-2 and TGF-β signalling in liver.

7 Conclusions

Present study examines function of ADAM17, ADAM10 and MMP19 in liver pathological processes such as liver fibrosis and liver regeneration. Presented results clarify the role of ADAM17 and ADAM10 in cooperative regulation of cMet and EGFR signalling by both proteases, while points out their contradictory function in TNF RI signalling in liver. In addition, we determined hepatoprotective effect of MMP-19 ablation in liver fibrosis in mice.

- **Ablation of ADAM10 in mice causes enhanced susceptibility to liver damage**. We showed that that ADAM10 deficiency in liver cells causes spontaneous fibrosis with defects in bile acid secretion. Moreover, mice in which ADAM10 deficiency was not in 100% of hepatocytes, were more prone to toxic damage, leading to enhanced pericentral fibrosis.

- **ADAM17 inhibition can ameliorate biliary liver damage.** We showed that ablation of ADAM17 can revert exacerbating effect of ADAM10 deficiency in liver fibrosis. Double deficient mice with ablation of both ADAM10 and ADAM17 developed comparable level of fibrosis as control littermates upon CCl_4 intoxication, in contrast to ADAM10 deficient mice. Importantly, damage of biliary epithelium, observed in ADAM10 deficient mice was not observed in double deficient strain. Moreover, we showed that inhibition of ADAM17 maturation is part of the hepatoprotective effect of UDCA, substance registered for treatment of certain liver diseases that affects biliary epithelium.

- **Hepatic ADAM17 and ADAM10 influence levels of soluble TNF RI in serum.** It is known that ADAM17 is protease responsible for TNF RI shedding. We showed, that ablation of ADAM10 caused increased levels of TNF RI in serum, having opposing effect to ablation of ADAM17.

- **Hepatic ADAM10 and ADAM17 cooperatively regulate growth factor signalling in liver regeneration.** We showed that both ADAM10 and ADAM17 contribute to shedding of EGFR ligands and shedding of cMet receptor after partial

hepatectomy. In result, simultaneous deficiency of both proteases led to hampered EGFR signalling, but this negative effect on liver regeneration was reversed by enhanced signalling through cMet receptor.

- **MMP-19 affects liver fibrosis development.** We showed that MMP-19 deficiency ameliorated CCl_4 induced fibrosis in mice and consequently led to faster recovery. MMP-19 deficient mice had different course of MMP-2 activation, which together with lacking MMP-19 activity led to reduced degradation of collagen IV. Furthermore, ablation of MMP-19 caused lower responsiveness to TGF-β.

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Zunke, F., and Rose-John, S. (2017). The shedding protease ADAM17: Physiology and pathophysiology. Biochim Biophys Acta Mol Cell Res *1864*, 2059-2070.

9 List of publications

9.1 Publications that form basis for this thesis

ADAM10 and ADAM17 in liver regeneration and fibrosis: regulation of EGFR, c-Met and TNF RI signalling. Zbodakova O, Chalupsky K, Sarnova L, Kasparek P, Beck I, Jirouskova M, Gregor M and Sedlacek R. (manuscript in preparation)

Contribution: Main project of my PhD study. I co-designed, performed and analyzed almost all of the experiments in this article. I wrote the manuscript and made all the figures.

A disintegrin and metalloprotease 10 (ADAM10) is a central regulator of murine liver tissue homeostasis. Müller M, Wetzel S, Köhn-Gaone J, Chalupsky K, Lüllmann-Rauch R, Barikbin R, Bergmann J, Wöhner B, Zbodakova O, Leuschner I, Martin G, Tiegs G, Rose-John S, Sedlacek R, Tirnitz-Parker JE, Saftig P, Schmidt-Arras D. Oncotarget. 2016 Apr 5;7(14):17431-41**.** (IF=5.17 (2016))

Contribution: In this article I participated in mouse sample collection and reviewed the article.

Liver protective effect of ursodeoxycholic acid includes regulation of ADAM17 activity. Buryova H, Chalupsky K, Zbodakova O, Kanchev I, Jirouskova M, Gregor M, Sedlacek R. BMC Gastroenterol. 2013 Oct 30;13:155. (IF=2.25)

Contribution: In this work I contributed to cell culture experiment, in which hepatocyte cell lines were treated with UDCA and release of ADAM17 substrates in medium was measured by ELISA. I did experimental model of cholestasis in mice, by performing microsurgical bile duct ligation. At the end of experiment I did the necropsies and analysed serum from animals by ELISA. In manuscript preparation, I only reviewed the manuscript.

ADAM10/17-dependent release of soluble c-Met correlates with hepatocellular damage. Chalupský K, Kanchev I, Žbodáková O, Buryová H, Jiroušková M, Kořínek V, Gregor M, Sedláček R. Folia Biol (Praha). 2013;59(2):76-86. (IF=1.07)

Contribution: In this article I participated in mouse sample collection, helped with figures and reviewed the article.

Hepatoprotective effect of MMP-19 deficiency in a mouse model of chronic liver fibrosis. Jirouskova M, Zbodakova O*, Gregor M, Chalupsky K, Sarnova L, Hajduch M, Ehrmann J, Jirkovska M, Sedlacek R. PLoS One. 2012;7(10):e46271. (IF=2.78)

* Shared co-first authorship

Contribution: I contributed to the design of experiments, performed or contributed to the most of the experiment in this article. I treated the experimental animals, performed necropsies, did all the immunoblots and zymographies and participated in primary hepatocytes isolation. In manuscript preparation I wrote part of Materials and Methods and prepared all figures.

9.2 Publications unrelated to thesis

Profiling system for skin kallikrein proteolysis applied in gene-deficient mouse models. Horn M, Zbodakova O*, Kasparek P, Srp J, Haneckova R, Hradilek M, Mares M, Sedlacek R. Biol Chem. 2018 Sep 25;399(9):1085-1089. (IF=3.01)

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Kallikrein-related peptidase 5 and seasonal influenza viruses, limitations of the experimental models for activating proteases. Magnen M, Elsässer BM, Zbodakova O, Kasparek P, Gueugnon F, Petit-Courty A, Sedlacek R, Goettig P, Courty Y. Biol Chem. 2018 Sep 25;399(9):1053-1064. (IF=3.01)

KLK5 and KLK7 Ablation Fully Rescues Lethality of Netherton Syndrome-Like Phenotype. Kasparek P, Ileninova Z, Zbodakova O, Kanchev I, Benada O, Chalupsky K, Brattsand M, Beck IM, Sedlacek R. PLoS Genet. 2017 Jan 17;13(1):e1006566. (IF=5.22)

MMP-19 deficiency causes aggravation of colitis due to defects in innate immune cell function. Brauer R, Tureckova J, Kanchev I, Khoylou M, Skarda J, Prochazka J, Spoutil F, Beck IM, Zbodakova O, Kasparek P, Korinek V, Chalupsky K, Karhu T, Herzig KH, Hajduch M, Gregor M, Sedlacek R. Mucosal Immunol. 2016 Jul;9(4):974-85. (IF=7.35)

Efficient gene targeting of the Rosa26 locus in mouse zygotes using TALE nucleases. Kasparek P, Krausova M, Haneckova R, Kriz V, Zbodakova O, Korinek V, Sedlacek R. FEBS Lett. 2014 Nov 3;588(21):3982-8. (IF=2.67)

10 Supplements

Supplement 1 – **ADAM10 and ADAM17 in liver regeneration and fibrosis: regulation of EGFR, c-Met and TNF RI signalling.** (Zbodakova et al., manuscript in preparation)

Supplement 2 – reprint of: **A disintegrin and metalloprotease 10 (ADAM10) is a central regulator of murine liver tissue homeostasis.** (Muller et al., 2016)

Supplement 3 – reprint of: **Liver protective effect of ursodeoxycholic acid includes regulation of ADAM17 activity.** (Buryova et al., 2013)

Supplement 4 – reprint of: **ADAM10/17-dependent release of soluble c-Met correlates with hepatocellular damage.** (Chalupsky et al., 2013)

Supplement 5 – reprint of: **Hepatoprotective effect of MMP-19 deficiency in a mouse model of chronic liver fibrosis.** (Jirouskova et al., 2012)