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Fibroblast activation protein in liver fibrosis

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1. ABSTRACT

Fibroblast activation protein (FAP) belongs to the dipeptidyl peptidase IV (DPP4; CD26) gene family. Other related genes in this family of enzyme include DPP4, 8 and 9. The FAP serine protease has the rare property of both dipeptidyl peptidase and endopeptidase activities capable of cleaving the post-proline bond at two or more residues from the N-terminus. FAP is involved in a variety of biological processes but its expression in healthy tissues is low. In contrast, FAP is significantly elevated in pathological conditions such as at sites of tissue remodelling and repair. Its differential pattern of expression in diseases supports the emerging concept for FAP as a potential disease biomarker as well as a useful therapeutic target for drug intervention. This review summarizes the current knowledge of FAP, particularly its diagnostic and pathological significance in liver fibrosis.

2. INTRODUCTION

Proteases are responsible for protein degradation by hydrolyzing the peptide bond(s) of a protein substrate. They are important in many post-translational modification events for the control of protein structure and function. They influence protein turnover critical for many biological as well as pathological processes such as developmental biology, tissue repair, tissue remodeling, inflammation, cardiovascular disease and cancer.

The DPP4 gene family consists of DPP4, FAP, DPP8, DPP9, DPP10 and DPP6/DPP-X. DPP6/DPP-X and DPP10 do not have enzymatic activity. The DPP4 family of post-proline serine proteases is a subfamily within the prolyl oligopeptidase (POP) family, which also includes prolyl endopeptidase (PEP). Members of this enzyme family have several

characteristic features that set them apart from the classical serine peptidases. They are specialized in cleaving the post-proline bond, which is resistant to proteolytic cleavage due to its cyclic nature, and the presence of an imino rather than an amino group. Furthermore, the catalytic pocket of the POP family enzymes is shielded inside the protein, a feature that restricts substrate size (1). As most hormones and neuropeptides comprise at least one proline residue, the POP family enzymes are particularly useful for processing and degrading peptide hormones. For these reasons, members of the POP family enzymes are increasingly recognized as important targets of drug design. The focus of this review is FAP and in particular its role in the pathogenesis of liver fibrosis.

3. FAP

Discovered in 1993, FAP, also known as FAPα and as seprase, is a homodimeric type II integral membrane serine protease with unique proteolytic capability and possesses a variety of biological functions. FAP and DPP4 share many features, with 52% amino acid sequence homology and a similar catalytic region with comparable enzymatic activity. FAP and DPP4 genes, each containing 26 exons, are located immediately adjacent to each another at 2q24.3 and 2q24.2 and have similar gene sizes of 72.8 kb and 81.8 kb respectively, suggesting they represent a product of gene duplication. Co-expression of FAP and DPP4 has been reported (2) but, unlike DPP4, FAP is not ubiquitously expressed and its expression is restricted to sites of tissue repair.

FAP has attracted tremendous interest as a potential drug target in recent years. This is in part due to its unique expression in tissue remodelling sites associated with tumours, fibrosis, atherosclerosis and arthritis. For instance, FAP is a well established marker of activated fibroblasts (3) and FAP expression is strongest in activated tumour stromal fibroblasts, activated hepatic stellate cells (aHSC) and myofibroblasts but not in healthy cells (4-6). These cells are key players in many pathological conditions, particularly in the development and progression of liver fibrosis.

3.1. Structure of FAP

FAP contains 760 amino acid residues comprised of a short cytoplasmic tail of only 6 amino acids, a single transmembrane domain of 18 amino acids and an extracellular domain of 736 amino acids. The crystal structure determination showed the extracellular domain of FAP contains two domains (Figure 1), an α/β-hydrolase domain (residues 27-53 and 493-760) and an eight-blade β-propeller domain (residues 54–492), that enclose a large cavity of ~30-45Å in diameter. A small pocket within this cavity at the interface of the α/β hydrolase and β–propeller domains, contains the catalytic triad, composed of residues Ser624, Asp702 and His734 (7). In addition to the catalytic triad, residues Ala657, Asn704, Arg123, Glu203 and Glu204 are all necessary for FAP catalytic activity. Access to this cavity is through a side opening (~15Å) allowing only elongated peptides or unfolded or partly unfolded protein fragments to reach the active site cavity. FAP is less heavily glycosylated than DPP4 (8, 9). FAP contains six potential *N*-linked glycosylation sites (motif Asn-X-Ser/Thr), at Asn residues 49, 92, 99, 227, 314 and 679. Most of these sites are located on the β-propeller surface with only one on the hydrolase domain, which is proximal to the cell surface. All but Asn99 are glycosylated in the baculovirus-expressed soluble human FAP (10).

3.2. Catalytic mechanism of FAP

In addition to the catalytic triad, Glu203 and Glu204 contained in the α-helix of the β-propeller domain are essential for catalytic activity (6). They align the substrate peptide by forming salt bridges to its N-terminus, leaving room for only two amino acids before the peptide reaches the active serine residue, thus explaining its dipeptide cleaving activity. These glutamic acids are conserved in DPP4 and DPP8 (11-13).

The crystal structures of FAP and DPP4 have one major difference in the vicinity of this Glu motif within the active site (Figure 1). FAP has lower acidity in this active site due to the presence of a neutrally charged Ala657 whereas DPP4 has a negatively charged Asp663. This amino acid substitution lowers FAP affinity for N-terminal amines by 100-fold compared to DPP4. Concordantly, the kinetic analysis of the mutant FAP Ala657Asp shows an approximately 60-fold increase in catalytic efficiency for the cleavage of dipeptide substrates, and an approximately 350-fold reduction for cleavage of the endopeptidase substrate Z-Gly-Pro-7-amino-4-methylcoumarin (7, 14). Tyr656 is essential for catalysis and Asn704 and Arg123 greatly influence activity (15). All five conserved non-catalytic triad residues, at 123, 203-204, 656 and 704, appear to confer transition state stabilisation (15).

3.3. FAP activity

Both the dipeptidyl peptidase activity (17-19) and the endopeptidase activity (7, 14) of FAP rely on the tertiary structure of FAP to dimerise (17, 19, 20). Recently, we reported a variant of a single substitution at Ser363 to Leu that is necessary for maintaining FAP tertiary structure. Interruption of FAP tertiary structure by Ser363 substitution consequently led to loss of FAP enzymatic activity (21). The k_{cat}/K_m values for cleavage of H-Ala-Pro-pNA by FAP are about 100-fold less than DPP4 (7). But, unlike DPP4, the endopeptidase activity of FAP is restricted to Gly-Pro

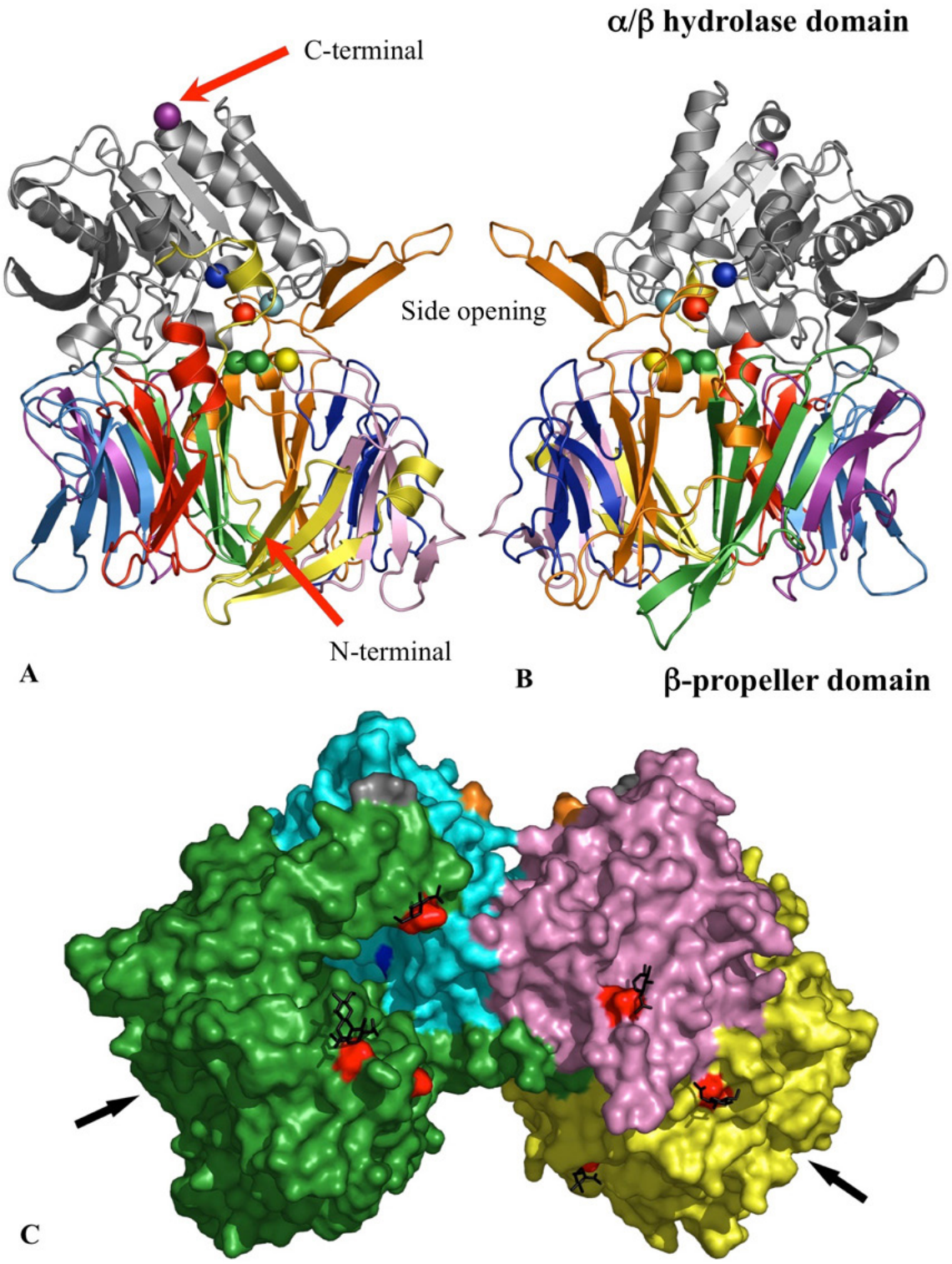

Figure 1. (A-B) Ribbon diagram of the front (A) and the back (B) view of FAP monomer with spheres depicting important residues for FAP catalytic activity. Ser624 (dark blue), Ala657 (red), and Asn704 (cyan) of the α/β hydrolase domain and Arg123 (gold), Glu203 and Glu204 (green) of the β-propeller domain. The N-terminal transmembrane and cytoplasmic portions of FAP are not shown; they would be above the molecule. (C) Space-filled representation of the FAP dimer highlighting potentially glycosylated Asn residues (red) and sugar (black) and is orientated to show the side opening. The hydrolase (blue and pink) and the propeller (green and gold) domains. The N- and C-termini are shown in grey and orange, respectively. Arrows point towards the opening in each beta propeller lower face. Image construction used PDB coordinates 1XFD (3.0. Angstrom resolution) and PyMOL. These depictions are derived from (16).

containing substrates (14). Thus, FAP has a unique endopeptidase activity on Gly-Pro derived substrates.

Beside its enzymatic function, certain FAP-driven roles are independent of its proteolytic activities. We found that *in vitro* effects of FAP on cell adhesion, migration, proliferation and apoptosis do not require FAP's enzyme activity (6). In a human breast cancer xenograft model, Kelly and co-workers demonstrated that breast cancer expressing wild type or S624A catalytic mutant FAP exhibited similar behaviour. Both tumours have rapid growth with similar degree of invasiveness (22). Furthermore, the role of FAP in promoting bone marrow mesenchymal stem cells migration has been shown to be independent of its peptidase activity (23).

FAP interacts with a number of surface molecules including $\alpha_3\beta_1$ integrin (24), DPP4 (25, 26) and urokinase plasminogen activator receptor (uPAR) (27). It has been postulated that these interactions help localise FAP to the ECM to promote cell invasion and migration (6), and implicate FAP in cell signalling (28, 29). Further studies will be necessary to reveal the relevance in FAP enzymatic activity in liver diseases.

3.4. Expression of FAP

In normal human tissues, both FAP mRNA and protein levels are low but detectable in breast tissue, cervix, endometrium, pancreas, placenta, and skin (30). However, intense FAP expression is found on activated fibroblasts and mesenchymal cell during embryogenesis (31) and in pathological conditions such as wound healing (32, 33), fibrosis (6, 17, 34, 35), and stromal fibroblasts of epithelial tumors (2, 4, 5, 32), bone marrow-derived mesenchymal stem cells (23) and tumour associated macrophages (36). FAP is also known to co-localize with MMP1, MMP13, CD44 and alpha-smooth muscle actin (α-SMA) in rheumatoid arthritis synovium (37). *In vitro,* FAP expression in cell lines includes some sarcoma and glioma cell lines, phorbol ester-stimulated melanocytes and cultured fibroblasts (17, 33, 38). FAP has also been localised to the advancing portion (invadopodia) of cultured melanoma cells in conjunction with MMP2 (19, 39). These findings collectively suggest FAP is implicated in cell migration, invasion and tissue remodelling.

FAP is not readily detectable in healthy adult liver, (6, 17). In contrast, FAP-positive cells are present in early stages of liver injury and FAP immunostaining intensity strongly correlates with the histological severity of fibrosis in cirrhotic liver (40).

In particular, FAP is present near lipid accumulation or liver steatosis, on myofibroblasts and aHSC at the portal-parenchymal interface of cirrhotic liver (17, 40).

There is currently no antibody available to reliably detect mouse FAP (41), thus it is unknown whether FAP expression in mouse liver differs from human, or whether FAP protein levels correlate with stage of liver disease in mice as was observed in human. Interestingly, the FAP gene knockout (KO) mouse has a normal phenotype for body weight, organ weights, histological examination of major organs and haematological analysis (42) suggesting that FAP is dispensable under physiological conditions. Similarly, humans appear to be unaffected when lacking FAP (21).

3.5. Circulating FAP (cFAP)

Increased expression of FAP in diseased tissues has prompted speculation that FAP might be a biomarker for diagnosis or prognosis, and for monitoring disease progression. FAP exists in both cell surface-bound and soluble forms that are both enzymatically active. Circulating FAP (cFAP) can be quantified at the antigen level using ELISA (43), or at the enzyme activity level using a specific FAP substrate (3144-aminomethylcoumarin) (44). These two assays are strongly correlated in both liver disease and coronary heart disease, as well as in healthy subjects (45). Serum and plasma contain comparable FAP activity that is measurable in non-diseased plasma from humans, mice and baboons (44). We showed that cFAP activity in mouse plasma is approximately 19- and 15- fold greater than in human and baboon plasma, respectively (44). Interestingly, in plasma from healthy subjects, men have significantly more cFAP than women, and there is a strong positive correlation between body mass index and cFAP (45, 46).

In chronic liver diseases, cFAP enzyme activity is almost doubled in alcoholic cirrhosis (44) and cFAP antigen is significantly increased with the severity of liver cirrhosis according to the Child-Pugh score (43). In both type 2 diabetes and morbid obesity, cFAP activity has been positively correlated with severity of liver fibrosis (47). Most importantly, lower cFAP activity has been found to have an excellent negative predictive value (95%) for clinically significant liver fibrosis in subjects at high risk of progressive non-alcoholic fatty liver disease (NAFLD) (47). Furthermore, in that study, cFAP activity adds substantial diagnostic value by re-classifying almost half of the 'indeterminate-risk' group, as classified by NAFLD fibrosis score, to 'low-risk' for current clinically significant fibrosis (47). In a hepatitis C virus infection cohort, both cFAP activity and antigen levels were significantly lower in patients after a liver transplant, toward levels of healthy individuals (45), which suggests that the increased cFAP was liver-derived in those patients.

Measuring cFAP in other diseases is intriguing. Despite upregulated FAP expression in tumour stroma, decreased cFAP antigen levels have been found in

patients with various malignancies when compared with controls (48). cFAP antigen and activity levels decrease in patients with colorectal cancer and pancreatic ductal adenocarcinoma, respectively (49, 50).

Nevertheless, combining cFAP with other biomarkers can increase the sensitivity for colorectal cancer diagnosis (49). This observation aligns with a recent study showing reduced cFAP antigen levels in esophageal squamous cell carcinoma and that cFAP improves the diagnosis when combined with other traditional biomarkers (51). These studies suggest that the release of cFAP is regulated in cell- and organ-specific manners. The shedding of hepatic cFAP has strong diagnostic relevance in liver disease.

FAP mediated cleavage of α2-antiplasmin (α2AP) enhances its activity and promotes incorporation of α2AP into the fibrin clot (52). In this setting, FAP has a pro-coagulant property. While the clinical relevance of cFAP level in liver disease is strong, the significance of cFAP in thrombotic related cardiovascular disease is less conclusive. Although cFAP is altered dynamically in coronary syndromes, cFAP antigen and activity levels remain unchanged in arterial thrombosis (45, 46). Furthermore, cFAP levels are downregulated in coronary heart disease (46, 53) and after acute ST-elevation myocardial infarction (STEMI) (54). Surprisingly, cFAP activity decreases in patients with stroke and inversely correlates with stroke severity, progression and outcome (55). These studies suggest that cFAP is an independent marker of thrombotic-associated cardiovascular diseases and reinforce its specificity in the setting of liver diseases.

Similarly, we have observed no positive correlation between tissue expression of FAP and cFAP activity in rheumatoid arthritis or systemic sclerosis (37), cFAP is negatively correlated with inflammatory markers in such patients (56). How cFAP is regulated in different inflammatory diseases, such as their expression pattern and their shedding mechanisms, are key questions to be addressed in the future.

Understanding how cFAP levels change in patients is very important for understanding how to use the FAP enzyme assay in diagnostic regimes. The potential of cFAP as an indicator for diagnosis has been strengthened in chronic liver diseases and cancers. When combined with other clinical parameters, cFAP quantitation will have application as a biomarker and may become a rapid and inexpensive adjunct in screening for specific diseases.

3.6. Substrates of FAP

FAP and DPP4 shared many key residues at substrate binding sites. In fact, some of the reported FAP substrates were initially identified because of their association with DPP4. A number of substrates that FAP cleaves include gelatin or type 1 collagen (CN-I) (18, 57), α2AP (58), Sprouty 2 (59), neuropeptide Y, substance P, peptide YY and B-type natriuretic peptide (60). To date, only some of these candidate substrates have been further characterised for their FAP-mediated biological roles. CN-I was the first FAP substrate to be identified (18). FAP-mediated cleavage of CN-I is important for ECM remodelling. Cleavage of α2AP by FAP enhances its binding to fibrin by 13-fold and promotes fibrin stabilization by protecting fibrin clots from plasmin-mediated degradation (58). Recently, fibroblast growth factor-21 (FGF-21) was identified as an FAP substrate (61-63). FGF-21 is an important regulator of glucose and lipid metabolism and is liver protective. Cleavage of FGF-21 by FAP inactivates FGF-21 activity, suggesting that FAP could influence glucose and lipid haemostasis implicated in high fat diet-induced liver injury.

In addition to its naturally occurring substrates, there are a number of synthetic compounds conjugated with 7-amino-4-methylcoumarin (AMC) available for measuring FAP enzymatic activity. The assay measures the release of AMC following hydrolysis by FAP. Although such assays provide quantitative measures of FAP activity, some are not specific to FAP (44). Alternatively, FAP specificity can be achieved with human samples by FAP enrichment using FAP-specific antibodies (18, 64).

4. HEPATIC EXTRACELLULAR MATRIX

The liver extracellular matrix (ECM) plays a critical role not only to provide a platform for cells and connective tissue but also to support the physiological functions of the liver. Unlike other epithelial organs, the liver has no basement membrane and only contains minimal ECM consisting mostly of structural glycoproteins. ECM proteins are large and structurally complex containing multiple domains with different functions and they are highly conserved across species (65).

Some of the known complex ECM proteins are collagen, fibronectin (FN), elastin, fibrillin-1, latent transforming growth factor (TGF)-β binding protein (LTBP-1) and thrombospondin-1 (65, 66). Beyond its structural support, the ECM proteins regulate the biological functions of integrins ($\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_3\beta_1$). Binding of integrin to ECM triggers signal transduction and promotes cell-matrix adhesion and drives polarisation, adhesion, migration, proliferation, survival and differentiation.

ECM proteins also serve as a storage or reservoir for a variety of growth factors, such as TGF-β, FGFs, hepatocyte growth factor, vascular endothelial growth factor, interleukin (IL)-3, tumour necrosis factor

(TNF)-α and platelet-derived growth factor. Binding of growth factor to ECM proteins is typically but not always to heparan sulphate proteoglycan (HSPG). For some growth factors, binding to its signalling receptor requires HSPG as a cofactor (67, 68). ECM proteins have both direct and indirect roles in cell signalling. Besides providing a docking platform for growth factors, they also directly contribute to growth factor-mediated cell signalling (69, 70)

ECM homeostasis is maintained by equilibrium between the rates of ECM synthesis and degradation. This matrix turnover is tightly regulated by the matrix metalloproteinases (MMPs) and the tissue inhibitors of matrix metalloproteinases (TIMPs) (71). Unlike FAP, MMPs are synthesised in pro-enzyme form requiring extracellular activation. The MMPs are a diverse family of zinc- and calcium- dependent endopeptidases involved in degrading a variety of ECM proteins. The interstitial collagenases (MMP1, 8 and 13) degrade CN-I, -II and -III, and the gelatinases (MMP2 and 9) digest denatured CN, along with CN-IV, -V and -VII (72, 73). The MMP expression levels are transcriptionally regulated by cytokine and growth factor signalling, including TGF-β, IL-1, IL-4, FGF, epidermal growth factor, connective tissue growth factor (CTGF; CCN2) (74) and insulin-like growth factor (IGF) (75). MMP regulation relies upon TIMPs and the matrix itself via integrin-linked pathways. The local microenvironment (health versus disease) is a very important and ultimately dictates the function of TIMPs and how they contribute to matrix turnover (76).

In response to injury, hepatic ECM proteins are capable of complete reconstitution. The initial phase involves the formation of granulation tissue containing FN, vitronectin, tenascin-C, and CN-III and CN-VI. Deposition of mature ECM proteins such as CN-I, CN-XIV and decorin follows. The most abundant ECM proteins in liver injury are the fibrillar collagens especially of type I and some types III and IV collagen). Further detail on the regulation of ECM proteins in liver injury is described in the liver fibrosis section.

5. LIVER FIBROSIS

Liver fibrosis is a wound-healing process characterised by excessive and aberrant accumulation of ECM deposition as a consequence of chronic liver injury of any cause. The trigger can be viral infection, alcoholic liver disease, metabolic diseases, haemochromatosis, autoimmune diseases and non-alcoholic steatohepatitis (NASH). Persistent fibrosis alters ECM production and compromises liver function leading to end stage liver disease such as cirrhosis and liver cancer (77).

In recent decades, cumulative research efforts have shed light on the mechanisms of liver fibrosis. Detailed knowledge of fibrotic mechanisms is key in the development of new concepts that fibrosis is in fact a reversible process (78). Such knowledge also provides exciting new perspectives on diagnosis and therapy to target fibrosis. Some of the key events pertinent in the pathogenesis of liver fibrosis will be discussed.

5.1. Hepatic stellate cell (HSC)

The HSCs constitute approximately 10% of liver resident cells. They are often referred to as Ito cells, Vitamin-A storing cells, fat-storing cells, interstitial cells or lipocytes. In healthy liver, the HSCs reside in the space of Disse and display a non-proliferative, non-contractile quiescent phenotype. They store retinoid and express quiescent markers such as desmin, glial fibrillar acidic protein (GFAP), synaptophysin and nerve growth factor receptor p75 (79).

An increasing body of evidence now supports the widely accepted sequence of events in HSC activation. The initiation phase is characterised by transdifferentiation of quiescent HSCs followed by an amplification phase of activated phenotypes. In response to injury, the quiescent HSCs undergo rapid activation and transform into the myofibroblast phenotype. Activated HSC are contractile, more motile, hyper-proliferative and pro-inflammatory. They are distinguished from other liver cell types by their unique markers and tissue distribution pattern. For example, they express α-SMA, platelet derived growth factor receptor β, lecithin retinol acyltransferase (LRAT), desmin, GFAP and heart- and neural crest derivatives- expressed protein 2 (HAND2). α-SMA positive HSCs are typically found within the areas of fibrotic bands and in the septum-parenchymal interface. Activated HSCs are the main source of ECM constituents in injured liver, so they are considered the main driver of liver fibrosis. However, aHSCs are not the only source of hepatic myofibroblasts. Other known sources of hepatic myofibroblasts include portal fibroblasts; bone marrow-derived fibrocytes and mesenchymal progenitor cells, and epithelial-to-mesenchymal transition (80-85). Human aHSCs express high levels of FAP. However, whether FAP is differentially expressed in myofibroblasts of different origins is unknown.

Activated HSCs produce a large repertoire of cytokines and chemokines (78). One of the well-characterised cytokines and the main contributor for liver fibrosis is TGF-β1. Liver TGFβ1 is synthesised and bound to ECM in a pro form, latent TGF-β (LTGFβ), and is locally activated in response to proteases. Once activated, TGFβ stimulates transcription of genes important for fibrogenesis, namely CTGF, leading to ECM gene transcription. TGF-β1 overexpression leads to increased matrix deposition.

Activated HSCs and myofibroblasts express a wide range of ECM molecules, and their synthetic capacity largely determines the composition of fibrotic matrix both quantitatively and qualitatively. Activated HSCs and myofibroblasts also secrete most of the MMP and TIMP. The final result of this process is that any liver injury that results in HSC activation, particularly if chronic, leads to an increase in overall numbers of myofibroblast-like aHSCs that are actively producing matrix, while simultaneously preventing degradation of the matrix through expression of TIMP1 and TIMP2.

The pathways regulating HSC activation have been extensively discussed (78). In brief, they include metabolic regulation, epigenetic regulation, immune signalling, as well as receptor-mediated regulation. A number of emerging pathways have also been described. yes-associated protein (YAP), endosialin, bromodomain-containing protein 4, galectin 3, and GATA4 are among the candidate proteins (78). In addition to the above-mentioned pathways of regulation, ECM stiffness has been shown to independently influence myofibroblast activation (86). Tissue stiffness is determined not only by the type of ECM proteins but also their degree of cross-linking. In this context, any proteases or their inhibitors involved in ECM remodelling can potentially affect myofibroblast activation and dictate fibrosis outcome. Examples of these proteolytic enzymes include lysyl oxidase (LOX) and lysyl oxidase like (LOXL) enzymes (87-89). LOX and LOXL2 are up regulated in fibrosis and facilitate the covalent cross-linking of fibrillar collagen and collagen, respectively.

5.2. FAP in liver fibrosis

5.2.1. FAP as a modulator of HSC activation

The expression of FAP has been shown to positively correlate with progression of liver disease in human (40). As discussed, FAP is only expressed by aHSCs in areas of tissue remodelling and is co-localised with fibrillar matrix, CN-I and FN (6, 17, 35). High levels of FAP are found in the portal - parenchymal interface in cirrhotic liver while absent in histologically normal parenchyma (17). *In vitro*, FAP overexpression in LX-2 (a human HSC cell line) enhances cell adhesion, migration, proliferation and apoptosis on ECM substrata (6). These emerging data suggest that FAP is implicated in liver fibrosis. We have found that chemical or genetic depletion of DPP4, the enzyme most similar to FAP, lessens liver fibrosis in mice (90). However, the pathological role of FAP in aHSCs is unclear. Furthermore, whether FAP directly affects aHSC function or indirectly acts through modification of ECM proteins remains an unexplored area of research. Thus, further studies are needed to fully reveal the pathological consequence of FAP expression in fibrogenesis and in chronic liver disease. A more robust study involving larger cohorts of patients will corroborate the clinical significance of FAP and with the potential to stimulate more research in the field.

5.2.2. FAP as a modifier of ECM proteins

Liver fibrosis represents a substantial change not only in the amount of complex ECM proteins but also the composition and the type of crosslinking. Thus, potential post-translational regulations of the ECM proteins are critical and will influence fibrogenesis outcomes. In addition, changed rates of protein synthesis and degradation dictate ECM turnover. Both the dipeptidyl peptidase and endopeptidase activity of FAP are important in the regulation of various ECM proteins. In the context of fibrosis, FAP-mediated MMP1 dependent cleavage of CN-I or collagen modifier proteins is of significance.

Another potential role of FAP in influencing ECM is through α2AP. FAP mediated cleavage of α2AP greatly enhances its inhibition of plasmin, leading to decreased fibrinolysis. Well-controlled fibrinolysis is crucial in the early phase of tissue repair. Fibrin clearance allows provisional matrix reorganization in addition to promoting downstream signaling events. Suppressed fibrinolysis is associated with some fibrosis (91). In this context, FAP-promoted decrease in fibrinolysis may be pro-fibrotic. However, there are still limited studies to support the importance of fibrinolysis in fibrosis.

These findings together imply that FAP has pro-fibrotic potential by orchestrating the post-translational modifications of ECM proteins as well as by modifying ECM protein organisation.

5.2.3. FAP in liver inflammation

It is well established that inflammation drives the hepatic wound healing response and is a major mediator of fibrogenesis. The findings that FAP is highly expressed in chronic inflammation and fibrotic conditions suggest that FAP has a pathological role within the inflammatory milieu (17, 37, 92, 93). It is known that fibroblasts play a significant role in attracting and retaining inflammatory leukocytes within sites of inflammation through their production of cytokines, chemokines, and other biologically active factors (94). FAP may have some effects on fibroblasts by modulating their soluble factor secretion and activities to influence leukocyte movement. However, we found that the outcome of influenza infection in mice is not significantly altered in the FAP KO mouse compared to wild type mice (95).

Chemokines are leukocyte chemoattractants and, together with pro-fibrotic cytokines, recruit inflammatory cells and myofibroblasts to sites of injury.

The recruitment of these cells is a fundamental process of the early stage of the wound healing response (96). In the context of fibrosis, the CC- and CXC-chemokine receptor families are important. Some chemokines require proteolytic processing for activation. Most type I transmembrane bound chemokines are proteolytically cleaved by a sheddase. For example, a disintegrin and metalloproteinase domains (ADAM)-10 and its relative ADAM-17 are well known sheddases for TNFα, TGFα, IL-6R among others (97). Many chemokines are substrates for DPP4, but are poor substrates for FAP (60). Further research in this area will shed light on whether FAP has a role in inflammation and, if so, whether they participate in the proteolytic cleavage of such cytokines.

FAP is highly expressed by a subset of macrophages within the breast cancer stroma (36). Although the pathological relevance of FAP in tumour associated macrophages is yet to be defined, this finding together with others in the field implicated FAP in inflammation and its potential role in mediating fibrogenesis.

5.2.4. FAP in metabolic disease

Increasing evidence is emerging that insulin resistance and NASH underlie liver fibrosis (98). NASH shares many pathological hallmarks associated with metabolic syndrome, which encompasses obesity, type 2 diabetes and dyslipidaemia. However, NASH can also progress without these underlying pathologies. As the prevalence of metabolic syndrome increases, so does the prevalence of NASH. This alarming trend in the spectrum of metabolic syndrome and its pro-fibrotic risk reinforces the clinical importance of understanding these conditions in order to effectively treat fibrosis.

FGF-21 is a non-mitogenic hepatokine with strong protective effects against several components of the metabolic syndrome. FGF-21 has anti-obesity and anti-diabetes roles (99-101) and is hepatoprotective (102, 103). FGF-21 KO mice are resistant to the insulin-sensitizing effects of thiazolidinediones (TZDs), suggesting that the anti-diabetic effect of TZDs is mediated by FGF-21 (104). FGF-21 is a substrate for FAP, rendering FGF-21 inactive. Preclinically, a FAP-resistant FGF-21 analogue has been shown to alleviate type 2 diabetes (105). We have found, in a diet induced obesity model, that FAP KO mice are protected from insulin resistance, steatosis and have improved glycaemic control compared to wild type control (106). The mechanism by which FAP promotes metabolic disease may involve FGF-21 (107).

5.2.5. FAP as a therapeutic target for liver fibrosis

The understanding of the cellular and molecular mechanisms underlying liver fibrogenesis has dramatically advanced in the last two decades. The discovery of aHSCs as the major fibrogenic cell type in the injured liver has facilitated the design of promising new anti-fibrotic therapies. These therapies are aimed at inhibiting the accumulation of aHSCs at the sites of liver injury and preventing the deposition of ECM. The development of liver fibrosis in most human liver diseases is preceded by chronic inflammation of the hepatic parenchyma, so treatments inhibiting liver inflammation may also attenuate the progression of liver fibrosis. As discussed, numerous levels of regulatory mechanisms exist in fibrogenesis. While this multifactorial pathophysiology offers therapeutic opportunity to target multiple signalling components, it is also very likely that no single therapy will be effective. Therefore, combinatorial approach therapy using agents with anti-fibrotic and anti-inflammatory effects should be considered for anti-fibrotic treatment.

An effective anti-fibrotic therapy would be one that is organ and cell specific, well tolerated with fewer side effects, and one that specifically target abnormal collagen deposition without affecting normal ECM synthesis.

As a therapeutic target FAP has several favourable features that cause it to be considered. Firstly, in liver FAP is only expressed by aHSCs, but not by quiescent HSCs in normal adult liver. Secondly, it is dominantly expressed in the tissue remodelling area, the regions with active fibrogenesis. Inhibition of FAP protease activity and disruption of the signalling of FAP complexes with other surface molecules is another potential therapeutic target. The effort of targeting FAP as potential therapeutic drug will continue to attract considerable interest. Additionally, the idea of FAP cleavable prodrugs for delivering drugs to HSCs in a precisely targeted fashion is attractive (108, 109).

6. CONCLUSION

Liver fibrosis is a multi-genic process. FAP is a multifunctional protein and has pro-fibrotic roles and possibly pro-inflammatory function in liver injury, as well as potential roles in energy and lipid metabolism. There may be several mechanisms by which FAP has an impact on liver fibrosis. Most likely, FAP executes its biological functions in a cell-context dependent manner through a combination of its protease activity and its ability to form complexes with other cell-surface molecules on HSCs and therefore influence fibrotic processes. Studies on tissue remodelling models of FAP deficient mice and on FAP associated signalling pathways may help to further elucidate its roles in ECM interactions and liver fibrosis.

Our understanding of the natural substrates of FAP is very limited. Identification of the natural substrates of FAP and the functions of FAP-hydrolysed

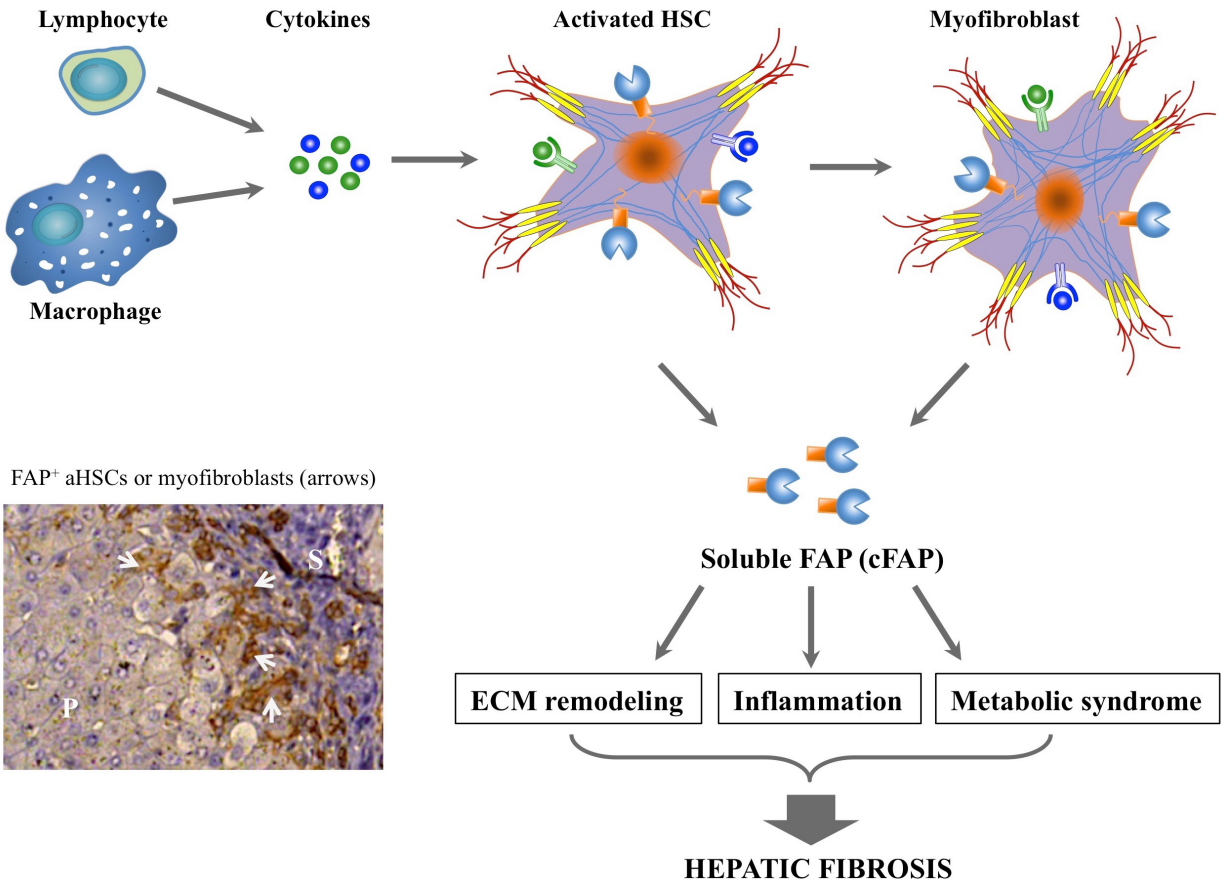

Figure 2. Working model of FAP in chronic liver injury. Inflammation drives HSC activation and trans-differentiation into the contractile hyperproliferative myofibroblast expressing high levels of FAP. Both soluble and membrane bound FAP have pro-fibrotic potential by modulating ECM remodelling, inflammation and the metabolic syndrome. FAP immunopositive aHSCs or myofibroblasts in cirrhotic liver (arrows), fibrotic septa (S); parenchymal tissue (P).

peptides remains an important area to be explored. The narrow endopeptidase activity of FAP is the main target for selective FAP inhibitors. Specific areas for *in vivo* testing of FAP-selective inhibitors will include not only fatty liver and liver fibrosis but also other biological processes in which FAP may act, such as cancer, haematopoiesis, arthritis, diabetes and cardiovascular diseases.

FAP and DPP4 may function co-ordinately via differing mechanisms to regulate pathological processes and both enzymes are appealing targets for therapeutics designed to inhibit liver fibrosis.

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8. REFERENCES

1. M. D. Gorrell: Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clinical Science*, 108(4), 277-292 (2005)
DOI: 10.1042/CS20040302

2. W. J. Rettig, P. Garin-Chesa, J. H. Healey, S. L. Su, H. L. Ozer, M. Schwab, A. P. Albino and L. J. Old: Regulation and heteromeric structure of the fibroblast activation protein in normal and transformed cells of mesenchymal and neuroectodermal origin. *Cancer Research*, 53(14), 3327-3335 (1993)

3. R. Kalluri and M. Zeisberg: Fibroblasts in cancer. *Nature Reviews Cancer*, 6(5), 392-401 (2006)
DOI: 10.1038/nrc1877

4. M. Chen, X. Lei, C. Shi, M. Huang, X. Li, B. Wu, Z. Li, W. Han, B. Du, J. Hu, Q. Nie,

W. Mai, N. Ma, N. Xu, X. Zhang, C. Fan, A. Hong, M. Xia, L. Luo, A. Ma, H. Li, Q. Yu, H. Chen, D. Zhang and W. Ye: Pericyte-targeting prodrug overcomes tumor resistance to vascular disrupting agents. *The Journal of Clinical Investigation*, 127(10), 3689-3701 (2017)
DOI: 10.1172/JCI94258

5. G.-M. Jiang, W. Xu, J. Du, K.-S. Zhang, Q.-G. Zhang, X.-W. Wang, Z.-G. Liu, S.-Q. Liu, W.-Y. Xie, H.-F. Liu, J.-S. Liu and B.-P. Wu: The application of the fibroblast activation protein α-targeted immunotherapy strategy. *Oncotarget*, 7(22), 33472–33482 (2016)
DOI: 10.18632/oncotarget.8098

6. X. M. Wang, D. M. T. Yu, G. W. McCaughan and M. D. Gorrell: Fibroblast activation protein increases apoptosis, cell adhesion and migration by the LX-2 human stellate cell line. *Hepatology*, 42(4), 935-945 (2005)
DOI: 10.1002/hep.20853

7. K. Aertgeerts, I. Levin, L. Shi, G. P. Snell, A. Jennings, G. S. Prasad, Y. Zhang, M. L. Kraus, S. Salakian, V. Sridhar, R. Wijnands and M. G. Tennant: Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein {alpha}. *Journal of Biological Chemistry*, 280, 19441 - 19444 (2005)
DOI: 10.1074/jbc.C500092200

8. K. Aertgeerts, S. Ye, L. Shi, S. G. Prasad, D. Witmer, E. Chi, B.-C. Sang, R. A. Wijnands, D. R. Webb and R. V. Swanson: N-linked glycosylation of dipeptidyl peptidase IV (CD26): Effects on enzyme activity, homodimer formation, and adenosine deaminase binding. *Protein Science*, 13(1), 145-154 (2004)
DOI: 10.1110/ps.03352504

9. M. Engel, T. Hoffmann, L. Wagner, M. Wermann, U. Heiser, R. Kiefersauer, R. Huber, W. Bode, H. U. Demuth and H. Brandstetter: The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 100(9), 5063-8 (2003)
DOI: 10.1073/pnas.0230620100

10. K. Aertgeerts, S. Ye, M. G. Tennant, B. Collins, J. Rogers, B. Sang, R. J. Skene, D. R. Webb and G. S. Prasad: Crystal structure of human Dipeptidyl Peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. *Protein Science*, 13(2), 412-21 (2004)
DOI: 10.1110/ps.03460604

11. C. A. Abbott, G. W. McCaughan and M. D. Gorrell: Two highly conserved glutamic acid residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. *FEBS Letters*, 458(3), 278-284 (1999)
DOI: 10.1016/S0014-5793(99)01166-7

12. K. Ajami, C. A. Abbott, M. Obradovic, V. Gysbers, T. Kähne, G. W. McCaughan and M. D. Gorrell: Structural requirements for catalysis, expression and dimerisation in the CD26/DPIV gene family. *Biochemistry*, 42(3), 694-701 (2003)
DOI: 10.1021/bi026846s

13. B. Ross, S. Krapp, M. Augustin, R. Kierfersauer, M. Arciniega, R. Geiss-Friedlander and R. Huber: Structures and mechanism of dipeptidyl peptidases 8 and 9, important players in cellular homeostasis and cancer. *PNAS*, 115(7), E1437-E1445. (2018)
DOI: 10.1073/pnas.1717565115

14. C. Y. Edosada, C. Quan, C. Wiesmann, T. Tran, D. Sutherlin, M. Reynolds, J. M. Elliott, H. Raab, W. Fairbrother and B. B. Wolf: Selective inhibition of fibroblast activation protein protease based on dipeptide substrate specificity. *Journal of Biological Chemistry*, 281(11), 7437-44 (2006)
DOI: 10.1074/jbc.M511112200

15. S. A. Meadows, C. Y. Edosada, M. Mayeda, T. Tran, C. Quan, H. Raab, C. Wiesmann and B. B. Wolf: Ala657 and conserved active site residues promote fibroblast activation protein endopeptidase activity via distinct mechanisms of transition state stabilization. *Biochemistry*, 46(15), 4598-605 (2007)
DOI: 10.1021/bi062227y

16. X. M. Wang, T.-W. Yao, N. A. Nadvi, B. Osborne, G. W. McCaughan and M. D. Gorrell: Fibroblast activation protein and chronic liver disease. *Frontiers in Bioscience*, 13, 3168-3180 (2008)
DOI: 10.2741/2918

17. M. T. Levy, G. W. McCaughan, C. A. Abbott, J. E. Park, A. M. Cunningham, E. Muller, W. J. Rettig and M. D. Gorrell: Fibroblast activation protein: a cell surface dipeptidyl peptidase and gelatinase expressed by

stellate cells at the tissue remodelling interface in human cirrhosis. *Hepatology*, 29(6), 1768-78 (1999)
DOI: 10.1002/hep.510290631

18. J. E. Park, M. C. Lenter, R. N. Zimmermann, P. Garin-Chesa, L. J. Old and W. J. Rettig: Fibroblast activation protein: A dual-specificity serine protease expressed in reactive human tumor stromal fibroblasts. *Journal of Biological Chemistry*, 274, 36505-36512 (1999)
DOI: 10.1074/jbc.274.51.36505

19. M. L. Pineiro-Sanchez, L. A. Goldstein, J. Dodt, L. Howard, Y. Yeh and W. T. Chen: Identification of the 170-kDa melanoma membrane-bound gelatinase (seprase) as a serine integral membrane protease. *Journal of Biological Chemistry*, 272(12), 7595-601 (1997)
DOI: 10.1074/jbc.272.12.7595

20. B. Wonganu and B. W. Berger: A specific, transmembrane interface regulates fibroblast activation protein (FAP) homodimerization, trafficking and exopeptidase activity. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1858(8), 1876-1882 (2016)
DOI: 10.1016/j.bbamem.2016.05.001

21. B. Osborne, T.-W. Yao, X. M. Wang, Y. Chen, L. D. Kotan, N. A. Nadvi, M. Herdem, G. W. McCaughan, J. Allen, D. M. Yu, A. K. Topaloglu and M. D. Gorrell: A rare variant in human fibroblast activation protein associated with ER stress, loss of function and loss of cell surface localisation. *BBA Proteins and Proteomics*, 1844(7), 1248-1259 (2014)
DOI: 10.1016/j.bbapap.2014.03.015

22. Y. Huang, A. E. Simms, A. Mazur, S. Wang, N. R. Leon, B. Jones, N. Aziz and T. Kelly: Fibroblast activation protein promotes tumor growth and invasion of breast cancer cells through non-enzymatic functions. *Clinical & Experimental Metastasis*, 28(6), 567-79 (2011)
DOI: 10.1007/s10585-011-9392-x

23. K. M. Chung, S. C. Hsu, Y. R. Chu, M. Y. Lin, W. T. Jiaang, R. H. Chen and X. Chen: Fibroblast Activation Protein (FAP) Is Essential for the Migration of Bone Marrow Mesenchymal Stem Cells through RhoA Activation. *Plos One*, 9(2) (2014)
DOI: 10.1371/journal.pone.0088772

24. S. C. Mueller, G. Ghersi, S. K. Akiyama, Q. X. A. Sang, L. Howard, M. Pineiro-Sanchez, H. Nakahara, Y. Yeh and W. T. Chen: A novel protease-docking function of integrin at invadopodia. *Journal of Biological Chemistry*, 274(35), 24947-24952 (1999)
DOI: 10.1074/jbc.274.35.24947

25. G. Ghersi, H. Dong, L. A. Goldstein, Y. Yeh, L. Hakkinen, H. S. Larjava and W. T. Chen: Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. *Journal of Biological Chemistry*, 277(32), 29231-41 (2002)
DOI: 10.1074/jbc.M202770200

26. G. Ghersi, Q. Zhao, M. Salamone, Y. Y. Yeh, S. Zucker and W. T. Chen: The protease complex consisting of dipeptidyl peptidase IV and seprase plays a role in the migration and invasion of human endothelial cells in collagenous matrices. *Cancer Research*, 66(9), 4652-4661 (2006)
DOI: 10.1158/0008-5472.CAN-05-1245

27. V. V. Artym, A. L. Kindzelskii, W. T. Chen and H. R. Petty: Molecular proximity of seprase and the urokinase-type plasminogen activator receptor on malignant melanoma cell membranes: dependence on beta1 integrins and the cytoskeleton. *Carcinogenesis*, 23(10), 1593-601 (2002)
DOI: 10.1093/carcin/23.10.1593

28. J. Jia, T. A. Martin, L. Ye and W. G. Jiang: FAP-alpha (fibroblast activation protein-alpha) is involved in the control of human breast cancer cell line growth and motility via the FAK pathway. *BMC Cell Biology*, 15 (2014)
DOI: 10.1186/1471-2121-15-16

29. H. Wang, Q. Wu, Z. Liu, X. Luo, Y. Fan, Y. Liu, Y. Zhang, S. Hua, Q. Fu, M. Zhao, Y. Chen, W. Fang and X. Lv: Downregulation of FAP suppresses cell proliferation and metastasis through PTEN/PI3K/AKT and Ras-ERK signaling in oral squamous cell carcinoma. *Cell Death and Disease*, 5, e1155 (2014)
DOI: 10.1038/cddis.2014.122

30. H. Dolznig, N. Schweifer, C. Puri, N. Kraut, W. J. Rettig, D. Kerjaschki and P. Garin-Chesa: Characterization of cancer stroma markers: in silico analysis of an mRNA expression database for fibroblast activation protein and endosialin. *Cancer Immunity*, 5, 10-18 (2005)

31. J. Niedermeyer, P. Garin-Chesa, M. Kriz, F. Hilberg, E. Mueller, U. Bamberger, W. J. Rettig and A. Schnapp: Expression of the fibroblast activation protein during mouse embryo development. *International Journal of Developmental Biology*, 45(2), 445-7 (2001)

32. P. Garin-Chesa, L. J. Old and W. J. Rettig: Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proceedings of the National Academy of Sciences of the United States of America*, 87(18), 7235-7239 (1990) DOI: 10.1073/pnas.87.18.7235

33. W. J. Rettig, S. L. Su, S. R. Fortunato, M. J. Scanlan, B. K. Raj, P. Garin-Chesa, J. H. Healey and L. J. Old: Fibroblast activation protein: purification, epitope mapping and induction by growth factors. *International Journal of Cancer*, 58(3), 385-392 (1994) DOI: 10.1002/ijc.2910580314

34. P. S. Acharya, A. Zukas, V. Chandan, A. L. Katzenstein and E. Pure: Fibroblast activation protein: a serine protease expressed at the remodeling interface in idiopathic pulmonary fibrosis. *Human Pathology*, 37(3), 352-60 (2006) DOI: 10.1016/j.humpath.2005.11.020

35. G. Cox, E. Kable, A. Jones, I. Fraser, F. Manconi and M. D. Gorrell: 3-dimensional imaging of collagen using second harmonic generation. *Journal of Structural Biology*, 141(1), 53-62 (2003) DOI: 10.1016/S1047-8477(02)00576-2

36. J. Tchou, P. J. Zhang, Y. Bi, C. Satija, R. Marjumdar, T. L. Stephen, A. Lo, H. Chen, C. Mies, C. H. June, J. Conejo-Garcia and E. Puré: Fibroblast activation protein expression by stromal cells and tumor-associated macrophages in human breast cancer. *Human Pathology*, 44(11), 2549-2557 (2013) DOI: 10.1016/j.humpath.2013.06.016

37. S. Bauer, M. C. Jendro, A. Wadle, S. Kleber, F. Stenner, R. Dinser, A. Reich, E. Faccin, S. Godde, H. Dinges, U. Muller-Ladner and C. Renner: Fibroblast activation protein is expressed by rheumatoid myofibroblast-like synoviocytes. *Arthritis Research & Therapy*, 8(6), R171 (2006) DOI: 10.1186/ar2080

38. M. A. Huber, N. Kraut, J. E. Park, R. D. Schubert, W. J. Rettig, R. U. Peter and P. Garin-Chesa: Fibroblast activation protein: differential expression and serine protease activity in reactive stromal fibroblasts of melanocytic skin tumors. *Journal of Investigative Dermatology*, 120(2), 182-8 (2003) DOI: 10.1046/j.1523-1747.2003.12035.x

39. W. T. Chen: Proteases associated with invadopodia, and their role in degradation of extracellular matrix. *Enzyme & Protein*, 49(1-3), 59-71 (1996) DOI: 10.1159/000468616

40. M. T. Levy, G. W. McCaughan, G. Marinos and M. D. Gorrell: Intrahepatic expression of the hepatic stellate cell marker fibroblast activation protein correlates with the degree of fibrosis in hepatitis C virus infection. *Liver Int*, 22(2), 93-101 (2002) DOI: 10.1034/j.1600-0676.2002.01503.x

41. M. Jacob, L. Chang and E. Puré: Fibroblast activation protein in remodeling tissues. *Current Molecular Medicine*, 12(10), 1220-43 (2012) DOI: 10.2174/156652412803833607

42. J. Niedermeyer, M. Kriz, F. Hilberg, P. Garin-Chesa, U. Bamberger, M. C. Lenter, J. Park, B. Viertel, H. Puschner, M. Mauz, W. J. Rettig and A. Schnapp: Targeted disruption of mouse fibroblast activation protein. *Molecular & Cellular Biology*, 20(3), 1089-1094 (2000) DOI: 10.1128/MCB.20.3.1089-1094.2000

43. S. Uitte de Willige, J. J. M. C. Malfliet, H. L. A. Janssen, F. W. G. Leebeek and D. C. Rijken: Increased N-terminal cleavage of alpha-2-antiplasmin in patients with liver cirrhosis. *Journal of Thrombosis and Haemostasis*, 11(11), 2029-2036 (2013) DOI: 10.1111/jth.12396

44. F. M. Keane, T.-W. Yao, S. Seelk, M. G. Gall, S. Chowdhury, S. E. Poplawski, J. H. Lai, Y. Li, W. Wu, P. Farrell, A. J. Vieira de Ribeiro, B. Osborne, D. M. T. Yu, D. Seth, K. Rahman, P. Haber, A. K. Topaloglu, C. Wang, S. Thomson, A. Hennessy, J. Prins, S. M. Twigg, S. V. McLennan, G. W. McCaughan, W. W. Bachovchin and M. D. Gorrell: Quantitation of fibroblast activation protein (FAP)-specific protease activity in mouse, baboon and human fluids and organs. *FEBS Open Bio*, 4, 43-54 (2014) DOI: 10.1016/j.fob.2013.12.001

45. S. Uitte de Willige, F. M. Keane, D. G. Bowen, J. J. M. C. Malfliet, H. E. Zhang,

B. Maneck, G. W. McCaughan, F. W. G. Leebeek, D. C. Rijken and M. D. Gorrell: Circulating fibroblast activation protein activity and antigen levels correlate strongly when measured in liver disease and coronary heart disease. *PLOS ONE*, 12(6), e0178987 (2017)
DOI: 10.1371/journal.pone.0178987

46. S. Uitte de Willige, J. J. M. C. Malfliet, J. W. Deckers, D. W. J. Dippel, F. W. G. Leebeek and D. C. Rijken: Plasma levels of soluble fibroblast activation protein in arterial thrombosis; determinants and cleavage of its substrate alpha-2-antiplasmin. *International Journal of Cardiology*, 178(1), 105-110 (2015)
DOI: 10.1016/j.ijcard.2014.10.091

47. K. H. Williams, A. J. Vieira De Ribeiro, E. Prakoso, A.-S. Veillard, N. A. Shackel, B. Brooks, Y. Bu, E. Cavanagh, J. Raleigh, S. V. McLennan, G. W. McCaughan, W. W. Bachovchin, F. M. Keane, A. Zekry, S. M. Twigg and M. D. Gorrell: Lower serum fibroblast activation protein shows promise in the exclusion of clinically significant liver fibrosis due to non-alcoholic fatty liver disease in diabetes and obesity. *Diabetes Research & Clinical Practice*, 108(3), 466-72 (2015)
DOI: 10.1016/j.diabres.2015.02.024

48. M. Javidroozi, S. Zucker and W. T. Chen: Plasma seprase and DPP4 levels as markers of disease and prognosis in cancer. *Disease Markers*, 32(5), 309-20 (2012)
DOI: 10.1155/2012/706745

49. S. Werner, F. Krause, V. Rolny, M. Strobl, D. Morgenstern, C. Datz, H. Chen and H. Brenner: Evaluation of a 5-Marker Blood Test for Colorectal Cancer Early Detection in a Colorectal Cancer Screening Setting. *Clinical Cancer Research*, 22(7), 1725-33 (2016)
DOI: 10.1158/1078-0432.CCR-15-1268

50. N. Wild, H. Andres, W. Rollinger, F. Krause, P. Dilba, M. Tacke and J. Karl: A combination of serum markers for the early detection of colorectal cancer. *Clinical Cancer Research*, 16(24), 6111-21 (2010)
DOI: 10.1158/1078-0432.CCR-10-0119

51. Y. Liao, S. Xing, B. Xu, W. Liu and G. Zhang: Evaluation of the circulating level of fibroblast activation protein alpha for diagnosis of esophageal squamous cell carcinoma. *Oncotarget*, 8(18), 30050-30062 (2017)
DOI: 10.18632/oncotarget.16274

52. S. Uitte de Willige, J. J. C. M. Malfliet, S. Abdul, F. W. G. Leebeek and D. C. Rijken: The level of circulating fibroblast activation protein correlates with incorporation of alpha-2-antiplasmin into the fibrin clot. *Thrombosis Research*, 166, 19-21 (2018)
DOI: 10.1016/j.thromres.2018.03.018

53. J. Tillmanns, C. Widera, Y. Habbaba, P. Galuppo, T. Kempf, K. C. Wollert and J. Bauersachs: Circulating concentrations of fibroblast activation protein α in apparently healthy individuals and patients with acute coronary syndrome as assessed by sandwich ELISA. *International Journal of Cardiology*, 168(4), 3926-3931 (2013)
DOI: 10.1016/j.ijcard.2013.06.061

54. J. Tillmanns, D. Fraccarollo, P. Galuppo, K. C. Wollert and J. Bauersachs: Changes in concentrations of circulating fibroblast activation protein alpha are associated with myocardial damage in patients with acute ST-elevation MI. *International Journal of Cardiology*, 232, 155-159 (2017)
DOI: 10.1016/j.ijcard.2017.01.037

55. L. Baerts, R. Brouns, K. Kehoe, R. Verkerk, S. Engelborghs, P. P. De Deyn, D. Hendriks and I. De Meester: Acute ischemic stroke severity, progression and outcome relate to changes in dipeptidyl peptidase IV and fibroblast activation protein activity. *Translational Stroke Research*, 8(2), 157-164 (2017)
DOI: 10.1007/s12975-016-0493-3

56. P. Sinnathurai, W. Lau, A. J. Vieira de Ribeiro, W. W. Bachovchin, H. Englert, G. Howe, D. Spencer, N. Manolios and M. D. Gorrell: Circulating fibroblast activation protein and dipeptidyl peptidase 4 in rheumatoid arthritis and systemic sclerosis. *International Journal of Rheumatic Diseases*, In Press (2016)
DOI: 10.1111/1756-185X.13031

57. P. O'Brien and B. F. O'Connor: Seprase: An overview of an important matrix serine protease. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, 1784(9), 1130-1145 (2008)
DOI: 10.1016/j.bbapap.2008.01.006

58. K. N. Lee, K. W. Jackson, V. J. Christiansen, K. H. Chung and P. A. McKee: A novel

plasma proteinase potentiates alpha2-antiplasmin inhibition of fibrin digestion. *Blood*, 103(10), 3783-8 (2004)
DOI: 10.1182/blood-2003-12-4240

59. C.-H. Huang, C.-S. Suen, C.-T. Lin, C.-H. Chien, H.-Y. Lee, K.-M. Chung, T.-Y. Tsai, W.-T. Jiaang, M.-J. Hwang and X. Chen: Cleavage-site Specificity of Prolyl Endopeptidase FAP Investigated with a Full-Length Protein Substrate. *Journal of Biochemistry*, 149(6), 685-692 (2011)
DOI: 10.1093/jb/mvr017

60. F. M. Keane, N. A. Nadvi, T.-W. Yao and M. D. Gorrell: Neuropeptide Y, B-type natriuretic peptide, substance P and peptide YY are novel substrates of fibroblast activation protein-α. *FEBS Journal*, 278(8), 1316-1332 (2011)
DOI: 10.1111/j.1742-4658.2011.08051.x

61. A. L. Coppage, K. R. Heard, M. T. DiMare, Y. Liu, W. Wu, J. H. Lai and W. W. Bachovchin: Human FGF-21 Is a substrate of fibroblast activation protein. *PLoS ONE*, 11(3), e0151269 (2016)
DOI: 10.1371/journal.pone.0151269

62. D. R. Dunshee, T. W. Bainbridge, N. M. Kljavin, J. Zavala-Solorio, A. C. Schroeder, R. Chan, R. Corpuz, M. Wong, W. Zhou, G. Deshmukh, J. Ly, D. P. Sutherlin, J. A. Ernst and J. Sonoda: Fibroblast Activation Protein cleaves and inactivates Fibroblast Growth Factor 21. *Journal of Biological Chemistry*, 291(11), 5986-96 (2016)
DOI: 10.1074/jbc.M115.710582

63. E. Y. Zhen, Z. Jin, B. L. Ackermann, M. K. Thomas and J. A. Gutierrez: Circulating FGF21 proteolytic processing mediated by fibroblast activation protein. *Biochemical Journal*, 473(5), 605–614 (2016)
DOI: 10.1042/BJ20151085

64. L. Juillerat-Jeanneret, P. Tafelmeyer and D. Golshayan: Fibroblast activation protein-alpha in fibrogenic disorders and cancer: more than a prolyl-specific peptidase? *Expert Opinion on Therapeutic Targets*, 21(10), 977-991 (2017)
DOI: 10.1080/14728222.2017.1370455

65. R. O. Hynes: The extracellular matrix: not just pretty fibrils. *Science*, 326(5957), 1216-9 (2009)
DOI: 10.1126/science.1176009

66. F. Klingberg, B. Hinz and E. S. White: The myofibroblast matrix: implications for tissue repair and fibrosis. *Journal of Pathology*, 229(2), 298-309 (2013)
DOI: 10.1002/path.4104

67. T. ROSKAMS, H. MOSHAGE, R. DE VOS, D. GuIDO, P. YAP and V. DESMET: Heparan Sulfate Proteoglycan Expression in Normal Human Liver *Hepatology*, 21(4), 950-958 (1995)
DOI: 10.1002/hep.1840210410

68. I. Matsuo and C. Kimura-Yoshida: Extracellular distribution of diffusible growth factors controlled by heparan sulfate proteoglycans during mammalian embryogenesis. *Philos Trans R Soc Lond B Biol Sci. 2014 Dec 5;369(1657).* 369(1657.), 1-9 (2014)
DOI: 10.1098/rstb.2013.0545

69. S. Kim, J. Turnbull and S. Guimond: Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *Journal of Endocrinology*, 209(2), 139-151 (2011)
DOI: 10.1530/JOE-10-0377

70. I. L. S. Chua, H.-W. Kim and J. H. Lee: Signaling of extracellular matrices for tissue regeneration and therapeutics. *Tissue Engineering and Regenerative Medicine*, 13(1), 1-12 (2016)
DOI: 10.1007/s13770-016-9075-0

71. S. Tsukada, C. J. Parsons and R. A. Rippe: Mechanisms of liver fibrosis. *Clinica Chimica Acta*, 364(1-2), 33-60 (2006)
DOI: 10.1016/j.cca.2005.06.014

72. R. C. Benyon and M. J. P. Arthur: Extracellular matrix degradation and the role of hepatic stellate cells. *Seminars in Liver Disease*, 21(3), 373-384 (2001)
DOI: 10.1055/s-2001-17552

73. R. Visse and H. Nagase: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circulation Research*, 92(8), 827-39 (2003)
DOI:10.1161/01.RES.0000070112.80711.3D

74. W. H. Fan and M. J. Karnovsky: Increased MMP-2 expression in connective tissue growth factor over-expression vascular smooth muscle cells. *J Biol Chem*, 277(12),

9800-5 (2002)
DOI: 10.1074/jbc.M111213200

75. D. Zhang, M. Bar-Eli, S. Meloche and P. Brodt: Dual regulation of MMP-2 expression by the type 1 insulin-like growth factor receptor: the phosphatidylinositol 3-kinase/Akt and Raf/ERK pathways transmit opposing signals. *Journal of Biological Chemistry*, 279(19), 19683-90 (2004) DOI: 10.1074/jbc.M313145200

76. V. Arpino, M. Brock and S. E. Gill: The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biology*, 44-46, 247-54 (2015) DOI: 10.1016/j.matbio.2015.03.005

77. C. Yang, M. Zeisberg, B. Mosterman, A. Sudhakar, U. Yerramalla, K. Holthaus, L. Xu, F. Eng, N. Afdhal and R. Kalluri: Liver fibrosis: insights into migration of hepatic stellate cells in response to extracellular matrix and growth factors. *Gastroenterology*, 124(1), 147-59 (2003) DOI: 10.1053/gast.2003.50012

78. T. Tsuchida and S. L. Friedman: Mechanisms of hepatic stellate cell activation. *Nature Reviews. Gastroenterology and Hepatology*, 14(7), 397-411 (2017) DOI: 10.1038/nrgastro.2017.38

79. D. A. Brenner, T. Kisseleva, D. Scholten, Y. H. Paik, K. Iwaisako, S. Inokuchi, B. Schnabl, E. Seki, S. De Minicis, C. Oesterreicher and K. Taura: Origin of myofibroblasts in liver fibrosis. *Fibrogenesis Tissue Repair*, 5(Suppl 1), S17 (2012) DOI: 10.1186/1755-1536-5-S1-S17

80. H. Robertson, J. A. Kirby, W. W. Yip, D. E. Jones and A. D. Burt: Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis. *Hepatology*, 45(4), 977-81 (2007) DOI: 10.1002/hep.21624

81. M. D. Gorrell: Liver Fibrosis: The Hepatocyte Revisited. *Hepatology*, 46(5), 1659-1661 (2007) DOI: 10.1002/hep.22003

82. N. C. Henderson and J. P. Iredale: Liver fibrosis: cellular mechanisms of progression and resolution. *Clinical Science*, 112(5), 265-80 (2007) DOI: 10.1042/CS20060242

83. J. Xu, X. Liu, Y. Koyama, P. Wang, T. Lan, I. G. Kim, I. H. Kim, H. Y. Ma and T. Kisseleva: The types of hepatic myofibroblasts contributing to liver fibrosis of different etiologies. *Frontiers in Pharmacology*, 5, 167 (2014) DOI: 10.10.3389/fphar.2014.00167

84. J. Brockhausen, S. S. Tay, C. A. Grzelak, P. Bertolino, D. G. Bowen, W. M. d'Avigdor, N. Teoh, S. Pok, N. Shackel, J. R. Gamble, M. Vadas and G. W. McCaughan: miR-181a mediates TGF-β-induced hepatocyte EMT and is dysregulated in cirrhosis and hepatocellular cancer. *Liver Int*, 35(1), 240-253 (2015) DOI: 10.1111/liv.12517

85. Y. L. Zhao, R. T. Zhu and Y. L. Sun: Epithelial-mesenchymal transition in liver fibrosis. *Biomedical Reports*, 4(3), 269-274 (2016) DOI: 10.3892/br.2016.578

86. F. Liu, J. D. Mih, B. S. Shea, A. T. Kho, A. S. Sharif, A. M. Tager and D. J. Tschumperlin: Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *Journal of Cell Biology*, 190(4), 693-706 (2010) DOI: 10.1083/jcb.201004082

87. S. B. Liu, N. Ikenaga, Z. W. Peng, D. Y. Sverdlov, A. Greenstein, V. Smith, D. Schuppan and Y. Popov: Lysyl oxidase activity contributes to collagen stabilization during liver fibrosis progression and limits spontaneous fibrosis reversal in mice. *FASEB Journal*, 30(4), 1599-609 (2016) DOI: 10.1096/fj.14-268425

88. N. Ikenaga, Z.-W. Peng, K. A. Vaid, S. B. Liu, S. Yoshida, D. Y. Sverdlov, A. Mikels-Vigdal, V. Smith, D. Schuppan and Y. V. Popov: Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. *Gut*, 66(9), 1697-1708 (2017) DOI: 10.1136/gutjnl-2016-312473

89. A. J. Lopez-Jimenez, T. Basak and R. M. Vanacore: Proteolytic processing of lysyl oxidase-like-2 in the extracellular matrix is required for crosslinking of basement membrane collagen IV. *Journal of Biological Chemistry*, 292(41), 16970-16982 (2017) DOI: 10.1074/jbc.M117.798603

90. X. M. Wang, L. E. Holz, S. Chowdhury, S. P. Cordoba, K. A. Evans, M. G. Gall, A. J. Vieira de Ribeiro, Y. Z. Zheng, M. T. Levy,

D. M. T. Yu, T.-W. Yao, N. Polak, C. J. Jolly, P. Bertolino, G. W. McCaughan and M. D. Gorrell: Profibrotic role of dipeptidyl peptidase 4 in a carbon tetrachloride induced experimental liver injury. *Immunology & Cell Biology*, 95(5), 443-453 (2017)
DOI: 10.1038/icb.2016.116

91. C. Ruppert, P. Markart, M. Wygrecka, K. T. Preissner and A. Gunther: Role of coagulation and fibrinolysis in lung and renal fibrosis. *Hamostaseologie*, 28(1-2), 30-2 (2008)

92. C. E. Brokopp, R. Schoenauer, P. Richards, S. Bauer, C. Lohmann, M. Y. Emmert, B. Weber, S. Winnik, E. Aikawa, K. Graves, M. Genoni, P. Vogt, T. F. Lscher, C. Renner, S. P. Hoerstrup and C. M. Matter: Fibroblast activation protein is induced by inflammation and degrades type i collagen in thin-cap fibroatheromata. *European Heart Journal*, 32(21), 2713-2722 (2011)
DOI: 10.1093/eurheartj/ehq519

93. R. Yazbeck, G. S. Howarth and C. A. Abbott: Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends in Pharmacological Sciences*, 30(11), 600-607 (2009)
DOI: 10.1016/j.tips.2009.08.003

94. M. R. Nazareth, L. Broderick, M. R. Simpson-Abelson, R. J. Kelleher, Jr., S. J. Yokota and R. B. Bankert: Characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of tumor-associated T cells. *Journal of Immunology*, 178(9), 5552-62 (2007)
DOI: 10.4049/jimmunol.178.9.5552

95. S.-Y. Tan, S. Chowdhury, N. Polak, M. D. Gorrell and W. Weninger: Fibroblast activation protein is dispensable in the anti-influenza immune response in mice. *PLoS ONE*, 12(2), e0171194 (2017)
DOI: 10.1371/journal.pone.0171194

96. H. Sahin and H. E. Wasmuth: Chemokines in tissue fibrosis. *Biochimica Biophysica Acta*, 1832(7), 1041-8 (2013)
DOI: 10.1016/j.bbadis.2012.11.004

97. K. J. Garton, P. J. Gough and E. W. Raines: Emerging roles for ectodomain shedding in the regulation of inflammatory responses. *Journal of Leukocyte Biology*, 79(6), 1105-16 (2006)
DOI: 10.1189/jlb.0106038

98. E. M. Brunt: Nonalcoholic steatohepatitis. *Seminars in Liver Disease*, 24(1), 3-20 (2004)
DOI: 10.1055/s-2004-823098

99. A. C. Adams, C. A. Halstead, B. C. Hansen, A. R. Irizarry, J. A. Martin, S. R. Myers, V. L. Reynolds, H. W. Smith, V. J. Wroblewski and A. Kharitonenkov: LY2405319, an Engineered FGF21 Variant, Improves the Metabolic Status of Diabetic Monkeys. *PLoS ONE*, 8(6), e65763 (2013)
DOI: 10.1371/journal.pone.0065763

100. N. Tanaka, S. Takahashi, Y. Zhang, K. W. Krausz, P. B. Smith, A. D. Patterson and F. J. Gonzalez: Role of fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline-deficient diet. *Biochimica Biophysica Acta*, 1852(7), 1242-52 (2015)
DOI: 10.1016/j.bbadis.2015.02.012

101. M. J. Potthoff: FGF21 and metabolic disease in 2016: A new frontier in FGF21 biology. *Nature Reviews Endocrinology*, 13(2), 74-76 (2017)
DOI: 10.1038/nrendo.2016.206

102. Y. Yu, F. Bai, Y. Liu, Y. Yang, Q. Yuan, D. Zou, S. Qu, G. Tian, L. Song, T. Zhang, S. Li, Y. Liu, W. Wang, G. Ren and D. Li: Fibroblast growth factor (FGF21) protects mouse liver against D-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/Akt pathways. *Molecular & Cellular Biochemistry*, 403(1-2), 287-99 (2015)
DOI: 10.1007/s11010-015-2358-6

103. B. Desai, G. Singhal, M. Watanabe, D. Stevanovic, T. Lundasen, F. Fisher, M. Mather, H. Vardeh, N. Douris, A. Adams, I. Nasser, G. FitzGerald, J. Flier, C. Skarke and E. Maratos-Flier: Fibroblast growth factor 21 (FGF21) is robustly induced by ethanol and has a protective role in ethanol associated liver injury. *Molecular Metabolism*, 6(11), 1395-1406 (2017)
DOI: 10.1016/j.molmet.2017.08.004

104. Paul A. Dutchak, T. Katafuchi, Angie L. Bookout, Jang H. Choi, Ruth T. Yu, David J. Mangelsdorf and Steven A. Kliewer: Fibroblast Growth Factor-21 Regulates PPARγ Activity and the Antidiabetic Actions of Thiazolidinediones. *Cell*, 148(3), 556-567 (2012)
DOI: 10.1016/j.cell.2011.11.062

105. R. Hecht, Y. S. Li, J. Sun, E. Belouski, M. Hall, T. Hager, J. Yie, W. Wang, D. Winters, S. Smith, C. Spahr, L. T. Tam, Z. Shen, S. Stanislaus, N. Chinookoswong, Y. Lau, A. Sickmier, M. L. Michaels, T. Boone, M. M. Veniant and J. Xu: Rationale-Based Engineering of a Potent Long-Acting FGF21 Analog for the Treatment of Type 2 Diabetes. *PLoS One*, 7(11), e49345 (2012) DOI: 10.1371/journal.pone.0049345

106. M. D. Gorrell, S. Song and X. M. Wang: Novel metabolic disease therapy. In: *Publication No. WO 2010/083570* Ed W. http://www.wipo.int/. University of Sydney, Australia (2010)

107. M. A. Sánchez-Garrido, K. M. Habegger, C. Clemmensen, C. Holleman, T. D. Müller, D. Perez-Tilve, P. Li, A. S. Agrawal, B. Finan, D. J. Drucker, M. H. Tschöp, R. D. DiMarchi and A. Kharitonenkov: Fibroblast activation protein (FAP) as a novel metabolic target. *Molecular Metabolism*, 5(10), 1015-1024 (2016) DOI: 10.1016/j.molmet.2016.07.003

108. W. N. Brennen, J. T. Isaacs and S. R. Denmeade: Rationale behind targeting fibroblast activation protein–expressing carcinoma-associated fibroblasts as a novel chemotherapeutic strategy. *Molecular Cancer Therapeutics*, 11(2), 257-266 (2012)

109. W. N. Brennen, D. M. Rosen, H. Wang, J. T. Isaacs and S. R. Denmeade: Targeting Carcinoma-Associated Fibroblasts Within the Tumor Stroma With a Fibroblast Activation Protein-Activated Prodrug. *Journal of the National Cancer Institute*, 104(17), 1320-1334 (2012)

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