BMP1.3 protein as potential target in treatment of fibrosis

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Abstract:

Bone morphogenetic protein 1 (BMP1) belongs to the procollagen C proteinase (PCP) family of proteases involved in development and pattern formation in various organisms. BMP1 proteinases mediate the cleavage of carboxyl peptides from procollagen molecules, which is a crucial step in fibrillar collagen synthesis. From six described alternatively spliced variants of human *Bmp1* gene, only BMP1.3 protein was detected in human plasma and elevated plasma levels of this protein were found in pathological conditions such as chronic kidney disease and acute myocardial infarction. Since BMP1 is required to convert pro-collagen to collagen, its inhibition is a potential intervention for treating fibrosis. Inhibition of BMP1.3 was shown to decrease the progression of liver fibrosis in an animal model of liver cirrhosis. One of the major inflammatory signaling molecules involved in fibrogenesis in various organs is transforming growth factor beta 1 (TGFβ1), which expression is elevated in various models of induced fibrosis. Many studies have revealed that BMP1 proteases play a key role in regulation of TGFβ activation. Here, we discuss BMP1.3 inhibition as a potential treatment in different pathological conditions related to the fibrosis. Testing BMP1.3 inhibition in these models indicates that the anti-BMP1.3 antibody targets relevant pathways in the development of fibrosis in different organs.

Keywords: Bone morphogenetic protein 1 (BMP1), fibrosis, procollagen, transforming growth factor, chronic kidney disease

Sažetak:

BMP1.3 protein kao potencijalna meta u liječenju fibroze

Koštani morfogenetski protein 1 (engl. BMP1) pripada obitelji C-proteinaza prokolagena (engl. PCP) uključenih u oblikovanje i razvitak različitih organizama. BMP1 proteinaze posreduju cijepanje peptida na karboksilnom kraju prokolagena, što je presudan korak u sintezi kolagena. Od šest opisanih varijanti humanog Bmp1 gena, samo BMP1.3 protein cirkulira u humanoj plazmi, a povišene razine ovog proteina otkrivene su u patološkim stanjima kao što su kronična bolest bubrega i akutni infarkt miokarda. Budući da je BMP1 protein potreban za pretvorbu pro-kolagena u kolagen , njegova inhibicija predstavlja potencijalnu metodu u liječenju fibroze. Pokazano je da inhibicija BMP1.3 smanjuje napredovanje fibroze jetre u animalnom modelu ciroze jetre. Transformirajući čimbenik rasta beta 1 (engl. TGFβ1) jedna je od glavnih upalnih molekula uključenih u fibrogenezu različitih organa, a njegova je ekspresija povišena u različitim modelima inducirane fibroze. Mnoge studije pokazale su da BMP1 proteaze imaju ključnu ulogu u regulaciji aktivacije TGFβ. U ovom radu opisujemo inhibiciju BMP1.3 kao moguću terapiju različitih patoloških stanja povezanih s fibrozom. Istraživanje inhibicije BMP1.3 u tim modelima pokazuje da protutijela specifična za BMP1.3 djeluju na zajedničke puteve važne za razvoj fibroze u raznim organima.

Ključne riječi: koštani morfogenetski protein 1 (BMP1), fibroza, pro-kolagen, transformirajući čimbenik rasta, kronična bolest bubrega

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BMP1 isoforms

Bone morphogenetic protein 1 (BMP1) was first isolated from bovine bone protein extract and appeared to be a regulatory molecule structurally and functionally different from other BMP proteins, which are mainly members of transforming growth factor (TGF) β protein family capable to induce cartilage formation *in vivo* (1). In contrast to the BMPs from the TGFβ family, BMP1 is a protein belonging to the family of proteases implicated in development and pattern formation in various organisms, namely procollagen C proteinase (PCP) family (2). Phylogenetically, gene encoding BMP1 is closely related to the evolutionary old family of astacin-like genes described in the nematode worm *Caenorhabditis elegans*, where 40 genes encoding astacin-like proteins were discovered. From these 40 nematode-astacin (NAS) genes, gene *nas-39* is structurally identical to the human *Bmp1* gene, whose domain structure remained conserved during the evolution, probably even in the common ancestor of nematodes and vertebrates (3).

The cDNA sequence of BMP1 showed high degree of similarity also to the *Drosophila* Tolloid metalloproteinase (4). In human and mice, the gene encoding BMP1 produces alternatively spliced transcripts with preserved domain structure, where the long isoform has an organization of domains identical to the *Drosophila* Tolloid and is designated mammalian Tolloid (mTLD) (5). Further study (6) described six alternatively spliced variants of human *Bmp1* gene, named *Bmp*1.1 do 1.6, where the *Bmp*1.3 isoform is the longest and corresponds to mTLD, as well as to the *nas-39* from *C. elegans* (3, 7). Of all BMP1 isoforms, only BMP1.3 protein was found to circulate in humans and it was identified in human plasma samples by liquid chromatography-mass spectrometry (LC-MS) (8).

Structurally, BMP1 proteins are members of the astacin subgroup of metzincin metalloproteases, which contain a N-terminal prodomain followed by a catalytic astacin-like protease domain and one or more EGF (epidermal growth factor-like) and CUB (complement subcomponents C1r/C1s – embryonic sea urchin protein Uegf – BMP1) domains (5, 9). EGF and CUB domains are non-catalytic domains which promote protein-protein interactions. Studies on recombinant truncated forms of protein revealed that CUB1 domain is necessary for BMP1 secretion, and CUB2 domain, together with the protease domain, for C-proteinase activity (10, 11). Besides BMP1 and mTLD, there are two genetically distinct proteins named tolloid-like (TLL)-1 and TLL-2. Together with BMP1 and mTLD, they belong to the family of mammalian BMP1/TLD-like proteases which share common domain structure, but differ in C-terminal amino acid sequences (12) (Fig.1).

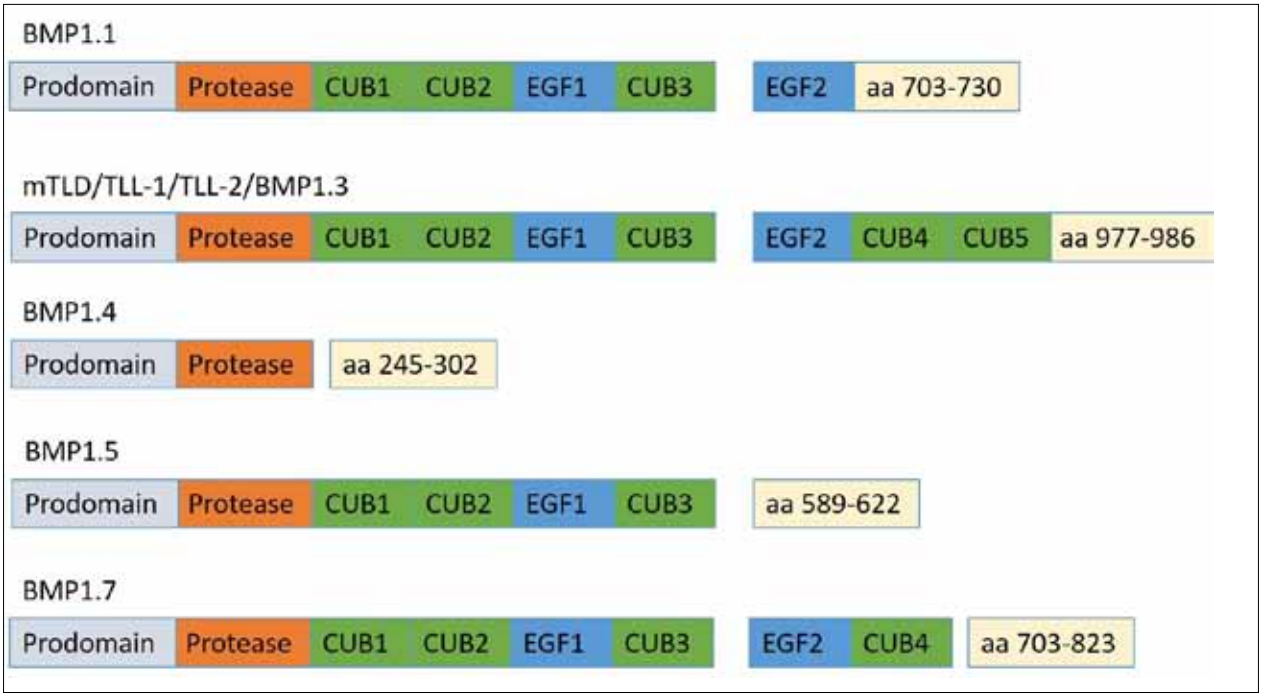

Figure 1. Schematic representation of BMP1 isoforms and their domain structure (adapted from (8))

BMP1/TLD-like proteases are involved in processing a wide range of extracellular matrix (ECM) precursors required for normal tissue assembly (13). Examples of such ECM precursor proteins are procollagens I-III, minor fibrillar procollagens, small leucine-rich proteoglycans (such as decorin, osteoglycin, biglycan), basement membrane components (laminin 332, collagen VII) and mineralization factors (dentin sialophosphoprotein, dentin matrix protein) (14). Further, BMP1/TLD-like proteases are engaged in release of TGFβ superfamily members from their inhibitory complexes (e.g. TGFβ1, BMP2, -4 and -7), which in turn regulates developmental patterning and tissue homeostasis (13). In mice, *Bmp1* gene appears to be required for normal embryo development, and homozygous mutants with complete deletion of *Bmp1* are lethal because of herniation of the gut combined with failure of ventral body wall closure (15, 16). Early lethality of conventional *Bmp1* knockout mice required development of conditional knockouts with tissue-specific ablations of *Bmp1* expression in adult organism (16).

After discovery of BMP1.3 protein isoform circulating in human plasma, separated from its pro-domain (8), elevated levels of BMP1.3 have been found in pathological conditions such as chronic kidney disease (8) and acute myocardial infarction (17). Moreover, inhibition of BMP1.3 by specific antibodies reduced extent of experimentally induced liver fibrosis (18). BMP1.3 is also involved in bone fracture repair, where its inhibition delayed fracture healing (19). These findings provided the rationale for developing and testing the efficacy of BMP1.3 inhibition for treating fibrosis, a condition where therapy targeted to the BMP1.3 inhibition could reverse adverse effects due to the fibrotic changes in the particular organ. For this purpose, a monoclonal antibody was generated in mice immunized with a specific BMP1.3 synthetic peptide (C-terminal amino acids 972 to 986 unique for this isoform; RYTSTKFQDTLHSRK) (17, 18). Additionally, polyclonal antibody against mature BMP1.3 was generated in rabbits immunized with synthetic BMP1.3 peptide (amino acids 759 to 772; TSPNWPDKYPSKKE) specific for mature domain of the protein (8). For monoclonal antibody production, the spleen from immunized Balb/c mice were used for *in vitro* hybridoma cell production. Cell culture supernatants from clones which produced the best monoclonal antibodies were collected and purified on Protein G affinity column. For polyclonal antibody production, New Zeland White rabbits were immunized with BMP1.3 peptide. Serum was collected between booster injections and antibody titer was determined. Appropriate aliquots of sera were affinity purified on Protein G column. These antibodies were used to explore the effects of BMP1 inhibition on liver fibrosis, chronic kidney disease, myocardial infarction and congenital muscule dystrophy in different animal models.

Fibrosis and the role of TGFβ

Fibrosis is defined as a reparative of reactive process characterized by the formation of excess of fibrous connective tissue, resulting in progressive remodelling of tissue or an organ (20). The process of wound healing after injury includes remodeling of extracellular matrix (ECM), where, in the early phase of regeneration, a provisional ECM is formed by cross-linking fibrin, fibronectin, fibrinogen and proteoglycans (21, 22). Although fibrogenic response is needed as a part of tissue repair process for restoring and maintaining an organ function after injury (scarring), an exacerbated fibrogenic response can shift this process to the chronic fibrosis which finally leads to destruction of normal organ architecture (21, 23).

Fibrosis is often triggered by an acute or chronic inflammatory process. Among major contributors to the induction of pathological fibrosis during chronic phase of inflammation are members of TGFβ protein family (namely, TGFβ1, -2 and -3), whose suppression was sufficient to block experimentally induced fibrogenesis in various models. Among them, TGFβ1 is most important factor in tissue repair during inflammation and fibrosis (20). During the early phase of wound healing, TGFβ1 promotes collagen and fibronectin production and ECM formation; however, it acts also as a general supressor of excessive inflammatory response, as seen from mice with inactivated *Tgfβ1* gene (20, 24). Blockade of TGFβ1 production by antisense RNA attenuated experimentally induced liver fibrosis *in vitro* and *in vivo* (25). Similar effect was observed in a model overexpressing BMP7, which in turn negative influences *Tgfβ1* expression via interference with several possible signalling mechanisms (26, 27). On the other hand, overexpression of *Tgfβ1* in the liver lead to the development of hepatic fibrosis with multiple tissue lesions (28).

TGFβ signalling pathways involve two types of receptors (TGFR1 and TGFR2) which act through so-called canonical (linked to intracellular Smad proteins) and non-canonical (non-Smad signalling molecules) pathways (29). For development of fibrosis, the most important is canonical signalling pathway which involves phosphorylation and activation of Smad2, Smad3 and Smad4 proteins (30), which in turn effect transcription of profibrotic genes (for example, α-smooth muscle actin (*α-SMA*), collagen I and tissue inhibitor of matrix deposition (*TIMP*)). Whereas Smad3 can bind directly to the Smad-binding elements within gene promoters, Smad2 and Smad4 need additional co-activators to act as regulators of gene transcription (31).

TGFβ activation is controlled by various inhibitory proteins, and for its regulation the crucial role play BMP1 proteases, which release active TGFβ protein from the latent inhibitory complex (32). This complex consists of the TGFβ linked to the prodomain (latency-associated peptide, LAP), which are together bound

to the latent TGFβ-binding protein (LTBP) and form the large latent complex (LLC) (33). BMP1 cleaves LTBP, enabling thus subsequent cleavage of LAP by matrix metalloproteinase (MMP) and activation of liberated TGFβ1 (32) (Fig. 2). A recent study using surface plasmon resonance assay demonstrated that CUB domain of BMP1 could be responsible for binding of TGFβ1, increasing thus its signalling pathway (34). It has also been shown that BMP1 enhances TGFβ activity not only by proteolysis of its latent precursor, but also by cleavage of matricellular glycoprotein thrombospondin (TSP-1) (14). BMP1 is not the only activator of TGFβ, but is a significant factor in regulating its activity.

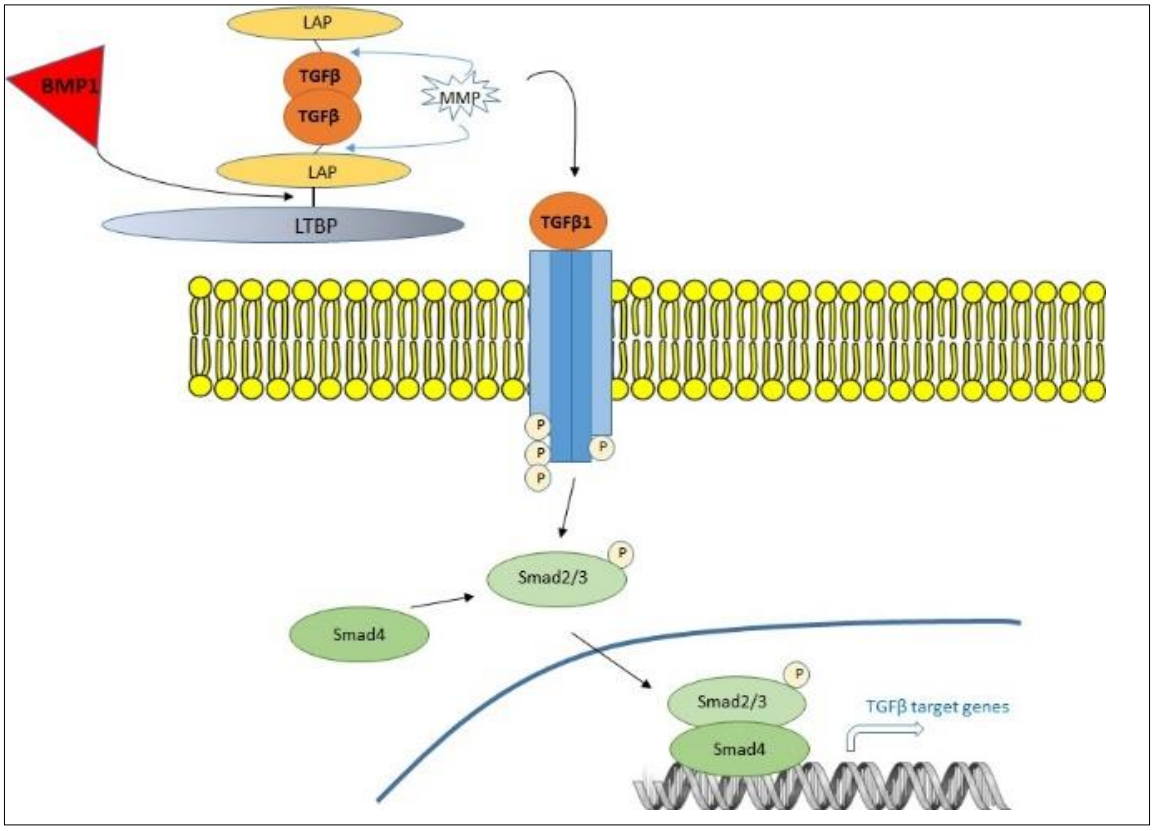

Figure 2. Activation of TGFβ and intracellular canonical signalling pathway. BMP1 cleaves latent TGFβ binding protein (LTBP), enabling thus subsequent cleavage of latency-associated peptide (LAP) by matrix metalloproteinase (MMP) and liberation and activation of TGFβ which then binds to receptor on cell membrane. Phosphorylation of TGFβ receptor upon binding of its ligand activates cannonical Smad2/3 signalling pathway which activates transcription of corresponding genes.

Liver fibrosis

Liver is among organs most frequently affected by fibrotic changes. Hepatic fibrosis (HF) usually results from an inflammatory process which first involves hepatocytes, but consequently leads to the activation of effector cells and excessive deposition of extracellular matrix. This process results finally in the liver cirrhosis, which can lead to the life-threatening complications (23). The most important role in development of liver fibrosis play hepatic stellate cells (HSCs), whose activation and proliferation during the liver injury leads to their transdifferentiation into myofibroblasts, which are characterized by expression of myogenic markers (*α-SMA*) (35) and increased ECM production (36). Activated HSCs are the major source of ECM in experimentally induced liver injury (37). Extensive studies on transgenic animal models revealed a number of key genes involved in liver fibrogenesis: genes regulating hepatocellular apoptosis/necrosis, genes regulating inflammatory response to the injury, genes regulating ROS generation, fibrogenic growth factors, vasoactive substances and adipokines (35). Among them, TGFβ1 has a crucial role in liver fibrogenesis in humans (38), stimulating transition of HSCs into myofibroblasts and inducing synthesis of ECM components (35) (Fig. 3). Quiescent HSCs are not the main source of TGFβ1; however, its expression is significantly upregulated in these cells upon liver injury. Besides HSCs, there are additional cellular sources of TGFβ1 in liver tissue (hepatocytes, macrophages, platelets) (39). In activated HSCs, TGFβ influences cytoskeletal organization and cellular migration through RhoA GTPase signalling (40), induces proliferative HSC response by a complex mechanism which involves PDGFβ and PI3 kinase pathways, but also acts on hepatocytes by inducing expression of profibrogenic mediators (*PDGF*, *IL-15*, *TIMP-1*, *EGFR*) (41, 42).

The role of BMP1 in liver fibrosis is scarcely described in the literature. A recent paper published by our group (18) showed that inhibition of BMP1-3 protein by specific polyclonal antibodies reduced plasma levels of TGFβ1, suppressing thus its profibrotic effect and preventing progression of experimentally induced liver fibrosis *in vivo*. Further, treatment of human stellate cell line LX-2 (a model for HSCs *in vitro*) with BMP1.3 antibodies attenuated increase in *Col1* expression induced by TGFβ treatment. In healthy liver, BMP1-3 is present mostly in sinusoidal epithelial cells, whereas in fibrotic liver it is found also in hepatocytes (18). This study suggested that BMP1.3 inhibition negatively influences release of TGFβ1 from its latent form, which in turn slows down the progress of fibrosis and could be considered as a new therapeutical approach.

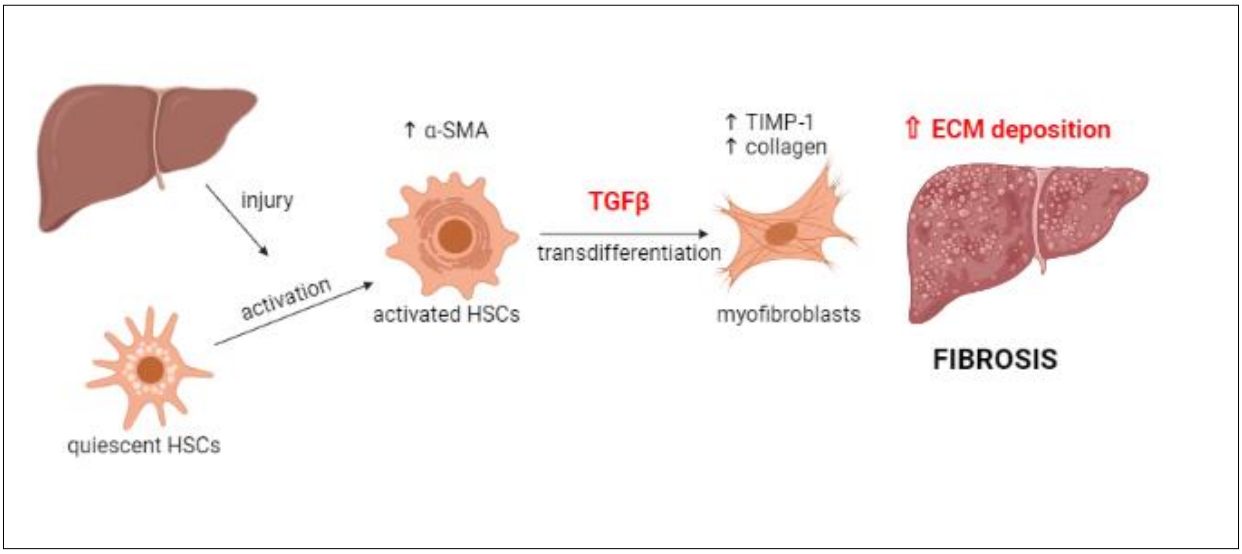

Figure 3. Schematic pathway of liver fibrosis development. Injury of liver parenchyma leads to the activation of quiescent hepatic stellate cells (HSCs), which, upon TGFβ1 signalling, transdifferentiate into myofibroblasts. Increased expression of TIMP-1 and collagen lead to the increased deposition of extracellular matrix (ECM) and development of liver fibrosis (image created with BioRender.com).

Chronic kidney disease

Chronic kidney disease (CKD) has a high prevalence (about 10% of worldwide population) and high mortality, usually developing progressively from chronic to end-stage renal disease, leading to kidney failure and finally requiring renal replacement therapies such as hemodialysis or kidney transplantation (43). CKD develops as a result of renal fibrotic process, characterized by increased ECM protein production and deposition of fibrotic matrix. Fibrosis affects all kidney compartments: glomeruli, tubulointerstitium and the vasculature (43), consequently resulting in the increased tissue stiffness and formation of scar tissue within the parenchyma, which ultimatively leads to the kidney failure (44, 45).

Two most frequent diseases underlying renal fibrosis are hypertension and diabetes, but CKD often results from inflammatory kidney diseases, such as glomerulonephritis or inappropriate use of medications (46). As in other organs, the process of fibrosis is triggered by the chronic inflammatory process, characterized by the simultaneous tissue repair and remodelling (47). Excessive accumulation of ECM proteins drives this process into fibrosis which disturbs normal organ function, interfering with normal regeneration of kidney structures. Similar to the fibrotic process in the liver described earlier, the main source of ECM in kidney are myofibroblasts. Myofibroblasts, which produce various types of ECM proteins (collagens, fibronectins, proteoglycans, etc.) can be derived from various cellular sources, but it seems that resident renal fibroblasts and hematopoietic cells which migrate to the kidney are their most important ancestors (48).

The initial trigger for renal fibrosis is infiltration of inflammatory cells which induce production of fibrogenic cytokines, such as IL-1, IL-8, TNFα, TGFβ and other chemokines (49, 50). Among them, TGFβ1 is considered to have a primary role in induction and progression of CKD, similarly to its role in other organs. Besides its action on inducing ECM synthesis and reducing ECM degradation, TGFβ1 also promotes transition of endothelial cells and resident fibroblasts into myofibroblasts, which in turn promote further ECM deposition (31) (Fig. 4).

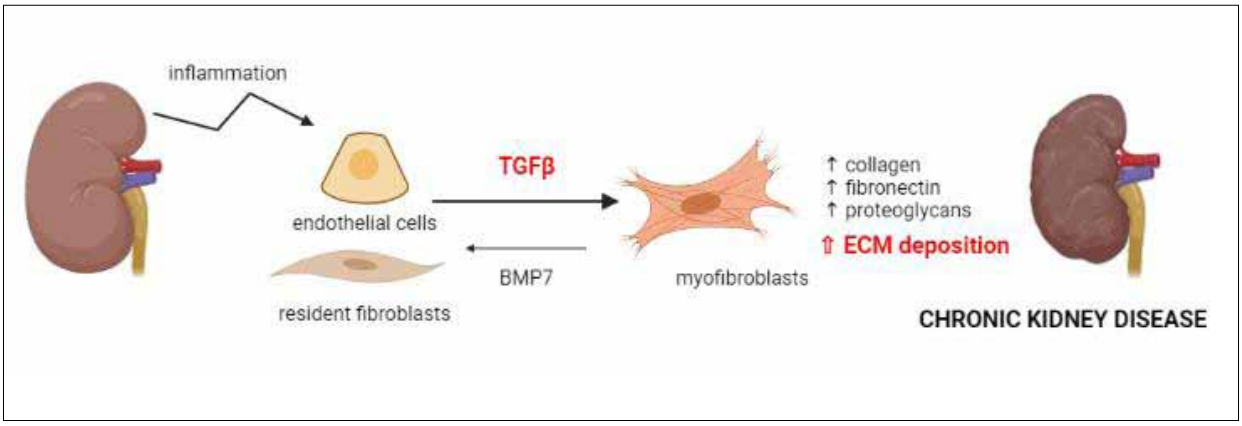

Figure 4. Schematic pathway of development of chronic kidney disease. Inflammatory process in kidney glomeruli and increased TGFβ expression lead to the transdifferentiation of endothelial cells and resident fibroblasts into myofibroblasts, whereas BMP7 acts in the opposite direction. Myofibroblasts express increased amounts of collagen, fibronectin and proteoglycans, which together contribute to the increased ECM deposition and development of chronic kidney disease (image created with BioRender.com).

Numerous studies have shown increased expression of TGFβ1 in human kidney disease (51-53), whereas several animal studies have identified TGFβ1 as the predominant pathogenic factor in fibrogenesis regulation (31, 54-56). On the other hand, another mediator in kidneys, bone morphogenetic protein 7 (BMP7), which belongs to the TGFβ superfamily of proteins, has an antifibrotic and anti-inflammatory effect and was found to prevent loss of kidney function in an animal model of acute renal failure (57). In kidneys, BMP7 is not only involved in regulation of fibrotic mechanisms, but also plays a role in kidney differentiation (58), acting via type II BMP receptors (59). Further studies revealed that BMP7 counteracts TGFβ by reversing the TGFβ-induced epithelial-to-mesenchymal transition and thus inhibits accumulation of myofibroblasts, which would otherwise lead to the development of fibrosis and CKD (60).

Although TGFβ1 can induce renal fibrosis via activation of different signalling pathways (29), the critical role in developing CKD has its canonical, Smad-dependent signalling pathway. Smad proteins mediate intracellular signal transduction which promotes deposition of ECM and inhibits its degradation, disturbing thus the balance between ECM aggregation and degradation and promoting the development of fibrosis (53, 55, 61). The primary downstream mediators of TGFβ1 are Smad2 and Smad3, which have opposite effect on development of kidney fibrosis. Phosphorylation of profibrotic Smad3 and suppression of antifibrotic Smad2 enhance fibroblast proliferation, differentiation of myofibroblasts and production of ECM in the kidney. In parallel, BMP7, which in kidney has an effect opposite to the TGFβ, acts via Smad1/5/8 signalling pathway (62).

Since there is currently no satisfactory strategy for treating CKD and preventing its progression to end-stage renal disease, new therapeutic approaches are needed. Considering the role of BMP1 in releasing an active form of TGFβ1, the main contributor to the fibrotic processes (31, 32), inhibition of BMP1 is potentially promising approach for treating kidney fibrosis. Recently, BMP1 inhibitor has been shown to block the accumulation of ECM components, collagens type I and III and fibronectin in both *in vitro* and *in vivo* models (63). Our group previously demonstrated that addition of specific polyclonal antibody raised against BMP1.3 (long) isoform attenuated fibrosis in experimentally induced CKD in rat model (8). Moreover, inhibition of BMP1.3 by specific antibodies increased expression of *Bmp7*, which could have an additional effect on reversal of fibrotic symptoms, regarding the role of BMP7 in kidney fibrosis, which is opposite to the TGFβ (8). These findings support the BMP1 antagonism as a potentially new therapeutical strategy for renal fibrosis and CKD treatment.

Heart failure

Acute myocardial infarction (AMI), which is the main cause of heart failure, affects more than 23 million people worldwide (64). Myocardial infarction often results in significant fibrotic scarring of the heart with concomitant loss of cardiac function. Progressive loss of contractile function following a cardiac injury is a consequence of the poor regenerative capacity of cardiomyocytes and their replacement by a collagen-based fibrotic tissue. Although mammalian heart has a regenerative potential for a brief period after birth, in adult organism, this capacity is lost (65). Multiple attempts have been made to regenerate the heart using various strategies (66-69). Remodelling of the myocardium surrounding the site of injury, which includes thickening (hypertrophy) and stiffening (fibrosis) of the ventricular wall, eventually results in impaired cardiac function (70). Initially, after MI, dead cardiomyocytes are cleared by macrophages and are progressively replaced by reparative cells, mainly fibroblasts. Cardiac fibroblasts undergo three phenotypic changes: diferentiation into myofibroblasts, proliferation and the production of extracellular matrix proteins (71). Among proteins produced by activated cardiac fibroblasts, BMP1 and lysyl oxidase (LOX) play a key role in collagen cross-linking (72, 73). It was demonstrated that BMP1 activates LOX precursor to mature active form which is responsible for the cross-linking of collagen. This process enables formation of mature, insoluble extracellular matrix less prone to degradation (72) (Fig. 5). Increased level and activity of LOX has been observed in the myocardium of patients with heart failure, which correlated with increased collagen cross-linking and collagen content (74).

As stated already in this review, members of TGFβ family play a significant role in processes of cell differentiation and proliferation, in particular after tissue damage, when TGFβ proteins are critical in wound healing and tissue regeneration. It is well-known that TGFβ is highly expressed in neonatal and adult murine heart, localized in both cardiomyocytes and the ECM (75), where it is implicated to have a significant role in an early angiogenesis and development of cardiovascular structures (76). Expression of all three TGFβ isoforms is significantly upregulated upon myocardial infarction in several animal models of AMI (77). In the myocardium, TGFβ is present in its latent form, and for the activation of its signal transduction cascade, it must be released from the latent complex. The main factor in cleavage of this latent complex is BMP1, which acts as a metalloproteinase and releases TGFβ from ECM, enabling thus its further activation through other enzymes (matrix metalloproteinases) (33). Besides TGFβ activation, BMP1 also converts pro-collagen into collagen, one of main constituents of ECM and also fibrotic tissue. It has been shown that the endogenous expression of BMP1 was significantly upregulated at the same time point when type I and type III procollagen and TGFβ were upregulated, which is consistent with the role of BMP1 as a key player in procollagen biosynthesis and maturation (78).

In infarcted heart, TGFβ modulates immune reaction and cardiomyocyte survival and regulates regenerative processes in

the heart following infarction (79). Activation of fibroblasts by TGFβ is an important part of this regenerative process, both by promoting transdifferentiation of fibroblasts into myofibroblasts and by stimulating ECM protein synthesis (80). Although this process is necessary for tissue regeneration, excessive myofibroblast activity and ECM deposition lead to the development of cardiac fibrosis, associated with increased stiffness and diastolic dysfunction, leading to heart failure (81).

Due to its role in regenerative and fibrotic processes, TGFβ is potentially interesting therapeutic target in myocardial infarction (79). Inhibition of one of its main activators, BMP1, could present a new therapeutic approach in treatment of cardiac fibrosis. Both BMP1.1 and its long isoform BMP1.3 convert many of ECM precursors into mature functional proteins which mediate collagen crosslinking (82). Recently, our group has found increased levels of circulating BMP1.3 in plasma of patients with AMI, suggesting the possible role of this protein in cardiac fibrosis, whereas studies in animal models of AMI strongly suggest that BMP1.3 inhibition could have a therapeutic benefit. The newly developed anti-BMP1.3 monoclonal antibody was tested in animal model of myocardial infaction and found to reduce the expression of *Lox* in the scars of treated mice, as well as other crosslinking indicators (17).

Congenital muscle dystrophy

The congenital muscular dystrophies (CMDs) are rare neuromuscular disorders, caused by allelic mutations in different genes and characterized by different pathologic features, primary affecting the skeletal muscle. CMDs vary in clinical features, but common are dystrophic features found by muscle biopsy, including variations in muscle fiber size, fiber degeneration and increased fibrosis (83). Chronic inflammatory processes in dystrophic muscle result in excessive accumulation of ECM components, leading to the replacement of muscle with fibrotic tissue (84). CMDs are classified in several groups, depending on source of protein defect. The most common form of CMD is laminin-α2 chain-deficient muscular dystrophy (LAMA2 MD), also called merosin-deficient CMD (MDC1A), which accounts for about 30% of all CMD patients in Europe (83, 85). Early-onset MDC1A is an autosomal recessive disorder caused by mutation in *LAMA2* gene which leads to loss of α2 subunit of laminin-211 protein. Laminins are heterotrimeric proteins composed of α, β and γ subunits, and laminin-211 (also called merosin) is predominantly expressed in skeletal muscle as an important tissue component of ECM (83, 86). Laminins are essential for basement membrane assembly and insufficient assembly of laminin network causes poor connection to the muscle fibers, which may be one of causes of MDC1A (87). Structural and functional integrity of basement membrane is crucial for regulation of cell interactions and processes which regulate differentiation of myofibroblasts, which are in turn critical for development of fibrosis (88).

Laminin-211 is expressed primarily in basement membranes of skeletal muscle and Schwann cells, but also in other tissues (heart, kidney, lung, stomach, placenta, testis) (89) Muscles lacking laminin-211 have signs of chronic inflammation and widespread fibrosis in the interstitial space (89), and there is substantially high expression of ECM genes in CMD, regardless of histological changes, supporting an early fibrosis in this disease (90). As the most significant driver of fibrosis appears TGFβ, whose activation depends on proteolysis of latent inhibitory complex by BMP1 (32), but also on activation of integrins (89). Indeed, TGFβ signalling increases early in CMD, stimulating fibroblasts to produce ECM components such as collagen and fibronectin

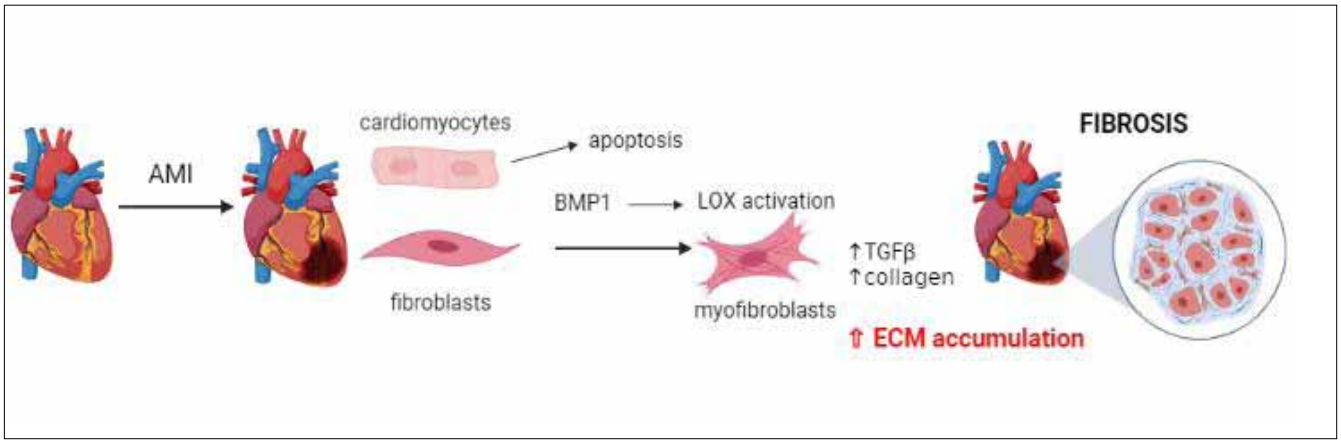

Figure 5. Schematic pathway of heart fibrosis development. Upon acute myocardial infarction (AMI), cardiomyocytes undergo apoptosis, and cardiac fibroblasts undergo transdifferentiation into myofibroblasts. Simultaneously, BMP1 activates lysyl oxydase (LOX) precursor into mature form, which is responsible for collagen cross-linking. Increased expression of TGFβ and collagen lead to the increased ECM accumulation and development of fibrosis (image created with BioRender.com).

and influencing negatively production of enzymes which degrade ECM (84, 91). TGFβ signalling pathway is stimulated through increased activity of phosphorylated of Smad2/3, as seen in mouse model for MDC1A (91). Chronic dysregulation of TGFβ signalling leads also to the transdifferentiation of myoblasts into myofibroblasts (Fig. 6). TGFβ1 seems to be a key player in this process through complex signalling mechanisms involving integrins (92) and sphingosine kinase pathway (93), along with increased expression of muscle fibroblast marker Tcf4 (94). Most therapeutical strategies for MDC1A are targeting TGFβ1 signalling pathway in order to alleviate fibrotic processes, for example, losartan, which is originally approved for treatment of hypertension as an AT_1R blocker (89). Losartan (or its derivatives) acts on angiotensin-renin system, which indirectly inhibits TGFβ1 signalling and leads to amelioration of fibrosis, as demonstrated in mouse models of MDC1A (95, 96). However, although treatment with losartan reduced inflammation and fibrosis, it did not increase muscle weight and could not be considered as a single mode therapy for this disease but should be supplemented with growth-inducing therapy (89, 97). Other therapeutical strategies which are currently explored include protein replacement therapy with addition of recombinant laminin-111 (98), use of linker proteins, targeting of intracellular regulatory systems or genetic approaches (for review, see (85)). Another therapeutical approach could also be inhibition of BMP1.3 by the use of specific antibodies, which already demonstrated its efficacy in reducing fibrosis in kidney (8) and liver (18). These studies are ongoing on animal models, by using DyW mice which are homozygous for the mutation in the laminin-α2 gene and are widely used as an animal model for MDC1A (99, 100). Initial studies on DyW mice suggest that treatment with anti-BMP1.3 antibodies could improve DyW mice mobility, quality of life and life expectancy in comparison with mice without therapy (unpublished data).

Concluding remarks

As proteolytic activators of TGFβ, one of main mediators of fibrotic processes, a group of BMP1 proteins has an universal function in mammalian organism. Among multiple BMP1 isoforms, only BMP1.3 (the long isoform) has been found to circulate in human blood. In search for appropriate targets for treating fibrosis, inhibition of BMP1.3 via synthetic inhibitors or specific antibodies could be a promising solution. Results on animal models of liver fibrosis, chronic kidney disease and myocardial infarction suggest that anti-BMP1.3 antibodies could alleviate fibrotic symptoms in these indications, and also to slow the progression of congenital muscular dystrophy (Fig. 7). More studies on animal models are needed, as well as clinical studies in humans, which would encourage development of specific biological therapies for these diseases.

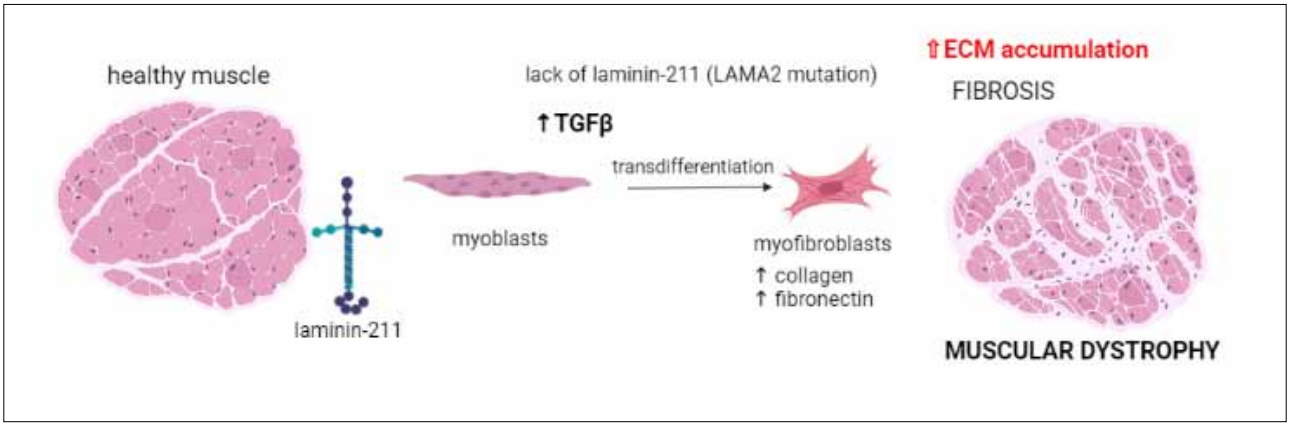

Figure 6. Development of muscular dystrophy. LAMA2 mutation causes lack of laminin-211, a protein crucial for proper basement formation in healthy muscle. Inreased TGFβ signalling in muscles lacking laminin-211 stimulate transdifferentiation of myoblasts into myofibroblasts which produce increased amounts of collagen and fibronectin and contribute to the ECM accumulation and development of fibrosis. Muscle fibers degenerate and muscular dystrophy develops (image created with BioRender.com).

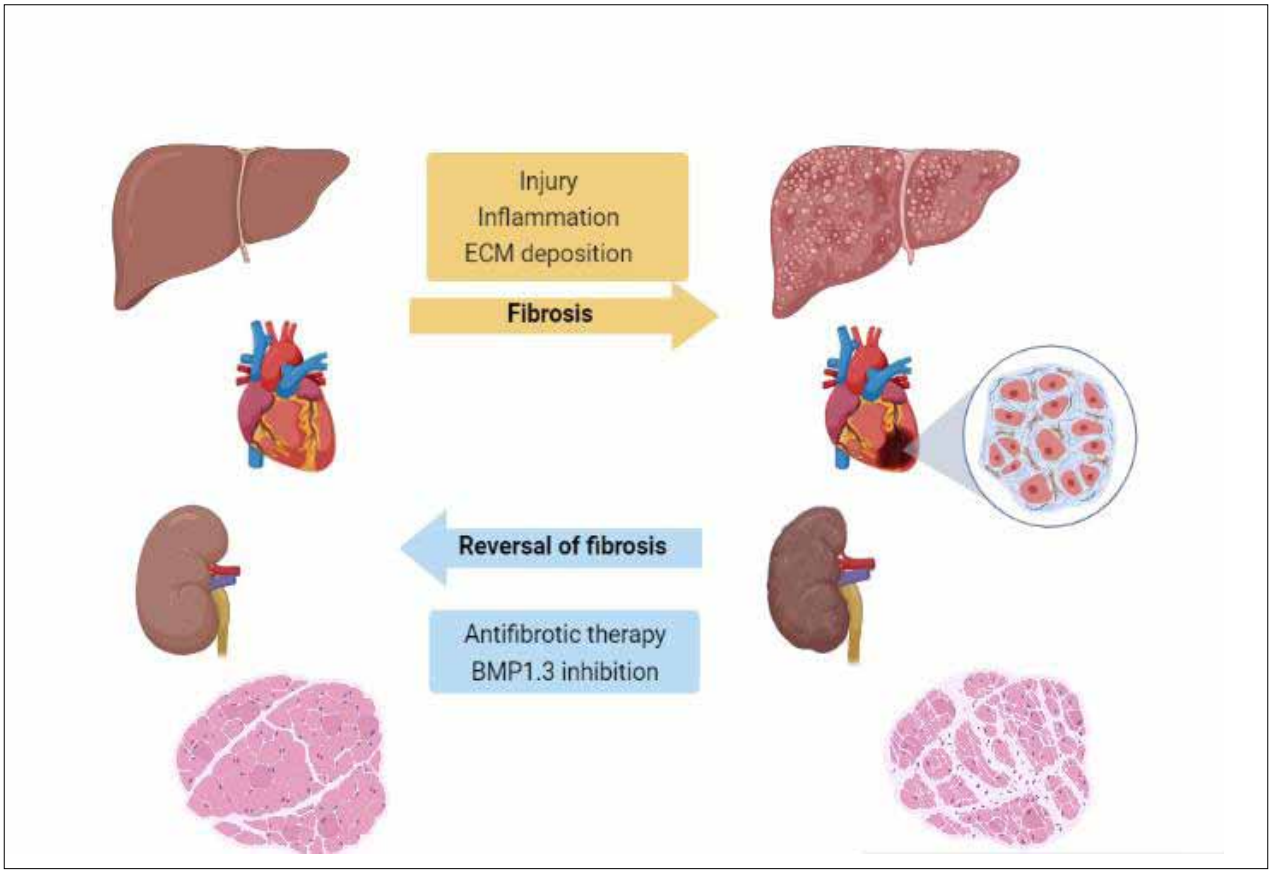

Figure 7. Examples of organs most frequently affected by chronic fibrosis. Injury and chronic inflammation accompanied by increased ECM deposition result in development of fibrosis, which finally lead to the loss of the function of particular organ (liver, heart, kidney, skeletal muscle). Antifibrotic therapy could relieve fibrotic symptoms and enable reversal of fibrosis. Among possible therapeutic approaches, inhibition of BMP1.3, which is one of the main activators of signalling cascade leading to development of fibrosis, appears to be a promising solution (image created with BioRender.com).

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