Summary. Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and fatal lung disease with no known etiology and treatment options. The hallmarks of the histopathology, which is characteristic of usual interstitial pneumonia (UIP) pattern, include interstitial fibrosis, honeycomb changes and fibroblast foci that develop owing to fibroblast proliferation and excessive matrix deposition. Although the complete pathomechanism is not yet understood, several molecular culprits, including transforming growth factor (TGF)-ß, Angiotensin (Ang) II, endothelin (ET)-1, matrix metalloproteinases (MMPs) and cytokines have been identified. IPF is increasingly believed to be an epithelial-driven disease; however, the literature does support an implication of altered immune response and inflammatory processes in the onset or progression of the disease (Song et al., 2009; Gilani et al., 2010). Although the complete pathomechanism is not yet fully understood, several molecular culprits, such as transforming growth factor (TGF)-ß,
Angiotensin (Ang) II, dimethylarginine dimethylamino-hydrolase (DDAH), chemokine stromal cell derived factor (SDF)-1, variety of proteases and growth factors, including platelet derived growth factor (PDGF) and connective tissue growth factor (CTGF) have been identified (Antoniades et al., 1990; Khalil and Greenberg, 1991; Xu et al., 2007; King et al., 2011; Kono et al., 2011; Pullamsetti et al., 2011; Uhal et al., 2012; Fernandez and Eickelberg, 2012). Moreover, it may be surmised that the pathogenesis of pulmonary fibrosis involves at least two different cellular routes: the epithelial and the inflammatory (Williams et al., 2004; Sisson et al., 2010; King et al., 2011).

Mast cells (MCs) are multifunctional tissue resident cells involved in the inflammatory and immune response. They were first described in 1878 by Paul Ehrlich based on the metachromatic phenomenon of their cytoplasmic granules in response to basic dyes such as toluidine blue. An accumulating body of literature suggests that persistent activation of MCs and release of their granules containing a large variety of mediators such as histamine, leukotriens, prostanoids, cytokines, chemokines and proteases may significantly influence inflammation and tissue remodeling (Krishnaswamy et al., 2001; Metz et al., 2007; Galli and Tsai, 2008; Trivedi and Caughey, 2010; Dahal et al., 2011). Among the MC mediators, cytokines and proteases are of particular interest with respect to the potential role of MCs in tissue remodeling (Pejler et al., 2010). Indeed, a growing number of studies indicate that the MC proteases, namely tryptase and chymase, may be involved in fibrotic tissue remodeling (Ruoss et al., 1991; Metz et al., 1995; Takai et al., 2004). The discovery of MC chymase dates back to the 1950s when researchers noticed an intense histochemical esterase activity, which was similar to chymotrypsin, in rat MCs (Trivedi and Caughey, 2010). Reimer et al have shown that MC chymase specificity has been evolutionarily conserved over 200 million years among mammals, indicating an important role of this enzyme in MC biology (Reimer et al., 2008). The crystal structure and the gene sequence of chymase were resolved in 1985 and 1990, respectively (Trivedi and Caughey, 2010).

Previous studies have shown that MCs are prevalent in fibrotic lungs (Fig. 1), they express profibrotic growth factor, namely, basic fibroblast growth factor (bFGF), and their count significantly correlates with the degree of fibrosis, suggesting the implication of MCs in chronic inflammation and pulmonary fibrosis (Pesci et al., 1993; Inoue et al., 1996; Kosanovic et al., 2011). Moreover, MC accumulation is predominant in lung interstitium including the areas closer to fibrotic foci (Fig. 1). Interestingly, the MCs that reside in the fibrotic regions display immunoreactivity to chymase (Fig. 2). Recently published studies suggest that chymase expressing pulmonary MCs are prevalent in the areas showing cellular inflammation or fibrosis (Andersson et al., 2011; Cha et al., 2012). Overall, the current findings imply the involvement of chymase in the process of fibrosis, which is characterized by an excessive deposition of fibrillar collagen. Indeed, it is recognized that MC chymase can exhibit procollagen peptidase activity by processing procollagen to fibril-forming collagen, and thus may have a potential role in the regulation of collagen biosynthesis (Kofford et al., 1997). Moreover, it is conceivable that MC chymase, due to its enzymatic activity, may participate in proteolytic processing of

Mast cell chymase in pathology of idiopathic pulmonary fibrosis (IPF)

Mast cell (MC) chymase is a serine protease produced and stored as an inactive enzyme within MC granules that have pH of around 5.5 (De Young et al., 1987; McEwen et al., 1995; Takai et al., 2004). Chymase starts its chymotrypsin-like enzymatic activity immediately upon its release into the tissue microenvironment (pH 7.4), as the pH conducive to chymase activity ranges between 7 and 9 (Urata et al., 1990; Takai et al., 2004). The discovery of MC chymase dates back to the 1950s when researchers noticed an intense histochemical esterase activity, which was similar to chymotrypsin, in rat MCs (Trivedi and Caughey, 2010). Reimer et al have shown that MC chymase specificity has been evolutionarily conserved over 200 million years among mammals, indicating an important role of this enzyme in MC biology (Reimer et al., 2008). The crystal structure and the gene sequence of chymase were resolved in 1985 and 1990, respectively (Trivedi and Caughey, 2010).

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Fig. 1. Mast cells accumulation in the lungs of IPF patients. Mast cells (MCs) were stained with toluidine blue and representative photomicrographs from donors and IPF patients are shown. Arrows indicate MCs. AS: airspace; L: lumen of pulmonary vessel. Scale bars: 50 µm.
cytokines, proteases and growth factors. In the following sections we will discuss how MC chymase is linked to and can potentially contribute to pulmonary fibrosis.

**Mast cell chymase and proinflammatory cytokines**

Interleukin (IL)-1 and IL-18 are members of the IL-1 family of cytokines and are involved in immune and inflammatory responses. Enhanced expression of IL-18 and its receptor was observed in patients with IPF. Moreover, experimental bleomycin-induced pulmonary injury and subsequent fibrosis were significantly attenuated in IL-18 and IL-18 receptor α knockout mice (IL-18(-/-) and IL-18Rα(-/-), respectively) as compared to the control wild type mice (Kitasato et al., 2004; Hoshino et al., 2009). These findings suggest that IL-18 and IL-18R are involved in the pathogenesis of pulmonary fibrosis. The importance of the cytokine IL-1 and its receptor IL-1R/MyD88 signaling in the development of pulmonary fibrosis is evident from the studies demonstrating that IL-1R1- and MyD88-deficient mice are protected against bleomycin-induced lung injury, and furthermore, increased levels of IL-1ß were observed in the bronchoalveolar lavage fluids of patients with IPF (Gasse et al., 2007; Wilson et al., 2010). In this context, it is worth noting that MC chymase is involved in processing the cytokines IL-18 and IL-1ß from their inactive forms into biologically active forms (Fig. 3) (Mizutani et al., 1991; Omoto et al., 2006), suggesting the implication of MC chymase in the process of pulmonary fibrosis. However, whether MC chymase exclusively accomplishes the processing of IL-1 and IL-18 during lung fibrosis development or other factors, such as caspase-1 come into play (Kordes et al., 2011), is not yet explored.

**Mast cell chymase and endothelin**

The pathobiology of endothelin in lung fibrosis has been recently reviewed (Fonseca et al., 2011). In this review, the authors summarized that endothelin (ET)-1, as a potent mitogen and mitogen for mesenchymal cells, can induce cell differentiation increasing both the synthesis and deposition of extracellular matrix components and contractile ability. A large body of *in vitro* and *in vivo* evidence supports the profibrotic role of ET-1 system in the pathogenesis of IPF (Uguccioni et al., 1995; Park et al., 1997; Abraham et al., 1997; Saleh et al., 1997; Mutiaers et al., 1998; Shahar et al., 1999; Shi-Wen et al., 2001, 2007; Fireman et al., 2001; Wendel et al., 2004). ET-1 is synthesized as a 212 amino-acid precursor, termed pre-proET-1, which is cleaved by a furin-like enzyme into the 38 amino-acid big-ET-1, which in turn is cleaved by the endothelin converting enzyme (ECE) to produce the mature, active 21 amino-acid ET-1, (1-21) (D'Orléans-Juste et al., 2008; Simard et al., 2009). The literature thus supports the hypothesis that the bioactive ET-1 generated in a chymase-dependent manner may contribute to the pathogenesis of pulmonary fibrosis. However, diminished ET-1 levels and ET-1-induced pathology *in vivo* in the caecal ligation and puncture (CLP) model of acute bacterial peritonitis have been attributed to the ET-1 degradation by chymase that is released as a result of ET-A-dependent MC activation (Maurer et al., 2004). These findings suggest a beneficial role of MC chymase in ET-1-induced toxicity, in contrast to its role as culprit in other pathological conditions. Future studies should therefore explore the role of chymase dependent ET-1 generation and its implication in the pathogenesis of pulmonary fibrosis.

**Mast cell chymase and matrix metalloproteinase**

As abnormal collagen deposition is the hallmark of lung fibrosis, the balance of collagen production and degradation is crucial to disease development. Accordingly, there arises a rational speculation that the dysregulation of matrix metalloproteinase (MMP) activities may act as a contributing factor to pulmonary fibrosis.
fibrosis (Dancer et al., 2011). A higher expression of tissue inhibitor of metalloproteinases (TIMP) compared with collagenases as observed in IPF (Selman et al., 2000) fits in with the ongoing fibrotic process. However, elevated levels of MMPs such as MMP-1, -2, -3, -7, -8 and -9 have also been reported (McKeown et al., 2009; Dancer et al., 2011), suggesting that the pathobiology of MMPs in pulmonary fibrosis is complex and goes beyond the simplistic view of mere matrix regulation. In this context, MC chymase may have a role in connecting MMPs, particularly the gelatinases MMP-2 and MMP-9, to pulmonary fibrosis, and this may be brought about by chymase-dependent processing of pro-MMP-2 and -9 into their active forms (Fig. 3) (Tchougounova et al., 2005; Oyamada et al., 2011). Indeed, an increased enzymatic activity in the bronchoalveolar lavage (BAL) fluid and pronounced pulmonary expressions of MMP-2 and MMP-9 have been demonstrated in IPF patients (Suga et al., 2000; García-Alvarez et al., 2006). Contrary to the literature suggesting a profibrotic role of MMP-9, a potential anti-fibrotic effect has been assigned to an increased activity of this matrix metalloproteinase owing to the alveolar macrophages overexpressing MMP-9 (Cabrera et al., 2007). Therefore, future studies are needed to elucidate the precise role of the MMP system in pulmonary fibrosis and the MMP-dependent profibrotic effects of chymase.

**Mast cell chymase and TGF-β**

Transforming growth factor beta (TGF-β) is a secreted pleiotropic protein that, by binding to and activating its receptors and downstream signaling molecules, participates in the regulation of various target genes. The TGF-β pathway is involved in multiple cellular processes such as cell growth, differentiation, apoptosis and cellular homeostasis, and dysregulation of this signaling pathway is associated with pathological processes including fibrogenesis (Jablonska et al., 2010; Biernacka et al., 2011). Both human IPF and experimental pulmonary fibrosis are characterized by an upregulation of TGF-β; moreover, inhibition of TGF-β signaling interferes with the development of experimental lung fibrosis (McCormick et al., 1999; Horan et al., 2008; Flechsig et al., 2012; Lepparanta et al., 2012). Although the underlying pathomechanisms of pulmonary fibrosis are not completely resolved, the ever-increasing body of literature unanimously recognizes TGF-β as a crucial player.

TGF-β may be processed by MC chymase from its inactive latent state to active form (Fig. 4) (Takai et al., 2003). It has been shown that the concentration of TGF-β in human fibroblast culture supernatants increases noticeably after application of chymase, while the addition of a chymase inhibitor abrogates this effect (Takai et al., 2003). Moreover, chymase supplementation results in increased proliferation of human fibroblasts, whereas it is inhibited by TGF-β neutralizing antibodies, suggesting the role of chymase in TGF-β-mediated fibroblast proliferation (Takai et al., 2003). In line with this contention, human chymase was found to generate mature TGF-β1 from its latent form and this has been suppressed by chymase inhibitor in a concentration dependent manner (Tomimori et al., 2003). MC chymase transforms latent TGF-β-binding protein (LTBP)-bound TGF-β propeptide to latent TGF-β and further to active TGF-β (Fig. 4) (Takai et al., 2003). Taking into consideration the crucial role of the TGF-β signaling pathway in the development of pulmonary fibrosis it is tempting to speculate that MC chymase dependent TGF-β activities are of particular importance in the pathological symphony of IPF.

![Fig. 3. Potential contribution of mast cell chymase to the pathogenesis of IPF.](image-url)
Mast cell chymase and angiotensin II

Angiotensin II (Ang II) is a component of the angiotensin system that also includes angiotensinogen, renin, angiotensin-converting enzyme (ACE), Ang II and the two Ang II receptors namely, Ang II receptor-1 (AT1) and 2 (AT2). It is now clear that many organs including lung have the local tissue angiotensin system that is independent of the classical endocrine ‘renin-angiotensin-aldosterone system’. In this context, MC chymase plays a significant role in the biosynthesis of Ang II from its precursor Ang I (Fig. 4) (Miyazaki et al., 2006a,b). Mammalian chymases, based on structure and substrate specificity, are categorized as α-chymase and β-chymase. Both α- and β-chymases are capable of converting Ang I to Ang II, but Ang II is further hydrolyzed by β-chymase to inactive peptide fragments (Sanker et al., 1997; Caughey et al., 2000; Doggrell and Wanstall, 2004). α-chymase is expressed in species such as humans, dogs, sheeps, monkeys and hamsters, while β-chymase is found in rats and mice (Caughey et al., 2000; Doggrell and Wanstall, 2004). Although ACE has been known for its angiotensinogen processing activities, it is interesting to note that 75% of all Ang II-generating activities in human cardiac tissue homogenate are chymase-dependent and only 25% are ACE-dependent (Takai et al., 2004). Moreover, inhibition of chymase resulted in a drop of all Ang II-generating activities by 90% in homogenates of human arteries. These findings thus suggest that Ang II production may be predominantly chymase-dependent in pathophysiology associated with MC activation and angiotensinogen upregulation.

Previous studies have revealed that Ang II plays an important role in tissue fibrosis (Reid et al., 2007; Sakamoto-Ihara et al., 2007; Komeda et al., 2008; Yang et al., 2009; Uhal et al., 2012). Moreover, a large body of evidence suggests the role of Ang II in the pathogenesis of lung fibrosis (Uhal et al., 2012). There is an increase of Ang peptides and AT1 and AT2 receptor expression in lung tissue from patients with IPF (Li et al., 2006; Königshoff et al., 2007). Moreover, Königshoff et al demonstrated that Ang II exerts a mitogenic activity for human lung fibroblasts via AT1 receptor. The profibrotic effects of Ang II also occur through its induction of procollagen production in human lung fibroblasts via AT1 receptor activation and, at least in part, via the autocrine action of TGF-β (Marshall et al., 2004). Furthermore, Lang et al have shown that chymase generates Ang II and enhances collagen expression in human lung fibroblasts culture (Lang et al., 2010). Taken together, the experimental evidence convincingly suggests the implication of chymase-AngII axis in the pathogenesis of pulmonary fibrosis.

Chymase as a therapeutic target: animal model studies

Among the available models, bleomycin-induced lung injury is the most widely accepted and used animal model to study the pathobiology and therapeutic strategies for pulmonary fibrosis (Moeller et al., 2008). Bleomycin is a chemotherapeutic antibiotic produced by the bacterium Streptomyces verticillus. However, bleomycin was recognized to induce pulmonary fibrosis in human as a side effect during cancer therapy, and therefore it was employed to induce experimental pulmonary fibrosis for the first time in dogs in 1971 and
later on in other species such as mice, hamsters and rats (Moeller et al., 2008). Upon instillation into the lungs of animals bleomycin causes inflammatory followed by fibrotic responses within a relatively short period of time (by 21 days after instillation pulmonary fibrosis is fully developed). The initial inflammatory response (about a week), is characterized by the accumulation of inflammatory cells and significant elevation of the levels of inflammatory mediators in the lung (Moeller et al., 2008). In the fibrotic phase there are increased levels of pro-fibrotic markers, such as TGF-β, fibronectin and collagen fibrils. Moreover, bleomycin-induced histopathological features that include excessive collagen accumulation, obliteration of alveolar space, and formation of fibrotic-like foci resemble those observed in the lungs of IPF patients (Moeller et al., 2008). Therefore, the model of bleomycin-induced lung fibrosis has been very useful in unraveling several profibrotic players, such as TGF-β and growth factors, phosphodiesterase etc., and some underlying molecular mechanisms (Moeller et al., 2008; Udalov et al., 2010). Despite its merits, the bleomycin-induced lung fibrosis model, like many other animal models of human diseases, is not without limitation. Unlike human IPF, bleomycin-induced pulmonary fibrosis is partially reversible (Izbicki et al., 2002).

In the past two decades different peptidic and non-peptidic chymase inhibitors have been developed and investigated at experimental level for various indications including fibrosis and cardiovascular diseases (Takai et al., 2004). The non-peptidic chymase inhibitors such as NK3201, BCEAB and SUN-C8077 are orally active compounds that have been used to explore the role of chymase in the development of tissue fibrosis (Takai et al., 2004). NK3201 is a competitive chymase inhibitor that selectively and specifically inhibits human, dog and hamster chymase by IC50 values of 2.5, 1.2 and 28 nM, respectively, but it does not interfere with the activity of MC serine protease tryptase (Takai et al., 2004). The therapeutic efficacy of NK3201 has been investigated in a hamster model of bleomycin-induced pulmonary fibrosis (Sakaguchi et al., 2004). Application of NK3201 attenuated bleomycin-triggered lung fibrosis, as evident by reduction of fibrotic scar and diminished collagen III gene expression. The beneficial effect of NK3201 was associated with significantly decreased activity of pulmonary chymase. Another inhibitor of chymase BCEAB was investigated in the hamster model of cardiomyopathy (Takai et al., 2003). BCEAB improved cardiac fibrosis and its beneficial effect was associated with considerably reduced cardiac chymase activity. The authors investigated the underlying mechanism in vitro, and found that the exposure of cardiac fibroblasts to chymase induces TGF-β expression and potentiates cell proliferation. The addition of BCEAB suppressed the chymase-induced fibroblast proliferation (Takai et al., 2003). The efficacy of chymase inhibitor SUN C8077 was investigated in a mouse model of bleomycin-induced pulmonary fibrosis (Tomimori et al., 2003).

They demonstrated that bleomycin-induced lung fibrosis was associated with a significantly increased pulmonary hydroxyproline content and chymase activity that were reversed by SUN C8077. Moreover, the authors showed in vitro that human chymase activates latent TGF-β to generate active form, which was inhibited by SUN C8077. Taken together, the findings from the animal models, mostly from bleomycin-induced lung injury, implicate chymase as a potential target and therefore chymase inhibitors may represent a promising strategy for the treatment of pulmonary fibrosis.

In summary, IPF is a chronic, progressive and fatal lung disease with no cure available. MC chymase may contribute to its pathogenesis potentially through regulating activity of various profibrotic molecules such as TGF-β, Ang II, ET-1, MMPs and cytokines. Moreover, animal model studies demonstrate that the chymase inhibitors may represent a promising strategy for the treatment of pulmonary fibrosis. However, future studies should focus on elucidating the MC chymase-dependent pathomechanisms of pulmonary fibrosis and on more preclinical studies on chymase inhibitors.

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