M10, a caspase cleavage product of the hepatocyte growth factor receptor, interacts with Smad2 and demonstrates antifibrotic properties in vitro and in vivo

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Hepatocyte growth factor receptor, also known as cellular mesenchymal–epithelial transition factor (c-MET, MET), is an important antifibrotic molecule that protects various tissues, including lung, from injury and fibrosis. The intracellular cytoplasmic tail of MET contains a caspase-3 recognition motif "DEVD-T" that on cleavage by caspase-3 generates a 10–amino acid peptide, TRPASFWETS, designated as "M10". M10 contains at its N-terminus the uncharged amino acid proline (P) directly after a cationic amino acid arginine (R) which favors the transport of the peptide through membranes. M10, when added to cell culture medium, remains in the cytoplasm and nuclei of cells for up to 24 hours. M10 effectively decreases collagen in both scleroderma and TGFβ-stimulated normal lung and skin fibroblasts. M10 interacts with the Mad Homology 2 domain of Smad2 and inhibits TGFβ-induced Smad2 phosphorylation, suggesting that the antifibrotic effects of M10 are mediated in part by counteracting Smad-dependent fibrogenic pathways. In the bleomycin murine model of pulmonary fibrosis, M10 noticeably reduced lung inflammation and fibrosis. Ashcroft fibrosis scores and lung collagen content were significantly lower in bleomycin-treated mice receiving M10 as compared with bleomycin-treated mice receiving scrambled peptide. We conclude that M10 peptide interacts with Smad2 and demonstrates strong antifibrotic effects in vitro and in vivo in an animal model of lung fibrosis and should be considered as a potential therapeutic agent for systemic sclerosis and other fibrosing diseases. (Translational Research 2016;170:99–111)

Abbreviations: HGF = Hepatocyte growth factor; c-MET, MET = mesenchymal-epithelial transition factor; systemic sclerosis, SSc = scleroderma; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; TGFβ = transforming growth factor beta; PLC = phospholipase C; PKC = protein kinase C; DAPI = 4′,6-diamidino-2-phenylindole; MYC = polypeptide protein tag consisted of EQKLISEEDL amino acids; DDK = polypeptide protein tag consisted of DYKDDDDK amino acids; ACR = American College of Rheumatology; EULAR = European League Against Rheumatism; IRB = Institutional Review Board; ATP = adenosine triphosphate; PBS = phosphate

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buffered saline; BSA = bovine serum albumin; SDS = sodium dodecyl sulfate; EDTA = ethylenediaminetetraacetic acid; ANOVA = analysis of variance; SFM = serum-free medium; MET = mesenchymal-epithelial transition factor; 5,6-TAMRA = 5,6- carboxytetramethyl-rhodamine, succinimidyl ester; IP = immunoprecipitation; IB = immunoblotting

AT A GLANCE COMMENTARY

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Background

The molecular mechanisms underlying the pathogenesis of fibrosis are not well understood, and treatment is often toxic and inefficacious. Earlier studies have identified hepatocyte growth factor as an antifibrotic agent that protects various tissues including lung from injury and fibrosis.

Translational Significance

Recently, we identified a C-terminal fragment of hepatocyte growth factor receptor, M10, as a peptide with strong antifibrotic properties. This is the first article to describe antifibrotic effects of M10 in vitro and in vivo that can be translated into a safe and effective treatment of pulmonary fibrosis and other fibrosing diseases.

INTRODUCTION

Scleroderma (systemic sclerosis, SSc) is an autoimmune disease characterized by fibrotic changes of skin and internal organs. The molecular mechanisms underlying the pathogenesis of SSc are not well understood, and treatment is often toxic and inefficacious.[1,2] Earlier studies have identified hepatocyte growth factor (HGF) as an antifibrotic agent that protects against tissue fibrosis in several animal models.[3-5] We previously demonstrated that antifibrotic effects mediated by the HGF receptor, also known as cellular mesenchymal–epithelial transition factor (c-MET, MET), are impaired in lung fibroblasts isolated from a subset of scleroderma patients with severe interstitial lung disease (ILD).[6] We recently observed that lung fibroblasts from some SSc-ILD patients with poor pulmonary outcomes express the D1398G variant of the MET receptor and that a D1398G MET receptor mutant generated in vitro does not exert antifibrotic effects of MET in lung fibroblasts.[7]

MET is a transmembrane protein and a member of the receptor tyrosine kinase class IV, transducing signals from extracellular matrix into the cytoplasm. The fully processed MET protein is composed of a 50 kD α-chain and a 145 kD β-chain linked by a disulfide bridge. The α-chain and the N-terminal part of the β-chain form the extracellular domain. The remainder of the β-chain forms transmembrane and tyrosine kinase domains and a C-terminal tail.[8,9]

MET is a high affinity receptor for HGF, a polypeptide growth factor from the plasminogen family. The binding of HGF to MET induces kinase catalytic activity that triggers autophosphorylation at the kinase domain and initiates receptor activation. Phosphorylated kinase domain works as a docking site for downstream signaling molecule and activates several signaling cascades including Ras-Erk, PI3 kinase-Akt, or PLC gamma-PKC.[9] HGF/MET signaling plays significant roles in embryo development, tissue repair, cell proliferation, migration, differentiation, mitogenesis, morphology, and survival.[9,10] MET-mediated pleiotropic signaling depends in part on the ability of MET to form heterodimers with other cell surface receptors such as CD44, β4-integrin, Fas-receptor, and semaphorin-receptor.[11-15]

Increased expression of MET in scleroderma fibroblasts and other myofibroblasts has been well documented.[16,17] Foveau et al[18] demonstrated that MET, when overexpressed, could activate endogenous cysteine-dependent aspartate-directed proteases (caspases) after stress conditions in several cell lines; other research groups showed that the MET receptor mediates HGF-induced apoptosis in lung and liver myofibroblasts.[4,19]

Caspase-3 plays a key effector role in apoptosis by cleaving specific substrates important for downstream apoptosis signaling[20] and has some additional functions including B-cell regulation and T-cell differentiation.[21,22] An autoantibody against caspase-3 is generated in SSc, and this antibody has been correlated to the severity of SSc-ILD, vascular damage, and inflammation.[23] Activated caspase-3 recognizes aspartic acid–containing motifs within MET and cleaves those generating several stable fragments of the MET receptor that have been implicated in regulation of cell apoptosis and MET expression.[18,24,25] On cleavage by caspase-3, the intracellular cytoplasmic tail of MET generates a 10–amino acid peptide, TRPASFWETS, designated as "M10". The present study was undertaken to investigate the signaling pathways underlying antifibrotic effects of M10 in lung and skin fibroblasts.