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Research Paper

Targeting the PDGF-B/PDGFR-β Interface with Destruxin A5 to Selectively Block PDGF-BB/PDGFR-ββ Signaling and Attenuate Liver Fibrosis

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ABSTRACT

PDGF-BB/PDGFR-ββ signaling plays very crucial roles in the process of many diseases such as liver fibrosis. However, drug candidates with selective affinities for PDGF-B/PDGFR-β remain deficient. Here, we identified a natural cyclopeptide termed destruxin A5 that effectively inhibits PDGF-BB-induced PDGFR-β signaling. Interestingly and importantly, the inhibitory mechanism is distinct from the mechanism of tyrosine kinase inhibitors because destruxin A5 does not have the ability to bind to the ATP-binding pocket of PDGFR-β. Using Biacore T200 technology, thermal shift technology, microscale thermophoresis technology and computational analysis, we confirmed that destruxin A5 selectively targets the PDGF-B/PDGFR-β interaction interface to block this signaling. Additionally, the inhibitory effect of destruxin A5 on PDGF-BB/PDGFR-ββ signaling was verified using *in vitro*, *ex vivo* and *in vivo* models, in which the extent of liver fibrosis was effectively alleviated by destruxin A5. In summary, destruxin A5 may represent an efficacious and more selective inhibitor of PDGF-BB/PDGFR-ββ signaling.

1. Introduction

Platelet-derived growth factor receptor-beta (PDGFR-β) is a cell surface receptor tyrosine kinase (Arora and Scholar, 2005). The biological effect of PDGFR-β is initiated through binding to its ligands PDGF-B or PDGF-D (Andrae et al., 2008; Borkham-Kamphorst et al., 2015; Kondo et al., 2013). Accumulating documents have revealed that PDGF-B, as the crucial ligand of PDGFR-β, binds to the extracellular domain of PDGFR-β to promote its dimerization, which initiates PDGF-BB/PDGFR-ββ signaling (Shim et al., 2010). The structure of the PDGF-B/PDGFR-β complex revealed that PDGFR-β is bound by two PDGF-B promoters, and the interface of the PDGF-B/PDGFR-β complex is predominantly hydrophobic (Shim et al., 2010).

Excessively enhanced PDGF-BB/PDGFR-ββ signaling is an important feature of liver fibrosis (Kocabayoglu et al., 2015). The underlying cellular mechanisms of liver fibrosis principally involve the activation of hepatic stellate cells (HSCs) which are the principal fibrogenic cell type in the liver (Bonner, 2004). Following chronic liver injury, HSCs differentiate from quiescent cells into proliferative myofibroblasts, which are an activated type of HSCs (Bataller and Brenner, 2005). During this transition, activated HSCs up-regulate the expression of PDGFR-β, whereas the level of PDGFR-β is low in healthy livers (Bonner, 2004; Kocabayoglu et al., 2015). Additionally, the paracrine signaling molecules released by other resident liver cells can also enhance the expressions of PDGF-B and PDGFR-β in activated HSCs (Bonner, 2004). However, the level of PDGFR-α expression by HSCs remains unchanged during liver injury (Bonner, 2004).

It is well known that PDGF-BB/PDGFR-ββ signaling is the most potent mitogenic pathway in HSC activation and fibrogenesis (Bonner, 2004; Kocabayoglu et al., 2015). Patients with liver cirrhosis caused by chronic hepatitis C exhibit more extensive platelet areas in the liver compared with normal livers, and HSCs expressing PDGFR-β are frequently observed in the areas with extensive platelets (Kondo et al., 2013). The PDGF-BB/PDGFR-ββ axis represents an appealing target for the treatment of liver fibrosis (Kocabayoglu et al., 2015; Prosser et al., 2006; Yoshida et al., 2014); specifically, antagonism of this axis has the potential to serve as an anti-fibrotic strategy. Previous studies have revealed that some receptor tyrosine kinase inhibitors, such as imatinib, sorafenib and sunitinib, whose targets include PDGFR-β, inhibit HSC activation and mitigate fibrosis (Majumder et al., 2013; Westra et al., 2014). However, these drugs are nonselective PDGFR-β inhibitors that were originally used to treat cancers in the clinic (Ehnman and

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile duct ligation; EGFR, epidermal growth factor receptor; FBS, fetal bovine serum; FLT3, FMS-like tyrosine kinase 3; HBV, hepatitis B virus; HAS, human serum albumin; HSC, hepatic stellate cell; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PI, propidium iodide; RU, relative units; SPR, surface plasmon resonance; VEGFR, vascular endothelial growth factor receptor.

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Östman, 2014), and their use may lead to unwanted side effects in patients affected only by the PDGFR-β-related diseases. In order to make anti-fibrotic drugs selectively target the activated HSCs, Brennand et al. constructed a cyclic peptide analog of PDGF-BB$^{73-81}$ (^{77}IVRKK^{81}-C-^{73}RKIE^{78}) that can prevent PDGF-BB from binding to its receptor and inhibit PDGF-BB-induced DNA synthesis in human fibroblasts (Brennand et al., 1997), whereas there are no data to support its *in vivo* effects. Beljaars et al. constructed a macromolecule with affinity for PDGFR-β by modification of human serum albumin (HSA) with a cyclic octapeptide (called pPB) that recognizes the PDGFR-β (Beljaars et al., 2003). This macromolecule (pPB-HSA) is able to reduce PDGF-BB-induced fibroblast proliferation *in vitro* (Beljaars et al., 2003). However, pPB alone has no ability to compete with the cellular binding of PDGF-BB within 0–125 μM (Beljaars et al., 2003). Notably, pPB-HAS and bicyclic pPB fail to exert anti-fibrotic effects on mice administered with CCl_4 (Bansal et al., 2011, 2014). Therefore, pPB and BipPB modified proteins are predominantly used as targeting devices to selectively interact with cells, predominantly (myo) fibroblasts, that express the PDGF-β receptor (Bansal et al., 2011, 2014; Beljaars et al., 2003). Therefore, more efficacious and selective drug candidates targeting the PDGF-BB/PDGFR-ββ axis are still lacking. These considerations impelled us to explore an alternative therapeutic approach to more selectively block the PDGF-BB/PDGFR-ββ axis. After screening of multiple compounds, we found that destruxin A5, a natural cyclopeptide which has insecticidal and anti-inflammatory effects (Krasnoff et al., 1996; Zhang et al., 2013), represents a therapeutic option for liver fibrosis. Herein, we describe a highly efficacious inhibitory approach involving destruxin A5 to selectively block PDGF-BB/PDGFR-ββ signaling by targeting PDGFR-β to occupy the protein–protein binding interface between PDGF-B and PDGFR-β.

2. Materials and Methods

2.1. Mice and Reagents

Destruxin A5 was isolated and identified as reported by us previously (Zhang et al., 2013). Detailed information on mice and reagents is provided in the Supplemental Experimental Procedures.

2.2. Cell Culture and Cell Proliferation Assay

The immortalized human HSC line LX-2 and activated rat HSC line CFSC-8B were maintained in new plastic culture dishes in Dulbecco's modified Eagle's medium supplemented with 100 μg/ml of streptomycin, 100 U/ml of penicillin and 10% fetal bovine serum (FBS) under a humidified 5% (v/v) CO_2 atmosphere at 37 °C. Cell proliferation was determined by MTT assay, as we have previously reported (Wang et al., 2014).

2.3. Surface Plasmon Resonance (SPR)

We performed SPR assays using the Biacore T200. Detailed information is provided in the Supplemental Experimental Procedures.

2.4. HSC Migration Assay

The migratory capacities of the cells were investigated as described previously (Liu et al., 2011). Detailed information is provided in the Supplemental Experimental Procedures.

2.5. HSC Wound Healing Assay

For the determination of cell migration during wound healing, a wound healing assay was performed as described previously (Rodriguez et al., 2005). Detailed information is provided in the Supplemental Experimental Procedures.

2.6. Western Blot

Proteins were extracted in lysis buffer. The proteins were then separated by SDS-PAGE (10%) and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were probed with antibodies overnight at 4 °C, and then incubated with a HRP-coupled secondary antibody. Detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK).

2.7. Cell Cycle Assay

This work was performed as we previously reported (Wang et al., 2015b). Detailed information is provided in the Supplemental Experimental Procedures.

2.8. Cell Apoptosis Assay

HSCs were seeded in 6-well plates in Dulbecco's modified Eagle's medium and treated with or without destruxin A5 for 24 h. Cell apoptosis was determined by flow cytometry after addition of FITC-conjugated annexin V and PI assay. Samples were analyzed by flow cytrometry on a FACScan (Becton Dickinson). Data were analyzed with CELLQuest software (BD Biosciences).

2.9. Immunohistochemistry

Paraffin-embedded liver sections were heat-fixed, blocked with 3% H_2O_2, and incubated with specific antibodies (1:100 diluted) overnight at 4–8 °C. Detection was done using Real Envision Detection kit from GeneTech Company (Shanghai, China) according to the manufacturer's instructions.

2.10. Real-time Quantitative PCR

This work was performed as we previously reported (Wang et al., 2015a). Detailed information is provided in the Supplemental Experimental Procedures.

2.11. Cultivation and Metabolites Isolation

Detailed information is provided in the Supplemental Experimental Procedures.

2.12. Models of Murine Liver Fibrosis

Liver fibrosis was induced by ligation of the common bile duct (BDL) (Liu et al., 2011). Mice (8–10 weeks, n = 8 in each group) were anesthetized. Following midline laparotomy, the common bile duct was double ligated and transected between the ligatures. The sham group was subjected to a similar operation without BDL. After BDL for 6 d, destruxin A5 was administered at 5–10 mg/kg/d for another 8 d. Mice that underwent BDL for 6 d and 14 d served as controls for the destruxin A5 treatment.

2.13. Statistical Analysis

All results shown represent means ± SEM. From triplicate experiments performed in a parallel manner. Data were statistically evaluated by one-way ANOVA followed by Dunnett's test between control group and multiple dose groups. The level of significance was set at a *P* value of 0.05.