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Induction of interleukin-8 secretion and activation of ERK1/2, p38 MAPK signaling pathways by thrombin in dermal fibroblasts

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Abstract

It was reported that thrombin could induce IL-8 secretion from human dermal fibroblasts (HDFs) through activation of proteinase activated receptor (PAR)-1. However, little is known of intracellular signaling pathways involved in the event. In the present study, expression of PARs in primarily cultured HDFs was determined by flow cytometry analysis and reverse transcription polymerase chain reaction (RT-PCR), levels of IL-8 were determined by using ELISA and signaling pathways were examined by using Western blot. It was found that HDFs express PAR-1 and PAR-3, and thrombin induces approximately 7.4-fold increase in IL-8 secretion from HDFs. Hirudin and a PAR-1 blocking antibody completely abolish the action of thrombin. It was also found that PD98059, a mitogen-activated protein kinase (MAPK) pathway inhibitor and U0126, an inhibitor of extracellular signal-regulated kinase (ERK) blocks thrombin-induced phosphorylation of ERK1/2 and IL-8 secretion, indicating the involvement of MAPK/ERK signaling pathway in thrombin-induced IL-8 secretion. p38 MAPK pathway appears also being involved as SB203580, a selective inhibitor of p38 MAPK inhibit phosphorylation of p38 MAPK and thrombin-induced IL-8 secretion. Furthermore, Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathway, but not phosphotidylinositol 3-kinase (PI3K)/Akt signaling pathway may also be activated by thrombin. In conclusion, thrombin potently induce IL-8 release via PAR-1 from HDFs. Thrombin elicited IL-8 release is predominantly conducted through MAPK/ERK and p38 MAPK signaling pathways. Discovery of the signaling pathways of thrombin in HDFs may help to understand the role of thrombin in inflammation and tissue remodeling.

Keywords: Thrombin; PAR-1; IL-8; Human dermal fibroblast; Signal transduction

1. Introduction

IL-8 is an inflammatory α-chemokine, which affects the function and recruitment of various inflammatory cells and fibroblasts (Moyer, Saggers, Allison, Mackay, & Ehrlich, 2002). The enhanced expression of IL-8 has been found in a number of skin diseases including systemic sclerosis (Fukasawa et al., 2003), rheumatoid arthritis (Georganas et al., 2000) and hypoxia (Galindo et al., 2001), indicating this pro-inflammatory cytokine may play a role in skin fibrosis.

Abbreviations: PAR, protease-activated receptors; HDF, human dermal fibroblast; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JAK, Janus kinase; STAT, signal transducer and activators of transcription; PI3K, phosphotidylinositol 3-kinase; IL, interleukin; FBS, fetal bovine serum; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair; TGF-β, transforming growth factorβ; ELISA, enzyme-linked immunosorbent assay

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In recent years, thrombin has been discovered to play an important role in inflammatory and tissue repair processes. It influences vascular and blood cells including endothelial cells (Chi et al., 2001), fibroblasts (Chambers, Leoni, Blanc-Brude, Wembridge, & Laurent, 2000), vascular smooth muscle cells (Weiss & Nuccitelli, 1992), T lymphocytes (Mari et al., 1996), eosinophils (Bolton, McNulty, Thomas, Hewitt, & Wardlaw, 2003) and monocytes (Colognato et al., 2003), and exerts a pro-inflammatory effect on these cells by stimulating pro-inflammatory cytokine release such as IL-1β release from monocytes (Naldini, Pucci, Carney, Fanetti, & Carraro, 2002), IL-8 release from fibroblasts (Bachili et al., 2003) and IL-6 release from fibroblasts and vascular smooth muscle cells (Hayakawa et al., 2005).

Since the last decade, PARs have been identified as receptors for serine proteinases. Among them, PAR-1 is a receptor of thrombin and trypsin (Vu, Hung, Wheaton, & Coughlin, 1991), PAR-2 is a receptor of trypsin and mast cell tryptase (He et al., 2004; Molino et al., 1997) and PAR-3 (Schmidt et al., 1998) and PAR-4 (Xu et al., 1998) are receptors of thrombin. Proteinase activates PAR via cleavage of its extracellular N-terminal domain, which then enables the new N terminus (now referred to as a tethered ligand) to interact distally within the same molecule to activate G protein-coupled signal transduction pathways (Brass & Molino, 1997). These receptors can also be activated without proteolytic cleavage using five to six residue peptides corresponding to the new N termini of the cleaved receptors (Vu et al., 1991).

Recent studies have suggested a role for PAR-1 in a variety of biological events including coagulation (Lidington, Haskard, & Mason, 2000), inflammation (Reiter et al., 2003), chemotaxis (Naldini, Sower, Bocci, Meyers, & Carney, 1998), mitogenesis (Tran & Stewart, 2003), apoptosis (Chalmers, Balmanno, Hadfield, Ley, & Cook, 2003), and angiogenesis (Yin et al., 2003). Fibroblasts are the most abundant cells in connective tissue, and damage to blood vessels places coagulation factors in contact with these and other types of cells. PAR-1 has been reported being expressed in fibroblasts from different origins, including lung fibroblast (Bachili et al., 2003), dental pulp fibroblast (Gruber et al., 2004), and human gingival fibroblast (Hou et al., 1998; Tanaka et al., 2003). PAR-1 was also expressed in serum-starved HDFs and through which thrombin-induced secretion of IL-8 (Bachili et al., 2003). However, little is known of the signaling mechanisms of thrombin induced IL-8 secretion from HDFs.

It was reported that PARs signaled via MAPK, a family of serine/threonine kinases that link receptor activation in the cell membrane with gene expression in the nucleus (Coughlin, 2000). Dental pulp fibroblast proliferation in response to thrombin involved MAPK signaling (Gruber et al., 2004), and dermal fibroblast cells releasing prolidase activity appeared to be associated with phosphorylation of ERK1/2 (Surazynski, Sienkiewicz, Wolczynski, & Palka, 2005). Some reports suggested that Gi proteins activated MAPKs through their $G_{\beta\gamma}$ subunits, an effect that was mediated via PI3K (Daub, Wallasch, Lankenau, Herrlich, & Ullrich, 1997; Kranenburg, Verlaan, Hordijk, & Moolenaar, 1997). In lung fibroblast, thrombin up-regulated expression of IL-8 via activation of PAR-1 and protein kinase C-γ, and the signal transduction was carried out via non-receptor tyrosine protein kinase (Ludwicka-Bradley et al., 2000). However, intracellular signaling pathways, which mediated thrombin-induced increase of expression of IL-8 in HDFs remain largely unknown. The aim of the present study is to elucidate the potential signal transduction pathways involved in thrombin-induced secretion of IL-8 in HDFs. To achieve this goal, the MAPK/ERK pathway inhibitor, p38 MAPK pathway inhibitor, the PI3K pathway inhibitor and the JAK/STAT pathway inhibitor were used to block the signal transduction mechanisms in HDFs upon thrombin stimulation.

2. Materials and methods

2.1. Reagents

Cell culture medium RPMI 1640, fetal bovine serum (FBS) were obtained from Gibco (Gibco BRL/Life-technologies, Rockville, MD, USA). 2-(2-diamino)-3 methoxyphenyl-4H-1-benzopyran-4-one (PD98059), 1,4-diamino-2,3-dicyano-1,4-*bis*(2-aminophynyltio) butadiene (U0126), 1,4-diamino-2,3-dicyano-1,4-*bis* (methylthio) butadiene (U0124), 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), tyrphostin (AG490), phospho-p44/42 MAPK antibody, unphospho-p44/42 MAPK antibody, phospho-p38 MAPK antibody, unphospho-p38 MAPK antibody, phospho-Akt antibody, unphospho-Akt antibody, phospho-STAT3 antibody and unphospho-STAT3 antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). Human thrombin (catalog number T6884), 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580), dimethyl sulphoxide (DMSO), biotin conjugated goat anti-rabbit immunoglobulins, biotin conjugated goat anti-mouse immunoglobulins, extr-Avidin-peroxidase, anti-β-actin and anti-TGFβ-1 monoclonal antibodies and antibiotics (a mixture of penicillin and streptomycin) were purchased from