New insights into the functions of intersectin-1s

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Intersectin-1s (ITSN) is a ubiquitously expressed multifunctional protein known as a scaffold and regulator of the general endocytic machinery as well as a critical integrator of cellular signaling pathways. We showed recently that ITSN deficiency triggers a transforming growth factor β (TGFβ)/Alk5 signaling switch, from the canonical Smad 2/3 to the Erk1/2 MAPK pathway; moreover, endocytic impairment induced by ITSN deficiency enhances Alk5 ubiquitination and degradation and elicits TGFβ-paracrine effects mediated by circulating microparticles, leading to endothelial cell survival and increased proliferation. The studies expand our understanding of how ITSN facilitates cross-regulation of signaling pathways and provide insights into the involvement of ITSN deficiency in human disease.

ITSN-1s and TGFβ-mediated Erk1/2 MAPK signaling
TGFβ is a ubiquitous and multifunctional cytokine that signals through heteromeric complex formation between 2 transforming growth factor β receptor I (TGFβRI) and 2 TGFβRII molecules; both receptors possess dual specificity as serine/threonine and tyrosine kinases.1 The heteromeric complex between Alk5, a broadly expressed TGFβ-RI, and the TGFβRII transduces the signal in multiple cell types and context-dependent manner from all 3 TGFβ Isoforms.2 It can either activate the Smad 2 and Smad 3 proteins via the canonical TGFβ signaling pathway or it can activate Smad-independent pathways.2 TGFβ has been shown to induce Erk1/2 MAPK signaling in endothelial and epithelial cells, fibroblasts, breast and colorectal cancer to promote disassembly of adherens junctions, cell migration and proliferation.3,4 Studies to understand the molecular mechanisms underlying TGFβ-dependent Erk1/2 MAPK activation indicated that plasma membrane-bound activated Alk5/TGFβRII heteromer recruits and phosphorylates the adaptor protein ShcA followed by the ShcA/Grb2/mSos complex assembly.5 Localization of mSos/Grb2 at the plasma membrane level is the primary mechanism for Ras activation.6 The guanine nucleotide exchange factor, mSos, activates Ras on the plasma membrane and triggers the c-Raf/MEK/Erk1/2 MAPK signaling cascade.5 Studies demonstrated that the general endocytic protein ITSN associates, with mSos in a protein complex that excludes Grb2.7 Thus, ITSN deficiency increases mSos availability for Grb2 interaction and leads to preferential formation of Alk5/mSos/Grb2 complex and activation of Erk1/2 MAPK signaling.4 Ras/Erk1/2 MAPK activation results in ineffective assembly of Alk5/Smad2/SARA (Smad Anchor for Receptor Activation) complex and subsequent alteration of the Smads2/3 - Erk1/2 signaling balance.4 TGFβ/Alk5-dependent Ras activation is an unresolved issue of huge interest due to its established role in TGFβ-induced epithelial-mesenchymal transformation8 and in providing a growth advantage.9 Our study identifies ITSN deficiency as a patho-physiological context that favors the formation of Alk5/mSos/Grb2 complex and TGFβ-dependent Ras/Erk1/2 MAPK signaling. ITSN deficiency can be encountered in a wide range of inflammatory diseases associated with increased levels granzyme B, since ITSN is a recently identified substrate of the cytotoxic protease.10,11 Altogether, the findings indicate that ITSN is a key plasma membrane scaffold able to

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modulate the localization and activity of TGFβ receptors family and to participate in the spatio-temporal regulation of TGFβ signaling necessary for endothelial cell and lung tissue homeostasis.

ITSN-1s and endocytic regulation of TGFβ/Alk5 signaling

In normal endothelial cells, Alk5 is internalized via clathrin-coated vesicles, leading to TGFβ-induced Smad2/3 activation, transcriptional responses and recycling to the plasma membrane and caveolae, which direct Alk5 to the ubiquitin proteasome and turn off TGFβ signaling.\(^1,2\) ITSN is a scaffold and regulator of the general endocytic machinery; through its strategic location at the neck region of caveolae and ability to bind simultaneously more dynamin-2 molecules, ITSN recruits dynamin-2 and regulates its ability to bind simultaneously and to bind to other endocytic sites, leading to TGFβ signaling.

Microparticles and TGFβ paracrine signaling

ITSN deficiency in a cultured cell system induces apoptotic cell death in a process that involves downregulation of Erk1/2 survival signaling, while in vivo, it causes endothelial cells phenotypic changes toward apoptosis-resistance and hyper-proliferation.\(^1,2\) Increased ITSN's versatility to organize plasma membrane–associated signaling complexes for controlling and integrating membrane trafficking and signaling events. Moreover, our study demonstrates the existence of additional in vivo mechanisms needed to compensate for enhanced Alk5 degradation when endocytosis via vesicular carriers is impaired and to explain the changes of endothelial cell phenotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Mu Y, Gudey SK, Landstrom M. Non-smad signaling pathways. Cell Tissue Res 2012; 347:11-20; PMID:22781809; http://dx.doi.org/10.1007/s00441-011-1201-y

2. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003; 425:577-84; PMID:14535477; http://dx.doi.org/10.1038/nature02006

3. Papagorgis P, Stylianopoulos T. Role of TGFbeta in regulation of the tumor microenvironment and drug delivery. Int J Oncol 2015; 46:933-43; PMID:25753464

4. Bardta C, Predescu DN, Sha F, Patel M, Balaji G, Predescu SA. Endocytic deficiency induced by intersectin-1s knockdown alters the Smad2/3-Erk1/2 signaling balance downstream of Alk5. J Cell Sci 2015; 128:1528-41; In press ; PMID:25720380

5. Lee MK, Pardoux C, Hall MC, Lee PS, Warburton D, Qing J, Smith SM, Derynck R. TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. EMBO J 2007; 26:3957-67; PMID:17673906; http://dx.doi.org/10.1038/sj.emboj.7601818

6. Aronheim A, Engelberg D, Li N, al-Alawi N, Schlesinger J, Karin M. Membrane targeting of the nucleotide exchange factor Sos is sufficient for activating the Ras signaling pathway. Cell 1994; 78:949-61; PMID:7923364; http://dx.doi.org/10.1016/0092-8674(94)90271-2

7. Tong X, Hussain NK, de Heuvel E, Kurakin A, Abijoude E, Quinn CC, Othon MF, Marais R, Baranes D, Kay BK, et al. The endocytic protein intersectin is a major binding partner for the Ras exchange factor sos1 in rat brain. EMBO J 2000; 19:1263-71; PMID:10710926; http://dx.doi.org/10.1093/emboj/19.6.1263

8. Davies M, Robinson M, Smith E, Huntley S, Prime S, Paterson I. Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-beta1 involves MAPK
Smad and AP-1 signalling pathways. J Cell Biochem 2005; 95:918-31; PMID:15861394; http://dx.doi.org/10.1002/jcb.20458
9. Suzuki K, Wilkes MC, Garamszegi N, Edens M, Leof EB. Transforming growth factor beta signaling via Ras in mesenchymal cells requires p21-activated kinase 2 for extracellular signal-regulated kinase-dependent transcriptional responses. Cancer Res 2007; 67:3673-82; PMID:17440079; http://dx.doi.org/10.1158/0008-5472.CAN-06-3211
10. Patel M, Predescu D, Tandon R, Bardita C, Pogoriler J, Bhorade S, Wang M, Comhair S, Hemnes AR, Chen J, et al. A novel p38 mitogen-activated protein kinase/Elk-1 transcription factor-dependent molecular mechanism underlying abnormal endothelial cell proliferation in plexogenic pulmonary arterial hypertension. J Biol Chem 2013; 288:25701-16; PMID:23893408; http://dx.doi.org/10.1074/jbc.M113.502674
11. Loob CR, Harris JL, Czak C3. Granzyme B proteolysis receptors important to proliferation and survival, tipping the balance toward apoptosis. J Biol Chem 2006; 281:28326-35; PMID:16798735; http://dx.doi.org/10.1074/jbc.M604544200
12. DiGuglielmo GM, Le Roy C, Goodfellow AF, Wrana JL. Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. Nat Cell Biol 2003; 5:410-21; PMID:12717440; http://dx.doi.org/10.1038/ncl975
13. Predescu SA, Predescu DN, Timblin BK, Stan RV, Malik AB. Intersectin regulates fusion and internalization of caveosomes in endothelial cells. Mol Biol Cell 2003; 14:4997-5010; PMID:12960435; http://dx.doi.org/10.1091/mbc.E03-01-0041
14. Predescu DN, Neamu R, Bardita C, Wang M, Predescu SA. Impaired caveolae function and upregulation of alternative endocytic pathways induced by experimental modulation of intersectin-1s expression in mouse lung endothelium. Biochem Res Int 2012; 2012:627205; PMID:225060115
15. Chen CL, Hou WH, Liu IH, Hsiao G, Huang SS, Huang JS. Inhibitors of clathrin-dependent endocytosis enhance TGFbeta signaling and responses. J Cell Sci 2009; 122:1863-71; PMID:19461075; http://dx.doi.org/10.1242/jcs.038729
16. Lu Z, Murray JT, Luo W, Li H, Wu X, Xu H, Backer JM, Chen YG. Transforming growth factor beta activates Smad2 in the absence of receptor endocytosis. J Biol Chem 2002; 277:29363-8; PMID:12034739; http://dx.doi.org/10.1074/jbc.M203495200
17. Predescu SA, Predescu DN, Knezevic I, Klein IK, Malik AB. Intersectin-1s regulates the mitochondrial apoptotic pathway in endothelial cells. J Biol Chem 2007; 282:17166-78; PMID:17405881; http://dx.doi.org/10.1074/jbc.M60896200
18. Bardita C, Predescu D, Justice MJ, Petrache I, Predescu S. In vivo knockdown of intersectin-1s alters endothelial cell phenotype and causes microvascular remodeling in the mouse lungs. Apoptosis 2013; 18:57-76; PMID:23054079; http://dx.doi.org/10.1007/s10495-012-0762-x