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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.

Keywords: circulating microparticles, endocytic deficiency, intersectin-1s, TGFβ, TGFβRI

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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 transformation[8] 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

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.[12] 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 GTPase and oligomerization activity at the neck region of caveolae, mediating the membrane fission process and caveolae internalization.[13] Thus, deficiency of ITSN interferes with dynamin-2 recruitment at the endocytic site, resulting in impaired clathrin-coated vesicles and caveolae endocytosis.[13,14] To compensate for deficient internalization of the endocytic vesicles, endothelial cells up regulate alternative transport pathways and their carriers (i.e., tubulo-vesicular structures, membranous rings).[4,14] These structures are caveolin-1 positive and are involved in TGFβ receptors internalization.[14] Internalization of Alk5 via the caveolin-1 positive tubulo-vesicular structures results in enhanced ubiquitination and degradation of Alk5, consistent with turning off TGFβ Signaling.[4] However, endothelial cells deficient of ITSN show Alk5-dependent Erk1/2 activation, consistent with the idea that TGFβ/Alk5 signaling can take place on the plasma membrane.[4] Previous studies aimed to define the subcellular site of TGFβ-induced signaling using a series of inhibitors of clathrin-mediated endocytosis have determined that all inhibitors used enhanced TGFβ signaling and responses and that receptor endocytosis is dispensable for TGFβ-mediated activation of Smad2.[15,16] Thus, our report that in ITSN-deficient endothelial cells the TGFβ receptor endocytosis via clathrin-coated vesicles is not necessary for TGFβ-dependent Erk1/2 MAPK activation, further confirms not only the cell type- and context-dependent endocytic regulation of TGFβ signaling but also 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.

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 hyperproliferation.[17,18] Given the growing attention to cell-to-cell communication via microparticles shed by apoptotic or activated cells, we hypothesized that endothelial cell survival and proliferation of mouse lung endothelial cells may involve the interactions with microparticles and their cargo. Favoring candidates of the microparticles cargo to "substitute" ITSN's absence and activate Erk1/2 signaling were the growth factors and their receptors. While the microparticles isolated from the ITSN-deficient mice were immunoreactive to several growth factors receptors, only Alk5 was significantly increased compared to microparticles isolated from wild-type mice. Interestingly, most Alk5 clusters present on the microparticles were accompanied by the TGFβRII, most likely, the assembled heteromeric complexes.[4] While both receptors possess dual specificity as serine/threonine and tyrosine kinases, TGFβRII is the "activator" and TGFβRI is the "signal propagating" component.[1] Microparticles were able to interact and transfer the Alk5/TGFβRII complexes to dysfunctional endothelial cells, but no control cells, via a mechanism dependent on the presence of phosphatidyl serine at the surface of the microparticles.[4] Once the Alk5/TGFβRII complex is transferred to dysfunctional endothelial cells, the plasma membrane-bound activated heteromer is in close proximity of its cytosolic substrates and triggers the Ras/Erk1/2 signaling cascade, leading to endothelial cell survival and increased proliferation. The observation strongly suggests a relevance of this interaction for pathological conditions and that the microparticles affect the phenotype and biology of target cells. It appears that TGFβ paracrine effects via microparticles, by promoting proliferation and apoptosis-resistance is emerging as an important mechanism in recovery and tissue repair of injured lung tissue. In this context, development of strategies to engineer microparticles' cargo (i.e., anti-apoptotic and pro-angiogenic factors able to promote neo-vascularization and tissue repair) may lead to new powerful therapeutics in regenerative medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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