DVL as a scaffold protein capturing classical GPCRs

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The classical G-protein-coupled receptors (GPCRs) are characterized by their ability to interact with heterotrimeric G proteins upon activation and by structural features such as seven transmembrane spanning domains. Frizzleds (Fzs) are comparable seven transmembrane receptors (7 TMRs) that are activated via seven transmembrane spanning domains. Frizzleds (Fzs) are association leading to internalization and desensitization. These upon activation by agonists, with subsequent receptor-

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The hallmark feature of classical G-protein-coupled receptors (GPCRs) is their ability to interact with heterotrimeric G proteins upon activation by agonists, with subsequent receptor-β-arrestin association leading to internalization and desensitization. These properties are present in addition to structural features such as seven transmembrane spanning domains. Frizzleds (Fzs) are comparable seven transmembrane receptors that are activated via Wnts and play a critical role in embryogenesis, tissue hemostasis and oncogenicity.1,2 It remains controversial, however, whether they may be considered GPCRs, and whether the coupling of Fzs to G-proteins for downstream signaling is obligatory. Hence, the ten members of Fzs constitute a distinct atypical family of seven-transmembrane receptors. Canonical Wnt/β-catenin signaling leads to the core process of β-catenin stabilization and, ultimately, to the translocation of β-catenin to the nucleus where it acts as a co-transcription factor and induces Wnt target gene transcription. We have documented that activation by proteinase-activated receptor1 (PAR1), a classical 7TMR, recruits dishevelled (DVL), an upstream Wnt signaling protein, to mediate β-catenin stabilization. DVL is selectively bound to activated Gα13 subunit, coupled to PAR, following activation. Formation of the PAR1-induced DVL-Gα13 axis is carried out independently of Wnt, Fz and the co-receptor LRPS/6 (low density lipoprotein—related protein 5/6) since neither siRNA-LRPS/6 co-receptors nor the presence of SFRPs; secreted Fz receptor proteins (Wnt antagonists) affect PAR1-induced β-catenin stabilization. Similarly, PAR1 induced placenta cytотrophoblast physiological invasion process was not affected by inhibiting Wnt, but was abrogated by siRNA-DVL. We propose that DVL serves as a central mediator protein that links classical GPCRs to β-catenin stabilization in both pathological (tumor) and physiological (placenta) invasion processes.

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Classical 7TM GPCRs and β-catenin Stabilization

Many of the classical GPCRs shown previously to lead to β-catenin stabilization act through traditional signaling
pathways triggered by 7TM receptors, such as the activation of protein kinase A induced by PGE2 (prostaglandin) binding to EP2 receptor. Another example is the lysophosphatidic acid (LPA) and its receptors, LPA1, LPA2 or LPA3, that act by a mechanism downstream of PKC, which eventually leads to the inhibition of glycogen synthase kinase 3β (GSK3β) (via serine phosphorylation for its inactivation). Further examples include α-adrenergic and endothelin-1 receptors in cardiomyocytes that, once activated, recruit Akt, subsequently leading to the inactivation of GSK3β. This group also includes the EP2 (mentioned already above) and EP4 prostanoid receptors. The involvement of the serine/threonine kinase Akt [protein kinase B (PKB)] in GPCR signaling is well established. In fact, Akt is a major effector of the PI3K pathway, which is activated by a wide spectrum of polypeptide growth factors. Interestingly, it has been shown that Wnt-activated DVL also increases the activity of Akt which, in the presence of DVL, instigates the disruption of the axin-GSK3β complex and the inactivation of GSK3β. Inactivated GSK3β is no longer capable of tag-phosphorylating β-catenin for degradation via the E3 ligase of the ubiquitin system.

Figure 1. DVL is a mediator of classical GPCRs for β-catenin stabilization. Wnt-induced β-catenin stabilization is mediated via frizzled receptors that are considered as atypical GPCRs. Classical GPCRs such as parathyroid hormone receptor (PTH1R) and proteinase-activated—receptor1 (PAR1) lead to β-catenin increased levels by recruiting DVL. Thus, DVL serves as a junction mediator routing the classical GPCR for β-catenin stabilization.

Figure 2. PAR1 induced β-catenin stabilization in tumor- and physiological placenta- invasion processes. PAR1 induces β-catenin stabilization in either pathological (tumor) or physiological (placenta invasion) processes. Both PAR1 induced pathways are independent of Wnt signaling.
DVL is emerging as a junction protein that is also capable of binding β-arrestins (e.g., β-arrestin1 and 2) for signaling. β-arrestins have long been recognized for their role in the internalization and desensitization of traditional GPCRs. Recently, β-arrestins have been identified as essential components of Wnt/β-catenin signaling capable of binding to DVL at the N-terminal PDZ site. This binding domain comprises the casein kinase 1 (CK1δ/ε) phosphorylation area. It is suggested that binding of β-arrestin to activated DVL directs efficient association with axin, consequently leading to DVL-linked disruption of the β-catenin “degradation complex” (e.g., axin-APC-GSK3β) and hence, to β-catenin accumulation.9,21 We have also demonstrated that β-arrestin-2 specifically binds DVL following PAR1 activation, and that activation of PAR1 initiates a chain of events that ultimately lead to β-catenin stabilization and trophoblast invasion independent of Wnt signaling. These functions of PAR1-induced placenta-EVT are abrogated in the presence of siRNA-DVL. We therefore regard DVL as a central upstream junction protein that connects PAR1 to trophoblast invasion and β-catenin stabilization (unpublished observations; Grisaru-Granovsky S and Turm H). It is proposed that while both pathways (Wnt and PAR1) act to induce β-catenin stabilization, they co-exist in a mutually exclusive manner and induce invasion in the appropriate context.

Essential Role for Gα13 and PAR1 in β-catenin Stabilization

G-proteins comprise a signal transducing system that is regulated through discrete transmembrane receptors upstream and impinges on downstream effectors.28 At present, Gα subunits are classified into four distinct sub-families.29 Gα12 and its sister protein Gα13 are the most recent identified sub class, sharing 66% amino acid homology. The transformation capabilities of the family members have been demonstrated via focus-forming activity in soft agar and oncogenic properties in 3T3 NIH cells.30,31 In fact, Gα13 was termed gpp oncogene since it was shown to induce neoplastic transformation of fibroblasts, as well as to stimulate mitogenic pathways in different cell types.32-35 It has been recently demonstrated that neoplastic transformation by the gpp oncogene involves activation of signal transducer and activator of transcription 3 (STAT3) via PDGFR.36 A third member of the family, the Cta- Concertina (cta) gene product of Drosophila, shares close homology with Gα12 and Gα13 (53–55%) and it’s absence results in the disruption of the ventral furrow during early Drosophila development.37 It appears that while Gα12 and Gα13 are part of the same family, they act in a different manner. While knock-out (KO) of the Gα13 gene causes lethality in mouse embryos at mid-gestation, in contrast, Gα12+/- mice appear viable and grow normally.38 An elegant study by the group of Coughlin SR39 showed that transgenic expression of Gα13 into the endothelium using a specific endothelial Tie promoter, rescued embryonic lethality associated with Gα13 or PAR1 gene deletion. This suggests that loss of Gα13 signaling in endothelial cells may account for the embryonic phenotype associated with PAR1 deficiency. It should be noted, however, that the rescue affected only the endothelial phenotype of Gα13-deficient mice, but did not rescue Gα13+/- mice in general, indicating that Gα13 function in cell types other than endothelial cells is important for embryonic development. In addition, unpublished observations from the group of Coughlin indicated that Gα13+/- embryos carrying the endothelial transgene are similar in phenotype to that resulting from a Wnt1-Cre-mediated deletion of Gα13. These findings indicate that Gα13 may play an as-yet undetermined role in developmental processes. Our studies on PAR1-activated Gα13 and the recruitment of DVL may be expanded in the future to

β-catenin Stabilization in a Physiological Invasion Process: The Placenta Trophoblasts

Placenta development is a tightly orchestrated process whereby anchoring to the uterus decidua is carried out by invasion of cells termed extravillous trophoblasts (EVT). These cells are a subpopulation of placenta cytotrophoblasts and are capable of forming anchoring villi that invade and reach the uterine wall, allowing direct contact with the maternal blood. The molecular machinery that governs trophoblast anchorage to maternal tissues remains largely unknown. It is however, well recognized that the molecular basis of trophoblast invasion shares many features with the process of tumor cell invasion.22,23 We have previously demonstrated that PAR1 is overexpressed in a spatiotemporally restricted manner during early gestation of placenta cytotrophoblasts and is shut off immediately thereafter when the need to invade is over.24,25 Therefore, in contrast to the continuous overexpression of PAR1 seen in malignant epithelia, PAR1 is strictly controlled in the physiological invasion process of placenta cytotrophoblasts. We have shown that activation of PAR1 markedly induces EVT invasion as well as β-catenin stabilization and its nuclear localization, demonstrated in an EVT-explant system.26 In parallel, studies by Sonderegger et al. have demonstrated that trophoblast invasion is regulated via Wnt3A, inducing the canonical Wnt signaling pathway.27 This was manifested via the application of Wnt3A to either a trophoblastic cell line SGHPL-5 or primary extravillous trophoblasts leading to increased phosphorylation of AKT and downstream GSK3β inactivation. Moreover, luciferase activity of a canonical Wnt/TCF reporter, as well as cell migration are also elicited. Our preliminary evaluations in placenta EVT explants have indicated that PAR1 mediated functions (e.g., β-catenin stabilization and trophoblast villi invasion) are effectively inhibited in the presence of siRNA-DVL. While Wnt3A-induced β-catenin stabilization and EVT invasion are attenuated after LRP5/6 silencing (siRNA-LRP5/6), PAR1-induced β-catenin stabilization and invasion are not affected. Hence, we propose that the activation of PAR1 initiates a chain of events that ultimately lead to β-catenin stabilization and trophoblast invasion, independent of Wnt signaling. These functions of PAR1-induced placenta-EVT are abrogated in the presence of siRNA-DVL. We therefore regard DVL as a central upstream junction protein that connects PAR1 to trophoblast invasion and β-catenin stabilization (unpublished observations; Grisaru-Granovsky S and Turm H). It is proposed that while both pathways (Wnt and PAR1) act to induce β-catenin stabilization, they co-exist in a mutually exclusive manner and induce invasion in the appropriate context.
demonstrate a fundamental role for the G\(_{\alpha13}\)-DVL axis in developmental processes. In this respect, it has recently been shown that activated G\(_{\alpha13}\) uniquely binds and activates integrin \(\beta_1\) to mediate “outside-in” signaling. This endows G\(_{\alpha13}\) with a unique property in mediating cell-extracellular matrix interaction during adhesion. It has also been shown that activation of Wnt signaling during embryonic development is important, demonstrating that DVL associates with the actin fibers and focal adhesion plaques in mesenchymal cells, thus impinging on the rearrangement of the cytoskeleton and cell adhesion during embryonic mouse kidney development. We suggest that, in addition to playing a role in tumor biology, PAR also has a function in development. The fact that the PAR gene sequence is highly conserved during evolution, sharing 55–60% homology (NCBI taxonomy blast) with both zebra fish and/or Xenopus, may indicate the important putative role of the gene in development. It seems that while both G\(_{\alpha12}\) and G\(_{\alpha13}\) play a role in tumor biology, a selective part is assigned to the activated G\(_{\alpha13}\)-DVL axis in \(\beta\)-catenin stabilization in the context of development.

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