Wnt signalling: What The X@# is WTX?

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The EMBO Journal (2011) 30, 1415–1417. doi:10.1038/emboj.2011.101

In this issue of *The EMBO Journal*, Tanneberger *et al* demonstrate that Amer1/WTX, a tumour suppressor protein previously known for promoting β -catenin degradation and thus antagonizing Wnt signalling, is also required for LRP6 receptor phosphorylation and therefore activation of Wnt signalling. Amer1/WTX is recruited by PtdIns(4,5)P₂ to the plasma membrane, and in turn recruits the Axin–GSK3 complex and CK1 γ to phosphorylate LRP6. While dichotomic players like Amer1/WTX are common in Wnt signalling, these new findings add more intrigues to this enigmatic protein and pathway.

Wnt/ β -catenin signalling controls embryogenesis and homeostasis, and has a central role in many diseases including cancer and osteoporosis (MacDonald *et al*, 2009). In the absence of an extracellular Wnt ligand, cytoplasmic β -catenin is phosphorylated and targeted for degradation via a complex assembled by the scaffolding protein Axin, the tumour suppressor APC (*adenomatous polyposis coli*), and kinases GSK3 and CK1 α . Binding of a Wnt ligand to a frizzled (Fz) serpentine receptor and its coreceptor, low density lipoprotein receptor-related protein 6 (LRP6, or its relative LRP5), inhibits β -catenin phosphorylation and degradation, leading to increased β -catenin and its association with LEF/TCF DNA-binding factors for transcription of Wnt target genes (MacDonald *et al*, 2009). Wnt-induced phosphorylation of

LRP6 represents a key step in receptor activation. Most critical are phosphorylation events at multiple PPPSPxS motifs, which provide docking sites for Axin and are sequentially carried out by GSK3 and CKI (Tamai *et al*, 2004, Zeng *et al*, 2005). Other phosphorylation events include those that are performed by CKI (likely CK1 γ) at a S/T-rich cluster (Davidson *et al*, 2005). This complex pattern of LRP6 phosphorylation is subjected to regulation by many components including Fz, Dishevelled (Dvl), and also Axin (Bilic *et al*, 2007; Zeng *et al*, 2008) (Figure 1). LRP6 phosphorylation also requires phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂), whose production is induced by Wnt through Dvl binding and activation of phosphatidylinositol 4-kinase type II α (PI4KII α) and phosphatidylinositol-4-phosphate 5-kinase type I (PIP5KI) (Pan *et al*, 2008). The mechanism by which PtdIns(4,5)P₂ promotes LRP6 phosphorylation has remained unclear and Tanneberger *et al* (2011) now demonstrate that Amer1/WTX (APC membrane recruitment 1/Wilms tumour gene on the X chromosome) is recruited by PtdIns(4,5)P₂ to the plasma membrane to promote LRP6 phosphorylation by Axin–GSK3 and CK1 γ .

Amer1 was initially identified as an APC-binding protein, which regulates APC cellular localization (Grohmann *et al*, 2007). Amer1 is identical to WTX, which is mutated in some cases of Wilms tumour, a paediatric kidney cancer (Rivera

Figure 1 Amer1/WTX and PtdIns(4,5)P₂ in LRP6 phosphorylation. (A) Fz/Dvl activates PI4KII α and PIP5KI for production of PtdIns(4,5)P₂ (in red colour), which recruits to the plasma membrane Amer1/WTX that recruits Axin–GSK3 and CK1 γ to phosphorylate LRP6. (B) Initiation–amplification model. Fz/Dvl directly recruits Axin–GSK3 to initiate LRP6 phosphorylation, which further recruits more Axin–GSK3 for LRP6 phosphorylation. Note that PI4K/PIP5K/PtdIns(4,5)P₂–Amer1/WTX appear to constitute a parallel branch from Dvl for Axin–GSK3 recruitment. (C) Signalosome model. Dvl polymerizes to form a platform to aggregate Fz and Axin–GSK3, promoting LRP6 phosphorylation. Only the Wnt stimulated condition is shown. See text for details.

et al, 2007). A molecular explanation for the WTX tumour suppressor function was uncovered by the observation that WTX is in complex with Axin, APC, and β -catenin and promotes β -catenin degradation (Major *et al*, 2007), although how Amer1/WTX acts in this capacity to antagonize Wnt signalling is yet to be delineated. The fact that Amer1/WTX harbours N-terminal PtdIns(4,5) P_2 binding motifs (Grohmann *et al*, 2007) prompted Tanneberger *et al* to evaluate its potential relationship with Wnt-induced PtdIns(4,5) P_2 production, uncovering a hidden role of Amer1/WTX in stimulation of Wnt signalling. Knockdown of Amer1/WTX via siRNAs decreased, whereas overexpression of Amer1/WTX elevated, Wnt-induced LRP6 phosphorylation (Tanneberger *et al*, 2011). Furthermore, Wnt stimulation recruited Amer1/WTX to the plasma membrane, and this recruitment depended on PI4KII α , PtdIns(4,5) P_2 , and the PtdIns(4,5) P_2 binding motifs of Amer1/WTX. The authors then provided data showing that PtdIns(4,5) P_2 involvement in LRP6 phosphorylation can be attributed to its recruitment of Amer1/WTX. First, an Amer1/WTX variant that is engineered to anchor to the plasma membrane rescued Wnt-induced LRP6 phosphorylation in PI4KII α , and thus PtdIns(4,5) P_2 , deficient cells; second, enhancement of LRP6 phosphorylation by PtdIns(4,5) P_2 depended on Amer1/WTX. These results suggest that PtdIns(4,5) P_2 recruitment of Amer1/WTX is required for LRP6 phosphorylation (Figure 1).

Previous studies have demonstrated a critical role of the Axin-GSK3 complex in LRP6 phosphorylation (Zeng *et al*, 2005, 2008). Consistent with the findings of Amer1/WTX association with the Axin complex (Major *et al*, 2007), Tanneberger *et al* found that Amer1/WTX recruited Axin and GSK3 to the plasma membrane, and that Amer1/WTX promotion of LRP6 phosphorylation relied on the Axin-binding domain of Amer1/WTX. The authors further illustrated the unusual scaffolding property of Amer1/WTX in its binding to CK1 γ and LRP6, providing a potential explanation for Amer1/WTX involvement in phosphorylation at PPPSPxS motifs (by GSK3 and CK1) and the S/T-rich cluster (by CK1 γ). Indeed a chimeric molecule between the LRP6 cytoplasmic domain and Amer1/WTX was sufficient to enhance LRP6 phosphorylation and signalling. The data provide a strong case for Amer1/WTX in mediating LRP6 phosphorylation through recruiting Axin-GSK3 and CK1 γ (Figure 1).

Amer1/WTX therefore appears to have two opposite functions in the Wnt pathway, analogous to those described for Axin, GSK3, and likely some CK1 members (Zeng *et al*, 2005, 2008). These proteins in the absence of Wnt stimulation promote β -catenin phosphorylation and thus antagonize Wnt signalling; but upon Wnt stimulation they switch sides to enhance LRP6 phosphorylation and activate Wnt signalling. These dual roles are nonetheless compatible with genetically defined tumour suppressor functions of WTX and Axin, as a loss of either results in elevated β -catenin levels and thereby tumourigenesis.

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- The involvement of PtdIns(4,5) P_2 and Amer1/WTX in LRP6 phosphorylation therefore adds additional layers of regulation to the two existing (and complementary) models of Wnt receptor activation (Figure 1) (MacDonald *et al*, 2009). In the ‘initiation–amplification’ model, Fz/Dvl recruitment of the Axin-GSK3 complex initiates PPPSPxS phosphorylation (initiation), which provides additional Axin-docking sites and thereby enhances further PPPSPxS phosphorylation (amplification) (MacDonald *et al*, 2009). In the ‘signalosome’ model, Dvl and Axin, via their ability to ‘polymerize’ through a unique domain (the DIX domain), provide a recruitment platform for Fz (hence LRP6) and Axin-GSK3 to form a receptor supercomplex in which LRP6 is phosphorylated (Bilic *et al*, 2007; Schwarz-Romond *et al*, 2007). PtdIns(4,5) P_2 and Amer1/WTX apparently share or facilitate some of the Axin recruitment function of Dvl (Tanneberger *et al*, 2011) (Figure 1). These results/models further raise an important question: as Dvl, Amer1/WTX, and phosphorylated LRP6 each can bind to Axin, are there discrete steps and roles of these Axin recruiting events/molecules? One speculation is that Dvl and Amer1/WTX/PtdIns(4,5) P_2 may account for the first step and most of Axin recruitment to the plasma membrane, from which Axin is then recruited to phosphorylated LRP6 that leads to inhibition of β -catenin phosphorylation (MacDonald *et al*, 2009). Consistently, Axin recruitment to the plasma membrane primarily depends on Dvl and/or Amer1/WTX but not GSK3 and its phosphorylation of LRP6 (Cliffe *et al*, 2003; Tanneberger *et al*, 2011).
- While the Wnt/ β -catenin pathway and core components are conserved among invertebrates and vertebrates, Amer1/WTX is present only in vertebrates. Invertebrates such as *Drosophila* may rely on Dvl to recruit Axin whereas in vertebrates Amer1/WTX appears to delegate a part of the task in parallel (Figure 1). One may ask whether the role of PtdIns(4,5) P_2 and PI4K/PIP5K in Wnt signalling is shared by invertebrates? Related to this issue, WTX loss-of-function mutations underlie the X-linked dominant disease osteopathia striata with cranial sclerosis (OSCS), which is characterized by excessive and hardened bone and other anomalies (Jenkins *et al*, 2009). A male patient who is almost certainly null for WTX is still alive in teens (Perdu *et al*, 2010). Thus, at least in some cases a lack of WTX provides quasi-normal Wnt signalling compatible with embryonic development and the postnatal life. Another puzzle is that OSCS patients do not appear to have increased cancer risk, implying that sporadic WTX loss in somatic cells and inherited loss of WTX from the germline have distinct consequences (Jenkins *et al*, 2009). Amer1/WTX represents likely a vertebrate innovation for fine-tuning Wnt signalling both positively and negatively, and exhibits critical roles that are yet to be understood in cancer and disease pathogenesis.

Conflict of interest

The authors declare that they have no conflict of interest.

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