Review

Genomic insights into WNT/β-catenin signaling

Joseph Rosenbluh[1,2,3], Xiaoxing Wang[1,2], and William C. Hahn[1,2,3]

[1] Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, MA 02142 USA
[2] Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215 USA
[3] Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115 USA

The canonical WNT pathway regulates the stability of the proto-oncogene β-catenin and is aberrantly activated in many cancer types. Studies in a wide range of experimental models confirm that β-catenin activity is required for tumor initiation in cancers where this pathway is deregulated. However, to date this pathway has proven to be challenging to target therapeutically. Moreover, several lines of evidence suggest that other components and regulators of β-catenin exist. Here we will describe recent structural and functional studies describing genomic alterations and new regulators of β-catenin that lead to aberrant activation of the WNT/β-catenin pathway. These findings provide new insights into the biology of WNT/β-catenin signaling and suggest potential therapeutic opportunities.

The canonical WNT pathway

WNT signaling is involved in diverse processes including embryonic development, maintenance of tissue homeostasis, and cancer pathogenesis. The Wingless (*WNT*) gene was first identified in a random mutagenesis screen in *Drosophila melanogaster* [1]. Mutations in *WNT* resulted in loss of wing development and defects in larval segmentation. Subsequent experiments demonstrated that mutations in *WNT* or *ARM* (the *D. melanogaster* β-catenin ortholog) result in a similar segmentation phenotype [1]. Genetic complementation screens and biochemical studies led to the finding that WNT signaling inactivates a cytosolic protein complex (the destruction complex) that regulates β-catenin stability [1].

Binding of WNT ligands to the FZD/LRP6 receptor inactivates the destruction complex, which stabilizes β-catenin [2]. The destruction complex, composed of APC, AXIN1, GSK3β, and CSNK1A1, phosphorylates serine residues in β-catenin leading to its ubiquitination by βTRCP and degradation by the proteasome. Activation of the WNT pathway was previously thought to result in disassembly of the destruction complex. However, recent work has shown that upon binding of WNT to the FZD/LRP6 receptor, the destruction complex remains intact and bound to AXIN1. In this state, newly synthesized β-catenin is not recognized by the destruction complex [3]. Stabilized β-catenin directly binds to nucleoproteins Nup62, Nup153, and RanBP2 facilitating its nuclear translocation [4]. In the nucleus, a β-catenin TCF/LEF complex regulates transcription of specific target genes [2] (Figure 1).

WNT in development

Early studies implicated WNT signaling as a critical regulator of early vertebrate development [5]. Injection of *Xenopus* embryos with mRNA encoding positive regulators of the WNT pathway such as *WNT1*, β-catenin, or *LEF1* inhibited formation of the anterior posterior axis and resulted in body axis duplication [5]. In consonance with these observations, β-catenin deletion results in early lethality due to defects in the formation of the anterior posterior axis [5]. Studies in flies revealed that a gradient of WNT ligand found throughout the developing embryo determines the anterior–posterior axis by deferential activation of β-catenin [6]. The WNT pathway also regulates cell fate and is essential for differentiation of embryonic stem cells into the endoderm and mesoderm lineages [5]. In adult animals and humans, WNT signaling is also essential to maintain the stem cell compartment in self-renewing organs such as the intestine and hair follicle [5].

During development, β-catenin regulates transcription by forming a complex with *TCF7L2* (also known as *TCF4*) or *LEF1* [2]. Indeed, *TCF7L2* null mice fail to develop the small intestine and die within 24 h of birth [7]. Moreover, both *TCF7L2* and β-catenin have been shown to co-occupy many promoters [8].

WNT activity in cancer pathogenesis

WNT signaling plays an important role in the pathogenesis of several types of human cancers. In a seminal paper, Nusse and Varmus showed that integration of the mouse mammary tumor virus (MMTV) in the mammary epithelium induces mammary tumors by forcing the expression of the proto-oncogene Wnt1 [9]. Moreover, individuals carrying a germline *APC* mutation develop familial adenomatous polyposis (FAP). Patients affected by FAP develop hundreds of colonic polyps, which progress inevitably to malignant colon cancer [10]. Subsequent studies identified recurrent mutations in components of the WNT signaling pathway in sporadic colon cancers [11].

Corresponding author: Hahn, W.C. (William_Hahn@dfci.harvard.edu).
Keywords: WNT; cancer; β-catenin; YAP1; synthetic lethality.

0165-6147/$ – see front matter
 http://dx.doi.org/10.1016/j.tips.2013.11.007

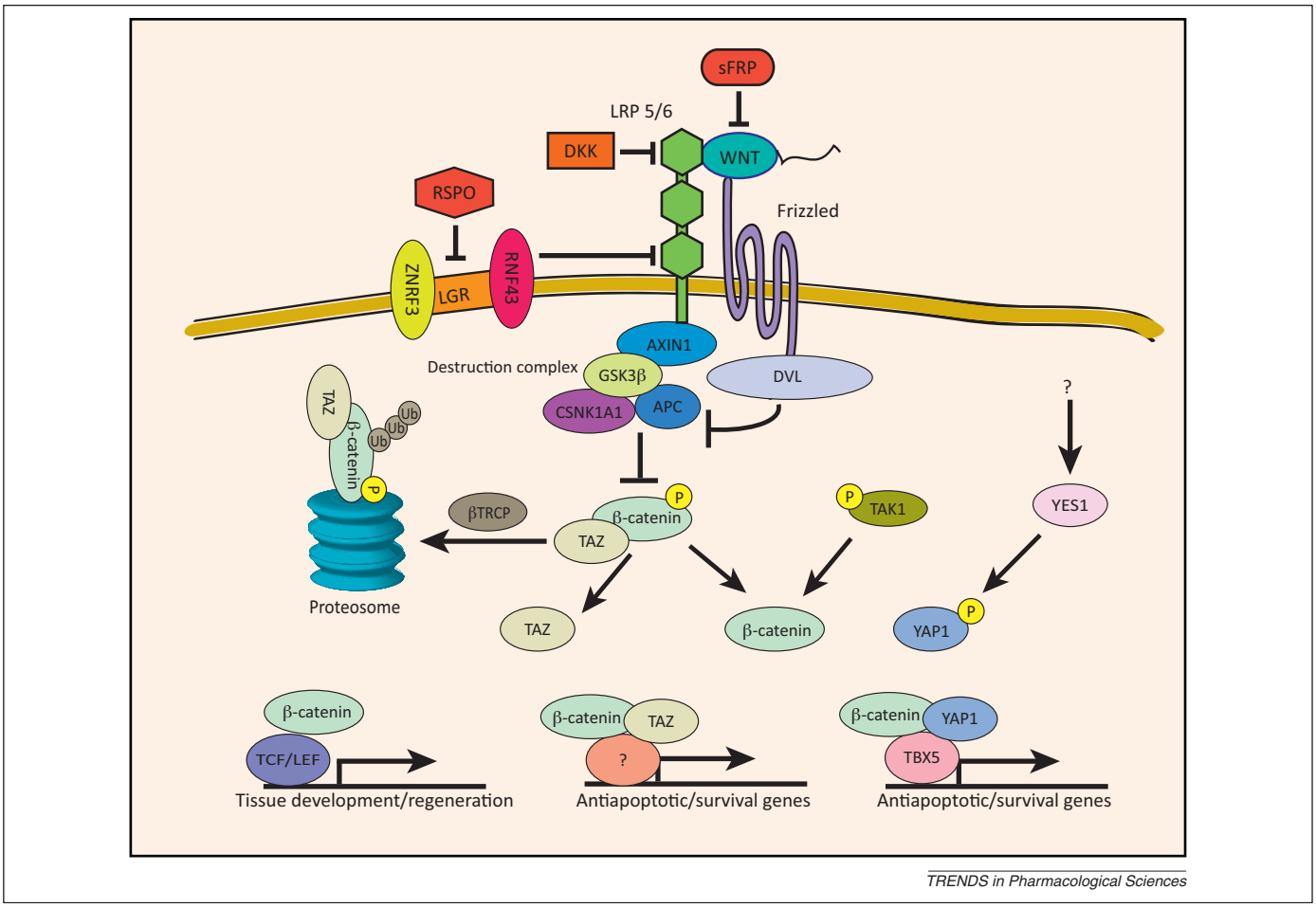

Figure 1. Multiple pathways regulate β-catenin signaling. In normal homeostasis, the destruction complex regulates β-catenin stability (top). Mutations in components of the destruction complex lead to stabilization of β-catenin and activation of various context-dependent transcriptional complexes.

Recent large-scale sequencing efforts have identified several new recurrent mutations in components of the WNT signaling pathway [11]. Surprisingly, these efforts have identified co-occurrence of mutations in positive and negative regulators of the WNT pathway, suggesting that WNT signaling in cancer is more complex than was previously appreciated. In this review, we will describe emerging evidence suggesting that β-catenin is a modular transcription factor activating distinct context-dependent transcriptional programs.

Genomic alterations in components of the WNT/β-catenin pathway

WNT *ligands*

WNTs are an evolutionary conserved family of secreted glycoproteins [12]. There are 19 distinct human WNTs that bind specific receptors and activate β-catenin-dependent and -independent pathways [12], in part explaining the diverse pathways and biological processes regulated by WNT signaling [12]. Indeed, forced expression of 14 of the 19 WNT ligands in human cell lines stabilizes β-catenin [13].

Several WNTs have been reported to be involved in cancer initiation and progression through autocrine or paracrine mechanisms. For example, expression of Wnt1 in murine mammary tissue leads to development of mammary tumors [9]. *WNT3A* and *WNT10B* are overexpressed in triple negative breast cancer [14]. In multiple myeloma, secreted WNT3A has been reported to induce migration and invasion through activation of RhoA kinase and PKC [15].

In contrast to these WNTs, other WNT ligands do not appear to contribute to cancer initiation. A commonly used model to assess WNT-transforming activity is the mouse mammary cell line C57MG. When transformed, C57MG cells undergo morphological changes and are able to form foci (clusters of cells) when seeded at low densities. A transformed phenotype was observed in C57MG cells following expression of *Wnt1*, *2*, *3A*, and *5B* but not *Wnt6*, *4*, or *5A* [16].

Why do some WNT ligands promote β-catenin stabilization yet not promote cancer? One possible explanation is that, in addition to control of β-catenin signaling, specific WNTs in particular contexts activate or inhibit pathways that contribute to or inhibit transformation. In support of this view, Green *et al*. reported that *WNT3A* promotes or inhibits tumor growth in a context-dependent manner [17]. Specifically, co-injection of fibroblasts overexpressing *WNT3A* and patient-derived triple negative breast cancer into immunodeficient mice resulted in accelerated growth of some cancers, whereas others were inhibited. It is clear that further work will be necessary to identify the key factors that specify the response to WNT signaling.