Cellular Signalling 26 (2014) 570–579

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

Wnt signaling in adult intestinal stem cells and cancer ☆

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ARTICLE INFO

Article history:
Received 4 November 2013
Accepted 26 November 2013
Available online 2 December 2013

Keywords:
APC
Colorectal cancer
Gut
Mouse models
Organoids
Tumor

ABSTRACT

Signaling initiated by secreted glycoproteins of the Wnt family regulates many aspects of embryonic development and it is involved in homeostasis of adult tissues. In the gastrointestinal (GI) tract the Wnt pathway maintains the self-renewal capacity of epithelial stem cells. The stem cell attributes are conferred by mutual interactions of the stem cell with its local microenvironment, the stem cell niche. The niche ensures that the threshold of Wnt signaling in the stem cell is kept in physiological range. In addition, the Wnt pathway involves various feedback loops that balance the opposing processes of cell proliferation and differentiation. Today, we have compelling evidence that mutations causing aberrant activation of the Wnt pathway promote expansion of undifferentiated progenitors and lead to cancer.

The review summarizes recent advances in characterization of adult epithelial stem cells in the gut. We mainly focus on discoveries related to molecular mechanisms regulating the output of the Wnt pathway. Moreover, we present novel experimental approaches utilized to investigate the epithelial cell signaling circuitry *in vivo* and *in vitro*. Pivotal aspects of tissue homeostasis are often deduced from studies of tumor cells; therefore, we also discuss some latest results gleaned from the deep genome sequencing studies of human carcinomas of the colon and rectum.

Contents

1. Introduction

Extracellular Wnt proteins act in metazoans as morphogens to regulate diverse processes throughout embryonic development, such as cell proliferation, differentiation, cell migration and cell polarity. In adulthood, Wnt signaling is essential for the maintenance of somatic stem cell and committed progenitor cell compartments. The pathway is also involved in tissue regenerative processes following injury [1,2]. Overall, there are at least three distinct "branches" of Wnt signaling (reviewed in [3]). The best studied so called "canonical" pathway depends on β-catenin as its key effector (Fig. 1). Besides its engagement in cadherin-based adherens junctions [4], β-catenin associates with DNA-binding

Abbreviations: APC, Adenomatous Polyposis Coli; Axin, axis inhibition protein; β-TrCP, beta-transducin repeat containing protein; BMP, bone morphogenic protein; CBC, crypt base columnar; CRC, colorectal carcinoma; CSCs, cancer stem cells; Dkk, Dickkopf; FAP, Familial Adenomatous Polyposis; GI, gastrointestinal; GSK3, glycogen synthase kinase 3; ISCs, intestinal stem cells; LEF/TCF, lymphoid enhancer-binding factor/T-cell factor; LGR, leucine-rich G protein coupled receptor; LRCs, label-retaining cells; LRP, low density lipoprotein receptor-related protein; MSI, microsatellite instability; MSS, microsatellite-stable; Rnf43, ring finger 43; Rspo, R-spondin; TAZ, transcriptional co-activator with PDZ-binding motif; YAP, Yes-associated protein; Znfr3, zinc and ring finger 3.

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http://dx.doi.org/10.1016/j.cellsig.2013.11.032

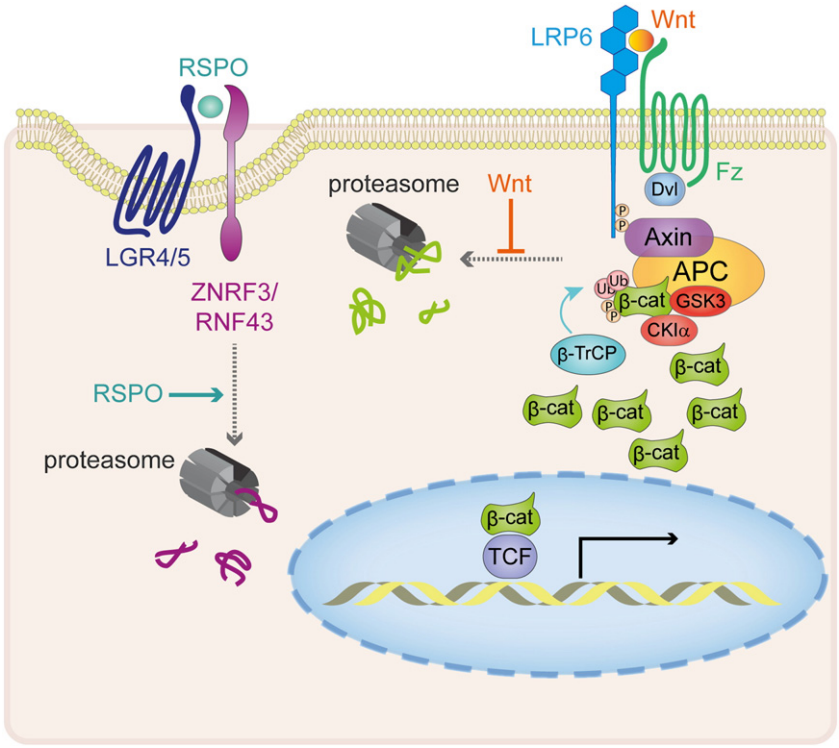

Fig. 1. Canonical Wnt signaling. In the absence of the Wnt signal, cytosolic β-catenin is bound by a multimeric destruction complex that includes Axin and APC. Beta-catenin is then phosphorylated by serine/threonine kinases CK1α and GSK3α/β. The N-terminal phosphorylated amino acid residues are recognized by β-TrCP, a component of SKP1-cullin-F-box (SCF) ubiquitin ligase, and the ubiquitinylated protein is destroyed by the proteasome. The presence of Wnt ligand bridges the Frizzled-LRP proteins, leading to recruitment of the destruction complex to cytosolic tails of clustered LRP receptors (simplified for clarity). The complex can still bind β-catenin, but its ubiquitinylation is inhibited. Consequently, newly synthesized β-catenin molecules accumulate in the cytoplasm and shuttle to the cell nucleus to transactivate expression of TCF-dependent target genes. In an alternative model, active Wnt signaling disrupts the β-catenin destruction complex and inhibits β-catenin phoshorylation (not depicted, see the text for additional details). The secreted Wnt pathway agonists, the RSPO proteins, augment the Wnt circuit by promoting stabilization of Frizzled and LRP proteins. RSPOs form a ternary complex with their LGR4/5 receptor and transmembrane E3 ubiquitin ligase proteins ZNRF3 and RNF43, thereby inhibiting turnover of the Wnt receptors.

proteins of the lymphoid enhancer-binding factor/T-cell factor (LEF/TCF) family (further referred to as TCFs) to modulate expression of context-specific target genes. In the absence of the Wnt stimulus, cytosolic β-catenin is marked for degradation by a protein complex that includes serine/threonine kinases casein kinase 1 alpha (CK1α) and glycogen synthase kinase 3 (GSK3) [5]. Scaffolding of the kinases and β-catenin is mediated by axis inhibition protein (Axin) [6] and adenomatous polyposis coli (APC) tumor suppressors [7]. Ultimately, N-terminally phosphorylated β-catenin is ubiquitinated by F-box-containing beta-transducin repeat containing (β-TrCP) E3 ubiquitin protein ligase and subsequently destroyed by the proteasome [8,9]. In unstimulated cells the TCF proteins are associated with transcriptional repressors of the groucho/transducin-like enhancer of split (TLE) family and block expression of Wnt-responsive genes [10]. Wnt molecules bind to a receptor complex composed of a seven-transmembrane receptor of the Frizzled family and co-receptor low density lipoprotein receptor-related protein (LRP) [11]. The ligand–receptor engagement triggers a cascade of events that include phoshorylation of the adaptor protein Disheveled (Dvl) by CK1ε [12]. In addition, the intracellular portion of LRP is phosphorylated by CK1γ and GSK3, and the LRP–Axin complex is subsequently formed [13]. Simultaneously, the phosphorylated amino acid residues in Axin (these modifications are catalyzed by GSK3) are removed by protein phosphatase 1 (PP1) [14] or PP2A [15]. The dephosphorylated protein constitutes "closed" conformation that is unable to interact with β-catenin and, consequently, β-catenin phosphorylation is inhibited. In an alternative model proposed by Li and colleagues, the β-catenin destruction complex remains intact in the Wnt signal receiving cell; however, active signaling suppresses β-catenin ubiquitination, which leads to the saturation of the complex with phosphorylated β-catenin [16]. In any case, β-catenin accumulates in the cell cytoplasm and nucleus, where it displaces the groucho/TLE proteins from TCFs. Beta-catenin contains a transactivation domain, and TCF-β-catenin complexes thus act as bipartite transcriptional activators of specific target genes such as *c-myc* [17], *cyclin D1* [18,19], *CD44* [20] and *Axin2* [21]—for a comprehensive list of Wnt target genes, please refer to the Wnt homepage www.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes.

The β-catenin-independent, 'noncanonical' Wnt signaling cascades utilize distinct signaling mechanisms to relay the signal from the Wnt receptor complex. The planar cell polarity (PCP) pathway activates small GTPases ras-related C3 botulinum toxin substrate 1 (Rac1) and ras homolog gene family member A (RhoA). The pathway also includes protein kinases rho-associated, coiled-coil-containing protein kinase (ROCK) and c-Jun N-terminal kinase (JNK) that in turn induce cytoskeletal remodeling or elicit a transcriptional response, respectively [22]. PCP signaling is implicated in the establishment of cell polarity and cell migration [23,24]. The second relatively well-characterized noncanonical pathway, Wnt/Ca^{2+} signaling, stimulates phospholipase C (PLC) through the action of heterotrimeric G proteins. The resulting mobilization of intracellular Ca^{2+} activates Ca^{2+}-dependent effectors that include calcium calmodulin mediated kinase II (CAMKII), protein kinase C (PKC) and calcineurin (reviewed in [25]). The Wnt/Ca^{2+} signaling branch is implicated in inflammation and promotion of cancer [26]. Wnt ligands can also engage tyrosine kinase-like orphan receptor 2 (ROR2) [27,28] and receptor-like tyrosine kinase (RYK) [29] as receptors; however, there is only little insight into these alternative Wnt signaling pathways. Importantly, despite the general consensus on the key role of β-catenin-independent Wnt signaling in development and cancer, the precise molecular mechanisms of the noncanonical pathways remain mostly unknown. This is mainly due to the fact that in contrast to Wnt/β-catenin signaling, the field of the noncanonical pathways suffers from the lack of suitable reagents and robust functional assays.