Wnt Signaling Pathways, Inflammation and Carcinogenesis

Subjects: Dermatology

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Wnt signaling is responsible for the regulation of different intracellular signal transduction pathways, which are essential for embryogenic development, cellular migration, polarization and differentiation as well as stem cell biology control and growth. Wnt signaling activation is related to the binding of Wnt ligands to a specific cell surface receptor which belongs to the Frizzled (Fzd) family, thus inducing the canonical (β-catenin-dependent) or non-canonical (β-catenin-independent) pathway.

Keywords: Wnt signaling ; basal cell carcinoma ; squamous cell carcinoma

1. Wnt Signaling

The Fzd receptor interacts with other coreceptors, such as the receptor-like tyrosine kinase (Ryk), the receptor tyrosine kinase-like orphan receptor 2 (Ror2) or the low-density lipoprotein receptor-related protein (Lrp)-5/6, with the consequent activation of Disheveled (Dvl) and the downstream pathways [1]. Generally, cytoplasmatic β-catenin is phosphorylated by a complex formed by glycogen synthase kinase 3β (Gsk3β), adenomatous polyposis coli (Apc), casein kinase 1α (Ck1α) and Axin; β-catenin phosphorylation leads to its degradation by proteasome [2][3]. β-catenin is stabilized when the degradation complex is inhibited by Dvl following the canonical Wnt pathway activation [4]. β-catenin translocation into the nucleus allows the interaction with transcription factors, such as lymphoid-enhancing factor/T-cell factor (Lef/Tcf) transcription proteins, thus promoting Wnt target genes transcription [5]. Canonical Wnt signaling role starts from stem cell differentiation until cell proliferation, both during embryogenesis and adult tissue homeostasis [6].

The non-canonical Wnt signaling pathways are divided into Wnt/Calcium (Ca^{2+}) and Wnt/Planar cell polarity (PCP) pathways [1]. In particular, in the Wnt/Calcium (Ca^{2+}) pathway, the activated Fzd-co-receptor-Dvl complex induces phospholipase C γ, which converts phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3), resulting in the increased release of intracellular Ca^{2+}. The release of Ca^{2+} leads to the activation of calcium-dependent kinases, such as Ca^{2+}-dependent phosphatase calcineurin (CaN), Ca^{2+}-calmodulin dependent kinase II (CAMKII) or protein kinase C (PKC) [7]. The activation of CaMKII may induce the phosphorylation of TGFβ-activated kinase 1 (TAK1), resulting in Nemo-like kinase (NLK) activation. TAK1-NLK pathway stimulation antagonizes the canonical Wnt/β-catenin pathway [8]; on the other hand, CaN induces the translocation of nuclear factor of activated T-cells (NFAT) family proteins into the nucleus, thus inducing the transcription of their target genes. In the Wnt/PCP pathway, activated Fzd-co-receptor-Dvl complex induces the activation of Rho family small GTPases, including RhoA, Rac and Cdc42 [9]. Cdc42 and Rac promote the c-Jun N-terminal kinase (JNK) signaling, resulting into the activating protein-1 (AP-1) complex activation [10]. On the other hand, RhoA induces the activation of Rho-associated kinase (ROCK) [11]. These pathways result in the regulation of cell motility and polarity [12][13].

2. Wnt Pathway, Inflammation and Carcinogenesis

Chronic inflammation is a widely recognized risk factor for several skin cancers, especially cutaneous squamous cell carcinoma (cSCC) [14]. The role of Wnt signaling in inflammation is complex, because it includes both anti- and proinflammatory functions [15]. Particularly, Wnt signaling would exert its immunomodulation acting on important inflammatory cytokines, such as the nuclear factor kappa B (NF-kB) and its target genes *IL6*, *IL8* and *TNFA*, encoding tumor necrosis factor-α (TNF-α) [15][16][17][18]. Wnt signaling alterations are involved in the pathogenesis of cutaneous chronic inflammatory diseases, as well as those affecting the skin, such as psoriasis, and autoimmune diseases [16][19][20]. In psoriasis, non-canonical Wnt pathway increases keratinocyte proliferation and secretion of pro-inflammatory cytokines, such as TNF-α, interleukin-12 (IL-12) and (IL-23) [21]. Moreover, it is important to mention the role of canonical and non-

canonical Wnt pathways in regulation of immune cells, particularly T cells and dendritic cells. The non-canonical Wnt pathway promotes migration of T cells via the CXC chemokine ligand-12 (CXCL12)–CXC chemokine receptor-4 (CXCR4) signaling [22]. Nevertheless, Wnt5a, via non-canonical Ca(2+)/CaMKII /NF-κB signaling, may also induce an abnormal phenotype of dendritic cells, which show an altered response to Toll-like receptor (TLR) ligands [23]. This phenotype is tolerogenic and characterized by increased production of IL-10 [23]. On the other hand, the canonical Wnt pathway represses the T regulatory cells function, promoting autoimmune response [24]. The imbalance of immune responses mediated by Wnt pathways may result in psoriatic inflammation but also in autoimmune disease, such as systemic lupus erythematosus (SLE) or systemic sclerosis (SSc) [21]. Canonical Wnt pathway is hyperactivated in SLE and plays a role in renal fibrosis of lupus nephritis, promoting the differentiation of T cell in Th17 clones [21]. An increased activation of Wnt signaling is present also in skin fibroblasts of patients with SSc, leading to skin fibrosis [21]. The deficiency of Wnt inhibitory factor-1 (WIF1) in fibroblasts of SSc patients is correlated with an hyperactivation of Wnt/*β*-catenin and, thus, with an increased production of collagen [25]. It is well known that reactive oxygen species (ROS) play an important role in fibrotic processes in SSc [26]. Preventing the accumulation of ROS in cultured SSc patient cells restored WIF-1 expression, thus avoiding collagen accumulation [25]. In conclusion, ROS promote Wnt activation, which contributes to fibrosis [25].

As in cutaneous inflammatory and autoimmune diseases, the Wnt pathway may modulate inflammatory responses in cutaneous cancers and influence tumor microenvironment (TME), thus promoting tumor development [27]. Wnt pathway and cancer are in relation to the complement system, which is involved not only in anti-tumor but also in pro-tumorigenic immune responses [14]. Complement component 3 (C3) and its active form, complement anaphylatoxin (C3a), may promote the epithelial-mesenchymal transition (EMT), thus giving invasive properties by decreasing E-cadherin expression [28]. C3a effects are also mediated by the transcription factor Twist [28], which induces the EMT through β-catenin [29]. In vitro experiments showed that exposure to C3 induces cyclin D1 and metalloproteases up-regulation which promote proliferation and migration of cSCC cells [30]. Moreover, C3a exposure stimulates β-catenin and Sox-2, a transcription factor which regulates cell stemness and induces pluripotent stem cells; in fact, C3a receptor silencing led to the down-regulation of β-catenin and Sox-2 with the consequent decrease of tumor volume [30]. The most important contribution of Wnt signaling to pro-tumorigenic inflammation is not limited to the complement system but also concerns T cells function: the thymic stromal lymphopoietin (TSLP) is a pro-inflammatory cytokine which antagonizes skin carcinogenesis by regulating the functions of CD8 and CD4 T cells [31]. TSLP-deficient mice show pro-tumorigenic inflammation and a predisposition to develop tumor growth through Wnt/β-catenin signaling involvement [31]. In fact, Wnt signaling may influence cancer immune-surveillance and facilitate a tumor immune escape, by reducing the recruitment of dendritic cells and by impairing the activity of T regulatory cells and cytotoxic T lymphocytes [32][33]. Tumors responding better to immunotherapy are characterized by a T cell-inflamed tumor microenvironment (TME), namely infiltrating antigen-specific T cells [33]. The TME may influence the growth and progression of tumors [33]. The activation of Wnt/β-catenin pathway prevent T-cell infiltration and activity in TME [33]. This results in progression of tumors and resistance to immunotherapy [33]. There are three main mechanisms underlying tumor immune escape by the Wnt/β-catenin pathway. First, the canonical Wnt pathway induces the activating transcription factor 3 (ATF3), thus inhibiting the transcription of *C-C* motif chemokine ligand 4 (CCL4) in a mouse model. This results in defective infiltration and activation of dendritic cells and T-cells. Another mechanism involved in immune escape concerns the crosstalk between tumor cells and tumor-associated macrophages (TAMs). Tumor cells may stimulate IL-1β production in TAMs via Snail, a transcription factor of canonical Wnt pathway. In turn, IL-β may increase the availability of β-catenin in tumor cells. Finally, canonical Wnt pathway may influence the activity of T regulatory cells, enhancing their survival [33]. A combination therapy based on the use of immune check point inhibitors plus Wnt signaling inhibitors may improve antitumor immunity [34].

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