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Secreted Wnt antagonists in leukemia: A road yet to be paved

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ABSTRACT

Wnt signaling has been a topic of research for many years for its diverse and fundamental functions in physiological (such as embryogenesis, organogenesis, proliferation, tissue repair and cellular differentiation) and pathological (carcinogenesis, congenital/genetic diseases, and tissue degeneration) processes. Wnt signaling pathway aberrations are associated with both solid tumors and hematological malignancies. Unregulated Wnt signaling observed in malignancies may be due to a wide spectrum of abnormalities, from mutations in the genes of key players to epigenetic modifications of Wnt antagonists. Of these, Wnt antagonists are gaining significant attention for their potential of being targets for treatment and inhibition of Wnt signaling. In this review, we discuss and summarize the significance of Wnt signaling antagonists in the pathogenesis and treatment of hematological malignancies.

1. Introduction

The first description of deregulated Wnt signaling in a hematological malignancy was reported in chronic myeloid leukemia (CML) [1]. CML is a clonal hematopoietic stem cell (HSC) disease characterized clinically by an increase in myeloid lineage cells at all stages of differentiation. The translocation −t(9;22)(q34;q11)- leading to the formation of the Philadelphia chromosome (derivative 22) is the hallmark of CML. This translocation results in the fusion of the proto-oncogene ABL located on the long arm of chromosome 9, with the BCR gene on chromosome 22 [2]. The BCR-ABL oncoprotein possesses an unregulated increased tyrosine kinase activity that has been shown to drive the disease in terms of cell proliferation and resistance to programmed cell death [3]. There are several signaling pathways that have been determined to play a prominent role in CML progression. Among them the Wnt signaling pathway stands out with its unique role in the formation of a second leukemic stem cell population derived from granulocyte-macrophage progenitors. The neoplastic transformation of a HSC, results in the overproduction of granulocytes; thus forming the initial BCR-ABL positive leukemic stem cell (LSC) pool [4]. In the normal HSC, nuclear accumulation of β-catenin has been shown to be the driving force of self-renewal; its nuclear accumulation is therefore limited to HSCs in the bone marrow [5]. It is established that granulocyte-macrophage progenitors from advance stage CML patients display self-renewal capacity (a capability they normally do not possess), as a result of an overly active β-catenin/canonical Wnt signaling pathway, leading to two pools of Ph(+) cells with self-renewing capacity.

The importance of the Wnt signaling pathway in hematological malignancies is not limited to CML. Over-expression and aberrant regulation of Wnt signaling, mutations in downstream pathway members and silencing of Wnt antagonists by epigenetic regulation have all been reported in different hematological malignancies [6–8]. The epigenetic silencing of Wnt antagonists is of special interest and they are considered promising targets which carry the potential to be exploited in designing new agents and therapeutic strategies.

In this review, we focus on the role of Wnt antagonists in leukemia, with a special focus on CML; and discuss potential therapeutic opportunities presented by Wnt antagonists in hematological malignancies.

1.1. The Wnt signaling pathway

Wnt signaling is involved in many biological processes, including cell adhesion, migration, apoptosis, polarity, proliferation, development and organogenesis [9]. Secreted Wnt proteins act as ligands for the Frizzled (Fzd) receptor family and trigger paracrine/autocrine signaling through the Fzd proteins in the cell [9,10]. The ligand-receptor specificity between the 19 Wnt ligands and more than 15 receptors/co-receptors leads to the activation of intracellular Wnt signaling that is classified as canonical and non-canonical pathways; in which the

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canonical pathway is responsible for preventing the degradation of β-catenin [11]. When Wnt signaling is not activated, β-catenin accumulation is prevented by its degradation by a multiprotein complex that is composed of Axin and adenomatous polyposis coli (APC) proteins (both defined as tumor suppressors), the Ser/Thr kinases GSK-3 and CK1, protein phosphatase 2A (PP2A), and the E3-ubiquitin ligase β-TrCP. A β-TrCP recognition site is generated by this degradation complex through the phosphorylation of β-catenin on a conserved Ser/Thr-rich sequence near its amino terminus; thus marking it for degradation by the proteasome [12].

Canonical Wnt signaling is triggered by the binding of the Wnt ligand to the Fzd receptor in the presence of the co-receptor LRP5 or 6 (Low-density lipoprotein receptor-related protein) which in turn transfers the signal into the cell [5,9]. Receptor-ligand coupling results in the phosphorylation of Dishevelled (Dvl) proteins. The resulting Dvl/Fzd receptor complex stimulates the formation of LRP aggregates in the cell membrane and CK1γ phosphorylation of the intracellular domains of the LRP receptors. These signaling events lead to Axin being trapped by the receptor complex. Phosphorylated Dvl disrupts the Axin-APC-GSK3β degradation complex, inhibiting the phosphorylation of β-catenin and suppressing proteosomal degradation. As a result, β-catenin levels rise in the cytoplasm and translocate to the nucleus. Nuclear β-catenin interacts with members of the Tcf/Lef transcription factor family and binds to co-activator proteins converting them into transcriptional activators of Wnt target genes [13].

Non-canonical Wnt pathways are defined as pathways that do not involve β-catenin stabilization. Presently eleven different non-canonical Wnt pathway has been identified (eg Wnt/RAP1, Wnt/Ror2, Wnt/PKA, Wnt/GSK3, Wnt/RYK, Wnt/mTOR ect.). Some of these pathways also use/interact with Fzd transmembrane proteins as receptor. Non-canonical pathways in which Fzd receptors are involved are divided into Wnt/planar cell polarity (PCP) and Wnt/Ca^{2+} pathways [14].

In Wnt/Ca^{2+} signaling, intracellular calcium release is triggered by the binding of the Wnt ligand to its Fzd receptor, followed by G protein signaling leading to PKC-mediated cleavage of phosphatidolinoside to diacylglycerol and inositol 1,4,5 triphosphate. Activation of secondary messengers triggers intracellular calcium release resulting in calcium dependent kinase activation such as CamKII and CaCN. These kinases activate the expression of transcriptional co-activators such as NFAT and NFkB [15]. This pathway is known to play an important role in regulating cell adhesion, cell migration, embryonic development of dorso-ventral patterns and development of the heart [16].

The Wnt/PCP pathway plays important roles in regulating morphogenic polarization, cell fate, embryonic morphogenesis and cellular mobility [16]. It's activated by the binding of ligands that function in non-canonical signaling such as Wnt5a, Wnt5b, Wnt11, to Fzd family proteins or ROR2, ROR1 and Ryk receptors [17]. Similar to LRP5/6, ROR1 and ROR2 are phosphorylated by GSK3β and interact with Dsh proteins after Wnt5a binding. As a result a Fzd/ROR active receptor complex is formed [15]. This complex activates Dvl which in turn transduces the signal to small GTPases and JNK (C-Jun NH2-terminal kinase) via R protein, G protein, RhoA, Rac and Cdc42 [18].

Recent studies have identified a new "alternative Wnt pathway" in which β-catenin is replaced by YAP/TAZ proteins. YAP/TAZ proteins are transcriptional regulators of the hippo pathway, the key regulator of organ size and tissue homeostasis [19]. In the absence of Wnt ligands, YAP/TAZ allows aggregation of β-TrCP and acts as a negative regulator of the Wnt pathway. When Wnt signaling is activated, YAP/TAZ and β-TrCP are removed from the degradation complex by Axin1. Free YAP/TAZ on the contrary, acts as a positive transcriptional regulator of the Wnt pathway [20]. Park et al. have shown YAP/TAZ accumulates in the nucleus through Wnt5a/b and Wnt3a and transcriptionally regulates the expression of various genes [19].

Recent and ongoing studies are constantly identifying new molecules associated with Wnt signaling. Of these, newly identified Wnt agonists, Norrin and R-Spondin are worth mentioning [21]. Norrin binds directly to the Fzd4/LRP5 complex and activates signal transduction. R-Spondin binds to G-protein-coupled receptors (Lgr4-6) and inhibits the ubiquitination and degradation of Fzd proteins [22,23]. Both proteins are described as positive regulators of the Wnt pathway.

1.2. Wnt signaling in Hematological Malignancies

It is well established that the Wnt/β-catenin pathway controls the proliferation, survival and differentiation of hematopoietic cells [24]. Continuous stimulation of Wnt signaling results in the neoplastic transformation of myeloid and lymphoid lineages. Physiologically the pathway is regulated stringently [25]. In healthy cells β-catenin levels are tightly controlled by secreted and/or intracellular located inhibitory proteins. Epigenetic abnormalities and silencing suppressors of the pathway trigger the increase of β-catenin levels, leading to the uncontrolled activation of the Wnt signaling pathway [26,27].

Canonical Wnt signaling is significant for the maintenance and establishment of fetal HSCs [28]. Wnt signaling can directly promote HSC self-renewal and has the ability to reconstitute the hematopoietic system of lethally irradiated mice [29]. Wnt signal strength was shown to regulate normal hematopoiesis [30]. Research over the past years has led to our understanding that Wnt signaling is a critical regulator of distinct aspects of self-renewal and differentiation in stem cells of the hematopoietic system [28]. The differential expression of canonical Wnt signaling was shown to have opposing effects on HSCs. Constitutive activation of β-catenin has been demonstrated to induce HSC re-population ability and inhibited differentiation [31]. On the other hand non-canonical Wnt signaling also plays an important role in HSC physiology. Non-canonical Wnt signaling maintains quiescent long-term HSCs the bone marrow niche and has been shown to have a role in the aging of HSCs [32,33].

Being a central element of HSC development and maintenance, it is not surprising that the deregulation of this pathway plays a role in leukemia development and progression. Clinical and experimental studies have shown that Wnt signal transduction is impaired in hematological malignancies. The most apparent result of deregulated Wnt signaling is the increase of intracellular β-catenin levels. Sadras et al. have shown that β-catenin expression is upregulated in primary AML cells and is associated with enhanced clonogenic and self-renewal capacity [34]. The aberrant activation of Wnt/β-catenin has also been implicated in the development and progression of acute lymphoblastic leukemia (ALL). The expression levels of Wnt ligands, effects dose dependent regulation of Wnt signaling in hematopoiesis [31]. Wnt ligands WNT2B, WNT5A, WNT10B, WNT16B and Wnt receptors FZD7 and FZD8 were reported to be overexpressed in B cell progenitor ALL cells and primary B-ALL cells. LEF1 is also shown to be overexpressed in ALL, CLL and malignant lymphoma [35].

Wnt signaling is known to prolong leukemic stem cell (LSC) survival. Similar to its effects on HSC's, Wnt/β-catenin signaling is required for the self-renewal of LSCs [36]. Additionally canonical Wnt signaling has anti-apoptotic effects in the LSCs of leukemia models when challenged with anti-cancer drugs [6]. Wnt signaling especially has a pivotal role in the different compartments of CML-LSC development [37]. β-catenin overexpression is observed in the LSCs during the accelerated stage of disease and blast crisis [1]; pointing to the canonical Wnt pathway as a good therapeutic target in CML-LSCs.

Epigenetic dysregulation is another cause of the Wnt/β-catenin pathway dysfunction in solid tumors and hematological malignancies [38]. Promotor methylation of Wnt antagonists were detected in multiple types of hematological malignancies and correlated with poor prognosis. The epigenetic silencing of one or multiple Wnt antagonists results in the constitutive activation of the pathway. The presence of chimeric oncogenes such as AML1/RUNX1, MLL/PTD, PML/RARα, has been reported to correlate with the hyper-methylation and silencing of Wnt inhibitors [35].

Although most research conducted on Wnt signaling in