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Review Article

Normal Hematopoiesis and Hematologic Malignancies: Role of Wnt/β-catenin and PI3K/AKT Cell Signaling Cascades

Armel Hervé Nwabo Kamdje[1]*, Richard Tagne Simo[2] and Hetvet Paulain Dongmo Fogang[1]

[1]Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Garoua, Garoua, Cameroon

[2]Department of Biomedical Sciences, University of Ngaoundere, Cameroon

***Corresponding Author:** Armel Hervé Nwabo Kamdje, Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Garoua, Garoua, Cameroon.

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Abstract

Wnts are a family of evolutionary-conserved secreted signaling molecules and PI3K-Akt is an intracellular signal transduction. These signaling pathways play significant roles in stromal microenvironment control of the balance between hematopoietic stem cell self-renewal and differentiation. An increasing body of evidence also indicates Wnt and PI3K signaling involvement in the disruption of this balance in hematologic malignancies, where the stromal microenvironment niche favors the infiltration and homing of cancer cells in the bone marrow, as well as leukemia stem cell development and chemoresistance. In the present review, we summarize and discuss the role of the canonical Wnt and PI3K/AKT signaling pathways in normal hematopoiesis and hematologic malignancies, in regards to recent findings on stromal microenvironment involvement in these processes.

Keywords: Canonical Wnt Signaling Pathway; PI3K-Akt Signaling Pathway; Hematopoiesis; Hematologic Malignancies; Leukemia Stem Cells; Chemoresistance; Cancer

Abbreviations

HMs: Hematologic Malignancies; BM: Bone Marrow

Introduction

Wnts are a family of evolutionary-conserved secreted signaling molecules that play significant roles in embryonic development [1], bone remodeling [2], and normal blood cell formation [3,4]. Relatively recent studies have provided evidence in support of a major role of canonical Wnt signaling in neoplasia and in the pathogenesis of several cancers, including hematologic malignancies (HMs), cancer types affecting the blood, bone marrow (BM), and lymph nodes. Besides the reported overexpression of this Wnt signaling pathway, mutations in downstream pathway members such as β-catenin and in molecules involved in β-catenin regulation such as axin or APC (adenomatous polyposis coli) have been shown to contribute to aberrant signaling activation in HMs. In addition, other mechanisms favoring canonical Wnt signaling expression have been reported as well, including the silencing of negative regulators of that pathway such as Dkk (dickkopf) and WIF-1 (Wnt inhibitory factor 1) by promoter hypermethylation. Moreover, stromal cells have been implicated in the significant enhancement of Wnt signaling expression in the hematopoietic niche, as recent findings have shown for instance that their membrane-proximal signaling events involving leucine-rich

repeat-containing G-protein coupled receptor 5 (LGR5) and LGR4 [5] significantly enhance Wnt signaling in the niche [6]. As the Wnt signaling pathway, the PI3K-Akt pathway has been extensively studied in normal and malignant cells [7]. Given all these observations and considering also recent reports indicating that Wnt and PI3K signaling strength regulate normal hematopoiesis, and that the deregulation of this signaling pathway expression is involved in leukemogenesis and chemoresistance [8], in the present review, we discuss the role of canonical Wnt and PI3K/AKT signaling pathways in normal hematopoiesis and HMs in the bone marrow (BM) stromal microenvironment.

Wnt signaling pathways

Wnt pathways in Mammals are complex and involve at least 19 Wnt proteins, 10 receptors, and two co-receptors, with more than 10 intracellular signaling pathways proposed [3,4], and multiple modifying molecules. Wnt family molecules are classified as canonical and non-canonical pathway members. For instance, Wnt-1, -3a, -8 and -8b belong to the canonical pathway, whereas non-canonical Wnt include at least Wnt-5a and -11 [1,9]. Wnt proteins bind to the Fz (Frizzled)/ LRP (low density lipoprotein receptor-related protein) complex on target cell surface. Fz are seven-pass transmembrane receptors also classified according to their involvement in canonical (e.g. Fz-1, -8, -9) and non-canonical (e.g. Fz-2, -3, -4, -6) Wnt signaling pathways. Non-canonical Wnt signaling pathways mainly include the planar cell polarity pathway and the Wnt–Ca_2+ pathway, and are beyond the scope of the present treatise.

The canonical Wnt signaling pathway is mediated through β-catenin, a protein encoded in humans by the CTNNB1 gene. In absence of Wnts, cytoplasmic levels of β-catenin are kept very low by the action of a protein complex termed as "destruction complex", which actively phosphorylate β-catenin, resulting in protein sequestration in the cytoplasm and proteasomal degradation (Figure 1). This complex is made, at least in part, of negative regulatory kinases such as glycogen synthase kinase 3β (GSK-3β), anchor proteins such as axin-1 and -2 that also function as tumor suppressor proteins, and APC (adenomatous polyposis coli) protein. Upon Wnt ligation, the destruction complex break up, resulting in β-catenin accumulation in the cytoplasm and subsequent migration to the nucleus. In the nucleus, β-catenin binds to members of the TCF/LEF transcription factor family and converts them from transcriptional repressors into transcriptional activators (Figure 1) [3,10].

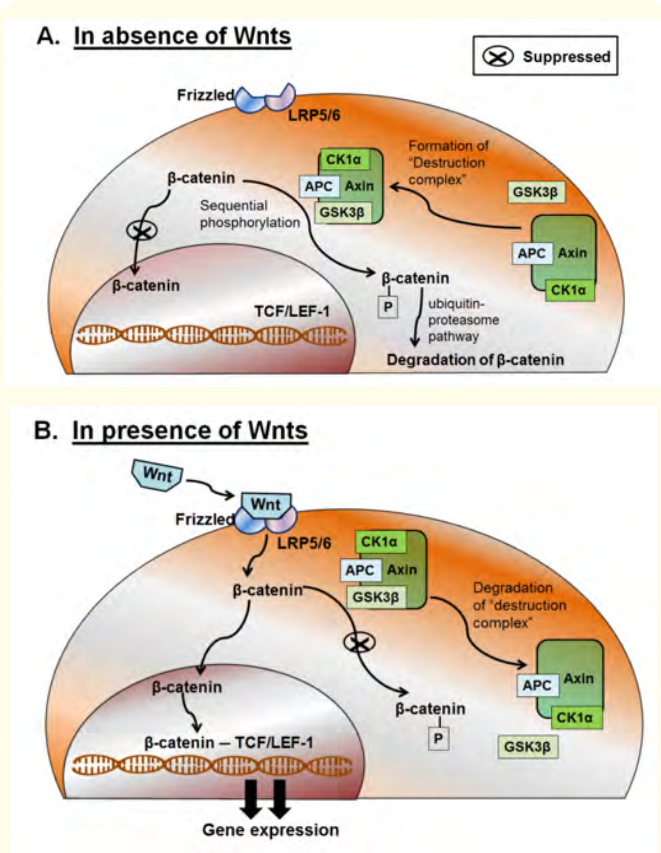

Figure 1: Canonical Wnt signaling pathway. In the absence of Wnt signaling (A), β-catenin is phosphorylated by a destruction complex made of GSK-3β, APC, and axin-1 proteins. Wnt signaling disrupts the destruction complex (B), enabling the dephosphorylation of β-catenin protein that functions then as a co-activator for the transcription factor TCF/LEF.

Physiologic roles of canonical Wnt signaling pathway: bone remodeling and hematopoiesis

Bone remodeling and repair

Canonical Wnts are major regulators of the bone mass. The canonical Wnt signaling pathway has been reported crucial roles in bone remodeling and repair. Wnts inhibit osteoclast formation through β-catenin-dependent modulation of OPG/RANKL/RANK

system, i.e. the final mediator of osteoclastogenesis [11]. As revealed by recent studies, Wnt signaling may also interact with transforming growth factor-β (TGF-β)/bone morphogenic protein (BMP) signaling on mesenchymal osteoblast progenitors and differentiated osteoblasts during osteoblast differentiation and bone formation [2,12,13]. Altogether, these Wnt signaling effects result in the suppression of bone resorption. Thus, particular attention has been given to canonical Wnt signaling in osteoblasts, as it may provide new therapeutic approaches for bone loss-associated diseases such as osteoporosis.

Bone marrow cells and hematopoiesis

Hematopoiesis is a continuous process in which progenitor cells develop into mature blood cells. In hematopoietic organs such as the spleen, hematopoietic populations can self-renew or generate mature blood cells due to the favoring microenvironment created by stromal cells, which provide cytokines, chemokines, adhesion molecules, and extracellular matrix molecules [14,15]. BM is the major hematopoietic site in the adult, and supports both myeloid and lymphoid lineage development [16,17]. Osteoblasts are important constituents of the BM niche capable of maintaining long-term stem cell activity and self-renewal [18]. Wnts stimulate osteoblast precursor growth and early events in osteoblast differentiation [19]. Staal and colleagues [3] hypothesized that Wnt signaling affects stromal components, at least in part, by regulating the architecture of the hematopoietic niche. These investigators observed lineage-committed progenitor dedifferentiation when exposed to mesenchymal stem cell (MSC) lines that express the canonical Wnt-3a gene. Recent studies have shown that MSCs overexpressing the Wnt-3a gene support hematopoiesis via Wnt-dependent production of extracellular matrix proteoglycans [20]. In addition, findings from in vitro studies have indicated that osteoblasts are potential sources of Wnts in the BM niche, as they express Wnt-1, -4, -7b and -14 [21]. Surprisingly, although early studies provided evidence for the influence of Wnt signaling pathways in the BM niche, the effects of this signaling pathway on niche cells such as osteoblasts, MSCs, endothelial cells, and hematopoietic stem cells (HSCs), as well as in hematopoiesis, have attracted the attention of the scientific community only recently [22].

Hematopoietic stem cell maintenance

Many reports have indicated crucial roles of Wnt signaling in the hematopoietic niche. It is widely accepted that canonical Wnt signaling pathway is a key player in the self-renewal of many stem cell types, including HSCs. Early studies showed that the overexpression of activated β-catenin expands the pool of HSCs in long-term cultures by both phenotype and function [3], suggesting that HCSs also respond to canonical Wnt signaling pathway in their normal microenvironment in vivo. Furthermore, experimental evidence indicates that Wnts are regulated in a dose-dependent fashion at key checkpoints in various lineages of the hematopoietic system [23]. The activation of canonical Wnt pathway results in the loss of HSC re-population ability, and in the blockade of multilineage differentiation [24]. It has been demonstrated, for example, that following Wnt expression human HSCs are retained, and B-cell development is inhibited [3]. In addition, fetal liver HSCs deficient for Wnt-3a completely loss canonical Wnt signaling and self-renewal ability [25], and adult HSCs that loss Wnt signaling following the overexpression of Wnt inhibitory protein Dkk-1 are also unable to self-renew [10]. Similarly, in a study where the Vav–Cre system has been used to achieve the deletion of β-catenin in HSCs, reduced self-renewal capacity has been observed as well [8].

Canonical Wnt signaling has been reported as crucial in the development of both myeloid and lymphoid cell lines [26]. However, reports from gain- and loss-of-function studies attempting to pinpoint the role of canonical Wnt signaling in HSC biology and hematopoiesis are controversial. For instance, whereas HSCs of transgenic mice lacking *Sfrp1* gene, a natural inhibitor of Wnt signaling pathways, has been reported to display niche-dependent self-renewal defects [27], transgenic mice lacking Wif1, another natural Wnt inhibitor, also display alterations in both the niche cells and HSCs, i.e. quiescence loss and self-renewal impairment, but these changes are observed only under stress conditions [28]. In addition, in transgenic mice lacking Wif1 the alterations has been associated with aberrant expressions of other signaling molecules in the niche, including cell fate determination major players like hedgehog and Notch. These observations provide strong evidence for a critical role of canonical Wnt signaling pathway in HSC biology, as well as in BM stromal cell function, but indicate also that mechanisms used by canonical Wnt signaling

to influence and control the normal hematopoietic niche are complex and, therefore, deserve further studies. Recent findings have also indicated cardinal roles of the canonical Wnt signaling in the BM niche aberrant behaviors characteristic of malignant hematopoiesis.

Wnt signaling and hematologic malignancies

An increasing body of evidence indicates that canonical Wnt signaling is required for the self-renewal of leukemia stem cells (LSCs), which may derive from both HSCs and more differentiated granulocyte-macrophage progenitors [29,30]. As other cancer stem cells, LSCs are capable of limitless self-renewal and are responsible for the maintenance of leukemia. BM microenvironment may contribute to deregulated signals that lead to the transformation of HSCs in LSCs.

BM niche and cancer cell homing

BM stroma is not just a bystander in HMs, as it has an active role in both clinical course and response to therapy of clonal HSC disorders such as myeloid neoplasms [31]. Other reports suggest that altered gene expression in osteoprogenitors induce BM dysfunction, with subsequent myelodysplasia and leukemia [30]. MSCs has been reported to protect infiltrating LSCs against chemotherapy-induced apoptosis [32,33]. And in addition, osteoblasts have been implicated in the fate of the leukemia blast, as these cells promote leukemic cell homing into the BM [26,34]. Several reports from various lymphoproliferative disorders indicate that BM infiltration is a common feature of HMs, and interestingly, specific patterns of infiltrating cells may characterize each disease [35,36].

Specific interactions of infiltrating LSCs with BM microenvironmental factors may determine the tissue architecture, but also the stromal organization, and fibrosis [35,36]. Such factors are numerous and include cytokines, chemokines, adhesion molecules, and endothelial factors. For instance, the expression of BM-homing molecules like the hyaluronan receptor (CD44) or the lectin CD22 in the absence of adhesion molecules such as P-selectin or E-selectin is not sufficient to insure a stable adhesion of infiltrating cells to the sinusoidal endothelium, resulting in the characteristic intrasinusoidal infiltration observed in many lymphomas and leukemias; conversely, HMs associated with the expression of a broad spectrum of adhesion molecules such as some lymphomas display nodules in the BM interstitium due to the spread of infiltrating cells. Overall, BM infiltration in low-grade lymphomas displays differences in the distribution of infiltrating and stromal cells with angiogenesis as pathogenetic hallmark; infiltrating cell aggregates seem to maintain contact, at least initially, with the BM stroma in follicular and lymphoplasmacytic lymphoma; whereas in marginal zone lymphomas and chronic lymphocytic leukemia, infiltrating aggregates tend to displace the pre-existing stroma [37].

In other HMs where malignant cells infiltrate the BM, such as non-Hodgkin lymphomas derived from germinal centers, a specific paratrabecular pattern is observed in the osteoblastic niche, with large infiltrates, megakaryocytic hyperplasia, and fibrosis in most cases [38-40]. Additional examples include follicular lymphoma, where infiltrating cell aggregates contain T-lymphocytes recruited through CCL17/CCL22 signaling [41], T-cell lymphomas of follicular derivation like angioimmunoblastic T-cell lymphoma [42], and B-cell malignancies such as T-cell/histiocyte-rich diffuse large B-cell lymphoma [43]. Interestingly, in these diseases the infiltrating cells share several signaling molecules and adhesion factors with the osteoblastic niche, such as integrin-β1 and Notch stromal ligands Delta-like-1 and Jagged-1 [44]. These cells also interact with the microvascular and stromal follicular dendritic cell networks [45,46]. From these observations, it appears that the BM stroma may condition the degree and pattern of cancer cell infiltration, and probably is a major player in neoplastic cell survival. In addition, crosstalks of neoplastic cells with BM niche cells probably allow the first to actively promote BM stroma modifications suitable for their maintenance and proliferation such as changes in the adhesion profile of the stromal meshwork, blood vessel formation, and changes in the expression of growth factors [47]. For instance, in xenograft models of B-cell and T-cell acute lymphoblastic leukemia, LSCs promote angiogenesis and modify the BM niche by expressing or inducing the expression of pro-angiogenic factors like vascular endothelial growth factor (VEGF), angiopoietins, basic fibroblast growth factor (bFGF), metalloproteinases, interleukin (IL)-6/-8 [48]. Moreover, whereas such leukemic niche is favorable to LSC maintenance, the HSC support and engraftment capabilities of this niche are drastically decreased because of a characteristic downregulation of CXCL12 expression [48].

MSC-HSC/cancer cell crosstalk and Wnt signaling

In vitro studies performed on MSC lines have indicated that Wnts mediate MSC-HSC crosstalk by producing extracellular matrix proteoglycans such as decorin that causes changes in niche shape upon binding to collagen and other proteoglycans [3]. An abnormal hematopoietic phenotype has been observed in decorin-deficient mice (Dcn-/-), with extramedullar hematopoiesis and increased numbers of Lin(-) Sca1(+) kit(-) CD150(+) cells, i.e. mouse equivalent of Lin(-) cKit(+) Sca1(+) CD34() CD135(-) HSCs. Given that MSCs provide hematopoietic support, in regards to the successful use of these cells in the treatment of various diseases, and considering that genetic modifications of MSCs dramatically alter their functions and differentiation properties [26], the use of genetically altered MSCs may represent a novel therapeutic approach in diseases associated with aberrant hematopoiesis, and accordingly deserves further investigation.

MSC anti-proliferative effects on blood cancer cell lines were reported. Zhu and collaborators [49] showed MSC ability to inhibit the proliferation of chronic myeloid leukemia K562 cell line in a humoral microenvironment, using CELLMAX artificial capillary modules that eliminate the immunosuppressive properties of MSCs, indicating that MSCs have a general inhibitory effect on their neighboring cells, even malignant ones. Interestingly, these investigators also observed that either Dkk-1 neutralization by anti-Dkk-1 antibodies, or Dkk-1 expression downregulation by RNA interference, result in the attenuation of MSC inhibitory effects on the proliferation of K562 cells, indicating that the secretion of this negative regulator of Wnt signaling accounts at least in part in MSC anti-proliferative effects. Other recent studies have reported that crosstalk between BM niche and myeloma cells triggers the release of Dkk1 and interleukin-6, which may play crucial roles in tumor progression and in the development of lytic bone disease in multiple myeloma [50]. Similarly, Wnt biology investigations have shown that Dkk-3 is genetically and epigenetically modulated in several human cancers [49,50], suggesting that this secreted Wnt antagonist and its regulators constitute novel therapeutic targets in these diseases. Besides, the canonical signaling molecule Wnt-4 has been reported to prevent the dexamethasone-induced upregulation of adipose differentiation-related aging markers in thymic epithelial cells [49,50], indicating a protective role of canonical Wnt signaling against senescence.

Additional links between BM stromal cell function and Wnt signaling has been provided by other recent reports, which have revealed a crucial role of canonical Wnt signaling in MSC glutamatergic marker gene expression [39]. Specifically, canonical Wnt signaling effects appeared to be mediated via the activation of the glutamatergic selector gene Tlx3 (T-cell leukemia 3), pointing out Tlx3 as a novel target for canonical Wnt signaling induction of sensory neuron phenotype in somatic stem cells upon neural induction.

Aberrant Wnt signaling and HM pathogenesis

Although in many cases the underlying mechanisms still are to be unraveled, over the last couple of years it has become clear that deregulated canonical Wnt signaling plays a key role in the development of HMs. Epigenetic changes in Wnt molecules and mutations resulting in the overexpression of Wnt signaling, including mutations in key Wnt-signaling molecules, have been reported, although, the functional effects of these changes during cancerogenic processes remain unclear [49,50]. Canonical Wnt pathway function is epigenetically regulated by the methylation of Wnt antagonists [50]. The inactivation of genes of the sFRP (secreted frizzled-related proteins) Wnt antagonist family by promoter hypermethylation has been found in acute myeloid leukemia and acute lymphoblastic leukemia [49,50]. The methylation of Wnt antagonists has been reported to have a prognostic relevance in acute myeloid leukemia [49,50]. In addition, small molecule inhibitors of canonical Wnt signaling have been shown to effectively induce apoptosis in acute myeloid leukemia cells [50], indicating that aberrant signaling through this pathway contribute to leukemogenesis. In addition, whereas in normal stem cell niche canonical Wnt signaling regulates hematopoiesis in a dose-dependent fashion by restricting self-renewal of HSCs and myeloid and T-lymphoid precursors [23], such regulation ability is lost in HM-affected niche, as observed for instance in acute myeloid leukemia where LSCs have undergone mutations rendering them independent of Wnt signaling [34].

Mutations of Wnt molecules have been reported in several HMs, and aberrant activations of Wnt signaling pathways have been implicated in the pathogenesis of leukemia [50]. In chronic myeloid leukemia caused by the [10,23] translocation that leads to the production of the abnormal BCR–ABL fusion protein, Wnt signaling is activated during blast crises, and the underlying mechanisms

accounting for the activation of such abnormal Wnt signaling are unknown. In acute lymphoblastic leukemia B-cell precursors, a GSK-3β inactivation associated with β-catenin activation was demonstrated [8]. Gelebart and colleagues [50] have reported that chronic myeloid leukemia tumors and cell lines highly and consistently express Wnt-3 and Wnt-10. These investigators also observed transcriptionally-activated β-catenin in more than half of the chronic myeloid leukemia cell lines and tumor examined, with concomitant expression of the phosphorylated/inactive form of GSK-3β (pGSK-3β). Although very rare in humans amino-terminal β-catenin mutations have been also reported in the disease experimental models [50]. In addition, clinical studies have shown a significant correlation of continuous pGSK-3β status with absolute lymphocyte count in the blood [50]. In these studies negative pGSK-3β expression has been associated with a longer survival of chronic myeloid leukemia cells, indicating a role of canonical Wnt pathway in tumorigenesis. The molecular mechanisms underlying these observations are unknown. Additional illustrations of canonical Wnt pathway involvement in the pathogenesis of HMs are provided for instance by reports from cutaneous lymphomas where frequently observed β-catenin overexpression may plays crucial roles in disease development [50], and extranodal marginal zone lymphoma where nuclear localization of β-catenin has been also detected [50]. Furthermore, Wnt genes are overexpressed in other neoplastic transformations of mature B cells, such as chronic lymphocytic leukemia, where the aberrant activation of Wnt signaling may contribute to defects in apoptosis that characterize the latter disease [49,50]. Similarly, canonical Wnt pathway effects on multiple myeloma cell biology have been reported to contribute to enhanced proliferative and metastatic properties of the malignancy [49,50].

Interestingly, β-catenin small interfering RNA treatment has been reported to successfully inhibit the growth of multiple myeloma tumors in a xenograft model [50], indicating that β-catenin represents an attractive target for the treatment of multiple myeloma. Yeung and colleagues [49,50] have recently reported that the inhibition of β-catenin suppresses the oncogenic potential of affected cells, suggesting that canonical Wnt signaling mediates the establishment of LSCs in mixed-lineage leukemia. The investigators have also observed that LSCs that had acquired resistance against GSK-3β inhibitor anti-cancer drugs have been re-sensitized after suppression of β-catenin expression, suggesting a crucial role of β-catenin in the chemoresistance of mixed-lineage leukemia cells. Comparable observations have been reported in other mixed-lineage leukemia preclinical models [49,50]. In in vitro studies performed on three lymphoma cell lines (OCI-LY8-LAM-53, SU-DHL- 4 and Raji) and a myeloma cell line (OPM-2), chemoresistant cancer cells entered in apoptosis following treatment with a combination of classical anticancer drugs with canonical Wnt pathway inhibitors such as ethacrynic acid (EA) or the antifungal agent ciclopiroxolamine [29]. In a conditional mouse model, Heidel and colleagues [49,50] have observed that associations of genetic inactivation of β-catenin with anticancer drug imatinib result in LSC death, and significantly delay disease recurrence after imatinib discontinuation. These effects where mimicked by pharmacologic inhibition of β-catenin via modulation of prostaglandin signaling; treatment with the cyclooxygenase inhibitor indomethacin reduced β-catenin levels and led to a reduction in leukemia cell population [49,50]. Similarly, stably integrated inducible RNA interference vectors have been reported to effectively suppress β-catenin expression and the transcriptional activity of β-catenin/TCF [49,50], rendering the cancer cells more sensitive to pro-apoptotic signaling molecules. Further investigations aiming at identifying the crucial components for chemoresistance along Wnt signaling pathways in HMs would provide new pharmacological targets for the treatment of these aggressive diseases.

Given the large amount of evidence indicating that aberrant activation of the canonical Wnt signaling accounts in the molecular bases of several leukemias, it has been hypothesized that Wnt signaling may confer to LSCs their stem cell properties, as observed for HSCs in embryos [49,50]. Thus, LSCs may have a higher requirement of Wnt activity than adult HSCs, raising the chances of success of pro-drugs specifically targeting Wnt/β-catenin-mediated transcriptional activity in leukemia. Such pro-drugs include for instance the pro-apoptotic small molecule CWP232291 presently entering in phase I clinical trial in acute myeloid leukemia and multiple myeloma. In vitro studies revealed CWP232291 anti-proliferative properties mediated through silencing of β-catenin target genes, whereas in vivo studies in acute myeloid leukemia models showed pro-drug ability to inhibit tumor progression [49,50]. It may be interesting to determine whether various HMs where Wnt signaling is involved have different and specific requirements of Wnt-signaling strength.

Wnt signaling and apoptosis

It has been reported that β-catenin activation is crucial for the development of cancer stem cells in various HMs, including LSCs of mixed-lineage leukemia [50] and treatment-insensitive BCR–ABL(+) chronic myeloid leukemia [45]. Surprisingly, despite the significant progresses made in the understanding of the canonical Wnt downstream signaling cascade, little is known about the pathway effects on cellular apoptosis. In a conditional mouse model of chronic myeloid leukemia for instance, fully developed LSC survival has not been affected by the suppression of β-catenin signaling [45-50], indicating that the anti-apoptotic effects of the canonical Wnt signaling originate from complex mechanisms that may also include indirect effects mediated via other signaling pathways. Recent studies have revealed that changes in Wnt signaling expression in the hematopoietic niche influence the fine-tuning of other pathways with proto-oncogene properties such as the hedgehog (Hh) and Notch pathways [28]. These cell apoptotic signaling pathways has been abundantly investigated during the last decade, and proved to be crucial in HM development [33], as well as in normal hematopoiesis, although with controversial roles. A body of evidence for interactions of canonical Wnt pathway with Hh and Notch signaling pathways has been found. For instance, the activation of Wnt signaling in HSCs cause increased expressions of the genes of the transcription factor HoxB4, previously implicated in self-renewal of HSCs, and of the transmembrane receptor Notch1 [45-50]. Genes involved in Hh and Notch pathways, such as PTEN-PI3K-AKT, NF-κB, and p53 show significant alterations in their expression following the knockout of β-catenin in HeLa cells [45-50]. It has been hypothesized that Wnt interactions with other signaling pathways occur through the regulation of the transcription of members of these pathways by the β-catenin/TCF/LEF complex, or through physical interactions with pathway members [45-50]. Thus, alterations in cardinal Wnt pathway would also affect other pathways implicated in the HSC self–renewal and quiescence. On the same hand, and interestingly, canonical Wnt signaling transcription factor LEF-1 has been recently reported as a pro-survival factor acting through β-catenin-dependent mechanisms in chronic lymphocytic leukemia [45-50]. Such mechanisms still are to be unraveled, but for instance, LEF-1 can function as an architectural context-dependent transcription factor and interact with alternative co-activator factors, including intracellular Notch [45-50]. Further investigations on the interactions of canonical Wnt signaling molecules with anti- and pro-apoptotic signaling pathways may provide better understanding of Wnt-mediated apoptosis evasion observed in presence of anticancer drugs.

Wnt signaling, cancer stemness, and malignant behavior

Canonical Wnt signaling has been reported to play a major role in the modulation of the delicate balance between stemness and differentiation in several adult stem cell niches such as the hair follicles in the skin, the mammary gland, and the intestinal crypt. Accordingly, constitutive Wnt signaling activation, resulting from mutations in genes encoding its downstream components, underlies tumorigenesis in these tissues. Stem cells are defined by their intrinsic capacity to self-renew and differentiate. Cancer stem cells retain both these features but without the homeostatic mechanisms that maintain normal cell numbers [45-50]. Identifying the signaling pathways that control the development of LSCs, for instance, may provide means for selective eradication of these malignant cells, and for a more effective treatment of leukemia and related diseases. The deletion of β-catenin during fetal HSC development results in the impairment of self-renewal [45-50], and β-catenin is crucial in HSC cell life in adults as well [45,50]. In a similarly way, Wnt signaling may confer self-renewal and other stem cell properties to LSCs -[]. In mouse models of acute myeloid leukemia induced either by co-expression of the oncogenes Hoxa9 and Meis1a or by the fusion of oncoprotein MLL-AF9, Wang and colleagues have recently shown that, as already mentioned, the canonical Wnt signaling is required for self-renewal of LSCs derived from either HSCs or more differentiated granulocyte-macrophage progenitors [29]. Given that the canonical Wnt signaling pathway is normally active in HSCs but not in granulocyte-macrophage progenitors, these results suggest that the reactivation of β-catenin signaling is required for the transformation of progenitor cells by these oncogenes. As β-catenin is not absolutely required for self-renewal of adult HSCs [29], the targeting of the canonical Wnt signaling pathway clearly represent a novel therapeutic target relatively safe against acute myeloid leukemia and related HMs. In addition, considering that most of the current anti-cancer drugs are not effective for specific targeting of cancer stem cells, therapy approaches specifically targeting aberrant Wnt signaling would be a way to directly affect LSCs, and may represent a drastic improvement to cancer treatment strategy.

In the endemic form of Burkitt's lymphoma (BL), Epstein-Barr Virus (EBV) is consistently detected. Increases in both free and total β-catenin have been reported in EBV -infected Burkitt's lymphoma cells compared to EBV-negative cells [45-50]. In addition, in the majority of sporadic colorectal cancer cases, the rate-limiting event is either the loss of antigen presenting cell function or the oncogenic β-catenin mutations [45-50]. However, although the presence of these mutations may predict nuclear β-catenin accumulation throughout the tumor mass, heterogeneous intracellular distributions of this signaling molecule are mainly observed within primary tumors and metastases. Moreover, tumor cells located at the invasive front and those migrating into the adjacent stromal tissues show β-catenin nuclear accumulation [45-50]. Hence, different levels of Wnt signaling activity probably reflect tumor heterogeneity, and are therefore likely to account for distinct cellular activities, i.e. proliferation and epithelial-mesenchymal transitions that respectively prompt tumor growth and malignant behavior. Several intrinsic and tumor microenvironment-dependent factors may explain such heterogeneity in canonical Wnt signaling activity within the tumor mass [45-50]. Illustrations in HMs include the observation of autocrine stimulations in T-cell malignancies like β-catenin mutation-originating abnormal Wnt pathway activation, not yet observed in acute and chronic myeloid leukemia [45-50]. Although beyond the scope of the present review, it must be mentioned that in chronic myeloid leukemia a non-canonical Wnt signaling pathway, i.e. Wnt/Calcium/NFAT signaling pathway, has been recently reported as crucial for the maintenance and chemoresistance of LSCs [45-50], further emphasizing the complexity of Wnt signaling in the BM niche.

Physiologic roles of PI3K/AKT signaling pathway

PI3K-Akt Pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth and angiogenesis in response to extracellular signals.

PI3K/Akt signaling and protein synthesis and cell growth

One of the best-conserved functions of Akt is its role in promoting cell growth through inhibition of TSC2 and indirectly activation of mTOR complex 1 (mTORC1). mTORC1 is a critical regulator of translation initiation and ribosome biogenesis and plays an evolutionarily conserved role in cell growth control. It can activate S6K and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), S6K can activate ribosomal S6 and promote the protein synthesis and cell growth [45-50].

PI3K/Akt signaling and cell proliferation

In general, the function of PI3K-AKT signaling pathway is to stimulate cell to proliferation and growth, and simultaneously inhibit cell apoptosis. Indeed, activated Akt protein modulates the function of numerous substrates related to the regulation of cell proliferation, such as glycogen synthase kinase-3 (GSK3), membrane translocation of the glucose transporter GLUT4, Cyclin-dependent kinase inhibitors, P21/Waf1/Cip1 and P27/Kip2, mammalian target of rapamycin (mTOR), and tuberous sclerosis complex 2 (TSC2) [45-50].

PI3K/Akt signaling and Cell apoptosis/Survival

Akt enhances the survival of cells by blocking the function of proapoptotic proteins and processes. Akt negatively regulates the function or expression of Bcl-2 family members, Bax protein, and Bim protein. Akt also inhibits the expression of BH3-only proteins through effects on transcription factors, such as FOXO and p53. p53 is also an oncogene that mediate cell apoptosis. Akt can promote the p53 degradation through phosphorylation of MDM2. It can also phosphorylates GSK3 isoforms on a highly conserved N-terminal regulatory site, and inactivates the kinase, so that regulate the apoptosis and glucose metabolism via GSK3 [45-50].

PI3K/Akt signaling and angiogenesis

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor whose effects on endothelial cells are mediated in part by the PI3K pathway. The VEGF ligands can bind to three type III receptor tyrosine kinases: VEGFRl/FLT1, VEGFR2/KDR, and VEGFR3/FLT4, of which VEGFR2 has the most important role in VEGF-induced angiogenesis. PI3K activation may occur via RAS mutation, by increased expression of growth factor receptors such as EGFR or by loss of PTEN, and can increase VEGF secretion. The PI3K/AKT pathway also modulates the expression of other angiogenic factors such as nitric oxide and angiopoietins [45-50].

PI3K-AKT signaling and hematologic malignancies

By counteracting the PI3K/AKT/mTOR pathway, PTEN plays an essential role in regulating hematopoietic stem cells (HSCs) self-renewal, migration, lineage commitment, and differentiation

[45-50]. As a matter of tact, Polak and Buitenhuis [45-50] have been reported the critical role of PI3K in both HSC maintenance and lineage development. However, this pathway is aberrantly upregulated in cancers including hematologic malignancies. Alterations in the components of PI3K/AKT signaling pathway have been observed in T-cell acute lymphoblastic leukemia (T-ALL), especially in the pediatric T- ALL. Approximately 29%–88% of human pediatric T-ALL patients have hyperactivated PI3K/AKT pathways, which could be the result of PTEN, PIK3R1, and AKT1 mutations [102]. In this cancer, recurrently affected genes deregulate pivotal cell processes, such as cycling (CDKN1B, RB1, TP53), signaling transduction (RAS pathway, IL7R/JAK/STAT, PI3K/AKT), epigenetics (PRC2 members, PHF6), and protein translation (RPL10, CNOT3). Moreover, PI3K-Akt-mTOR pathway is among one of the intracellular pathways aberrantly upregulated in cancers including acute myeloid leukemia [45-50].

Crosstalk between PI3K/Akt pathway and Wnt/β-catenin signaling pathway

Previous studies reported that no pathways exist independently. Although the Wnt/β-catenin and PI3K/Akt pathways have different carcinogenic mechanisms, they have been shown to be associated. The Wnt pathway is an important factor maintaining intestinal homeostasis; it regulates the self-renewal of stem cells and increases the proliferation of intestinal epithelial cells, and overactivation of the Wnt pathway may eventually lead to cancer. Some studies have shown an association between the Wnt/β-catenin and PI3K/Akt pathways in cancer. Wnt/ β-catenin pathway activation is mediated by phospholipase D1PLD1 (PLD1), which downregulates ICAT via the PI3K/Akt signaling axis.

Moreover, in breast cancer, activation of the PI3K/Akt pathway by Nectin-4 induces the activation of the Wnt pathway and then affects the proliferation of tumor stem cells, which is an important mechanism by which cancer stem cells achieve self-renewal. Communication between the Wnt/β-catenin and PI3K/Akt pathways has been observed in different types of human cancer, and PI3K/Akt pathway activation leads to Wnt/β-catenin pathway inhibition. In contrast, when the PI3K/Akt pathway is inhibited, the Wnt/β-catenin pathway is overactivated [45-50].

Concluding Remarks

Over the last decades, many investigators have attempted to mimic the hematopoietic niche signals in order to manipulate HSCs for clinical purposes [45-50]. In this long-standing and ongoing challenge the biology of induced pluripotent stem cells able to differentiate into blood cells have also been thoroughly investigated. Wnt and PI3K signaling molecules have been very attractive, because of their crucial roles in the self-renewal and maintenance of HSCs [45-50]. However, a major limitation has been the fact that alterations of Wnt and/or PI3K signaling expression result in drastic changes in the niche delicate balances and lead to immunodeficiency, autoimmunity, or HMs [45-50]. More specifically, in the latter diseases, the BM stroma is not an innocent bystander, as it favors neoplastic cell growth and maintenance in the evolving tumor microenvironment. Given the increasing body of evidence indicating a crucial role of canonical Wnt and PI3K signaling in these BM stroma effects, new therapeutic strategies specifically targeting aberrant Wnt and/or PI3K signaling are being explored in HMs and would drastically improve treatment outcomes. For instance, promising small-molecule inhibitors of Wnt and PI3K signaling are currently tested in various leukemia models. Further investigations are needed to translate this basic knowledge into clinical applications, and would aim at unraveling the molecular mechanisms of Wnt and PI3K signaling chemoresistance and the interactions of these pathways with other cell fate determining pathways such as hedgehog and Notch.

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All authors contributed equally to the design, preparation, and editing of the document. AHNK and HPDF were responsible for the final review and ethics approval.

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Bibliography

1. Li Z., *et al*. "The Amotl2 gene inhibits Wnt/beta-catenin signaling and regulates embryonic development in zebrafish". *Journal of Biological Chemistry* 287 (2012): 13005-13015.

2. Chen G., *et al*. "TGF-beta and BMP signaling in osteoblast differentiation and bone formation". *International Journal of Biological Sciences* 8 (2012): 272-288.

3. Yu S., *et al*. "The Wnt/β-catenin signaling pathway in Haematological Neoplasms". *Biomark Research* 10 (2022): 74.

4. Malhotra S and Kincade PW. "Wnt-related molecules and signaling pathway equilibrium in hematopoiesis". *Cell Stem Cell* 4 (2009): 27-36.

5. Barker N and Clevers H. "Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells". *Gastroenterology* 138 (2010): 1681-1696.

6. Carmon KS., *et al*. "R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling". *Proceedings of the National Academy of Sciences of the United States of America* 108 (2011): 11452-11457.

7. Kornblau SM., *et al*. "Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia". *Blood* 108 (2006): 2358-2365.

8. Luis TC., *et al*. "Wnt signaling strength regulates normal hematopoiesis and its deregulation is involved in leukemia development". *Leukemia* 26 (2012): 414-421.

9. Khan NI., *et al*. "Activation of Wnt/beta-catenin pathway mediates growth and survival in B-cell progenitor acute lymphoblastic leukaemia". *British Journal of Haematology* 138 (2007): 338-348.

10. Luis TC., *et al*. "Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation". *Blood* 113 (2009): 546-554.

11. McGrath EE. "OPG/RANKL/RANK pathway as a therapeutic target in cancer". *Journal of Thoracic Oncology* 6 (2011): 1468-1473.

12. Glass DA., *et al*. "Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation". *Development Cell* 8 (2005): 751-764.

13. Sabbagh Y., *et al*. "Repression of osteocyte Wnt/beta-catenin signaling is an early event in the progression of renal osteodystrophy". *The Journal of Bone and Mineral Research* (2012).

14. Lucas D. "Structural organization of the bone marrow and its role in hematopoiesis". *Current Opinion on Hematology* 28 (2021): 36-42.

15. Kokkaliaris K., *et al*. "Adult blood stem cell localization reflects the abundance of reported bone marrow niche cell types and their combinations". *Blood* 136 (2020): 2296-2307.

16. May M., *et al*. "Dynamic regulation of hematopoietic stem cells by bone marrow niches". *Current Stem Cell Reports* 4 (2018): 201-208.

17. Ding L., *et al*. "Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches". *Nature* 495 (2013): 231-235.

18. Calvi LM., *et al*. "Osteoblastic cells regulate the haematopoietic stem cell niche". *Nature* 425 (2003): 841-846.

19. Zhang J., *et al*. "Identification of the haematopoietic stem cell niche and control of the niche size". *Nature* 425 (2003): 836-841.

20. Santiago F., *et al*. "Noncanonical Wnt signaling promotes osteoclast differentiation and is facilitated by the human immunodeficiency virus protease inhibitor ritonavir". *Biochemical and Biophysical Research Communications* 417 (2012): 223-230.

21. Kato M., *et al*. "Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor". *Journal of Cell Biology* 157 (2002): 303-314.

22. Suda T and Arai F. "Wnt signaling in the niche". *Cell* 132 (2008): 729-730.

23. Luis TC., *et al*. "Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion". *Cell Stem Cell* 9 (2011): 345-356.

24. Kirstetter P., *et al*. "Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block". *Nature Immunology* 7 (2006): 1048-1056.

25. Fleming HE., *et al*. "Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo". *Cell Stem Cell* 2 (2008): 274-283.

26. Ichii M., *et al*. "The canonical Wnt pathway shapes niches supportive of hematopoietic stem/progenitor cells". *Blood* 119 (2012): 1683-1692.

27. Renstrom J., *et al*. "Secreted frizzled-related protein 1 extrinsically regulates cycling activity and maintenance of hematopoietic stem cells". *Cell Stem Cell* 5 (2009): 157-167.

28. Schaniel C., *et al*. "Wnt-inhibitory factor 1 dysregulation of the bone marrow niche exhausts hematopoietic stem cells". *Blood* 118 (2011): 2420-2429.

29. Schmidt M., *et al*. "Targeting Wnt pathway in lymphoma and myeloma cells". *British Journal of Haematology* 144 (2009): 796-798.

30. Raaijmakers MH., *et al*. "Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia". *Nature* 464 (2010): 852-857.

31. Tripodo C., *et al*. "The bone marrow stroma in hematological neoplasms--a guilty bystander". *Nature Reviews Clinical Oncology* 8 (2011): 456-466.

32. Iwamoto S., *et al*. "Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase". *Journal of Clinical Investigation* 117 (2007): 1049-1057.

33. Nwabo Kamdje AH., *et al*. "Notch-3 and Notch-4 signaling rescue from apoptosis human B-ALL cells in contact with human bone marrow-derived mesenchymal stromal cells". *Blood* 118 (2011): 380-389.

34. Lane SW., *et al*. "Differential niche and Wnt requirements during acute myeloid leukemia progression". *Blood* 118 (2011): 2849-2856.

35. Mishra A., *et al*. "Homing of cancer cells to the bone". *Cancer Microenvironment* 4 (2011): 221-235.

36. Fend F and Kremer M. "Diagnosis and classification of malignant lymphoma and related entities in the bone marrow trephine biopsy". *Pathobiology* 74 (2007): 133-143.

37. Vega F., *et al*. "The stromal composition of malignant lymphoid aggregates in bone marrow: variations in architecture and phenotype in different B-cell tumours". *British Journal of Haematology* 117 (2002): 569-576.

38. Roodman GD. "Mechanisms of bone metastasis". *The New England Journal of Medicine* 350 (2004): 1655-1664.

39. Kondo T., *et al*. "Wnt signaling promotes neuronal differentiation from mesenchymal stem cells through activation of Tlx3". *Stem Cells* 29 (2011): 836-846.

40. Guise TA., *et al*. "Basic Mechanisms Responsible for Osteolytic and Osteoblastic Bone Metastase". *Clinical Cancer Research* 12 (2006): 6213s-6216s.

41. Piccaluga PP., *et al*. "Gene expression analysis provides a potential rationale for revising the histological grading of follicular lymphomas". *Haematologica* 93 (2008): 1033-1038.

42. Burger M., *et al*. "Small peptide inhibitors of the CXCR4 chemokine receptor (CD184) antagonize the activation, migration, and antiapoptotic responses of CXCL12 in chronic lymphocytic leukemia B cells". *Blood* 106 (2005): 1824-1830.

43. Khokhar FA., *et al*. "Angioimmunoblastic T-cell lymphoma in bone marrow: a morphologic and immunophenotypic study". *Human Pathology* 41 (2010): 79-87.

44. Yoon SO., *et al*. "Notch ligands expressed by follicular dendritic cells protect germinal center B cells from apoptosis". *Journal of Immunology* 183 (2009): 352-358.

45. Takam KP., *et al*. "The Role of Notch and Wnt Signaling in MSC Communication in Normal and Leukemic Bone Marrow Niche". *Frontiers in Cell and Developmental Biology* 8 (2021): 599276.

46. Li Q., *et al*. "Potential roles of follicular dendritic cell-associated osteopontin in lymphoid follicle pathology and repair and in B cell regulation in HIV-1 and SIV infection". *The Journal of Infectious Diseases* 192 (2005): 1269-1276.

47. Ayala F., *et al*. "Contribution of bone microenvironment to leukemogenesis and leukemia progression". *Leukemia* 23 (2009): 2233-2241.

48. Colmone A., *et al*. "Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells". *Science* 322 (2008): 1861-1865.

49. Zhu Y., *et al*. "Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1". *Leukemia* 23 (2009): 925-933.

50. Gunn WG., *et al*. "A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma". *Stem Cells* 24 (2006): 986-991.