



Review Article

## The Role of Wnt/ $\beta$ -Catenin Signaling in Normal and Malignant Hematopoiesis

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### Abstract

The Wnt/ $\beta$ -catenin signaling plays indispensable roles in embryonic development and adult homeostasis. Through diverse mechanisms which are partially defined, abnormal activation of  $\beta$ -catenin occurs frequently in neoplastic disease. A critical function for Wnt/ $\beta$ -catenin in solid tumors, in particular colorectal cancer, has been well characterized. Increasing evidence indicates that this pathway is also deregulated in hematological malignancies. In acute myeloid leukemia, enhanced  $\beta$ -catenin levels are observed in a large proportion of patients, and correlates with increased blast clonogenicity and poor outcome. Similarly, in chronic myeloid leukemia (CML), Wnt/ $\beta$ -catenin activation is observed in advanced disease, and inhibition of  $\beta$ -catenin *in vivo* potentiates the effect of tyrosine kinase inhibitors in mouse models of CML. While studies suggest that  $\beta$ -catenin may be dispensable for steady-state maintenance of adult hematopoietic stem cells, a critical function for this pathway in regulation of specialized leukemic stem cells (LSC) is emerging. Here we review the currently understood mechanisms and roles of  $\beta$ -catenin in hematological malignancies, and describe the evidence outlining the contribution of  $\beta$ -catenin activation in mediating the self-renewal and drug-resistant properties of LSC. In addition, given the implication of LSC in disease relapse, we discuss current approaches and limitations of targeting the Wnt/ $\beta$ -catenin axis in the clinic.

### Keywords

Wnt/ $\beta$ -Catenin; Hematopoietic stem cell; Self-renewal; Leukemic stem cell; Myeloid leukemia; Drug-Resistance

### Introduction

The highly conserved canonical Wingless-type (Wnt)/ $\beta$ -catenin signaling pathway is a key mediator of proliferation, survival, differentiation and migration of cells during development, and plays a critical role in maintaining homeostasis in a range of adult tissues [1-3].  $\beta$ -catenin is a multi-functional protein; its role largely determined by its subcellular localization. At the plasma membrane  $\beta$ -catenin is an important component of the adherens junctions and links cadherin adhesion proteins to the actin cytoskeleton. A second, cytoplasmic pool of  $\beta$ -catenin, acts as the primary effector of the canonical Wnt signaling pathway [1,3-6].

Vitaly, disruptions in this pathway have been implicated in the

development of many diseases, most notably cancer [7-9]. In this article we outline the molecular regulation of the Wnt/ $\beta$ -catenin pathway, and review the current evidence supporting a role for activated  $\beta$ -catenin signaling in hematological malignancies with an emphasis on myeloid leukemia's. We highlight the varied mechanisms leading to  $\beta$ -catenin activation in this setting, and the accruing data indicating a crucial role for this pathway in the maintenance of leukemic stem cells and its associated significance in disease relapse and drug resistance.

### Molecular Regulation of the Canonical Wnt/ $\beta$ -catenin Pathway

In the absence of Wnt signaling, free cytoplasmic  $\beta$ -catenin is rapidly targeted for degradation by a multiprotein complex containing (i) the scaffold proteins axin and adenomatous polyposis coli (APC), and (ii) the serine/threonine kinases casein kinase 1  $\alpha$  (CK1 $\alpha$ ) and glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) [10,11]. After binding axin and APC,  $\beta$ -catenin is initially phosphorylated by CK1 $\alpha$  at Ser45, which primes  $\beta$ -catenin for subsequent GSK3 $\beta$ -dependent phosphorylation of Ser33, Ser37 and Thr41 residues [10,11]. Ser/Thr-phosphorylated  $\beta$ -catenin is then recognized by an E3 ubiquitin ligase complex containing  $\beta$ -transducin repeat containing protein ( $\beta$ -TrCP), which marks  $\beta$ -catenin for proteosomal mediated degradation (Figure 1A).

Upon binding to their cell-surface receptor, consisting of Frizzled (FZ) and the associated low-density lipoprotein receptor-related protein 5/6 (LRP 5/6), the cysteine-rich Wnt proteins induce the stabilization of  $\beta$ -catenin via the recruitment of axin and the inhibition of GSK3 $\beta$  (Figure 1B) [10,12]. Stabilized  $\beta$ -catenin then accumulates in the cytoplasm, and translocates to the nucleus where it interacts with members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) subfamily of transcription factors to induce expression of target genes including cyclin D1 and c-myc and fra-1 [1,2]. In the absence of  $\beta$ -catenin, the high-mobility group (HMG)-box containing TCF/LEF transcription factors associate with co-repressors such as Groucho and CtBP, and repress the expression of Wnt/ $\beta$ -catenin target genes. Binding of  $\beta$ -catenin to the N-terminal domain of TCF/LEFs, is thought to displace any bound co-repressors and recruit a series of transcriptional co-activators such as CREB-binding protein (CBP)/p300, pygopus (Pygo) and legless (LGS)/BCL9, hence resulting in an active transcription-promoting complex (Figure 1B) [10,11].

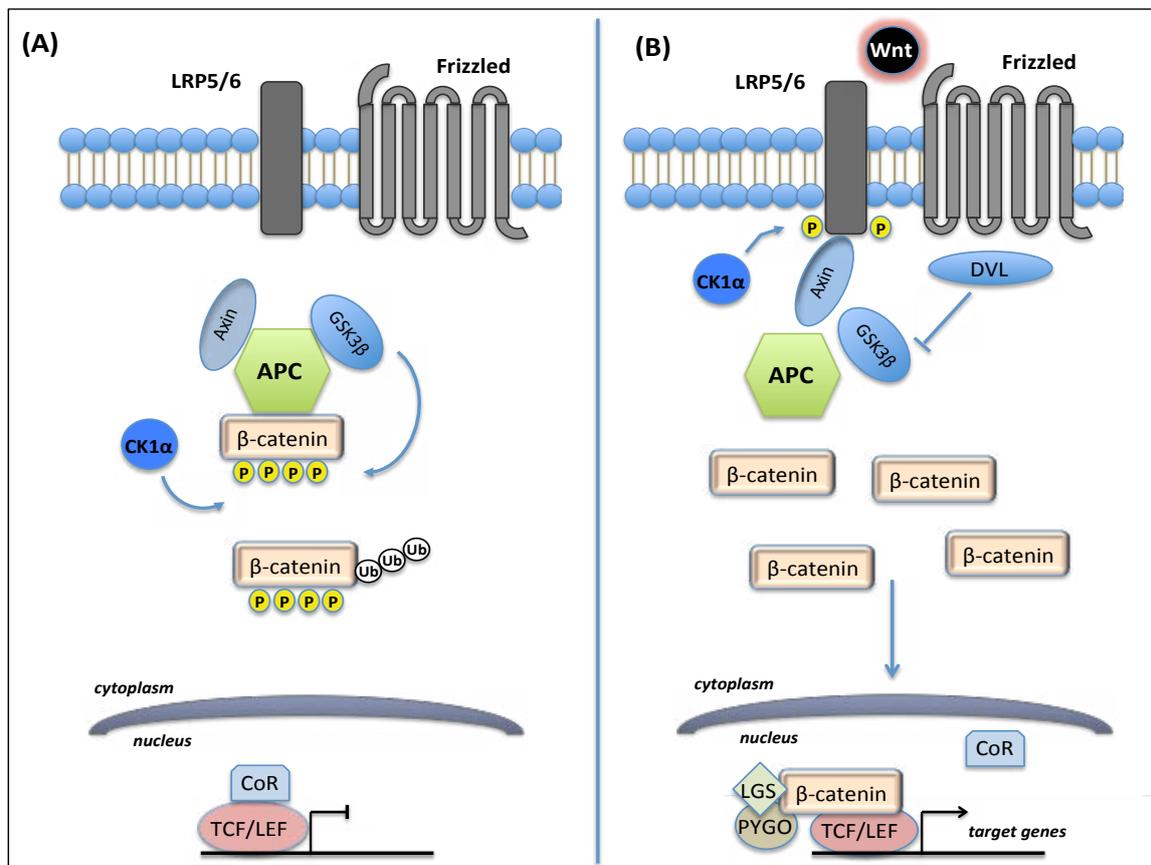
It is now evident, that in addition to the core factors mentioned above, there are many other cellular proteins that can directly or indirectly interact with  $\beta$ -catenin and other components of the Wnt pathway to modulate their localization and activity [10,13-15]. Based on the versatility of  $\beta$ -catenin as a transcription factor and its critical role in distinct cell types and developmental stages, it is likely that the expression and activity of the bio-molecular components regulating Wnt/ $\beta$ -catenin signaling display a marked diversity amongst tissues. Furthermore, normal or abnormal fluctuations in regulatory proteins and pathways may alter the intensity of  $\beta$ -catenin signaling and have a profound effect on the fate of a cell.

### Activation of $\beta$ -catenin in Cancer: A Focus on Hematological Malignancies

Consistent with a critical role for Wnt/ $\beta$ -catenin signaling in the

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Received: June 17, 2016 Accepted: June 24, 2016 Published: June 30, 2016



**Figure 1:** The canonical Wnt/ $\beta$ -catenin signaling pathway. (A) In the absence of Wnt signaling,  $\beta$ -catenin is phosphorylated in N-terminal Ser/Thr residues and marked for degradation by a regulatory complex containing axin, adenomatous polyposis coli (APC), and the serine/threonine kinases casein kinase 1  $\alpha$  (CK1 $\alpha$ ) and glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ). Binding of Wnt proteins to transmembrane Frizzled/LRP 5/6 receptors. (B) Induces the stabilization of  $\beta$ -catenin by inhibiting the  $\beta$ -catenin regulatory complex. Stabilized  $\beta$ -catenin then translocate to the nucleus where it interacts with HMG-box containing TCF/LEF transcription factors, and activates transcription of target genes via the displacement of co-repressors (CoR) and the recruitment of transcriptional co-activators such as pygopus (PYGO) and legless (LGS).

regulation of self-renewal, proliferation and differentiation of cells in stem-cell niches, including the skin and hair follicle, the mammary gland, the intestinal crypt and the bone marrow [1-3], aberrant activation of  $\beta$ -catenin signaling has been widely associated with tumorigenesis in these tissues [7,14,16-18].

The prototypic example of aberrant activation of Wnt/ $\beta$ -catenin signaling in neoplastic disease is colorectal cancer. Inactivating mutations in APC, which lead to the stabilization and accumulation of  $\beta$ -catenin, are observed in up to 85% of patients diagnosed with sporadic colorectal cancer [19,20]. Importantly, constitutive activation of  $\beta$ -catenin has now been recognized as the rate-limiting and initiating event of this disease [21,22]. The aberrant activation of  $\beta$ -catenin mediated transcriptional signaling in the intestinal crypt disturbs the tightly regulated balance between epithelial cell proliferation and self-renewal/differentiation paving the way for tumorigenesis. Remarkably, the small fractions of colorectal cancers which lack APC mutations have identifiable mutations in  $\beta$ -catenin which render the protein resistant to GSK3 $\beta$ -mediated phosphorylation and subsequent proteolytic degradation [22].

In addition, mutations in  $\beta$ -catenin and other components of the Wnt signaling pathway including axin and TCF4, all which result in constitutive  $\beta$ -catenin signaling, have been detected in a range of other cancers including hepatocellular carcinoma, melanoma, and gastric, and ovarian cancers [11,23]. Numerous studies have recently focused on the role of Wnt/ $\beta$ -catenin signaling in leukemia, and compounding evidence suggests that aberrant activation of this pathway may be a common event in aberrant hematopoiesis. Of interest, in contrast to solid cancers, mutations in  $\beta$ -catenin or its central regulatory proteins are rarely identified in leukemias [24-27] indicating that additional non-canonical mechanisms underlie  $\beta$ -catenin stabilization in this setting.

### A Role for $\beta$ -catenin in Normal Hematopoiesis

Initial evidence for a role of Wnt signaling in hematopoiesis came from a series of studies which demonstrated that exposure of primitive hematopoietic cells to Wnt ligands significantly increased their proliferation *in vitro* and maintained the immature phenotype of these cells [28-30]. Accordingly, Wnt ligands and receptors were shown to be expressed in the hematopoietic stem cells (HSC) and primitive hematopoietic progenitors of both mice and humans, and

these proteins are also expressed in the cells that make up the bone marrow microenvironment [29,31].

These early findings were extended by the work of Reya et al. [32], who directly examined the role of Wnt/ $\beta$ -catenin signaling in purified mouse HSC. In this study, HSC were isolated from mice overexpressing the anti-apoptotic Bcl-2 gene and retro virally transduced with a constitutively active form of  $\beta$ -catenin [32]. Overexpression of  $\beta$ -catenin extensively increased the proliferation of HSC *in vitro* and maintained the immature phenotype of HSC in long term cultures. In addition, cultured HSC expressing constitutively active  $\beta$ -catenin were capable of engrafting irradiated mice in contrast to the un-transduced counterparts. Similarly, increased proliferation and also the maintenance of an immature phenotype and function was observed in human and mouse HSC induced to express  $\beta$ -catenin by the inhibition of GSK3 $\beta$  [33]. Furthermore, using a TCF/LEF-1 reporter, it was shown that HSC normally activate Wnt/ $\beta$ -catenin signaling *in vivo* and that signaling is reduced as these stem cells give rise to more committed myeloid progenitor cells [34]. In accordance with this, enforced expression of constitutively active  $\beta$ -catenin in normal lymphoid or myeloid progenitors led to an increase in self-renewal and a block in differentiation [35], further supporting a role for  $\beta$ -catenin signaling in the maintenance of 'stem-like' properties. Lastly, activation of Wnt/ $\beta$ -catenin signaling in normal HSC was shown to up regulate the expression of transcription factors and cell-cycle regulators implicated in HSC self-renewal including Notch1 and HoxB4 [33,36].

While the studies described above support a role for Wnt/ $\beta$ -catenin signaling in HSC function, these investigations have mainly focused on the effects of elevated levels of  $\beta$ -catenin in the hematopoietic system. The reciprocal knockout studies however, have generated controversial results. The deletion of  $\beta$ -catenin, the  $\beta$ -catenin homologue  $\gamma$ -catenin (plakoglobin) or both  $\beta$ -catenin and  $\gamma$ -catenin in adult mice using Mx1-Cre showed no defects in hematopoiesis of lymphopoiesis [37,38], and similar observations were observed by an independent group [39]. These initial results led the authors to exclude a role for the canonical Wnt pathway in hematopoiesis. However, it was subsequently shown that TCF/LEF-dependent transcription remained intact in the combined absence of  $\beta$ -catenin and  $\gamma$ -catenin, and it has been suggested that partially functional  $\beta$ -catenin protein may persist in these animals [39,40].

In contrast, a study by Zhao et al. [41] which used Vav-Cre to induce  $\beta$ -catenin deletion in HSC, found that while the formation of HSC was normal in the absence of  $\beta$ -catenin, the self-renewal of these cells was significantly impaired when transplanted into recipient mice. Furthermore, Wnt3a deficiency, which correlates with reduced canonical Wnt signaling *in vivo*, led to a five-fold reduction in the numbers of HSC and progenitor cells in the fetal liver (FL) and a large reduction in self-renewal potential [42,43]. One explanation for the discrepancies between these knockouts studies described above may lie in the developmental stage at which  $\beta$ -catenin and/or  $\gamma$ -catenin genes were deleted. While the IFN-induced deletion of  $\beta$ -catenin or  $\gamma$ -catenin in the Mx1-Cre mice occurs in adulthood, the Vav-Cre system used by Zhao et al. drives the deletion of  $\beta$ -catenin from embryonic life. This suggests that a requirement for  $\beta$ -catenin may be pronounced in fetal stem cells in line with the strong demand for proliferation and expansion of this cell population, an effect which may be otherwise obscured in adults and during homeostasis. The idea that  $\beta$ -catenin may be critical in conditions where extensive self-renewal and proliferation are required, is also supported by the

observation that stem cell renewal defects in models with deficiencies in Wnt/ $\beta$ -catenin signaling were only apparent in secondary transplantation assays. It has also become clear that hematopoietic cells are highly sensitive to the 'signal strength' and relative levels of Wnt/ $\beta$ -catenin signaling, which likely accounts for some of the discrepancies observed in previous studies [44].

In addition to intrinsic mechanisms, extrinsic signals emanating from the microenvironment niche are crucial in the regulation of HSC self-renewal and quiescence [45-47]. The HSC niche is located within the bone marrow cavity and comprises osteoblasts, osteoclasts, stromal fibroblasts, and elements of the extracellular matrix, which synthesize or secrete a range of signaling molecules, growth factors and cytokines [47,48]. Recent studies have shown that Wnt/ $\beta$ -catenin signaling between HSC and the bone marrow microenvironment plays an important role in the functional maintenance of these stem cells and activation of Wnt/ $\beta$ -catenin in the bone marrow niche appears to be crucial for the preservation of HSC self-renewal and quiescence [49-51].

While more work is required to delineate the precise role of  $\beta$ -catenin in adult HSC, data from multiple perspectives support an important function for this pathway in hematopoiesis, and importantly, these studies in normal HSC development have prompted the exploration of Wnt/ $\beta$ -catenin pathway in the pathogenesis of leukemia. It is now clear, that the aberrant activation of  $\beta$ -catenin signaling occurs in a range of hematological malignancies, with emerging associations with maintenance of leukemic stem cells and drug resistance, and the evidence supporting a role for this pathway in the pathogenesis of leukemia is described in the remainder of this review.

### **$\beta$ -catenin Activation in Acute Myeloid Leukemia**

Despite substantial advances in the diagnosis of the different subtypes of Acute Myeloid Leukemia (AML) and the development of therapeutics, AML remains particularly difficult to treat. This is largely due to the immense phenotypic and genetic heterogeneity associated with the disease. The current overall success rate for AML treatment still remains low with 5-year survival rates between 10-70% depending on the leukemia subtype. To date, over 200 chromosomal aberrations including chromosomal translocations and deletions, as well as a wide range of karyotypic abnormalities and cryptic lesions affecting proteins such as cell surface receptors, intracellular signaling proteins and transcription factors, have been linked with the development and progression of AML [52-54]. The prognosis and genetics of AML are tightly linked, and pre-treatment karyotype has long been recognized as the most important independent predictor of clinical outcome in this disease [55-58]. Furthermore, differential treatment of certain molecular subgroups appears to markedly improve prognosis [59-61], emphasizing the importance of understanding the genetics underlying AML for the development of strategic and more proficient therapeutics.

Early studies on primary AML samples revealed that these cells express significantly higher levels of  $\beta$ -catenin mRNA and protein than normal hematopoietic progenitors [62] and studies on AML cohorts, have similarly shown that constitutively active  $\beta$ -catenin can be detected in a large proportion (~60%) of patients [24,63]. Moreover, aberrant expression of additional Wnt-signaling components including Wnt-1, Wnt-2b and LEF-1 are observed in the majority of these cases indicative of a positive feedback loop [24]. Following is an elegant study by Ysebaert et al. [63] demonstrated that increased

$\beta$ -catenin expression in primary AML cells correlates with enhanced clonogenic and self-renewal capacity, and poor patient prognosis. Subsequent studies on large patient cohorts further documented expression of  $\beta$ -catenin as an important prognostic factor in AML, and overexpression of this signaling molecule is associated with both shortened relapse-free survival and poor overall survival [63-65], suggesting that AML associated with aberrant  $\beta$ -catenin signaling may represent a particularly aggressive disease.

AML is organized in a hierarchy of functionally distinct cells which arises from a small pool of specialized leukemic stem cells (LSC), which have the idiosyncratic ability to self-renew [66,67]. Despite the long-term proliferative capacity of LSC which drives continual expansion of the population of malignant cells, these cells remain largely quiescent allowing them to evade traditional chemotherapy which targets rapidly dividing tumor cells [37-39]. The resistance of these potent LSC to chemotherapy and customary cancer therapeutics is likely to account for the high rates of disease recurrence associated with AML, and targeted eradication of these stem cells will be crucial for permanent elimination of the disease and improved overall outcome. In line with this, the LSC frequency at diagnosis has been shown as a relevant prognostic factor, as AML patients with increased frequencies of CD34<sup>+</sup>CD38<sup>-</sup> LSC display significantly higher levels of minimal residual disease and decreased long term survival [68,69].

Although the ability to self-renew is believed to be essential for the leukemic potential of LSC, the molecular pathways which regulate this process are still being understood. Nonetheless, the sustained expansion of the leukemic clone suggests that self-renewal may be deregulated in LSC, and transplantation studies have indicated that LSC possess significantly higher self-renewal potential than normal HSC [66].

Critically, genome-wide expression analysis comparing the profiles of highly enriched HCS and AML LSC identified Wnt/ $\beta$ -catenin as a key pathway deregulated in AML LSC [70], and consistent with an active role in the LSC compartment, imaging flow cytometry confirmed increased  $\beta$ -catenin nuclear localization occurs in AML LSC compared to normal progenitors [71]. More recently, numerous comprehensive studies indicate that  $\beta$ -catenin activity may be particularly critical for the maintenance and drug-resistant properties of AML LSC in patients with mixed-lineage leukemia (MLL) translocations [72,73]. MLL-fusions can aberrantly activate Wnt/ $\beta$ -catenin in HCS and more committed progenitors, and recent evidence suggests this may be in part through regulation of the G protein-coupled receptor GPR84 [74]. *In vivo* mouse experiments have further shown that  $\beta$ -catenin is critical in leukemic transformation of granulocyte macrophage (GM) progenitors driven by Meis1/HoxA9 or MLL-AF9 [72], and inducible deletion of  $\beta$ -catenin in MLL pre-LSC impedes leukemia development in syngeneic mice, indicating that  $\beta$ -catenin is not only essential for the long-term maintenance of LSC, but also for the establishment of these cells *in vivo* [73]. MLL-AML represents a subset of patients with exceptionally poor outcome, and there has been increasing interest in understanding the pathogenesis of this disease since the demonstration that certain MLL fusion proteins possess the ability to confer stem cell renewal properties to normal myeloid progenitors [75,76]. *In vitro* analysis of primary AML samples further supports a critical role for  $\beta$ -catenin in the self-renewal potential of MLL LSC, and introduces a novel window for therapeutic targeting of LSC in patients with MLL leukemia [73].

In addition to a key role in MLL-driven AML, it is clear that a range of mechanisms may underlie the activation of  $\beta$ -catenin in other AML subtypes, and that normal Wnt regulatory elements may be bypassed or altered in disease development. The oncogenic fusion proteins AML1-ETO t (8;21) and PML-RAR $\alpha$  t (15;17), and the close structural and functional homolog of  $\beta$ -catenin,  $\gamma$ -catenin, have also been associated with increased  $\beta$ -catenin activity in leukemic cells [77,78]. Furthermore, activating internal tandem duplication (ITD) mutations in the FLT3 receptor can induce  $\beta$ -catenin tyrosine phosphorylation and nuclear translocation [79,80] and we have previously shown that signaling downstream of the interleukin-3 receptor promotes  $\beta$ -catenin stabilization in primary AML cells [81]. Abnormal promoter methylation of specific Wnt/ $\beta$ -catenin inhibitors has also been reported in myeloid malignancies and correlates with a poor prognosis in patients [82], highlighting that  $\beta$ -catenin activation is a common downstream target of a variety of AML associated lesions (Table 1), and posing  $\beta$ -catenin as an attractive therapeutic target in this disease.

### $\beta$ -catenin Activation in Chronic Myeloid Leukemia

The aberrant activation of Wnt/ $\beta$ -catenin has also been implicated in the development and progression of Chronic Myeloid Leukemia (CML). In contrast to the complex molecular genetics of AML, CML displays an overt and well-defined pathology and is consistently associated with the t (9;22) chromosomal translocation and the presence of the BCR-ABL1 oncoprotein which drives the malignant expansion of leukemic cells [83].

CML originates as an indolent disease, and its initial chronic phase is characterized by the BCR-ABL1-mediated hyperproliferation of transformed myeloid cells without a distinctive block in differentiation. In the absence of treatment, or failed response to tyrosine kinase inhibitors (TKIs), CML progresses through an accelerated phase to blast crisis (BC) [84]. BC is strongly associated with the upregulation of BCR-ABL1 mRNA and protein, and the prominent expansion of committed GM precursors, which unlike normal GM progenitors, display self-renewal capacity *in vitro* [85]. Importantly,  $\beta$ -catenin levels are significantly increased during the transition to BC, and nuclear  $\beta$ -catenin signaling is linked to the enhanced self-renewal and leukemic potential of these GM precursors [85].

Evidence suggests that BCR-ABL can directly interact with  $\beta$ -catenin and promote its stabilization and nuclear accumulation [26], and silencing of  $\beta$ -catenin by targeted siRNAs reduces the proliferation and clonogenicity of Bcr-Abl<sup>+</sup> CML cells *in vitro* indicating a functional dependency of Bcr-Abl on  $\beta$ -catenin activation [26]. It is not clear, however, whether the accumulation of  $\beta$ -catenin observed in BC is a direct result of increased BCR-ABL1 levels; and interestingly, missplicing of GSK3 $\beta$  in BC GM progenitors has been linked to increased  $\beta$ -catenin expression in these cells [86], suggesting that multiple mechanisms may contribute to the stabilization of  $\beta$ -catenin in CML.

In line with *in vitro* data, Zhao et al. [41] demonstrated that  $\beta$ -catenin signaling is required for the progression of Bcr-Abl mediated CML *in vivo*. In this study conditional  $\beta$ -catenin<sup>-/-</sup> mice were generated using the Cre/LoxP system under the control of *vav* regulatory elements, to induce the deletion of  $\beta$ -catenin predominantly in the hematopoietic system. In contrast to control Bcr-Abl<sup>+</sup> CML leukemic cells which generated leukemia in the

**Table 1:** Lesions and pathways leading to activation of  $\beta$ -catenin in leukaemia.

Mediator of $\beta$ -catenin activation	Disease	Cell type	Contributing mechanism/s	Ref
MLL-AF9 (and other MLL fusions)	AML	mHSC, mGMP	upregulation of GPR84	[72-74]
FLT3-ITD	AML	primary AML cells, m/h cell lines	tyrosine phosphorylation of $\beta$ -catenin	[79,80]
AML1-ETO	AML	mHSC, m/h cell lines	upregulation of COX-2	[77,110]
PML-RAR $\alpha$	AML	m/h cell lines	unknown	[77]
IL-3 signaling	AML	primary AML cells, h cell lines	unknown	[81]
Wnt antagonist downregulation	AML	primary AML cells, h cell lines	Wnt antagonist promoter methylation	[82]
$\gamma$ -catenin	AML	primary AML cells, h cell lines	unknown	[78]
Bcr-Abl	CML	primary CML cells, h cell lines	tyrosine phosphorylation of $\beta$ -catenin	[26]
Inactivation of GSK3 $\beta$	CML	primary CML cells	missplicing of GSK3 $\beta$	[86]
c-Kit mutations	MCL	h cell lines	tyrosine phosphorylation of $\beta$ -catenin	[103]

**Note:** GMP: granulocyte-macrophage progenitor; h: human; m: mouse; MCL: mast cell leukaemia.

majority of recipient mice following transplantation, leukemic cells from  $\beta$ -catenin $^{-/-}$  mice failed to establish CML *in vivo*. Consistent with a role in self-renewal, subsequent analysis revealed that loss of  $\beta$ -catenin prevented CML development by reducing the long-term propagation of these cells [41]. Paradoxically while deletion of  $\beta$ -catenin blocked CML formation, Bcr-Abl $^{+}$  leukemic cells from  $\beta$ -catenin $^{-/-}$  mice were capable of inducing acute lymphoblastic leukemia (ALL) *in vivo*, indicating a distinct requirement for  $\beta$ -catenin downstream of Bcr-Abl in myeloid leukemia, possibly a reflection of differing cell-of-origin and resulting transcriptional contexts.

To further determine whether  $\beta$ -catenin plays a role in maintenance of the CML phenotype, and whether  $\beta$ -catenin expression in CML LSC may impact response to TKI, Heidel et al. [87] performed similar experiments using a tamoxifen inducible model to allow deletion of  $\beta$ -catenin following disease establishment *in vivo*. While there was no difference in disease latency in primary recipients in which genetic deletion of  $\beta$ -catenin was induced following CML onset, these mice had a marked reduction in Bcr-Abl $^{+}$  LSC, and displayed impaired disease propagation in sequential transplantation experiments. This is consistent with a key role for  $\beta$ -catenin in the maintenance of self-renewal in this population. Supporting the view that TKI treatment may not fully eradicate the leukemic clone due to the persistence of a small LSC pool which is inherently resistant to these targeted therapies, genetic inactivation of  $\beta$ -catenin resulted in delayed recurrence of CML after imatinib discontinuation, and similarly pharmacological inhibition of  $\beta$ -catenin potentiated the therapeutic effect of imatinib *in vivo*, resulting in a significant reduction in disease burden.

In line with a potential role for  $\beta$ -catenin activation in clinical response to TKI, gene expression studies on CML patient cohorts have shown that chronic phase patients who fail front line imatinib therapy have increased expression of Wnt/ $\beta$ -catenin signaling networks, and gene-expression profiles more similar to those with advanced disease [88,89].

Increased  $\beta$ -catenin signaling has also been associated with acquired secondary acquired resistance to TKI *in vitro* and *in vivo*, in part due to enhanced leukemic cell survival and self-renewal [90-92]. Together with overexpression of BCR-ABL1 and kinase-domain mutations which circumvent TKI binding, an increase in drug efflux has been well documented as a player in resistance to BCR-ABL1 inhibition, and intriguingly, there is some evidence that  $\beta$ -catenin activation may also contribute to TKI resistance by inducing expression of the ABCB1 drug transporter in leukemic cells [90,93,94].

### $\beta$ -catenin Activation in Lymphoid Neoplasms and Other Hematological Malignancies

In addition to a role for aberrantly activated Wnt/ $\beta$ -catenin in the pathogenesis of myeloid leukemias described above, emerging studies suggest that  $\beta$ -catenin may also be implicated in lymphoid neoplasia. Activation of Wnt/ $\beta$ -catenin in both B- and T- cell acute lymphoblastic leukemia (ALL), has been associated with the expansion of these malignancies, and the upregulation of canonical pathway genes including Wnt16, Frizzled 3 and TCF-3 is commonly observed in these leukemias [95-97]. More in-depth studies have recently shown that activated Wnt/ $\beta$ -catenin signaling is enriched in the LSC fraction in T-ALL, and cooperates with Notch-1 to maintain LSC self-renewal and survival, in part via the upregulation of MYC [98,99]. Importantly, and similar to studies in AML and CML, the deletion of inhibition of  $\beta$ -catenin in *in vivo* models of T-ALL demonstrates a detrimental effect on LSC frequency and impaired transplantation potential in secondary xenografts [99,100]. Consistent with these findings, overexpression studies have shown that ectopic activation of  $\beta$ -catenin in mouse thymocytes blocks normal T-cell development at the double-positive stage, induces a leukemic phenotype and is associated with increased genomic instability [101,102].

Aberrant  $\beta$ -catenin signaling has also been linked to activating mutations in c-Kit in mast cell-leukemia, which were shown to induce tyrosine-phosphorylation an nuclear accumulation of  $\beta$ -catenin [103] and, several reports have demonstrated increased  $\beta$ -catenin signaling in patients with chronic lymphocytic leukemia [104,105]. The precise role  $\beta$ -catenin in these neoplasms, however, is still been understood.

Lastly, the overexpression of  $\beta$ -catenin has been documented in multiple myeloma (MM) [25,106], a hematological malignancy characterized by the overproduction of malignant secretory plasma cells within the bone marrow. Supporting a role in the development of this disease, blocking  $\beta$ -catenin transcriptional activity in MM cells led to a decrease growth and survival *in vitro* [25,107], and siRNA-mediated inhibition of  $\beta$ -catenin *in vivo* was shown to reduce MM progression in a xenograft model [106]. More work is now required to understand the mechanisms which may induce  $\beta$ -catenin activation in this cancer.

### Targeting the Wnt/ $\beta$ -catenin Pathway in Leukemia: The Achilles Heel of LSC?

The widespread action of oncogenic Wnt/ $\beta$ -catenin signaling in diverse hematological malignancies, and its resounding role in LSC maintenance, particularly in AML, CML and T-ALL has postured

targeting of this pathway as an attractive therapeutic strategy in leukemia.

The activation of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by its upstream regulator cyclooxygenase (COX) has been previously shown to induce the stabilization of  $\beta$ -catenin in colon cancer, HSC and leukemic cells suggesting this may be an active mechanism for  $\beta$ -catenin regulation in disease development [108-110]. Fittingly, treatment of MLL-AF9 induced leukemia with the COX2 inhibitor indomethacin effectively reduced  $\beta$ -catenin levels, and LSC frequency in treated mice, and of interest, it was observed that the therapeutic effect of  $\beta$ -catenin inhibition by indomethacin was greater in the setting of minimal disease as compared to treatment of more extensive disease, and may be more efficient in combination with chemotherapy or TKI treatment [72,87]. While it is not clear whether this pathway may be functional across all AML subtypes, similar results have been recapitulated using indomethacin and other COX inhibitors in pre-clinical models of AML1-ETO driven AML [110], and CML [87].

The development of Wnt/ $\beta$ -catenin targeting drugs has been a challenging task in part due to the complexities of the signaling components and the pleiotropic nature of this pathway in a range of biological processes; however the broad involvement of this pathway in cancer has fostered intensive efforts towards development of therapeutics targeting  $\beta$ -catenin. The use of relevant pathway members as target structures, and high throughput screening of small molecule compound libraries against cells transduced with Wnt reporters, have led to the development of a range of natural and synthetic inhibitors [111-113]. In addition to a potential role for COX inhibitors and other NSAIDs, developed therapeutic strategies include: (a) neutralizing monoclonal antibodies and peptides targeting Wnt receptors or ligands (b) small molecule inhibitors which activate/stabilize negative regulators of  $\beta$ -catenin including pyriminyl a selective activator of CK1 $\alpha$ , and tankyrase inhibitors such as XAV939 which result in stabilization of axin and (c) compounds that interfere with  $\beta$ -catenin associated transcription factors and co-activators, most notably PRI-724 (also named ICG-001) and iCRT3, iCRT5, iCRT14 which block the interaction of  $\beta$ -catenin with CBP and TCF respectively.

Many of these compounds have shown varying degrees of effectiveness in pre-clinical models of solid tumors such as colorectal, breast, prostate glioblastoma and pancreatic cancer, and a number of drugs have reached Phase I clinical trials in these diseases [111-113]. These trials will provide critical insight into the side effects, drug interactions and clinical efficacy of Wnt/ $\beta$ -catenin inhibition, and provide a strong platform for subsequent trials which if successful may have important clinical implications, in particular for patients with advanced disease. Promisingly, recent studies suggest that undesirable effects on normal tissue homeostasis resulting from Wnt/ $\beta$ -catenin inhibition could be reversible [114-116], and could hence be minimized assuring proper dosing and scheduling of Wnt inhibitors.

The use of Wnt/ $\beta$ -catenin inhibitors in hematological malignancies has been less characterized, however studies over the last few years demonstrate a potential role for some newly developed blocking compounds including the tankyrase inhibitor XAV939 and iCRT drugs in *in vitro* and *in vivo* models of myeloid and lymphoid leukemia [98,99,117,118]. In addition, a recent report by Ma et al. [119] describes the identification and characterization of a novel dual FLT3 and  $\beta$ -catenin inhibitor, SKLB-677 which suppressed AML LSC potential *in vitro*, and warrants further exploration for

Wnt/ $\beta$ -catenin combination therapy in leukemia. Although lagging behind trials in solid tumors, a Phase I/II dose escalation clinical trial (NCT01606579) exploring the tolerability and primary efficacy of PRI-724 in patients with advanced myeloid malignancies is underway and is expected to be completed by December 2016. Encouragingly, PRI-724 has reached a Phase II trial of combination chemotherapy in patients with newly diagnosed metastatic colorectal cancer following an acceptable safety profile in a preceding trial [120], although the efficacy for this compound in disease regression is still to be fully determined. Nonetheless, blocking Wnt/ $\beta$ -catenin in leukemia remains an exciting prospect for patients with relapsed/refractory disease.

Lastly, given the range of non-canonical lesions associated with  $\beta$ -catenin accumulation in leukemia described in this review and summarized in Table 1, further understanding of the molecular mechanisms underlying these processes may provide additional avenues for disease-specific targeting of the  $\beta$ -catenin axis in hematological malignancies.

## Concluding Remarks

The Wnt/ $\beta$ -catenin pathway is a highly conserved network which plays a fundamental role during development, and also in the maintenance of homeostasis across adult tissues. The essential role  $\beta$ -catenin in maintenance of self-renewal in normal stem cell populations has been extensively explored in matching neoplastic disease, and aberrant activation of this pathway has been established as a recurrent event in solid cancers and hematological neoplasms.

To date a range on 'non-canonical' mechanisms, largely converging on activated fusion proteins and receptors/kinases have been associated with the aberrant accumulation of  $\beta$ -catenin in leukemia, however more work is still required to fully understand some of the key underlying regulatory pathways in this setting. Importantly, the deletion or pharmacological inhibition of Wnt/ $\beta$ -catenin in *in vivo* mouse models of myeloid and lymphoid leukemia correlates with a significant reduction in LSC numbers, and impaired transplantability in secondary recipients. Consistent with a critical role for this pathway in LSC integrity, evidence suggests that the combination of  $\beta$ -catenin inactivation with standard therapies, which primarily target the leukemia population 'bulk' but spare the quiescent LSC pool, can markedly reduce leukemic burden and delay disease reoccurrence.

While clinical trials investigating the efficacy of Wnt/ $\beta$ -catenin inhibitors in hematological malignancies are still in their infancy, strong evidence from pre-clinical studies provide promising expectation that pharmacologic inhibitors of this pathway may improve clinical outcomes, particularly for patients with refractory or relapsed disease following conventional therapies.

## Conflict of Interest Statement

The material contained in this manuscript has not been previously published and has not been submitted for publication elsewhere. All authors concur with the submission of this manuscript, and declare no conflict of interest.

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