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The canonical Wnt signaling pathway plays an important role in lymphopoiesis and hematopoiesis

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Abstract

The evolutionarily conserved canonical Wnt- β -catenin-T cell factor (TCF)/lymphocyte enhancer binding factor (LEF) signaling pathway regulates key checkpoints in the development of various tissues. Therefore, it is not surprising that a large body of gain-of-function and loss-of-function studies implicate Wnt- β -catenin signaling in lymphopoiesis and hematopoiesis. In contrast, recent papers have reported that Mx-Cre-mediated conditional deletion of β -catenin and/or its homolog γ -catenin (plakoglobin) did not impair hematopoiesis or lymphopoiesis. However, these studies also report that TCF reporter activity remains active in β -catenin- and γ -catenin-deficient hematopoietic stem cells and all cells derived from these precursors, indicating that the canonical Wnt signaling pathway was not abrogated. Therefore, these studies in fact show that the canonical Wnt signaling pathway is important in hematopoiesis and lymphopoiesis, even though the molecular basis for the induction of the reporter activity is currently unknown. In this perspective, we provide a broad background to the field with a discussion of the available data and create a framework within which the available and future studies may be evaluated.

Keywords

Development; Stem cell; Thymus; Wnt signaling

Introduction

The Wnt family of lipid-modified secreted factors consists of 19 family members that regulate the so-called canonical and non-canonical Wnt signaling pathways. Here we will largely limit our discussion to the canonical Wnt pathway. In the absence of a Wnt ligand, β -catenin is found in a cytoplasmic ‘destruction complex’ where it is phosphorylated and degraded by ubiquitin-mediated mechanisms [1,2] (Fig. 1). When a Wnt protein binds to the receptor complex consisting of a member of the frizzled family of seven transmembrane proteins and a low-density lipoprotein receptor-related protein (LRP5 or LRP6), β -catenin is no longer phosphorylated and not targeted for degradation. This results in accumulation of the N-

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Note added in proof: The article cited in the text by Hossain *et al.* as “in press” has the following reference: Hossain, M. Z., Yu, Q., Xu, M. and Sen J. M., ICAT expression disrupts β -catenin-TCF interactions and impairs survival of thymocytes and activated nature T cells. *International Immunology*. 2008. DOI 10.1093/intimm/dxn051

terminally dephosphorylated β -catenin protein and its translocation to the nucleus [3]. In the nucleus, β -catenin regulates gene expression in cooperation with members of the T cell factor (TCF) and lymphocyte enhancer binding factor (LEF) family of transcription factors [4]. β -Catenin also functions at the cell surface in association with α -catenin and the cadherin family of proteins [5].

Many recent reviews have described the Wnt- β -catenin-TCF/LEF signaling cascade in the context of lymphopoiesis and/or hematopoiesis [2,6–15]. In this perspective we will assess the available evidence for and against a role for the canonical Wnt- β -catenin-TCF/LEF signaling pathway in the development of hematopoietic stem cells (HSC) and lymphocytes with the aim of providing a framework in which existing controversies can be evaluated.

We note that transcription factors TCF and LEF were both cloned from lymphocytes [16,17] and have been shown to play a role in both T and B cells [18–20]. However, it was only when TCF was shown to be directly down-stream of the Wnt- β -catenin signaling pathway [21–23] that the question of signaling to TCF and LEF *via* Wnt and β -catenin in hematopoiesis and lymphopoiesis became pertinent. It is important to acknowledge that the field is still struggling to find a resolution, 10 years after the question was first raised.

Wnt- β -catenin-TCF signaling in lymphopoiesis

Normal lymphocyte development

Lymphoid cells develop from bone marrow-derived HSC. T cells mature in the thymus while B cells develop in the bone marrow. Progression of T cell development in the thymus is commonly defined by the expression of cell surface markers CD4 and CD8. CD4[−]CD8[−] double-negative (DN) thymocytes mature to the CD4⁺CD8⁺ double-positive (DP) stage and finally to the CD4⁺CD8[−] or CD4[−]CD8⁺ single positive (SP) stage [24]. B cell maturation is defined by the cell surface expression of markers such as B220 and CD19. Pro-B cells develop into pre-B (CD19⁺ surface IgM[−]) cells followed by transitional cell intermediates and mature B cells [25]. The development of both T and B cells is under strict molecular control of various transcription factors that are at least in part controlled by extracellular signals [24,26].

Loss-of-function manipulations in the thymus

Tcf1-deficient mice demonstrate multiple defects at DN stages of T cell development and a block at the immature SP stage, which increases in severity with age [19,27]. *Lef1* partially compensates for *Tcf* function because double deficiency results in an acute block in T cell development at the immature SP stage [18]. The first evidence for a role for Wnt and β -catenin in signaling to TCF in the immune system was provided by Staal and Clevers [28]. These authors demonstrated that loss of Wnt- β -catenin-TCF signaling, by the introduction of soluble frizzled receptors as decoys for Wnt proteins, significantly inhibited thymocyte differentiation in fetal thymic organ cultures. Under these conditions thymocyte proliferation was significantly affected. Subsequently, Staal and colleagues [11] directly demonstrated TCF reporter activity in thymocytes. Accordingly, Send and colleagues found that thymuses in Wnt1 $\times$ Wnt4 double-deficient mice showed low thymic cellularity [29]. Because TCF and LEF also act as transcriptional repressors, the requirement of Wnt and co-factor β -catenin to convert the repressor activity of TCF/LEF transcription factors into transcriptional activators supported the notion that canonical Wnt signaling may provide crucial proliferative signals to developing thymocytes [24,27,29].

Additional loss-of-function studies include manipulating the expression of naturally occurring inhibitors of the Wnt signaling pathway. Jenkinson and colleagues [30] showed that increased expression of the naturally occurring inhibitor of β -catenin and TCF interaction (ICAT), which inhibits Wnt signaling by preventing binding of β -catenin to TCF, blocked the transition of

DN thymocytes to the DP stage in thymic organ cultures. Similarly, the secreted Wnt inhibitor DKK1, which blocks binding of Wnt to LRP coreceptor thereby preventing Wnt signaling, inhibits thymocyte differentiation at the DN stage. The DN stage is further divided into four stages (DN1–4) that bear a precursor-product relationship. Weerkamp *et al.* [11] showed that high levels of DKK1 led to complete inhibition of T cell development at the earliest DN1 stage. Finally, Sen and colleagues [31] demonstrated that T cell-specific deletion of β -catenin using Lck-Cre resulted in impaired maturation of DN3 cells to the DP stage of T cell development and reduced proliferation of pre-DP thymocytes. It is important to note that the deletion at the DN3 stage was incomplete; therefore the phenotype is likely to underestimate the function of β -catenin in DN thymocytes [31].

The differences in phenotype between *Tcf1*-deficient and β -catenin (*ctnnb*)-deficient thymocytes may be explained by considering the following possibilities. First, deletion of *Tcf* in the HSC may allow for functional compensation in the cells that populate the thymus while deletion of β -catenin in DN3 cells may not allow the compensatory pathways to be activated in time for efficient development. Second, both TCF and β -catenin function in cooperation with other proteins and the differences in phenotype between the two knockout mice may point towards the influence of other pathways. In other words, a simple one-on-one relationship between these two factors may not reflect the complexity *in vivo*. Collectively, these studies suggest a role for Wnt- β -catenin-TCF signaling during thymocyte maturation.

Gain-of-function manipulations in thymocytes

Activation of the canonical Wnt pathway by enforced expression of stabilized β -catenin also demonstrated a role for β -catenin at multiple stages of T cell development. It is important to note that the outcomes varied depending on the level of β -catenin protein accumulation and the stage of thymocyte development when it was first stabilized. Gounari, von Boehmer and colleagues [32] showed that high levels of β -catenin expression, generated by deletion of exon 3 sequences that encode the glycogen synthase kinase-3 β phosphorylation domain, bypassed pre-TCR signals and induced generation of TCR β^+ DP thymocytes in a RAG2-deficient background. Expression of stabilized β -catenin also induced expression of proliferation-associated genes in immature DN thymocytes [33]. Thus, gain-of-function studies and loss-of-function [31] studies in DN thymocytes show concordant results, and demonstrate that Wnt- β -catenin signaling regulates thymocyte proliferation and β -selection at immature stages of intrathymic T cell development.

DP thymocytes, which are downstream of these early intrathymic progenitors, also benefit from TCF- β -catenin interactions. TCF-deficient DP thymocytes are prone to apoptosis which can be rescued by transgenic expression of full-length TCF but not by a mutant TCF unable to bind β -catenin [34]. Similar observations were reported with over-expression of stabilized β -catenin specifically at the DP stage using CD4 promoter [35]. More recently, Sen and colleagues (Hossain *et al.*, *Int. Immunol.* in press) have demonstrated that transgenic over-expression of ICAT, which impedes β -catenin-TCF interactions, impairs DP cell survival due to diminished expression of Bcl-x_L, suggesting that interaction between the two transcriptional partners is essential for the expression of Bcl-x_L. Together these data indicate an important function for β -catenin and TCF in the survival of DP thymocytes.

The maturation of DP thymocytes into SP thymocytes is accompanied by commitment to the CD4 or CD8 SP lineage and positive selection of committed cells that ensures the generation of adequate numbers of self MHC-restricted T cells. Sun and colleagues [36] showed that β -catenin and TCF controlled, in part, expression of the CD4 gene. In transgenic mice expressing stabilized β -catenin, two aspects of positive selection of thymocytes were regulated by β -catenin. First, β -catenin expression changed the timing of positive selection of CD8 SP thymocytes, such that the kinetics of CD4 and CD8 SP thymocyte generation was

synchronized, in contrast to normal mice, where generation of CD8 SP thymocytes lag behind CD4 SP thymocytes [37]. Second, β -catenin expression augmented IL-7R signaling during positive selection, thereby enhancing the development of CD8 SP thymocytes [38]. Together these studies show that DP and SP thymocytes can utilize β -catenin for survival and maturation.

In addition, moderate over-expression of β -catenin from the Lck promoter accelerated thymic involution [39]. At a mechanistic level, this was due to induction of oncogene-induced senescence, growth arrest and apoptosis upon expression of oncogenic (stabilized) β -catenin in thymocytes [40]. In contrast, when β -catenin was specifically over-expressed in DP thymocytes, where intrathymic signals down-regulate β -catenin expression, c-Myc-dependent lymphomas developed [41]. Together these data suggest that when intrathymic signals induce stabilization of β -catenin, as a part of the developmental signaling process, 'safety' mechanisms remove cells that fail to down-regulate oncogenic β -catenin and have lymphomagenic potential [40]. Taken together with the observation that intrathymic signals regulate the abundance of β -catenin in a developmentally significant manner [11,37], these manipulations suggest a role for β -catenin signaling at multiple stages of thymocyte development.

Studies on Wnt signaling in B lymphocytes

Several studies support the notion that the canonical Wnt signaling pathway regulates aspects of B cell development. *Lef1*-deficient mice have a mild block in fetal, but not adult, B lymphopoiesis and show defects in B cell proliferation [20]. Mice deficient for the Wnt receptor frizzled-9 show a specific defect in the clonal expansion of pre-B cells during development as well as an accumulation with age of plasma cells in the lymph nodes [42]. Wnt5a signaling through the non-canonical Wnt/Ca signaling pathway negatively regulated B cell proliferation. Moreover, deletion of Wnt5a gene results in B cell lymphomas in mice and humans, suggesting that it works as a tumor suppressor [43]. In addition, treatment of human B cell progenitors in stromal cell co-culture assays with Wnt3a negatively regulated cell proliferation [44]. Together these studies suggest a role for the Wnt signaling pathway in B cell development.

Wnt- β -catenin-TCF/LEF signaling in HSC biology

Seminal work by Weissman, Nusse and coworkers [12,45], using TCF-GFP reporters, demonstrated that Wnt- β -catenin-TCF/LEF signaling was active in HSC. Wnt signaling was also documented in HSC *in vitro* after treatment with purified Wnt3a [1,45]. These data suggest that the Wnt- β -catenin-TCF/LEF signaling pathway is active during proliferation of HSC. These investigators also showed that retroviral expression of a constitutively active form of β -catenin in murine Bcl-2-transgenic HSC led to an increase in the numbers of HSC with enhanced ability to reconstitute lethally irradiated recipient mice [1,45]. Conversely, retroviral expression of the Wnt signaling inhibitor Axin in the same system showed reduced reconstitution. These studies suggested that increased signaling from the canonical Wnt pathway led to increased hematopoiesis and lymphopoiesis.

Recently, these findings have come under scrutiny due to reports from the laboratories of Achim Leutz [46] and Claus Nerlov [47]. These studies, also using a gain-of-function approach, showed that constitutive activation of β -catenin impaired multilineage differentiation and caused exhaustion of the HSC pool, after a transient increase in the numbers of HSC. In addition, data from the laboratory of Paul Kincade [48,49] indicated that expression of stabilized β -catenin in HSC slowed and even reversed differentiation of committed hematopoietic cells. Taken together, these results show that stabilization of β -catenin affects HSC in a variety of ways and underscores the importance of the correct level of Wnt signaling. We suggest that additional experimental manipulations will reveal mechanistic aspects of Wnt- β -catenin-TCF/LEF signaling and thereby clarify the role of this pathway in HSC biology.

The controversy on the role of β - and/or γ -catenin-deficiency

As outlined above, many studies from several laboratories suggest that components of Wnt- β -catenin-TCF/LEF signaling pathway impact HSC, hematopoiesis and lymphopoiesis. Therefore it was surprising that HSC, in which β -catenin was deleted using Mx1-Cre, when transplanted in irradiated mice, showed no defects in hematopoiesis or lymphopoiesis [50]. Freddy Radtke and coworkers [50] postulated that a β -catenin homologue, γ -catenin (plakoglobin), compensated for the lack of β -catenin. Redundancy between γ - and β -catenin has often been suggested; however, some important differences between these proteins have also been noted. The pattern of expression of the two proteins is distinct; γ -catenin binds TCF/LEF factors at an overlapping but distinct site from β -catenin binding site and the two proteins activate expression of a non-overlapping set of target genes. Furthermore, Avri Ben-Ze'ev and coworkers [51] have demonstrated that γ -catenin is a weak activator of TCF/LEF-dependent transcription and is unlikely to compensate for β -catenin. Finally, the non-immune phenotypes of γ -catenin- or β -catenin-deficient mice argue against a compensatory role for these proteins.

Nevertheless, this possibility was investigated by deleting β -catenin using Mx-Cre, in γ -catenin-deficient HSC, and transplanting double-deleted HSC into recipient mice. Hematopoiesis and lymphopoiesis proceeded normally, leading the authors to exclude a role for Wnt signaling in both hematopoiesis and lymphopoiesis [52]. The same approach to delete both β - and γ -catenin was also taken by Held and colleagues [53] with similar results. However, using reporter assays that had previously been used to demonstrate TCF/LEF transcriptional activity [11,45], Held and colleagues [53] documented that TCF reporter activity remained intact when both β - and γ -catenin were deleted. In light of these data the authors suggested that additional β -catenin-like proteins could substitute for β - and γ -catenin in the HSC.

In contrast, a recent report using Vav-Cre to delete β -catenin in HSC, showed that while the formation of HSC was normal in the absence of β -catenin, HSC self renewal, especially when extensive self renewal was required, was impaired [54]. In addition, as yet unpublished data from Staal and colleagues (Luis, T., Staal, F. J. T. *et al.*, submitted) show that Wnt3a-deficient mice have fivefold lower numbers of HSC with diminished self renewal, suggesting that Wnt signals regulate HSC self renewal. Understandably, in light of these disparate reports the role of Wnt signaling in HSC biology has become highly controversial.

While we do not have concrete answers to the controversies we have documented above, we have some suggestions that may be worth considering. One explanation for the maintenance of TCF reporter activity, but a lack of functional defect, may be due to the function of 52-kD protein produced by the deleted β -catenin allele after deletion of the floxed region [55]. This protein is expressed in thymocytes as well as mature Tcells after Cre-mediated deletion of the floxed gene. Indeed, cells with one deleted and one intact allele show two bands on a Western blot that react with anti- β -catenin antibody: one corresponding to the full-length β -catenin and the other to the truncated β -catenin (Sen, J. M. *et al.*, unpublished observation). The truncated β -catenin retains partial binding site for TCF and complete binding sites for chromatin remodeling protein BRG1 and histone acetyltransferase CBP/p300. If the major function of β -catenin in hematopoiesis and lymphopoiesis is to recruit these proteins to the DNA to facilitate gene expression, the 52-kD protein might be sufficient for these functions. Direct assessment of this possibility is essential before making final evaluation of the importance of β -catenin in hematopoiesis and lymphopoiesis.

Another explanation may lie in the developmental stage at which β -catenin and/or γ -catenin genes are deleted. HSC that delete β -catenin and/or γ -catenin genes may experience disadvantages that are overcome by alternate pathways that allow hematopoiesis and lymphopoiesis to proceed. In this case the outcome of the experiments would reveal no role

for the deleted proteins. In contrast, the fate of precursor cells that develop in the presence of β -catenin until they arrive in the thymus, undertake the T cell developmental program and then delete β -catenin after commitment to the T lineage may be very different. β -Catenin-deleted DN3–DN4 cells may not be able to activate compensatory signaling pathways and therefore may fail to develop efficiently. In this setting, when β -catenin is deleted in T lineage-committed cells, experiments would reveal stalled T cell development.

In conclusion, data from many laboratories (Fig. 2) point to an important function for the canonical Wnt signaling pathway in hematopoiesis and lymphopoiesis, although some studies observed no functional defects when key mediators of this pathway were eliminated. Because the overall evidence points to a role for the canonical Wnt signaling pathway in HSC and lymphocyte biology, we encourage further investigation with the aim of resolving these issues to a satisfactory end.

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Abbreviations

DN	double-negative
DP	double-positive
ICAT	inhibitor of β catenin and TCF
LEF	lymphocyte enhancer binding factor
LRP	low-density lipoprotein receptor-related protein
SP	single-positive
TCF	T cell factor

Figure 1.

The canonical Wnt signaling pathway. (Left) In the absence of Wnt binding, β -catenin is sequestered in the cytoplasm by Axin and adenomous polyposis coli (APC). In this 'destruction complex', β -catenin is phosphorylated by serine/threonine kinases casein kinase (CK) and glycogen synthase kinase (GSK)3 β . Phosphorylated β -catenin is recognized by β -transducin repeat-containing protein (β -TRCP), targeted for ubiquitination and degraded by the proteasome pathway. In the absence of β -catenin, TCF family transcription factors bind co-repressors of the groucho family. (Right) Wnt proteins bind to a receptor complex consisting of a frizzled receptor (Fz) and LRP5 or LRP6. Wnt signals are transduced to the destruction complex via as yet unknown pathway involving Dishevelled (DSH). DSH may directly interact with Fz and LRP may interact with Axin. Signaling inhibits GSK3 β activity and β -catenin degradation. Accumulated β -catenin translocates to the nucleus to activate gene transcription in conjunction with TCF/LEF. γ -Catenin (plakoglobin), a structural homolog of β -catenin, also binds TCF/LEF transcription factors but is believed to provide a weaker transcriptional activation domain compared to β -catenin. This figure is adapted from the article by Weerkamp and colleagues [63].

Figure 2.

Overview of reports documenting Wnt signaling in T cell development and HSC biology.

References with a green bullet provide evidence favoring a role, while those labeled with a red bullet provide evidence against a role for canonical Wnt signaling.