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Significance of Wnt Signaling Pathway in the Pathogenesis of Acute Myeloid Leukemia

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ABSTRACT

Hematopoietic stem cells (HSCs) are responsible for the generation of various blood cell lineages, characterized by self-renewal property. Deregulation and differentiation of blood cells leads to leukemogenesis. In the present review, we mark out the emerging role of Wnt signaling as a critical regulator of distinct aspects of self-renewal and differentiation and importance of targeting the pathway to inhibit leukemia development. Aberrant activation of Wnt signaling and downstream effectors have been demonstrated in Acute Myeloid Leukemia (AML). Moreover, the chimeric transcription factors such as promyelocytic leukemia- retinoic acid receptor-α (PML-RARAα), promyelocytic zink finger protein (PLZF-RARα) and AML-ETO that are observed to play role in AML which induce downstream Wnt signaling events. Various studies suggest that AML associated fusion proteins contribute to leukemogenesis by enhancing self-renewal and inducing plakoglobin expression, activating the Wnt signaling pathway. The canonical Wnt pathway could be shown to be of major importance in pathogenesis of AML and seems to be a promising treatment strategy in AML

Background

A rare population of multipotent cells found in the adult bone marrow (BM) is responsible for continuous production of blood cells called Hematopoietic Stem Cells (HSCs) through the process hematopoiesis including platelets, erythrocytes, and all leukocytes throughout life (Orkin et al, 2008 [1]). HSCs have self-renewal type proliferation that undergoes a stepwise loss of multi-lineage potential and become progressively committed to a single hematopoietic lineage and thereby into fully mature blood cells (Luice et al, 2012 [2]). These properties of HSCS such as self-renewal, differentiation, proliferation, apoptosis and senescence of HSC are regulated by various pathways including Wnt, Notch, Hedgehog, TGFβ/SMAD and several others. Of these, Wnt (Wingless-Int)/β-catenin pathway is a crucial pathway which controls development and cell fate determination (Luice et al, 2012 [2])and by far the best characterized. The role of Wnt signaling in normal hematopoiesis and lymphocyte development is important and has been covered extensively in a number of recent reviews (William et al, 2013; Staal et al, 2008; Staal et al, 2008 [3-5]). Wnt/β-catenin pathway is shown to be dysregulated in many types of cancers and hence could provide an excellent therapeutic target (Valkenburg et al, 2011; Borah et al,2015; Dahmani et al, 2011 [6-8]). In recent years, knowledge about the role of Wnt signaling in hematopoiesis and leukemia has increased (Luice et al, 2012 [9]). A better understanding of this pathway pave the way to developing better therapeutic approaches for hematologic malignancies (William et al, 2013 [3]). Therefore, this review will recapitulate the contribution of Wnt/β-catenin pathway in cancer progression with a focus on AML.

AML is a clonal disease resulting from a malignant transformation of a hematopoietic stem or progenitor cell. It is characterized by an abnormal accumulation of hematopoietic progenitor cells leading to progressive insufficiency of normal hematopoiesis. There are different genetic causes resulting in variable clinical courses of AML that include cytogenetic abnormalities, gene mutations, deletions of certain chromosomes or abnormal karyotype making molecular causes of the disease highly heterogenous.

Cytogenetic abnormalities in AML

Cytogenetic abnormalities can be detected in approximately 50% to 60% of newly diagnosed AML patients (Kumar et al., 2016 [10]). The most of the cases of AML are associated with nonrandom chromosomal translocations that often result in gene arrangements. Cytogenetics

is the most important prognostic factor for predicting remission rate, relapse, and overall survival. Several chromosomal abnormalities such as monosomies or deletions of part or all of chromosomes 5 or 7 (–5/–7) and trisomy 8 are common in AML (Kumar et al, 2016 [10]). The chromosomal abnormalities also balanced translocations such as t(8;21), t(15;17), t(11;17), t(9;11); and inv(16)shows the most frequent chromosomal aberrations and their corresponding fusion genes in AML [10]. The most frequent onco fusion proteins such as AML1-ETO (Licht et al, 2001 [11]), PML-RARα and PLZF-RARα have the role in the pathogenesis of AML (Grignani et al, 1998 [12]).

Further, the mutations comprise alterations in myeloid transcription factors and activating mutations of signal transduction intermediates leading to inappropriate gene expression and aberrant signal transduction, respectively. Both mechanisms are highly interdependent, resulting in reduced apoptosis, increased stem cell self-renewal and blocked differentiation of AML cells (Steffen et al. 2005 [13]). The majority of AML patients still cannot be cured inspite of major progress in the treatment during the last couple of years. Thus, new therapeutic strategies are essential owing to high mortality rates and high relapse rates even among transplanted patients (Mawad et al, 2013 [14]). The Wnt/ β-catenin pathway has been shown to play an essential role in the development of AML by regulating the cell proliferation, differentiation, and apoptosis of HSCs (Gilliland et al, 2004; Lapidot et al,1994; Okuhashi et al, 2011 [15,16,17]). Thus, Wnt/ β-catenin signaling molecules can be attractive candidates for developing novel targeted therapies for this disease (Gilliland et al., 2004; Lapidot et al., 1994; Okuhashi et al., 2011; Lane et al., 2011; Mochmann et al., 2011 [15-19]).

Wnt signaling pathway

The Wnt pathway is a group of signal transduction pathways made of proteins that pass signals into a cell through cell surface receptors. Wnt pathway is initiated by evolutionarily conserved growth factors of the Wnt family. Wnts are encoded by 19 different wingless and integration site growth factor (Wnt) genes that share a high degree of sequence homology (Swarup et al, 2012 [20]). They bind to cell surface receptors Frizzled (Fzd) to activate the Wnt pathway, thus initiating the signaling cascades that are crucial in many physiological settings (Clevers et al, 2012 [21]). Wnt signaling pathway actively functions in embryonic development and helps in homeostasis in mature tissues by regulating diverse processes including cell proliferation, survival, migration and polarity, specification of cell fate, and

self-renewal property (Kim et al, 2013, Wang et al,2012 [22-23]).Wnt proteins comprise a major family of signaling molecules that orchestrate and influence a myriad of cell biological and developmental processes. These proteins are hydrophobic and are found in association with cell membranes and the extracellular matrix. They become palmitoylated in the endoplasmic reticulum of the Wnt-producing cells in the presence of acyltransferase porcupine. This palmitate modification is thought to assist in ligand reception on Wnt-responding cells. Once modified, the proteins are transported and secreted using secretory vesicles controlled by the multi-pass transmembrane protein Wntless/Evi (evenness interrupted), which is present in the Golgi and/or on the plasma membrane. This facilitates the release of Wnt protein from the cells to get associated with the seven-pass transmembrane receptor Fzd. Fzd is present on the surface of responding cells and possesses a large extracellular domain containing a conserved motif which comprises 10 cysteine residues called the cysteine-rich domain. There are various co-receptors – low-density lipoprotein receptor-related proteins 5 or 6 (LRP5/6) or ROR2 that aid in the binding of Wnt proteins to the receptor (Rosso et al, 2013 [24]). The co-receptor engaged then determines the downstream effect of the successful ligand binding, initiating either the canonical or the noncanonical pathways (Verkaar et al, 2010 [25]).

Wnt signalling pathway is divided into canonical pathway that involves Wnt/β-catenin and the noncanonical pathway that include planar cell polarity pathway (PCP) and Wnt/calcium pathway. These are activated by binding of Wnt protein ligand to the Fzd family receptor, which passes the biological signal to the Dishevelled protein inside the cell. The canonical Wnt pathway leads to regulation of gene transcription and the noncanonical PCP pathway regulates the cytoskeleton that is responsible for the shape of the cell. The noncanonical Wnt/calcium pathway regulates calcium inside the cell (Rao et al, 2010 [26]).

The canonical Wnt pathway

Canonical Wnt pathway also referred to as β-catenin-dependent Wnt pathway, is the best characterized of the three Wnt signaling pathways. In the absence of Wnt ligand, the cytoplasmic β-catenin is maintained at a low level through ubiquitin-proteasome-mediated degradation. It is regulated by a multiprotein destruction complex comprising Axin, adenomatous polyposis coli (APC), glycogen synthase kinase-3 β (GSK-3 β) and CK1 (casein kinase 1). As shown in Figure 1, the signaling pathway is initiated upon engagement

of the Wnt ligand with Fzd receptor protein in combination with either LRP5 or LRP6, forming a ternary complex on the extracellular membrane. LRP5/6 is a transmembrane receptor with a large extracellular domain critical for Wnt binding and a short intracellular tail. LRP5/6 also acts as the receptor for the secreted agonists of the Wnt pathway, R-spondin family of proteins. R-spondin uniquely synergizes with the Wnt proteins and leads to enhancement of the signal responses (De et al, 2012; Jin et al, 2012 [27, 28]). The upstream ligand binding then results in the activation of the kinases which induces phosphorylation of serine residues in the intracellular cytoplasmictail of LRP5/6, resulting in the initiation of the Wnt-mediated signaling cascade (Curtin et al, 2010 [29]). Consequently, phosphoprotein Dvl is recruited to the formed complex at the plasma membrane which leads to translocation of Axin and GSK-3 β from the cytoplasm to the receptor complex. As a result, the destruction complex dissociates and disrupts, due to which the cytoplasmic concentration of β-catenin increases. Hence, the accumulated β-catenin translocates into the nucleus where it forms a complex with members of the T-cell transcription factor/lymphoid enhancer-binding factor (LEF) family of transcription factors. Here, β-catenin acts as a transcription activator by displacing Grouchos and recruits the co-activators cAMP response element-binding protein binding protein (CBP) or its homolog p300 and also other components of the basal transcription machinery (such as CtBP, Foxo, TNIK, Bcl9, and Pygopus) (Takahashi et al, 2010 [30]). The binding of CBP and p300 activates the Wnt pathway. CBP-mediated Wnt signaling is shown to be associated with colonic cell proliferation, and p300-mediated Wnt activity promotes differentiation (Bordonaro et al, 2015; Lenz et al, 2014 [31, 32]). This results in the expression of the downstream target genes, c-jun, fra-1, c-myc, cyclin D1, etc., that are normally involved in developmental stages and adult tissue homeostasis [Figure 1].

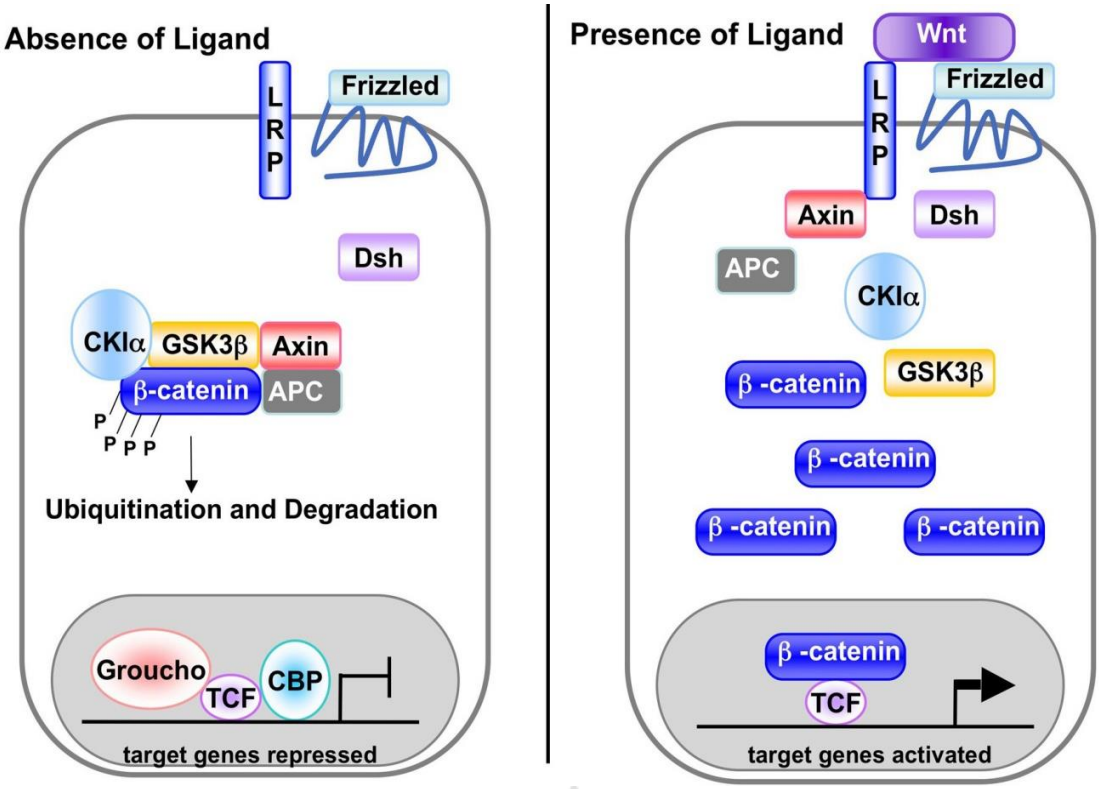

Figure 1. Schematic representation of canonical Wnt signaling pathway (http://www.wormbook.org/chapters/www_wntsignaling/wntsignaling.html)

The canonical Wnt pathway is also regulated tightly by members of several families of secreted antagonists that interfere with ligand–receptor interactions, such as members of the Dickkopf (DKK) family, the secreted frizzled-related protein (sFRP) family and Wnt inhibitory factor 1 (WIF1) (Valencia et al, 2009; Gasemi et al, 2016 [33, 34]). The four mammalian homologues of Dickkopf (DKK), DKK1–4 negatively regulate Wnt signaling by interacting with the Wnt co-receptor LRP5/6, thereby inhibiting signals that emanate from the Fzd-LRP complex. FRPs are naturally occurring secreted forms of Fzd, which contain the cysteine-rich domain of members of the Fzd-family but do not have a transmembrane region. Thus, they bind Wnt proteins and thereby abrogate the activity of Wnts. Also, WIF1 is a lipid-binding protein that binds to Wnt proteins, preventing them from passing signals to the cell (Liu et al, 2017 [35]). Furthermore, the transcriptional activity of the β-catenin-TCF complex is modulated within the nucleus (Valencia et al, 2009 [33]).

Dysregulated Wnt signaling has been identified as a key factor in the initiation of various malignancies. Several transcriptional targets of Wnt signaling are known oncogenes in the pathogenesis of epithelial cancers such as c-myc and cyclin D1 which are supposed to be involved in the oncogenic function of inappropriate Wnt signal activation (Polakis et al, 2000 [36]). In addition, Fzd, β-catenin and cyclin D1 are found to be the predictor of a poor outcome in poorly differentiated TNBC patients (Kazi et al, 2016; Kazi et al, 2018 [37, 38]. Dey et al [39] have also studied the tumor specimens from two breast cancer cohorts and together with meta-analysis of other breast cancer microarray studies confirmed the Wnt pathway activation in TNBC sub-types. Moreover, there are strong evidences that defects in the Wnt pathway are involved in the development of several types of solid tumors like colorectal, prostate, and oral cancer (Noguti et al, 2012; Lu et al, 2009; Chen et al,2010) [40-42]. It has been known that hematological malignancies, such as chronic myeloid and lymphocytic leukemia, mantle cell lymphoma, multiple myeloma, and AML may occur partly because of the constitutive activation of Wnt/β-catenin canonical signaling pathway (Xueling et al, 2010; Wang et al, 2009 [43, 44]). The Wnt signaling pathway has recently been implicated in self-renewal and proliferation of hematopoietic stem and progenitor cells [3]. Staal et al. [45] suggest that continuous activation of Wnt pathway result in the development of highly aggressive leukemias. Hence overexpression or mutation in any of the pathway components leads to malignant growth.

Wnt signaling in AML

Aberrant Wnt signaling is proposed to be a hallmark of many cancers (Pohl et al, 2017 [46]). The role of the Wnt pathway for survival, proliferation and differentiation of HSCs has raised the hypothesis that aberrant Wnt signaling might play a role in pathogenesis of leukemia. AML is frequently associated with mutations of the FLT3 receptor tyrosine kinase as well as with chromosomal translocations, such as t(8;21), t(15;17) and t(11;17) resulting in the generation of chimeric genes encoding specific transcription factors. Both, chromosomal translocations and FLT3 mutations have recently been reported to be associated with aberrant Wnt signaling in AML [Figure 2] (Mikesch et al, 2007 [47]).

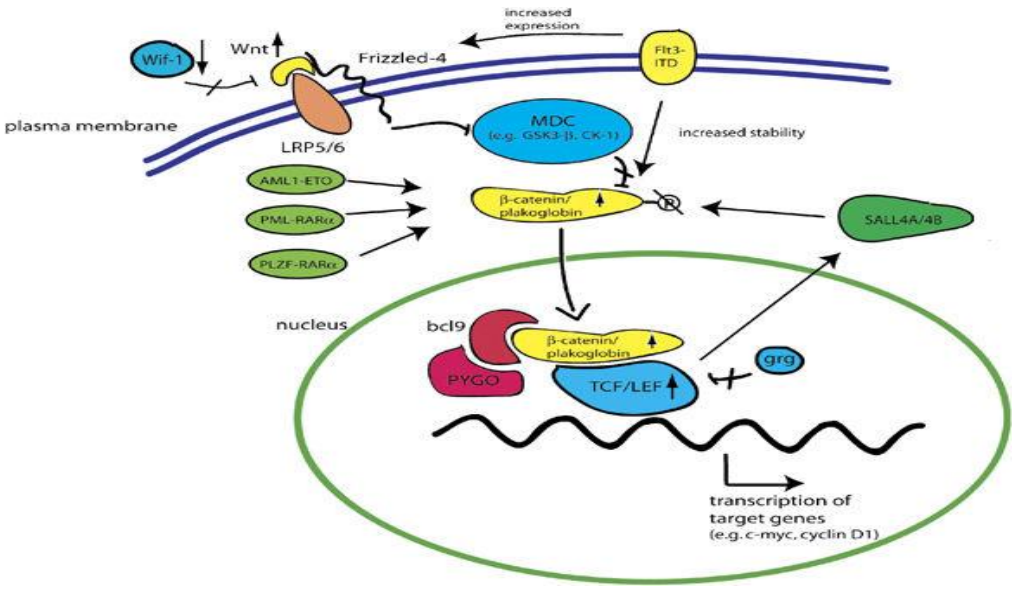

Figure 2. Schematic representation of Wnt signalling pathway in AML (Mikesch et al) [47]

A significant proportion of AML cases have been shown to have aberrant expression of Wnt pathway components including Wnt-1, Wnt-2b and LEF-1 (Simmon et al, 2005[48]). In addition, Wnt1, Wnt2B, and LEF1 mRNA are also overexpressed in CD34+ leukemic blast cells from AML patients (Ashihara et al, 2015 [49]). Moreover, deregulation of the expression of certain Wnt ligands (i.e., WNT2B, WNT6, WNT10A, WNT10B) are shown in AML cases (Beghini et al, 2012 [50]). Tickenbrock et al. demonstrated that FZD4 protein was expressed in about 80% samples from AML patients but rarely expressed in normal bone marrow (Trickembrock et al, 2008 [51]). Also, FZD4 expression modulated apoptosis and enhanced WNT3a-induced β-catenin stability in myeloid progenitor cells. Moreover, β-catenin is shown to be aberrantly expressed in patients with AML (Chung et al, 2002 [52]) and β-catenin activation in myeloid leukemia is associated with decreased apoptosis and differentiation and increased proliferation leading to poor prognosis (Ysebaert et al, 2006 [53]). Yuan et al. indicate that LEF1 contributes to the pathophysiology of AML and could serve as a novel predictor of better treatment response (Fu et al, 2014 [55]). On the contrary, Metzeler and co-authors observed that high LEF1 expression associates with favorable outcomes in cytogenetically normal AML patients[56]. MYC overexpression is a strong prognostic factor in untreated AML (Ohanian et al, 2016 [57]). Wang et al. suggested that the significant over-expressions of cyclin D1 exist in different subgroups of AML patients

indicating the Wnt/β-catenin pathway is aberrantly activated in AML. This activates downstream target cyclin D1 leads to disturbance in the regulation of cell cycle and abnormal proliferation of leukemic cells [54].

Many studies reveal that epigenetic inactivation of Wnt pathway inhibitors by CpG island methylation provides an additional mechanism for the observed Wnt-pathway activity in AML leukemic cells. The methylation status of Wnt antagonists, such as SFRP-1, 3, 4, and DKK1, was shown to be responsible for the activation of the Wnt pathway in AML cells and correlated with poor prognosis (Valencia et al, 2009; Griffiths et al, 2010 [33,58]). Ghasemi et al also showed that CpG island methylation of WIF1 and DKK-1 genes is a common event in AML patients [34].

These data demonstrate the importance of Wnt signaling pathway in AML patients and their outcome, which exhibits the promising therapeutic approach by targeting various components of Wnt pathway.

Activation of Wnt signaling by translocation products in AML

The AML-associated translocation products AML1-ETO, PML-RARα and PLZF-RARα encode abnormal transcription factors that induce self-renewal capacity and a differentiation block in susceptible hematopoietic cells.

The fusion proteins AML1–ETO, PML–RARα and PLZF–RARα share and regulate several target genes that have been found to be associated with Wnt signaling. Plakoglobin, a homologue of β-catenin is one of such target genes that is strongly induced by all three fusion proteins on the mRNA as well as on the protein level. It is a co-activator of TCF and LEF transcription factors (Zhurinsky et al, 2000 [59]) and mediates Wnt signaling. Plakoglobin binds to TCF/LEF transcription factors, enhancing formation of plakoglobin-LEF-1 complexes localizes to the nucleus and contributes to TCF- and LEF-dependent promoter transactivation of target genes like cyclin D1, c-myc and PPARδ (Muller et al, 2004 [60]). Plakoglobin binds to the c-myc promoter leading to its increased expression in AML cells carrying one of the fusion proteins. Activation of c-myc by plakoglobin has previously been reported by Rennoll et al [61]. The plakoglobin promoter was cloned and shown to be induced by AML1-ETO in 32D cells. Moreover, plakoglobin was also significantly over-expressed in AML patient samples with fusion protein compared to AML patient samples that did not express a fusion protein (Muller et al, 2004 [60]).

Further, the transcriptional regulation of plakoglobin results in the accumulation of endogenous β-catenin in the nucleus and thereby increased levels of β-catenin protein (Miller et al., 1997; Shtutman et al, 2002 [62, 63]). This localization within the nucleus could reduce the accessibility of plakoglobin to the cytosolic degradation machinery. In addition, plakoglobin interacts with the APC protein, possibly elevating the β-catenin levels by interfering with its degradation (Miller et al, 1997 [62]). In addition to direct transcriptional activation, plakoglobin might compete with β-catenin for APC/axin binding and as a consequence lead to increased β-catenin-mediated transcription. Plakoglobin appears to be important in Wnt signaling induced by AML associated translocation products, since the colony-forming capacity of HSCs transduced with fusion proteins is abrogated upon plakoglobin inhibition (Zeng et al, 2004 [64]. Moreover plakoglobin overexpression in myeloid 32D cells enhanced proliferation and clonal growth and injecting plakoglobin-expressing 32D cells into syngeneic mice significantly accelerated the development of leukemia [60]. Thus,β-catenin along with other molecules could be the key component in the pathogenesis in AML (Maqbool et al, 2016 [65]).

CONCLUSION

AML is often characterized by activation of Wnt signaling and downstream effectors. An important step forward will be to analyze the therapeutic potential of this pathway in AML. AML associated fusion proteins contribute to leukemogenesis by enhancing self-renewal and inducing plakoglobin expression, activating the Wnt signaling pathway. Also, the canonical Wnt pathway could be shown to be of major importance in the pathogenesis of AML. Therefore, inhibition of this pathway might be a promising therapeutic target for AML patients.

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