Mini Review

Stem Cell INVESTIGATION

Targeting chronic myeloid leukemia stem cells: can transcriptional program be a druggable target for cancers?

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Abstract: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm resulting from acquisition of constitutively active BCR-ABL protein tyrosine kinase in a hematopoietic stem cell (HSC). Though tyrosine kinase inhibitors (TKIs) have changed a fatal disease into manageable disease, most patients cannot discontinue TKI treatment due to persistence of TKI-resistant leukemia stem cells (LSCs). Much effort has been made to find out factors or pathways specifically operating in LSCs to selectively target LSCs, with some promising results at least in preclinical models. In this article, we briefly review the role of Wnt/β-catenin signaling and its related factors in CML LSCs, especially focusing on Tcf1/Lef1 transcription factors, major effectors of Wnt/β-catenin pathway, of which transcriptional program have recently been shown to be targetable with prostaglandin E1.

Keywords: Chronic myeloid leukemia (CML); leukemia stem cells (LSCs); Wnt/β-catenin pathway; transcriptional program; prostaglandin E1

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, characterized by clonal expansion and accumulation of differentiated myeloid cells in the blood and the bone marrow (1). CML usually begins with chronic phase, where expanded myeloid cells are almost normal in terms of function. Without effective therapy, most patients progress to accelerated phase and blast phase, as CML progenitors lose terminal differentiation capacity. CML stems from reciprocal translocation t(9;22)(q34;q11), called Philadelphia chromosome, generating constitutively active fusion kinase BCR-ABL. BCR-ABL is a sole oncogenic driver of CML, essentially believed to be sufficient to establish and sustain chronic phase CML (2), while a recent evidence suggests some additional changes may be required for development of CML (3). BCR-ABL confers proliferative and survival advantage to hematopoietic cells by activating several downstream signaling, including rapidly accelerated fibrosarcoma (RAF)/mitogen-activated activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and phosphatidylinositol 3-kinase (PI3K)/AKT pathways. Cumulative accumulation of chromosomal and molecular changes in addition to BCR-ABL is associated with disease progression to advanced phase diseases, often fetal conditions.

The advent of an orally active small-molecule tyrosine kinase inhibitor (TKI) in 2001 has revolutionized the treatment of CML, marking the start of the era of targeted cancer therapy (4). TKIs can block downstream signals of BCR-ABL effectively and eliminate most CML cells both *in vitro* and *in vivo*. In fact, TKIs have changed a fatal disease

into controllable disease, with 10-year survival over 80% (5). Most CML patients, however, cannot be cured solely by TKIs and committed to life-long TKI dependence. In fact, around half of the patients who achieved even complete molecular response upon TKI therapy experience CML relapse once they discontinue TKIs (6). Long-term TKI treatment is often associated with adverse effects, acquired resistance and high monetary costs, imposing a substantial burden on healthcare resources as well as individual patients. Furthermore, even under TKI therapy, there remains unignorable risk of progression to advanced stages (7), which cannot be cured without intensive therapy such as allogenic hematopoietic stem cell (HSC) transplantation.

Leukemia stem cells (LSCs)

As many studies using murine retroviral bone marrow transplantation models or xenograft transplantation models have consistently shown, CML is maintained by a very small number of CML LSCs, as is the case of hierarchically-structured normal hematopoietic system, sustained by self-renewing HSCs at the apex of developmental hierarchy (1,8,9). Molecular bases of persisting disease after TKI therapy have been extensively investigated, and many studies have attributed them to LSC persistence even under prolonged and apparently efficacious TKI treatment (10). CML LSCs, unlike their differentiated progenies, don't depend on BCR-ABL signaling for their survival, in other words, they are free from oncogene addiction (11). Importantly, CML LSCs are very similar to normal HSCs in many aspects, not only indicating the fact that CML LSCs are derived from HSCs, but also suggesting inherent difficulties in selectively eradicating LSCs without sparing HSCs (1,9).

Wnt/β-catenin signaling and its related factors in HSCs and LSCs

For the establishment of targeted therapy against CML LSCs, much effort has been devoted to comprehensively elucidate differences between LSCs and normal HSCs. These studies have illustrated that most key molecules essential for the maintenance of stemness are shared by LSCs and HSCs, including those implicated in transcription, regulation of cell cycle, metabolism, autophagy, signal transduction, and niche-associated factors, while LSCs are more dependent on some of these factors than HSCs, providing potential therapeutic opportunities (1). Among them, a long list of studies have probed into the role of Wnt/β-catenin signaling in normal and malignant hematopoiesis, including the pathogenesis of CML, as depicted in recent reviews (12,13). Wnt/β-catenin signaling is discerned into canonical and non-canonical pathways (*Figure 1*). In the canonical pathway, so-called destruction complex, consisted of two negative regulatory kinases, degrade β-catenin by targeting it for ubiquitination in the absence of Wnt ligands. After Wnt protein binds to Frizzled family receptor, β-catenin accumulates in the cytoplasm followed by translocation into the nucleus. In the nucleus, β-catenin, not having an ability to bind to DNA directly, modulates gene expression by interacting with members of TCF/LEF transcription factor family; the most extensively studied and widely acknowledged effectors of β-catenin. TCF/LEF factors, consisting of four members: Tcf1, Tcf3, Tcf4 and Lef1, generally act as transcriptional repressors, while binding to β-catenin turns them into transcriptional activators. β-catenin can exert its effects in some contexts through interactions with other transcription factors, including Prop1, forkhead box O (FOXO) factors and Krueppel-like factor 4. As non-canonical Wnt pathways independent of β-catenin, Planar Cell Polarity and Wnt/Ca^{2+}/ nuclear factor of activated T-cells (NFAT) pathways have been well characterized. Furthermore, Wnt signaling is modified by several classes of secreted factors; some inhibit binding of Wnt to its receptors and others isolate its co-receptors, composing multilayered regulation of Wnt activity.

Numerous studies have demonstrated substantial roles of Wnt/β-catenin signaling in the self-renewal of normal HSCs, with some opposing results depending on experimental systems utilized (12). A recent study illustrated that a differential, lineage-specific optimal Wnt dosage within a narrow window is required for robust hematopoietic function, including HSCs, myeloid precursors and T cell precursors (14), suggesting that apparent discrepancies of previous studies can be explained by different levels of Wnt/β-catenin signal activation. In addition, above-mentioned complex and redundant regulation of this pathway at multiple steps might complicate the problem.

In contrast to the conflicting results in the roles of Wnt/β-catenin signaling in normal HSCs, past studies have consistently supported its essential roles in CML, in terms of maintenance of CML LSCs (15,16), TKI resistance (17) and disease progression to blast phase CML (18,19). BCR-ABL directly stabilizes β-catenin through its kinase

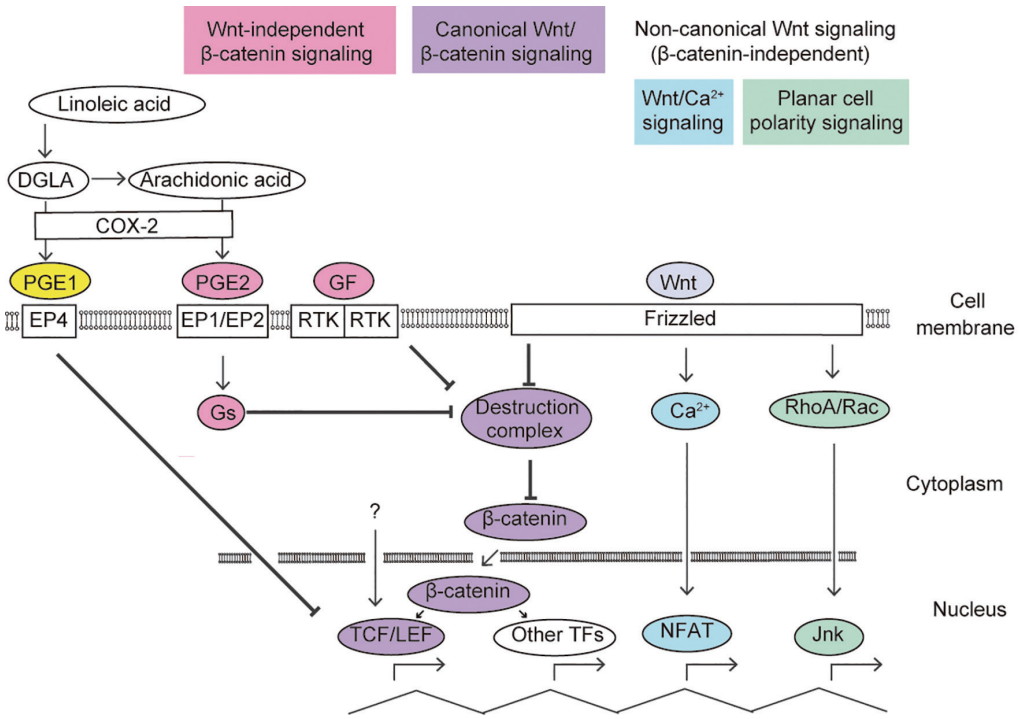

Figure 1 Simplified model of Wnt/β-catenin and related signaling and proposed model of PGE1 activity in CML. Binding of Wnt to its receptor Frizzled induces different downstream signaling, depending on various factors including different co-receptors: Wnt/β-catenin, Planar Cell Polarity and Wnt/Ca^{2+}/NFAT pathways. In CML, canonical (β-catenin-dependent) and Wnt/Ca^{2+}/NFAT signal pathways have been known to be involved in the pathogenesis. β-catenin level is also regulated in a mechanisms independent of Wnt, examples of which are COX-2/PGE2- and RTK-dependent fashions. COX-2/PGE2 signal induces increase in β-catenin level via EP1 in CML and EP2 in colon cancer. β-catenin exerts its role via TCF/LEF family transcription factors in most cases, while other transcription factors are also involved in some cases. Activation of TCF/LEF is usually induced by nuclear accumulation of β-catenin, but β-catenin-independent mechanisms have been also reported. In CML, PGE1 triggers transcriptional programs similar to those caused by loss of Tcf1/Lef1 via β-catenin-independent manner, leading to eradication of LSCs. CML, chronic myeloid leukemia; LSCs, leukemia stem cells; COX, cyclooxygenase; DGLA, dihomo-γ-linolenic acid; EP, prostaglandin E receptor; GF, growth factor; Gs, G protein Gs alpha subunit; PG, prostaglandin; RTK, receptor tyrosine kinase; TF, transcription factor.

activity, being an essential process in CML development in mice models (15). Both genetic ablation of β-catenin (catenin beta 1) and pharmacologic inhibition of COX2, which reduces β-catenin level by inhibiting prostaglandin E2 (PGE2) production (20), contribute to decreasing in CML cells resistant to TKIs, indicating β-catenin is also required for the maintenance of TKI-resistant LSC (16). Wnt/Ca^{2+}/NFAT pathway, one of a major component of non-canonical Wnt pathway, is also involved in the survival of CML cells independently of β-catenin, when BCR-ABL is inhibited (21). Constitutive TCF/LEF transcriptional activity in the absence of β-catenin stabilization has been reported in several human hematological tumor cells including K562 cells, a cell line derived from CML, which points to the presence of β-catenin independent activation of TCF1/LEF1 (22). These findings suggest the potential difficulty in targeting Wnt pathways, as well as the complexity of the cellular network regulated by Wnt and related factors in CML.

Tcf1/Lef1 and their associated transcriptional programs as a therapeutic target in LSCs

In 2016, it has been reported that genetic ablation of Tcf1 and Lef1, downstream transcription target of β-catenin, severely compromised CML LSCs, represented by impaired leukemia initiation and maintenance in CML mice models, while normal HSC functions are modestly compromised,

which is observed only under the settings of competitive transplantation and regenerative stress (23). In this study, downstream targets of Tcf1 and Lef1 in CML have remained elusive, though functional defects of Tcf1/Lef1-deficient HSCs in hematopoietic reconstitution have been attributed to Egr1 and Tcf3, transcription factors positively regulated by Tcf1 and Lef1. To investigate the molecular background of the differential dependence of LSCs on Tcf1 and Lef1, the same group has performed RNA-sequencing (RNA-seq) analyses comparing between wild-type (WT) and Tcf1/Lef1 double-knockout (DKO) hematopoietic stem and progenitor cells (HSPCs), in addition to corresponding LSCs from CML model mice receiving $P210^{BCR-ABL}$-transduced bone marrow transplant (24). Cross-comparison of differentially expressed genes between WT and DKO HSPCs and those between WT and DKO LSCs has shown that Tcf1/Lef1 regulates a distinct transcriptional program only in LSCs, with multiple members of activator protein 1 (AP-1) transcription factors enriched in either common or LSC-specific differentially expressed genes. Furthermore, two gene sets, upregulated in human quiescent CML LSCs compared with normal HSPCs, were downregulated in DKO LSCs, suggesting that Tcf1/Lef1 deficiency partly impairs the transcriptional program that maintains LSCs. By using Connectivity Map (CMAP) database, the authors tried to find compounds that can induce gene expression changes recapitulating those caused by Tcf1/Lef1 loss in LSCs (25). Among a couple of candidate compounds, PGE1 significantly impaired colony forming activity of CML LSCs. PGE1 and PGE2 are closely related 20-carbon fatty acid derivatives produced by cyclooxygenase (COX)-dependent oxygenation, of dihomo-γ-linolenic acid (DGLA) for PGE1 and arachidonic acid, a derivative of DGLA, for PGE2. In spite of the structural similarities, their biological functions are strikingly different; PGE1 is characterized by vasodilative effect, usually used to treat conditions such as pulmonary hypertension and peripheral artery occlusive disease, while PGE2 has been involved in more diverse physiological phenomena, including inflammation, bone resorption and parturition, and it can also be utilized for *in vitro* expansion of human cord-blood HSPCs. In contrast to the fact that pharmacological inhibition of PGE2 signaling is effective against CML through suppressing β-catenin in LSCs (16), the roles of PGE1 in Wnt/β-catenin signaling as well as hematopoietic diseases including CML hadn't been elucidated. The authors revealed that PGE1, but not PGE2, greatly diminished CML LSCs and prolonged survival of CML mice without affecting the onset of CML. PGE1 abrogated LSCs more effectively than blocking overall PGE production by COX2 inhibitor indomethacin. Furthermore, PGE1 diminished CML LSCs as efficiently as tamoxifen-induced ablation of Tcf1/Lef1, showing a synergistic therapeutic effect upon a TKI, which preferentially affects more differentiated cells.

In an attempt to elucidate mechanistic basis of the anti-CML activity of PGE1, further transcriptomic analyses of DKO and PGE1- and PGE2-stimulated LSCs have been performed, revealing that PGE1 and PGE2 activate distinct pathways and target genes in LSCs; about 26% of downregulated genes in DKO LSCs, including Egr1 and AP-1 factors such as FosB, were repressed by PGE1 but not by PGE2 (24). Ectopic expression of these downregulated AP-1 factors rendered LSCs less sensitive to PGE1, indicating that AP-1 factors are major targets for PGE1-mediated LSC dysfunction. Among four E-prostanoid receptors, designated EP1, EP2, EP3 and EP4, the authors revealed that PGE1 exerts its antileukemic activity through the EP4 receptor independently of β-catenin, by use of $Vav1\text{-}Cre^{+}/EP4^{flox/flox}$ and $Vav1\text{-}Cre^{+}/\beta\text{-catenin}^{flox/flox}$ LSCs. Misoprostol, a widely-used analog of PGE1, impaired LSC activity in murine primary, secondary and serial transplant CML models, as well as in murine xenograft models using human CML cells. Its activity was again more pronounced in conjugation with TKIs. Importantly, PGE1 was also effective against CML LSCs from patients on advanced stages such as accelerated and blast phase, less sensitive to TKIs, suggesting that PGE1-mediated AP-1 repression is a conserved regulatory pathway in targeting LSCs at different stages of CML.

Future prospects

By targeting a specific transcriptional program that specifically targets CML LSCs, the study by Li *et al.* (24) provided promising therapeutic approach to LSCs, including those of near intractable advanced disease. Some open questions, however, remains to be solved. Firstly, the whole picture of β-catenin-independent regulation and activity of Tcf1/Lef1 have been still obscure. The difference in the mechanisms and consequences between β-catenin-dependent and -independent activation of Tcf1/Lef1 remain to be clarified, as well as the physiological role of above mentioned PGE1-induced signaling. Li *et al.* suggested that PGE1-mediated *FosB* and *Fos* down-regulation was completely abrogated or even reversed by pharmacologically inhibiting any one of MEK, PI3K and Ca^{2+}, provoking a question how these

pathways are interconnected and integrated into β-catenin-independent Tcf1/Lef1 signaling. Secondly, the mechanism how Tcf1/Lef1 play a specific role in CML LSCs and its possible relationship to BCR-ABL and/or other oncogenic signals should be further investigated. Possible functional association of Tcf1/Lef1 with BCR-ABL may lead to a novel therapeutic opportunity for the treatment of Philadelphia chromosome positive acute lymphoblastic leukemia. In addition, because constitutive activation of Wnt/β-catenin signal is a nearly universal features common to many malignancies, clarifying whether Tcf1/Lef1-mediated transcriptional regulation has specific role also in other malignancies, especially in the maintenance of cancer stem cells, might lead to novel therapeutic opportunities.

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Footnote

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