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Chronic myeloid leukemia - Section 1

Novel approaches to eradicate chronic myeloid leukemia stem cells

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Take-home messages

- CML stem cells utilize multiple cell-intrinsic pathways, together with microenvironmental and immune cell interactions to evade current therapies.
- Identification of clinically relevant targets on CML stem cells, e.g. PPARγ, p53, c-MYC, BCL-2 and EZH2, is likely to lead to improved therapies for patients with all phases of CML in the future.

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder, derived from a hematopoietic stem cell (HSC), which acquires the *BCR-ABL* fusion oncogene. Despite the huge success of tyrosine kinase inhibitors (TKIs) in treating CML, resulting in the majority of patients obtaining a major molecular response (MMR) on sustained therapy,[1-3] there is strong evidence that these drugs are ineffective against the CML leukemic stem cell (LSC).[4-6] This can lead to molecular disease persistence and relapse, both on TKI therapy and after TKI cessation. It is clear from TKI discontinuation studies, e.g. STIM, TWISTER and EUROSKI,[7-9] that only a minority of optimally responding patients can safely stop their TKI without evidence of molecular recurrence. A number of recent studies have identified novel approaches that may eliminate CML LSCs. This short review will evaluate some of these potential LSC eradication strategies (Figure 1).

Current state of the art

LSC quantification

Two recent studies have focused on estimating the size of the LSC population in CML, and how this alters with therapy. Werner *et al* presented a mathematical model which describes the relative increase in LSCs over time on therapy in comparison to overall tumur burden, together with the slow decline in absolute LSC numbers.[10] Thielen *et al.* used multiparameter flow cytometry and fluorescence in situ hybridization for *BCR-ABL* to correlate LSC numbers (CD34+38-) with established prognostic markers and response to therapy.[11]

LSC heterogeneity

Evidence is accumulating that CML LSCs are heterogeneous. In a murine model of CML, Zhang *et al* described the heterogeneity of leukemia-initiating capacity of CML LSCs, with high levels of MPL expression correlating with superior engraftment and enhanced leukemogenesis[12] Our own recent studies in myeloid blast-phase CML clearly show that multiple non-hierarchically arranged immunophenotypically-defined stem and progenitor cell populations have functional LSC capacity.[13] Furthermore, blast-phase-associated additional chromosomal abnormalities are detected in all stem and progenitor cell populations.

The importance of the stem cell niche in CML

In addition to CML LSC-intrinsic factors, it is becoming increasingly clear that the immune system and bone marrow (BM) microenvironment have very important roles in CML LSC persistence.[14-18] CML LSCs have altered proliferation, differentiation and localization within the BM niche. While aberrant cytokine expression gives CML LSCs a growth advantage, abnormal localization of LSCs is due, at least partly, to reduced CXCL12 expression. Studies have shown that TKI treatment only partially corrects these alterations in the CML BM microenvironment, and targeting these microenvironmental pathways can enhance LSC eradication.[14,15] Recently, the importance of gonadal adipose tissue as an LSC niche has been described,[16] supporting LSC metabolism and enhancing chemoresistance, particularly in CML LSCs expressing the fatty acid transporter CD36.

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Aberrant interleukin-1 signaling

Two recent studies have identified the interleukin 1 receptor (IL-1R) pathway as a potential therapeutic target in CML LSCs. Increased expression of the IL-1R complex, via upregulation of survival pathways including NF-kB, JNK and p38MAPK, promotes the growth and survival of CML LSCs.[19,20] These two preclinical studies adopted different, but effective, strategies for eliminating CML LSCs. The first used an IL-1R antagonist (used clinically for the treatment of rheumatoid arthritis[21]) to inhibit growth of CML LSCs and increase sensitivity to nilotinib.[19] The second utilized antibody-dependent cellular cytotoxicity against IL-1 receptor accessory protein (IL-1RAP), a surface molecule shown previously to be expressed on CML LSC but not normal HSC,[22] and demonstrated increased survival in murine xenograft models of chronic- and blast-phase CML.[20]

Targeting alternative cell surface molecules

Other groups are also seeking to exploit aberrant expression of cell surface molecules on CML LSCs to improve therapeutic responses. CD26 (dipeptidylpeptidase IV), CD33, and CD93 have all been shown to be overexpressed on CML LSC,[23-25] and have the potential to be exploited therapeutically. CD26 expression discriminates CML LSCs from normal HSCs and, furthermore, numbers of CD26+ LSCs correlate with response to TKI therapy.[26] Preclinical *in vivo* studies indicate that gliptins, a family of anti-diabetic drugs, may reduce CML LSCs.[24] CD33+ CML LSC may be targeted by the antibody-drug conjugate gemtuzumab ozogamicin,[23] but clinical utility is likely to be limited by the unacceptable side effect profile for most patients with CML. Although CD93 is overexpressed on CML LSC compared to normal HSC, it is also expressed on endothelial cells, platelets and more mature myeloid cells,[27] making it a less attractive option for therapy.

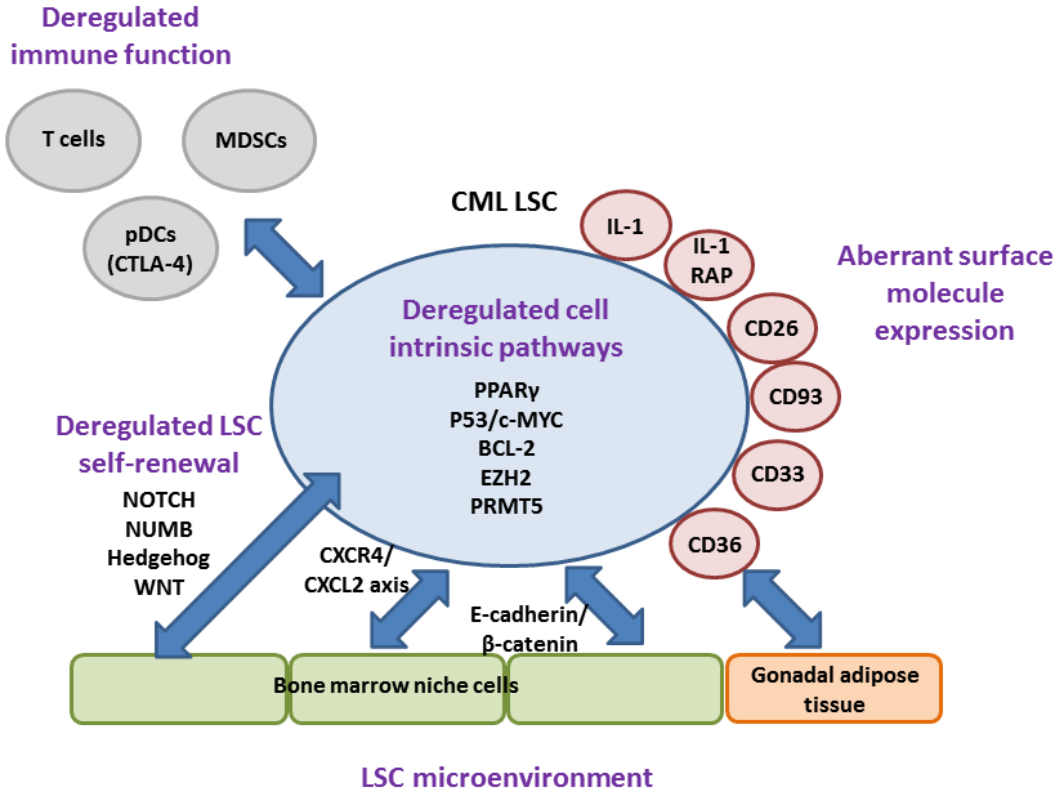

Figure 1. Potential leukemia stem cell (LSC) eradication strategies. This review focuses on a number of recently published original articles exploiting different approaches for eliminating CML LSCs. Four different approaches are considered which focus on LSC microenvironment, aberrant cell surface marker expression, deregulated LSC self-renewal and deregulated cell-intrinsic pathways. MDSCs; myeloid-derived suppressor cells, pDCs; plasma dendritic cells.

Self-renewal pathways as therapeutic targets in CML LSC

Interest continues to focus on self-renewal pathways as a potential route to eliminate CML LSCs. We have recently shown that NOTCH is silenced in chronic-phase CML, with activation of NOTCH leading to reduced self-renewal capacity in CML LSCs.[28] Recent studies have also demonstrated that NUMB inactivation results in imatinib resistance in CML.[29] Thus, activation of NOTCH or NUMB may be potential therapeutic strategies against CML LSCs. Although the Hedgehog pathway is de-regulated in CML,[30] clinically available SMO antagonists have proven to have an unacceptable side effect profile when used alone or in combination with TKIs in clinical trials. Previous studies have shown that WNT signaling from the bone marrow niche contributes to LSC persistence.[15] Secreted WNT ligands are modified by the O-acyl transferase, porcupine (PORCN). Recently, Agarwal *et al.* demonstrated that the potent and selective PORCN inhibitor WNT974, either alone or in combination with nilotinib, effectively targeted CML LSCs.[31]

Targeting CML LSC-intrinsic pathways

A number of recent studies have described strong preclinical evidence for cell-intrinsic pathways that may be exploited to eradicate CML LSCs. Peroxisome proliferator-activator (PPAR)-γ agonists, including the anti-diabetic drug pioglitazone, reduce the CML LSC pool in preclinical studies by bringing quiescent cells into cell-cycle and rendering them sensitive to TKIs.[32] The ACTIM study, a proof-of-concept study, comparing imatinib plus pioglitazone with a historical imatinib-only cohort indicated a superior MR4.5 rate in the combination arm (56% *versus* 23%).[33] A randomized study to confirm this is now required.

Using a novel bioinformatics approach, incorporating proteomics, transcriptomics and network analyses in primary chronic-phase CML, Abraham *et al.* identified p53 and c-MYC as critical signaling hubs.[34] Upregulation of p53 using an MDM2 inhibitor, combined with downregulation of c-MYC using a BET inhibitor, led to selective and potent elimination of CML LSCs.

BCL-2 is over-expressed in advanced phase CML. The combination of BCL-2 inhibitor, ABT-199, with TKI-mediated inhibition of BCL-XL and MCL-1 effectively eliminated CML LSCs in murine models and blast-phase patient samples by inducing apoptosis of quiescent LSCs.[35]

There is an increasing focus on epigenetic therapies in leukemia. Two groups have recently described the over-expression of EZH2, the catalytic subunit of the polycomb repressive complex (PRC)-2 in CML LSCs.[36,37] These preclinical *in vitro* and *in vivo* studies have demonstrated that the combination of EZH2 inhibitor with TKI enhances eradication of CML LSCs. Further recent studies have also demonstrated that targeting the methyltransferase PRMT5, which is over-expressed in CML LSCs, reduced self-renewal, possibly via reduction of the WNT/β-catenin pathway molecule disheveled homolog 3 (DVL3).[38]

Conclusions/future perspectives

Remarkable progress continues to be made in defining and identifying potential therapeutic strategies to eliminate CML LSCs. It is likely that a number of the approaches described here will proceed to clinical trial and the long term goal is improved elimination of CML LSCs in all phases of CML; to reduce resistance in advanced phase patients and increase the number of optimally responding patients capable of permanently discontinuing TKI therapy.

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