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REVIEW

Strategies For Targeting Chronic Myeloid Leukaemia Stem Cells

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Giovanna Carrà
Antonio Cartellà
Beatrice Maffeo
Alessandro Morotti

Department Of Clinical And Biological Sciences, University Of Turin, Orbassano 10043, Italy

Abstract: Chronic Myeloid Leukaemia is a myeloproliferative disorder driven by the t (9;22) chromosomal translocation coding for the chimeric protein BCR-ABL. CML treatment represents the paradigm of molecular therapy of cancer. Since the development of the tyrosine kinase inhibitor of the BCR-ABL kinase, the clinical approach to CML has dramatically changed, with a stunning improvement in the quality of life and response rates of patients. However, it remains clear that tyrosine kinase inhibitors (TKIs) are unable to target the most immature cellular component of CML, the CML stem cell. This review summarizes new insights into the mechanisms of resistance to TKIs.

Keywords: chronic myeloid leukaemia, stem cells, tyrosine kinase inhibitors

Introduction

Chronic Myeloid Leukaemia (CML) is the paradigm of bench-to-bedside translational research.[1–6] CML was among the first cancers to be clearly associated with a genetic lesion, namely the Philadelphia Chromosome, able to generate the chimeric BCR-ABL protein. A plethora of studies with cellular and murine models[7] converged on the assumption that one single oncogenic gene - BCR-ABL - can drive a potent leukaemogenic signal.[3] For this reason, BCR-ABL has been intensively studied as a perfect druggable target, leading to the development of imatinib, which quickly raised the clinical arena.[8] CML remains the most successfully treated disease with a TKI,[9] while in other cancers, responses to other specific TKIs are less pronounced. Therefore, understanding the unique biological features of CML should provide new insights into the management of other cancers. In this respect, resistance to TKIs has been generally considered a consequence of the insensitivity of cancer stem cells to these drugs[10] and, therefore, CML remains a perfect battlefield to investigate biological behaviours of these elusive cells.[11] A lot of evidence has clearly demonstrated that CML stem cells remain unaffected by BCR-ABL TKIs, as extensively reviewed.[10,12–14] In particular, TKIs are able to enter CML stem cells, to inhibit BCR-ABL, but are not able to promote their apoptosis.[14]

The resistance of CML stem cells to TKIs is a very challenging issue that has been investigated in great depth over the years.[15] Resolving this problem may not affect CML patients,[16] who highly benefit from TKI therapy, but may significantly improve our knowledge on leukaemia stem cells, and may improve cancer therapies in general, specifically in those tumours where kinase inhibitors or other molecular approaches fail to achieve convincing clinical results.

This review focuses on mechanisms that affect CML stem cells.

Correspondence: Alessandro Morotti
Department Of Clinical And Biological Sciences, University Of Turin, Regione Gonzole 10, Orbassano 10043, Italy
Email alessandro.morotti@unito.it

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Cooperating Oncogenes

For many years, various groups have focused their attention on different pathways that may cooperate with BCR-ABL or may act independently from BCR-ABL in promoting resistance of CML stem cells to TKIs. Here, we will review the most well-known pathways.

NF-kB

The contribution of the NF-kB signalling pathway has been intensively investigated in CML, and in many other cancers.[17] NF-kB is a transcriptional pathway able to promote various biological processes, favouring cell growth, survival, metastatization and resistance to chemotherapy.[18] The most common form of NF-kB is the heterodimer p65/p50, which becomes entrapped in the cytoplasm by the IkB-alpha protein, therefore blocking its transcriptional activity. Upon stimulation, the IkB-alpha protein is phosphorylated at serine residues by the IKK-kinase complex, promoting its proteosomal degradation, and enabling NF-kB to shuttle into the nucleus. Various studies have attributed an essential role for NF-kB in BCR-ABL-mediated signalling,[19,20] as we have also recently reviewed.[17]

Besides playing a pivotal role in the bulky population of CML cells, NF-kB has been also investigated in the stem cell compartment. In particular, two groups have shown that CML stem cells are able to produce and secrete both transforming growth factor-β (TGF-β)[21,22] and tumour necrosis factor-α (TNF-α),[23] which - in turn - support the survival status of the same cells. While these observations suggest that NF-kB can play a remarkable role in stem cells, it is not known whether NF-kB inhibitors may play a specific role in promoting CML stem cell eradication.

Hedgehog Pathway

Among the many pathways able to modulate stem cell maintenance, hedgehog signalling undoubtedly plays a pivotal role.[24] Three Hedgehog homologues, namely Desert (DHH), Indian (IHH) and Sonic (SHH), bind to the hedgehog receptor - Patched (Ptc) - promoting cell proliferation and survival in a complex mechanism. While the DHH and IHH pathways have been found to be deregulated in various tumours, SHH signalling has been found to be altered in CML and in leukaemia progenitor cells. Targeting this signalling pathway offers a chance to eliminate CML stem cells, while sparing normal haematopoietic stem cells (HSC).[25] However, to our knowledge, to date, no data from clinical trials have been published with Hedgehog inhibitors in the CML context.

Beta-Catenin

The Wnt/β-catenin pathway is, historically, a major stem cell pathway, able to modulate both quiescence and maintenance, as extensively reviewed.[26] It was demonstrated that β-catenin is involved in various aspects of CML biology, including maintenance of CML stem cells, thus promoting the study of the beta-catenin pathway as a druggable pathway in CML.[27]

PP2A

The tumour suppressor PP2A has been extensively studied in the context of CML stem cells. In particular, BCR-ABL was shown to inhibit the phosphatase activity of PP2A and its reactivation was found to be associated with a marked growth suppression and apoptosis induction.[28]

Remarkably, a PP2A activator named FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride) displayed potentially relevant implications from a therapeutic standpoint.[29,30] Similar conclusions were achieved with the clinically validated PP2A inhibitors LB100 and LB102.[31]

Additional Pathways

Various signal transduction pathways have been shown to modulate BCR-ABL signalling[8,32] and to promote resistance to TKI in a BCR-ABL independent manner. Among them, the CK2[33,34] and Alox5[35] pathways, along with others, were also associated with the possibility of specific inhibition. It should, however, be noted that most of these pathways have not been investigated in CML stem cell settings.

Tumour Suppressors

While no tumour suppressors have been consistently found mutated or deleted in the chronic phase of CML, in recent years, various tumour suppressors have been found to be functionally inactive in CML, as we have previously reviewed.[36] Identifying inactive tumour suppressors and the mechanisms of inactivation may open new therapeutic opportunities. Indeed, restoring the function of an inactive tumour suppressor may represent a strong pro-apoptotic signal.

PML

The promyelocytic leukaemia (PML) tumour suppressor protein is an essential component of nuclear bodies and is

involved in various cellular processes. PML has been shown to play an important role in regulating CML stem cells.[37] PML is a key regulator of the quiescence of these cells, irrespective of the BCR-ABL signal. The relevance of these observations has been associated with the ability of arsenic trioxide to target PML to degradation. Arsenic trioxide has been demonstrated to promote the exit of CML stem cells from the quiescent status, rendering these cells susceptible to apoptosis. For this rational, clinical trials are still ongoing.

PTEN

PTEN is a tumour suppressor involved in regulating various cellular processes, such as the maintenance of genomic stability, cell survival, migration, proliferation and metabolism.[38] While originally described as a phosphatase, able to dephosphorylate PIP3, it also displays phosphatase-independent functions. HSC rely on functional PTEN, as extensively studied.[39,40] In addition, PTEN integrity is mandatory in CML.[41] The involvement of PTEN in CML is strictly connected to its correct cellular compartmentalization. We observed that, while in CML progenitor cells PTEN is mostly expressed in the cytoplasm, in the CML stem cell compartment, PTEN is retained in the nuclear pool.[42] Regulating PTEN compartmentalization depends on a functional PML/HAUSP network, which is maximized in the stem cell compartment. Shuttling of PTEN is indeed associated with changes in cellular behaviours of various cancers.[42,43] PTEN cellular compartmentation can be modulated by arsenic -trioxide, which affects the PML/HAUSP network, as we previously described. More recently, strategies for targeting the Enhancers of zeste homologue 2 (EZH2), a core catalytic subunit of polycomb repressive complex (PRC2) were shown to modulate PTEN expression in the stem cell compartment, with important therapeutical implications.[44]

FoxO

Forkhead box subgroup O (FoxO) is a family of transcription factors (TFs) that play an essential role in regulating cancer stem cells.[45] As described for PTEN and p53, FOXOs are mainly deregulated through functional modification of cellular compartmentalization. It has been widely demonstrated that inhibition of FoxO1 and 3a, through cytoplasmic shuttling, supports the growth and inhibition of cell death in CD34+ CML cells. Conversely, leukaemia-initiating cells (LICs) are enriched in FoxO3a nuclear localization mediated by a decrease in Akt phosphorylation.[46,47]

Morgana

Morgana/chp-1 plays an essential role in mouse embryonic development, involved in the regulation of centrosome duplication and genomic stability.[18,48] Morgana binds to ROCKI and ROCK II, favouring inhibition of ROCKII kinase activity. Recently, we have demonstrated that morgana ± mice developed a fatal myeloproliferative disorder, resembling atypical CML.[49] In an extended analysis, we also demonstrated that some CML patients may also display reduced morgana protein levels in the most immature cellular compartment. Therefore, ROCK activity was increased in these patients with a reduced response to TKI treatment.

TP53

TP53 is one of the most studied and well-known tumour suppressors.[50,51] The role of p53 in CML was originally assessed when searching for genetic inactivation. TP53 was indeed discovered to be mutated/deleted in a fraction of CML blast crisis.[52] However, it was also clear that the role of p53 in cancer is more complex, including functional inactivation through delocalization and post-translational modifications.

P53 has been shown to be functionally inactivated through direct binding with IkB-alpha in the CML context.[53] More recently, by investigations with proteomics, transcriptomics and network analyses, p53 was shown to be deregulated in the stem cell compartment, together with c-Myc.[54] Pharmacological modulation of both p53 and c-Myc levels has been associated with a marked induction in CML stem cell apoptosis.

Epigenetics And Modifiers

Histone deacetylase inhibitors (HDACi) are epigenetic modifiers that, in vitro, promote growth arrest and apoptosis of myeloid tumour cells.[55] For this reason, many HDACi have been tested in CML and in CML stem cells,[56] and various trials have been designed to test HDCA in association with TKI. To our knowledge, no relevant data have, however, been published following these trials.

Yet, epigenetic reprogramming remains a challenging topic in CML stem cells, as recently observed with EZH2.[57] Taken together, these data suggest that further investigations are necessary to identify the best epigenetic modifiers in CML and the best combinatorial approach.

CML Stem Cell Metabolism

Cellular metabolism reprogramming is an emerging hallmark for cancer survival and cancer stem cell biology.[58] While normal cells use glucose to produce energy by mitochondrial

oxidative phosphorylation, cancer cells have been shown to increase glucose uptake and promote aerobic glycolysis, originally described as the Warburg effect. This metabolic shift has also been described in CML, where it appears to be therapeutically modulated.[59] The mTOR pathway has been extensively studied as a major determinant of the anabolic and catabolic processes in normal and cancer cells,[60] and the mTOR pathway has also been intensively studied in the CML setting.[61] The kinase - AMPK - has been described as an essential regulator of mTOR and cellular metabolism and shown to play an important role in CML pathogenesis[62] and resistance to TKIs.[63] Notably, AMPK appeared to be targetable in CML.[64] Finally, and in agreement with the metabolic reprogramming of CML cells, antidiabetic drugs have also been shown to play important roles in this disease. In addition, Metformin,[65,66] glitazones have been recently studied due to their ability to target CML stem cells.[67,68]

microRNAs

Various microRNAs (miRNA) have been reported to play a role in CML and, in particular, in the stem cell compartment:[69] miR-126,[70] miR-29a-3p, miR-494-3p and miR-660-5p,[71] and others. More recently, a challenging hsa-mir183/EGR1/E2F1 axis has been reported to directly control CML stem cell behaviour.[72] Most of these miRNAs have been extensively reviewed elsewhere.[69,73–77]

Cluster Of Differentiation

Various biomarkers defined as Cluster of Differentiation (CD) have been recognized as specifically expressed in the stem cell compartment.[78] The cytokine targeting surface enzyme dipeptidylpeptidase-IV (DPPIV/CD26) has been shown to be mostly expressed in the stem cell compartment of CML.[78–83] Interestingly, CD26 expression appears to be associated with the expression of Polycomb BMI1 protein.[84] The IL2 receptor CD25 was also shown to be over-expressed in CML cells and - in particular - in the stem cell compartment.[85–87] While the function of most of these biomarkers remains to be defined in the CML stem cell population, selective expression in these cells may offer a challenging therapeutic implication. Monoclonal antibodies may possibly be developed to specifically target these CD and potentially reach the stem cells of CML.

Stroma

Interaction of HSC and leukaemia stem cells with bone marrow microenvironments is indispensable for the initiation, maintenance, and progression of CML, and may also affect the sensitivity to therapies.[88] Furthermore, the role of the stem cell niche in CML leukaemogenesis has been investigated in-depth, highlighting the role of Cxcl12 in the regulation of quiescence of CML.[89]

Autophagy

Autophagy is a process that is evolutionally conserved to allow recycling of cytoplasmic components through the formation of the autophagosome. These vesicles are driven into lysosomes where they are degraded.[90,91] Inhibiting autophagy has been shown to play an important role in cancer therapy.[92] In line with these observations, autophagy inhibitors have been tested as strategies to target CML stem cells alone[93] or in combination with mTOR inhibitors[61] and PARP inhibitors.[94]

Immune System

Aberrant immune-inhibitory responses have been observed in CML patients at diagnosis,[95] and the immune cellular context has been shown to impact CML therapy[96] and/or affect immune surveillance.[97] Similarly, TKIs have been shown to affect the immune system.[98] Various cytokines known to play a role in the immune system, such as CXCR2 and CXCL4, have been shown to regulate the survival of CML stem cells.[99] While further investigations are needed in this context, these observations indicate a pivotal role of the immune system in each phase of CML maintenance and, therefore, suggest the variegation of therapeutical implications. In this respect, following the increase in the use of Chimeric Antigen Receptor - engineered T cells - in the clinical scenario, it was also shown that CAR-T directed toward IL1RAP could represent an efficient approach for targeting CML stem cells.[100] In addition, checkpoint inhibitors have been discussed as potential relevant targets in CML therapy.[101]

Parallel to the role of the immune system, the intriguing role of inflammation (a mixture of immune system regulators and cytokines) has also been investigated as a determinant for CML stem cell maintenance and/or development.[102]

Discussion

In this review we have reported various pathways and/or mechanisms that have been recognized to modulate CML stem cell behaviour. For each of these pathways, specific inhibitors have been identified, allowing the proposal of a combinatorial therapy with TKIs with the aim of eradicating CML stem cells. Various clinical trials have been designed for this purpose,[10] but results are still pending. Data obtained from the clinical scenario will allow identification of

pathways with relevant roles in the CML stem cell compartment from the plethora of cooperating pathways. CML response rates suggest that combinatorial therapies will only be proposed to those patients resistant to TKIs; however, deciphering mechanisms of insensitivity of CML stem cells to TKIs may shed new light on how to efficiently treat other cancers. It is worth noting that other Philadelphia-positive types of Leukaemia, such as Acute Lymphoblastic Leukaemia, are much less sensitive to BCR-ABL TKIs, and the reason is far from being understood. Moreover, other cancers with other active tyrosine kinases did not respond to specific TKIs with the same efficacy described for CML. Knowing the correct approach for targeting stem cells should enhance the response rates in various tumours.

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Disclosure

The authors report no conflicts of interest in this work.

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