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REVIEW ARTICLE

Natural course and biology of CML

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Abstract Chronic myeloid leukaemia (CML) is a myeloproliferative disorder arising in the haemopoietic stem cell (HSC) compartment. This disease is characterised by a reciprocal t(9;22) chromosomal translocation, resulting in the formation of the Philadelphia (Ph) chromosome containing the BCR-ABL1 gene. As such, diagnosis and monitoring of disease involves detection of BCR-ABL1. It is the BCR-ABL1 protein, in particular its constitutively active tyrosine kinase activity, that forges the pathogenesis of CML. This aberrant kinase signalling activates downstream targets that reprogram the cell to cause uncontrolled proliferation and results in myeloid hyperplasia and 'indolent' symptoms of chronic phase (CP) CML. Without successful intervention, the disease will progress into blast crisis (BC), resembling an acute leukaemia. This advanced disease stage takes on an aggressive phenotype and is almost always fatal. The cell biology of CML is also centred on BCR-ABL1. The presence of BCR-ABL1 can explain virtually all the cellular features of the leukaemia (enhanced cell growth, inhibition of apoptosis, altered cell adhesion, growth factor independence, impaired genomic surveillance and differentiation). This article provides an overview of the clinical and cell biology of CML, and highlights key findings and unanswered questions essential for understanding this disease.

Keywords Chronic myeloid leukaemia · BCR-ABL · Philadelphia chromosome · Tyrosine kinase inhibitors · Signal transduction

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Introduction

The discovery of the Philadelphia (Ph) chromosome in chronic myeloid leukaemia (CML) patients marked the first time that a chromosomal abnormality was linked to a particular disease. The next major breakthrough for understanding CML was the identification of the BCR-ABL1 fusion gene, which is formed in the Ph chromosome. Subsequent functional studies demonstrated that BCR-ABL1 was central to driving early disease. This led to the development and success of a BCR-ABL1-targeted therapy. Currently, the key biological questions for CML surround the understanding of how this leukaemia transforms from a relatively indolent chronic phase (CP) to an aggressive blast crisis (BC), and at dissecting the biology of the leukaemic stem cell (LSC) (where BCR-ABL1 originates) and early progenitors, which are vital for establishment and maintenance of CML.

Clinical overview

Diagnosis

CML is usually diagnosed in CP [1]. The main symptoms and signs at presentation are fatigue, anaemia, splenomegaly, abdominal discomfort and episodes of infections [1]. However, a significant proportion of patients are asymptomatic, with diagnosis occurring after unrelated medical examination [1]. Males show an increased incidence of CML at a male/female ratio of 1.3–1.5:1 [1–3]. The only proven risk factor is exposure to high-dose ionising radiation [4]. The average age at presentation is region-dependent. For example, in Africa and Latin America, CML patients are diagnosed at least 15 years younger compared to Australia (median age 55 years), Europe and the USA [1–3]. Differences in life

expectancy do not entirely explain the age of onset, thus future investigation could identify additional determinants of CML.

The BCR-ABL1 gene is observed in all cases of CML and detection of this gene, together with karyotyping to identify the Ph chromosome, is used to confirm the diagnosis [1]. Screening for BCR-ABL1 is usually performed if a full blood cell count reports an abnormally high granulocyte count. Measurement of BCR-ABL1 transcript levels by quantitative PCR allows for monitoring initial treatment response, and predicting treatment failure and/or disease progression [5]. Interestingly, the BCR-ABL1 transcript can also be detected, by specially sensitive PCR methods, in healthy individuals without CML symptoms [6]. It is hypothesised that in these cases, the translocation occurs in a haemopoietic cell or environment that is unable to support leukaemia transformation. The BCR-ABL1 signal from this phenomenon is very low and thus does not pose a concern for diagnostic laboratories [6].

Disease evolution and prognosis

Without therapeutic intervention, CML progresses from CP (generally after 3–5 years) to BC, often via an accelerated phase (AP). Disease progression is defined by the blast cell count in the peripheral blood as of 10–20 % in AP and >20 % in BC. The phenotype of BC can be myeloid or lymphoid or, in rare cases, both. Myeloid-BC is predominantly observed (on a 2:1 ratio) over lymphoid-BC [7].

Leukaemic cells in advanced disease lose the ability to undergo terminal differentiation, resulting in an expansion of primitive cells rather than mature granulocytes. The exact mechanism for disease progression is unknown. However, mutations in genes other than BCR-ABL1 are commonly detected following BC transformation [8], which suggests that a second hit is important for the transformation into acute leukaemia.

BC-CML nearly invariably leads to patient mortality from infection, thrombosis or anaemia—a consequence of bone marrow failure due to the lack of cell differentiation and massive infiltration with immature blasts [9]. Prior to successful treatment, the median survival of CML patients after diagnosis was approximately 3 years [1, 10].

Treatment

Introduction of interferon-α therapy and stem cell transplantation marked the first era when survival and quality of life noticeably improved for a large proportion of patients [1, 10]. During the peak of interferon treatment, the median survival doubled to 6 years [10]. Previously, cytotoxic agents (e.g., arsenic, radiotherapy, busulfan and hydroxyurea) were primarily used to treat the symptoms of CML, but did not alter the course of the disease. The recent development of tyrosine kinase inhibitors (TKIs) has greatly improved patient outcome. The TKI imatinib is currently the first-line therapy for CML. Its inhibition of the BCR-ABL1 kinase significantly reduces the frequency of progression to BC and eliminates the symptoms of CP [11]. TKI treatment has led to overall survival rates of >80 % after 8 years [12]. Moreover, deep responses achieved in some patients have allowed clinicians to consider therapy cessation—a new goal for the treatment of CML [13].

Despite successful advances in CML treatment, imatinib resistance is observed in approximately 25 % of patients [14]. Of these patients, the most common known resistance mechanism are mutations in the BCR-ABL1 protein, which are observed in 25–30 % of early CP and 70–80 % of BC patients [15]. Current strategies to circumvent resistance include the use of second-generation BCR-ABL1 TKIs, such as nilotinib, dasatinib and bosutinib [16], and targeting other cellular pathways. In addition, the treatment options in BC-CML remain dismal. Therefore, there is still a necessity to refine the treatment of CML, as discussed in detail on the subsequent articles in this issue.

The molecular biology of CML

The t(9;22) translocation and the BCR-ABL1 gene

The Ph chromosome is formed by a reciprocal t(9;22)(q34;q11) translocation between the long arms of chromosomes 9 and 22, causing the juxtaposition of the BCR and ABL1 genes. The BCR-ABL1 fusion gene consists of the 5′ end of the BCR (breakpoint cluster region) gene and the 3′end of the ABL1 gene (also known as Abelson). The location of the BCR and ABL1 genomic breakpoints is highly variable [17], but the recombination usually involves fusion of intron 13 or 14 of BCR with a 140-kilobase (kb) region of ABL1 between exons 1b and 2 (Fig. 1a) [17]. Regardless of the breakpoint location on the ABL1 gene, mRNA splicing gives rise to major BCR-ABL1 transcripts with e13a2 (BCR exon 13 and ABL1 exon 2) or e14a2 junctions. These transcripts were originally referred to as b2a2 and b3a2, respectively. Both transcripts result in the expression of a 210-kDa BCR-ABL1 protein. There has been much debate regarding the consequence of a patient expressing either the e13a2 or e14a2 transcript [18]. The position of the BCR breakpoint has been correlated with patient prognosis [19–21], platelet count [22–25] and response to therapy [19, 23, 26], but there are other reports refuting the importance of the BCR breakpoint [27–30].

Protein structure

The 210-kDa BCR-ABL1 protein observed in CML contains more than ten protein domains (Fig. 1b). BCR-ABL1 retains the Ser/Thr kinase, Rho/GEF and dimerisation (coiled-coil)

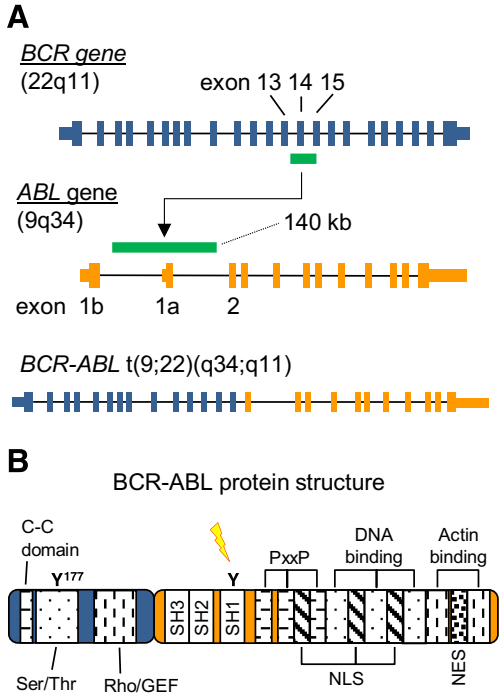

Fig. 1 The gene and protein structure of BCR-ABL. **a** The BCR-ABL fusion gene consists of the 5′ end of the BCR gene and the 3′end of the ABL1. The location of the translocation usually involves fusion of intron 13 or 14 of BCR with a 140-kilobase (*kb*) region of ABL1 between exons 1b and 2. **b** The BCR-ABL protein contains the dimerisation or coiled-coil (*C-C*) domain, Ser/Thr kinase domain and the Rho/GEF domain of BCR, as well as the SH-domains, proline-rich (*PxxP*), nuclear localisation signal (*NLS*), DNA-binding, nuclear export signal (*NES*) and Actin-binding domains from ABL. The tyrosine residues in the Ser/Thr and SH1 kinase domains have been highlighted with a *Y*. The diagrams in **a** and **b** are not to scale

domains from BCR, and is fused to the SH, proline-rich (PxxP), DNA- and actin-binding domains, and nuclear localisation and entry signals from ABL1. The SH1 tyrosine kinase region is the most studied BCR-ABL1 domain due to its inherent role in CML pathogenesis. However, other features such as tryrosine-177 in the Ser/Thr kinase domain are equally integral for the function of BCR-ABL1 [31–34].

Although BCR-ABL1 contains the majority of the ABL1 gene, it lacks the sequence coding for ABL1's N-terminal myristoylation site. It is thought that the loss of this moiety is in part responsible for BCR-ABL1's constitutive kinase activity. For the normal ABL1 protein, myristoylation of its N-terminus and subsequent cis-binding within ABL1's myristoylation binding pocket causes autoinhibition of SH1 kinase activity [32]. Since BCR-ABL1 retains the myristoylation binding pocket, compounds targeting this motif have been trialed to inhibit BCR-ABL1 kinase activity. These compounds exhibit promising allosteric inhibition of BCR-ABL1 activity and may enhance the capabilities of therapeutic targeting of BCR-ABL1 [35].

The consequence of BCR-ABL1

The BCR-ABL1 protein gives rise to aberrant activation of cell-signalling pathways and a shift to a cellular environment that supports leukaemia. This atypical pathway activation has been linked to changes in growth factor dependence, apoptosis, proliferation and cell adhesion. These attributes cause hyper-proliferation of granulocytes and clinical features observed in CP-CML (Fig. 2) [36]. The importance of BCR-ABL1 signalling (particularly via the tyrosine kinase activity) is demonstrated by the success of TKI therapy. The ability to target BCR-ABL1 signalling has also given scientists the ability to further dissect the biology of CML.

Early CML models focused on BCR-ABL1's primary mechanism of disease. Reconstitution of mouse bone marrow with BCR-ABL1 expressing haemopoietic stem cells (HSCs) caused affected mice to display a CML-like phenotype [36–38]. Additional work established BCR-ABL1's ability to transform cells, cause growth factor-independent cell growth and block apoptosis [39–41]. Specific targeting of BCR-ABL1 by antisense oligonucleotides [42–44] and disruption of BCR-ABL1 kinase activity [45] showed that BCR-ABL1 was essential for maintenance of leukaemia. These initial observations underpinned the function of BCR-ABL1 and affirmed this gene as the driver of CP-CML. Since expression of the BCR-ABL1 coding sequence in a HSC is sufficient to generate CML-like disease, it is generally accepted that BCR-ABL1 is the sole lesion required for CML. It would be unlikely that an additional event is required to

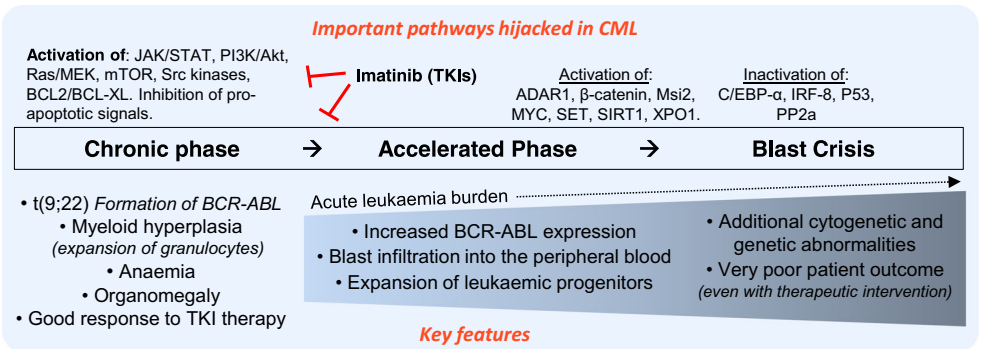

Fig. 2 Important events in CML outlined in this review. The colour gradient depicts increasing acute leukaemia burden and severity of listed features (five) found in accelerated phase and blast crisis

generate CML. As will be discussed herein, BCR-ABL1 has remarkable properties that can control almost every cellular event to function in its favour for promoting CP-CML. Furthermore, there are no consistent abnormalities that accompany BCR-ABL1. However, the requirement of an additional event to cause CML has not been formally ruled out.

How does BCR-ABL1 work?

After understanding the resultant phenotype of BCR-ABL1 expression, the focus of research shifted to identifying the targets of BCR-ABL1. A substantial body of work has since dissected a complex network of pathways that are hijacked by BCR-ABL1 to promote CP-CML. Thus, it was found that Jak/STAT, PI3K/Akt and Ras/MEK are at the forefront to pathogenic signalling via BCR-ABL1 [46]. The next section outlines some of the important genes that are required for BCR-ABL1's leukaemogenic reprogramming.

JAK/STAT

Signalling from the JAK/STAT pathway is commonly augmented in leukaemia [47]. STAT proteins are transcription factors activated by the JAK cell receptor [48]. CML models demonstrated that BCR-ABL1 kinase activity directly enhances JAK2/STAT activation to promote cell growth/survival [49, 50]. Moreover, JAK2 can phosphorylate BCR-ABL1 tyrosine-177, a key component of BCR-ABL1 activity [51]. Conditional knockout of STAT5 prior to and following BCR-ABL1-induced CML in mice demonstrated that STAT5 is essential for both CML development and maintenance. STAT5 knockout mice failed to display a CML phenotype in the presence of BCR-ABL1, and their donor cells did not engraft secondary recipient mice. Furthermore, STAT5 was not essential for normal haemopoiesis or the induction of lymphoid leukaemia [52, 53], which makes it a good therapeutic target for CML. Interestingly, whilst JAK2 is upstream of STAT5, it was reported that deletion of JAK2 was not essential for myeloid (but was required for lymphoid) leukaemia in BCR-ABL1 mouse models [54]. It was proposed that BCR-ABL1 may directly activate STAT5 [54] and bypass endogenous regulation by JAK2 to promote leukaemogenesis [55, 56]. Nevertheless, JAK inhibitors exhibit efficacy against primary CML cells, including TKI-resistant cells [57], and recent work suggests that JAK-signalling is important for stem cell biology (discussed later).

PI3K/AKT and autophagy

PI3K proteins communicate extracellular signals to modulate transcription factor activation and programming that favours cell growth/survival and inhibition of cell death [58]. AKT is a downstream effector of PI3K and plays a major role in its signalling [58]. BCR-ABL1 can stimulate PI3K signalling via the Grb2/Gab2 [59] and CBL [60] adaptor proteins. One of the first reports revealing PI3K's role in CML was the observation that PI3K was required for BCR-ABL1-mediated transformation of haemopoietic cells [61]. Subsequent work found that PI3K/AKT is also important for CML maintenance, and inhibition of PI3K signalling can circumvent BCR-ABL1 oncogenesis and kill primary CML cells [62]. Another consequence of PI3K activation is stimulation of the mTOR pathway [63], which is responsible for controlling protein synthesis, cell growth/size and autophagy.

Autophagy involvement in CML is a new area of interest. Autophagy can occur following cell stress (i.e., loss of BCR-ABL1 signalling) to send the cell into hibernation rather than apoptosis, and can be reversed when the environment becomes favourable again. Recent studies have observed that whilst BCR-ABL1 inhibits autophagy, TKI treatment restores this pathway and may allow for protection of leukaemia cells and resistance to therapy [64]. Co-inhibition of autophagy and BCR-ABL1 considerably enhances eradication of primitive CML cells compared to TKI alone [65]. Therefore, this approach appears to be a promising method to counter the unwanted TKI-mediated inhibition of autophagy.

Ras/MEK pathway

Activation of Ras GTPases/MEK kinases stimulates cell growth via membrane receptor-binding cascade to activate transcription of a number of growth factor genes, and is a key pathway deregulated in cancer [66]. BCR-ABL1 activates Ras via Grb2/Gab2 phosphorylation to promote cell growth [31, 67]. Disruption of Ras signalling impairs development of BCR-ABL1-induced CML-like disease in mice [60, 68]. In addition, small molecule inhibitors against MEK can target primitive CML cells [69, 70]. However, there is limited knowledge of how the Ras-effector repertoire contributes to disease, and which effectors in particular are important. One exception is NF-κB, which is a transcription factor activated by BCR-ABL1/Ras [71] and required for BCR-ABL1-induced CML [72].

Src kinases

The Src-family kinases (SFKs) are another group of widely studied downstream targets of BCR-ABL1. Their role is to coordinate cell growth, differentiation and motility in response to extracellular signals [73]. Initial CML cell line models showed that BCR-ABL1 expression significantly activated the Hck and Lyn SFKs [74]. Subsequent studies demonstrated that Hck, Lyn and Fyn were required for BCR-ABL1 cell line transformation, as well as functionally phosphorylating several BCR-ABL1 tyrosines [75, 76]. One mechanism by which SFKs contribute to disease is in assisting BCR-ABL1 in its

activation of STAT5 and AKT [77, 78]. Interestingly, knockdown of Lyn exhibited impressive killing of BC cells and its upregulation in BC-CML suggested a potential role for promoting disease progression [79, 80]. However, their importance in CML remains unclear because CML mouse models show that SFKs are not required for initiation of CML and support the generation of acute lymphoid leukaemia [81, 82].

Crkl

The adaptor protein Crkl is constitutively activated by BCR-ABL1 [83]. Protein networks involving BCR-ABL1 and Crkl include Cbl, STAT, PI3K, Paxillin and Ras [84]. Indeed, loss of the interaction between Crkl and BCR-ABL1 impaired BCR-ABL1-induced transformation in mice [85]. Potent phosphorylation of Crkl by BCR-ABL1 allows the measurement of the percentage of phospho-Crkl as a surrogate to BCR-ABL1 phosphorylation levels (which are more difficult to measure) in order to experimentally examine patient response to TKI therapy and to predict outcome [86].

Long non-coding RNA-BGL3

The first functional role for a long non-coding (lnc)RNA in CML has just been described. The general mechanism by which lncRNAs function is not yet fully understood, but lncRNA-BGL3 was reported to play an important role in BCR-ABL1 transformation. In K562 and primary CML cells, BCR-ABL1 inhibits the expression of this lncRNA in a kinase-dependent manner via the MYC transcription factor [87]. Forced expression of lncRNA-BGL3 in K562 cells induced apoptosis and reduced the ability of these cells to engraft in mice. It was subsequently found that this lncRNA acted as a decoy for several microRNAs that target the tumour suppressor gene PTEN, leading to PTEN stabilisation and associated inhibition of leukaemogenesis.

Apoptosis deregulation

In addition to promoting cell proliferation, BCR-ABL1 can disrupt cell death. An example of this involves a BCR-ABL1, Bad, BCL2 and BCL-X_L circuit. Expression of BCR-ABL1 can inhibit apoptosis by increasing expression of the anti-apoptotic proteins BCL2 and BCL-X_L [88]. Both STAT5 and PI3K signalling are important mediators of BCR-ABL1's anti-apoptotic function. STAT5 activation by BCR-ABL1 causes increased BCL-X_L expression [89, 90]. Furthermore, phosphorylation of the pro-apoptotic protein Bad by PI3K/Akt facilitates the interaction between the chaperone protein 14-3-3 and Bad, which restricts Bad to the cytoplasm [91]. This prevents Bad opposing BCL2 and BCL-X_L inhibition of apoptosis in the mitochondrion.

CML stem cells

There are now substantial observations that quiescent LSCs within the CD34+ population are resistant to TKIs [92–94]. This phenomenon is believed to be responsible for relapse in approximately half of all patients eligible for therapy cessation [13]. As a result, CML stem cells have been thrust into the limelight. Prior to this, research was focused on characterising the differences between normal HSCs and LSCs. One goal was to understand exactly how BCR-ABL1 altered normal haemopoiesis to drive CP-CML, leading to the identification of several haemopoietic markers and oncogenes that differed between the two populations (reviewed in [95]). The improvement of strategies to isolate primitive cells increased accessibility to this very rare (less than 2 % of PB-MNCs) cell population [96, 97]. These early studies also acknowledged the importance of LSCs in the quest for a cure in CML [98], which became a more viable possibility after the availability of potent TKIs.

LSCs are refractory to TKIs

A seminal paper from the Holyoake laboratory showed that kinase inhibition reduced LSC proliferation, but did not kill quiescent LSCs [99]. Further work from the same group demonstrated that LSCs were also insensitive to more potent second-generation TKIs, even though the BCR-ABL1 kinase activity was silenced [93, 94]. These studies warned of the possibility of early relapse, but long-term TKI usage has quelled these concerns. Subsequent studies have strengthened the notion that LSCs do not rely on BCR-ABL1 kinase activity for survival [100, 101]. They showed that potent TKIs failed to wipeout CML-LSCs, that the bone marrow environment may offer sanctuary against TKIs and that withdrawal of TKIs leads to reconstitution of leukaemic expansion [100, 101]. It was recently reported that therapy-refractory LSCs exhibit a bias for low BCR-ABL1 expression [102, 103]. So, LSCs that 'keep their kinase activity down' may survive TKI therapy and, perhaps, a non-kinase BCR-ABL1-dependent mechanism may protect LSCs (which has yet to be ruled out). Several pathways have been shown to play key roles in stem cell biology, and targeting them could lead to a promising strategy to eliminate the LSC in CML.

β-catenin

β-catenin signalling is important for HSC and LSC development and self-renewal [104]. β-catenin is a component of the Wnt signalling pathway. When Wnt is bound to its receptor Frizzled, β-catenin is protected from ubiquitin-mediated degradation and is free to translocate to the nucleus and activate its target genes [105]. β-catenin tyrosine phosphorylation by

BCR-ABL1 also leads to its stabilisation and increased levels and activity in CML [102]. Although dispensable for maintenance of LSCs and HSCs [106–108], therapeutic targeting of this pathway can cooperate with TKIs to delay disease onset and deplete CML-LSCs in CML mouse models [107]. The β-catenin pathway has also been implicated in BC-CML. Enhanced β-catenin signalling in BC-CML is thought to confer stem cell-like properties to progenitor cells leading to their expansion—a prerequisite for advanced disease [109].

Smo

The Smoothened (Smo)/hedgehog pathway governs developmental and homeostasis decisions conserved from *Drosophila* to humans. Smo is a membrane receptor for the hedgehog ligand. Analogous to Wnt/β-catenin, activation of Smo causes activation of the Gli transcription factor, which activates its downstream transcriptional targets [110]. Whilst there is debate surrounding the role of this pathway in HSC development, there is a consensus that it is important for CML-LSCs [111]. Smo signalling is enhanced in CML compared to normal HSCs and both the loss and inhibition of Smo impairs the development and maintenance of BCR-ABL1-induced CML in mice [112]. The availability of Smo inhibitors and their synergy with TKIs has allowed for clinical trials to determine the efficacy of Smo/TKI therapy [111].

PP2A-JAK2-SET

BCR-ABL1 was reported to circumvent the requirement of JAK2 for activation of STAT5 [54], but a recent study rekindled the issue on the possible importance of JAK2 in CML. A network involving PP2A/JAK2/Set/GSK-3β was shown to play a critical role in LSC survival [113]. At the centre is PP2A, a tyrosine phosphatase whose activity is impaired in CML. Activated PP2A has the ability to silence key pathways that are activated by BCR-ABL1, including BCR-ABL1 itself [114]. In CML-LSCs, BCR-ABL1/JAK2 signalling overcomes PP2A activity by enhancing the activity of SET, a PP2A-inhibitor. Blocking SET-inhibition of PP2A restores PP2A function and impairs the self-renewal and survival of CML-LSCs, but not normal HSCs [113]. A major mechanism by which PP2A activation affects LSC maintenance is thought to be the loss of β-catenin signalling via GSK-3β-mediated ubiquitination. This is coupled with PP2A silencing of BCR-ABL1 to allow for LSC turnover and reduced leukaemic potential.

FoxO

The FoxO transcription factors, in particular FoxO3a, have also been linked to LSC biology. BCR-ABL1 promotes nuclear export and deactivation of these transcription factors via PI3K/Akt [115]. In mature cells, Akt signalling is strong and assists propagation of BCR-ABL1's proliferative advantage. However, in LSCs, Akt signalling is inhibited by PTEN [116] and TGF-β [117]. This reverses BCR-ABL1 inactivation of FoxO3a and allows for BCL6 transcription, which favours quiescence and self-renewal [116]. Targeting this mechanism with BCL6 or TGF-β inhibitors together with TKIs perturbed CML development and induced cell death/turnover of primitive CML cells [116, 117].

Bone marrow microenvironment

HSCs reside in the bone marrow, which provides an environment that controls haemopoiesis by coordinating HSC renewal and differentiation into functional blood cells. The bone marrow supportive environment comprises the osteoblast and vascular niches [118, 119]. The former promotes self-renewal and quiescence, whilst the vascular niche is permissive of differentiation into progenitor and then functional cells. Furthermore, signalling molecules and membrane receptors are also vital for legitimate haemopoiesis. In CML, it is thought that the osteoblast niche nurtures LSCs and may explain why LSCs do not require BCR-ABL1 kinase activity to survive TKI exposure [120, 121]. This may also contribute to BC. Since progenitor cells attain stem cell-like properties (discussed later), a progenitor-contingent may retreat towards the osteoblast niche for protection against TKIs, whilst retaining cycling properties that allow for faster accumulation of mutations (compared to LSCs) required for transformation.

Biology of blast crisis

It is currently unknown exactly how the transition to BC occurs. This stage of the disease is characterised by the expansion of haemopoietic progenitors that can no longer differentiate and can invade the peripheral blood. These progenitor cells gain self-renewal capacity, differentiation arrest and survival properties that lead to their uncontrolled proliferation [109]. Thus, BC progenitors exhibit more stem cell-like characteristics compared to CP-progenitors. This is partially attributed to increased β-catenin activity, which is also thought to drive their capacity to initiate leukaemia in mice [109]. Genomic and genetic instability is another feature of advanced disease [122, 123]. Extra chromosomal abnormalities are observed in approximately 80 % of BC patients (e.g., Ph duplication, trisomy 8 or 19, loss of 17p) [124]. Pathogenic alterations of tumour suppressor and oncogenes have also been detected in advanced CML [125]. Thus, it is hypothesised that these additional hits are responsible in part for the transition into BC [123, 125]. The changes in cell biology in BC may explain why TKIs have diminished efficacy in BC, reflecting reduced reliance on BCR-ABL1 activity in the presence of

other mutations, and/or stem cell-like progenitors becoming refractive to TKIs similar to CP-LSCs.

BCR-ABL1 and BC-CML

Inhibition of BCR-ABL1 kinase activity effectively delays the onset of BC, but does not eliminate the primitive population that establishes advanced disease. One interpretation is that BCR-ABL1 signalling must be a prerequisite for transition to BC, especially since progression to BC is rare in TKI-responsive patients. A number of studies have found increased expression of BCR-ABL1 in BC compared to CP. This increase was observed when comparing matched CP and BC samples (from the same patient) at both the mRNA [126–129] and protein levels [114, 126, 130]. Additionally, it has been shown that cells expressing higher amounts of BCR-ABL1 have an increase in genomic instability as well as perturbed differentiation, which are intrinsic properties of BC-CML [123, 131]. These findings imply more than a passenger role for BCR-ABL1 in BC transformation, but this has yet to be determined.

DNA damage/repair

BCR-ABL1 has been shown to facilitate genomic instability via disrupting DNA repair pathways, generating reactive oxygen species and inhibiting DNA damage-induced apoptosis, which may lead to retention of genomic mutations [132–136]. These events are in part tied to the level of BCR-ABL1 expression [137]. CML CD34+ cells express high levels of BCR-ABL1 as compared to mature cells [128], and they are highly susceptible to genomic instability as compared to their healthy counterparts [100]. Although not formally shown, it is reasonable to suggest that BCR-ABL1 provides progenitor cells with the genomic plasticity required for malignant transformation [123, 138, 139].

C/EBPα and hnRNP-E2

Required for myeloid differentiation [140], C/EBPα expression is reduced in cell lines expressing BCR-ABL1 [141]. These lines responded poorly to growth factor-induced differentiation [131], but ectopic expression of C/EBPα and BCR-ABL1 kinase inhibition were able to reverse this differentiation block [141]. Further experiments revealed that BCR-ABL1 negatively regulates the expression of C/EBPα via upregulation of hnRNP-E2, an RNA-binding protein which inhibits C/EBPα expression [131]. Analysis of CML-patient cells found that loss of C/EBPα and expression of hnRNP-E2 was restricted to BC [131]. In addition, hnRNP-E2 upregulation and C/EBPα downregulation were directly proportional to increasing levels of BCR-ABL1 [131]. To add extra complexity to this pathway, it was recently shown that the microRNA miR-328 acts in a non-canonical way to block hnRNP-E2 regulation of C/EBPα and promotes myeloid differentiation [142]. The expression of miR-328 negatively correlates with BCR-ABL1 expression levels, and is thus downregulated in BC [142]. These experiments provide evidence of a sophisticated circuit by which enhanced BCR-ABL1 expression can facilitate a switch to BC by disrupting myeloid differentiation.

IRF-8: the antithesis of BCR-ABL1?

Remarkably, genomic deletion of interferon-regulatory factor 8 (IRF-8) (also known as ICSBP) in mice was sufficient to generate a CML-like myeloproliferative disease [143]. The mice developed splenomegaly, WBC counts consistent with those of CML patients and one third of them succumbed to a BC-like pathology. It is thus unexpected that loss of IRF-8 has not been observed to generate cancer in its own right in humans. IRF-8 is downregulated in CML patients [144], which occurs via the BCR-ABL1/STAT5 signalling axis [145]. Over-expression of IRF-8 produces the opposite effect—induction of apoptosis in myeloid cell lines [146]. A mouse model co-expressing BCR-ABL1 and IRF-8 demonstrated IRF-8's tumour suppressor role in vivo. Mice transplanted with BCR-ABL1/IRF-8 cells survived much longer than those with BCR-ABL1 alone, but the former showed increased incidence of lymphoid leukaemia [147, 148]. IRF-8's reversal of BCR-ABL1's anti-apoptotic effects partially explained IRF-8's suppressor role [146, 149]. As interferon activates IRF-8 expression [144], IRF-8 may underpin the mechanism behind interferon treatment efficacy in CML.

Recent work has identified a putative role for IRF-8 in CML progenitor cells and disease progression via β-catenin disruption. In the absence of BCR-ABL1, IRF-8 destabilises the β-catenin protein via the GAS2 protease to promote normal haemopoiesis [150, 151]. Interestingly, expression of a mutant form of β-catenin which cannot be degraded was toxic and impaired myelopoiesis. However, upon IRF-8 deletion, the mutant β-catenin caused acute leukaemia in mice [151]. That study suggests that β-catenin signalling is toxic in the presence of IRF-8, and loss of IRF-8 is required for myeloproliferation and BC-like disease. In the presence of BCR-ABL1, IRF-8 expression is reduced and β-catenin activity enhanced, which according to this model should prime for disease progression.

Other pathways involved in BC-CML

MYC

The MYC proto-oncogene was one of the first genes implicated in disease progression. MYC is a transcription factor

which governs the expression of genes enabling cell growth and proliferation and, thus, commonly activated in cancer [152]. It was originally observed that patients with BC exhibited higher levels of MYC as compared to CP patients [153]. This was followed by reports that ABL1 expression enhances MYC expression and that MYC is required for BCR-ABL1-induced transformation [154, 155]. Although excess MYC can induce apoptosis [156], early cell line models show that BCR-ABL1 activation of BCL2 can inhibit MYC apoptotic activity whilst retaining its proliferative advantage [157]. This is one of many examples by which BCR-ABL1 creates 'a perfect storm' to promote leukaemogenesis.

BCR-ABL1 can control MYC expression via PI3K, JAK2 and the transcription factor E2F1 [61, 158–160], and protein stability via MEK and hnRNP-K [161]. The latter is of interest because the hnRNP-K stabilisation of MYC is enhanced in BC, and disruption of hnRNP-K restores myeloid differentiation in BCR-ABL1-expressing cells [161]. A recent CML mouse model demonstrated that MYC expression is required for CML maintenance and progression. They further showed that high levels of MYC are harmful for LSCs, and ubiquitination (degradation) of MYC by ubiquitin ligase Fbw7 keeps MYC levels in check in LSCs [162]. This provides a rationale for the constrained BCR-ABL1 kinase activity observed in quiescent LSCs [113] and selection of low BCR-ABL1 expression in TKI-refractive LSCs [102, 103] (suggesting that enhanced BCR-ABL1 signalling is toxic for quiescent cells). These findings, coupled with MYC's established role in myeloid differentiation [163], present deregulation of MYC as a strong candidate for BC transformation in CML.

p53

The normal function of p53 is to respond to cell stress events, where it becomes activated and drives transcription of genes that decide cell fate (apoptosis, DNA repair, cell cycle arrest or senescence) [164]. Early CML genetic studies observed inactivating mutations of p53 in approximately 20 % of patients who progressed to BC-CML [165, 166]. Regulation of p53 by BCR-ABL1 is complex and unclear, with p53 activation [167, 168] and inactivation [169, 170] being reported. However, loss or inhibition of p53 promotes BC-like disease in mice [170–172], and stabilisation of p53 in BC cells induces apoptosis [168, 169]. It has also been shown that MYC over-expression is only toxic to LSCs if p53 is present [162], which might explain why p53 mutations are observed in about 20 % of BC-CML [166].

Musashi-2 (Msi2)

Musashi-2 (Msi2) is an RNA-binding protein [173], which has been recently linked to HSC development [174]. Two groups simultaneously reported the involvement of Musashi-2 in promoting BC-like disease [175, 176]. These studies found that RNA-binding protein's expression is markedly elevated in BC compared to CP. In addition, CML mouse models demonstrated that enhanced Msi2 expression promoted aggressive leukaemia via impaired myeloid differentiation and progenitor expansion [175, 176]. It was initially thought that high Msi2 expression inhibited the Msi2's downstream target NUMB, leading to disruption of cell differentiation [175, 176]. However, a new study identified direct mRNA targets of Musashi-2 and discovered an interactome consisting of genes associated with self-renewal. Surprisingly, this study found no connection between Msi2 and NUMB and, instead, proposed that Msi2 cooperates with TGF-β to propagate self-renewal signals important for promoting advanced disease [177].

XPO1

The nuclear export protein, XPO1 is another novel candidate for regulation of BC. Pharmacological blockade of XPO1 function was sufficient to kill both CP and BC-primary CD34+ cells and synergise with imatinib in cell line models [178]. XPO1 inhibition in BCR-ABL1 cell lines demonstrated that impaired nuclear transport could explain XPO1-inhibition lethality. For example, both SET and p53 were abnormally enriched in the nucleus leading to their inactivation [178]. Additional experiments revealed that long-term XPO1 inhibition caused BCR-ABL1 degradation (via loss of SET control of PP2A activity), whereas short-term inhibition shutdown STAT5, AKT and MEK signalling prior to affecting BCR-ABL1 activity [178]. This suggests that both BCR-ABL1-dependent and BCR-ABL1-independent cell death results upon XPO1 inhibition. Remarkably, an XPO1 inhibitor reversed CML symptoms (WBC count/splenomegaly) in a patient who was resistant to TKI therapy and had progressed to AP-CML—highlighting an exciting strategy to treat advanced disease [178].

SIRT1

Recently, the protein-deacetylase SIRT1 was implicated in CML LSC function. Expression of SIRT1 is enhanced in CML, and is in part regulated by BCR-ABL1/STAT5 [179]. SIRT1 suppression of p53/FoxO-controlled LSC maintenance is believed to prolong the survival of CML-LSCs [179, 180]. In contrast, knockout or inhibition of SIRT1 impairs CML development and disease progression in mice [179, 180]. SIRT1 regulation of the DNA repair protein Ku70 in CML cell lines causes enhancement of less faithful non-homologous end joining to enhance

DNA mutations [181]. This may have an impact on promoting second hits required for BC transformation. The ability to prolong LSC survival and increase genomic instability are indicative of SIRT1 having a major role in BC-CML development.

ADAR1

BCR-ABL1's diverse repertoire now includes the RNA-editing machinery, represented by its capacity to regulate ADAR1 in BC-LSCs [182, 183]. ADAR1 is an RNA editor whose enzymatic activity converts adenosine to inosine in RNA, resulting in these nucleotides being interpreted as guanine in the ribosome, thus altering RNA behaviour and protein amino acid composition. Analysis of ADAR1 expression in CML found enhanced expression in the progression to BC, and this was dependent on BCR-ABL1 levels. As a result, an increase in A to I RNA editing (by ADAR1) in BC cells caused altered expression of RNA-edited genes [182]. These studies speculate that ADAR1 editing allows for the progenitor self-renewal properties that are observed in BC-CML. Following disruption of ADAR1 expression in CML mouse models, CML development, maintenance and BC onset were impaired due to the loss of primitive leukaemic cells. In contrast, ADAR1 over-expression caused myeloid progenitor expansion [182]. Specific deletion of ADAR1's RNA-editing moiety demonstrated that RNA editing is vital for CML progenitor self-renewal [183].

Concluding remarks

The biology of CML is initially centred on BCR-ABL1's kinase activity, which is sufficient to cause the clinical features of CP-CML. The ability to readily model CML in both cell lines and mice has allowed for a large accumulation of knowledge regarding the molecular network of CML. These studies have shown that BCR-ABL1 is implicated in altering almost every process within the cell to drive CML pathogenesis. Current literature has shown that STAT5 stands out as a vital component for BCR-ABL1's induction of CML as demonstrated by the 'gold-standard' conditional knockout model [52, 53]. The recent investigation of primitive CML cell biology has resulted in the utilisation of new and powerful techniques to identify a number of genes important within this compartment. The most studied are p53, MYC and β-catenin, which have prominent roles in both stem cell biology and BC transformation.

The LSC and progenitor populations are currently at the forefront of CML biology. Improved methods to examine these cell types have uncovered vital information that is both mechanistically and therapeutically important for understanding this disease. Of particular interest is the finding that LSCs do not rely on BCR-ABL1 kinase activity for survival. It is unknown if another protein domain of BCR-ABL1 confers LSC survival properties. Another possibility is that BCR-ABL1 can programme LSCs in such a way that the kinase activity is no longer required. This is consistent with the observation that BCR-ABL1 signalling is tempered in LSCs, that TKI-resistant LSCs express low levels of BCR-ABL1 and enhanced signalling may even be toxic. Primitive CML cells also contain the answer to the mechanisms of disease progression. It remains elusive whether the HSC or progenitor compartment gives rise to the clone(s) responsible for BC-CML. Pinpointing the cell(s) responsible is important because each of these compartments have discrete biological properties and thus require alternate therapeutic strategies.

The advent of next-generation sequencing and powerful experimental modelling tools will no doubt provide a flood of information regarding CML biology. Rapid and accurate sequencing of whole genomes, exomes and epigenomes is becoming increasingly accessible to most laboratories. This should generate evidence for novel recurrent mutations and epigenetic marks that favour or hinder CML pathogenesis or response to treatment. One example is a polymorphism in the *Bim* gene, which perturbs apoptosis induced by imatinib to impair TKI efficacy [184]. This mutation was uncovered by next-generation sequencing and examined in mouse models using new gene editing techniques.

In the proteomics field, improved methods to study proteins (SILAC, [185]) and more powerful mass spectrometers have the potential to uncover post-translational modifications and protein interactomes. The study of proteome-networks is relatively untapped in CML (although elegant examples do exist, [186, 187]), making this an attractive area of interest to improve the knowledge of CML biology. The same can be said of non-coding RNA (ncRNA) involvement in CML. It is known that ncRNA deregulation occurs in CML, for example in CP vs. BC, and in primitive cells vs. granulocytes [188, 189]. However, most functional work is limited to a single microRNA and target. Further work is required to understand the global ncRNA circuitry in key areas within this disease. It is also anticipated that the recent lncRNA-BGL3 study will spark interest into researching the impact of lncRNAs in CML.

Finally, availability of pathway inhibitors and genome editing (TALEN and crispR) systems [190] are powerful options to functionally validate pathways identified by next-generation sequencing/proteomic studies in both cell lines and mouse models. These technologies will make for an exciting time to uncover novel mechanisms behind CML pathogenesis and the potential for application to other diseases.

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