

Blast Transformation of Chronic Myeloid Leukemia Caused by Genetic Alterations, Epigenetic Dysfunctions and Aberrantly Regulated Factors in Bone Marrow Microenvironment

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Abstract

Chronic myeloid leukemia (CML), a refractory leukemia stem cell (LSC) disease, is characterized by the Philadelphia chromosome (Ph) t(9;22)(q34;q11.2) that generates the gene fusion between *BCR* and *ABL1* genes. The *BCR::ABL1* fusion gene products have a constitutively activated tyrosine kinase that causes leukemogenesis of CML. Tyrosine kinase inhibitors (TKIs) are effective in chronic phase of CML (CML-CP) but ineffective in blast phase of CML (CML-BC). The mechanisms of blast crisis (BC) in CML are highly complicated. This review aims to depict several mechanisms of BC induction in CML-BP. Blast transformation is caused by various mechanisms such as genetic alterations (*IKZF1*, *TP53*, *INK4-ARF* and *Rb*), epigenetic dysfunctions (aberrant methylation of *CEBPα* and *PU.1*, and underexpression of histone demethylase RBP2), suppression of differentiation-mediated transcription factors (*CEBPα*, *CEBPβ*, *PU.1* and *UBE2a*), abnormal splicing (*IKZF1* and *GSK3β*) and aberrantly regulated HIF-1α in bone marrow microenvironment. To develop novel therapies against blast cells in CML-BP based on the mechanisms of blast transformation, further studies are required.

Keywords: CML, Blast Crisis, Genetic Alterations, Epigenetic Dysfunction, Differentiation Arrest.

ABBREVIATIONS

ABL1=Abelson protooncogene 1, **aCML**=atypical chronic myeloid leukemia, **ALL**=acute lymphoblastic leukemia, **AML**=acute myeloid leukemia, **AP**=accelerated phase, **ARF**=alternative reading frame, **ARNT**=aryl hydrocarbon receptor nuclear translocator, **ASXL1**=additional sex combs-like 1, **B-ALL**=B-cell acute lymphoblastic leukemia, **BCR**=breakpoint cluster region, **BMM**=bone marrow microenvironment, **BP**=blast phase, **CA9**=carbonic anhydrase IX, **CBP**=cyclic adenosine monophosphate response element-binding protein, **CEBP**=CCAAT/enhancer-binding protein, **CIC**=cancer-initiating cell, **CML**=chronic myeloid leukemia, **CML-N**=neutrophilic chronic myeloid leukemia, **CNL**=chronic neutrophilic leukemia, **CNV**=copy number variation, **CP**=chronic phase, **CSC**=cancer stem cell, **ERK**=extracellular signal-regulated kinase, **GATA-2**=GATA-binding factor-2, **GSK3β**=glycogen synthase kinase-3β, **hnRNP E2**=heterogeneous nuclear ribonucleoprotein E2, **HIF**=hypoxia-inducible factor, **HRE**=hypoxia responsive element, **HSC**=hematopoietic stem cell, **IKZF1**=Ikaros family zinc finger protein 1, **INK4**=inhibitor of cyclin-dependent kinase 4,

indel=insertion/deletion, **LBC**=lymphoid blast crisis, **LSC**=leukemia stem cell, **MBC**=myeloid blast crisis, **Mdm2**=murine double minute 2, **Ph**=Philadelphia chromosome, **PHD2**=prolyl hydroxylase domain protein 2, **PI3K**=phosphatidylinositol 3-kinase, **PTEN**=phosphatase and tensin homolog, **pVHL**=von Hippel-Lindau tumor suppressor protein, **Rb**=retinoblastoma tumor suppressor gene, **RBP2**=retinol-binding protein, **RUNX**=runt-related transcription factor, **SNV**=single-nucleotide variant, **STAT**=signal transducers and activators of transcription, **SV**=structural variation, **TKI**=tyrosine kinase inhibitor, **T-ALL**=T-cell acute lymphoblastic leukemia, **TME**=tumor microenvironment, **UBE2A**=ubiquitin-conjugating enzyme E2A,

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INTRODUCTION

Chronic myeloid leukemia (CML), a refractory leukemia stem cell (LSC) disease [1-4], is characterized by the Philadelphia chromosome (Ph) t(9;22)(q34;q11.2) [5] that generates the gene fusion between *breakpoint cluster region* (*BCR*) and *Abelson protooncogene 1* (*ABL1*). The *BCR::ABL1* fusion gene products have a constitutively activated tyrosine kinase that causes leukemogenesis of CML [6,7]. Tyrosine kinase inhibitors (TKIs) targeting the *BCR::ABL1* tyrosine kinase are quite effective in chronic phase of CML (CML-CP) [3,8-11]. CML progresses from CML-CP through accelerated phase (CML-AP) and finally to blast phase (CML-BP). In CML-BP, differentiation arrest of CML LSCs is induced and blast cells accumulate in bone marrow ($\geq 30\%$) and peripheral blood ($\geq 30\%$) [2]. In CML-BP, blast cell expansion and therapy resistance are serious problems, resulting in poor prognosis. Thus elucidation of the mechanisms of blast transformation is quite important to the development of novel therapies against blast cells in CML-BP. However, the mechanisms of blast crisis (BC) in CML-BP are highly complicated [12-18]. In solid tumors, induction of cancer stem cells (CSCs) is promoted by cooperation of genetic alterations, epigenetic dysfunctions and aberrantly regulated factors in tumor microenvironment (TME) [19-22]. Cellular components of TME consist of CSCs, cancer-initiating cells (CICs), mesenchymal stem cells, endothelial cells, cancer-associated fibroblasts, tumor-associated macrophages, myeloid-derived suppressor cells, extracellular matrix, vasculature, T-lymphocytes and B-lymphocytes [23,24], while non-cellular factors of TME include transcription factors, signaling pathways, post-transcriptional controls (RNA methylation, RNA-binding protein, RNA alternative splicing and noncoding RNAs), cytokines, immune responses, metabolic controls (glycolysis, glutaminolysis and lipogenesis), hypoxic

Table 1. *BCR::ABL1* fusion genes.

<i>BCR::ABL1</i> proteins	Gene fusions	Transcripts	References
p210	<i>BCR</i> exon 13 or 14 with <i>ABL1</i> exon 2 (e13a2) (e14a2)	M-BCR	[38]
p190	<i>BCR</i> exon 1 with <i>ABL1</i> exon 2 (e1a2)	m-BCR	[39]
p230	<i>BCR</i> exon 19 with <i>ABL1</i> exon 2 (e19a2)	μ -BCR	[40]

Abbreviations: *ABL1*=*Abelson protooncogene 1*, *BCR*=*breakpoint cluster region*.

are observed in different *BCR::ABL1* positive leukemias in Table 2 [31,34,35,37]. The p210-*BCR::ABL1* (M-BCR) is found in almost all the cases of CML [31,41,42], whereas the p190-*BCR::ABL1* (m-BCR) is found frequently in *BCR::ABL1* positive acute lymphoblastic leukemia (ALL) [37,43,44], occasionally in acute myeloid leukemia (AML) [45-47] and rarely in CML [48]. However, there are

response (induction of hypoxia-inducible factors [HIFs]) and acidosis correction (induction of carbonic anhydrase IX [CA9] [20-22,25]. Accordingly, blast transformation in CML-BP is promoted by cooperation of genetic alterations, epigenetic dysfunctions and aberrantly regulated factors in bone marrow microenvironment (BMM) [12,15,17,26]. Indeed, various additional genetic mutations [27,28] are observed in CML-BP, and several epigenetic dysfunctions are involved in blast transformation in CML-BP [17,18,29], whereas many differentiation-mediated transcription factors are suppressed in blast cells of CML-BP [17,29], and other mechanisms of blast transformation are proposed [15,30]. This review aims to depict several mechanisms of blast transformation in CML-BP by elucidating genetic alterations, epigenetics dysfunctions, suppression of differentiation-mediated transcription factors, abnormal splicing and aberrantly regulated factors in BMM from an integrated perspective.

BCR::ABL1 fusion genes and BCR::ABL1 positive leukemias

The Ph chromosome is generated by the reciprocal translocations t(9;22)(q34;q11.2) and this translocation generates the *BCR::ABL1* fusion genes [6,7]. There are three *BCR::ABL1* fusion gene products, i.e., p190, p210 and p230 in Table 1 [31-37]. There are several breakpoints in both *BCR* and *ABL1*. The fusion of *BCR* exon 13 or exon 14 with *ABL1* exon 2 are designated as e13b2 or e14b2, and these fusion genes encode a p210 protein with the major *BCR::ABL1* transcript (M-BCR) [38], while the fusion of *BCR* exon 1 with *ABL1* exon 2 (e1a2) constitutes a p190 product with the minor *BCR::ABL1* transcript (m-BCR) [39]. In addition, the fusion of *BCR* exon 19 with *ABL1* exon 2 (e19a2) generates a p230 protein with the micro (μ) *BCR::ABL1* transcript (μ -BCR) [40].

These *BCR::ABL1* transcripts (M-BCR, m-BCR and μ -BCR)

rare cases of atypical CML (aCML) that lacks the p210-*BCR::ABL1* [49-52]. Furthermore, the p190-*BCR::ABL1* positive AML [45-47] or p210-*BCR::ABL1* positive AML [45,53,54] (*de novo* AML with *BCR::ABL1*) and the p190-*BCR::ABL1* positive *de novo* ALL [37,43,44] are also observed. In this regard, it is important to distinguish the *de novo* AML or *de novo* ALL with *BCR::ABL1* from the blast

crisis of CML [32, 55]. In addition, the p230-BCR::ABL1 (μ -BCR) is typically found in neutrophilic CML (CML-N) [31,35,56]. This CML-N should be distinguished from chronic neutrophilic leukemia (CNL) [2] because CNL has no *BCR::ABL1* fusion gene [57,58]. These *BCR::ABL1* fusion

gene tyrosine kinases constitutively activate downstream JAK/signal transducers and activators of transcription (STAT), phosphatidylinositol 3-kinase (PI3K)/AKT and RAS/extracellular signal-regulated kinase (ERK) signaling pathways [1,34,36].

Table 2. *BCR::ABL1* positive leukemias.

<i>BCR::ABL1</i> proteins (transcripts)	<i>BCR::ABL1</i> positive leukemias	References
p210 (M-BCR)	CML AML (rare)	[31,41,42] [45,53,54]
p190 (m-BCR)	ALL (frequent) AML (occasional) CML (rare)	[37,43,44] [45,46,47] [48]
p230 (μ -BCR)	CML-N (typical)	[56]

Abbreviations: *ABL1*=Abelson protooncogene 1, *ALL*=acute lymphoblastic leukemia, *AML*=acute myeloid leukemia, *BCR*=breakpoint cluster region, *CML*=chronic myeloid leukemia, *CML-N*=neutrophilic chronic myeloid leukemia.

BLAST CRISIS IN CML-BP

CML LSCs are evolved from hematopoietic stem cell (HSC) by the *BCR::ABL1* fusion gene and have abilities of self-renewal, differentiation to aberrant hematopoietic cells, cell survival and resistance to apoptosis [15,26]. In contrast, blasts in CML-BP are characterized by differentiation arrest. Introduction of TKI has drastically decreased the percentage of patients with CML-AP or CML-BP from more than 20% to 1~1.5% per year [59], but blast cells in CML-BP are resistant to PKIs mainly due to secondary mutations in the *BCR::ABL1* tyrosine kinase domain [27,60-62]. Blast cells in adult patients with CML-BP are morphologically classified into several lineages, i.e., myeloid BC (up to 70%) [4,17,62], lymphoid BC (up to 30%) [55,63-65], erythroid BC (rare) [66-68] and megakaryocytic blast crisis (rare) [69-72]. Lymphoid BC is further divided into B-cell acute lymphoblastic leukemia (B-ALL) [63,65] and T-cell acute lymphoblastic leukemia (T-ALL) [64].

ADDITIONAL GENETIC MUTATIONS IN CML-BP

In blast cells of CML-BP, various genetic mutations are observed, i.e., somatic nonsynonymous and nonsense single-nucleotide variants (SNVs), small insertion/deletion (indel), copy number variations (CNVs), and structural variations (SVs = fusions). In 39 patients with CML-BP reported by Branford et al. [27], additional SNVs are *ABL1* kinase domain (48.7%), *Ikaros* family zinc finger protein 1 (*IKZF1*) (33.3%), *runt-related transcription factor 1* (*RUNX1*, also known as *AML1*) (30.1%), *additional sex combs-like 1* (*ASXL1*) (23.1%), *BCORL1* (12.8%), *IDH1/IDH2* (7.7%), *BCOR* (5.1%), *GATA-binding factor-2* (*GATA-2*) (5.1%), *PHF6* (5.1%), *SETD1B* (5.1%), *ubiquitin-conjugating enzyme E2A* (*UBE2A*) (5.1%), and *TP53* (2.6%). In 134 patients with CML-BC reported by another study [28], similar

additional SNVs are found, including *ABL1* kinase domain (14.9%), *IKZF1* (12.0%), *RUNX1* (24.6%), *ASXL1* (23.1%), *BCORL1* (7.5%), *IDH1* (2.2%), *IDH2* (2.2%), *BCOR* (3.4%), *GATA-2* (3.0%), *PHF6* (2.2%), *SETD1B* (3.0%), *UBE2A* (3.7%), *TP53* (7.5%), *WNT1* (3.4%), *CDKN2AB* (6.7%) and *TET2* (5.9%). In CML-BC, CNVs such as -7/del(7p), +8, del(17p), amp (17q), +21, and an extra Ph chromosome (+Ph) are frequently found [27,28]. Frequent SVs (fusions) found in CML-BC are *RUNX1-ETS2*, *NUP214-XKR3* [28], *CBFB-MYH11* [27,28], *PAX5-ZCCHC7*, *RUNX1-MX1*, *MBNL1-MECOM*, and *MSI2-PRMT2* [27].

GENETIC ALTERATIONS

Ikaros, encoded by the *IKZF* gene [73,74], is a transcription factor necessary for not only lymphoid differentiation [74-76] but also myeloid, erythroid and megakaryocytic differentiation [74,77,78]. The *Ikaros* family consists of five zinc-finger proteins, *IKZF1-IKZF5* [74], and *IKZF1* is further classified into eight isoforms, *IK1-IK8*. *IK1* is a full-length isoform, while due to alternative splicing, *IK6* lacks exons 4-7 that encode the N-terminal DNA-binding domain but retains the C-terminal dimerization domain that has the ability to interact with the full-length *IK1*. Thus *IK6* acts as a dominant-negative inhibitor of *IKZF1* [74-76]. The *IK6* is expressed exclusively in lymphoid BC of CML-BP [73,75,79] and *BCR::ABL1*-positive B-ALL [73-76], but not in myeloid BC of CML-BP nor in CML-CP [73,75]. For instance, *IK6* expression is found in 5 of 5 (100%) patients with lymphoid BC of CML, in 7 of 7 (100%) patients with *BCR::ABL1*-positive B-ALL, but neither in 0 of 26 (0%) patients with CML-CP nor in 0 of 5 (0%) patients with myeloid BC of CML [73]. The high expression of *IK6* leads to suppression of the *IKZF1* function. This indicates association of the *IKZF1* inhibition due to *IK6*

with lymphoid differentiation arrest in CML-BP (Table 3). In addition, deletion of *IKZF1* is also found in *BCR::ABL1*-positive B-ALL and lymphoid BC of CML-BP, but not in CML-CP [80]. This also means that loss of *IKZF1* causes lymphoid blast transformation in CML-BP (Table 3).

p53 protein, encoded by the *TP53* gene, acts as a tumor suppressor that inhibits cell cycle and promotes apoptosis [81,82]. In CML-CP, alterations of *TP53* gene are not found [27,28,83,84], but during the transition from CP to BC, alterations of *TP53* such as deletion and point mutations accumulate [27,28,82-85]. For instance, *TP53* gene alterations are found in 5 of 31 patients with CML-BC (4 myeloid and 1 lymphoid), but not in 80 patients with CML-CP [83]. Thus inactivation of *TP53* that induces cell cycle progression or apoptosis inhibition [86,87] promotes blast crisis in CML-BC [27,28,88]. The alterations of *TP53* gene are found in myeloid BC, lymphoid BC and erythroid BC [87] (Table 3).

Inhibitor of cyclin-dependent kinase 4 (INK4)-alternative reading frame (ARF) locus encodes two inhibitors (p16-INK4A and p5-INK4B) and p14-ARF. p16-INK4A and p15-INK4B maintain *retinoblastoma (Rb)* tumor suppressor gene product in a hypophosphorylated form, while p14-

ARF inhibits murine double minute 2 (Mdm2) and activates p53 [89]. The *INK4-ARF* gene products are involved in cell cycle arrest. Deletion of *INK4-ARF* loses its tumor suppressor function, and is frequently found in lymphoid BC of CML-BC and *BCR::ABL1*-positive B-ALL, but not in myeloid BC of CML-BC nor in CML-CP [12,90,91]. For instance, deletion of *INK4-ARF* is found in 3 of 8 patients with lymphoid BC of CML, but neither in 9 patients with myeloid BC of CML nor in 22 patients with CML-CP [91]. Thus deletion of the tumor suppressor gene locus *INK4-ARF* loses cell cycle arrest and promotes lymphoid BC in CML-BC (Table 3). *Rb* tumor suppressor gene is also involved in cell cycle arrest [91]. Mutations and deletion of *Rb* gene is associated with blast transformation of CML [12]. Expression of abnormal *Rb* gene structure promotes myeloid BC of CML (6 of 17 patients) and lymphoid BC of CML (1 of 9 patients) and is found in 4 of 17 patients with AML (M4 and M5 of FAB classification) and 3 of 11 patients with *BCR::ABL1*-positive ALL [92]. Alterations of *Rb* gene is also found in 6 patients with CML-AP [93] and in 2 patients with biphenotypic BC (myeloid and B-lymphoid) of CML [94]. In addition, deletion of *Rb* is found in 5 of 5 (100%) patients with megakaryocytic BC of CML, but not in 17 patients with myeloid or lymphoid BC of CML [95].

Table 3. Blast transformation caused by genetic alterations in CML-BP.

Mechanisms	Effects	Results	References
Expression of <i>IK6 isoform</i>	Inhibition of IKZF1-mediated lymphoid differentiation	Lymphoid BC	[73,75]
Deletion of <i>IKZF1</i>	Loss of IKZF1-mediated lymphoid differentiation	Lymphoid BC	[80]
Deletion and point mutation of <i>TP53</i>	Cell cycle progression and apoptosis inhibition	Myeloid, lymphoid and erythroid BC	[27,28,87]
Deletion of <i>INK4-ARF</i>	Cell cycle progression	Lymphoid BC	[12,90,91]
Mutations of <i>Rb</i>	Cell cycle progression	Myeloid and lymphoid BC	[92]
Deletion of <i>Rb</i>	Cell cycle progression	Megakaryocytic BC	[95]

Abbreviations: ARF=alternative reading frame, BC=blast crisis, BP=blast phase, CML=chronic myeloid leukemia, IK6=Ikaro family zinc finger protein 1 isoform 6, IKZF1=Ikaro family zinc finger protein 1, INK4=inhibitor of cyclin-dependent kinase 4.

EPIGENETIC DYSFUNCTIONS

Epigenetic modulations such as DNA methylation and histone modification also contributes to blast transformation in CML-BP [17,18,29,96]. For instance, transcription factor CCAAT/enhancer-binding protein- α (CEBP α) is required for myeloid differentiation [97-99]. Aberrant methylation in the CpG island of CEBP α promoter region, observed in 32 of 100 patients with CML, represses transcription of CEBP α mRNA, resulting in myeloid differentiation arrest [100] (Table 4). In addition, aberrant methylation in the promoter region of another differentiation-mediated transcription

factor PU.1 is also indicated, leading to suppression of PU.1-mediated myeloid differentiation arrest [101] (Table 4). Furthermore, expression of histone H3 lysine 4 (H3K4) demethylase retinol-binding protein 2 (RBP2) is negatively correlated with *PCR::ABL1* expression in CML. RBP2 directly downregulates phosphatase and tensin homolog (PTEN), and PTEN targets protein phosphatase activity of BCR-ABL1, leading to its dephosphorylation. mRNA expression of RBP2 is positively correlated with that of PTEN. Taken together, underexpression of RBP2 promotes blast crisis transition in CML by activating an RBP2/PTEN/BCR-ABL1 cascade [102] (Table 4).

Table 4. Epigenetic dysfunctions in CML-BP.

Mechanisms	Effects	Results	References
Aberrant methylation in <i>CEBPα</i> promoter	Repression of <i>CEBPα</i> mRNA transcription	Myeloid differentiation arrest	[100]
Aberrant methylation in <i>PU.1</i> promoter	Repression of <i>PU.1</i> mRNA transcription	Myeloid differentiation arrest	[101]
Underexpression of histone demethylase RBP2	Activation of RBP2/PTEN/BCR-ABL1 cascade	Promotion of BC transition	[102]

Abbreviations: *ABL1*=Abelson protooncogene 1, *BC*=blast crisis, *BCR*=breakpoint cluster region *CEBP*=CCAAT/enhancer-binding protein, *PTEN*=phosphatase and tensin homolog, *RBP2*=retinol-binding protein 2.

DIFFERENTIATION ARREST

Blasts in CML-BP is characterized by differentiation arrest [1,12,17,34]. Various mechanisms of differentiation arrest in CML-BP via aberrantly regulated transcription factors that induce differentiation of HSCs have been proposed. A transcription factor *CEBPα* is involved in myeloid differentiation [97-99]. In CML-BC, expression of *CEBPα* is suppressed, resulting in myeloid differentiation arrest [12,17] (Table 5). Suppression of *CEBPα* expression is caused by interaction of the poly(rC)-binding protein heterogeneous nuclear ribonucleoprotein E2 (hnRNP E2) with *CEBPα* mRNA at the translational level [103,104]. In contrast, miR-328 inhibits the hnRNP E2 interaction with *CEBPα* mRNA. Thus loss of miR-328 induces activation of

hnRNP E2, leading to suppression of the *CEBPα*-mediated differentiation [105]. A transcription factor c-Myb that is highly expressed in CML-BP cells via the PI3K/AKT/glycogen synthase kinase-3β (GSK3β) signaling pathway [106] also represses transcription of *CEBPα* mRNA, leading to inhibition of the *CEBPα*-mediated myeloid differentiation [107]. Another transcription factor GATA2 inhibits in part the *CEBPα*-mediated differentiation by repressing its transcription [107]. A transcription factor *CEBPβ* also promotes myeloid differentiation [108]. In CML-CP, *CEBPβ* is highly expressed while in CML-BP, transcription of *CEBPβ* gene is repressed [109], indicating suppression of *CEBPβ*-mediated myeloid differentiation at BP [109] (Table 5).

Table 5. Differentiation arrest in CML-BP.

Mechanisms	Effects	Results	References
Interaction of hnRNP E2 with <i>CEBPα</i> mRNA	Suppression of <i>CEBPα</i> mRNA translation	Myeloid differentiation arrest	[103,104]
Activation of hnRNP E2 by loss of miR-328	Suppression of <i>CEBPα</i> mRNA translation	Myeloid differentiation arrest	[105]
Activated expression of c-Myb	Repression of <i>CEBPα</i> mRNA transcription	Myeloid differentiation arrest	[107]
Activated expression of GATA-2	Repression of <i>CEBPα</i> mRNA transcription	Myeloid differentiation arrest	[107]
Repression of <i>CEBPβ</i> mRNA transcription	Inhibition of <i>CEBPβ</i> -mediated differentiation	Myeloid differentiation arrest	[109]
Repression of <i>PU.1</i> mRNA transcription by <i>GATA-2</i> -L359V	Inhibition of <i>PU.1</i> -mediated differentiation	Myeloid differentiation arrest	[113]
Loss-of-function mutations of <i>UBE2A</i>	Inhibition of <i>UBE2A</i> -mediated differentiation	Myeloid differentiation arrest	[115]
Expression of <i>IK6</i> isoform, deletion of <i>IKZF1</i>	Inhibition of <i>IKZF1</i> -mediated differentiation	Lymphoid differentiation arrest	[73,75,80]
Mutations of <i>TP53</i>	Promotion of erythroid BC	Erythroid differentiation arrest	[87]
Deletion of <i>Rb</i>	Promotion of megakaryocytic BC	Megakaryocytic differentiation arrest	[95]

Abbreviations: *BC*=blast crisis, *CEBP*=CCAAT/enhancer-binding protein, *GATA-2*=GATA-binding factor 2, *hnRNP E2*=heterogeneous nuclear ribonucleoprotein E2, *Rb*=retinoblastoma tumor suppressor gene, *UBE2A*=ubiquitin-conjugating enzyme E2A.

PU.1 is another transcription factor that promotes myeloid differentiation [110-112]. However, a gain-of-function mutation *GATA-2-L359V* inhibits myeloid differentiation [113,114] by repressing transcription of PU.1 mRNA by *GATA-2-L359V* [113] (Table 5). In addition, *UBE2A* induces myeloid differentiation [115]. However, loss-of-function mutations of *UBE2A* in CML-BP causes differentiation arrest [115] (Table 5).

In addition, as already indicated, expression of the *IK6* isoform lacking exons 4-7 due to alternative splicing [73,75] and deletion of *IKZF1* [80] induce inhibition of *IKZF1* protein function, resulting in lymphoid differentiation arrest (Table 5). Furthermore, as also indicated, the mutations of tumor suppressor gene *TP53* are involved in promotion of erythroid BC, indicating erythroid differentiation arrest by *TP53* gene mutations [87] (Table 5). Deletion of another tumor suppressor gene *Rb* also promotes megakaryocytic BC of CML [95]. This suggests that deletion of *Rb* induces megakaryocytic differentiation arrest (Table 5).

Table 6. Other mechanisms of blast transformation in CML-BP.

Mechanisms	Effects	Results	References
<i>IK6</i> (alternative splicing of <i>IKZF1</i>)	Inhibition of <i>IKZF1</i>	Lymphoid differentiation arrest	[73,75]
Mis-splicing of <i>GSK3β</i>	Activation of β -catenin	Promotion of myeloid BC	[30]
Genetic alterations of <i>TP53</i>	Inhibition of p53-mediated degradation of HIF-1 α	Promotion of BC	[27,28,88,124]

Abbreviation: BC=blast crisis, *GSK3β*=glycogen synthase kinase-3 β , *HIF-1 α* =hypoxia-inducible factor-1 α , *IK6*=Ikaros family zinc finger protein 1 isoform 6, *IKZF1*=Ikaros family zinc finger protein 1.

HYPOXIA

Hypoxia-inducible factors (HIF-1 α , HIF-2 α , HIF-3 α and HIF-1 β) play significant roles in adaptation to hypoxia [120]. Under normoxic conditions in BMM, two proline residues of HIF-1 α are hydroxylated by prolyl hydroxylase domain protein 2 (PHD2) [121]. Then HIF-1 α is degraded by the ubiquitin-proteasomal degradation pathway via its binding to the von Hippel-Lindau tumor suppressor protein (pVHL) [122,123]. HIF-1 α is also regulated by another p53-mediated ubiquitin-proteasomal degradation [124,125]. Under hypoxic conditions, HIF-1 α is rapidly induced and accumulates in the cytoplasm due to inhibition of the ubiquitin-proteasomal degradation, and HIF-1 α translocates to the nucleus and forms a heterodimer complex with constitutively expressed HIF-1 β (also known as aryl hydrocarbon receptor nuclear translocator [ARNT]). This HIF-1 α /HIF-1 β complex then binds to the hypoxia responsive element (HRE) of target genes [126] with recruitment of cyclic adenosine monophosphate response element-binding protein (CBP)/p300 [127,128] and activates transcription of numerous

ALTERNATIVE SPLICING AND MIS-SPLICING

Alternative splicing of relevant genes is also involved in blast transformation of CML. As already indicated, *IK6* is an alternatively spliced isoform of *IKZF1* that lacks exons 4-7. *IK6* expression in CML-BC promotes lymphoid differentiation arrest by inhibiting the *IKZF1*-mediated lymphoid differentiation, resulting in lymphoid BC [73,75] (Table 6).

In addition, mis-splicing is another mechanism to promote BC. High expression of β -catenin is observed in CML-BC [116], and β -catenin is supposed to promote BC [117,118]. β -Catenin is an effector of the Wnt/ β -catenin signaling pathway and GSK3 β negatively regulates β -catenin [119]. However, mis-splicing of *GSK3β* due to deletion of exons 8 and 9 is supposed to promote myeloid BC of CML by decreasing the inhibitory activity of GSK3 β , because the mis-spliced GSK3 β isoform (m-GSK3 β) does not inhibit β -catenin, resulting in activation of β -catenin [30] (Table 6).

target genes. Expression of HIF-1 α mRNA in primary cells from patients with CML is significantly higher than that in the control [129], and HIF-1 α maintains LSCs of CML [130-132]. HIF-1 α is activated by the BCR-ABL1/PI3K/AKT/mTORC1 signaling pathway via the translation activation of HIF-1 α mRNA [133]. However, the genetic alterations (deletion and point mutations) of *TP53* are additional gene mutations in CML-BP [27,28], and HIF-1 α is supposed to be activated by the alterations of *TP53* due to the loss of p53-mediated ubiquitin-proteasomal degradation. Indeed, loss of p53 activates expression and function of HIF-1 α [124] and promotes BC of CML [88]. Taken together, the alterations of *TP53* promote blast transformation of CML [27,28,88,124] (Table 6). Expression of CA9, one of the relevant targets induced by HIF-1 α , is also confirmed in CML cell lines [132].

INTEGRATION OF GENETICS, EPIGENETICS AND FACTORS IN BMM

The exponential advances in the single-cell technology have facilitated generation of large biological data sets, called multiomics data including genomics, epigenomics,

proteomics as well as transcriptomics and metabolomics [134-138]. However, it is required to integrate multiomics data into a whole integromics or panomics [134]. Accordingly, it is required to integrate genetic alterations, epigenetic dysfunctions and aberrantly regulated factors in BMM into a comprehensive understanding of blast transformation in CML. Our review article is a humble trial of this objective, as in several studies on the multistep-oncogenesis model of ATL [22,25,139-141].

In addition, elucidation of the blast transformation in CML-BP means trials to find new therapeutic target points as reviewed by several studies [17,18,26,32,59,132]. These review articles indicate that novel therapies against blast cells in CML-BP can be expected.

CONCLUSION

Blast transformation in CML is highly complicated and is caused by various mechanisms such as genetic alterations (*IKZF1*, *TP53*, *INK4-ARF* and *Rb*), epigenetic dysfunctions (aberrant methylation of *CEBPα* and *PU.1*, and underexpression of histone demethylase RBP2), suppression of differentiation-mediated transcription factors (*CEBPα*, *CEBPβ*, *PU.1* and *UBE2a*), abnormal splicing (*IKZF1* and *GSK3β*) and aberrantly regulated HIF-1α in BMM. To develop novel therapies against blast cells in CML-BP based on the mechanisms of blast transformation, further studies are required.

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