Received: 2 August 2022 | Accepted: 27 September 2022

DOI: 10.1002/ame2.12282

ORIGINAL ARTICLE

c-myb is involved in CML progression and is a therapeutic target in the zebrafish CML model

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Funding information
National Key R&D Program of China, Grant/Award Number: 2018YFA0801000; National Natural Science Foundation of China, Grant/Award Number: 32170830; Natural Science Foundation of Guangdong Province, China, Grant/Award Number: 2021A1515010422; South China University of Technology

Abstract

Background: Despite the success of tyrosine kinase inhibitors in chronic myeloid leukemia (CML) therapy, CML still faces the challenges of drug resistance and progression to blast crisis. Twenty-five percent of patients have imatinib resistance and treatment difficulties due to heterogeneity after progression, but little is known about the mechanism. A key transcription factor in hematopoiesis, *MYB*, has been reported to increase abnormally in several types of aggressive blood disorders including CML.

Methods: This study used a zebrafish model to explore the relationship between *BCR/ABL1* and *c-myb* in CML progression. A CML zebrafish model was crossed with a *c-myb* hyperactivity transgenic line.

Results: It was found that both exogenous *BCR/ABL1* and *c-myb* could up-regulate the expression of neutrophil-related genes. More seriously, neutrophil accumulation was observed when *BCR/ABL1* was combined with *c-myb* overexpression. Further studies showed that *c-myb* may be one of the downstream targets of *BCR/ABL1* and the effect of *BCR/ABL1* on neutrophils was *c-myb* dependent. Taking advantage of this inheritable in vivo model, it was shown that a combination of imatinib and flavopiridol, a cyclin-dependent kinase inhibitor targeting *MYB*, could more effectively alleviate the aggressive phenotype of the double transgene line.

Conclusion: In summary, this study suggests that *c-myb* acts downstream of *BCR/ABL1* and is involved in CML progression and is therefore a risk factor and a valuable target for the treatment of CML progression. The model used in the study could be helpful in high-throughput drug screening in CML transformation.

KEYWORDS
chronic myeloid leukemia, c-myb, flavopiridol, zebrafish model

1 | INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal hemopoietic stem cell disorder driven by the juxtaposition of *BCR* and *ABL1*.[1] The fusion gene *BCR/ABL1* encodes the aberrantly activated ABL kinase that activates multiple pathways, resulting in enhanced cell survival, inhibition of apoptosis and disturbance of cell adhesion and migration.[2] The discovery of imatinib, a first-generation tyrosine kinase inhibitor (TKI), has improved the outcome of CML patients in the chronic phase (CP). Despite the exciting results obtained with imatinib, resistance

is observed in approximately 25% of patients.[3] Current strategies to circumvent resistance include using second-generation *BCR/ABL1* TKIs, such as nilotinib, dasatinib and bosutinib, targeting other cellular pathways.[3] Nevertheless, TKI response rates are still insufficient for optimal survival of patients who have CML progression.

Accelerated phase (AP) and blast phase (BP) occur in a minority of patients and outcomes are significantly worse in these patients compared with CML-CP, with lower response rates and decreased duration of response to TKI.[4] The median overall survival and failure-free survival times of CML-BP are 12 and 5 months, respectively.[5] At present, allogeneic stem cell transplantation is the only curative therapy for CML-BP, but overall cure rate is only 10%–20%.[6] The underlying molecular mechanism of blastic transformation remains unclear. It is believed that multiple molecular changes, such as increased expression of *BCR/ABL1*, oncogenetic activation or amplification of pro-oncogenes and loss-of-function mutations in tumor suppressor genes may be involved in the progression to blast crisis.[7] As few treatment options are available and prognosis poor, there is a need to refine the treatment of CML.

A transcription factor, *MYB*, plays a pivotal role in hematopoietic cell development. It has been reported that *MYB* is not only a transcription factor but also an oncogene that can induce leukemia. *MYB* mRNA is expressed at high levels in most human myeloid and acute lymphoid leukemias,[8] including CML.[9,10] In CML patients, higher levels of *MYB* expression are detected in progression to AP or BP than in CP,[9,11] which implies that *MYB* may be involved in CML progression. Several studies have demonstrated that *MYB* is indispensable for colony formation in CML cells[9,12–16] and $p210^{BCR/ABL1}$-dependent leukemogenesis in mice,[16,17] but there is no inheritable CML progression model for drug screening and observation of blastic transformation. As a proposed therapeutic target in blastic transformation and drug resistance,[10,18] no personalized therapeutic strategy for *MYB* has yet been reported.

To assess the role of *c-myb* in CML progression, we overexpressed *c-myb* in a zebrafish CML model that closely mimics the natural progression of human CML.[19] The resulting data suggested that *BCR/ABL1* and *c-myb* synergistically promoted the expansion of neutrophils. In addition, it was found that *c-myb* may be a target of *BCR/ABL1* and that the regulation of neutrophils by *BCR/ABL1* depends, in part, on *c-myb*. More importantly, for the first time, the cyclin-dependent kinase (CDK) inhibitor flavopiridol that targets *MYB*[20] in the CML model was evaluated and combined treatment with imatinib was found to work better than imatinib alone. These results implied that *c-myb* may be a regulatory factor involved in CML progression and targeting *c-myb* would be an option for the treatment of CML patients in BP.

2 | METHODS

2.1 | Zebrafish husbandry

The following strains were used: AB, *Tg(hsp70:p210$^{BCR/ABL1}$)*,[19] and *c-myb^{hyper}*.[21] *Tg(hsp70:p210$^{BCR/ABL1}$)* expresses human *BCR/ABL1* (h*BCR/ABL1*) (b3a2) under the control of the zebrafish heat shock-inducible hsp70 promoter.[19] *c-myb^{hyper}* expressed a truncated *c-myb*, lacking the majority of the negative-regulatory region, fused with a nearly full-length *c-myb* under the control of the first core *c-myb* promoter.[21] Zebrafish were maintained according to the guidelines of the Animal Care and Use Committee of the South China University of Technology. All experimental protocols were approved by the Division of Cell, Developmental and Integrative Biology, School of Medicine, South China University of Technology.

2.2 | Heat-shock treatment

Tg(hsp70:p210$^{BCR/ABL1}$) and *Tg(hsp70:p210$^{BCR/ABL1}$)*;c-myb^{hyper} embryos were heat-shock treated at 39.5°C for 1 h during 10–12 hours post-fertilization (hpf), and then for 2 h twice per day after 22 hpf. The interval of heat-shock treatment was 6 h at least. Adult zebrafish were heat-shocked at 39.5°C for 2 h once per day.

2.3 | Whole-mount in situ hybridization

In vitro transcription of antisense digoxigenin-labeled RNA probes and whole-mount in situ hybridization were performed according to standard protocols.[22] The following probes were synthesized: *lyz*, *mpx*, *npsn*, *srgn*, *c-myb*, and *lcp1*. We used the multi-point tool in ImageJ software to count the number of whole-mount in situ hybridization signals.

2.4 | Sudan black staining

Embryos at 3 dpf were fixed with 4% paraformaldehyde (Macklin) in phosphate-buffered saline (PBS) for 2 h at room temperature. After being rinsed in PBS twice, fixed embryos were incubated in Sudan black (Macklin) solution for 30 min to mark neutrophils and then washed as described.[23]

2.5 | Cytological analysis

Kidney marrow (KM) was re-suspended in PBS with 5% fetal bovine serum, followed by Cytospin 4 (Thermo Scientific) at 400 rpm for 3 min. The cells were then stained with May-Grünwald-Giemsa (Merck) stain according to the manufacturer's instructions. Blood cells in KM were calculated manually based on their morphologies.[24,25]

2.6 | Drug treatment

After heat-shock treatment, embryos at 2.5 days post-fertilization (dpf) were soaked in egg water containing 1‰ dimethylsulfoxide (DMSO) (Sigma-Aldrich), 80 μmol/L imatinib (Selleck), and 0.1 μmol/L

flavopiridol (Selleck) for drug treatment. Embryos were treated with drugs for 48 h, and the egg water containing the drugs was changed after 24 h of treatment.

2.7 | Statistical analysis

Data were analyzed by GraphPad Prism 7 software. Student's *t* test was used for comparisons between two groups and one-way analysis of variance (ANOVA; with Fisher's LSD test) was applied to comparisons among multiple groups. Significance was accepted when $p < 0.05$ (ns indicates not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, and $^{****}p < 0.0001$). Data are shown as means ± standard deviation (SD) from at least three independent experiments with duplicate samples.

3 | RESULTS

3.1 | Both *BCR/ABL1* and *c-myb* overexpression can affect the expression of neutrophil-related genes

To first characterize the effect of *BCR/ABL1* and *c-myb* on neutrophils, lineage-specific markers of neutrophils were detected by whole-mount in situ hybridization. It was found that lysozyme (*lyz*) was significantly increased in *c-myb*^{hyper}, a transgenic zebrafish with *c-myb* overexpression, at 36 hpf compared with the control group, as seen in Figure 1A,B,M, concurring with a previous study.[21] Other neutrophil-related markers, such as myeloperoxidase (*mpx*) in Figure 1D,E,N, nephrosin (*npsn*) in Figure 1G,H,O, and serglycin (*srgn*) in Figure 1J,K,P, were further analyzed, showing comparable results. Like *c-myb*^{hyper}, all the genes mentioned above were increased in the heritable CML zebrafish model expressing human *BCR/ABL1*, as seen in Figure 1C,F,I,L, which indicated that both *BCR/ABL1* and *c-myb* overexpression affected the expression of neutrophil-related genes.[19]

3.2 | Neutrophils expansion in *Tg(hsp70:p210*$^{BCR/ABL1}$*)* depended on *c-myb*

As described above, zebrafish larvae expressing exogenous *BCR/ABL1* showed increased expression of neutrophil-related genes similar to that expressed *c-myb*, which suggested that *BCR/ABL1* and *c-myb* may form vertical or parallel regulatory networks in neutrophil development. The *c-myb* expression in *Tg(hsp70:p210*$^{BCR/ABL1}$*)* was first detected by whole-mount in situ hybridization, and indicated that the expression of *c-myb* mRNA increased in *Tg(hsp70:p210*$^{BCR/ABL1}$*)* at 60 hpf, as seen in Figure 2A–C, in line with the previous observation.[19] This result suggested that *BCR/ABL1* and *c-myb* might form a vertical genetic network related to hematopoietic regulation. To test this idea, the *c-myb* antisense oligonucleotide morpholino[26] was injected into a *Tg(hsp70:p210*$^{BCR/ABL1}$*)* embryo at the one cell stage to block *c-myb* translation in order to examine whether *c-myb* inhibition would affect the promotion of neutrophil production by *Tg(hsp70:p210*$^{BCR/ABL1}$*)*. The number of L-plastin (*lcp1*) positive myeloid cells significantly decreased after injection at 36 hpf, as shown in Figure 2D–F, and the numbers of SB^{+} neutrophils and *lyz*$^{+}$ neutrophils also decreased markedly after injection at 3 dpf, as shown in Figure 2G–L. Taken together, these data indicated that *c-myb* may function as a downstream regulator of *BCR/ABL1* in the CML zebrafish model.

3.3 | Exogenous *c-myb* activation could mimic CML progression in the zebrafish CML model

Aberrant elevation of *MYB* has been reported in CML-BP patients,[9] implying the potential role of *MYB* in CML progression. To identify the function of *c-myb* in CML progression, a *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper} double transgenic zebrafish was obtained by crossing *Tg(hsp70:p210*$^{BCR/ABL1}$*)* with *c-myb*^{hyper} to overexpress *c-myb* in *Tg(hsp70:p210*$^{BCR/ABL1}$*)*. Compared with *c-myb*^{hyper} or *Tg(hsp70:p210*$^{BCR/ABL1}$*)* alone, the numbers of *lyz*$^{+}$, *mpx*$^{+}$, *npsn*$^{+}$, *srgn*$^{+}$ neutrophils markedly increased in double transgenic zebrafish at 36 hpf, as shown in Figure 3A–L, indicating that *c-myb* overexpression can affect the neutrophil expansion of *Tg(hsp70:p210*$^{BCR/ABL1}$*)*. To further verify this result, kidney marrow cells were collected from *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper} adult zebrafish after inducing *BCR/ABL1* expression by continuous heat shock and subjected to cytological and white blood cell analyses. As expected, more neutrophils were observed in the kidney marrow of *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper} compared with *Tg(hsp70:p210*$^{BCR/ABL1}$*)*, as shown in Figure 3M,N. In addition, these data revealed that neutrophils exceeding 55% were found in 20% of *Tg(hsp70:p210*$^{BCR/ABL1}$*)* zebrafish, but in 80% of *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper} zebrafish, as seen in Figure 3O. These results indicated that *BCR/ABL1* combined with *c-myb* overexpression affected neutrophil expansion both in embryonic and adult zebrafish, which suggested that *c-myb* might be involved in CML progression.

3.4 | The combination treatment of imatinib and flavopiridol worked better than imatinib alone in *BCR/ABL1* combined with *c-myb* overexpression

It has been reported that different gene expression profiles can predict early molecular response and long-term outcomes of CML.[27] Given the increased expression of *MYB* in CML patients, especially in BP,[9] and that *c-myb* can aggravate the *BCR/ABL1* phenotype mentioned above, *c-myb* may serve as a predictive risk factor for preventing progression to blast crisis. The double transgenic zebrafish may therefore serve as an ideal model for drug screening. A previous study showed that the *MYB*-targeting drug flavopiridol[20] could alleviate the abnormal accumulation of neutrophils in *c-myb*^{hyper}. Therefore in search for better therapeutic strategies to prevent the

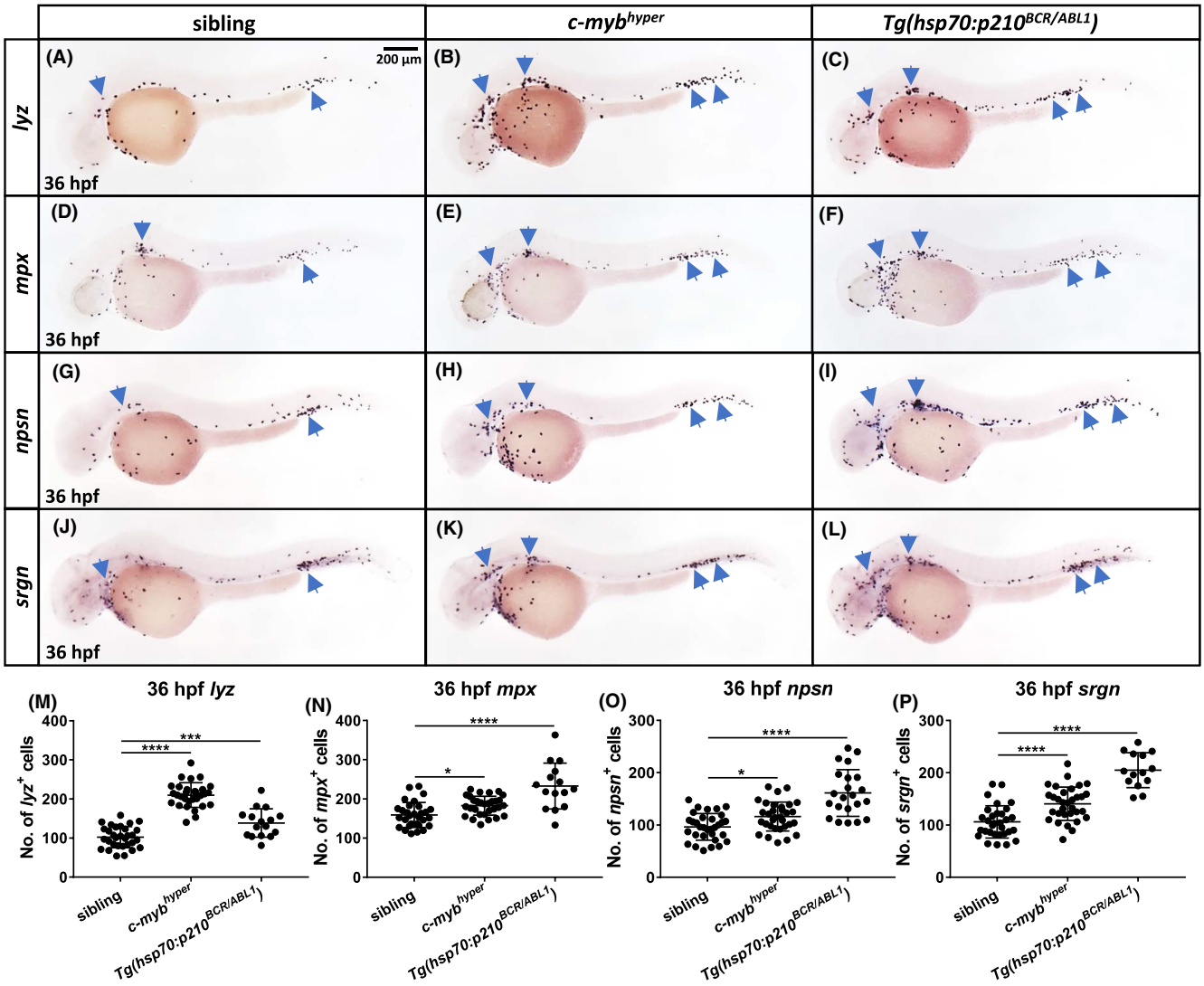

FIGURE 1 Both *BCR/ABL1* and *c-myb* overexpression can up-regulate neutrophil-related genes. (A–L) Whole-mount in situ hybridization (WISH) showed increases in *lyz* (A–C), *mpx* (D–F), *npsn* (G–I), and *srgn* (J–L) expression in *Tg(hsp70:p210*$^{BCR/ABL1}$*)* and *c-myb*^{hyper} at 36 hpf compared with controls. Blue arrowheads indicated *lyz*$^+$, *mpx*$^+$, *srgn*$^+$ and *npsn*$^+$ neutrophils in each row. (M–P) Quantification of numbers of 36 hpf *lyz*$^+$ (M) (sibling, $n = 31$; *c-myb*^{hyper}, $n = 30$; *Tg(hsp70:p210*$^{BCR/ABL1}$*)*, $n = 16$), *mpx*$^+$ (N) (sibling, $n = 30$; *c-myb*^{hyper}, $n = 30$; *Tg(hsp70:p210*$^{BCR/ABL1}$*)*, $n = 15$), *npsn*$^+$ (O) (sibling, $n = 32$; *c-myb*^{hyper}, $n = 32$; *Tg(hsp70:p210*$^{BCR/ABL1}$*)*, $n = 22$), *srgn*$^+$ (P) (sibling, $n = 31$; *c-myb*^{hyper}, $n = 32$; *Tg(hsp70:p210*$^{BCR/ABL1}$*)*, $n = 14$) cells. Scale bar, 200 μm; applies to panels (A–L).

risk of blastic transformation that may exist with abnormally elevated *MYB*, flavopiridol was utilized in this model. To evaluate whether flavopiridol treatment could relieve the abnormal expansion of myeloid cells in *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper}, embryos were incubated with flavopiridol alone and in combination with imatinib. In the control CML model embryos, imatinib reduced the number of myeloid cells while flavopiridol only had a slight but non-significant effect, as seen in Figure 4A,C,E,I, and there was no significant difference between imatinib treatment and combined treatment with flavopiridol (Figure 4E,G,I).

In *Tg(hsp70:p210*$^{BCR/ABL1}$*);c-myb*^{hyper} embryos, flavopiridol effectively reduced the number of abnormally increased *lcp1*$^+$ myeloid cells, as shown in Figure 4B,D,I, and the combination treatment of imatinib and flavopiridol had a better response (Figure 4B,D,F,H,I). The results suggested that *c-myb* might be a potential therapeutic target in CML progression.

4 | DISCUSSION

To date, few reports have provided a comprehensive description of the underlying pathogenesis of CML progression. *MYB*, a transcription factor associated with hematopoiesis, has been reported to be up-regulated in CML-BP patients[9] and relapsed CML patients,[10] suggesting the potential role of *MYB* in CML progression. The mechanism involved in the increased level of *MYB* expression in CML is still unclear. To investigate the role of *c-myb* in CML progression and the relationship between *BCR/ABL1* and *c-myb*, the impact of *c-myb* in the CML zebrafish model was

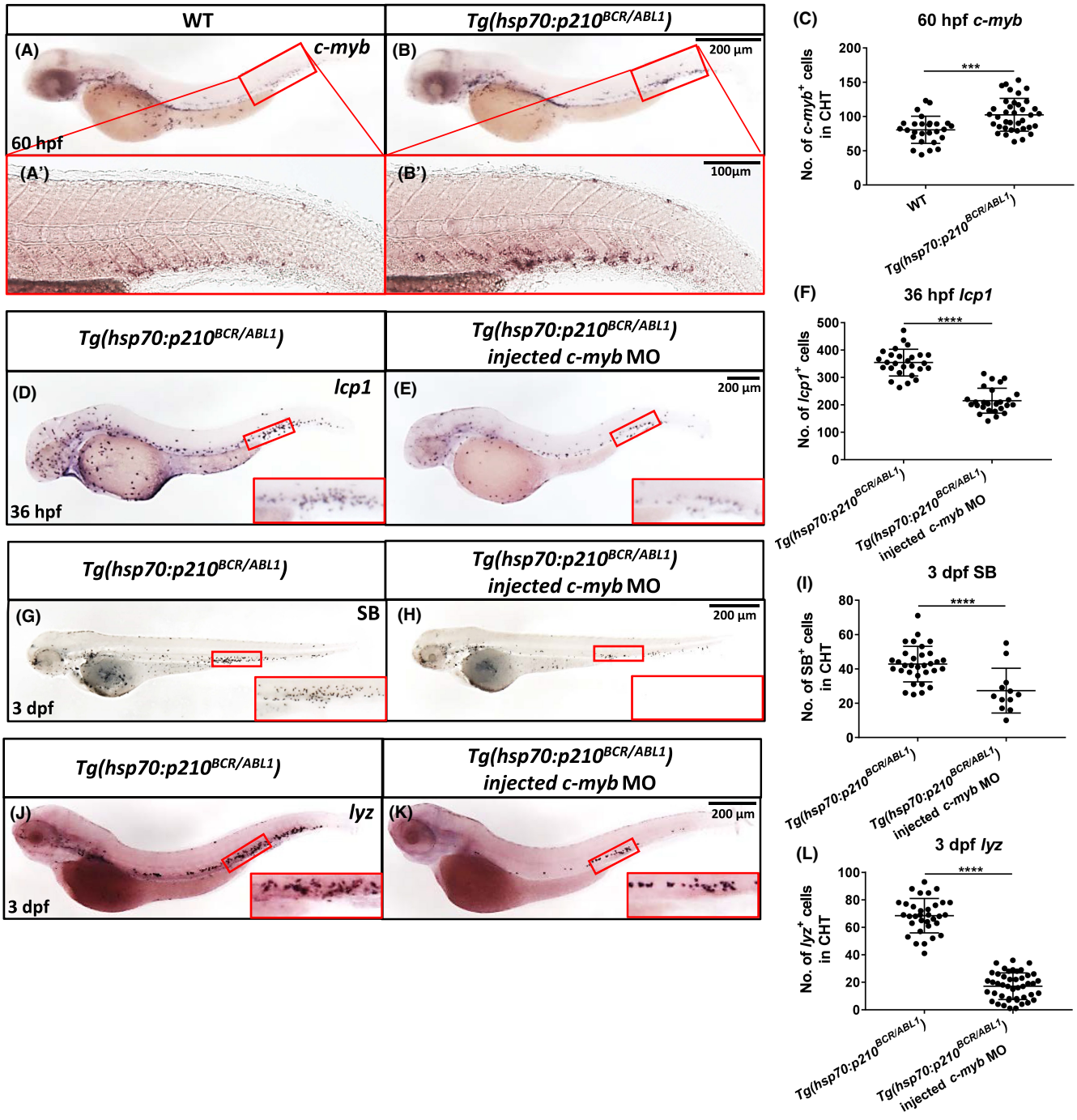

FIGURE 2 The impact of *BCR/ABL1* on neutrophil regulation depends on *c-myb*. (A,B), (A′,B′) WISH showed an increase of *c-myb* expression in *Tg(hsp70:p210$^{BCR/ABL1}$)* (B,B′) at 60 hpf compared with controls (A,A′). Red boxes showed enlarged details. (C) Quantification of numbers of 60 hpf *c-myb*$^+$ cells in caudal hematopoietic tissue (CHT) (WT, $n = 27$; *Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 37$). (D,E) WISH showed a decrease of *lcp1* expression in *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* antisense oligonucleotide morpholino (MO) at 36 hpf. (F) Quantification of numbers of 36 hpf *lcp1*$^+$ cells (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 27$; *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* MO, $n = 26$). (G,H) SB staining showed decreased number of SB$^+$ cells in *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* MO at 3 dpf. Red boxes showed enlarged detail of SB$^+$ cells in each group. (I) Quantification of numbers of 3 dpf SB$^+$ cells in CHT (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 32$; *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* MO, $n = 12$). (J,K) WISH showed a decrease of *lyz* expression in *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* MO at 3 dpf. Red boxes showed enlarged detail of *lyz*$^+$ cells in each group. (L) Quantification of numbers of 3 dpf *lyz*$^+$ cells (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 33$; *Tg(hsp70:p210$^{BCR/ABL1}$)* injected *c-myb* MO, $n = 45$). Scale bars, 200 μm (A, B, D, E, G, H, J, K) and 100 μm (A′,B′).

investigated by overexpression or knockdown and it was found that both *BCR/ABL1* and *c-myb* overexpression could play a role in regulating neutrophils in zebrafish. The expression of *c-myb* in *Tg(hsp70:p210$^{BCR/ABL1}$)* was therefore analyzed to illustrate the relationship between the genes. Given the increased expression of *c-myb* in *Tg(hsp70:p210$^{BCR/ABL1}$)*, similar to increased expression of *MYB* in CML patients,[9,10] it was

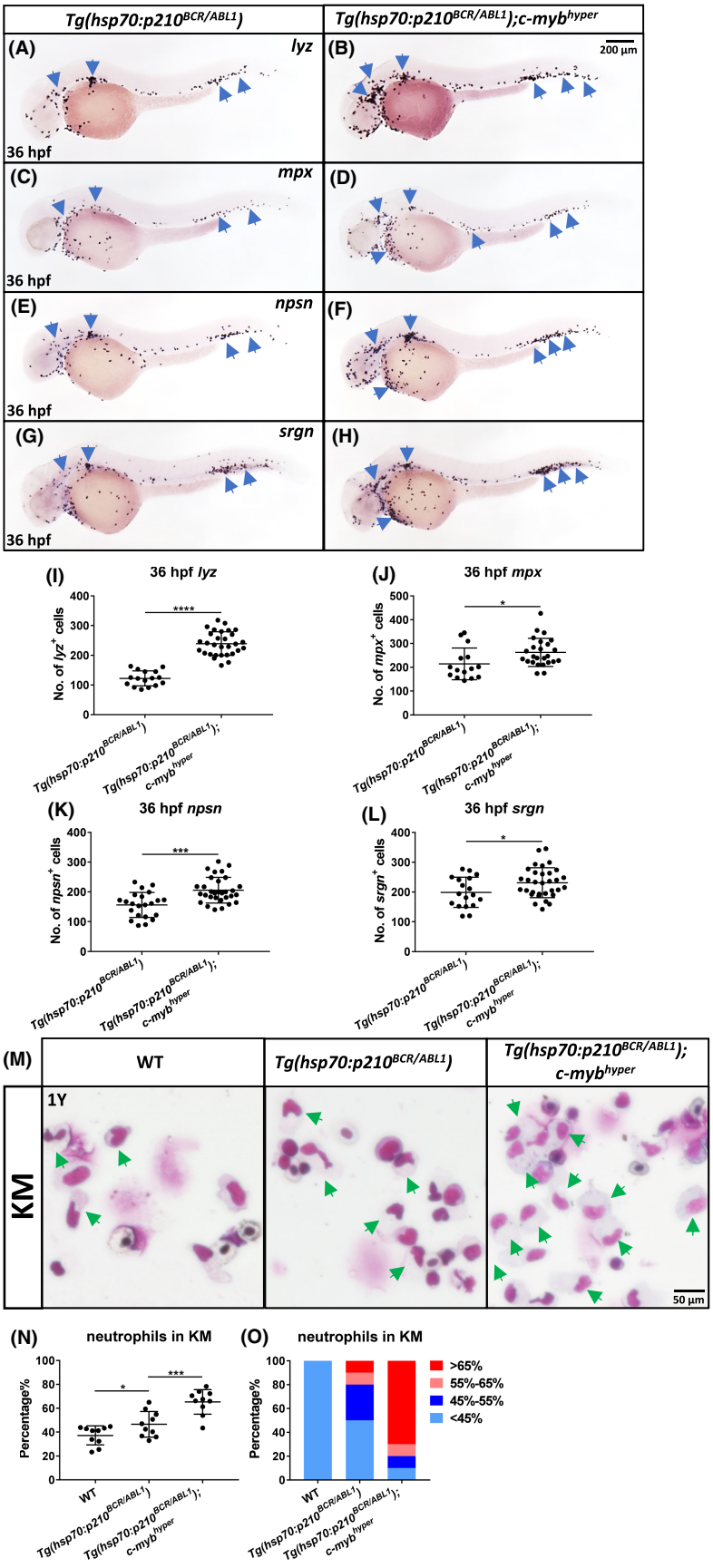

FIGURE 3 Exogenous *c-myb* activation can mimic CML progression in the zebrafish CML model. (A–H) WISH showed increase of *lyz* (A,B), *mpx* (C,D), *npsn* (E,F), and *srgn* (G,H) expression in *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* at 36 hpf compared with *Tg(hsp70:p210$^{BCR/ABL1}$)*. Blue arrowheads indicate *lyz*$^+$, *mpx*$^+$, *srgn*$^+$ and *npsn*$^+$ neutrophils in each row. (I–L) Quantification of numbers of 36 hpf *lyz*$^+$ (I) (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 16$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*, $n = 30$), *mpx*$^+$ (J) (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 15$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*, $n = 25$), *npsn*$^+$ (K) (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 22$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*, $n = 31$), *srgn*$^+$ (L) (*Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 18$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*, $n = 32$) cells. (O) May-Grünwald Giemsa staining of kidney marrow (KM) blood cells that were obtained from WT, *Tg(hsp70:p210$^{BCR/ABL1}$)*, and *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* adult zebrafish. Green arrowheads indicated neutrophils. After staining, 1000 cells were randomly chosen for further calculation. (N) The proportion of neutrophils in white blood cells in whole kidney marrow (WT, $n = 10$; *Tg(hsp70:p210$^{BCR/ABL1}$)*, $n = 10$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*, $n = 10$). (O) High proportions of neutrophils presented more in *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* than in *Tg(hsp70:p210$^{BCR/ABL1}$)*. Scale bars, 200 μm (A–H) and 50 μm (M).

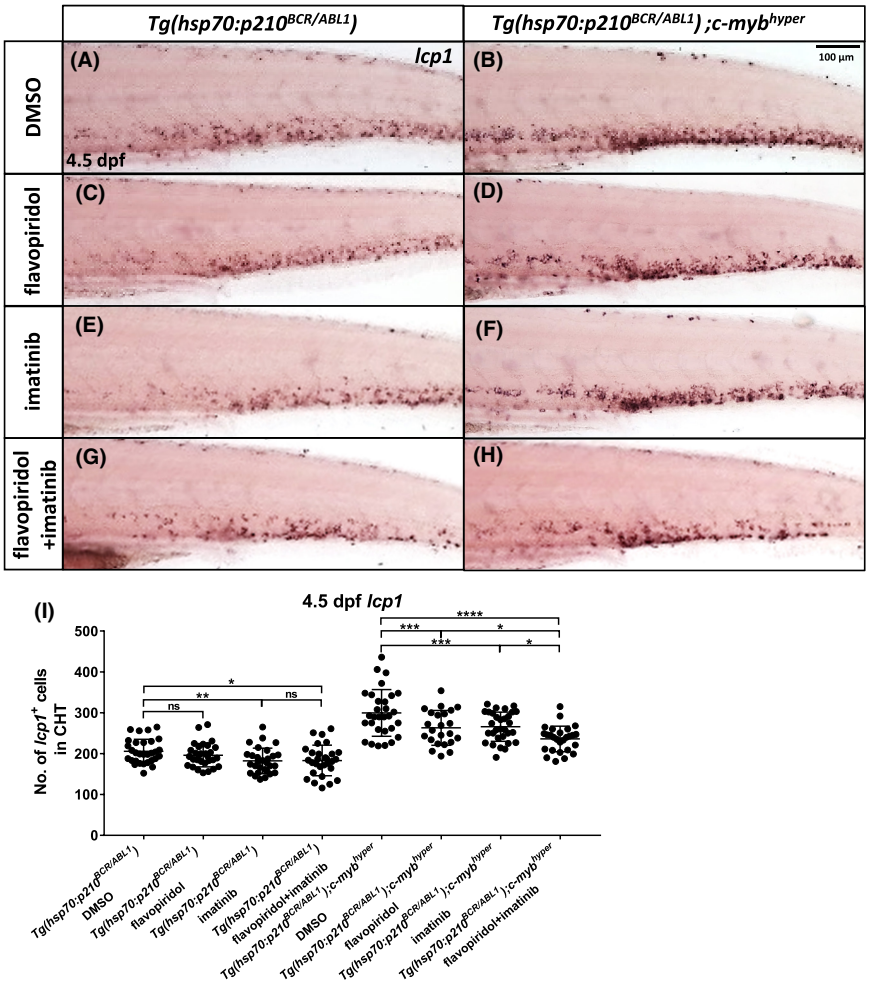

FIGURE 4 Combined treatment with flavopiridol and imatinib had a better response in *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}*. (A–H) WISH of *lcp1* expression in the drug-treated larvae at 4.5 dpf. After heat shock treatment, 2.5 dpf *Tg(hsp70:p210$^{BCR/ABL1}$)* and *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* were treated for 48 h with 1‰ DMSO control, 0.1 μmol/L flavopiridol, 80 μmol/L imatinib, or 0.1 μmol/L flavopiridol combined with 80 μmol/L imatinib. (I), Quantification of numbers of 4.5 dpf *lcp1*$^{+}$ cells in CHT (*Tg(hsp70:p210$^{BCR/ABL1}$)* DMSO, $n = 30$; *Tg(hsp70:p210$^{BCR/ABL1}$)* imatinib, $n = 29$; *Tg(hsp70:p210$^{BCR/ABL1}$)* flavopiridol, $n = 30$; *Tg(hsp70:p210$^{BCR/ABL1}$)*flavopiridol+imatinib, $n = 29$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* DMSO, $n = 30$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* imatinib, $n = 31$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* flavopiridol, $n = 30$; *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* flavopiridol+imatinib, $n = 27$). Scale bar, 100 μm; applies to panels (A–H).

hypothesized that *c-myb* acted downstream of *BCR/ABL1*, so the presence of abnormal neutrophil elevation in *Tg(hsp70:p210$^{BCR/ABL1}$)* after knockdown of *c-myb* was examined. Prior work demonstrated that *MYB* is required for *BCR/ABL1*-dependent leukemogenesis.[16,17] The function of *BCR/ABL1* relies on *c-myb*, implying that *c-myb* acts downstream of *BCR/ABL1* in the regulation of neutrophils in the CML zebrafish model, and suggesting that *c-myb* may be a potential therapeutic target for CML treatment. Induction of human *BCR/ABL1* in zebrafish combined with overexpression of *c-myb* could result in more severe neutrophil expansion in both zebrafish larvae and adult fish, suggesting that *c-myb* may participate in CML progression. However, owing the long BP transformation latency, the occurrence of blastic transformation in one-year-old adult fish has not found. This study provided in vivo evidence to show the function of *c-myb* in CML progression. This model could be used to observe blastic transformation in vivo, which would be an interesting area for further study, but there are still limitations in this study. Owing to the differences in blood cells between zebrafish and humans, precursor cells in zebrafish are difficult to distinguish, while those in humans can be well recognized by cell-specific surface antigens.

Since CML patients in BP have limited therapeutic options, blastic transformation is always followed by a poor prognosis. Once blastic transformation occurs, survival is less than one year with death due to infection or bleeding,[28] so it is much more important to prevent progression rather than treat it. Current methods of CML monitoring in CP patients include detection of Philadelphia chromosome, *BCR/ABL1* DNA, *BCR/ABL1* mRNA and mutations in the ABL1 kinase domain.[29] Elevated expression of *BCR/ABL1* and mutations in the ABL1 kinase domain generally indicate an increased risk of progression to blast crisis.[29] In addition, deregulated expression of some stem cells, and cell cycle and immune response-related genes can also predict the long-term prognosis of CML patients and those patients recognized as being at high-risk could benefit from the addition of timely novel interventional strategies.[27] These findings indicated that *MYB* may be a risk factor for blastic transformation, suggesting the targeting of *MYB* as an alternative therapeutic strategy. Although *MYB* is indispensable for the generation and normal functioning of all hematopoietic lineages, reduced *MYB* levels can be tolerated, indicating that therapeutic inhibition of *MYB* activity is also likely to be tolerated by patients.[8] Upon treatment with the *MYB*-targeting drug flavopiridol,[20] abnormally increased *lcp1*$^{+}$ myeloid cells were successfully reduced in *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* and the effect was increased in combination with imatinib. These studies are the first to utilize flavopiridol in CML therapy and to report that a combination of flavopiridol and imatinib may be a superior treatment strategy for *BCR/ABL1* combined with elevated *MYB* expression levels. Taken together, the results provide an alternative therapeutic option for CML patients with elevated expression of *MYB*.

Flavopiridol is a pan-CDK inhibitor and can cause either cell cycle arrest or apoptosis by inhibiting CDK7 and CDK9, which exist in a super-enhancer complex that is capable of promoting transcriptional processivity and elongation.[30] It has been reported that *MYB* transcription is inhibited by flavopiridol through its inhibition of CDK9.[20] Furthermore, flavopiridol has synergistic activity with most anti-cancer drugs and radiation.[31] More importantly, flavopiridol has already received orphan drug designation for the treatment of chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML),[32] indicating the possibility of application in other hematological malignancies, such as CML, and offering an interesting area for further investigation.

These results demonstrate the synergistic regulation of *BCR/ABL1* and *c-myb* on neutrophils in the CML zebrafish model and indicate that *c-myb* functions as a key regulator and risk factor in *BCR/ABL1*-dependent blastic transformation. The observed therapeutic effect on *Tg(hsp70:p210$^{BCR/ABL1}$);c-myb^{hyper}* conferred by combined treatment with imatinib and flavopiridol provides a valuable strategy for the therapy of CML. Importantly, this study has provided a novel disease model for drug screening and dynamic monitoring of disease progression and an excellent resource to further the understand blastic transformation and identify therapeutics for this aggressive disease.

AUTHOR CONTRIBUTIONS

Yin Ye and Zhibin Huang designed the study and contributed to data collection, interpretation, and manuscript writing. Xiaojun Yang performed the Sudan black staining and parts of whole-mount in situ hybridization. Feifei Li and Wei Liu participated in the discussion. Zhibin Huang and Wenqing Zhang revised the manuscript and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Yiyue Zhang, Jin Xu, and Jianchao Li from the South China University of Technology for their expert advice.

FUNDING INFORMATION

This work was supported by the National Key R&D Program of China (2018YFA0801000), National Natural Science Foundation of China (32170830). Natural Science Foundation of Guangdong Province, China (2021A1515010422).

CONFLICT OF INTEREST

All authors declare that they have no potential conflicts of interest with respect to this submission.

ETHICS STATEMENT

Zebrafish were maintained according to the guidelines of the Animal Care and Use Committee of the South China University of Technology. All experimental protocols were approved by the Division of Cell, Developmental and Integrative Biology, School of Medicine, South China University of Technology.

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REFERENCES

1. Apperley JF. Chronic myeloid leukaemia. *Lancet*. 2015;385(9976):1447-1459. doi:10.1016/s0140-6736(13)62120-0
2. Mughal TI, Radich JP, Deininger MW, et al. Chronic myeloid leukemia: reminiscences and dreams. *Haematologica*. 2016;101(5):541-558. doi:10.3324/haematol.2015.139337
3. Chereda B, Melo JV. Natural course and biology of CML. *Ann Hematol*. 2015;94(suppl 2):S107-S121. doi:10.1007/s00277-015-2325-z
4. How J, Venkataraman V, Hobbs GS. Blast and accelerated phase CML: room for improvement. *Hematology*. 2021;2021(1):122-128. doi:10.1182/hematology.2021000240
5. Wang L, Li L, Chen R, Huang X, Ye X. Understanding and monitoring chronic myeloid leukemia blast crisis: how to better manage patients. *Cancer Manag Res*. 2021;13:4987-5000. doi:10.2147/CMAR.S314343
6. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *Am J Hematol*. 2018;93(3):442-459. doi:10.1002/ajh.25011
7. Zhou T, Medeiros LJ, Hu S. Chronic myeloid leukemia: beyond BCR-ABL1. *Curr Hematol Malig Rep*. 2018;13(6):435-445. doi:10.1007/s11899-018-0474-6
8. Pattabiraman DR, Gonda TJ. Role and potential for therapeutic targeting of MYB in leukemia. *Leukemia*. 2013;27(2):269-277. doi:10.1038/leu.2012.225
9. Negi V, Vishwakarma BA, Chu S, et al. Hoxa9 and Hoxa10 induce CML myeloid blast crisis development through activation of Myb expression. *Oncotarget*. 2017;8(58):98853-98864. doi:10.18632/oncotarget.22008
10. Machova Polakova K, Lopotova T, Klamova H, et al. Expression patterns of microRNAs associated with CML phases and their disease related targets. *Mol Cancer*. 2011;10:41. doi:10.1186/1476-4598-10-41
11. Beck Z, Bácsi A, Kovács E, et al. Changes in oncogene expression implicated in evolution of chronic granulocytic leukemia from its chronic phase to acceleration. *Leuk Lymphoma*. 1998;30(3–4):293-306. doi:10.3109/10428199809057542
12. Giotopoulos G, van der Weyden L, Osaki H, et al. A novel mouse model identifies cooperating mutations and therapeutic targets critical for chronic myeloid leukemia progression. *J Exp Med*. 2015;212(10):1551-1569. doi:10.1084/jem.20141661
13. Ratajczak MZ, Hijiya N, Catani L, et al. Acute- and chronic-phase chronic myelogenous leukemia colony-forming units are highly sensitive to the growth inhibitory effects of c-myb antisense oligodeoxynucleotides. *Blood*. 1992;79(8):1956-1961. doi:10.1182/blood.V79.8.1956.1956
14. Waldron T, De Dominici M, Soliera AR, et al. c-Myb and its target Bmi1 are required for p190BCR/ABL leukemogenesis in mouse and human cells. *Leukemia*. 2012;26(4):644-653. doi:10.1038/leu.2011.264
15. Calabretta B, Sims RB, Valtieri M, et al. Normal and leukemic hematopoietic cells manifest differential sensitivity to inhibitory effects of c-myb antisense oligodeoxynucleotides: an in vitro study relevant to bone marrow purging. *Proc Natl Acad Sci U S A*. 1991;88(6):2351-2355. doi:10.1073/pnas.88.6.2351
16. Gewirtz AM. Potential therapeutic applications of antisense oligodeoxynucleotides in the treatment of chronic myelogenous leukemia. *Leuk Lymphoma*. 1993;11(suppl 1):131-137. doi:10.3109/10428199309047876
17. Lidonnici MR, Corradini F, Waldron T, Bender TP, Calabretta B. Requirement of c-Myb for p210(BCR/ABL)-dependent transformation of hematopoietic progenitors and leukemogenesis. *Blood*. 2008;111(9):4771-4779. doi:10.1182/blood-2007-08-105072
18. Srutova K, Curik N, Burda P, et al. BCR-ABL1 mediated miR-150 downregulation through MYC contributed to myeloid differentiation block and drug resistance in chronic myeloid leukemia. *Haematologica*. 2018;103(12):2016-2025. doi:10.3324/haematol.2018.193086

19. Xu M, Ye Y, Ye Z, et al. Human BCR/ABL1 induces chronic myeloid leukemia-like disease in zebrafish. *Haematologica*. 2020;105(3):674-686. doi:10.3324/haematol.2019.215939
20. Mitra P, Pereira LA, Drabsch Y, Ramsay RG, Gonda TJ. Estrogen receptor-alpha recruits P-TEFb to overcome transcriptional pausing in intron 1 of the MYB gene. *Nucleic Acids Res*. 2012;40(13):5988-6000. doi:10.1093/nar/gks286
21. Liu W, Wu M, Huang Z, et al. c-myb hyperactivity leads to myeloid and lymphoid malignancies in zebrafish. *Leukemia*. 2017;31(1):222-233. doi:10.1038/leu.2016.170
22. Narayanan R, Oates AC. Detection of mRNA by whole mount in situ hybridization and DNA extraction for genotyping of zebrafish embryos. *Bio Protoc*. 2019;9(6):e3193. doi:10.21769/BioProtoc.3193
23. Le Guyader D, Redd MJ, Colucci-Guyon E, et al. Origins and unconventional behavior of neutrophils in developing zebrafish. *Blood*. 2008;111(1):132-141. doi:10.1182/blood-2007-06-095398
24. Alghisi E, Distel M, Malagola M, et al. Targeting oncogene expression to endothelial cells induces proliferation of the myeloerythroid lineage by repressing the notch pathway. *Leukemia*. 2013;27(11):2229-2241. doi:10.1038/leu.2013.132
25. Traver D, Paw BH, Poss KD, Penberthy WT, Lin S, Zon LI. Transplantation and in vivo imaging of multilineage engraftment in zebrafish bloodless mutants. *Nat Immunol*. 2003;4(12):1238-1246. doi:10.1038/ni1007
26. Soza-Ried C, Hess I, Netuschil N, Schorpp M, Boehm T. Essential role of c-myb in definitive hematopoiesis is evolutionarily conserved. *Proc Natl Acad Sci U S A*. 2010;107(40):17304-17308. doi:10.1073/pnas.1004640107
27. Kok CH, Yeung DT, Lu L, et al. Gene expression signature that predicts early molecular response failure in chronic-phase CML patients on frontline imatinib. *Blood Adv*. 2019;3(10):1610-1621. doi:10.1182/bloodadvances.2019000195
28. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia*. 2020;34(4):966-984. doi:10.1038/s41375-020-0776-2
29. Radich J, Yeung C, Wu D. New approaches to molecular monitoring in CML (and other diseases). *Blood*. 2019;134(19):1578-1584. doi:10.1182/blood.2019000838
30. Zeidner JF, Karp JE. Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia. *Leuk Res*. 2015;39(12):1312-1318. doi:10.1016/j.leukres.2015.10.010
31. Blagosklonny MV. Flavopiridol, an inhibitor of transcription: implications, problems and solutions. *Cell Cycle*. 2004;3(12):1537-1542. doi:10.4161/cc.3.12.1278
32. Wiernik PH. Alvocidib (flavopiridol) for the treatment of chronic lymphocytic leukemia. *Expert Opin Investig Drugs*. 2016;25(6):729-734. doi:10.1517/13543784.2016.1169273

How to cite this article: Ye Y, Yang X, Li F, Liu W, Zhang W, Huang Z. *c-myb* is involved in CML progression and is a therapeutic target in the zebrafish CML model. *Anim Models Exp Med*. 2024;7:136-144. doi: 10.1002/ame2.12282