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Hippo/STK4 is downregulated in imatinib-resistant chronic myeloid leukemia and its restoration enhances apoptosis

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ABSTRACT: Chronic myeloid leukemia (CML) is a clonal hematologic disorder characterized by the presence of the *BCR::ABL1* fusion gene and is frequently associated with imatinib mesylate (IM) treatment failure. Aberrant expression of some key genes in the Hippo signaling pathway has been reported in CML and is involved in its pathogenesis and drug resistance. However, the entire core components of the Hippo pathway have not been elucidated in IM-sensitive and IM-resistant CML patients. We then compared the gene expression levels of the core mediators in the Hippo signaling pathway in normal subjects and CML patients with IM-sensitive and IM-resistant phenotypes. Compared to the normal group, *KIBRA*, *STK4*, and *YAP1* were significantly downregulated, while *LATS1* and *LATS2* were increased in CML patients. Intriguingly, the correlation analysis indicated that decreased *STK4* expression was associated with anemia in CML patients, particularly those with IM-resistance. The selected gene, *STK4*, was ectopically overexpressed in the CML-derived K562 cell line to demonstrate the therapeutic potential. Overexpression of *STK4* significantly enhanced IM-induced apoptosis of CML cells. These findings suggest that expression of the gene-encoding Hippo pathway could be used as an optional prognostic marker in CML patients and rescue of Hippo/*STK4* can provide a therapeutic way for CML treatment.

KEYWORDS: chronic myeloid leukemia, Hippo pathway, imatinib resistance, *STK4*

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal hematologic malignancy originating from reciprocal translocation between chromosomes 9 and 22. It creates a consecutively activated *BCR::ABL1* oncogenic fusion tyrosine kinase that can activate and crosstalk with many cellular pathways, providing leukemic cells with pro-survival and apoptosis resistance benefits [1]. Tyrosine kinase inhibitors (TKIs), including imatinib mesylate (IM), are frontline drugs commonly used in treating CML patients. Despite its effectiveness, some patients develop IM resistance; therefore, next-generation TKIs are considered to treat these patients resistant to IM [2, 3]. Alternatively, the other therapeutic approaches to improve CML treatment by enhancing IM sensitivity need to be elucidated.

The Hippo signaling pathway is an evolutionarily conserved pathway that controls organ size by regulating cell growth, proliferation, and apoptosis. Aberrant regulation of Hippo pathway mediators can initiate cellular transformation, leading to the development of tumors [4]. The Hippo pathway is related to other pathways that favor survival and progression in cancer cells [5]. Several reports have shown that key Hippo components such as large tumor suppressor family (*LATS1* and *LATS2*), Yes-associated protein-1 (*YAP1*), and WW domain-containing transcription regulator 1 (*WWTR1*) are aberrantly expressed in CML [6] and myeloproliferative neoplasms [7]. Apart from these key mediators, Serine/Threonine Kinase 4 (*STK4* or *MST1*), an essential upstream component that phosphorylates the LATS family [8], is also involved in crosstalk with other signaling pathways, namely AKT signaling [9] and JAK/STAT pathway [10]. *STK4* has been studied in several types of cancers and may be used as a diagnostic marker for colon cancer and related to colon cancer-lymph node metastasis [11, 12]. A recent study demonstrated that the downregulation of *STK4* in colon cancer was associated with distal metastasis and poor survival [13].

However, the expression of STK4 in hematologic malignancy, especially in CML patients who are IM-sensitive and IM-resistant, has not been elucidated. Therefore, our study aims to completely investigate the expression of the entire core Hippo pathway components and their associations with other clinical parameters in CML patients who are sensitive and resistant to IM compared to healthy controls. The differential gene expression in the Hippo pathway could be an optional prognostic marker in CML patients and choose the appropriate treatment. The function of *STK4* shown

in this study may lead to the novel targeted therapy of IM-resistant CML patients.

MATERIALS AND METHODS

Preparation of primary specimens

This study was conducted under the ethical approval of the Siriraj Hospital Ethics Committee (COA number Si101/2015) following the Declaration of Helsinki. Written informed consent was obtained from all subjects enrolled in the study. Peripheral blood samples were collected from 15 normal subjects and 50 CML patients, including 23 IM-sensitive and 37 IM-resistant patients, at the Hematology Clinic, Department of Medicine, Faculty of Medicine, Siriraj Hospital. The hematologic parameters of the healthy donors and CML patients are shown in Table S1.

Mononuclear cells were isolated from an EDTA-anticoagulated blood collection tube (BD Biosciences, USA) using the Ficoll-Paque (GE Healthcare, Marlborough, MA, USA) density gradient centrifugation method. The cell pellets were washed with PBS before being subjected to RNA extraction.

Quantitative RT-PCR

Total RNA was extracted from the cell pellet using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer. Two micrograms of RNA were used for cDNA preparation using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). qPCR was performed using a BioRad CFX384 real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The reaction mixture contained 5 µl of 2X SYBR Select Master Mix (Applied Biosystems, Waltham, MA, USA), 0.5 µl of each primer, 2 µl of RNase-free water, and 2 µl of cDNA template. The PCR cycle conditions were as follows: 50 °C for 2 min; 1 cycle of 95 °C for 2 min; 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 30 s (annealing/extension). The sequences of the primers used in this study are provided in Table S1. *GAPDH* was used as the housekeeping gene for relative quantification using the $2^{-\Delta\Delta Ct}$ method.

Ectopic expression of *STK4*

To demonstrate the functional role of *STK4* in CML therapy, a human pJ3H-*MST1* plasmid (#12203, Addgene, Cambridge, MA, USA) was used for overexpression in the CML-derived K562 cell line (obtained from JCRB, Japan) using Lipofectamine 3000 reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Briefly, 10^6 K562 cells were mixed with 5 µg of plasmid in Opti-MEM™ medium (Gibco, Life Technologies, NY, USA) in the presence of Lipofectamine 3000 reagent before seeding in RPMI1640 medium (Gibco, Thermo Fisher Scientific) with 2% fetal bovine serum (FBS, PAN-Biotech, Aidenbach, Germany) for 12 h. Then, the medium was replaced with a complete RPMI1640 medium supplemented with 10% FBS, and cells were used for IM treatment.

IM treatment and apoptosis detection

K562 cells at a density of 2×10^5/ml were plated in RPMI1640 medium supplemented with 10% FBS in a 24-well plate. Then, 2 µM of IM (Sigma-Aldrich, USA) were added for 48 h, and cells were harvested for apoptosis detection. Cells were washed twice in PBS and incubated with PE-conjugated annexin-V and 7-AAD reagents (BD Biosciences) in binding buffer for 15 min at room temperature in the dark. Data acquisition was performed using a FACS Canto cytometer (BD Biosciences).

Western blot analysis

Protein lysates were extracted from cell pellets using RIPA buffer containing protease and phosphatase inhibitors. The concentration of the extracted protein was quantified using a Pierce™ BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IN, USA). Equal amounts of protein were resolved on a 7.5% polyacrylamide gel and transferred onto a PVDF membrane. The membranes were blocked in 5% skim milk/TBST buffer before being incubated with primary antibodies (Table S2) at 4 °C overnight. After washing the membranes, they were incubated with HRP-conjugated secondary antibodies, and immune complexes were detected using an enhanced chemiluminescence (ECL) substrate (Merck Millipore, Burlington, MA, USA) on an ImageQuant LAS 4010 biomolecular imager (GE Healthcare).

Statistical analysis

Differences in gene expression between groups were analyzed using the One-Way ANOVA test. The simple linear regression and Spearman's correlation coefficient (r^2) were also determined. A p-value < 0.05 was considered statistically significant in all analyses. All statistical analyses were performed using GraphPad Prism software version 9.0.0.

RESULTS

Hematological characteristics

As shown in Table 1, the RBC indices of IM-sensitive patients indicate macrocytic anemia compared to those of normal subjects and IM-resistant patients. Furthermore, there were no significant differences in WBC count between the groups, but mild thrombocytopenia was found in patients sensitive to IM. In addition, the demographic data, treatment characteristics, and responses of patients with CML are given in Table S3. The blast count, level of fusion *BCR::ABL1* transcript,

Table 1 Association between the expression levels of the Hippo component gene and hematologic parameters in patients with IM-sensitive CML.

	KIBRA	*STK3*	*STK4*	*LATS1*	*LATS2*	*YAP*	*WWTR1*
WBC count	p **= 0.014** $r^2 = 0.220$	$p = 0.601$ $r^2 = -0.034$	$p = 0.085$ $r^2 = 0.093$	p **= 0.014** $r^2 = 0.220$	p **= 0.005** $r^2 = 0.283$	p **<0.001** $r^2 = 0.542$	$p = 0.209$ $r^2 = 0.030$
RBC count	p **= 0.047** $r^2 = 0.135$	$p = 0.441$ $r^2 = -0.018$	p **= 0.022** $r^2 = 0.189$	$p = 0.099$ $r^2 = 0.082$	$p = 0.254$ $r^2 = 0.017$	$p = 0.114$ $r^2 = 0.073$	p **= 0.013** $r^2 = 0.224$
PLT count	$p = 0.463$ $r^2 = -0.021$	$p = 0.155$ $r^2 = 0.051$	$p = 0.737$ $r^2 = -0.042$	$p = 0.706$ $r^2 = -0.040$	$p = 0.637$ $r^2 = -0.036$	$p = 0.225$ $r^2 = 0.025$	$p = 0.077$ $r^2 = 0.101$
Hb	$p = 0.309$ $r^2 = 0.004$	$p = 0.179$ $r^2 = 0.041$	p **= 0.039** $r^2 = 0.148$	$p = 0.461$ $r^2 = -0.020$	$p = 0.231$ $r^2 = 0.023$	p **= 0.044** $r^2 = 0.141$	$p = 0.204$ $r^2 = 0.032$
HCT	$p = 0.270$ $r^2 = 0.013$	$p = 0.118$ $r^2 = 0.070$	p **= 0.034** $r^2 = 0.157$	$p = 0.540$ $r^2 = -0.029$	$p = 0.241$ $r^2 = 0.020$	$p = 0.054$ $r^2 = 0.126$	$p = 0.116$ $r^2 = 0.071$
MCV	$p = 0.072$ $r^2 = 0.105$	$p = 0.345$ $r^2 = -0.003$	$p = 0.554$ $r^2 = -0.030$	$p = 0.103$ $r^2 = 0.080$	$p = 0.995$ $r^2 = -0.048$	$p = 0.820$ $r^2 = -0.045$	$p = 0.086$ $r^2 = 0.093$
MCH	$p = 0.136$ $r^2 = 0.060$	$p = 0.521$ $r^2 = -0.027$	$p = 0.681$ $r^2 = -0.039$	$p = 0.276$ $r^2 = 0.011$	$p = 0.835$ $r^2 = -0.045$	$p = 0.808$ $r^2 = -0.045$	$p = 0.074$ $r^2 = 0.104$
MCHC	$p = 0.136$ $r^2 = -0.048$	$p = 0.521$ $r^2 = -0.043$	$p = 0.681$ $r^2 = -0.037$	$p = 0.276$ $r^2 = 0.010$	$p = 0.835$ $r^2 = -0.038$	$p = 0.808$ $r^2 = 0.012$	$p = 0.074$ $r^2 = -0.027$
BCR::ABL1	$p = 0.339$ $r^2 = -0.002$	$p = 0.354$ $r^2 = -0.004$	$p = 0.194$ $r^2 = 0.035$	p **= 0.033** $r^2 = 0.160$	$p = 0.192$ $r^2 = 0.036$	$p = 0.336$ $r^2 = -0.001$	$p = 0.156$ $r^2 = 0.050$
% IS	$p = 0.339$ $r^2 = -0.002$	$p = 0.354$ $r^2 = -0.004$	$p = 0.194$ $r^2 = 0.035$	p **= 0.033** $r^2 = 0.160$	$p = 0.192$ $r^2 = 0.036$	$p = 0.336$ $r^2 = -0.001$	$p = 0.156$ $r^2 = 0.050$

and percentage of international scale (% IS) in IM-resistant patients were significantly higher than those in IM-sensitive group.

Differential expression of Hippo pathway genes

The quantification of genes encoding components of the Hippo pathway cascade (Fig. 1A) was performed by real-time PCR analysis. *KIBRA* (Fig. 1B) and *STK4* (Fig. 1D) were significantly downregulated in both IM-sensitive and IM-resistant patients. Increase of *LATS1* (Fig. 1E) was found in both IM-sensitive and IM-resistant CML patients, while *LATS2* (Fig. 1F) was significantly overexpressed in IM-sensitive patients. On the other hand, the level of *YAP1* (Fig. 1G) was dramatically decreased in both CML groups. However, we did not notice the difference in the level of *STK3* (Fig. 1C) and *WWTR1* (Fig. 1H) among groups. We also measured the STK4 protein levels in mononuclear cells isolated from primary CML patients and normal subjects. We found an absence of STK4 protein in CML samples compared to normal subjects (Fig. 1I), indicating a loss of STK4 involved in CML pathogenesis.

Associations between Hippo pathway component gene levels and blood parameters in CML patients

Given that aberrant expression of Hippo pathway genes could be used as a prognostic marker for patients with CML, we tested the association between the expression of genes that encode components of the Hippo pathway and the clinical data of patients with CML. Table 1 and Table 2 show the statistical significance and adjusted coefficient of determination (r^2) of IM-sensitive and IM-resistant patients, respectively. For example, downregulation of *KIBRA* and *YAP1* and upregulation of the *LATS* family were associated with slightly increased WBC counts in the IM-sensitive group. Interestingly, the lower *STK4* expression was significantly correlated with anemia, as indicated by lower RBC parameters in both the IM-sensitive and IM-resistant groups.

Ectopic expression of *STK4* promoting IM sensitivity of CML cells

We used the K562 cell line to model the therapeutic role of STK4 by ectopic expression. RT-qPCR analysis showed increased *STK4* in overexpressed K562 (*STK4*-K562) cells (Fig. 2A), and Western blot analysis revealed the increased STK4 protein and its phosphorylated form in *STK4*-K562 cells (Fig. 2B). Gene expression analysis showed that overexpression of *STK4* induced downregulation of Hippo pathway components including *LATS1*, *LATS2*, *YAP1* and significant decrease of *CYCR61* target gene. The exposure of K562 cells to IM treatment extended the effect of *STK4* in changing levels of genes tested (Fig. 2C). For the apoptosis result, *STK4*-K562 cells exhibited more cell death as determined by increased positivity to annexin-V binding. The cytotoxic effect of IM treatment was significantly pronounced in *STK4*-K562 cells compared to mock K562 cells (Fig. 2D,E).

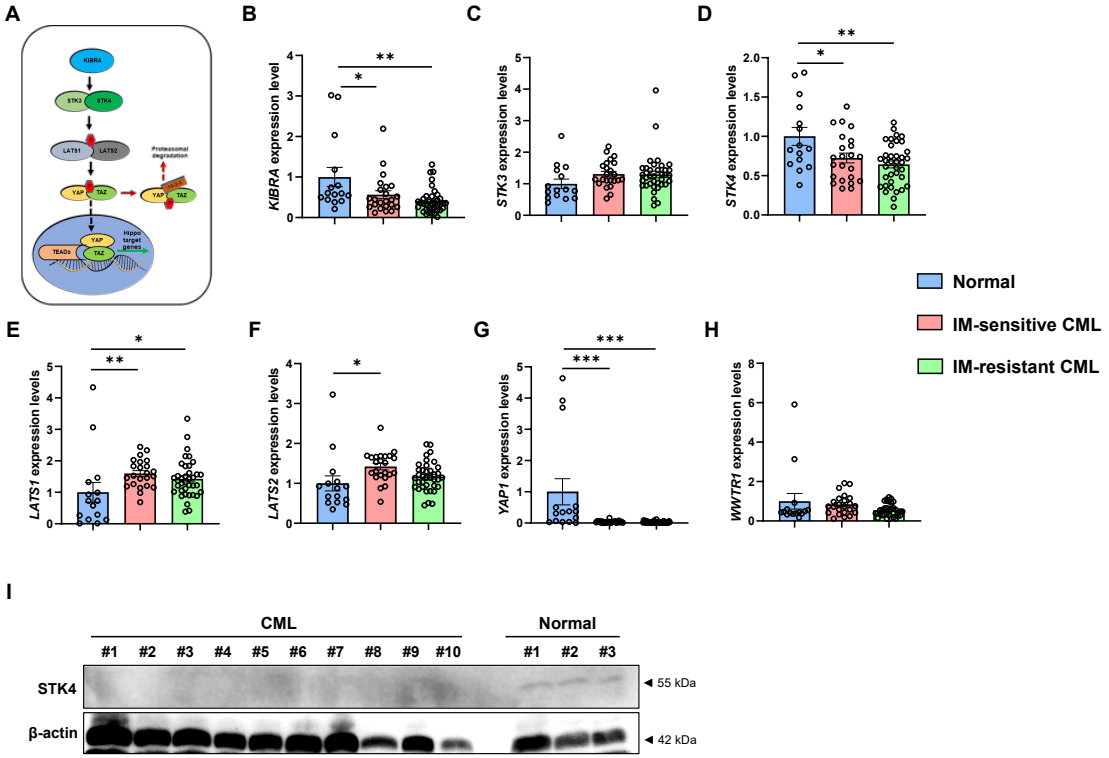

Fig. 1 Expression of Hippo pathway components in CML specimens. (A) The diagram depicting the Hippo signaling cascade. (B–H) mRNA levels of key Hippo pathway mediators, including (B) *KIBRA*; (C) *STK3*; (D) *STK4*; (E) *LATS1*; (F) *LATS2*; (G) *YAP1*; and (H) *WWTR1* in healthy individuals and IM-sensitive and IM-resistant CML patients. (I) Western blot analysis of STK4 protein in CML samples compared to healthy subjects. (* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$).

DISCUSSION

IM is the main TKI used for CML treatment; however, chemoresistance hinders the achievement of complete remission. Although numerous genes are known to be frequently aberrant in CML, the full list of key components in the Hippo signaling pathway has not been thoroughly explored. Previous works demonstrated significantly greater expression of only the *LATS1*, *LATS2*, and *WWTR1* genes in CML patients, particularly those in the chronic and advanced phases [6]. This raises the question of how other key components of the Hippo pathway impact the pathogenesis of CML. To fill this knowledge gap about the core components of the Hippo pathway, the present study investigated the dysregulated expression of all key mediators of the Hippo pathway in patients with CML in both the IM-resistant and IM-sensitive groups compared to normal individuals. To the best of the authors' knowledge, the present study demonstrated that the *LATS* family was highly expressed in patients with CML, which is consistent with the findings of previous publications [6, 14], suggesting their oncogenic potential. However, *YAP1* is significantly negatively regulated in our CML cohort, implying a tumor suppressive role in the CML setting. *KIBRA* is upstream of the Hippo pathway and is one of the Hippo cascade initiators that phosphorylates the STK3/4 protein kinase. *KIBRA* was reported to be epigenetically silent through DNA methylation in lymphocytic leukemia [15, 16]. We observed a lower expression of this gene in IM-resistant CML patients.

To utilize the expression of Hippo-encoding genes as the prognostic biomarker, we then explored the association of their mRNA levels and hematologic parameters in CML patient groups. Interestingly, significantly positive correlations were observed between decreased *STK4* expression and anemia indices, including low hemoglobin levels, low hematocrit levels, and low mean corpuscular volume according to red blood cell parameters, in both groups of patients with CML. With respect to its prognostic significance, *STK4* is associated with an unfavorable outcome in several cancers, including colorectal cancer [11], breast cancer [17] , and renal cell carcinoma [18]. In our hands, we found a low level of *STK4* in IM-resistant patients and an absence of STK4 protein in CML samples, implying that STK4 is directly involved in drug resistance and, in part, anemic status in CML patients. The role of *STK4* in regulating erythropoiesis was demonstrated in *Mst1/2*-knockout mice, in which significantly impaired red cell production in bone marrow was observed [19]. Although our cohort has a small sample size and

Table 2 Association between the expression levels of the Hippo component gene and hematologic parameters in patients with IM-resistant CML.

	KIBRA	*STK3*	*STK4*	*LATS1*	*LATS2*	*YAP*	*WWTR1*
WBC count	$p = 0.313$ $r^2 = -0.001$	$p = 0.904$ $r^2 = -0.027$	$p = 0.229$ $r^2 = 0.024$	$p = 0.433$ $r^2 = -0.008$	$p = 0.562$ $r^2 = -0.014$	$p = 0.936$ $r^2 = -0.028$	$p = 0.030$ $r^2 = 0.111$
RBC count	$p = 0.865$ $r^2 = 0.028$	$p = 0.475$ $r^2 = -0.013$	$\mathbf{p = 0.004}$ $r^2 = 0.195$	$p = 0.094$ $r^2 = 0.052$	$p = 0.227$ $r^2 = 0.014$	$p = 0.399$ $r^2 = -0.008$	$p = 0.776$ $r^2 = -0.026$
PLT count	$p = 0.751$ $r^2 = -0.026$	$p = 0.564$ $r^2 = -0.019$	$p = 0.992$ $r^2 = -0.029$	$p = 0.954$ $r^2 = -0.028$	$p = 0.959$ $r^2 = -0.028$	$p = 0.963$ $r^2 = -0.029$	$p = 0.305$ $r^2 = 0.003$
Hb	$p = 0.348$ $r^2 = -0.003$	$p = 0.478$ $r^2 = -0.014$	$\mathbf{p = 0.019}$ $r^2 = 0.122$	$p = 0.160$ $r^2 = 0.029$	$p = 0.285$ $r^2 = 0.005$	$p = 0.597$ $r^2 = -0.020$	$p = 0.313$ $r^2 = 0.001$
HCT	$p = 0.406$ $r^2 = -0.008$	$p = 0.491$ $r^2 = -0.015$	$\mathbf{p = 0.015}$ $r^2 = 0.133$	$p = 0.159$ $r^2 = 0.029$	$p = 0.284$ $r^2 = 0.005$	$p = 0.451$ $r^2 = -0.012$	$p = 0.336$ $r^2 = -0.001$
MCV	$p = 0.064$ $r^2 = 0.069$	$p = 0.662$ $r^2 = -0.023$	$p = 0.199$ $r^2 = 0.019$	$p = 0.370$ $r^2 = -0.005$	$p = 0.662$ $r^2 = -0.023$	$p = 0.636$ $r^2 = -0.022$	$p = 0.293$ $r^2 = 0.004$
MCH	$\mathbf{p = 0.047}$ $r^2 = 0.082$	$p = 0.754$ $r^2 = -0.026$	$p = 0.381$ $r^2 = -0.006$	$p = 0.510$ $r^2 = -0.016$	$p = 0.874$ $r^2 = -0.028$	$p = 0.611$ $r^2 = -0.021$	$p = 0.226$ $r^2 = 0.014$
MCHC	$p = 0.329$ $r^2 < -0.001$	$p = 0.912$ $r^2 = -0.028$	$p = 0.388$ $r^2 = -0.007$	$p = 0.575$ $r^2 = -0.019$	$p = 0.604$ $r^2 = -0.021$	$p = 0.433$ $r^2 = -0.010$	$p = 0.433$ $r^2 = -0.010$
% Blast	$p = 0.779$ $r^2 = 0.002$	$p = 0.154$ $r^2 = 0.057$	$p = 0.078$ $r^2 = 0.086$	$p = 0.318$ $r^2 = 0.028$	$p = 0.233$ $r^2 = 0.040$	$p = 0.465$ $r^2 = 0.015$	$p = 0.112$ $r^2 = 0.071$
BCR::ABL1	$p = 0.701$ $r^2 = -0.024$	$p = 0.677$ $r^2 = 0.023$	$p = 0.772$ $r^2 = -0.026$	$p = 0.659$ $r^2 = -0.023$	$p = 0.061$ $r^2 = 0.071$	$p = 0.410$ $r^2 = -0.010$	$p = 0.863$ $r^2 = -0.028$
% IS	$p = 0.962$ $r^2 = -0.028$	$p = 0.304$ $r^2 = 0.002$	$p = 0.370$ $r^2 = -0.005$	$p = 0.213$ $r^2 = 0.017$	$p = 0.997$ $r^2 = -0.029$	$p = 0.055$ $r^2 = 0.077$	$p = 0.051$ $r^2 = 0.079$

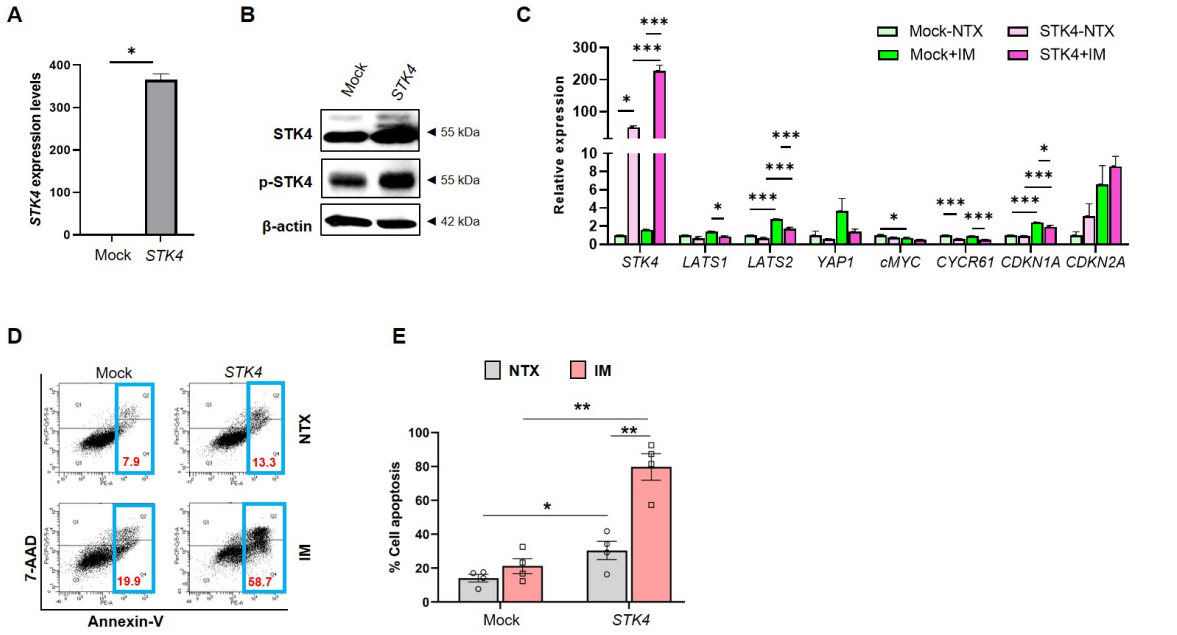

Fig. 2 IM sensitivity of CML cells restored by *STK4* overexpression. (A–B) STK4 expression at mRNA and protein levels in K562 cells after ectopic expression by lipofection determined by RT-qPCR analysis (A) and Western blot analysis (B). (C) mRNA levels of the downstream STK4 signaling pathway in mock and *STK4*-K562 cells after exposure to 2 μM IM quantified by RT-qPCR analysis. (D–E) Flow cytometric analysis of total apoptotic cells (blue square) in annexin-V/7-AAD binding assay of IM-treated K562 cells for 48 h. (IM: Imatinib; NTX: non-treated; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$).

the results may be influenced by predisposing factors such as age and comorbidities, the study demonstrates significant findings in many cases. Future research involving larger and more diverse populations would help to further validate and expand upon our findings.

What is the functional significance underlying the downregulation of *STK4* in CML? The possibility of silent *STK4* in cancer could be the consequence of dysregulated epigenetic regulation in addition to DNA methylation. For example, microRNA-18a-mediated suppression of *STK4* leads to cervical cancer transformed by human papillomavirus [20], EZH2 histone methyltransferase, together with MYC, attenuate *STK4* expression in prostate cancer [21], and SIRT7, a histone desuccinylase, inhibits *STK4* transcription by inducing histone deacetylation at its promoter in hepatocellular carcinoma [22]. Several reports show the tumor suppressor role of *STK4* in hematologic disorders. *Mst1* (human STK4) increased the chance of genetically and chemically induced lymphoma development in a mouse model, and notable downregulation of *STK4* was found in samples of acute human lymphoblastic leukemia [23]. STK4 was shown to be activated by caspase-3 and mediate H2AX phosphorylation, leading to IM-induced CML cell apoptosis [24]. Furthermore, STK4, which is induced by the natural compound shikonin, cooperates with YAP to inhibit glycolysis by blocking c-MYC and GLUT1 expression, leading to leukemic cell apoptosis [25]. The expression levels of *STK4* vary across different hematological malignancies. For example, decreased *STK4* levels were found in newly diagnosed acute leukemia patients compared to healthy controls [26], while Safari et al [27] observed no difference in *STK4* expression in acute myeloid leukemia samples. Another study highlighted elevated *STK4* levels during disease progression in multiple myeloma [28]. These discrepancies suggest that the role of *STK4* may differ depending on the type of hematological malignancy.

Our observations and these findings led us to select *STK4* as a candidate to further investigate its therapeutic potential in CML. We tried overexpressing *STK4* in the CML cell line to demonstrate the drug sensitization of IM. In our experiment, *STK4*-overexpressed cells exhibit increased cell death, which may be mediated by the downregulation of several downstream targets of *STK4* such as the *LATS* gene family, *YAP1*, and the *cMYC* oncogene. This effect is further amplified when the cells are treated with IM, as *STK4* levels are elevated, suggesting a potential tumor-suppressive role of *STK4* in CML. K562 cells are commonly used to study drug resistance in chronic myelogenous leukemia (CML); however, they do not fully capture the disease's complexity due to disease heterogeneity. Since CML exhibits a wide range of genetic and molecular variations, relying solely on K562 cells is insufficient to represent all aspects of the disease. To better understand CML biology, additional CML-derived cell lines and primary CML samples are needed.

Given that *STK4* serves as a potential therapeutic target for CML treatment and its tissue-dependent function, systemic modulation of *STK4* may lead to some unintended consequences. Although current studies on *STK4*-targeted therapies are still at an experimental stage [29], there is evidence from murine models suggesting that complete loss of STK4 function can impair immune function and hematopoiesis. For example, depletion of *Mst1/2* resulted in abnormality of Xenopus shape, including smaller eyes, short axis, and abnormal epidermis. Importantly, *Mst1/2* morphants displayed decreased differentiation markers of erythroid, myeloid, and endothelial lineages, suggesting its role as a differentiation switch of hematopoietic-endothelial cells [30]. Restoring *STK4* expression in IM-resistant CML presents a promising therapeutic avenue for CML; however, translating this concept into a viable clinical strategy presents several challenges. First, non-specific restoration could disrupt normal hematopoiesis or provoke adverse effects in other organ systems. Second, there is currently a lack of clinically approved agents that can specifically and safely upregulate STK4 activity. Gene therapy, small-molecule modulators, epigenetic drugs, and synthetic or natural compounds may offer potential routes in targeting the Hippo pathway [31], but each comes with technical hurdles, including delivery specificity, off-target effects, and long-term safety. Besides, patient-to-patient variability in the underlying mechanisms of STK4 downregulation—whether epigenetic, post-transcriptional, or due to upstream regulatory mutations—could influence therapeutic response and complicate treatment design.

CONCLUSION

Our data support the use of the Hippo pathway as a valuable prognostic biomarker to predict the severity or clinical complications of CML patients, and targeting key components of this pathway offers a therapeutic advantage for CML.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.060.

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Appendix A. Supplementary data

Table S1 Sequence of qPCR primers used in this study.

Gene	Forward	Reverse
KIBRA	GCGCCCAGGAAAGATACC	CTGGGCCGTATTCACAGC
STK3	TGGTCCCTTGGCATTACTTC	TTGTGGGAATCATAAAAATAGCC
STK4	AGTGGACCAGGACGATGAAG	AGTGGACCAGGACGATGAAG
LATS1	GGCACAAACACCATTAGAAACA	AGAAGCTTCAGGACTGAGTTTAGC
LATS2	AGCAAGAAATGGCCAAAGC	GGTAGAGGATCTTCCGCATCT
YAP1	ATCCCAGCACAGCAAATTCT	TGGATTTTGAGTCCCACCAT
WWTR1	TGGATTTTGAGTCCCACCAT	TGGATTTTGAGTCCCACCAT
CYCR61	AAACCCGGATTTGTGAGGT	GCTGCATTTCTTGCCCTTT
CMYC	CCTCCCTCCACTCGGAAG	TCTGACACTGTCCAACTTGACC
CDKN1A	TGGCGTAAAGGACCTGAACC	CTCAGACACTGGCATGGTGT
CDKN2A	CTTCGGCTGACTGGCTGG	TCATCATGACCTGGATCGGC
GAPDH	GAAGGTGAAGGTCGGAGTCA	GGGGTCATTGATGGCAACAATA

Table S2 List of antibodies used in the present study.

Antibody	Dilution	Catalog No./ Company
STK4	1:1,000	#HPA015270, Merck
p-STK4 (Thr183)	1:1,000	#49332, Cell Signaling Technology
β-actin	1:10,000	#A3854, Merck

Table S3 Hematological demographics of the subjects enrolled in this study.

Parameter	Normal subject	CML patient	
		IM-sensitive	IM-resistant
Gender (M/F)	3/12	12/11	29/8
Age	27.8 ± 4.8	54.7 ± 17.9	45.4 ± 21.9
WBC count	5839 ± 1136	6148 ± 1803	$8741 \pm 5361^{**}$
RBC count	4.69 ± 0.49	$3.86 \pm 0.81^{***,\#}$	$4.11 \pm 0.92^{*}$
Hb (g/dl)	13.3 ± 1.2	$11.6 \pm 2.2^{*,\#}$	$11.5 \pm 2.4^{*}$
HCT (%)	41.2 ± 2.8	$35.8 \pm 5.8^{***,\#}$	$35.8 \pm 7.2^{*}$
MCV (fL)	86.7 ± 7.2	$94.3 \pm 12.5^{*}$	87.4 ± 10.1
MCH (pg)	27.9 ± 2.9	$30.6 \pm 4.5^{*}$	28.2 ± 3.7
MCHC (%)	32.3 ± 1.1	32.4 ± 1.7	32.1 ± 1.3
PLT count	259533 ± 54951	$209652 \pm 1803^{*}$	216432 ± 89582
CML phase	–	Chronic phase = 100%	Chronic phase = 83.8% Accelerated phase = 8.1% Blast crisis = 8.1%
% Leukemic blast	–	0	1.3 ± 4.4
BCR::ABL1 copy number	–	$2649 \pm 10258^{\#\#\#}$	4816 ± 10255
% IS	–	$4.08 \pm 15.82^{\#\#\#}$	9.42 ± 18.57
Recent TKI used	–	Imatinib	Ascriminib, Nilotinib, Dasatinib
Molecular response (cases)	–	Undetectable = 18 CMR4.5 = 4 No MMR = 1	Undetectable = 1 MMR = 5 CMR4.5 = 4 CCyR = 9 MCyR = 8 No MMR = 10

Note: $^{*}\ p < 0.05$, $^{**}\ p < 0.01$, $^{***}\ p < 0.001$ vs. the normal group. $^{\#}\ p < 0.05$, $^{\#\#\#}\ p < 0.001$ vs.$ the IM-resistant group.