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Over-expression of FoxM1 is associated with adverse prognosis and FLT3-ITD in acute myeloid leukemia

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ABSTRACT

Forkhead box M1 (FoxM1) drives cell cycle progression and the prevention of growth arrest and is over-expressed in many human malignancies. However, the characteristics of FoxM1 in acute myeloid leukemia (AML) are not clearly understood. We investigated the expression level of FoxM1 and analyzed the correlation of FoxM1 expression with AML patient characteristics and prognoses. Changes in FoxM1 expression were detected after MV4–11 cells, which have an internal tandem duplication (ITD) of the fms-like tyrosine kinase 3 gene (FLT3-ITD), and control THP1 cells (encoding wild-type FLT3) were treated with the FLT3 receptor tyrosine kinase inhibitor AC220 (quizartinib) or FLT3 ligand (FL). Finally, we determined the apoptosis rates after the addition of the FoxM1 inhibitor thiostrepton (TST) to AML cells with or without FLT3-ITD. The expression of FoxM1 in AML patients was correlated with the presence of FLT3-ITD, genetic groups, and possibly overall survival. Inhibition of FLT3-ITD by AC220 down-regulated FoxM1 expression in MV4–11 cells, and stimulation of FLT3 by FL up-regulated FoxM1 expression in MV4–11 and THP1 cells. TST induced the apoptosis of MV4–11 and THP1 cells in a dose-dependent manner. Thus, FoxM1 is a potential prognostic marker and a promising therapeutic target in AML.

1. Introduction

Forkhead box protein M1 (FoxM1), also known as HFH-11, MPP-2, and WIN, belongs to the forkhead superfamily of transcription factors, which share an evolutionary conserved "winged helix" DNA-binding domain. FoxM1 protein expression is normally restricted to proliferating cells and is absent in quiescent or terminally differentiated cells. By binding the consensus sequence TAAACA in target genes [1], FoxM1 regulates a wide spectrum of biological processes, including cell cycle progression, cell proliferation, cell differentiation, DNA damage repair, tissue homeostasis, and apoptosis. FoxM1 transcription is regulated by estrogen receptor (ER), E2F, and FOXO [1,2]. FoxM1 is a downstream target of the epidermal growth factor receptor (EGFR)–phosphoinositide 3-kinase (PI3K)–AKT–FOXO pathway. The interaction of FOXO with the putative forkhead response element (FHRE) down-regulates FoxM1 transcription [1].

However, elevated expression of FoxM1 has also been observed in various malignancies [1]. In human solid tumors, genome-wide gene expression profiling of cancers has independently and consistently identified FoxM1 as one of the most commonly up-regulated genes [3]. By regulating the G1/S and G2/M transitions and M phase progression, antagonizing cellular senescence, stimulating stem cell-like characteristics (including self-renewal), promoting multiple steps of cancer progression by inducing mitogenic and survival signals, and promoting tumor invasion, migration, and angiogenesis, FoxM1 plays an important role in tumorigenesis and cancer progression [1]. In addition, recent research also links FoxM1 deregulation to the development of cancer drug resistance [4]. Multiple studies [5–7] have demonstrated that FoxM1 could be a useful marker for predicting poor prognosis in patients with solid tumors. However, there have been relatively few studies [8,9] of the role of FoxM1 in leukemia, particularly in acute myeloid leukemia (AML).

Internal tandem duplication (ITD) of the fms-like tyrosine kinase 3 gene (FLT3-ITD) is a common genetic alteration in AML. FLT3-ITD is a gain-of-function mutation and occurs in nearly 30% of AML cases [10]. FLT3 is a class III receptor tyrosine kinase consisting of 5 extracellular immunoglobulin-like domains, 1 transmembrane domain, 1 juxtamembrane domain, and 2 tyrosine kinase domains (TKD1 and TKD2) [11]. After binding to the FLT3 ligand (FL), FLT3 dimerizes and autophosphorylates, resulting in the activation of tyrosine kinase activity [12]. FLT3-ITD results in

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an in-frame duplication of 3–400 base pairs at the juxtamembrane or TKD1 domain, resulting in changes in cellular signaling, including the constitutive activation of FLT3 independent of ligand binding, activation of signal transducer and activator of transcription 5 (STAT5) via SRC kinase, phosphorylation of the transcription factor FOXO3A via the PI3K-AKT pathway, down-regulation of the equilibrative nucleoside transporter 1 for cytarabine, and induction of reactive oxygen species production [13]. These molecular consequences result in damage to and defective repair of DNA, increased cellular proliferation, and resistance to apoptosis [13]. AML with FLT3-ITD has a higher relapse rate and, consequently, inferior disease-free and overall survival, particularly in AML with a larger ITD size, higher allelic burden, and multiple ITDs [13].

Because FoxM1 and FLT3 both function through the PI3K–AKT–FOXO3A signaling pathway and FoxM1 has prognostic significance in solid tumors, we evaluated whether FoxM1 is a downstream target of FLT3 or the FLT3-ITD-like receptor tyrosine kinase EGFR/HER2 as well as whether FoxM1 could be a prognostic marker and therapeutic target in AML.

2. Materials and methods

2.1. Patients samples and cell lines

Bone marrow cells or bone marrow biopsy samples were obtained from newly diagnosed patients at the time of bone marrow puncture or bone marrow biopsy at Tongji Hospital for diagnostic purposes according to a protocol approved by the Institutional Review Board for Human Research at Tongji Hospital. The diagnosis of AML was made based on a multiparametric approach, including examination of clinical characteristics, morphological features, immunophenotype, and cytogenetic and molecular findings. After lysing red blood cells using red blood cell lysis buffer (8.99 g of NH_4Cl, 1 g of $KHCO_3$, 0.037 g of EDTA, dissolved in 1 L of water), bone marrow mononuclear cells were separated and stored at −80 °C. The FLT3-ITD cell line MV4–11 was kindly provided by the Cancer Biology Research Center of Tongji Hospital (purchased from American Type Culture Collection). The THP1 cell line (which expresses the wild-type FLT3 receptor) was purchased from American Type Culture Collection.

2.2. Reverse transcription–polymerase chain reaction

Total RNA was extracted from AML bone marrow samples using the TRIzol reagent (Takara Bio Inc., Japan) according to the manufacturer's instructions. Standard reverse transcription (RT) was performed using 2 μg of total RNA, oligo(dT), and the RevertAid™ Minus First Strand cDNA Synthesis Kit (Fermentas, K1622, Canada) according to the manufacturer's instructions. The resulting cDNAs were used as templates for PCR with primers for NPM1 mutation A (NPM1 mA), FLT3-ITD, FoxM1, and GAPDH. The primer sequences and PCR conditions are listed in Table 1. PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and visualized under UV light.

2.3. Immunohistochemical staining

Bone marrow biopsy samples were fixed in 10% formaldehyde solution, embedded in paraffin blocks, cut in 4-μm-thick sections, and mounted on glass slides. Immunohistochemical stain was performed as described [14]. Polyclonal rabbit anti-human FoxM1 antibody (Bioss, Beijing, China; 1:200 dilution) was used.

Immunohistochemical staining for FoxM1 was evaluated using a semi-quantitative scoring system for both staining intensity and the percentage of positive immature cells. The immunohistochemistry score was calculated by multiplying the intensity (negative scored as 0, mild scored as 1, moderate scored as 2, and strong scored as 3) by the percentage of stained cells (0, <5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100%). These products were graded as follows: −, 0; +, 1–3; ++, 4–8; +++, 9–12. In addition, for statistical analysis, the − and + cases were pooled into the low-expression group, and the ++ and +++ cases were pooled into the high-expression group.

2.4. Western blot assay

Western blot assay was performed as described [15]. Anti-FoxM1 (Bioss, Beijing, China; 1:200 dilution) and anti-β-actin (Abcam, UK; 1:2000 dilution) antibodies were used.

2.5. Quantitative real-time PCR

A total of 1×10^6 MV4–11 or THP1 cells were cultured with dimethylsulfoxide (DMSO, control, Sigma, USA), 5 nmol/L AC220 (Biovision, USA), 5 μmol/L thiostrepton (TST, Enzo, USA), or 100 ng/mL FL (PeproTech, USA) for 12, 24, or 48 h. Total RNA was isolated from the harvested cells using TRIzol according to the manufacturer's protocol. First-strand cDNAs were synthesized using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, K1622, Canada). Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green PCR Master Mix (Toyobo, Japan) on a StepOne™ Real-Time PCR System (Applied Biosystems). GAPDH was used as the reference gene. The following primers were used: GAPDH, 5′-GCACCGTCAAGGCTGAGAAC-3′ (forward), 5′-TGGTGAAGACGCCAGTGGA-3′ (reverse); FoxM1, 5′-TGCCCAGCAGTCTCTTACCT-3′ (forward), 5′-CTACCCACCTTCTGGCAGTC-3′ (reverse); CyclinB1, 5′-GAAATGTACCCTCCAGAAATTGGT-3′ (forward), 5′-CCATCTGTCTGATTTGGTGCTTAG-3′ (reverse); Cdc25B, 5′-CCCTATGGACCCCCACATG-3′ (forward), 5′-ATGGCAAACTGCTCGTTTCG-3′ (reverse). The PCR program was 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s. Each reaction was performed in triplicate and analyzed individually. The results were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6. Flow cytometric analysis of apoptosis

A total of 1×10^6 MV4–11 or THP1 cells were cultured with an increasing concentration of AC220 or TST alone or in combination for 24 h. DMSO was used as the negative control. Apoptosis rates were measured by flow cytometry after staining with fluorescein-conjugated annexin-V and propidium iodide using the Annexin V-FITC Kit (Key GENE, Nanjing, China) according to the manufacturer's instructions.

2.7. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences, Version 16.0 (SPSS Inc., Chicago, IL, USA). The relationships between FoxM1 mRNA or protein expression and clinical and genetic characteristics were analyzed using the χ^2 test or Fisher's exact test (genetic groups were divided according to European LeukemiaNet [16]). After excluding patients without chemotherapy based on a DA regimen (daunorubicin 40–45 mg/m^2 per day on days 1–3 and cytarabine 100 mg/m^2 per day on days 1–7), the survival data for 37 patients were compared using the log-rank test and graphically presented in Kaplan–Meier curves.

The means of FoxM1 mRNA expression were compared using Student's *t*-test. Cell apoptosis rates in the AC220 or TST groups were compared with those of the AC220 + TST groups by the paired-sample *t*-test. *P* values <0.05 were considered statistically

Table 1
Primers sequences and PCR conditions used for RT-PCR.

Name	Sequence	PCR conditions
GAPDH-F	5′-CCACCCATGGCAAATTCCATGGCA-3′	Preheating at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 50 s; final extension at 72 °C for 10 min
GAPDH-R	5′-TCTAGACGGCAGGTCAGG-3′	
FoxM1-F	5′-CGAAAGATGAGTTCTGATGG-3′	Preheating at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, and extension at 72 °C for 45 s; final extension at 72 °C for 10 min
FoxM1-R	5′-GAAAGGTTGTGGCGGATG-3′	
FLT3-F	5′-TGTCGAGCAGTACTCTAAACA-3′	Preheating at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min, and extension at 72 °C for 2 min; final extension at 7 °C for 10 min
FLT3-R	5′-ATCCTAGTACCTTCCCAAACTC-3′	
NPM1-F	TGGAGGTGGTAGCAAGGTTC	Preheating at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 50 s; final extension at 72 °C for 10 min
NPM1-R	CTTCCTCCACTGCCAGACAGA	

significant. Drug synergism for AC220 and TST was determined by factorial analysis.

3. Results

3.1. FoxM1 is highly expressed in human acute myeloid leukemia cells

We first examined the mRNA expression of FoxM1, NPM1 mA, and FLT3-ITD in 120 bone marrow samples from AML patients (excluding M3) by reverse transcription–PCR. High expression of FoxM1 mRNA was observed in the AML samples (91/120, 75.83%). Immunohistochemical staining of 45 AML bone marrow biopsy samples was then performed to investigate FoxM1 protein expression. We observed high expression of the FoxM1 protein in 75.56% of the AML samples (34/45). The mRNA and protein expression of FoxM1 in the AML samples are partly shown in Fig. 1.

3.2. The high expression of FoxM1 may be related to adverse prognosis

We next determined the relationships between FoxM1 expression and clinical parameters (gender, age), FAB subtype, immunophenotype (by flow cytometry: CD13, CD33, CD34, CD117, HAL-DR, CD15, CD64, CD11b, CD11c, CD14, CD9, CD56, CD36, CD7, and MPO), cytogenetics (cytogenetic G-banding analysis), molecular findings (FLT3-ITD and NPM1 mA), and prognosis.

As Table 2 shows, the FoxM1 mRNA level was significantly correlated with gender, FLT3-ITD, and genetic groups, while FoxM1 protein expression was non-significantly correlated with FLT3-ITD and genetic groups.

The prognostic value of FoxM1 for overall survival in AML patients was evaluated by comparing the patients with high and low FoxM1 protein expression. A total of 37 AML patients treated with standard chemotherapy based on a DA regimen were included in the Kaplan–Meier survival analysis. AML patients with high FoxM1 expression had a slightly lower overall survival rate than those with low FoxM1 expression, but this difference was not significant (11.27 ± 1.16 vs. 18.86 ± 3.63, $P = 0.120$). However, as shown in Fig. 2, the difference in the overall survival rate between the two groups gradually increased as the follow-up time increased. Due to the study limitations of a small patient sample and short follow-up (range, 6–30 months), future studies with larger sample sizes and longer follow-up are needed to confirm this trend in the overall survival rate.

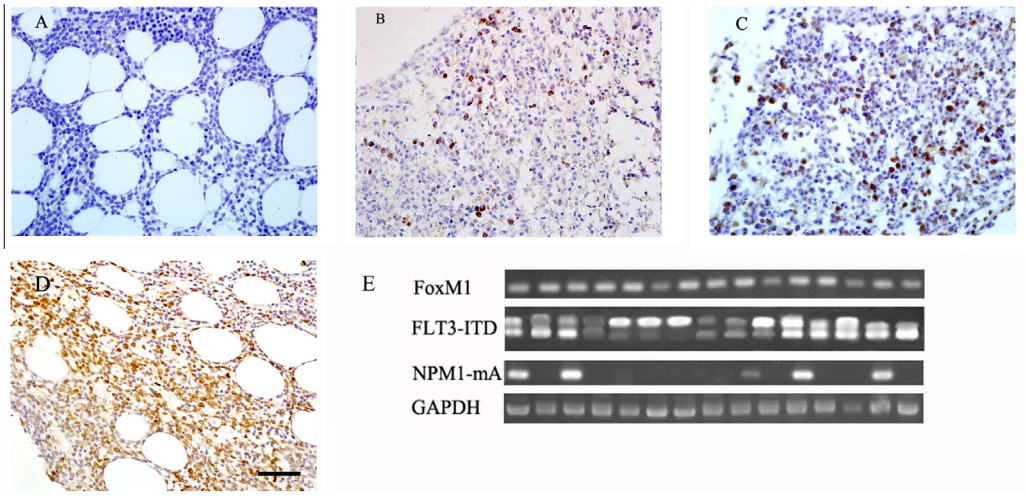

Fig. 1. FoxM1 expression in AML patients (excluding acute promyelocytic leukemia). In total, 45 bone marrow biopsy samples were stained by immunohistochemistry using an anti-FoxM1 antibody. The four images (A–D) show immunohistochemical analysis of FoxM1 protein expression: − in (A), + in (B), ++ in (C), and +++ in (D). Magnification, all ×400. Size bar (A–D), 100 μm. The mRNA expressions in 120 bone marrow samples from AML patients (excluding M3) were examined by RT-PCR, with primers for FoxM1, FLT3-ITD, NPM1-mA, and GAPDH (E). PCR products with an aberrant band indicated FLT3-ITD; otherwise, wild-type FLT3 was indicated. GAPDH was assayed as an internal control. AML: acute myeloid leukemia; FLT3-ITD: internal tandem duplication of the fms-like tyrosine kinase 3 gene; NPM1-mA: NPM1 mutation A; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Table 2
FoxM1 mRNA and protein expression in AML patients determined by RT-PCR and immunohistochemistry.

Variable	FoxM1-mRNA expression		FoxM1-protein expression	
	Positive	Negative	High	Low
Gender				
Male	47	21	18	7
Female	44	8	16	4
P-value	0.049*		0.532	
FLT3-ITD				
Positive	20	1	10	0
Negative	71	28	24	11
P-value	0.022*		0.089	
*Genetic groups***				
Favorable	18	13	6	5
Intermediate	56	16	19	5
Adverse	17	0	9	1
P-value	0.004*		0.147	

* $P < 0.05$.
** Genetic groups were divided according to European LeukemiaNet (2010); intermediate includes intermediate 1 and intermediate 2.

3.3. FLT3-ITD inhibition decreases the expression of FoxM1

Based on the significant association between FoxM1 expression and FLT-ITD indicated by the clinical data, we hypothesized that FoxM1 might be a downstream target of the FLT3–PI3K–AKT–FOXO pathway, similar to EGFR [2]. Therefore, we assessed FoxM1 expression in MV4–11 and THP1 cells after inhibition of FLT3-ITD using a selective FLT3-ITD inhibitor, AC220, or stimulation of FLT3 by FL.

As shown in Fig. 3A and B, qRT-PCR analysis demonstrated that FLT3-ITD inhibition significantly decreased the mRNA expression of FoxM1 in a time-dependent manner in MV4–11 cells compared with control THP1 cells (FoxM1 mRNA, THP1 vs. MV4–11: 12 h, 0.976 vs. 0.722, $P < 0.05$; 24 h, 1.077 vs. 0.334, $P < 0.05$; 48 h, 0.786 vs. 0.095, $P < 0.05$). Similar results were observed by western blot analysis (Fig. 3C). Next, we examined whether the stimulation of FLT3 by FL led to up-regulation of FoxM1 and downstream target mRNAs. As shown in Fig. 3A and B, the mRNA expression of FoxM1 mRNA was up-regulated in both MV4–11 and THP1 cells, although at different times (FoxM1 mRNA, THP1: 12 h, 0.867; 24 h, 1.740; 48 h, 1.019; MV4–11: 12 h, 2.564; 24 h, 1.094; 48 h, 1.152). The mRNA expressions of downstream targets (CylinB1 and Cdc25B) of FoxM1 had similar change trend (not shown). These results suggest that FoxM1 might be an important downstream target of FLT3/FLT3-ITD.

3.4. Thiostrepton induces apoptosis in both MV4–11 and THP1 cells

Our data indicate that FoxM1 is expressed in a large percentage of AML patients and is an important downstream target in FLT3-ITD AML cells. Finally, we determined whether FoxM1 could be a therapeutic target and whether TST has a synergistic effect with the FLT3-ITD inhibitor AC220. Apoptosis of MV4–11 and THP1 cells was measured by flow cytometry after culturing with increasing concentrations of AC220, TST, or AC220 + TST for 24 h. TST induced apoptosis of both MV4–11 and THP1 cells in a dose-dependent manner (Fig. 4), while AC220 had no apoptotic effect on THP1 cells (Fig. 4A). The apoptosis rate of 1 nmol/L AC220-treated MV4–11 cells peaked at 35.19 ± 8.06% (Fig. 4B). For MV4–11 cells, the apoptosis rate of the AC220 + TST group was significantly higher than that of the AC220 group or TST group (AC220 + TST vs. AC220 or TST, $P = 0.008$ or <0.001, respectively), while for THP1 cells, the apoptosis rate of the AC220 + TST group was slightly lower than that of the TST group (24.42% vs. 30.36%, $P = 0.035$). In addition, AC220 and TST had no synergistic effect ($P = 0.991$).

4. Discussion

AML has been demonstrated by previous studies to have high clinical and biological heterogeneity [17]. But up to now, there are a relatively small number of cytogenetic and molecular lesions which could be sufficiently characterized to influence clinical practice [18]. The prognostic relevance of cytogenetic and

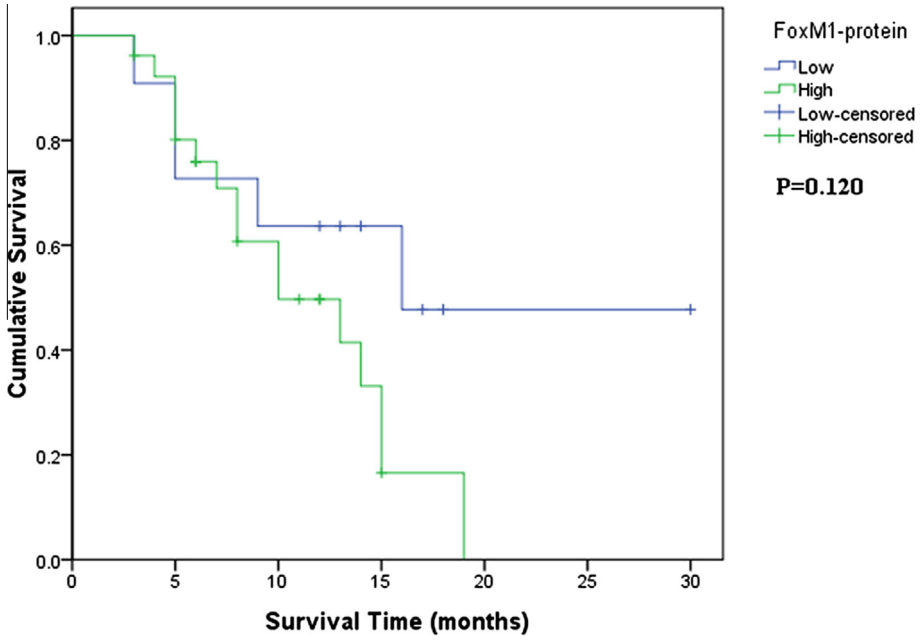

Fig. 2. Kaplan–Meier analysis of AML patients with high and low FoxM1 protein expression. Kaplan–Meier analysis of the overall survival of 37 AML patients treated with standard chemotherapy based on a DA regimen revealed that there was no significant difference between the high and low FoxM1 protein expression groups ($P = 0.120$), but as the follow-up time increased, the difference in the overall survival rate between the two groups gradually increased.

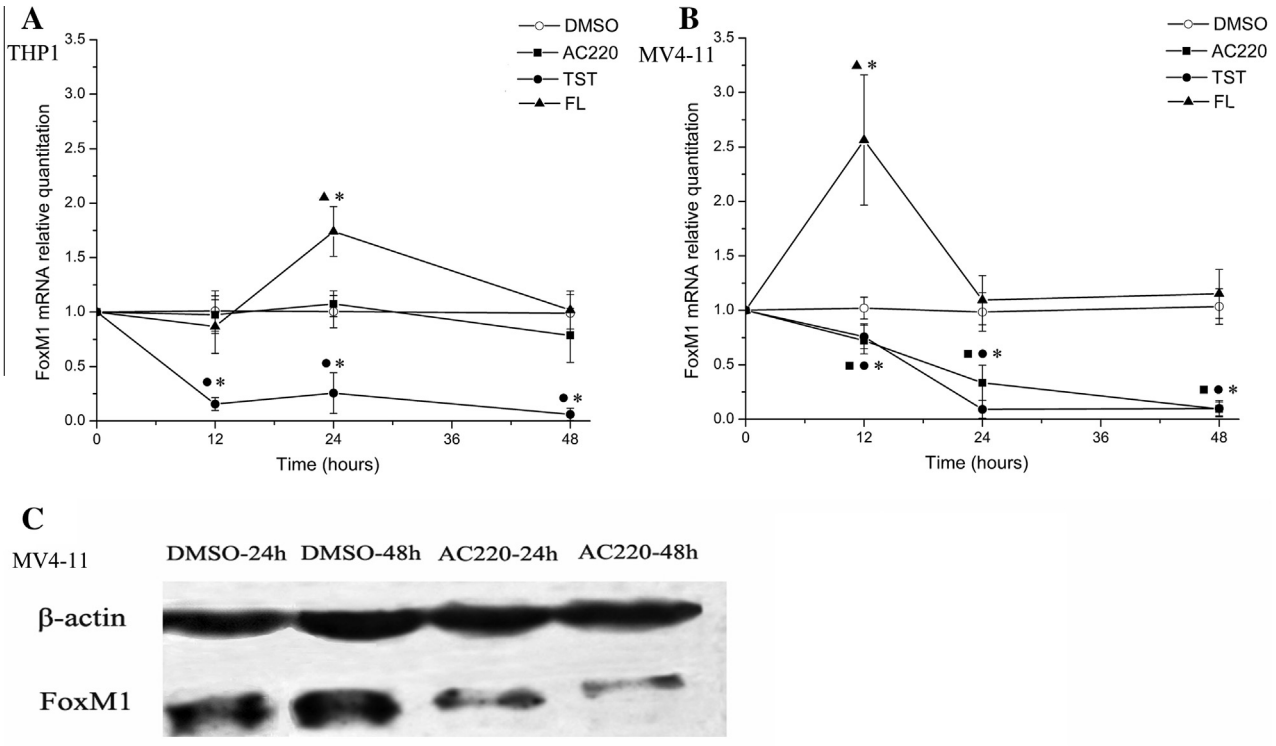

Fig. 3. Changes in the expression of FoxM1 in THP1 and MV4–11 cells. A total of 1×10^6 THP1 or MV4–11 cells were cultured with DMSO (control), 5 nmol/L AC220, 5 μmol/L TST, or 100 ng/mL FL for 12, 24, or 48 h. qRT-PCR was performed on FoxM1. A and B show the change in FoxM1 mRNA after THP1 (A) or MV4–11 cells (B) were cultured with AC220, TST, or FL for 12, 24, or 48 h. *: $P < 0.05$ was considered significant compared with the DMSO group. Western blotting (C) was used to detect FoxM1 protein expression in MV4–11 cells cultured with AC220 for 24 or 48 h (β-actin was the internal control). DMSO: dimethylsulfoxide; TST: thiostrepton; FL: FLT3 ligand.

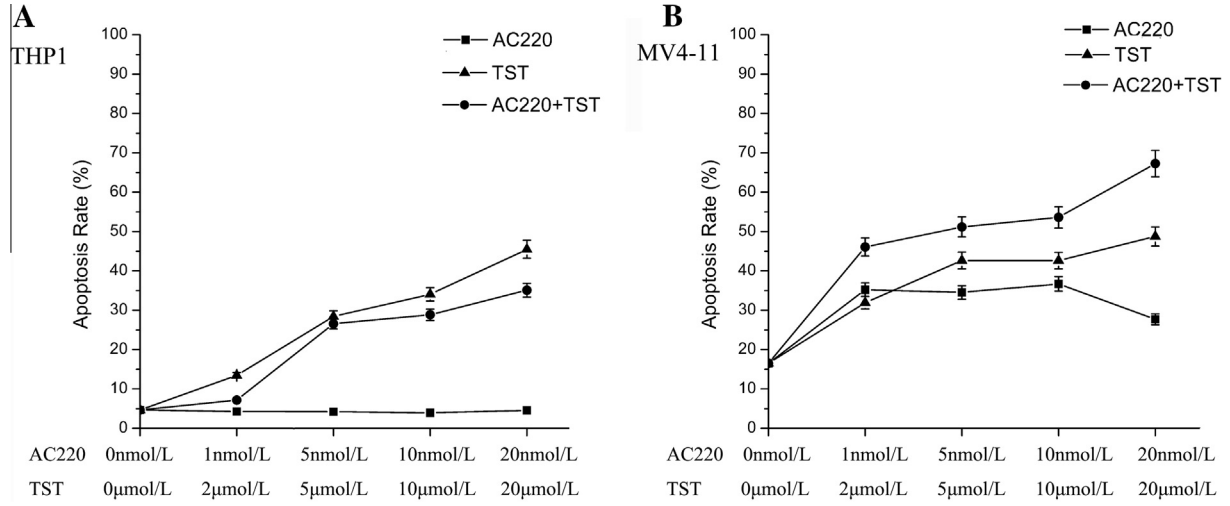

Fig. 4. TST induces apoptosis of both THP1 and MV4–11 cells. THP1 (A) and MV4–11 (B) cells were treated respectively with increasing concentrations of AC220, TST or AC220 + TST for 24 h. Their apoptosis rates were measured by PI-FACS analysis ($n = 2$, mean ± SEM). The apoptotic effect of TST and AC220 + TST on THP1 cells increased in a dose-dependent manner. The apoptotic effect of TST and AC220 + TST on MV4–11 cells increased in a dose-dependent manner, but the apoptotic effect of AC220 peaked at 1 nmol/L. The apoptosis rate was significantly higher in the AC220 + TST group than in the TST and AC220 groups, but AC220 and TST had no synergistic effect on the induction of apoptosis. TST: thiostrepton.

molecular lesions has been widely adopted to divide patients into 3 or 4 defined-risk genetic groups with significant differences in overall survival [16,19]. Although progress has been made in defining prognostic markers for AML, a substantial percentage of patients lack a specific abnormality of prognostic significance. In addition, there is considerable heterogeneity in the outcome for individual patients in each risk group. Although the identification of novel mutations with prognostic significance in AML has been the focus of intense research [20–22], the results are far from satisfactory and must be further validated by detailed clinical and mutational annotation in large, homogeneously treated cohorts of patients with AML.

Deregulation of FoxM1 has been implicated in the pathogenesis of many cancers because of its ability to drive cell cycle progression and prevent growth arrest [4]. Elevated FoxM1 expression has been implicated in carcinogenesis of tumor development in various

solid tumors. A few studies have examined the role of FoxM1 in leukemia. We observed high FoxM1 expression in AML samples; this high FoxM1 expression was correlated with FLT3-ITD, genetic groups, and possibly gender. However, the correlations of these factors with mRNA and protein expression differed, possibly due to insufficient bone marrow biopsy numbers and the lower accuracy of immunohistochemical staining compared to RT-PCR. However, immunohistochemical staining can be performed easily in basic hospitals and provides another means to evaluate AML prognosis. In addition, although the Kaplan–Meier survival analysis revealed that AML patients with high FoxM1 protein expression had overall survival rates similar to those with low FoxM1 protein expression, the difference in the overall survive rate between these two groups gradually increased with follow-up time. Based on these findings, the detection of FoxM1 by immunohistochemical staining of bone marrow biopsy samples might be a promising new prognostic marker in AML, independent of genetic analysis.

The standard induction regimen of AML is administration of anthracycline given for 3 days in combination with continuous infusion of cytarabine for 7 days (3 + 7). The complete remission (CR) rate achieved by 3 + 7 is approximately 70% in patients younger than 60. Numerous trials have sought to improve the rate and quality of CR, but important and consistent improvements in outcome have not been observed [23]. FLT3 is the only mutation identified thus far that can be pharmacologically targeted in AML, and clinical trials of FLT3 inhibitors have been ongoing for a decade. To date, more than 20 small-molecule inhibitors of FLT3 have been reported, of which 8 have been evaluated in clinical trials [13]. However, there is now compelling clinical evidence that resistance to FLT3 inhibitors may arise from the emergence of FLT3-ITD$^+$ clones carrying additional TKD mutations, like resistance to imatinib mesylate in CML [24].

In this study, FoxM1 expression was observed in nearly all samples from AML patients with FLT3-ITD. Inhibition of FLT3-ITD in an AML cell line led to the down-regulation of FoxM1, while stimulation of FLT3 led to the up-regulation of FoxM1. These findings suggest that FoxM1 is transcriptionally up-regulated downstream of FLT3/FLT3-ITD. However, the mechanisms by which FLT3/FLT3-ITD regulates FoxM1 expression and stimulation of MV4–11 and THP1 cell lines by FL induced up-regulation at different times remain to be fully elucidated. Because both FLT3 and EGFR/HER2 are receptor tyrosine kinases and FLT3/FLT3-ITD can phosphorylate the transcription factor FOXO3A via PI3K-AKT, we speculate that, like EGFR/HER2, FLT3/FLT3-ITD promotes the expression of FoxM1 via the PI3K–AKT–FOXO3A pathway. However, further studies are needed to confirm this hypothesis. We also have demonstrated that the addition of a FoxM1 inhibitor to AML cell lines with or without FLT3-ITD induces significant apoptosis in a dose-dependent manner, suggesting that FoxM1 might be a potential, promising therapeutic target in AML patients, particularly in patients with poor prognosis or FLT3-ITD.

The FoxM1 inhibitor TST [25] has been extensively studied in solid tumors. TST can cause breast cancer cells to undergo proliferative arrest and apoptosis and to become less migratory and invasive [25]. The exact mechanism of action of TST has not been defined, but it could be related to the ability of most thiazole antibiotics to function as proteasome inhibitors [26]. Notably, TST does not exert any anti-growth or apoptotic effects on untransformed cells, making TST an extremely attractive molecule for further development into specific therapeutics against cancer [25,26] and suggesting a new approach for AML treatment.

In summary, we identified relationships between FoxM1 and both poor prognosis and FLT3-ITD in AML, thereby indicating that FoxM1 is a potential prognostic marker and therapeutic target, particularly in poor-prognosis and FLT3-ITD AML patients. However, the small sample size and short follow-up are limitations of our study. Future large-cohort studies with extended follow-up should be performed to elucidate the relationship between FoxM1 and prognosis in AML.

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