Aberrantly expressed transcription factors C/EBP and SOX4 have positive effects in the development of chronic myeloid leukemia

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Abstract. The aim of the present study was to examine the expression and significance of CCAAT/enhancer binding protein α (C/EBPα) and SRY-related high mobility group box containing transcription factor 4 (SOX4) in chronic myeloid leukemia (CML). Bone marrow samples were collected from patients with CML, and peripheral blood mononuclear cells were collected from healthy controls. Protein and mRNA were extracted from the collected samples, and analyzed using western blotting and reverse transcription-quantitative polymerase chain reaction analyses, respectively. Spearman's method was used to evaluate the correlation between the expression levels of these two genes, with $P<0.05$ considered to indicate a statistically significant difference. A total of 79 patients, including 57 patients with newly diagnosed CML and 22 patients treated with imatinib therapy, and 30 controls were enrolled. The expression of SOX4 was upregulated in the patients with CML, whereas the expression of C/EBPα was downregulated ($P<0.05$). However, no differences were observed among the chronic, accelerated and blastic CML phases, respectively ($P>0.05$). In addition, no associations were found between the changes in expression and age, gender, white blood cells or the expression of breakpoint cluster region/abelson in patients ($P>0.05$). However, the expression of SOX4 was negatively correlated with the expression of C/EBPα ($P<0.01$). Following imatinib treatment, the expression of SOX4 was downregulated in the progression-free patients, but upregulated in the blastic phase patients, whereas the expression of C/EBPα showed the opposite trend. Therefore, C/EBPα and SOX4 were important and negatively associated with the process of CML, and the C/EBPα-SOX4 axis may be a novel potential therapeutic target for the treatment of CML.

Introduction

Chronic myeloid leukemia (CML) is a malignant myeloproliferative disorder characterized by reciprocal chromosomal translocation between chromosomes 9 and 22, which leads to the formation of the breakpoint cluster region-abelson (BCR/ABL) oncoprotein with constitutively active tyrosine kinase (1). According to its progression, CML can be divided into three phases: Chronic phase (CP), accelerated phase (AP) and blastic phase (BP), and the majority of cases are diagnosed in the CP (2). It has been demonstrated that, following allogeneic cell transplantation, the survival rate of patients with CML is significantly prolonged and may be cured; however, this treatment is only suitable for young patients with CP CML and fully matched human leukocyte antigen donors (3), whereas the mean age of diagnosis of CML is 64 years old (4). Due to the specific characteristic of CML, tyrosine kinase inhibitors targeting BCR/ABL have been developed, including imatinib. However, drug resistance has been found in patients with CML (5). To date, CML accounts for 15% of all cases of leukemia and affects 1/100,000 individuals per year in Western countries (6). The prevalence of this disease is likely to increase in the future; therefore, it is essential to improve current understanding of the pathogenesis of CML.

Previous studies have demonstrated that the investigation of transcription factors is a useful and crucial method for understanding the mechanism of diseases. CCAAT/enhancer binding protein α (C/EBPα) is a prototypical basic region-leucine zipper transcription factor, which acts as an oncogene in several types of cancer (7). C/EBPα has been reported to be involved in acute lymphoblastic leukemia (ALL) (8). Several studies have demonstrated that C/EBPα can regulate genes, which are key to cell differentiation in AML, and can inhibit cell cycle and

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Abbreviations: C/EBPα, CCAAT/enhancer binding protein α; CML, chronic myeloid leukemia; CP, chronic phase; AP, accelerated phase; BP, blastic phase; ALL, acute lymphoblastic leukemia; SOX4, SRY-related high mobility group box containing transcription factor 4; CML-CP, chronic phase CML; CML-AP, accelerated phase CML; CML-BP, blastic phase CML; WBC, white blood cell; PB, peripheral blood; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

Key words: chronic myeloid leukemia, SRY-related high mobility group box containing transcription factor 4, CCAAT/enhancer binding protein α, target therapy

apoptosis (9,10). Zhang *et al* revealed that SRY-related high mobility group box containing transcription factor 4 (SOX4) is a direct downstream target and important mediator of C/EBPα in ALL (11), however, its mechanism in CML remains not to be elucidated. SOX4, which belongs to the SoxC class of transcription factors, primarily contributes to regulation of the proliferation and survival of mesenchymal and neural progenitors (9), B and T cell maturation (12), cardiac outflow formation and myeloid differentiation (13,14). The aberrant expression of SOX4 in adult tissues has been linked to the occurrence and progression of cancer in humans and mice, and the overexpression of SOX4 is usually found in the majority of types of cancer, including bladder, hepatic, lung, gastric, prostate and hematopoietic cancer (15,16). Ramezani-Rad *et al* demonstrated that SOX4 can act as a central mediator to enable oncogenic survival signals via phosphoinositide 3-kinase (PI3k)/AKT and mitogen-activated protein kinase (MAPK) signaling, which results in a poor clinical outcome in ALL (17).

In the present study, in order to investigate changes in SOX4 and C/EBPα in CML, 79 patients with CML were enrolled. The expression of SOX4 and C/EBPα were compared between patients with CML and healthy controls, and the expression prior to and following imatinib treatment were compared in patients with CML. The aims of these investigations were to provide novel insight into CML targeted therapy.

Materials and methods

Patients and study design. Between January 2014 and October 2015, a total of 79 patients with CML, confirmed by morphology, immunology, cytogenetics and molecular biology (MICM) were enrolled at Yantai Yuhuangding Hospital Affiliated to Qingdao University Medical College (Yantai, China) for the present study. Among these, 57 patients had primary CML and had not received treatment. The male/female ratio of these patients was 31/26 and their median age was 56 years old. Based on the diagnostic results, the CML stages were confirmed as follows: 41 cases of newly diagnosed chronic phase CML (CML-CP), six cases of accelerated phase CML (CML-AP), 10 cases of blastic phase CML (CML-BP). Of the 79 patients, 22 patients were treated with imatinib, all of which had CML-CP. In addition, the white blood cell (WBC) levels and the expression levels of BCR/ABL in the patients with CML were determined. The peripheral blood (PB) mononuclear cells of 30 healthy individuals were also collected and used as controls. The Ethics Committee of Qingdao University Medical College authorized the present study and all patients provided signed informed consent.

Inclusion and exclusion criteria. Patients were enrolled if they met the following criteria: i) confirmation by MICM; ii) no other types of cancer or severe functional disease present; ii) no other blood disease present; iv) newly diagnosed and untreated, with the exception of those treated with imatinib; v) voluntarily participation and ability to complete treatment. Patients were excluded if they were <18 years of age or if their clinical information was incomplete.

Imatinib treatment. To receive imatinib therapy, patients were required to meet the following terms: i) Philadelphia chromosome-positive on bone marrow chromosome cultivation; ii) BCR/ABL mutant positive; iii) T315I mutant negative. The specification of imatinib in this study was 100 mg per tablet. CML-CP and CML-AP patients orally received 4 tablets of imatinib once a day (400 mg/day), and CML-BP patients orally received 6 tablets of imatinib once a day (600 mg/day). During treatment, all patients were required to follow the advice of their doctor and underwent hematological examination every week, with genetic diagnosis and molecular diagnosis every 3 months. The observation period was adjusted until significant blood, genetic or molecular activity was present, and the drug was present from the initiation of treatment. Bone marrow samples were collected from the patients at initial diagnosis and 3 months following imatinib treatment.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. To determine the mRNA levels of C/EBPα and SOX4, lymphocyte separation medium (Tianjin Hao Yang Biological Manufacture Co., Ltd., Tianjin, China) was used to separate mononuclear cells from the bone marrow and PB samples. TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to extract RNA from the separated mononuclear cells and an Easy Taq® PCR kit (Beijing Transgen Biotech Co., Ltd., Beijing, China) was used for cDNA production. Primers of SOX4, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), C/EBPα and β-actin were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. (Table I), and the RT-PCR kit was purchased from Applied Biosystems; Thermo Fisher Scientific, Inc. The reactive sample for SOX4 was as follows: 10 μl 2X Mix, 0.5 μl each primer (10 μmol/l), 1.0 μl cDNA, and double distilled water to 20 μl; and the reactive conditions were as follows: 95˚C for 10 min; 39 cycles of 95˚C for 31 sec, 60˚C for 1 min; 65˚C for 31 sec; and 72˚C for 10 min. The reactive sample for C/EBPα was as follows: 10.5 μl 2X Mix, 0.5 μl each primer (10 μmol/l), 1.0 μl cDNA and double distilled water to 25 μl; and the reactive conditions were as follows: 94˚C for 5 min; 35 cycles of 94˚C for 45 sec; 6˚C for 45 sec; 72˚C for 30 sec; and 72˚C for 10 min. The reactive systems of GAPDH and β-actin were as follows: 10 μl 2X Mix, 0.5 μl each primer (10 μmol/l), 1.0 μl cDNA and double distilled water to 20 μl; and the reactive conditions were as follows: 94˚C for 5 min; 35 cycles of 94˚C, 30 sec; 55˚C for 30 sec; 72˚C for 1 min; and 72˚C for 10 min. Amplification was performed on a ABI 7500 PCR equipment (Applied Biosystems, USA), and the recorded data were subjected to statistical analysis to analyze the relative expression levels of the genes using $2^{-\Delta\Delta Cq}$ method (18). GAPDH was used as the internal reference for SOX4 and β-actin was used as the internal reference for C/EBPα.

Western blot analysis. In order to investigate the protein levels of C/EBPα and SOX4, the separated mononuclear cells were dissociated in protein lysis buffer to extract proteins, and the protein concentrations were measured using the bicinchoninic acid method (Thermo Fisher Scientific, Inc.). The electrophoresis of proteins (20 ng) was performed on a 10% SDS-PAGE gel and then transferred onto a nitrocellulose membrane. Following blocking with 5% skim milk for 2 h, the nitrocellulose membrane was incubated with rabbit anti-human SOX4 (cat. no. ab85204; 1:500; Abcam, Cambridge, MA,

Table I. Primers and forecasted product sizes of genes detected using reverse transcription-quantitative polymerase chain reaction analysis.

Gene	Primer	Product size (bp)
SOX4 (NM_003107.2)	F: 5'-TTCAGCAACCAGCATTC-3' R: 5'-TCCCTCTCTCTCGCTCTCTC-3'	104
GAPDH (NM_001289746.1)	F: 5'-CCCACTCCTCCACCTTTGAC-3' R: 5'-ATGAGGTCCACCACCCTGTT-3'	115
β-actin (NM_001101.3)	F: 5'-GATCTGGCACCACACCTTCTAC-3' R: 5'-AGGCATACAGGGACAGCACA-3'	182
C/EBPα (NM_004364.4)	F: 5'-CACCGCTCCAATGCCTAC-3' R: 5'-CCCATCGCAGTGAGTTCCG-3'	372

SOX4, SRY-related high mobility group box containing transcription factor 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; C/EBPα, CCAAT/enhancer binding protein α; F, forward; R, reverse.

USA) or rabbit anti-human GAPDH (cat. no. ab9485; 1:1,000; Abcam) at 4˚C overnight and then washed three times with phosphate-buffered saline with Tween-20 (PBST). The nitrocellulose membrane was then incubated with goat anti-rabbit IgG (H+L)-HRP (cat. no. ab6721; 1:5,000; Abcam) at room temperature for 1 h and washed with PBST three times. Finally, an electro-chemiluminescence method was used to reveal the protein bands and Quantity One software (version 4.6.3; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was utilized to analyze the gray values of protein bands.

Statistical analysis. Statistical analysis was performed using SPSS software (version 19.0; IBM SPSS, Armonk, NY, USA). Comparison of measurement data was performed using Student's t-test and the results are expressed as the mean ± standard deviation. The comparison of enumeration data was performed using a χ^2 test, and the correlation between two samples in patients with primary CML was determined using Spearman's method. P<0.05 was considered to indicate a statistically significant difference.

Results

C/EBPα in patients with primary CML. Based on the results of the RT-qPCR, the relative mRNA level of C/EBPα was significantly lower in the patients with primary CML, compared with that in the control group (0.253±0.034, vs. 0.811±0.0563; P<0.01; Fig. 1).

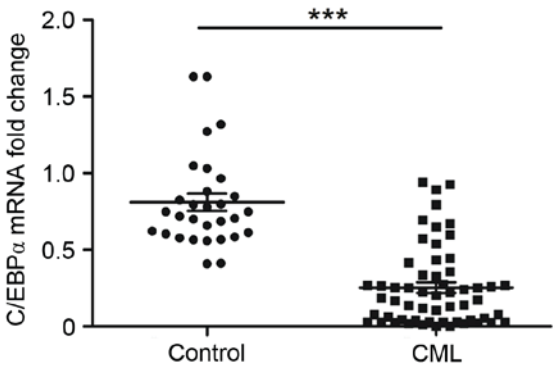

Figure 1. mRNA expression of C/EBPα detected using reverse transcription-quantitative polymerase chain reaction analysis. The mRNA expression of C/EBPα was significantly lower in patients with CMR, compared with that in healthy controls. ***P<0.001 compared with the control group. CML, chronic myeloid leukemia; C/EBPα, CCAAT/enhancer binding protein α.

Associations between the expression of C/EBPα and gender, age, WBC and BCR/ABL in primary CML. To further elucidate the associations between the expression of C/EBPα and known factors, including gender, age, WBC levels and expression levels of BCR/ABL, the patients with primary CML were divided into two groups according to C/EBPα/β-actin<0.5, which was shown in all patients in the control group, and Pearson's χ^2 test was performed. The results showed no significant association between the expression of C/EBPα and gender (P>0.05). In addition, no significant association was found between the expression of C/EBPα and age when the patients were separated into two groups by their median age of 51.5 years (P>0.05). No significant association was identified between the expression of C/EBPα and WBC levels when the patients were divided into two groups by WBC>$1x10^{10}$ (P>0.05). In addition, no significant association was found between the expression of C/EBPα and BCR/ABL when the patients were divided into two groups according to the expression of BCR/ABL (P>0.05). The above results indicated that there were no significant associations between the expression of C/EBPα and gender, age, WBC levels or the expression of BCR/ABL in primary CML (Table II).

Changes in the expression of C/EBPα in primary CML following imatinib treatment. Among the enrolled patients, a total of 22 CML patients accepted imatinib treatment. Following treatment, no improvements in condition were observed in 13 patients with CML, however, the expression of C/EBPα was significantly increased, compared with that prior to treatment (0.314±0.0565, vs. 0.111±0.0242; P<0.01; Fig. 2A). However, following treatment with imatinib, the expression of C/EBPα remained lower than that in the control group (P<0.05). In addition, nine cases of CML transformed into BP CML and the expression of C/EBPα was significantly

Table II. Correlations between the expression of C/EBPα and gender, age, WBCs and BCR/ABL.

Index	Group	C/EBPα/β-actin <0.5	C/EBPα/β-actin ≥0.05	χ^2	P-value
Gender	Male	27	6	0.063	0.802
	Female	19	5		
Age (years)	≤51.5	22	7	1.774	0.183
	>51.5	25	3		
WBCs (n)	$\leq 1x10^{10}$	11	0	2.900	0.089
	$>1x10^{10}$	36	10		
BCR/ABL (%)	≤100	25	4	0.574	0.449
	>100	22	6		

C/EBPα, CCAAT/enhancer binding protein α; WBCs, white blood cells; BCR/ABL, breakpoint cluster region-abelson.

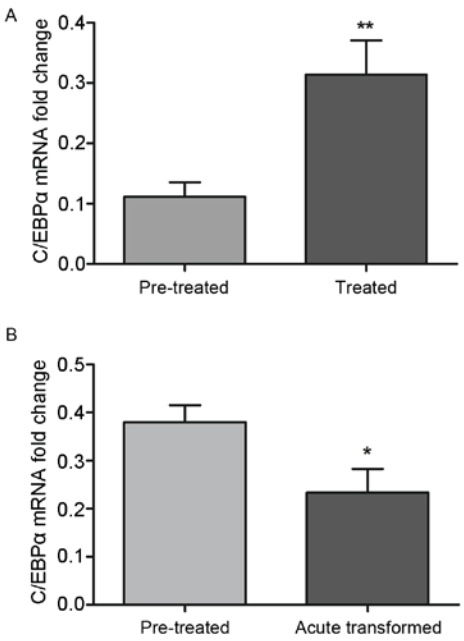

Figure 2. mRNA expression levels of C/EBPα prior to and following imatinib treatment. Following treatment with imatinib, the mRNA expression of C/EBPα was (A) significantly increased in 13/22 patients with progression-free CML and (B) decreased in 9/22 patients with CML transformed into the blastic phase. *P<0.05 and **P<0.01 compared with the pre-treated group. CML, chronic myeloid leukemia; C/EBPα, CCAAT/enhancer binding protein α.

decreased following treatment, compared with the control (0.234±0.0493, vs. 0.3798±0.0356; P<0.05; Fig. 2B).

SOX4 in patients with primary CML. According to the results of the RT-qPCR analysis, the mRNA level of SOX4 in the patients with primary CML was significantly higher, compared with that in the control group (6.546±1.495, vs. 0.0596±0.0187; P<0.01; Fig. 3A). Further subgroup investigation found no significant differences in the mRNA levels of SOX among the CML-CP, CML-AP and CML-BP patient groups (P>0.05; Fig. 3B). The protein level of SOX4 was also determined using western blot analysis. Similar to the mRNA levels, the protein expression of SOX4 in the primary CML group was significantly higher, compared with that in the control group (P<0.05; Fig. 3C and D).

Associations between the expression of SOX4 and gender, age, WBC and BCR/ABL in primary CML. To investigate the associations between the expression of SOX4 and known factors, including gender, age, WBC levels and expression levels of BCR/ABL, the patients with primary CML were divided into two groups according to SOX4/GAPDH <1, which was shown in all patients in the control group. Pearson's χ^2 test was performed, and the results revealed there was no significant association between the expression of SOX4 and gender (P>0.05). In addition, no significant association was found between the expression of SOX4 and age when the patients were separated into two groups by the median age of 51.5 years (P>0.05). In addition, no significant association was observed between expression of SOX4 and WBC levels when the patients were divided into two groups by WBC $>1x10^{10}$ (P>0.05). There was also no significant association between the expression of SOX4 and BCR/ABL when the patients were divided into two groups according to the expression of BCR/ABL (P>0.05). The above data suggested that there were no significant associations between the expression of SOX4 and gender, age, WBC levels or the expression levels of BCLR/ABL in primary CML (Table III).

Changes in the expression of SOX4 in primary CML following imatinib treatment. As with C/EBPα, the expression of SOX4 was also detected following imatinib treatment. The expression of SOX4 was significantly decreased following treatment, compared with expression prior to treatment, in the 13 patients with CML who were treated with imatinib (0.601±0.315, vs. 2.669±0.758; P<0.05; Fig. 4A). However, following treatment with imatinib, the expression of SOX4 was higher, compared with that in the control group (P<0.05). The remaining nine patients with CML developed BP CML following treatment, and the expression of SOX4 was significantly increased compared with the expression prior to treatment (0.648±0.157, vs. 0.128±0.0338; P<0.01; Fig. 4B).

Correlation between the expression of SOX4 and C/EBPα. To further determine the relevance of the expression of SOX4 and C/EBPα, Spearman's correlation analysis was performed in the 57 cases of primary CML. The analytical outcome revealed that the expression of SOX4 was significantly negatively

Table III. Correlations between the expression of SOX4 and gender, age, WBCs and BCR/ABL.

Index	Group	SOX/GAPDH <1	SOX/GAPDH ≥1	χ^2	P-value
Gender	Male	15	16	0.211	0.646
	Female	11	15		
Age (years)	≤51.5	15	14	0.888	0.346
	>51.5	11	17		
WBCs (n)	$\leq 1x10^{10}$	3	9	2.604	0.107
	$>1x10^{10}$	23	22		
BCR/ABL (%)	≤100	12	18	0.805	0.370
	>100	14	13		

SOX4, SRY-related high mobility group box containing transcription factor 4; WBCs, white blood cells; BCR/ABL, breakpoint cluster region-abelson.

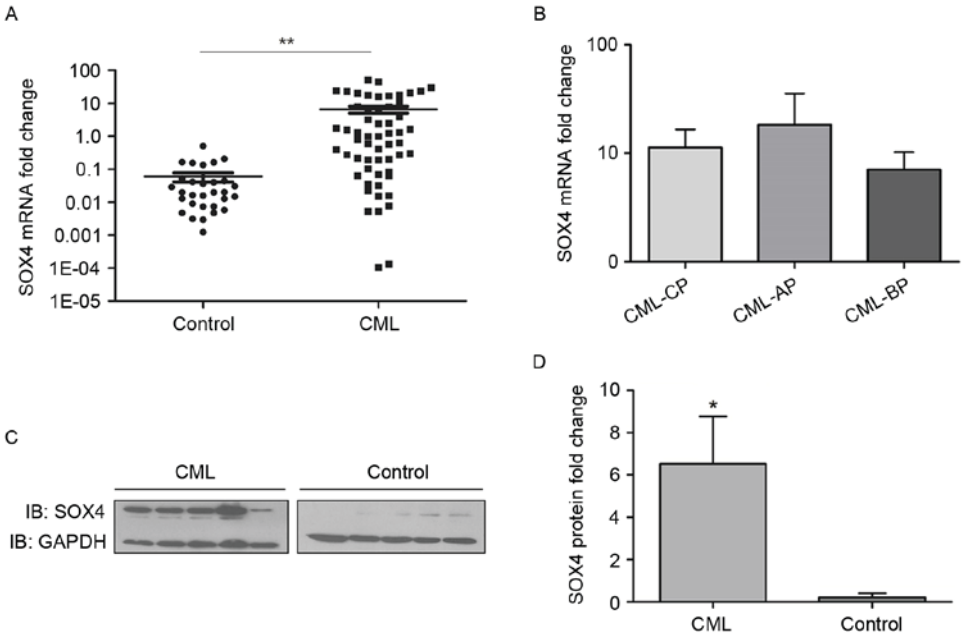

Figure 3. Expression of SOX4 in healthy controls and patients with CML. The results of the reverse transcription-quantitative polymerase chain reaction analysis showed that (A) the mRNA expression of SOX4 was higher in patients with CML, compared with that in healthy controls. (B) No significant difference in SOX4 was observed among patients with CML-CP, CML-AP and CML-BP. (C) Protein expression of SOX4 was increased in patients with CML, compared with that in healthy controls. (D) Quantification of protein expression revealed a significant difference. *P<0.05 and **P<0.01 compared with the control group. CML, chronic myeloid leukemia; CP, chronic phase; AP, accelerated phase; BP, blastic phase; SOX4, SRY-related high mobility group box containing transcription factor 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

correlated with the expression of C/EBPα in patients with CML (Fig. 5).

Discussion

CML is a myeloproliferative disorder originating from hematopoietic stem cells with constitutive expression of the BCR/ABL oncoprotein (19). Imatinib was the first target drug approved by the US Food and Drug administration for the treatment of CML, and remains a commonly used drug in clinical therapy (20). In the present study, C/EBPα was found to be markedly downregulated in patients with CML, compared with healthy controls, whereas the opposite was found for SOX4. The changes in the expression of C/EBPα and SOX4 were not associated with the age, gender, WBC level or the expression of BCR/ABL. Following treatment with imatinib, the expression of C/EBPα was increased and the expression of SOX4 was decreased in 13/22 patients with CML, which were progression-free. The opposite was observed in the expression levels of C/EBPα and SOX4 in the remaining nine patients with CML, which developed into CML-BP. In addition, the relative expression analysis showed that the expression of C/EBPα was negatively correlated with that of SOX4 in CML.

Myelopoiesis is the process by which myeloid progenitor cells differentiate into myeloid cells, including eosinophils, monocytes and granulocytes. C/EBPα is crucial in addition to other myeloid transcription factors in this process (21). A previous study reported that mutation of the C/EBPα gene was present in

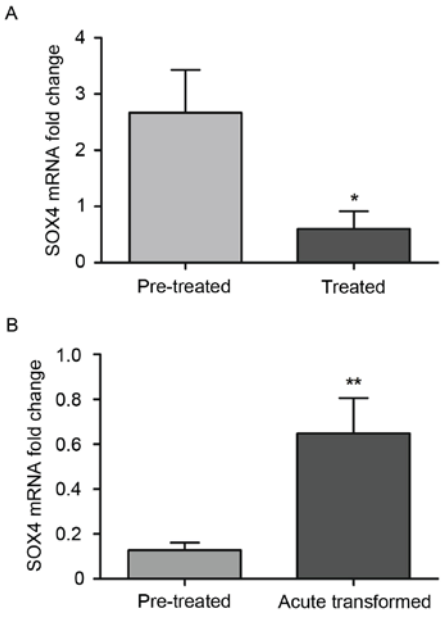

Figure 4. mRNA expression levels of SOX4 prior to and following imatinib treatment. Following treatment with imatinib, the mRNA expression of SOX4 was (A) significantly decreased in 13/22 patients with progression-free CML and (B) significantly increased in 9/22 of patients with CML transformed into the blastic phase. *P<0.05 and **P<0.01 compared with the pre-treated group. CML, chronic myeloid leukemia; SOX4, SRY-related high mobility group box containing transcription factor.

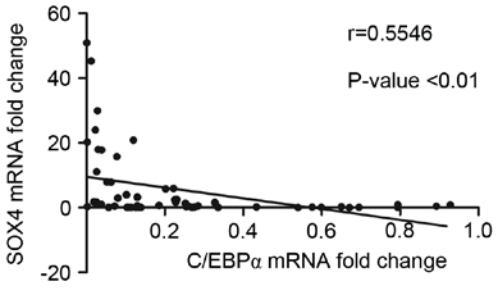

Figure 5. Correlation in the expression of C/EBPα and SOX4 in CML. Based on Spearman's correlation, a significant negative correlation was identified between the expression of C/EBPα and the expression of SOX4 in patients with CML. CML, chronic myeloid leukemia; C/EBPα, CCAAT/enhancer binding protein α; SOX4, SRY-related high mobility group box containing transcription factor 4.

~5-14% of patients with AML (10). The mutation or deletion of C/EBPα may result in arrest of the transit of common myeloid progenitors into granulocyte-monocyte progenitors, and lead to a reduction in granulocytes and monocytes (22). In addition, deficiency of C/EBPα in mice may induce disorder in myeloproliferation (19). In the present study, significantly lower expression of C/EBPα was detected in patients with CML, compared with that in normal controls. Epigenic modification is recognized as an important mechanism in regulating the expression of specific genes, which are associated with leukemogenesis (23), and Chim *et al* (24) reported that C/EBPα was hypermethylated in its promoter in patients with AML. Therefore, methylation may cause the downregulation of C/EBPα. This was confirmed by Annamaneni *et al* (25), who reported that the aberrant methylation of the C/EBPα promoter is a common event in CML. Theil *et al* (26) demonstrated that EZH2 acts as an oncogene to improve the methylation of C/EBPα, and thus inhibit myeloid differentiation. Ubiquitination is another essential mechanism for the downregulation of C/EBPα. Trib1 and 2 are two identified Tribbles family members, which can act as adapters to recruit E3 ligases to mediate ubiquitin degradation and inactivation (27). Therefore, the downregulation of C/EBPα may be a result of methylation and ubiquination, and this change may be crucial during the process of CML.

SOX4 has been reported to be directly regulated by C/EBPα, and to be important in the normal differentiation of myeloid and lymphoid lineages (14). It has been shown that C/EBPα can suppress the expression of SOX4 via directly binding to its promoter, and the transition of leukemia caused by C/EBPα mutation can be partially reversed by downregulating SOX4 (11,28). The present study also revealed significantly upregulated mRNA and protein expression levels of SOX in patients with CML, compared with levels in normal controls, and SOX4 was negatively correlated with C/EBPα. This suggested that the upregulated mRNA and protein levels of SOX4 may have been caused by the downregulation of C/EBPα. Aue *et al* (29) demonstrated that the overexpression of SOX4 induces myeloid leukemia via cooperating with the haplo-insufficiency of PU.1, which is an important regulator of the proliferation and differentiation of hematopoietic stem cells. The overexpression of SOX4 can inhibit the differentiation of myeloid progenitor cells (30). Ramezani-Rad *et al* (17) revealed that SOX4 activates the PI3K/AKT and MAPK signaling pathways to enhance survival signaling, and these signaling pathways are required for the survival, progression and proliferation of pre-B ALL. However, whether this signaling pathway is associated with CML remains to be elucidated and further investigations are required. Taken together, these results indicate that aberrant hypermethylation of the C/EBPα promoter may lead to the downregulation of C/EBPα, and this downregulation may have a positive effect on the expression of SOX4. The resulting excessive expression of SOX4 may lead to the dys-proliferation of myeloid and lymphoid lineages, and result in the occurrence and development of CML. In the present study, this molecular pathway was referred to as the C/EBPα-SOX4 axis.

To further elucidate the correlation between the C/EBPα-SOX4 axis and BCR/ABL, comparisons were made in 22 CML patients who received imatinib treatment. The results showed that the expression of SOX4 was decreased in the progression-free patients, but increased in the acute transformed patients. By contrast, the expression of C/EBPα was increased in the progression-free patients, but decreased in the acute transformed patients following treatment. Although the expression levels of C/EBPα and SOX4 in 13 cases of CML improved, the stages of the patients remained unchanged or worsened. It is known that imatinib is a BCR/ABL-targeting drug, and the conditions of patients with CML can be improved following therapy (31). However, the diagnostic results were not in accordance with this. This indicates that the signaling pathway of the C/EBPα-SOX4 axis involved in CML was not in accordance with the signaling pathway of BCR/ABL. In addition, the analyses of individual factors demonstrated that the expression levels of C/EBPα and SOX4 were not correlated with the expression of BCR/ABR. Therefore, the C/EBPα-SOX4 axis may be a novel therapeutic target in CML, differing from the BCR/ABL target.

Another change in gene expression following imatinib treatment requires mention. Following treatment for 3 months, the expression of C/EBPα was significantly increased, compared with the expression prior to treatment. However, the mean level remained lower than that in the healthy controls. Although the expression of SOX4 was decreased, the mean level remained higher than that in the healthy controls. These results may indicate that SOX4 and C/EBPα served critical roles in the treatment of CML with imatinib. However, due to the short treatment duration, the overall condition of the patients remained below that in the healthy controls. Therefore, long-term evaluations of C/EBPα and SOX4 require consideration in subsequent investigations.

In conclusion, the C/EBPα-SOX4 axis was found to be important in the process of CML. Due to methylation, the expression of C/EBPα was downregulated in CML and this downregulation induced an upregulation in the expression of SOX4. However, these changes were not correlated with the expression of BCR/ABL. Therefore, the C/EBPα-SOX4 axis may be a novel therapeutic target for the treatment of CML.

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