

SOX30 methylation correlates with disease progression in patients with chronic myeloid leukemia

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Ting-Juan Zhang^{1-3,*}
Xiang-Mei Wen^{2-4,*}
Jing-Dong Zhou^{1-3,*}
Yu Gu¹⁻³
Zi-Jun Xu²⁻⁴
Hong Guo²⁻⁴
Ji-Chun Ma²⁻⁴
Qian Yuan¹⁻³
Qin Chen²⁻⁴
Jiang Lin²⁻⁴
Jun Qian¹⁻³

¹Department of Hematology, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China; ²Zhenjiang Clinical Research Center of Hematology, Zhenjiang, Jiangsu, People's Republic of China; ³The Key Lab of Precision Diagnosis and Treatment of Zhenjiang City, Zhenjiang, Jiangsu, People's Republic of China; ⁴Laboratory Center, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jun Qian
Department of Hematology, Affiliated People's Hospital of Jiangsu University, 8 Dianli Road, Zhenjiang 212002, People's Republic of China
Fax +86 5 118 523 4387
Email qianjun0007@hotmail.com

Jiang Lin
Laboratory Center, Affiliated People's Hospital of Jiangsu University, 8 Dianli Road, Zhenjiang 212002, People's Republic of China
Fax +86 5 118 523 4387
Email 2651329493@qq.com

Background: Our previous study has reported that aberrant *SOX30* methylation was associated with poor prognosis in AML, and it correlated with disease progression in MDS. Herein, we further determined *SOX30* methylation and its clinical significance in the other myeloid malignance – chronic myeloid leukemia (CML).

Methods: *SOX30* methylation was examined by real-time quantitative methylation-specific PCR and bisulfite sequencing PCR, whereas *SOX30* expression was detected by real-time quantitative PCR.

Results: *SOX30* methylation was identified in 11% (10/95) CML patients. *SOX30* methylation was associated with lower hemoglobin and platelets ($P=0.006$ and 0.032 , respectively). Importantly, significant differences were observed in the distributions of clinical stages and cytogenetics ($P=0.006$ and 0.002 , respectively). The frequency of *SOX30* methylation in chronic phase (CP) stage occurred with lowest frequency (4/74, 5%), higher in accelerated phase (AP) stage (1/7, 14%), and the highest in blast crisis (BC) stage (12/31, 39%). In addition, *SOX30* methylated patients tended to have a higher level of *BCR-ABL* transcript than *SOX30* non-methylated patients ($P=0.063$). In two paired CML patients, *SOX30* methylation showed lower density in CP stage (19% and 17%, respectively), and was significantly increased in BC stage (89% and 69%, respectively) during disease progression. Additionally, *SOX30* methylated CML patients presented a lower *SOX30* transcript level than *SOX30* non-methylated CML patients ($P=0.046$).

Conclusion: Our study revealed that *SOX30* methylation correlated with disease progression in chronic myeloid leukemia.

Keywords: *SOX30*, methylation, chronic myeloid leukemia, progression

Introduction

Chronic myeloid leukemia (CML) was the first human cancer to be associated with a consistent chromosomal abnormality, namely the Philadelphia (Ph) chromosome, which generates from a reciprocal translocation between chromosome 9 and 22.¹ In its natural history, CML is usually diagnosed in chronic phase (CP) and then progresses through an accelerated phase (AP) to a nearly invariably fatal blast crisis (BC).¹ Without effective treatment, CML patients in CP progress to advanced stages, the prognosis for which is poor.¹ However, until now, the underlying mechanisms of CML progression are varied and not entirely understood. So far the best characterized includes the accumulation of molecular and chromosomal abnormalities.^{2,3} Recently, aberrant DNA methylation through the activation of

tumor suppressor genes playing a role in the progression of CML has been aroused great attention.⁴

SOX30, a relatively new member of the SOX family, encodes transcription factor belonging to the high mobility group (HMG) superfamily.⁵ It has been considered to be involved in spermatogonial differentiation and spermatogenesis.⁶ For years, the potential role of *SOX30* in human cancers remains poorly defined. Recently, *SOX30* has been identified as a key tumor suppressor gene mediated by promoter methylation in tumorigenesis including lung cancer, acute myeloid leukemia (AML), and myelodysplastic syndromes (MDS).^{7,8} In clinics, *SOX30* had a favorable prognostic impact on lung adenocarcinoma patients.⁹ Moreover, aberrant *SOX30* methylation was associated with poor prognosis in AML, whereas it correlated with disease progression in MDS.⁸ In this study, we further determined *SOX30* methylation and its clinical significance in CML.

Patients and methods

Patients and samples

The study was approved by the Ethics Committee and Institutional Review Board of the Affiliated People's Hospital of Jiangsu University in accordance with the Declaration of Helsinki. After signing the written informed consents, bone marrow (BM) was collected from 95 CML patients at diagnosed time as well as 28 healthy donors seen as controls. The main clinical and laboratory features of

CML patients were presented in Table 1. Treatment of CML patients at CP stage received tyrosine kinase inhibitors (TKI)-based therapy, whereas CML patients at AP/BC stage received chemotherapy together with TKI-based therapy. BM mononuclear cells (BMMNCs) were separated by density-gradient centrifugation using Lymphocyte Separation Medium (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). When karyotypes were analyzed, BM cells were harvested after 1 to 3 days of unstimulated culture in RPMI 1640 medium containing 20% fetal calf serum (ExCell Bio, Shanghai, China).

RNA isolation, reverse transcription and RQ-PCR

Total RNA isolation and reverse transcription were as reported previously.¹⁰ The expression of *SOX30* was detected by real-time quantitative PCR (RQ-PCR) as reported previously.⁸ The housekeeping gene *ABL* detected by RQ-PCR using 2×SYBR Green PCR Mix (Multisciences, Hangzhou, China) was used to calculate the abundance of *SOX30* transcript. Relative *SOX30* transcript level was calculated by $2^{-\Delta\Delta CT}$ methods.

DNA isolation, bisulfite modification, and RQ-MSP

Genomic DNA isolation and modification were performed as reported previously.¹⁰ The methylation level of *SOX30* was examined by real-time quantitative methylation-specific

Table 1 Comparison of clinical and laboratory features between CML patients with and without *SOX30* methylation

Patients' parameters	Methylated (n=10)	Non-methylated (n=85)	P-value
Sex, male/female	3/7	52/33	0.089
Median age, years (range)	55 (32–67)	47 (15–88)	0.446
Median WBC, ×10 ⁹ /L (range)	64 (21.7–144.8)	71.7 (16.4–413.8)	0.431
Median hemoglobin, g/L (range)	66.2 (50–96)	101 (57–152)	0.006
Median platelets, ×10 ⁹ /L (range)	60 (16–48)	359.5 (38–1489)	0.032
Stage			0.006
CP	4 (40%)	70 (82%)	0.007
AP	1 (10%)	6 (7%)	0.553
BC	5 (50%)	9 (11%)	0.006
Cytogenetics			0.002
t(9;22)	2 (20%)	61 (72%)	0.002
t(9;22) with additional alteration	4 (40%)	7 (8%)	0.015
Normal karyotype	2 (20%)	4 (5%)	0.119
No data	2 (20%)	13 (15%)	0.656
<i>BCR-ABL</i> transcript (relative copy)	1100 (94.1–14464.7)	166.9 (13.8–2030.3)	0.063

Abbreviations: WBC, white blood cells; CP, chronic phase; AP, accelerated phase; BC, blast crisis; CML, chronic myeloid leukemia.

PCR (RQ-MSP) using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscataway, NJ, USA) as reported previously.⁸ Relative *SOX30* methylation level was calculated by $2^{-\Delta\Delta CT}$ methods.

BSP

Bisulfite sequencing PCR (BSP) and clone sequencing were conducted as reported previously.⁸ Six independent clones of BSP products were sequenced.

Statistical analysis

SPSS software version 20.0 and GraphPad Prism 5.0 were applied to perform statistical analysis. Mann-Whitney's U test was carried to compare the difference of continuous variables between two groups, whereas Pearson Chi-square analysis/Fisher exact test was applied to compare the difference of categorical variables between two groups. Correlation analysis was performed by Spearman test. All tests were two sided, and $P < 0.05$ was defined as statistically significant.

Results

Correlation of *SOX30* methylation with clinical/laboratory characteristics in CML

Our previous study has reported aberrant *SOX30* methylation in AML and MDS, and *SOX30* hypermethylation correlated with poor prognosis in AML, also associated with disease progression in MDS.⁸ Herein, we further determined *SOX30* methylation in the other myeloid malignance CML using RQ-MSP. According to the previously set cut-off value of 1.024, *SOX30* methylation was identified in 11% (10/95) CML patients, and was a bit higher than controls (0%, 0/28) (Figure 1, $P=0.115$). In order to analyze the clinical significance in CML, we

further compared the clinical and laboratory features between *SOX30* methylated and non-methylated patients (Table 1). No significant differences was shown in age and white blood cells ($P > 0.05$). However, *SOX30* methylation was associated with lower hemoglobin and platelets ($P=0.006$ and 0.032 , respectively). Importantly, significant differences were observed in the distributions of clinical stages and cytogenetics ($P=0.006$ and 0.002 , respectively). Among different clinical stages, the frequency of *SOX30* methylation in chronic phase (CP) stage occurred with lowest frequency (4/74, 5%), higher in accelerated phase (AP) stage (1/7, 14%), and the highest in blast crisis (BC) stage (12/31, 39%). For cytogenetics, t(9;22) with additional alterations patients showed the highest incidence of *SOX30* methylation (4/11, 36%), whereas cases with t(9;22) patients presented the lowest incidence of *SOX30* methylation (2/63, 3%). In addition, *SOX30* methylated patients tended to have a higher level of *BCR-ABL* transcript than *SOX30* non-methylated patients ($P=0.063$). All the positive results indicated that *SOX30* methylation was associated increased clinical stage and may play a role in disease progression in CML.

SOX30 methylation alterations during disease progression in paired CML patients

To confirm whether *SOX30* was associated with CML progression, we next determined *SOX30* methylation at different clinical stages of two paired CML patients during disease progression. Detected by BSP, both two CML patients showed low density of *SOX30* methylation in CP stage (19% and 17%, respectively), and the density of *SOX30* methylation was significantly increased in BC

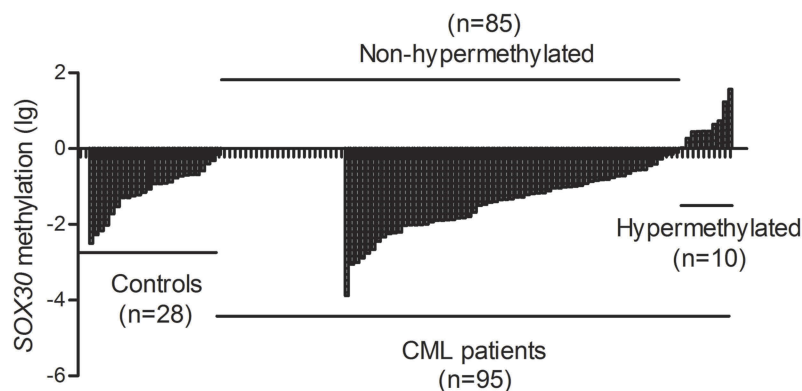


Figure 1 *SOX30* methylation level in controls and CML patients. *SOX30* methylation level in 95 CML patients at diagnosed time as well as 28 healthy donors seen as controls was examined by RQ-MSP, and showed in lg conversion.

Abbreviation: CML, chronic myeloid leukemia.

stage during disease progression (89% and 69%, respectively) (Figure 2).

Association of *SOX30* methylation with its expression in CML

As is well known, DNA methylation in promoter-associated CpG islands plays a direct role in gene silencing that is one of the drivers of neoplastic transformation through the inactivation of critical tumor-suppressor pathways. We further determined *SOX30* transcript level in 52 CML patients with available mRNA samples. Although we did not observe the significantly negative association between *SOX30* methylation and *SOX30* expression ($R=-0.169$, $P=0.232$, $n=52$, Spearman test), *SOX30* methylated CML patients presented a lower *SOX30* transcript level than *SOX30* non-methylated CML patients ($P=0.046$, Figure 3).

Discussion

Following our previous study, herein, we further for the first time reported *SOX30* methylation in CML. Although it was not a frequent event in CML, *SOX30* methylation was associated with advanced clinical stage, and may have a crucial role in CML progression. The mechanisms of CML progression are

varied and not entirely understood. So far the best characterized includes differentiation arrest, genomic instability, telomere shortening and loss of tumor-suppressor functions.² Mechanically, cytogenetic abnormalities and molecular alterations, such as double Ph chromosome, trisomy chromosome 8, i(17q), trisomy chromosome 19, t(3;21)(q26;q22), t(7;11)(p15;p15), p53 mutations, RAS mutation, and increased *BCR-ABL* transcript, are pathogenetically linked to the progression of CML.³ Recently, epigenetic changes especially aberrant DNA methylation associated with gene silencing are also identified to play crucial roles in the disease progression of CML.⁴ For instance, *SHP-1* hypermethylation was involved in the progression in CML through dysregulating BCR-ABL1, AKT, MAPK, MYC and JAK2/STAT5 signaling pathways.¹¹ In addition, our previous studies have revealed that hypermethylation of *ID4* and *DLX4* was related to disease progression in CML, and *ID4* also had a direct role in affecting cell proliferation and apoptosis in K562 cell-line.^{12,13}

Although we have proved that *SOX30* methylation was associated with CML progression in clinics, the direct role of *SOX30* in the pathogenesis of CML was not studied. Recently, *SOX30* has been validated to be a tumor suppressor gene in several solid cancers with diagnostic and prognostic value. Han et al demonstrated that *SOX30* was silenced caused by

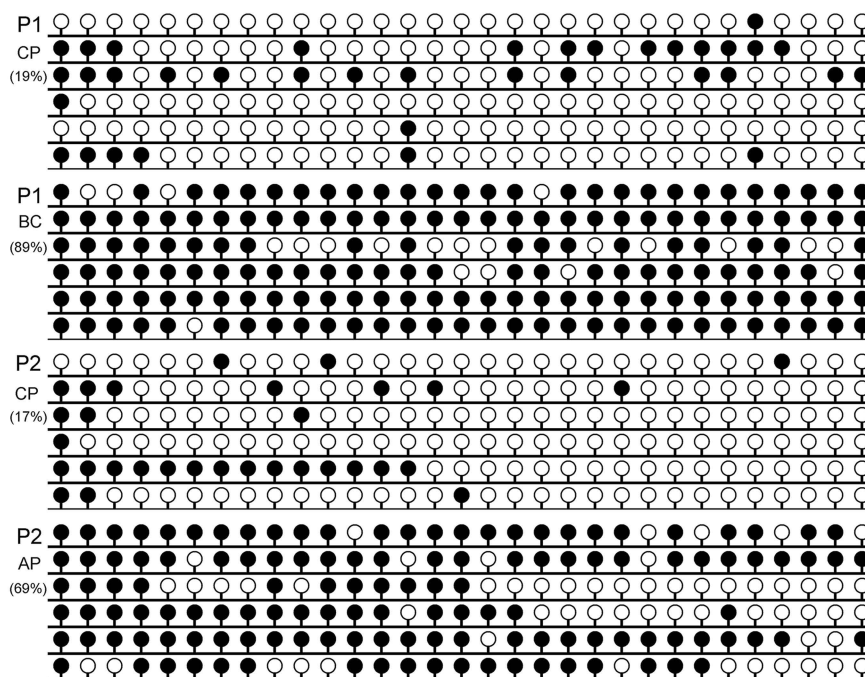


Figure 2 Dynamic changes of *SOX30* methylation density in two paired CML patients during the progression. White cycle: unmethylated CpG dinucleotide; Black cycle: methylated CpG dinucleotide. Patient 1: the mean density of six clones in CP and BC stage was 19% and 89%, respectively. Patient 2: the mean density of six clones in AP and BC stage was 17% and 69%, respectively.

Abbreviations: CP, chronic phase; AP, accelerated phase; BC, blast crisis; CML, chronic myeloid leukemia.

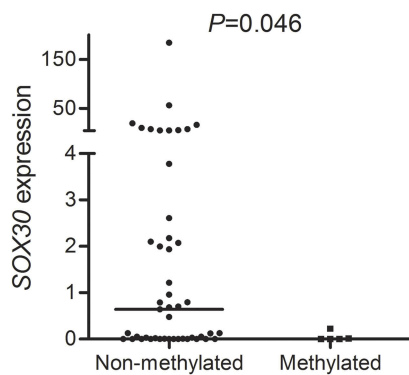


Figure 3 *SOX30* expression level in *SOX30* methylated and non-methylated CML patients. *SOX30* methylation level and expression level in 52 CML patients were examined by RQ-MSP and RQ-PCR, respectively.

promoter methylation and functioned as a novel tumor suppressor partly by transcriptional activating p53 or activating the transcription of desmosomal genes in lung cancer.^{7,14} In addition, *SOX30* could also inhibited tumor metastasis through attenuating Wnt signaling via the regulation of β -Catenin in a transcriptional and posttranslational manner in lung cancer.¹⁵ In clinics, the expression of *SOX30* was verified to be closely associated with clinical outcomes in lung cancer patients.⁹ Moreover, *SOX30* expression was identified as prognostic biomarkers in several other human cancers such as bladder cancer and advanced-stage ovarian cancer.^{16,17} In colon cancer and hepatocellular carcinoma, the anti-cancer effects of *SOX30* rescued the tumor-promoting effect mediated by *miR-645* overexpression.^{18,19} However, no functional studies showed the role of *SOX30* in hematological malignancies. Moreover, the current study could not further determine the impact of *SOX30* methylation in CML due to limited cases with survival data. Obviously, further studies are needed to determine the direct role of *SOX30* during CML progression and its clinical implication.

In summary, *SOX30* methylation correlated with disease progression in CML, and provided novel insights into CML biology acting as a potential therapeutic target against disease progression.

Ethics statements

This study approved by the Ethics Committee and Institutional Review Board of the Affiliated People's Hospital of Jiangsu University in accordance with the Declaration of Helsinki.

Acknowledgments

This study was supported by National Natural Science Foundation of China (81270630), Medical Innovation Team

of Jiangsu Province (CXTDB2017002), 333 Project of Jiangsu Province (BRA2016131), Six Talent Peaks Project in Jiangsu Province (2015-WSN-115), Zhenjiang Clinical Research Center of Hematology (SS2018009), Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX18_2281, KYCX17_1821), Social Development Foundation of Zhenjiang (SH2017040, SH2018044), Clinical Medical Science Development Foundation of Jiangsu University (JLY20160011), Natural Science Foundation of Jiangsu Province for Youths (BK20180280) and Scientific Research Foundation of Affiliated People's Hospital of Jiangsu University for Ph.D. (KFB201603).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Apperley JF. Chronic myeloid leukaemia. *Lancet*. 2015;385(9976):1447–1459. doi:10.1016/S0140-6736(13)62120-0
2. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nat Rev Cancer*. 2007;7(6):441–453. doi:10.1038/nrc2147
3. Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood*. 2004;103(11):4010–4022. doi:10.1182/blood-2003-12-4111
4. Heller G, Topakian T, Altenberger C, et al. Next-generation sequencing identifies major DNA methylation changes during progression of Ph+ chronic myeloid leukemia. *Leukemia*. 2016;30(9):1861–1868. doi:10.1038/leu.2016.143
5. Bowles J, Schepers G, Koopman P. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev Biol*. 2000;227(2):239–255. doi:10.1006/dbio.2000.9883
6. Osaki E, Nishina Y, Inazawa J, et al. Identification of a novel Sry-related gene and its germ cell-specific expression. *Nucleic Acids Res*. 1999;27(12):2503–2510. doi:10.1093/nar/27.12.2503
7. Han F, Liu W, Jiang X, et al. *SOX30*, a novel epigenetic silenced tumor suppressor, promotes tumor cell apoptosis by transcriptional activating p53 in lung cancer. *Oncogene*. 2015;34(33):4391–4402. doi:10.1038/onc.2014.370
8. Zhou JD, Wang YX, Zhang TJ, et al. Identification and validation of SRY-box containing gene family member *SOX30* methylation as a prognostic and predictive biomarker in myeloid malignancies. *Clin Epigenetics*. 2018;10:92.
9. Han F, Liu W, Xiao H, et al. High expression of *SOX30* is associated with favorable survival in human lung adenocarcinoma. *Sci Rep*. 2015;5:13630. doi:10.1038/srep13630
10. Zhang TJ, Zhou JD, Zhang W, et al. H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia. *Clin Epigenetics*. 2018;10:47. doi:10.1186/s13148-018-0486-z
11. Li Y, Yang L, Pan Y, Yang J, Shang Y, Luo J. Methylation and decreased expression of *SHP-1* are related to disease progression in chronic myelogenous leukemia. *Oncol Rep*. 2014;31(5):2438–2446. doi:10.3892/or.2014.3098
12. Zhou JD, Zhang TJ, Li XX, et al. Epigenetic dysregulation of *ID4* predicts disease progression and treatment outcome in myeloid malignancies. *J Cell Mol Med*. 2017;21(8):1468–1481. doi:10.1111/jcmm.13073
13. Zhou JD, Wang YX, Zhang TJ, et al. Epigenetic inactivation of *DLX4* is associated with disease progression in chronic myeloid leukemia. *Biochem Biophys Res Commun*. 2015;463(4):1250–1256. doi:10.1016/j.bbrc.2015.06.095

14. Hao X, Han F, Ma B, et al. SOX30 is a key regulator of desmosomal gene suppressing tumor growth and metastasis in lung adenocarcinoma. *J Exp Clin Cancer Res.* 2018;37(1):111. doi:10.1186/s13046-018-0778-3
15. Han F, Liu WB, Shi XY, et al. SOX30 inhibits tumor metastasis through attenuating Wnt-signaling via transcriptional and posttranslational regulation of β -catenin in lung cancer. *EBioMedicine.* 2018;31:253–266. doi:10.1016/j.ebiom.2018.04.026
16. Liu Y, Wang H, Zhong J, et al. Decreased expression of SRY-box containing gene 30 is related to malignant phenotypes of human bladder cancer and correlates with poor prognosis. *BMC Cancer.* 2018;18(1):642. doi:10.1186/s12885-018-4242-8
17. Han F, Liu WB, Li JJ, et al. SOX30 is a prognostic biomarker and chemotherapeutic indicator for advanced-stage ovarian cancer. *Endocr Relat Cancer.* 2019;303–319. doi:10.1530/ERC-18-0529
18. Guo ST, Guo XY, Wang J, et al. MicroRNA-645 is an oncogenic regulator in colon cancer. *Oncogenesis.* 2017;6(5):e335. doi:10.1038/oncsis.2017.37
19. Tao J, Liu Z, Wang Y, et al. MicroRNA-645 represses hepatocellular carcinoma progression by inhibiting SOX30-mediated p53 transcriptional activation. *Int J Biol Macromol.* 2019;121:214–222. doi:10.1016/j.ijbiomac.2018.10.032

OncoTargets and Therapy

Dovepress

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>