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c-myb activates *CXCL12* transcription in T47D and MCF7 breast cancer cells

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Chemokine C-X-C motif ligand 12 (CXCL12) is a potent chemotactic and angiogenic factor that has been proposed to play a role in organ-specific metastasis and angiogenic activity in several malignancies. In this study, we found that the overexpression of *c-myb* could elevate *CXCL12* mRNA level and *CXCL12* promoter activity in human T47D and MCF-7 breast cancer cells. Chromatin immunoprecipitation assay demonstrated that c-myb could bind to the *CXCL12* promoter in the cells transfected with *c-myb* expression vector. *c-myb* siRNA attenuated *CXCL12* promoter activity and the binding of c-myb to the *CXCL12* promoter in T47D and MCF-7 cells. These results indicated that c-myb could activate *CXCL12* promoter transcription.

Keywords c-myb; CXCL12 promoter; transcription regulation

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Introduction

Tumor angiogenesis, the sprouting of new capillaries from the preexisting vascular network that involves in proliferation of capillary endothelial cells and their migration toward the angiogenic stimulus, is an absolute requirement for the growth, progression, and metastasis of solid tumors [1,2]. Chemokine C-X-C motif ligand 12 (CXCL12) is a chemokine of the CXC family. This chemokine potently plays a role in organ-specific metastasis and angiogenic activity in several malignancies [3,4]. The anomalous elevation of CXCL12 is related to the specialized microenvironments created by the persistent growth of blood vessels [5]. Elevated CXCL12 not only promotes the growth of tumor cells, but also enhances the tumor angiogenesis and metastasis [6]. Consequently, the inhibition of CXCL12 signaling abrogates the development of a wide variety of tumors. Expression of *CXCL12* was silenced by promoter hypermethylation in non-small cell lung cancer, breast cancer cell lines, and primary mammary tumors [7,8]. Epigenetic silencing of CXCL12 increases the metastatic potential of mammary carcinoma cells [9].

CXCL12 gene expression is directly induced by hypoxia-inducible factor-1 (HIF-1) in direct proportion to reduced oxygen tension [10,11]. Some transcription factors can influence *CXCL12* transcription. It is reported that the effects of AP1 on *CXCL12* expression in human cancer cells involves in a Sp1 motif located between −57 and −39 upstream of the main transcription start site [12]. The proto-oncogene c-myb is also a transcription factor, and so far there is no report as to whether c-myb can activate *CXCL12* transcription.

The aim of the present study was to explore the activity of c-myb on *CXCL12* transcription and to reveal the role of c-myb involved in CXCL12-mediated angiogenesis. Our results showed that c-myb played an important role in inducing *CXCL12* promoter activity by directly binding to the *CXCL12* promoter, which is helpful to understand the angiogenic mechanism. The inhibition of CXCL12 signaling may provide potential targets for antiangiogenic therapy in several malignancies.

Materials and Methods

Cell lines, cell culture, plasmids, and cell transfection

Human breast cancer cell lines, T47D and MCF-7 were maintained in RPMI 1640 containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were checked routinely and found to be free of contamination by *Mycoplasma* or fungi.

CXCL12 promoter/luciferase gene construct pCMVLUC-SDF1010 (−1010 to +122) and its negative control plasmid pDPROMLUC were kindly provided by Dr Antonio Caruz (Immunogenetics Unit, Faculty of Sciences, University of Jaen, Campus Las Lagunillas SN, Jaen, Spain) [12]. *c-myb* expression vector (pCMV-c-myb) was purchased from Origene Company (Rockville, USA).

Transfections were conducted by Lipofectamine method. Briefly, for transient transfection, cells were seeded in

6-well plates at a density of 4×10^5 cells/well. The following day, cells were transfected with 4 μg of *c-myb* expression vector or pcDNA3 using Lipofectamine 2000 (Gibco BRL, Carlsbad, USA). Following transfection, cells were maintained in RPMI 1640 containing 10% FBS and cultured for 48 h.

Reverse transcription-PCR

Total RNA was extracted from cells with Trizol reagent (Invitrogen, Carlsbad, USA) and quantified by UV absorbance spectroscopy. The reverse transcription reaction was performed using the Superscript First-Strand Synthesis System (Invitrogen) in a final volume of 20 μl containing 5 μg of total RNA, 200 ng of random hexamers, 1 × reverse transcription buffer, 2.5 mM $MgCl_2$, 1 mM deoxynucleoside triphosphate mixture, 10 mM DTT, RNaseOUT recombinant ribonuclease inhibitor (Invitrogen), 50 units of superscript reverse transcriptase, and diethylpyrocarbonate-treated water. After incubation at 42°C for 50 min, the reverse transcription reaction was terminated by heating at 85°C for 5 min. The newly synthesized cDNA was amplified by PCR. The reaction mixture contained 2 μl of cDNA template, 1.5 mM $MgCl_2$, 2.5 U of *Tag* polymerase, and 0.5 μM of *CXCL12* primer (5′-AGAGCCAACGTCAAGCATCT-3′; 5′-CGTCTTTGCCCTTTCATCTC-3′), or *c-myb* primer (5′-GCCAATTATCTCCCGAATCGA-3′; 5′-ACCAACGTTTCGGACCGTA-3′), or *GAPDH* primer (5′-GCCAAAAGGGTCATCATCTC-3′; 5′-GTAGAGGCAGGGATGATGTTC-3′). *GAPDH* was used as an internal control. PCR conditions were: 94°C for 3 min, then 33 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min, followed by 72°C for 10 min. Aliquots of PCR product were electrophoresed on 1.5% agarose gels, and PCR fragments were visualized by ethidium bromide staining.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were carried out according to the manufacturer's protocol (Active Motif, Carlsbad, USA). Briefly, cells in 150 mm tissue culture dishes were fixed with 1% formaldehyde and incubated for 10 min at 37°C. The cells were then washed twice with ice-cold phosphate-buffered saline (PBS), harvested and re-suspended in ice-cold TNT lysis buffer (20 mM Tris–HCl, pH 7.4, 200 mM NaCl, 1% Triton X-100, 1 mM PMSF, and 1% aprotinin). The lysates were sonicated to shear the DNA to fragments of 200 − 600 bp, and subjected to immunoprecipitation with the following antibodies, respectively, c-myb or IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, USA). Antibodies (3 μg) were used for each immunoprecipitation. The antibody/protein complexes were collected by Protein G beads and washed three times with ChIP washing buffer (5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, 40 mM $NaHPO_4$, pH 7.2). The immune complexes were eluted with 1% SDS and 1 M $NaHCO_3$, and the cross links were reversed by incubation at 65°C for 4 h in the presence of 200 mM NaCl and RNase A. The samples were then treated with proteinase K for 2 h, and then DNA was purified by mini-column, ethanol precipitation, and re-suspended in 100 ml of H_2O. The primer corresponding to the *CXCL12* promoter region (−395 to −213; sense: 5′-TCAGTTCCCGCCATCGAAAGG-3′; antisense: 5′-CTCCGGCCTTTGACCTTCTCAG-3′) was used for PCR to detect the presence of the *CXCL12* promoter DNA.

Small-interfering RNA preparation and transfection

c-myb small-interfering RNA (siRNA) is a target-specific 19 nt siRNA (5′-UGUUAUUGCCAAGCACUUAAA-3′ and 5′-UAAGUGCUUGGCAAUAACAGAA-3′) designed to knock down *c-myb* expression. The siRNA was synthesized by Shanghai GeneChem (Shanghai, China). The cells in the exponential phase of growth were seeded in 6-well plates at a concentration of 5×10^5 cells/well. After incubation for 24 h, the cells were transfected with siRNA specific for *c-myb* and non-targeting siRNA at a final concentration of 100 nM using oligofectamine and OPTI-MEMI reduced serum medium (Invitrogen), according to the manufacturer's protocol. Silencing was examined 48 h after transfection.

Western blot analysis

Cells were washed twice with PBS containing 1 mM phenylmethylsulphonyl fluoride, lysed in mammalian protein extraction buffer (Pierce, Rockford, USA). The lysates were transferred to Eppendorf tubes and clarified by centrifugation at 12,000 *g* for 40 min at 4°C. Equal amounts (50 mg of protein) of cell lysates were resolved by SDS-PAGE. The proteins were transferred to nitrocellulose membranes. Membranes were incubated in blocking solution consisting of 5% powered milk in PBST (PBS plus 0.1% Tween-20) at room temperature for 1 h, then immunoblotted with anti-c-myb antibody (Santa Cruz Biotechnology, Inc.) (1:1000) or anti-tubulin antibody (Sigma-Aldrich, St. Louis, USA) (1:5000), respectively. Detection by enzyme-linked chemiluminescence (Amersham Pharmacia Biotech, Piscataway, USA) was performed according to the manufacturer's protocol.

CXCL12 ELISA

Cells were seeded in 6-well plates. Then cells were transfected with *c-myb* expression vector. After 48 h of growth, 1.5 ml of medium was collected from each well to evaluate CXCL12 levels by ELISA. The supernatants of 4 wells from each time point were collected and analyzed for *CXCL12* expression using a commercially available ELISA

kit (R&D Systems Inc., Minneapolis, USA) according to the manufacturer's instructions. The plates were read at 450 nm. CXCL12 concentrations in conditioned media were calculated from a standard curve generated by adding recombinant CXCL12 to the specific unconditioned media.

Mutagenesis

CXCL12 promoter/luciferase gene construct pCMVLUC-SDF1010 was used as template. Methylated plasmid DNA with DNA methylase at 37°C for 1 h. Amplify the plasmid in a mutagenesis reaction with two overlapping primers, one of which contained the target mutation. The product was linear, double-stranded DNA containing the mutation. Transform the mutagenesis mixture into wild-type *E. coli*. The host cells circularized the linear mutated DNA, and *Mcr*BC endonuclease in the host cells digested the methylated template DNA, leaving only unmethylated and mutated product. For individual mutations, the sequence of c-myb-binding sites TTCAGTTC was converted to TTCATATC.

Luciferase reporter gene assay

T47D or MCF-7 cells were seeded in 6-well plates at a density of $1-2 \times 10^5$ cells/well and cultured for 24 h. Cells were then co-transfected wild-type (pCMVLUC-SDF1010) or *c-myb* mutant (pCMVLUC-SDF1010mut) and *CXCL12* reporter construct (0.5 μg/well), or co-transfected with 0.5 μg of pcDNA3.0 or *c-myb* expression vector together with 20 ng of control *Renilla* luciferase reporter gene construct, pRL-TK (Promega, Madison, USA). The total amount of DNA per well was adjusted to 1.5 μg by the addition of sonicated salmon sperm DNA. Luciferase assays were performed as recommended by the manufacturer (Promega) and normalized relative to protein concentration determined by bicinchoninic acid protein assay (Pierce).

Results

Overexpression of *c-myb* induced *CXCL12* expression in T47D and MCF-7 cells

To explore the role of c-myb in regulating *CXCL12* transcription, the *c-myb* expression vector or pcDNA3 was transfected into T47D cells and MCF-7 cells for 48 h, then *CXCL12* mRNA and protein levels were detected. **Figure 1(A,B)** showed that the level of *CXCL12* mRNA in the cells transfected with *c-myb* expression vector increased as determined by RT-PCR, compared with control cells transfected with pcDNA3. **Figure 1(C,D)** showed that as compared with control cells transfected with pcDNA3, the level of CXCL12 secretion in the cells transfected with *c-myb* expression vector increased as determined by ELISA. In this experiment, exogenous c-myb could induce

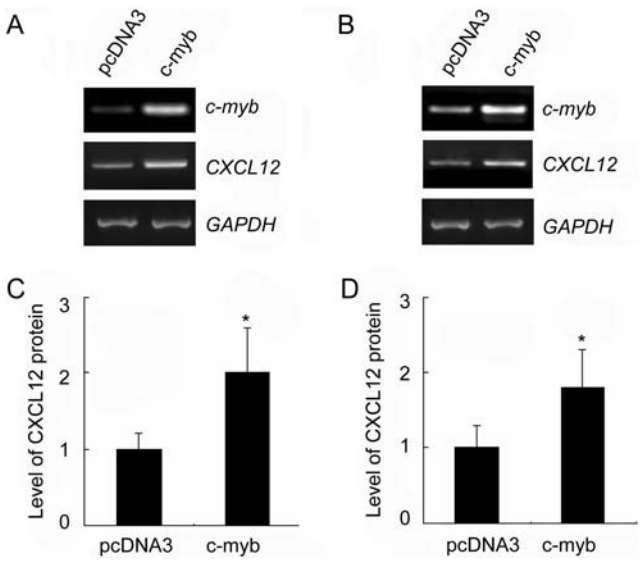

Figure 1 Overexpression of *c-myb* induced *CXCL12* mRNA and protein levels in T47D and MCF-7 cells T47D (A,C) or MCF-7 (B,D) cells were plated in 6-well tissue culture plates, then transfected with 4 μg of c-myb expression vector, and cultured for 48 h. mRNA expression levels of c-myb and CXCL12 were detected by RT-PCR (A,B), and protein expression levels of CXCL12 were detected by ELISA analysis (C,D). pcDNA3 was used as a control. $n = 3$. $^*P < 0.05$ vs pcDNA3.

CXCL12 expression, indicating c-myb played a role in regulating *CXCL12* transcription.

c-myb activated *CXCL12* promoter activity in T47D and MCF-7 cells

To identify the role of c-myb in regulating *CXCL12* transcription, we co-transfected the *CXCL12* promoter/luciferase gene construct with *c-myb* expression vector or pcDNA3 in T47D and MCF-7 cells and detected CXCL12 promoter activity. **Figure 2(A,B)** showed that the luciferase activity was enhanced by c-myb both in T47D and in MCF-7 cells, respectively, further indicating that c-myb could activate *CXCL12* promoter activity. In this experiment, exogenous c-myb could activate *CXCL12* promoter activity, suggesting that c-myb played a role in regulating *CXCL12* transcription.

To identify the potential-binding site of c-myb on *CXCL12* promoter, *c-myb* was co-transfected with either a wild-type (pCMVLUC-SDF1010) or c-myb-site-mutated mutant (pCMVLUC-SDF1010mut) *CXCL12* construct into MCF-7 or T47D cells. Though the overexpression of *c-myb* activated the wild-type promoter more than 6 folds, it had no effect on the c-myb mutant [**Fig. 2(C,D)**].

c-myb bounds to the *CXCL12* promoter in *c-myb*-overexpressed T47D and MCF-7 cells

To investigate if c-myb bounds to the *CXCL12* promoter in the cells transfected with *c-myb* expression vector, we performed ChIP experiments. The results showed that c-myb

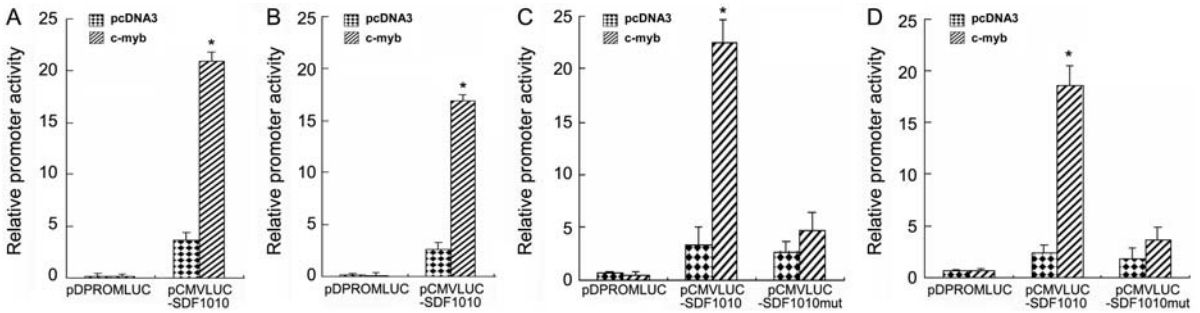

Figure 2 c-myb activated *CXCL12* promoter activity in T47D and MCF-7 cells (A,B) c-myb activated *CXCL12* promoter activity in T47D and MCF-7 cells T47D (A) or MCF-7 (B) cells were plated in 6-well tissue culture plates, then co-transfected with 0.5 μg of pCMVLUC-SDF1010 with 0.5 μg of *c-myb* expression vector or pcDNA3 control vector for 48 h. Luciferase activity was detected as described in 'Materials and Methods'. $n = 3$. $^*P < 0.05$ vs controls. (C,D) c-myb requires an intact c-myb site for *CXCL12* promoter activation. 0.5 μg of wild-type (pCMVLUC-SDF1010) or *c-myb* site mutated (pCMVLUC-SDF1010mut) *CXCL12* construct was transfected into T47D (C) or MCF-7 (D) cells with or without 0.5 μg of *c-myb* expression vector. *Renilla* luciferase reporter construct pRL-TK (20 ng) was used as an internal control for transfection efficiency. Luciferase activity was determined and normalized to protein concentration. $n = 3$. $^*P < 0.05$ vs pcDNA3.

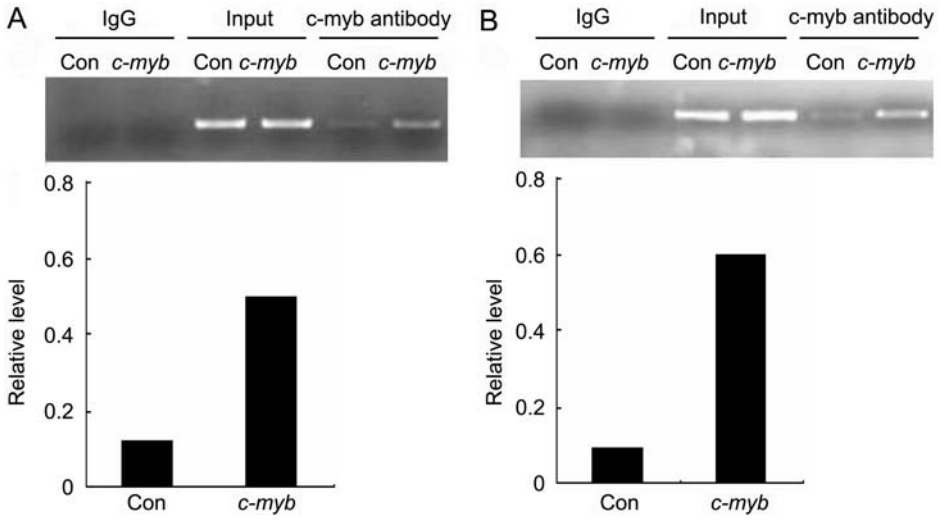

Figure 3 c-myb bounds to the *CXCL12* promoter after overexpression of *c-myb* in T47D and MCF-7 cells T47D (A) or MCF-7 (B) cells were plated in 6-well tissue culture plates, then transfected with 4 μg of *c-myb* expression vector, and cultured for 48 h. Nucleic extracts were prepared from T47D or MCF-7 cells with or without *c-myb* transfection. ChIP assays were performed using antibody against c-myb or IgG, as described in 'Materials and Methods'. The primers corresponding to the *CXCL12* promoter region (−395 to −213) upstream of the transcriptional start site was used for PCR to detect the presence of the *CXCL12* promoter DNA. Con: cells transfected with the empty pcDNA3 vector. $n = 3$.

could bind to the *CXCL12* promoter both in T47D and in MCF-7 cells transfected with c-myb expression vector (**Fig. 3**). In this experiment, c-myb could bind to the *CXCL12* promoter in c-myb-overexpressed T47D or MCF-7 cells, indicating that c-myb activated *CXCL12* transcription by binding directly to the *CXCL12* promoter.

c-myb siRNA inhibited *c-myb* mRNA, *CXCL12* mRNA and protein in T47D and MCF-7 cells

To further identify the role of c-myb in regulating *CXCL12* transcription, we knocked down the expression of c-myb with a gene-specific siRNA and measured *c-myb* mRNA. As shown in **Fig. 4(A,B)**, *c-myb* siRNA inhibited *c-myb* mRNA significantly in T47D and MCF-7 cells after transfection with *c-myb* siRNA for 48 h, respectively. And it also inhibited *CXCL12* mRNA in the T47D and MCF-7 cells. Also *c-myb* siRNA inhibited CXCL12 protein secretion significantly in T47D and MCF-7 cells after transfection with *c-myb* siRNA for 48 h [**Fig. 4(C,D)**]. This experiment indicated that *c-myb* siRNA could knock down *c-myb* expression efficiently and decrease CXCL12 protein secretion significantly.

c-myb siRNA repressed *CXCL12* promoter activity in T47D and MCF-7 cells

To determine if the decrease of c-myb would reduce *CXCL12* gene transcription, we knocked down the expression of *c-myb* and measured *CXCL12* promoter activity. As shown in **Fig. 5**, *c-myb* siRNA attenuated *CXCL12* promoter activity in T47D and MCF-7 cells (without incubating any

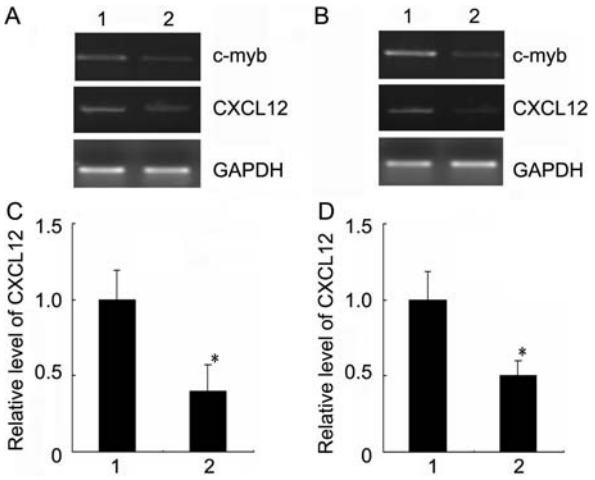

Figure 4 *c-myb* siRNA inhibited *c-myb* mRNA, *CXCL12* mRNA and protein in T47D and MCF-7 cells (A,B) *c-myb* siRNA inhibited *c-myb* mRNA and *CXCL12* mRNA in T47D or MCF-7 cells, respectively. (C,D) *c-myb* siRNA inhibited CXCL12 protein in T47D or MCF-7 cells, respectively. T47D (A,C) or MCF-7 (B,D) cells were treated with 100 nM of *c-myb* siRNA or non-targeting siRNA for 48 h. RT-PCR (A,B) and ELISA (C,D) analysis were performed as described in 'Materials and Methods'. *GAPDH* was used as an internal control in the RT-PCR and tubulin was used as an internal control in the ELISA. 1, cells transfected with non-targeting siRNA; 2, cells transfected with *c-myb* siRNA. $n = 3$. $^*P < 0.05$ vs cells transfected with non-targeting siRNA.

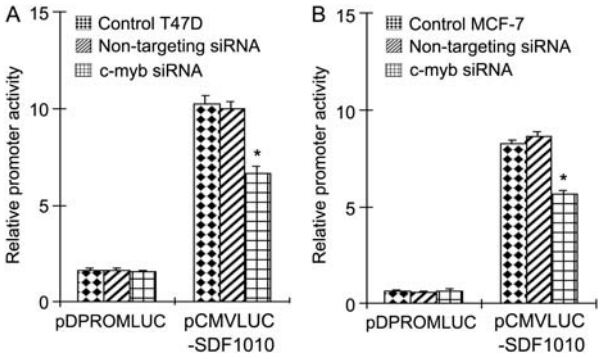

Figure 5 *c-myb* siRNA repressed *CXCL12* promoter activity in T47D and MCF-7 cells T47D (A) or MCF-7 (B) cells were plated in 6-well tissue culture plates. pDPROMLUC or *CXCL12* promoter construct (pCMVLUC-SDF1010) was co-transfected with *c-myb* siRNA or non-targeting siRNA, and cultured for 48 h. Luciferase activity was detected as described in Materials and Methods. $n = 3$. $^*P < 0.05$ vs controls (T47D or MCF-7 cells without incubating any siRNA).

siRNA) after transfection with *c-myb* siRNA for 48 h. This experiment indicated that when endogenous c-myb was knocked down by siRNA, the promoter activity of endogenous CXCL12 also decreased.

c-myb siRNA attenuated the binding of c-myb to the *CXCL12* promoter

To determine if the decrease of c-myb would influence the binding of c-myb on the *CXCL12* promoter, we knocked down the expression of *c-myb* and measured the binding status of c-myb to the *CXCL12* promoter. As shown in **Fig. 6**, c-myb siRNA attenuated the binding of c-myb to the *CXCL12* promoter in T47D and MCF-7 cells after transfection with *c-myb* siRNA for 48 h. Result showed that when endogenous c-myb was knocked down by siRNA, the binding of c-myb to the *CXCL12* promoter was decreased, and it also indicated that c-myb regulated *CXCL12* transcription by binding directly to the *CXCL12* promoter.

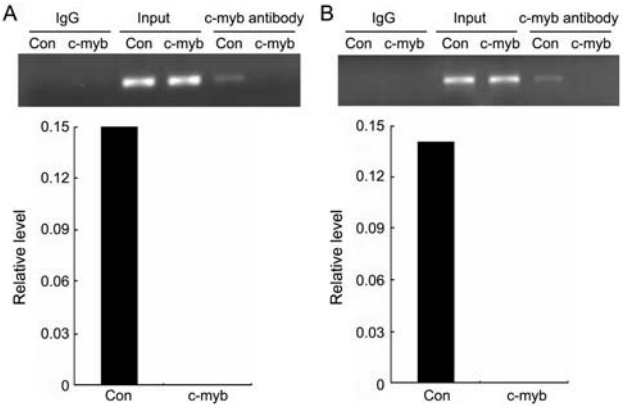

Figure 6 *c-myb* siRNA attenuated the binding of c-myb to the *CXCL12* promoter T47D (A) or MCF-7 (B) cells were treated with 100 nM *c-myb* siRNA or non-targeting siRNA for 48 h. Then ChIP assay was performed as described in 'Materials and Methods'. $n = 3$.

Discussion

The proto-oncogene *c-myb* is the cellular homolog of the *v-myb* oncogene carried by the avian myeloblastosis viruses [13]. It encodes a 75-kDa transcriptional factor protein c-myb [14]. c-myb function can be regulated by many factors including different RNA splicing [15], C/EBP [16], cyclin D1 [17], and so on [18,19]. The c-myb protein plays an essential role in the regulation of cell growth, survival, and differentiation of hematopoietic cells [20]. These results suggested that the c-myb protein is required for proliferation of cells. In fact, proliferation is often associated with cancer progression. Although *c-myb* expression was initially thought to be related to the hematopoietic cells, it has subsequently been reported in non-hemopoietic tissues and cell lines including the breast, colon, lung carcinomas, neuroblastomas, and so on [21]. Ramsay *et al*. [22] reported c-myb could activate COX-2 transcription in colorectal cancer. McHale *et al*. [23] found that c-myb protein was increased in invasive breast cancers compared with normal tissues via immunohistochemistry evaluation. c-myb affected estrogen/ER signaling pathway, functioned as a STAT5a co-activator and potentiated STAT5a-driven gene expression in human breast cancer [24,25]. These data at least can explain the potential role of the c-myb protein in myeloid leukaemias and in solid tumors. Disrupting the

function of c-myb may be an effective target for cancer treatment. All these studies indicate that c-myb is related to cancer progression and invasion, but there are no reports about the relationship between c-myb and CXCL12 transcription in breast cancer.

In our studies, overexpression of c-myb could increase *CXCL12* mRNA and protein levels in T47D and MCF-7 cells. In order to analyze the putative effects of c-myb on *CXCL12* transcription, we performed luciferase assay. Our results demonstrated that c-myb activated *CXCL12* promoter activity. ChIP assay demonstrated that c-myb could bind to the *CXCL12* promoter in the *c-myb*-overexpressed cells. Bioinformatic analysis of the 5′-flanking region of the human *CXCL12* gene showed that there existed a c-myb-binding site (TT**CAGTTC**) in the *CXCL12* promoter region from −396 to −389. It has been reported that c-myb could bind to the c-myb-binding site (CAGTTC) in GSTP1 promoter [26]. In our study, point mutant of c-myb-binding site in the *CXCL12* promoter construct abrogated the activation effect of c-myb on *CXCL12* promoter activity, indicating that c-myb activated *CXCL12* promoter activity by directly binding to the c-myb-binding site of *CXCL12* promoter.

In the following experiments, we found that *c-myb* siRNA attenuated CXCL12 secretion and *CXCL12* promoter activity in T47D and MCF-7 cells; at the same time, c-myb siRNA attenuated the binding of c-myb to the *CXCL12* promoter. These results further showed that c-myb could affect *CXCL12* promoter activity by binding to the *CXCL12* promoter.

We conclude that c-myb plays an important role in inducing *CXCL12* promoter activity by directly binding to the *CXCL12* promoter. These investigations are important and offer potential for defining angiogenic mechanism regulated by CXCL12 and c-myb. With this information it will be possible to demarcate potential targets and define appropriate reagents, such as antisense or small molecule antagonists, for inhibiting or preventing cancer progression and metastasis.

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References

1 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995, 1: 27–31.
2 Battegay EJ. Angiogenesis: mechanistic insights, neovascular diseases, and therapeutic prospects. J Mol Med 1995, 73: 333–346.
3 Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME and McClanahan T, *et al*. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001, 410: 50–56.
4 Mashino K, Sadanaga N, Yamaguchi H, Tanaka F, Ohta M, Shibuta K and Inoue H, *et al*. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Res 2002, 62: 2937–2941.
5 Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG and Batchelor TT. Angiogenesis in brain tumors. Nat Rev Neurosci 2007, 8: 610–622.
6 Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R and Carey VJ, *et al*. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005, 121: 335–348.
7 Suzuki M, Mohamed S, Nakajima T, Kubo R, Tian L, Fujiwara T and Suzuki H, *et al*. Aberrant methylation of CXCL12 in non-small cell lung cancer is associated with an unfavorable prognosis. Int J Oncol 2008, 33: 113–119.
8 Sowińska A and Jagodzinski PP. RNA interference-mediated knockdown of DNMT1 and DNMT3B induces CXCL12 expression in MCF-7 breast cancer and AsPC1 pancreatic carcinoma cell lines. Cancer Lett 2007, 255: 153–159.
9 Wendt MK, Cooper AN and Dwinell MB. Epigenetic silencing of CXCL12 increases the metastatic potential of mammary carcinoma cells. Oncogene 2008, 27: 1461–1471.
10 Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME and Capla JM, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004, 10: 858–864.
11 Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME and Capla JM, *et al*. Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue. Trends Cardiovasc Med 2005, 15: 57–63.
12 García-Moruja C, Alonso-Lobo JM, Rueda P, Torres C, González N, Bermejo M and Luque F, *et al*. Functional characterization of SDF-1 proximal promoter. J Mol Biol 2005, 348: 43–62.
13 Klempnauer KH, Ramsay G, Bishop JM, Moscovici MG, Moscovici C, McGrath JP and Levinson AD. The product of the retroviral transforming gene v-myb is a truncated version of the protein encoded by the cellular oncogene c-myb. Cell 1983, 33: 345–355.
14 Plaza S, Turque N, Dozier C, Bailly M and Saule S. c-myb acts as transcriptional activator of the quail PAX6 (PAX-QNR) promoter through two different mechanisms. Oncogene 1995, 10: 329–340.
15 O'Rourke JP and Ness SA. Alternative RNA splicing produces multiple forms of c-myb with unique transcriptional activities. Mol Cell Biol 2008, 28: 2091–2101.
16 Lei W, Liu F and Ness SA. Positive and negative regulation of c-myb by cyclin D1, cyclin-dependent kinases, and $p27^{Kip1}$. Blood 2005, 105: 3855–3861.
17 Tahirov TH, Sato K, Ichikawa-Iwata E, Sasaki M, Inoue-Bungo T, Shiina M and Kimura K, *et al*. Mechanism of c-Myb-C/EBP beta cooperation from separated sites on a promoter. Cell 2002, 108: 57–70.
18 Andersson KB, Kowenz-Leutz E, Brendeford EM, Tygsett AH, Leutz A and Gabrielsen OS. Phosphorylation-dependent down-regulation of c-myb DNA binding is abrogated by a point mutation in the v-myb oncogene. J Biol Chem 2003, 278: 3816–3824.
19 Kitagawa K, Kotake Y and Kitagawa M. Ubiquitin-mediated control of oncogene and tumor suppressor gene products. Cancer Sci 2009, 100: 1374–1381.
20 Introna M, Luchetti M, Castellano M, Arsura M and Golay J. The myb oncogene family of transcription factors: potent regulators of hematopoietic cell proliferation and differentiation. Semin Cancer Biol 1994, 5: 113–124.
21 Thompson MA and Ramsay RG. Myb: an old oncoprotein with new roles. BioEssays 1995, 17: 341–350.

22 Ramsay RG, Friend A, Vizantios Y, Freeman R, Sicurella C, Hammett F and Armes J, *et al*. Cyclooxygenase-2, a colorectal cancer nonsteroidal anti-inflammatory drug target, is regulated by c-myb. Cancer Res 2000, 60: 1805–1809.

23 McHale K, Tomaszewski JE, Puthiyaveettil R, Livolsi VA and Clevenger CV. Altered expression of prolactin receptor-associated signaling proteins in human breast carcinoma. Mod Pathol 2008, 21: 565–571.

24 Drabsch Y, Hugo H, Zhang R, Dowhan DH, Miao YR, Gewirtz AM and Barry SC, *et al*. Mechanism of and requirement for estrogen-regulated MYB expression in estrogen-receptor-positive breast cancer cells. Proc Natl Acad Sci USA 2007, 104: 13762–13767.

25 Fang F, Rycyzyn MA and Clevenger CV. Role of c-myb during prolactin-induced signal transducer and activator of transcription 5a signaling in breast cancer cells. Endocrinology 2009, 150: 1597–1606.

26 Rønneberg JA, Tost J, Solvang HK, Alnaes GI, Johansen FE, Brendeford EM and Yakhini Z, *et al*. GSTP1 promoter haplotypes affect DNA methylation levels and promoter activity in breast carcinomas. Cancer Res 2008, 68: 5562–5571.