CCCTC-Binding Factor Controls Its Own Nuclear Transport *via* Regulating the Expression of Importin 13

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CCCTC-binding factor (CTCF), a multivalent zinc-finger protein, is involved in different aspects of regulation including promoter activation or repression, gene silencing, chromatin insulation, gene imprinting, X-chromosome inactivation, cell growth or differentiation and tumor genesis. However, the molecular mechanisms of CTCF nuclear import remains unclear. In this study, we showed that the expression of CTCF influenced the intracellular distribution of itself, which might go through transport receptor - import 13 (IPO13). We further confirmed that there is a CTCF target site in *ipo13* -774~-573 bp promoter region and CTCF regulates the expression of IPO13. Besides, GST pull-down and Co-IP experiments demonstrated that CTCF interacts with IPO13. Immunofluorescence staining showed that IPO13 influenced intracellular distribution of CTCF. In all, we conclude that CTCF regulates the expression of IPO13, which, in turn, mediates the nuclear import of CTCF.

INTRODUCTION

CCCTC-binding factor (CTCF) is a ubiquitously expressed and evolutionarily conserved zinc finger (ZF) protein that binds target sites through combinatorial use of its 11 ZFs in eukaryote, which plays diverse roles including promoter activation or repression, gene silencing, chromatin insulation, gene imprinting and X-chromosome inactivation (Bell et al., 1999; Chao et al., 2002; Klenova et al., 2002; Lutz et al., 2000; Szabo et al., 2000; Zlatanova and Caiafa, 2009). As an important transcription regulation factor interrelated to cell proliferation and tumorigenesis, CTCF nuclear translocation is an important process. However researchers concentrated their attention to the function of CTCF, the molecular mechanism by which CTCF enters the nucleus is unknown.

Previous researches indicate that CTCF is localized in the nucleus in the majority of cells, independently of its phosphorylation state (Klenova et al., 1993; 2001). The CTCF subcellular distribution during the cell cycle is dynamic and it has been found associated with mitotic chromosomes, mitotic centro-

somes as well as the midbody of the cytokinesis, suggesting different roles for CTCF at different stages of the cell cycle (Burke et al., 2005; Zhang et al., 2004). Besides, Torrano et al. (2006) found nucleolar localization of CTCF is associated with growth arrest through a poly(ADP-ribosyl)ation-dependent mechanism. Further, investigations of CTCF expression pattern in 344 cases of invasive breast carcinoma showed that intracellular distribution of CTCF is significantly correlated with the histological grades. Cytoplasmic distribution was associated with grade III tumors and vascular invasion (Rakha et al., 2004). Our previous studies also showed that CTCF was mostly located in nucleus in grade I and II tumors. However, CTCF was mostly located in the cytoplasm in grade III tumors. Interestingly, Docquier et al. found that CTCF levels were elevated in 15 breast cancer cell lines compared to normal human breast cell lines (Docquier et al., 2005). However, the CTCF expression level was not consistent in these breast cancer cell lines. CTCF expression level in highly invasive breast cancer cells was much lower than that in the low invasive breast cancer cells. Therefore, we hypothesize that the expression level of CTCF affects the intracellular distribution of CTCF, which, in turn, influences the invasive capability of tumor cells. With regard to the mechanism by which CTCF expression influences its intracellular distribution, we speculate that CTCF regulates the expression of one or several proteins that are related to the transport of proteins with high molecular weight, which consequently regulates the nuclear import of CTCF. To identify the target genes regulated by CTCF, we used the CHIP-on-chip technique, and detected a target gene encoding Importin 13 (IPO13) which is one of members of Karyopherins superfamily which plays an important role in nucleocytoplasmic transport.

Nucleocytoplasmic transport is an essential activity in eukaryotic cells. For example, mRNAs and tRNAs are produced in the nuclear compartment and need to be exported to the cytoplasm where they function in translation. Conversely, all nuclear proteins are imported from the cytoplasm where they are produced. Nuclear transport proceeds through nuclear pore complexes (NPCs). Small molecules go through NPCs directly, while molecules larger than 50 kDa have to be actively transported via soluble nuclear transport receptors (NTRs) bind spe-

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cific amino acid residues or sequence elements within the cargo protein (Cook et al., 2007; Görlich and Kutay, 1999; Terry et al., 2007; Weis, 2003). Members of Karyopherins superfamily are important NTRs. Almost nuclear transport pathways except mRNAs are mediated by Karyopherins superfamily with 14 family members in the yeast *Saccharomyces cerevisiae* and probably > 20 in human. These family members are evolutionarily conservative with similar molecular mass, isoelectric point and structural domain. Karyopherins superfamily is comprised by 2 subclasses: Karyopherins α and Karyopherins β . Family members of Karyopherins α (Importin α or IMP α) mainly act as adapters in nucleocytoplasmic transport; while family members of Karyopherins β are responsible for nucleocytoplasmic transport. Some members of Karyopherins β (Importin β or IMP β) mediate nuclear import, and others (Exportin β or EXP β) mediate nuclear export. Most members are just responsible for one-way transport, except Msn5 in the yeast *Saccharomyces cerevisiae* and IPO13 in human, which for both of nuclear import and export (Mattaj and Englmeier, 1998; Na-kielny and Dreyfuss, 1999; Wozniak et al., 1998).

As one of members of Karyopherins superfamily, it has been known that IPO13 mediates nuclear import of NF-Y, myopodin, hUBC9, RBM8(Y14)-MGN, GR (glucocorticoid receptor) and nuclear export of eIF-1A (Bono et al., 2010; Giagtoglou et al., 2009; Kahle et al., 2005; Liang et al., 2008; Tao et al., 2006; Walker et al., 2009). Therefore, we hypothesized that IPO13 might also mediate the nuclear import of CTCF. In this paper, our results demonstrates that there is a CTCF target site in *ipo13* -774--573 bp promoter region and CTCF regulates the expression of IPO13. GST pull-down and Co-IP experiments demonstrates that CTCF interacts with IPO13. Immunofluorescence staining shows that IPO13 influenced intracellular distribution of CTCF. We conclude that CTCF regulates the expression of IPO13, which, in turn, mediates the nuclear import of CTCF.

MATERIALS AND METHODS

Cell lines

Human breast cancer cell lines (MDA-MB-453, MDA-MB-231, MDA-MB-157, BT474, MCF7, MCF7B and LCC1), human hepatoma carcinoma cell lines (SMMC-7721 and HepG2), human uterine cervix cancer cell line (HeLa) and human embryo kidney cell line (293FT) were purchased from the cell culture center of Institute of Basic Medical Sciences (IBMS). MDA-MB-231 and MDA-MB-157 cell lines were maintained in L-15 medium supplemented with 15% fetal calf serum (FCS). SMMC-7721 cell line was maintained in RPMI 1640 medium supplemented with 10% FCS, and other cell lines were maintained in DMEM medium supplemented with 10% FCS (all from GIBCO). These cell lines were grown in a humidified 5% CO₂-containing atmosphere at 37°C.

Expression vectors, siRNA and primers

The plasmids expressing CTCF protein (pCMV6-XL5-CTCF) and IPO13 protein (pCMV-SPORT6-IPO13) were purchased from ORIGENE. Glutathione-S-transferase (GST) fusion with IPO13 was generated by inserting ORF of *ipo13* (L: 5'-TTGAATTCGAGCGGCGGGAGGAGCAGCCG-3'; R: 5'-GCCTCAGATTATCAGTAGTCAGCTGTGTAA-3'; *EcoRI* and *XhoI*) into pGEX-4T-1 vector (Amersham Biosciences). The plasmid expressing CTCF protein in 293FT was constructed by inserting the ORF of *ctcf* (L: 5'-CCGGAATTCGGATGGAAGGTGATGCAGTCGA-3'; R: 5'-AGAAATGCGGCCGCTCACCGGTCC

ATCATGCTGA-3'; *EcoRI* and *NotI*) into pCMV-HA.

The oligonucleotide sequences for RNAi of *ctcf* and *ipo13* were shown below. The preparation for siRNA was described previously (Zhu et al., 2005).

luciferase : caCTTACGCTGAGTACTTCTGA
CTCF #1 : aaGGAAAGGAGAGAAAGACTT
CTCF #2 : aaCGTCACATTCGCTCTCATA
IPO13 #1 : aaGTAGAGCACTGGCAGAGTT
IPO13 #2 : aaGGAGCAGTTCCGAATTTAC
IPO13 #3 : aaGTGGAGGAGATCCTTAAGA

The primers for RT-PCR were shown below:

CTCF L 5'-CTTGCGAAAGCAGCATTCCTA-3'
CTCF R 5'-GGCATAGCGCTCATGAAACAC-3'
IPO13 L 5'-CTGCCTGAGGAGTTCAGAC-3'
IPO13 R 5'-CTCCACACTGCTGTCCGAAGA-3'
Beta-actin L 5'-CTGACTGACTACCTCATGAAG-3'
Beta-actin R 5'-ATCCACATCTGCTGGAAGGTG-3'

GST pull-down

GST fusion proteins were incubated with magnetic glutathione particles (Promega, USA), which were balanced in 1× PBS (pH 7.4) for 0.5 h at room temperature. After washing, beads were incubated with CTCF, which was over expressed in 293FT by transient transfection, for 1 h at room temperature. After washing, proteins on beads were eluted by boiling in 1× SDS-PAGE sample buffer and examined by Western blot.

Co-immunoprecipitation (Co-IP)

Extracts of 293FT cells transfected with plasmids encoding CTCF and IPO13, or HepG2 cells were prepared in RIPA lysis buffer containing 50 mmol/L Tris (pH 7.6), 150 mmol/L sodium chloride, 1% Triton X-100 and 2 mmol/L PMSF. The cell lysate was pre-cleaned by incubating with 50 μ l Protein G-Agarose (Roche) at 4°C for at least 3 h. The pre-cleaned cell lysate was incubated with 5 μ g anti-CTCF antiserum or anti-IPO13 polyclonal antibody or Normal Rabbit IgG at 4°C for 1 h. Subsequently, 50 μ l Protein G-Agarose was added and incubated at 4°C for approximately 16 h. The beads were spun down, washed with Wash Buffer I, II, III (Roche) for several times. Finally, proteins on the beads were eluted by boiling in 1× SDS-PAGE sample buffer and examined by Western blot.

Chromatin immunoprecipitation (CHIP)

CHIP was performed using CHIP-IT Express Enzymatic Kit (Active Motif) following the manufacturer's instruction. Using a sonicator with 25% power, chromatin was sheared by ten pulses (20 s for each pulse) with a 30-second rest on ice between each pulse. CHIP reaction contained 25 μ l Protein G Magnetic Beads, 10 μ l CHIP Buffer 1, 0.6 μ g sheared chromatin, 1 μ l PIC, 3 μ g antibody (anti-CTCF antiserum or Normal Rabbit IgG), and dH₂O to achieve a total volume of 100 μ l.

The primers used for CHIP-PCR were shown below:

IPO13 5L 5'-AGCAAAGTCCTGCTCCTAGC-3'
IPO13 5R 5'-CACCACCCAGAGAAGAGGAG-3'

Immunofluorescence (IF)

Transfected cells were trypsinized into cell suspension, and then transferred to glass slide in a culture dish. After 24 h, the cells were fixed in fixation solution (methanol: acetone = 1:1) for 10 min at room temperature. After washing with 1× PBS, the cells were incubated with primary antibody (anti-CTCF antiserum, 1:200 in 3% BSA/PBS) for overnight at 4°C. After washing with 1× PBS, the cells were incubated with secondary antibody

Fig. 1. The intracellular distribution of CTCF is correlated with the CTCF expression level. (A) The CTCF protein level in low invasive cells was much higher than that in the highly invasive cells. (B) CTCF was mostly located in cellular nucleus of low invasive BT474 and MDA-MB-453 cells, however, in cytoplasm of highly invasive MDA-MB-157 and MDA-MB-231 cells. Green fluorescence illustrates location of CTCF in cells; Blue fluorescence illustrates location of nucleus stained by DAPI; Merge means the overlay effect of Green fluorescence and Blue fluorescence. (C) Knocking down the CTCF expression level in low invasive SMMC-7721, HepG2 and HeLa cells decreased the distribution ratio of CTCF in nucleus and increased the distribution ratio in cytoplasm. Green fluorescence illustrates location of CTCF in cells; Red fluorescence illustrates location of cellular nucleus stained by PI; Merge means the overlay effect of Green fluorescence and Red fluorescence.

(FITC-labeled Goat Anti-Rabbit IgG, 1:50 in 3% BSA/PBS) for 2 h at room temperature. Subsequently, the cells were incubated with DAPI (1 μ g/ml in 1 $\times$ PBS) or PI (10 μ g/ml in 1 $\times$ PBS) for 15 min at room temperature in the environment protected from light. After 4 times of washes with 1 $\times$ PBS, the cells were observed by confocal microscopy.

RESULTS

Intracellular distribution of CTCF is correlated with the CTCF expression level

According to the introduction, the CTCF expression level was not consistent in breast cancer cell lines, while CTCF expression level in highly invasive breast cancer cells was much lower than that in the low invasive breast cancer cells. We hypothesize that the expression level of CTCF affects the intracellular distribution of CTCF, which, in turn, influences the invasive capability of tumor cells. To confirm the statement, we detected the CTCF protein levels in highly invasive cell lines (MDA-MB-157, MCF7B, MDA-MB-231 and LCC1) and low invasive cell lines (MDA-MB-453, BT474, and MCF7 cells) (Fig. 1A). The results confirmed that the CTCF expression level in low invasive cells was much higher than that in the highly invasive cells (Fig. 1A). Whether the expression level of CTCF affects the intracellular distribution of CTCF, immunofluorescence analysis showed that CTCF was mostly localized in the nucleus of low invasive cells (BT474 and MDA-MB-453), however in the cytoplasm of highly invasive cells (MDA-MB-157 and MDA-MB-231) (Fig. 1B). These results indicated intracellular localization of CTCF might be correlated with the CTCF expression level. To test this possibility, we determined the intracellular distribution of CTCF after knocking down the CTCF protein level by transient transfection. The results showed that knocking down the

CTCF expression level in low invasive cells (SMMC-7721, HepG2 and HeLa) reduced the distribution ratio of CTCF in the nucleus, but increased the distribution ratio in the cytoplasm (Fig. 1C). These results confirmed that the CTCF expression level indeed influences its intracellular distribution. We hypothesized that reduction of CTCF expression level might down-regulate the expression of one or multiple proteins that are related to the transport of proteins with high molecular weight, which results in the inhibition of the CTCF nuclear import.

CTCF binds *ipo13* promoter and regulates the IPO13 expression

To identify the target genes regulated by CTCF, we used the CHIP-on-chip technique and found that *ipo13* encoding Importin 13 (IPO13) was a target gene (the result wasn't shown). To avoid false positives, CHIP-PCR was performed using the primers of *ipo13* -774~-573 bp promoter region. And it was confirmed that CTCF bound *ipo13* promoter region in MDA-MB-453, SMMC-7721, HepG2 and HeLa cells (Fig. 2A). The CTCF binding site was located in *ipo13* -774~-573 bp promoter region. However, could transcription factor CTCF which binds *ipo13* promoter regulate the expression of target gene- *ipo13*? To address this question, CTCF was over-expressed in MDA-MB-157 by transient transfection. Following the increases of CTCF, IPO13 transcription and translation were increased as determined by real time-PCR (Fig. 2B) and Western blot (Fig. 2C). Besides, knocking down the CTCF expression in HepG2, SMMC-7721 and HeLa cells by transient transfection decreased IPO13 protein level as determined by Western blot (Fig. 2D). These results demonstrated that CTCF regulate the expression of IPO13 probably via binding *ipo13* -774~-573 bp promoter region.

Fig. 2. CTCF binds the ipo13 promoter and regulates the IPO13 expression. (A) The outcome of CHIP-PCR. (B) Over-expression of CTCF by transient transfection up regulated the expression of IPO13 MDA-MB-157 and MDA-MB-231 cells as determined by real time-PCR. (C) Over-expression of CTCF by transient transfection in MDA-MB-157 up regulated the expression of IPO13 in translational level. (D) Knocking down CTCF expression using siRNA down-regulated the expression of IPO13 in translational level.

Fig. 3. IPO13 interacts with CTCF. (A, B) The results of Co-immunoprecipitation experiments with overexpression of CTCF and IPO13 in 293FT. (C, D) The results of Co-immunoprecipitation experiments using lysate of HepG2. (E, F) CTCF and CTCF-M could be precipitated by IPO13 and IPO13-N as determined by GST pull-down experiment. (G) Illustrations for the deletion mutants of IPO13 and CTCF.

Fig. 4. IPO13 mediates the nuclear import of CTCF. (A) Knocking down the IPO13 expression using siRNA in HepG2 and SMMC-7721 cells. (B) Over-expression IPO13 in MDA-MB-157 by transient transfection. (C) When IPO13 was over expressed in MDA-MB-231 and MDA-MB-157 cells, the distribution ratio of CTCF in nucleus was increased and the distribution ratio in cytoplasm was decreased. (D, E) When the IPO13 expression was knocked down in HepG2, SMMC-7721 and HeLa cells, the distribution ratio of CTCF in nucleus was decreased and the distribution ratio in cytoplasm was increased.

IPO13 interacts with CTCF

According to the introduction, we hypothesized that IPO13 might mediate the nuclear import of CTCF. However, Import of proteins with high molecular weight into nucleus normally requires interaction with their transport receptors. Therefore, we hypothesized that nucleus import of CTCF was mediated by interaction between CTCF and IPO13. Co-IP analysis by over expressing CTCF and IPO13 in 293FT cells showed that CTCF interacted with IPO13 *in vivo* (Figs. 3A and 3B). Co-IP using HepG2 cell lysate also detected physical interaction between CTCF and IPO13 (Figs. 3C and 3D). To detect the binding region, two deletion mutants of IPO13 (IPO13-N, IPO13-C) were expressed in fusion with GST in *E. coli* and three deletion mutants of CTCF (CTCF-N, CTCF-M, CTCF-C) were over expressed in 293FT with HA-tag (Fig. 3G). According to the GST pull-down assays, N-terminal portion of IPO13 and middle part of CTCF contribute to the interaction (Figs. 3E and 3F). The interaction between CTCF and IPO13 provided physical foundation for the nuclear import of CTCF.

IPO13 mediates the nuclear import of CTCF

To determine if IPO13 regulates nuclear import of CTCF, we modulated the IPO13 expression level by transient transfection (Figs. 4A and 4B) and detected intracellular distribution of CTCF by immunofluorescence staining. The results showed that over expression of IPO13 in MDA-MB-231 and MDA-MB-157 cells increased the distribution ratio of CTCF in nucleus, but reduced the distribution ratio in cytoplasm (Fig. 4C), indicating that over expression of IPO13 promoted the nuclear import of CTCF. In contrast, knocking down the IPO13 expression in HepG2, SMMC-7721 and HeLa cells reduced the distribution ratio of CTCF in nucleus, but enhanced the distribution ratio in cytoplasm (Figs. 4D and 4E), indicating that knocking down the IPO13 expression inhibited the nuclear import of CTCF. To further determine the mechanisms by which CTCF nuclear import is mediated by IPO13, we knocked down CTCF expression and over expressed IPO13 simultaneously in HeLa and SMMC-7721 cells. Consistent with the results shown in Fig. 1, knocking down the CTCF expression level reduced the distribu-

Fig. 5. The outcome of rescue experiment in HeLa and SMMC-7721 cells. (A) The results of immunofluorescence. Vector, cells were transfected with control vector; IPO13, cells were transfected with pCMV-SPORT6-IPO13 to over express IPO13; siControl, cells were transfected with luciferase siRNA as control; siCTCF#1, cells were transfected with CTCF siRNA-1; siCTCF#2, cells were transfected with CTCF siRNA-2; Green fluorescence illustrates location of CTCF in cells; Red fluorescence illustrates location of cellular nucleus stained by PI; Merge means the overlay effect of Green fluorescence and Red fluorescence. (B, C) The histograms of HeLa and SMMC-7721 cells. The blue bar means the ratio of cells in which CTCF was mostly located in cellular nucleus. The prunus bar means the ratio of cells in which CTCF was mostly located in cytoplasm. 1, Vector + siControl; 2, IPO13 + siControl; 3, Vector + siCTCF#1; 4, IPO13 + siCTCF#1; 5, Vector + siCTCF#2; 6, IPO13 + siCTCF#2.

Fig. 6. The possible mechanism of nuclear import of CTCF. CTCF controls its own nuclear transport by regulating the expression of IPO13.

tion ratio of CTCF in nucleus and increased distribution ratio in cytoplasm (Fig. 5A). However, in cells with simultaneous over expression of IPO13 and knocking down of CTCF, CTCF was mostly localized back to the nucleus (Fig. 5A). Because of the limited transfection efficiency, the intracellular distribution of CTCF was not changed in all cells. In order to quantitatively demonstrate the effect of transient transfection on intracellular distribution of CTCF, we randomly counted over 100 cells for each sample, and calculated the percentage of cells with nuclear or cytoplasm localization of CTCF. CTCF was located in

nucleus in the control cells and cells with over expression of IPO13 (Fig. 5B). Knocking down the CTCF expression reduced the distribution ratio of CTCF in nucleus (more than 60% cells have cytoplasm localization of CTCF). However, in cells with simultaneous over expression of IPO13 and knocking down of CTCF, CTCF was located in the nucleus of approximately 70% of the cells, which indicated IPO13 promoted the distribution of CTCF back into the nucleus in more than 30% cells (Figs. 5B and 5C). These results indicated that over expression of IPO13 can compensate the effect of CTCF knocking down on the

localization of CTCF.

DISCUSSION

As a multivalent transcriptional factor, CTCF plays an important role in promoter activation or repression, gene silencing, chromatin insulation, gene imprinting, X-chromosome inactivation and regulation of cell growth or differentiation. Dysfunction of CTCF can lead to the occurrence of multiple malignant tumors (Ohlsson et al., 2001; 2009). CTCF has to enter the nucleus through nuclear pore complex (NPC) to perform these functions. Abnormal transport of CTCF between nucleus and cytoplasm may result in the dysfunction of CTCF. It was reported that cytoplasmic distribution of CTCF was associated with grade III tumors (Rakha et al., 2004). Therefore, nucleocytoplasmic transport is vital for CTCF to achieve its appropriate functions. However, the mechanisms by which CTCF enters the nucleus are still unclear.

To elucidate the regulation network of CTCF, we used the CHIP-on-chip technique discovered that *ipo13* is one of the target genes. Importin 13 (IPO13) encoded by *ipo13* is one of Karyopherins superfamily members and mediates large protein transport between cytoplasm and nucleus. It has been known that IPO13 mediates nuclear import of NF- κ B, myopodin, hUBC9, RBM8(Y14)-MGN, GR (glucocorticoid receptor) and nuclear export of eIF-1A. Our results demonstrated that IPO13 can also mediate the nuclear import of CTCF (Fig. 4). Our results also showed CTCF can regulate the expression of IPO13 via binding to *ipo13* -774~-573 bp promoter region. Furthermore, we detected the direct interaction between CTCF and IPO13 using GST pull-down and Co-IP (Fig. 3), which supplied physical foundation to the nuclear import of CTCF. Over expression of IPO13 promoted the nuclear import and knocking down the IPO13 expression inhibited the nuclear import of CTCF. These results revealed a potential novel mechanism of nuclear import of CTCF (Fig. 6). In the cytoplasm, IPO13 recognizes and binds the CTCF, which results in the transport of CTCF into the nucleus. Within the nucleus, IPO13 was separated from CTCF and the released CTCF binds -774~-573 bp promoter region of *ipo13* to regulate the expression of IPO13. Therefore, we propose a novel mechanism in which CTCF controls its own nuclear transport by regulating the expression of IPO13.

We hypothesize that the intracellular distribution of CTCF might be correlated with the CTCF expression level, and the intracellular distribution of CTCF might influence the invasive capability of tumor cells. When the CTCF expression level is low, CTCF is mostly located in cytoplasm, and the invasive capability of tumor cells is strong. In contrast, when the CTCF expression level is high, CTCF is mostly located in the nucleus, and the invasive capability of tumor cells is weak. The invasion and migration of tumor is a multi-procedure and multistage process including shedding from tumors from primary focus of infection, degradation of extracellular matrix (ECM), invasion into the blood vessel or absorbent vessel, transferring through the circulation system, escaping from the blood vessel or absorbent vessel, and formation of fresh focus of infection through proliferation and angiogenesis. This process requires complex molecular mechanisms including the participation of multiple cytokines, growth factors, adhesion factors and proteases (Lash et al., 2006; Mihaljevic et al., 2010; Nerlich et al., 2009; Wong and Gumbinger, 2003). Through wide-scale screening for CTCF binding site by CHIP, CTCF might also regulate the expression of proteins similar to cadherin like 26 and MMP23B (Arpanahi et al., 2009; Mukhopadhyay et al., 2004). Cadherin plays a role

in adhesion between cells or between cells with ECM. Functional disorder of Cadherin results in the decreased cell adhesion, increased cell migration and reduced cell contact inhibition. Consequently, tumor cell growth is enhanced and invasion of peripheral tissues occurs (Davies et al., 2001; Fransvea et al., 2008; Hsu et al., 2000). MMP23B belongs to the superfamily matrix metalloproteinases (MMPs) which possesses more than 20 members. MMPs is a class of endogenous proteases that are recognized as major critical molecules in tumor invasion and degradation of ECM, natural barrier of tumor invasion (Poincloux et al., 2009; Woenne et al., 2010). It has been known that MMP23B promotes liver development and hepatocyte proliferation through the tumor necrosis factor pathway in zebrafish (Qi et al., 2010). These results indicate that CTCF might influence the invasion capability of tumor cells through regulating the expression of Cadherin and MMPs.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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