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Correlation between cellular localization of TEL/AML1 fusion protein and repression of AML1-mediated transactivation of CR1 gene

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Abstract

Human chromosome translocation t(12;21)(p12;q22) is the most frequent chromosome rearrangement in childhood B-lineage acute lymphoblastic leukemia (ALL), and produces the TEL/AML1 fusion protein. The chimeric protein, TEL/AML1 contains the first 336 amino acids of TEL that is linked to residues 21–480 of AML1 and the fusion protein is generally known as a transcription repressor to the various target genes. Furthermore, TEL/AML1 has been shown to interfere with AML1-mediated transactivation on the CR1 gene. To understand the mechanism of the TEL/AML1-mediated repression, we used transient-transfection assay and immunofluorescence to monitor subcellular localization of TEL/AML1. Here, we show that TEL/AML1 is localized in the cytoplasm and the transcriptional activities of CR1 promoter are affected by the subcellular localization of TEL/AML1 fusion protein.

Keywords: TEL/AML1; Subcellular localization; Transcription; Leukemia; CR1

The fusion gene, TEL/AML1 is generated by the chromosomal translocation between chromosomes 12 and 21 associated with acute lymphoblastic leukemia. This gene encodes a chimeric protein, TEL/AML1 contains the 336 amino-terminal region of an Ets family protein, TEL, fused to the residues 21–480 of tissue-specific transcription factor, AML1 [1–3]. TEL and AML1 are also associated with other various chromosomal translocations including AML1/Evi1, AML1/MDS1, AML1/EAP [4,5], AML1–ETO [6,7], and TEL–PDGFRβ [8]. As both TEL and AML1 are putative transcription factors, one might speculate that the acquisition of the t(12;21) alters the regulation of hematopoietic-specific gene expression that results in unregulated growth of leukemic cells. In addition, transgenic mice heterozygously knocked-in with chimeric genes have been shown to be defective in definitive hematopoiesis [9,10]. These observations have led to the general notion that the formation of chimeric genes is primarily responsible for leukemogenesis.

Previously, others and we showed that the TEL/AML1 fusion protein act as a repressor for the transcription of various target genes and interferes with AML1-mediated transactivation [11–14]. However, the transcription repression mechanism of TEL/AML1 is not clear and although some possible mechanisms are suggested to explain the negative effect of the chimeric TEL/AML1 protein, no direct biochemical evidence has been demonstrated. Here we show that the RUNT and HLH domains of the TEL/AML1 fusion protein are required for inhibiting AML1-mediated transactivation of CR1 gene and the cellular localization of TEL/AML1 fusion protein and repression of AML1-mediated transactivation by fusion protein is correlated.

Materials and methods

Cell culture. HEL (human erythroleukemia) cells were maintained in RPMI1640 containing 10% fetal bovine serum and ECV304 (human endothelial cell) cells were cultured in Medium 199 with 10% fetal bovine serum. The cells were grown in an atmosphere of 5% CO_2 at 37 °C.

Plasmid construction. Mammalian expression plasmids for His-tagged AML1, TEL, and TEL/AML1 fusion proteins were previously

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constructed in our laboratory [14] and HA-tagged AML1 and TEL/AML1 expression vectors were provided by Bae, SC (Chungbuk National University, Cheongju, Korea). A series of deletion mutant of TEL/AML1 expression plasmid were constructed by using internal restriction enzyme sites and then subcloned into pcDNA3.1/His vector (Invitrogen). The lucifererase reporter pCR(Δ376) has already been described [15].

Transfection and luciferase assays. The HEL cells were transfected with a reporter plus expression plasmid via electroporation and the relative luciferase activities were assayed as described previously [15]. The compositions of the transfected plasmids are described in the figure legend. Transfection efficiencies were normalized to the level of β-galactosidase activities expressed from co-transfected pCMV-βgal plasmid containing cytomegalo-virus promoter directing bacterial β-galactosidase gene expression.

Immunofluorescence microscopy. HEL and ECV304 cells grown on coverslips were transfected with expression plasmid. Forty-eight hours after transfection, the cells were fixed and permeabilized with cold 100% methanol, followed by incubation with 500-fold diluted either mouse monoclonal His-tag antibodies or rabbit polyclonal HA-tag antibodies. For staining PEBP2β, 125-fold diluted mouse monoclonal antibodies against PEBP2β were incubated. The cells were stained by sequential incubation with fluorescence-labeled secondary antibodies and visualized by using a BIO-RAD MRC1024 confocal microscope (BIO-RAD Laboratories, Richmond, CA).

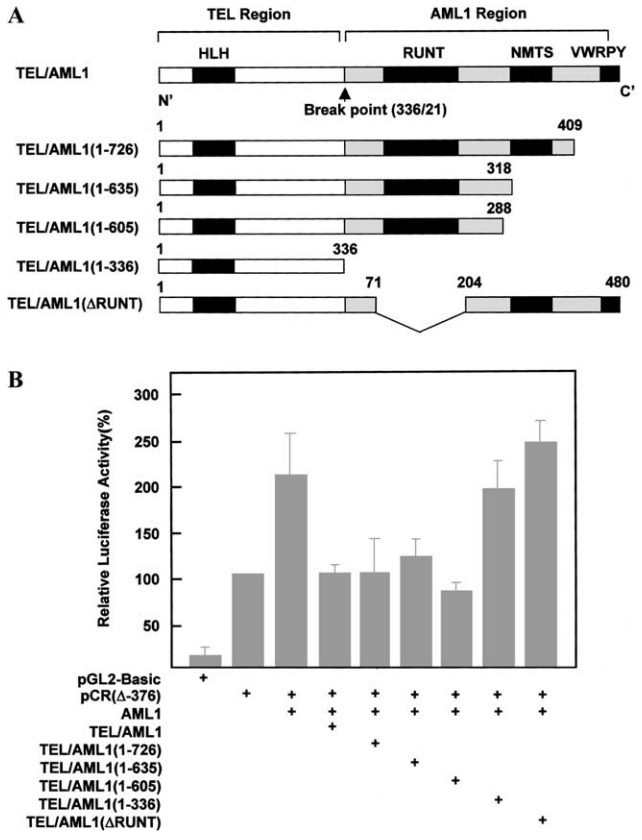

Fig. 1. The RUNT domain region of TEL/AML1 is required for TEL/AML1-mediated repression. (A) Schematic diagram of the structures of full length and deletion derivatives of the TEL/AML1 fusion protein. Numbers denote the positions of amino acids of each fusion partner. Highly conserved domains are denoted in the top line. The helix–loop–helix domain (HLH), DNA binding and CBF interacting domain (RUNT), nuclear localization signal (NLS), nuclear matrix targeting signal (NMTS), and conserved VWRPY penta-peptide are indicated. (B) Transcriptional repression by TEL/AML1 and deletion mutant proteins of TEL/AML1 to the AML1-mediated transactivation. HEL cells were transfected with the indicated plasmids and the luciferase activity was measured 48 later. Values were normalized to the levels of β-galactosidase expressed from CMV immediate-early promoter, which was used as an internal control for transfection efficiency. Relative luciferase activity represents the promoter activity from cells transfected with expression plasmid compared to those transfected pCR(Δ-376), which defined 100% activity.

Results and discussion

The RUNT and HLH domain regions of TEL/AML1 fusion protein are required for inhibit AML1-mediated transactivation

The TEL/AML1 fusion gene, created by the t(12:21), is the most common genetic alteration in childhood acute lymphoblastic leukemia. The TEL/AML1 fusion is unique in that the AML1 RUNT and transactivation domains are preserved in the chimeric protein and the fusion transcript is expressed from the TEL promoter [16].

To identify the region that is required for repression of transcription, we have generated serial deletion mu-

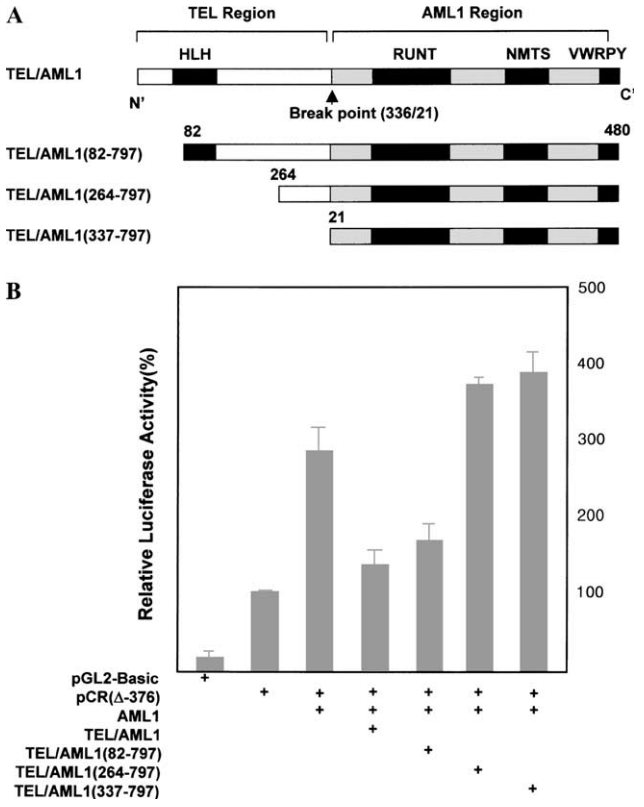

Fig. 2. The HLH domain region of TEL/AML1 is required for TEL/AML1-mediated repression. (A) Illustration of the N-terminal deletion mutants of TEL/AML1 used in these experiments. (B) The effect of N-terminal deletion of TEL/AML1 on the AML1-mediated transactivation was analyzed by transient transfection and luciferase assay. Transfected expression plasmids were indicated in the figure.

tants of TEL/AML1 and investigated their effects on the AML1-mediated transactivation of the CR1 gene. As shown in Fig. 1B, TEL/AML1, TEL/AML1 (1–726), TEL/AML1 (1–635), and TEL/AML1 (1–605) repressed the enhanced AML1-mediated transactivation. In contrast, TEL/AML1 (1–336) and TEL/AML1 (ΔRUNT) did not exhibit any significant effect on the AML1-mediated transactivation of CR1 gene. These results indicate that the RUNT domain is required for transcriptional repression by TEL/AML1 fusion protein. To determine the effect of N-terminal mutant of TEL/AML1 on AML1-mediated transactivation of CR1 gene, we also generated N-terminal deletion mutants, as shown in Fig. 2A and transfected them in HEL cells with pCR(Δ376) reporter plasmid. As shown in Fig. 2B, deletion of N-terminal 81 amino acid [TEL/AML1 (82–797)] had moderate effect on AML-mediated transactivation of CR1 gene, but deletion of HLH domain (TEL/AML1 (264–797) and TEL/AML1 (337–797) abolished the dominant interfering ability of TEL/AML1. Taken together, these results suggest that the RUNT and HLH domain regions of TEL/AML1 fusion protein are required for inhibiting AML1-mediated transactivation.

Subcellular localization of AML1, TEL, and TEL/AML1

An important but unanswered question is how TEL/AML1 interferes with the AML1-mediated transactivation without inhibiting the basal transcription activity.

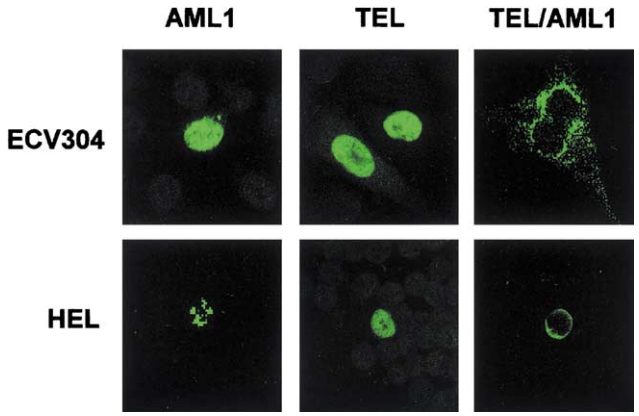

Fig. 3. Subcellular localization of AML1, TEL, and TEL/AML1 proteins. His-tagged AML1, TEL, and TEL–AML1 expression plasmids were transfected into either ECV304 or HEL cells, and then were detected the proteins with antibodies against His-tag, followed by staining with FITC-labeled secondary antibodies.

As attempt to answer the question, subcellular localization of the plasmids containing full-length coding sequences TEL, AML1, and TEL/AML1 was examined by transient transfection into HEL or ECV304 cells. Expression and localization of these proteins were detected by incubation with primary antibodies against His-tag, followed by incubation with fluorescein-labeled secondary antibodies.

Transiently expressed AML1 and TEL were localized completely in the nuclei with diffused pattern in ECV304

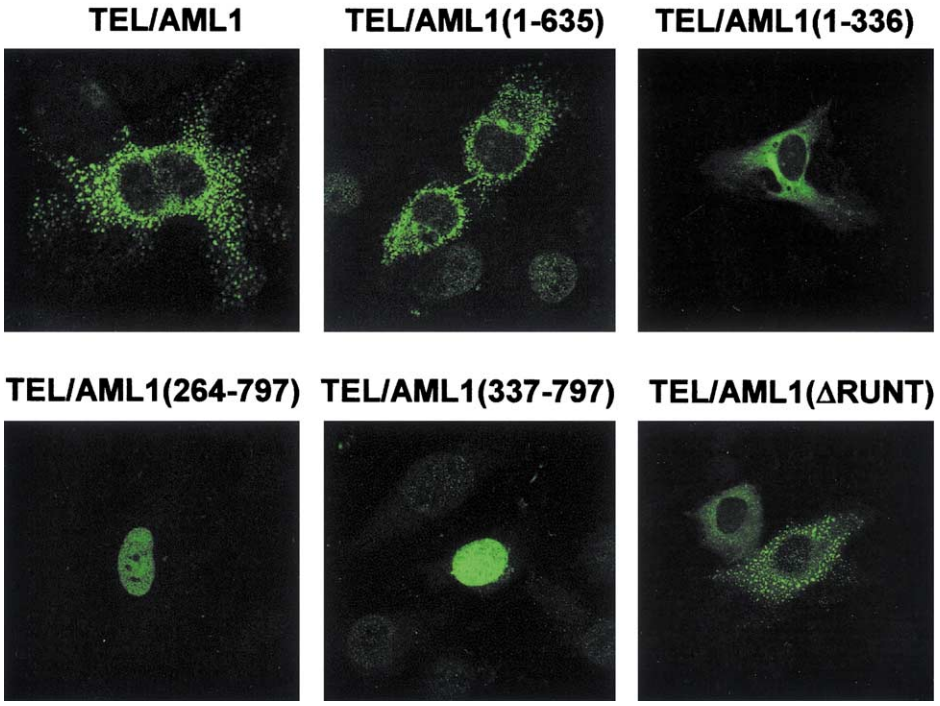

Fig. 4. Subcellular localization of TEL/AML1, TEL/AML1 (1–635), TEL/AML1 (1–336), TEL/AML1 (264–797), TEL/AML1 (337–797), and TEL/AML1 (ΔRUNT) in transiently transfected cells. Expression plasmids for TEL/AML1 and the deletion mutants were transfected into ECV304 and sequentially stained as described in Materials and methods.

and HEL cells (Fig. 3). These results are consistent with the previous findings that the TEL and AML1 proteins contain putative nuclear localization signal or NMTS (nuclear matrix targeting signal), respectively [17,18]. However, the TEL/AML1 fusion protein was found to exist in the cytoplasm of ECV304 and HEL cells (Fig. 3). This result was surprising, because the TEL/AML1 fusion protein retains the almost full length of AML1 protein, thus, containing NMTS. To further study, plasmids containing deletion mutant of TEL/AML1 [TEL/AML1 (1-635), TEL/AML1 (1–336), TEL/AML1 (264–797), TEL/AML1 (337–797), and TEL/AML1 (ΔRUNT)] as well as wild-type TEL/AML1 were transiently transfected into ECV304 cells and immunofluorescence labeling was carried out as described above. The TEL/AML1 and C-terminal deleted TEL/AML1 mutant proteins were detected in the cytoplasm (Fig. 4). However, the N-terminal mutant proteins that lack most of TEL region in the TEL/AML1 fusion protein were localized in the nucleus (Fig. 4). In addition, the TEL/AML1 (ΔRUNT), which lacked the RUNT domain of TEL/AML1 fusion protein, was detected in the cytoplasm. Next, we analyzed the TEL/AML1 effects on the subcellular localization of AML1, TEL, or PEBP2β proteins by co-transfection assay. As shown in Fig. 5, the TEL/AML1 fusion protein was localized in the cytoplasm constantly, in any combination with AML1, TEL, or PEBP2β proteins. The subcellular localization of TEL, however, was dramatically changed when the cells were co-transfected with TEL and TEL/AML1. The TEL proteins were moved to the cytoplasm by the TEL/AML1 fusion protein and the distribution of the TEL protein was co-localized with TEL/AML1 protein. This result could be explained by the fact that TEL and TEL/AML1 proteins multimerize and form a large complex and the aggregation of this complex inhibits nuclear localization of the TEL protein.

This analysis demonstrates that TEL/AML1 distributes in the cytoplasm and its localization is independent with that of AML1. Importantly, however, the subcellular localization of TEL/AML1 and its mutant protein appears well correlated with their ability in repressing AML1-mediated transcription.

To explain the negative effect of the chimeric TEL/AML1 protein, one possible mechanism is that TEL/AML1 recruits co-repressor complex such as histone deacetylase or blocks the recruitment co-activator molecule such as p300 within promoters of target genes and alters the regulation of gene expression. In this point of view, recent data from other laboratory showed that AML1 and TEL interact with mSin3A and the TEL sequences present in TEL/AML1 stabilize the interaction between AML1 and mSin3A [12]. In contrast, another laboratory suggests that the p300 co-activator molecule is able to bind to the transcriptional activating domain of AML1 and the fusion protein TEL/AML1

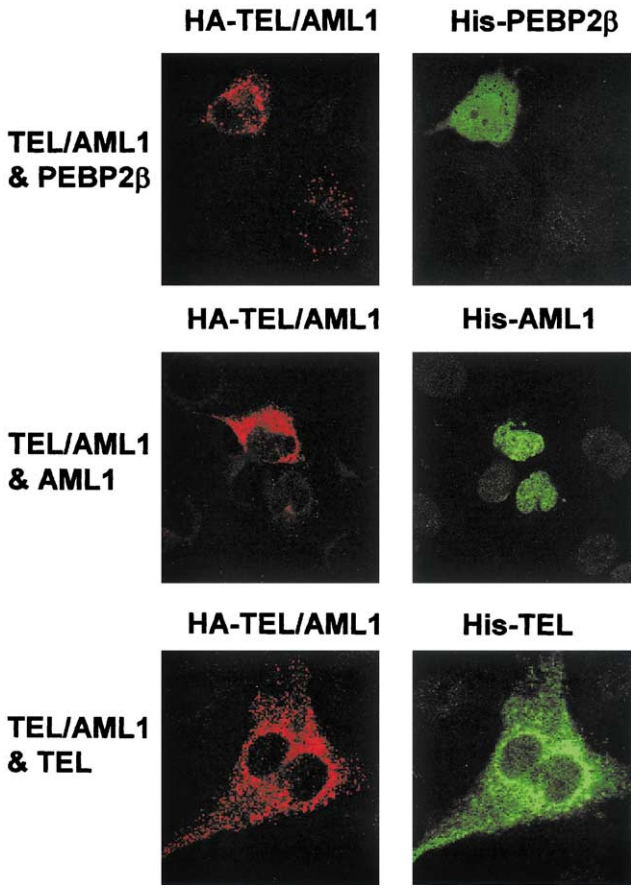

Fig. 5. Subcellular localization of AML1, TEL, or PEBP2β proteins in the presence of TEL/AML1 fusion protein. ECV304 cells were co-expressed with HA-tagged TEL/AML1 either His-tagged AML1, His-tagged TEL, or His-tagged PEBP2β by transient transfection. The expressed protein was detected. For staining the co-transfected cell, 1:1 mixture of His-tag mouse monoclonal antibodies and HA-tag rabbit polyclonal antibodies was incubated. The cells were stained by sequential incubation with 1:1 mixture of FITC-labeled mouse secondary antibodies and Rhodamine-labeled rabbit secondary antibodies. By using two different fluroscence-labeled secondary antibodies, TEL/AML1 localization is shown by the red signal, while the AML1, TEL, and PEBP2β are shown by the green fluorescent signal.

would prevent the interaction between AML1 and p300 co-activator molecules, resulting in decreasing the transcription activation by AML1 protein [13]. However, the other overlooked possible mechanism could explain the dominant negative effect of the TEL/AML fusion protein that TEL/AML1 sequestrating the co-activators into nonfunctional complex in the cytoplasm thus altered the regulation of transcription of target genes. Taken together, the most feasible interpretation to our results in this study is that TEL/AML1 interferes with AML1-dependent transactivation by cytoplamic sequestration of co-activator molecule or molecular chaperone, which may be limiting in cells. In support of this speculation, CBFβ–SMMHC fusion protein has been reported to sequester CBF in the cytoplasm [19,20]. Factors interact functionally with TEL/AML1 and

molecular mechanisms of cytoplasmic retention by TEL/AML1 wait to identify and characterize with a view of general understanding for molecular mechanisms of leukemogenesis mediated by the chimeric protein.

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