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Mechanisms of transcriptional repression by the t(8;21)-, t(12;21)-, and inv(16)-encoded fusion proteins

Abstract *AML-1* is one of the most frequently translocated genes in human leukemia. AML-1 binds DNA and activates or represses transcription, while the chromosomal translocation fusion proteins in acute myeloid leukemia subvert these functions. The t(8;21) is the second most frequent translocation in acute myeloid leukemia and creates a fusion between the DNA binding domain of AML-1 and the ETO (also known as MTG8) corepressor. The t(12;21) is found in up to 25% of pediatric B cell acute lymphoblastic leukemias and fuses the ETS family transcription factor TEL to the amino terminus of AML-1. In addition, the inv(16), the most frequent translocation in acute myeloid leukemia, fuses the AML-1 cofactor CBFβ to the smooth muscle myosin heavy chain MYH11. Both the t(8;21) and t(12;21) create transcriptional repressors that impair AML-1 target gene expression. We demonstrated that the fusion proteins encoded by these translocations contact the nuclear hormone corepressors (N-CoR/SMRT), mSin3A, and histone deacetylases. We have also found that both TEL and AML-1 interact with mSin3A. TEL also binds N-CoR and histone deacetylase-3, indicating that wild-type TEL is a transcriptional repressor. The t(12;21) fuses the mSin3A interaction domain of TEL to AML-1 to transform AML-1 from a regulated to an unregulated transcriptional repressor. The recognition that AML-1 interacts with mSin3A to repress transcription suggested that the inv(16) fusion protein might also repress the transcription of AML-1-target genes. In fact, the inv(16) encodes a protein that cooperates with AML-1 to repress transcription. The inv(16) fusion protein was found in a ternary complex with AML-1 and mSin3A, suggesting that the inv(16) also acts by recruiting transcriptional corepressors and histone deacetylases.

Keywords t(8/21) · t(12/21) · inv(16) · AML-1 · CBFβ · TEL

Introduction

Acute leukemia is characterized by recurring chromosomal translocations, which are closely associated with specific forms of the disease. The t(8;21), t(12;21), and inv(16) represent some of the most frequent chromosomal translocations in acute myeloid and lymphoblastic leukemias. The t(8;21) is found in up to 12% of acute myeloid leukemias and disrupts the *AML-1* (acute myeloid leukemia-1, also known as *RUNX1*) and the *ETO* (eight-twenty-one, also known as myeloid tumor gene-8) genes (Fig. 1A) [10, 16, 17]. The chimeric mRNA that is produced encodes the DNA binding domain of AML-1 fused to the majority of the coding region of ETO. The inv(16) disrupts a gene encoding an AML-1 cofactor, core-binding factor β (CBFβ) and the smooth muscle myosin heavy chain gene MYH11 (Fig. 1C) [9]. The inv(16) is also associated with acute myeloid leukemia and is possibly the most frequent translocation found in leukemia [10]. The t(12;21) is found in pediatric cases of B cell acute lymphoblastic leukemia and represents up to 25% of these cases [19]. It fuses the transcriptional control region and the first 336 codons of translocation-ets-leukemia (TEL also known as ETV6) to nearly all of AML-1 (Fig. 1B) [4, 18]. Although TEL is a DNA-binding transcription factor, its DNA binding domain is lost in the fusion. Thus TEL/AML-1 regulates transcription mediated by the AML-1 DNA-binding domain.

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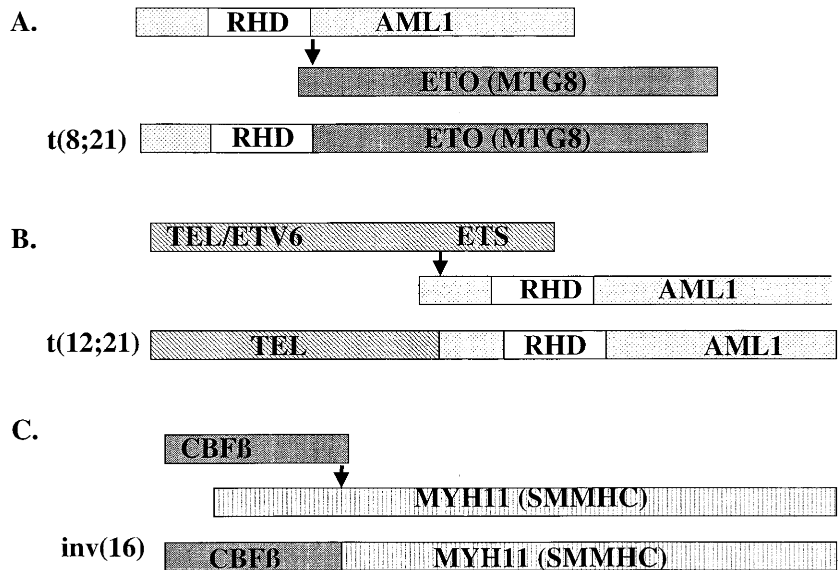

Fig. 1A–C Schematic diagram depicting the wild-type proteins and the chromosomal translocation fusion proteins created by the (**A**) t(8;21), (**B**) t(12;21), and (**C**) inv(16) (*RHD* runt-homology domain, is the domain of AML-1 homologous to the *Drosophila* Runt protein, *ETS* DNA binding domain of TEL; *arrows* indicate the chromosomal translocation breakpoint)

While AML-1 can both activate and repress transcription [11, 14, 21], the t(8;21), t(12;21), and inv(16) translocation fusion proteins block AML-1-dependent transactivation [6, 7, 13, 15]. The finding that TEL/AML-1 and AML-1/ETO could block both basal and activated transcription of several different promoters indicated that these fusion proteins actively repress transcription [6, 11]. In addition, the inv(16) fusion protein cooperates with AML-1 to repress transcription [13]. In this review, the mechanisms of transcriptional repression for these important chromosomal translocation fusion proteins will be addressed.

Materials and methods

Immunoprecipitation assay

Typically, Cos-7 cells ($3{\times}10^6$) were transfected using Superfect (Qiagen, Valencia, Calif.) or Lipofectimine (Life Technologies, Rockville, Md.) with 5–6 μg (total) of the indicated expression constructs. Cells were lysed in phosphate-buffered saline (PBS) supplemented with 1 m*M* EDTA, 1× protease inhibitor cocktail (Roche, Basel, Switzerland) and various combinations of detergents ranging from 0.5% Triton X-100, to 0.5% Triton X-100, 0.5% sodium deoxycholate, and 0.5% SDS. Before immunoprecipitation, cell lysates were precleared with Pansorbin (Calbiochem, San Diego, Calif.). Antibodies used for immunoblotting and immunoprecipitation were anti-mSin3A (K-20; Santa Cruz Biotechnology, Santa Cruz, Calif.), anti-FLAG (M2 antibody; Sigma, St. Louis, Mo.), anti-TEL [3], anti-ETO (Calbiochem), anti-GAL4 (Babco, Berkeley, Calif.), anti-AML-1 (Calbiochem), or anti-CBFβ [13].

Transient transcription assay

NIH3T3 cells (35-mm plates) were transfected with Superfect reagents with 1.5 μg plasmid DNA including the luciferase reporter plasmid, the effector plasmid, and a plasmid expressing a secreted form of alkaline phosphatase as an internal control for transfection efficiency [3]. Cells and supernatants were harvested 40 h after transfection using 100–200 μl reporter lysis buffer (Promega, Madison, Wis.). Aliquots (10–80 μl) were assayed for luciferase activity using the Luciferase Reagent Assay (Promega) according to the manufacturer's instructions. Secreted alkaline phosphatase (SEAP) activity was quantitated as described previously [14]. Luciferase activities were then normalized with respect to SEAP activity.

In vitro transcription and translation reactions and glutathione agarose precipitation assays

N-CoR or mSin3A was transcribed and translated in vitro in the presence of ^{35}S-methionine and cysteine (PROMIX; Amersham, Little Chalfont, UK) using the T7-coupled reticulocyte lysate system (Promega). The pGEX-AML-1 constructs were as described previously [8]. GST fusion proteins were extracted from *Escherichia coli* (DH5a) after sonication in 1×PBS containing 0.1% NP-40, 1 m*M* PMSF, and 10 μg/ml aprotinin, and purified with glutathione sepharose-4B beads (Pharmacia, Uppsala, Sweden). For precipitation assays, equal amounts of the GST fusion proteins were incubated with 5 μl of the in vitro transcription/translation reaction in a final volume of 200 μl immunoprecipitation buffer. After 1 h at 4 °C, the beads were washed with immunoprecipitation buffer and bound proteins were analyzed by SDS-PAGE.

Results and discussion

Although there are several possible mechanisms for transcriptional repression, deletion studies of AML-1/ETO and TEL/AML-1 ruled out competition for DNA binding sites because nuclear localization and DNA binding were not sufficient for repression [6, 11]. In addition, titration experiments indicated that transcriptional squelching was unlikely [15]. Based on these observations, a candidate approach was undertaken to search for potential corepressors that might interact with the domains required for repression in ETO. The nuclear hormone corepressor N-CoR specifically associated with a domain containing two zinc fingers termed the MYND domain [12] (Fig. 2). The central domain of N-CoR was sufficient to interact with the MYND domain of ETO in

yeast two-hybrid assays, and in coimmunoprecipitation purification studies N-CoR appeared to contact ETO more than once [12].

N-CoR is a component of a corepressor complex and can interact with the mSin3 corepressors and histone deacetylases [5]. Therefore the ability of these proteins to interact with ETO was assessed. In yeast two-hybrid assays, in vitro binding, and coimmunoprecipitation purification studies, ETO associated with mSin3A [12]. Furthermore, ETO copurified with histone deacetylases-1 (HDAC), -2, [12] and -3 (unpublished observations). Unexpectedly, the four domains of ETO that are conserved with its *Drosophila* homologue *Nervy* were not responsible for mSin3A binding. In fact, two nonconserved domains of ETO contact mSin3A (unpublished observations).

In attempting to define the regions of ETO that interact with mSin3A, we tested whether AML-1 could also contact mSin3A and, if so, whether mSin3A could mediate AML-1-dependent transcriptional repression. AML-1 contacted mSin3A directly as judged by coimmunoprecipitation of coexpressed or endogenous proteins, in vitro binding assays, and yeast two-hybrid studies [13]. In addition, deletion of a 30-amino acid domain in the immediate C-terminal direction of the AML-1 DNA binding domain eliminated the mSin3A interaction and AML-1-dependent repression. Thus the association between AML-1 and mSin3A contributes to AML-1-mediated repression.

The AML-1 mSin3A interaction domain mapped to a region of AML-1 that is deleted in the t(8;21), but that is retained in the t(12;21), suggesting that TEL/AML-1 might also associate with the mSin3 corepressors to repress transcription. TEL/AML-1 coimmunoprecipitates with mSin3A [2]. Under stringent conditions (0.5% Triton X-100, 0.5% sodium deoxycholate, 0.5% SDS), more TEL/AML-1 binds mSin3A than AML-1 binds mSin3A [2]. When the AML-1 mSin3A interaction domain is deleted, mSin3A still copurifies with TEL/AML-1, indicating that TEL can also associate with mSin3A. In addition to mSin3A, TEL also associates with N-CoR [1] and histone deacetylase-3 and these contacts are required for TEL-mediated repression (unpublished observations). Thus TEL/AML-1 makes multiple contacts with corepressors to convert AML-1 from a regulated to an unregulated transcriptional repressor (Fig. 3).

The inv(16) fusion protein can both block AML-1-dependent transactivation [7] and stimulate AML-1-mediated transrepression [13], leading to the hypothesis that it also actively represses transcription. To determine whether the inv(16) contains a transcriptional repression domain, it was fused to the yeast GAL4 DNA binding domain and the chimeric protein was assessed for its ability to repress a promoter containing GAL4 DNA binding sites [13]. The GAL4-inv(16) fusion protein repressed transcription five- to sixfold. Since mammalian cells do not have a GAL4 site DNA binding activity, this repression was probably active. Therefore deletion mutagenesis has been used to map the minimal domain required for transrepression. Unexpectedly, the C-terminal 163 amino acids of the smooth muscle myosin heavy chain are sufficient to repress transcription. This is a region of MYH11 that has homology to the Ski and Sno oncogenic transcription factors that bind the mSin3 corepressors, leading to the hypothesis that the inv(16) fusion protein binds mSin3A or mSin3B to repress transcription (Fig. 4).

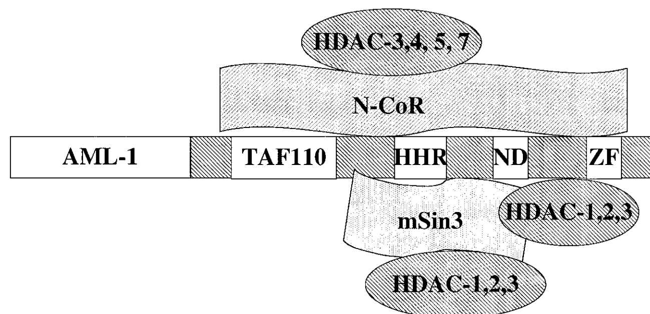

Fig. 2 Schematic diagram of the t(8;21) fusion protein and its molecular contacts with corepressors. Also shown are the contacts between the N-CoR and mSin3 corepressors and histone deacetylases. Open boxes are regions of homology with the *Drosophila* homologue of ETO termed Nervy (*TAF110* a domain with homology to the Drosophila TAF110 protein, *HHR* hydrophobic heptad repeat, *ND* nervy domain, *ZF* zinc finger, *HDAC* histone deacetylase)

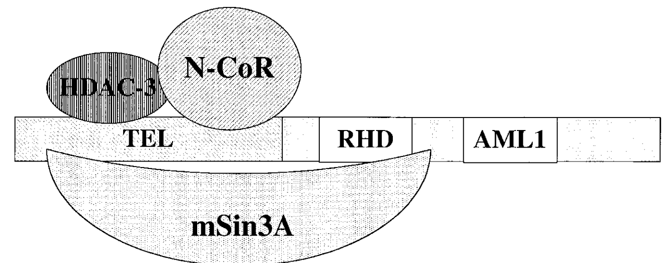

Fig. 3 Schematic diagram of the t(12;21) fusion protein and its molecular contacts with corepressors. Both TEL and AML-1 contain mSin3A interaction domains that create a stable interaction with mSin3A (*RHD* runt homology domain, *HDAC* histone deacetylase, *N-CoR* nuclear hormone corepressor)

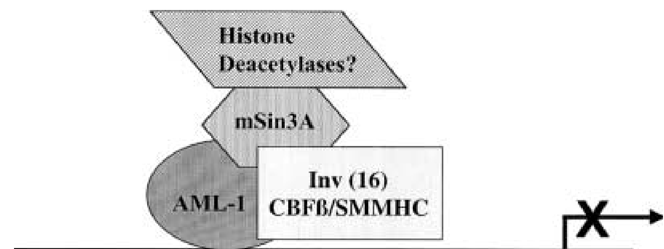

Fig. 4 Schematic diagram of a model of how the inv(16) fusion protein may act as an AML-1 corepressor. The *line* indicates a promoter containing AML-1 binding sites; the *arrow* indicates the transcriptional start site

The work presented here indicates that the t(8;21) and the t(12;21) fusion proteins repress transcription by interacting with the nuclear hormone corepressor mSin3A or mSin3B and histone deacetylases. However, there are some distinctions. The t(12;21) fusion protein retains nearly all of AML-1 and forms an apparently stable, unregulated complex with mSin3A. Meanwhile, the t(8;21) fusion protein replaces the C-terminal two-thirds of AML-1 with ETO, a putative corepressor. Although AML-1/ETO does not bind corepressors with the apparent affinity that TEL/AML-1 does, it makes multiple contacts with corepressors. For the t(8;21), it is likely that the loss of the C-terminal domain of AML-1, which is required for transactivation [22], creates a constitutive transcriptional repressor. In this respect, the inv(16) is unusual in that it is the smooth muscle myosin heavy chain portion of the fusion protein that contains a repression domain.

Why this protein might contain a repression domain is a major question to be addressed. Is it unfortunate homology to the repression domains of Ski and Sno? Or do myosins play an unrecognized role in normal transcriptional control? Although less well developed, it is hypothesized that the inv(16) might act in a manner similar to the t(8;21) and t(12;21) by trapping AML-1 in a complex with corepressors, such as mSin3A [13].

Since these are the most frequent translocations in acute myeloid leukemia and childhood B cell acute lymphocytic leukemia, the targeting of these downstream corepressors may be a viable direction for novel therapeutic approaches. Clinical trials are underway using histone deacetylase inhibitors for the treatment of a variety of neoplasms including acute promyelocytic leukemia [20]. Our work might suggest that these agents will have some beneficial effects in leukemias associated with the t(8;21) and t(12;21). By further exploring the components of the corepression complexes bound by AML-1/ETO and TEL/AML-1, we may uncover other targets for therapeutic intervention.

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