AML1-ETO Decreases ETO-2 (MTG16) Interactions with Nuclear Receptor Corepressor, an Effect That Impairs Granulocyte Differentiation

Vinzon Ibañez,1 Arun Sharma,1 Silvia Buonomici,1 Amit Verma,1 Sudhakar Kalakonda,2 Jianxiang Wang,4 ShriHari Kadkol,3 and Yogen Saunthararajah1

1Section of Hematology/Oncology, Department of Medicine, and 2Department of Pathology, University of Illinois, Chicago, Illinois; 3Department of Ophthalmology, University of Maryland, Baltimore, Maryland; and 4Laboratory of Hematological Malignancy, State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences

ABSTRACT

The t(8;21) chromosome abnormality in acute myeloid leukemia targets the AML1 and ETO genes to produce the leukemia fusion protein AML1-ETO. Another member of the ETO family, ETO-2/MTG16, is highly expressed in murine and human hematopoietic cells, bears >75% homology to ETO, and like ETO, contains a conserved MYND domain that interacts with the nuclear receptor corepressor (N-CoR). AML1-ETO prevents granulocyte but not macrophage differentiation of murine 32Dc13 granulocyte/macrophage progenitors. One possible mechanism is recruitment of N-CoR to aberrantly repress AML1 target genes. We wished to examine another mechanism by which AML1-ETO might impair granulocyte differentiation. We demonstrate that AML1-ETO decreases interactions between ETO-2 and N-CoR. Furthermore, overexpression of ETO-2 relieves AML1-ETO-induced granulocyte differentiation arrest. This suggests that decreased interactions between ETO-2 and N-CoR may contribute to granulocyte differentiation impairment. The MYND domain of ETO-2 and N-CoR inhibits interactions between ETO-2 and N-CoR, presumably by occupying the ETO-2 binding site on N-CoR. This inhibition of ETO-2 interactions with N-CoR is specific because the MYND domain does not inhibit retinoic acid receptor interactions with N-CoR. To examine the effect of decreasing interactions between ETO-2 and N-CoR in hematopoietic cells, without effects of AML1-ETO such as direct repression of AML1 target genes, the MYND domain was expressed in 32Dc13 and human CD34+ cells. The MYND domain prevented granulocyte but not macrophage differentiation of both 32Dc13 and human CD34+ cells, recapitulating this effect of AML1-ETO. In conclusion, decreasing interactions between ETO-2 and N-CoR, an effect of AML1-ETO, inhibits granulocyte differentiation.

INTRODUCTION

MTG16 (murine homologue ETO-2) is a member of the ETO family; the other members are MTG8 (ETO) and MTGR1. MTG16/ETO-2 is expressed in hematopoietic cells. Murine ETO-2 bears 75% identity with murine ETO, 86% identity with human MTG16, and 77% identity with human ETO (1). Both MTG16 and ETO are targets of chromosomal translocations in acute myeloid leukemia, the t(16;21) and t(8;21) abnormalities, respectively. Both ETO-2 and ETO interact with nuclear receptor corepressor (N-CoR); the usual function of this interaction is not known.

There are four regions conserved among the ETO family members, typically referred to as the nervy homology regions (NHR) after nervy, the ETO homologue found in Drosophila. NHR1 has sequence similarity with a central domain found in human TAF105, human TAF130 and Drosophila Taf110—it is also known as the TAF homology domain. NHR2 is an amphipathic helix structure that mediates homodimerization and binding of ETO to homologous family members (2). NHR3 is a coiled-coiled region that plays a role in interactions with N-CoR (3). NHR4 contains two MYND zinc finger motifs, which are necessary for binding to N-CoR (3–7); it is also known as the MYND domain.

ETO-2, like ETO, demonstrates corepressor function (8). This function is likely to be mediated through the interactions of ETO-2 with histone deacetylases 1, 2, 3, 6, and 8 and the ubiquitously expressed N-CoR. N-CoR is a large (2440 amino acids) platform protein known to mediate transcriptional repression by unliganded nuclear receptors and many other classes of transcription factors. N-CoR function is mediated at least in part through its interactions with Sin3-histone deactetylase (9, 10). Some of N-CoR function in hematopoiesis is mediated by its interactions with the retinoic acid receptor (RAR) and the thyroid hormone receptor (11, 12). N-CoR interaction with unliganded RAR is suggested to block maturation of granulocytes (12, 13).

MTG16 is highly expressed in human heart, pancreas, skeletal muscle, spleen, thymus, and peripheral blood leukocytes (14). ETO-2 is expressed in murine heart, brain, spleen, lung, liver, muscle, and kidney (1).

Acute myeloid leukemia containing chromosomal translocations involving ETO and MTG16 usually have an acute myeloblastic leukemia with maturation (FAB M2) phenotype. We demonstrate that AML1-ETO, the leukemia fusion protein produced by the t(8;21) abnormality, decreases interactions between ETO-2 and N-CoR. After confirming that a 77-amino acid ETO fragment containing the MYND domain could decrease ETO-2 interactions with N-CoR in 293T cells (presumably by competing with ETO-2 for binding to the same interaction site on N-CoR), we expressed this fragment in hematopoietic cells. The MYND domain was sufficient to inhibit granulocyte differentiation of murine 32Dc13 and human CD34+ hematopoietic cells, recapitulating this effect of AML1-ETO. This suggests that disruption of endogenous ETO-2 interactions with N-CoR may be a relevant leukemogenic effect of the AML1-ETO leukemia fusion protein.

MATERIALS AND METHODS

Reverse Transcriptase-PCR. Total RNA was extracted by the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. On-column DNase I digestion was performed to eliminate genomic DNA contamination. Absence of DNA in purified RNA was confirmed by PCR amplification without reverse transcription. ETO, ETO-2, and glyceraldehyde-3-phosphate dehydrogenase expression was analyzed using the One Step reverse transcription-PCR kit (Qiagen) in a MJ Research PTC 200 DNA Engine thermocycler. Each reaction contained 1 µg of total RNA; 1 × reverse transcription-PCR buffer (with 2.5 mM MgCl2); 0.4 mM each of dATP, dCTP, dTTP, and dGTP; 1 × Q solution; 30 pmol each of forward and reverse primers; 10 units of RNase inhibitor (Promega); and 2 µl of enzyme mix containing reverse transcriptase and HotStarTaq DNA polymerase. Primer sequences were designed and optimized using Oligo Version 6.0 software (Molecular Biology Insights, CO). Primer sequences were: ETO (human and murine) 5′-AGG GTG AGG CAA TCT GCT TC-3′; and 5′-CAA CAA ACA AGA ACC ACA CCA AC-3′; ETO-2 (human and murine) 5′-GTC TGG TGG TTT TGC TCC TC-3′; and 5′-GAG ATA TCC TGC AGG AGC CAC-3′; and glyceraldehyde-3-phosphate dehydrogenase 5′-ACC ACA GTC TCA GAC GC-3′; and 5′-GAC CAA GTC GAC AAA GA-3′.

Received 11/25/03; revised 4/9/04; accepted 4/26/04.

Grant support: Illinois Department of Public Health/UC IUC Sickle Cell Center and the American Cancer Society (Y. Saunthararajah), National Natural Science Foundation of China Grant 3037093 (J. Wang), National Science Fund for Distinguished Young Scholars Grant 30025019, and Tianjin Natural Science Fund Grant 00380321.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Yogen Saunthararajah, MBBR room 3150 (MC734), 900 South Ashland Avenue, Chicago, IL 60607-7173. Phone: (312) 413-7963; Fax: (312) 413-7963; E-mail: yogen@uic.edu.
A nuclear-localizing signal fused to a myc-tag was subcloned from the pCMV/myc/nuc expression vector (Invitrogen) into the SalI/BamHI site on pRES-EGFP2 (Clontech). The ETO MYND zinc finger domain from amino acids 484–560 (GenBank accession no. D14289) was cloned into the XhoI/Sall site of the modified pRES-EGFP2 (forward primer, 5′-agt tgc tgg aat tgt ctc gag aag agg ggt agg-3′; reverse primer, 5′-gg agg gtt tgt cct cga cgt gga-3′). The ETO MYND zinc finger domain was also cloned into the MSCV retroviral vector (Clontech; forward primer, 5′-aaa aaa gaa ttc atg aaa gcg agt gaa acc tgc agt ggc-3′; reverse primer, 5′-aaa aaa ctc gag tta gga agg gtt tcc ggt ggt g-3′) modified to replace the G418 resistance cassette with an internal ribosome entry site (IRES)-yellow fluorescent protein (YFP) motif.

Fig. 1. ETO-2 is expressed in 32Dcl3 cells, human CD34+ hematopoietic cells, HL60 cells, and Kasumi-1 cells. A, using reverse transcription-PCR (RT-PCR) with primers specific for ETO or ETO-2, ETO-2 signal was readily detected in 32Dcl3 wild-type (32Dwt) cells. 32Dcl3 cells transfected with an expression vector for ETO (32D ETO) were used as a positive control. B, 32Dcl3 lysates were immunoprecipitated (IP) with nonspecific polyclonal antibody, anti-ETO-2, and anti-ETO. The blot was probed with anti-ETO-2. C, RNA was extracted from human CD34+ cells cultured in IMDM supplemented with bovine growth serum and cytokines at D6, D7, and D12, from the myeloid leukemia cell line HL60 and the AML1-ETO-containing cell line Kasumi-1. MTG16 (ETO-2) signal was detected in all of the samples tested. ETO was detected in HL60 and Kasumi cells only. In Kasumi cells, the primers for ETO would also amplify from AML1-ETO transcripts.

RESULTS

ETO-2 (MTG16) Is Expressed in Murine and Human Hema-
topoietic Cells. The 32Dcl3 cell line is a murine granulocyte/macrophage progenitor cell line. It is MLL-3 dependent for survival and proliferation. Upon removal of MLL-3, it terminally differentiates into granulocytes in G-CSF and macrophages and granulocytes in mGM-CSF (15). In 32Dcl3 cells (Fig. 1A), ETO-2 expression was demonstrated by reverse transcription-PCR (Fig. 1A) and by Western blot (Fig. 1B). ETO signal was not detected in the 32Dcl3 cells by reverse transcription-PCR (Fig. 1A). In human CD34+ hematopoietic cells,
HL60, and Kasumi-1 myeloid leukemia lines, MTG16 (ETO-2) expression was demonstrated by reverse transcription-PCR (Fig. 1C). ETO signal was not detected in the human CD34+/H11001 cells but was detected in the HL60 and Kasumi-1 cells. In the Kasumi-1 cells, the detected ETO signal could also have arisen from AML1-ETO transcripts (Fig. 1C).

AML1-ETO Decreases the Interaction of ETO-2 with N-CoR. Both AML1-ETO and ETO-2 bind to N-CoR (8). We hypothesized that one action of AML1-ETO might be to decrease ETO-2 interactions with N-CoR, through binding/sequestering of ETO-2 or by competing for the same binding site on N-CoR. 293T cells were transfected with equivalent microgram quantities of expression vectors for HA-ETO-2, Flag-NCoR, and either AML1-ETO or its empty vector. The cell lysates were immunoprecipitated with anti-N-CoR, and the blots were probed for HA tagged ETO-2. AML1-ETO prevented the coimmunoprecipitation of ETO-2 with N-CoR. The decreased coimmunoprecipitation of ETO-2 with N-CoR in the presence of AML1-ETO was not a consequence of lower expression of ETO-2 or N-CoR in the AML1-ETO-containing samples (Fig. 2). As reported by others (16), AML1-ETO coimmunoprecipitates with ETO-2 (Fig. 2, Lane 8).

ETO-2 Overexpression Relieves the Granulocyte Differentiation Arrest Induced by AML1-ETO Expression in 32Dcl3 Cells. Unlike wild-type 32Dcl3 cells, 32Dcl3 cells transduced with AML1-ETO (32D AML1-ETO) do not terminally differentiate in G-CSF but instead continue to proliferate (15, 17). 32Dcl3 cells were transduced with MSCV AML1-ETO-IRES-YFP (both AML1-ETO and YFP are translated from the same mRNA transcript) or the empty vector. We noted that 32D AML1-ETO cells do not terminally differentiate in G-CSF, as observed by others, but do terminally differentiate into macrophages in mGM-CSF (data not shown). ETO-2 was overexpressed in 32D AML1-ETO cells through transfection with an expres-
AML1-ETO DECREASES ETO-2 INTERACTIONS WITH N-CoR

The MYND Domain Alone [ETO/N-CoR Interaction-Domain Decoy (ENIDD)] Binds to N-CoR and Specifically Decreases the Bidirectional Coimmunoprecipitation of ETO-2 with N-CoR. Previously, we and others demonstrated that the MYND domain of ETO (Fig. 4A) could bind to N-CoR in two-hybrid assays (5, 18). We confirmed that the MYND domain alone (amino-acids 484–560; GenBank accession no. D14289) could coimmunoprecipitate with N-CoR in 293T cells (Fig. 4B). We reasoned that the MYND domain, if expressed in cells, would compete with endogenous ETO-2 for binding to the same interaction site on N-CoR. This would allow us to specifically examine the consequences of disrupting endogenous ETO-2 interactions with N-CoR without other effects of full-length AML1-ETO such as direct repression of AML1 target genes. To reflect our goal of competitively inhibiting endogenous ETO-2 interactions with N-CoR, we termed the MYND zinc finger domain of ETO that we expressed in cells the ENIDD.

To confirm that ENIDD would decrease ETO-2 interactions with N-CoR, 293T cells were transfected with equivalent microgram quantities of expression vectors for HA-ETO-2, Flag-NCoR, and either ENIDD-Myc or its empty vector. The cell lysates were immunoprecipitated with antiflag or anti-ETO-2, ENIDD decreased the bidirectional coimmunoprecipitation of ETO-2 with N-CoR. The decreased coimmunoprecipitation of ETO-2 with N-CoR (and vice versa) was not a consequence of lower expression of ETO-2 or N-CoR in the ENIDD-containing samples (Fig. 4C). ENIDD did not decrease the bidirectional coimmunoprecipitation of RARα with N-CoR. Therefore, ENIDD was a specific antagonist of ETO-2 interactions with N-CoR (Fig. 4D). ENIDD expression was confirmed by anti-myc Western blot of cell lysates (Fig. 4E).

is the domain used to competitively inhibit ETO-2 interaction with N-CoR in these experiments. B. Myc-tagged ENIDD (the ETO MYND domain) coimmunoprecipitates bidirectionally with Flag-N-CoR from transfected 293T cells (Lanes 1 and 3). ctrl, nonspecific polyclonal mouse antibody (Lane 2). C. 293T cells were transfected with expression vectors for Flag-N-CoR, HA-ETO-2, and either ENIDD-Myc (Lanes 3 and 6) or empty vector (Lanes 2 and 5). With antiflag immunoprecipitation, there is an equivalent amount of Flag-N-CoR in the 293T lysates but less coimmunoprecipitation of ETO-2-HA in the presence of ENIDD (Lane 3). With anti-ETO-2 immunoprecipitation from the same cell lysates, there is an equivalent amount of HA-ETO-2 but less coimmunoprecipitation of Flag-N-CoR in the presence of ENIDD (Lane 6). D. 293T cells were transfected with expression vectors for Flag-N-CoR, RAR, and either ENIDD-Myc (Lanes 3 and 6) or empty vectors (Lanes 2 and 5). With antiflag immunoprecipitation, there is an equivalent amount of RAR coimmunoprecipitation, even in the presence of ENIDD (Lane 6). The same results are seen with anti-RAR immunoprecipitation (Lane 3). E, ENIDD expression was confirmed by anti-Myc Western blot of cell lysates.

Fig. 4 ENIDD decreases the bidirectional coimmunoprecipitation of Flag-N-CoR with ETO-2-HA but not with the RAR. A, human ETO (GenBank accession no. D14289) and human MTG16a (accession no. ABB010419) showing conserved domains (NHR, nervy homology region; TAFH, TAF homology domain; amphi, amphipathic helix; CC, coiled-coiled region). The MYND domain of ETO, or ENIDD (amino acid numbers 484–560),...
Expression of ENIDD in 32Dcl3 Cells Prevents Terminal Granulocyte but not Macrophage Differentiation. To examine the consequences of disrupting endogenous ETO-2 interactions with N-CoR on myeloid differentiation, we expressed ENIDD in 32Dcl3 cells. Stable transfectants were selected in G418 and then sorted for GFP positivity. 32Dcl3 cells transfected with ENIDD (32D ENIDD) or pIRES-myc-nuc-EGFP2 (32D Empty) were cultured in G-CSF or mGM-CSF. In G-CSF, 32D Empty undergo terminal differentiation into neutrophils as demonstrated by increased GR-1 expression on flow cytometry and on cytospin preparations (Fig. 5, A–C). In contrast, 32D ENIDD continue to proliferate in G-CSF (Fig. 5A). 32D ENIDD did demonstrate some increase in GR-1 expression, and although abundant granule formation was noted on examination of Giemsa-stained cytospin preparations (Fig. 5, B and C). In mGM-CSF, both 32D Empty and 32D ENIDD terminally differentiate into macrophages (Fig. 5, D–F). The inhibition of differentiation in G-CSF but not in mGM-CSF is similar to the effect noted with expression of full-length AML1-ETO in 32Dcl3 cells. Experiments were performed three times using independently generated polyclonal populations of stable transfectants.

In mIL-3, there is no difference in the proliferation rate or GR-1 expression between 32D Empty and 32D ENIDD (data not shown).

Expression of ENIDD Blocks Granulocyte and Increases Macrophage Differentiation of Human CD34-Positive Cord Blood Cells. hCD34+ cord blood cells were transduced with MSCV-ires-YFP (CD34 Empty) or MSCV-ENIDD-IRES-YFP (CD34 ENIDD). YFP-positive cells (ENIDD and YFP are translated from the same mRNA transcript) were flow sorted and then cultured in IMDM 20% fetal bovine serum containing IL-3, stem cell factor, and G-CSF to promote granulocyte differentiation. CD34 Empty underwent terminal differentiation into neutrophils with increased CD11b myeloid differentiation marker expression and abundant segmented forms on Giemsa-stained cytospin preparations (Fig. 6, A and B). In contrast, there was less CD11b expression and no terminal granulocyte differentiation of CD34 ENIDD on examination of Giemsa-stained cytospin preparations (Fig. 6, A and B). However, CD34 ENIDD did undergo terminal differentiation into macrophages: CD13 expression (CD13 is
seen on both granulocytes and monocytes; monocytic differentiation is associated with greater CD13 expression (19)] and the percentages of macrophages were higher in CD34 ENIDD compared with CD34 Empty (Fig. 6, A and B).

In culture of transduced but unsorted cells, the proportion of YFP-positive cells did not appreciably increase or decrease in either the empty vector or ENIDD retroviral supernatant treated populations. This suggested that ENIDD expression did not confer a proliferative advantage (or disadvantage) on human cord blood cells over this time period (Fig. 6C).

At day 15 of methylcellulose culture, there was no difference in the number or size of CFU-GM, CFU-mixed, CFU-E, or BFU-E derived from CD34 Empty versus CD34 ENIDD (Fig. 6D).

Similar to the effects of ENIDD noted in 32Dc13 cells, ENIDD prevents terminal neutrophil but not macrophage differentiation of hCD34+ hematopoietic cells.

DISCUSSION

ETO-2, a member of the ETO family of proteins, is expressed in both murine and human hematopoietic cells. Using reverse transcription-PCR, we did not detect ETO expression in either murine
AML1-ETO decreases ETO-2 interactions with N-CoR.

32Dcl3 granulocyte/macrophage progenitors or in human CD34+ hematopoietic cells. ETO-2, like ETO (and AML1-ETO), interacts with the ubiquitous corepressor N-CoR. Because both ETO-2 and AML1-ETO are likely to bind to the same site on N-CoR, we examined whether AML1-ETO decreased ETO-2 interactions with N-CoR. AML1-ETO decreases the co-immunoprecipitation of ETO-2 with N-CoR in 293T cells. Furthermore, ETO-2 overexpression relieved AML1-ETO-induced granulocyte differentiation arrest of 32Dcl3 cells in G-CSF. This suggested that inhibition of endogenous ETO-2 interactions with N-CoR may be one mechanism by which AML1-ETO could impair granulocyte differentiation. The MYND domain present in the ETO family is necessary for interactions with N-CoR (3–7). To examine the effect of inhibiting endogenous ETO-2 interactions with N-CoR without other effects of AML1-ETO such as direct interference with AML1 transactivation (17, 20), we determined whether the MYND domain of ETO could act as an ENIDD by binding to N-CoR and specifically antagonizing ETO-2 interactions with N-CoR. This was confirmed in 293T cells in which ENIDD co-immunoprecipitated with N-CoR and specifically decreased the bidirectional co-immunoprecipitation of ETO-2 with N-CoR but not of the RAR with N-CoR. To examine the functional role of endogenous ETO-2 interactions with endogenous N-CoR, we expressed ENIDD in 32Dcl3 murine granulocyte/macrophage progenitors and human CD34+ cells. ENIDD prevented terminal granulocyte but not macrophage differentiation, an effect similar to that seen with full-length AML1-ETO expression in 32Dcl3 cells. These results suggest that endogenous ETO-2 interactions with N-CoR have a usual role in facilitating granulocyte differentiation and that one mechanism by which AML1-ETO impairs granulocyte differentiation is through disruption of this interaction.

ETO-2 has multiple protein interactions and may have multiple cellular functions besides those mediated via interactions with N-CoR. By sequestering ETO-2 (16), AML1-ETO may disrupt a number of usual ETO-2 interactions. ENIDD only mimics the effect of disrupting ETO-2 interactions with N-CoR and may underestimate the contribution of disrupted ETO-2 activity to the abnormal hematopoesis in AML1-ETO leukemia. A decrease in the proportion of terminally differentiated granulocytes and an increase in the proportion of macrophages in the hCD34+ ENIDD cultures could arise through increased macrophage commitment of the common granulocyte/macrophage progenitor (causing a relative decrease in granulocytes) or inhibition of terminal granulocyte differentiation after granulocyte commitment by the common progenitor. Although the CD34 ENIDD culture system lacked terminally differentiated neutrophils, it did contain numerous granulated cells with promyelocyte morphology. This suggests that ENIDD inhibits terminal granulocyte differentiation after granulocyte commitment by the common progenitor. Similarly, 32D ENIDD in G-CSF continues to proliferate with a morphology of partial granulocyte differentiation. However, based on our current data, we cannot rule out the possibility that ENIDD also promotes macrophage commitment of bipotential granulocyte/macrophage progenitors.

In addition to the ETO family members ETO, ETO-2, and MTGRI(2), there are at least four other proteins in humans and mice that have been identified as containing the MYND domain (SET and MYND domain-containing 3; melanin-concentrating hormone receptor interacting zinc finger protein; protein kinase C-binding protein Rack7; and zinc finger, MYND domain-containing 10). Therefore, we cannot exclude the possibility that some or all of the AML1-ETO- or ENIDD-induced effect is related to interference with protein-protein interactions involving other MYND domain-containing proteins. Nonetheless, the relief of AML1-ETO-induced granulocyte differentiation arrest by overexpression of ETO-2 suggests the effects are related to inhibition of ETO-2 interactions with N-CoR.

AML1-ETO does not prevent macrophage differentiation of 32Dcl3 cells. AML1-ETO expression in primary murine hematopoietic stem cells also did not prevent terminal macrophage differentiation, although it did prevent terminal granulocyte differentiation (21). AML1-ETO up-regulates transcription of the macrophage colony-stimulating factor (M-CSF) receptor (22). In primary human cells transduced with AML1-ETO, there was an increase in CD14 expression indicating macrophage differentiation (23). Treatment of the AML1-ETO-containing human cell-line Kasumi-1 with the histone deacetylase inhibitor phenylbutyrate resulted in monocytoïd differentiation (24). These findings suggest that both murine and human AML1-ETO-containing cells may be amenable to macrophage differentiation. This may be a feature that can be exploited for therapeutic purposes.

In conclusion, AML1-ETO inhibits the interaction between ETO-2 and N-CoR. The functional consequence of disrupting ETO-2 interactions with N-CoR in hematopoietic cells is impaired granulocyte but not macrophage differentiation. AML1-ETO inhibition of endogenous ETO-2 interactions with N-CoR may be one mechanism by which the leukemia fusion protein impairs granulocyte differentiation.

ACKNOWLEDGMENTS

Flag-N-CoR vector was a kind gift of Michael Rosenfeld. ETO-2 vector was a kind gift of Ari Melnick. RAR vector was a kind gift of Kun-Sang Chang. Giuseppina Nucifora and Johnson Liu provided valuable advice and discussions. We thank Alvin Ayala, Annette Bruno, John McGuire, Michael J. Pacini, Paul Weiss, and Karen Hagen for technical assistance.

REFERENCES


AML1-ETO Decreases ETO-2 (MTG16) Interactions with Nuclear Receptor Corepressor, an Effect That Impairs Granulocyte Differentiation

Vinzon Ibañez, Arun Sharma, Silvia Buonamici, et al.

*Cancer Res* 2004;64:4547-4554.

| Updated version | Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/64/13/4547](http://cancerres.aacrjournals.org/content/64/13/4547) |

| Cited articles | This article cites 23 articles, 17 of which you can access for free at: [http://cancerres.aacrjournals.org/content/64/13/4547.full.html#ref-list-1](http://cancerres.aacrjournals.org/content/64/13/4547.full.html#ref-list-1) |

| Citing articles | This article has been cited by 8 HighWire-hosted articles. Access the articles at: [http://cancerres.aacrjournals.org/content/64/13/4547.full.html#related-urls](http://cancerres.aacrjournals.org/content/64/13/4547.full.html#related-urls) |

| E-mail alerts | Sign up to receive free email-alerts related to this article or journal. |

| Reprints and Subscriptions | To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org. |

| Permissions | To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org. |