

# AML1-ETO fusion protein up-regulates TRKA mRNA expression in human CD34<sup>+</sup> cells, allowing nerve growth factor-induced expansion

James C. Mulloy\*, Vladimir Jankovic<sup>†</sup>, Mark Wunderlich\*, Ruud Delwel<sup>‡</sup>, Jorg Cammenga<sup>†</sup>, Ondrej Krejci\*, Hui Zhao<sup>†</sup>, Peter J. M. Valk<sup>‡</sup>, Bob Lowenberg<sup>‡</sup>, and Stephen D. Nimer<sup>†§¶</sup>

\*Division of Experimental Hematology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229; <sup>†</sup>Sloan-Kettering Institute and <sup>‡</sup>Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021; and <sup>§</sup>Department of Hematology, Erasmus University Medical Center, Dr. Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands

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The AML1-ETO fusion protein, generated by the t(8;21) in acute myeloid leukemia (AML), exerts dominant-negative functions and a variety of gains of function, including a positive effect on the growth of primary human CD34<sup>+</sup> hematopoietic stem/progenitor cells. We now show that AML1-ETO expression up-regulates the level of TRKA mRNA and protein in these cells and that AML1-ETO-expressing CD34<sup>+</sup> hematopoietic cells grown in the presence of five early-acting hematopoietic cytokines further proliferate in response to nerve growth factor (NGF). These cells also show a unique response to NGF and IL-3; namely, they expand in liquid culture. To determine the biological relevance of our findings, we analyzed 262 primary AML patient samples using real-time RT-PCR and found that t(8;21)-positive AML samples express significantly higher levels of TRKA mRNA than other subtypes of AML. NGF, which is normally expressed by bone marrow stromal cells, could provide important proliferative or survival signals to AML1-ETO-expressing leukemic or preleukemic cells, and the NGF/TRKA signaling pathway may be a suitable target for therapeutic approaches to AML.

target gene | leukemia | t(8;21) | cellular context | cytokine receptor

The core-binding factor (CBF) transcription factor complex [composed of AML1 (also known as RUNX1) and CBF $\beta$ ] is frequently affected by chromosomal translocations in acute leukemia, leading to the formation of the AML1-ETO (RUNX1-CBFA2T1) [t(8;21)], RUNX1/MDS/EVI1 [t(3;21)], ETV6-RUNX1 [t(12;21)], and CBF $\beta$ /SMMHC [inv(16)] fusion proteins (reviewed in refs. 1–3). Mutations in the AML1 gene are also found in undifferentiated leukemias (4, 5) and therapy-related acute myeloid leukemia (AML) or myelodysplastic syndrome (6, 7). Haploinsufficiency of AML1 plays a role in the familial leukemia predisposition syndrome FPD/AML (8), suggesting that dominant-negative forms of AML1 proteins may not be required to promote leukemogenesis.

AML1 and its heterodimeric partner CBF $\beta$  are essential for definitive hematopoiesis, because mice lacking either AML1 or CBF $\beta$  die at  $\approx$ 11.5 days postcoitum because of CNS hemorrhage and the absence of definitive hematopoiesis (9–13). AML1 appears to function primarily as a transcriptional activator (reviewed in ref. 1), but it also acts as a transcriptional repressor in certain cellular and promoter contexts (14, 15). AML1-ETO functions mainly as a transcriptional repressor (16–18), binding corepressor molecules (e.g., N-COR and mSIN3) and recruiting histone deacetylases (19–21). AML1-ETO can also act as a transcriptional activator in certain cellular contexts, perhaps by up-regulating c-JUN activity (22), by blocking repression by the promyelocytic leukemia zinc finger protein (23), or by other not-yet-characterized mechanisms (24, 25). AML1-ETO target genes have been identified by scrutiny of promoter regions for AML1 consensus-binding motifs (26) and by overexpression experiments in leukemia cell lines (27–29). However, the overlap between these target gene lists has been poor, and the contribution of a particular gene to AML1-ETO leuke-

mogenesis has been difficult to assess, because the cells used for these analyses are often already leukemic.

Using human CD34<sup>+</sup> hematopoietic cells, we demonstrated that AML1-ETO promotes hematopoietic stem/progenitor cell (HSPC) proliferation while having inhibitory effects on more committed progenitor cells (e.g., colony-forming units) (30). Furthermore, expression of AML1-ETO in human HSPC consistently leads to the generation of factor-dependent, clonal outgrowths of CD34<sup>+</sup> cells that retain significant *in vitro* differentiating activity (31). However, AML1-ETO does not appear to be sufficient for leukemogenesis in either human or murine hematopoietic cells (32–37).

To understand the basis for the effects of AML1-ETO in human CD34<sup>+</sup> hematopoietic cells, we used Affymetrix oligonucleotide gene arrays and identified the tyrosine receptor kinase A (TRKA) nerve growth factor (NGF) receptor gene (NTRK1) as a target gene increased by the expression of AML1-ETO. Although NGF/TRKA signaling has been most intensively studied in the nervous system, it also participates in hematopoiesis, prostate cancer cell behavior, and angiogenesis (38–40). NGF is normally expressed by bone marrow stromal cells (41), whereas TRKA is expressed in hematopoietic progenitor cells (42).

We examined the NGF/TRKA pathway in regulating the behavior of AML1-ETO-expressing human hematopoietic cells and found that physiologic concentrations of NGF increase the proliferation of AML1-ETO-positive cells, even in the presence of five early-acting hematopoietic cytokines. Additionally, the combination of NGF and IL-3 promotes the *in vitro* growth of AML1-ETO-expressing CD34<sup>+</sup> hematopoietic cells but not the expansion of empty-vector-transduced CD34<sup>+</sup> cells. To define the clinical relevance of these *in vitro* findings, we examined a large number of primary AML samples and found that those containing the t(8;21) translocation express significantly higher levels of TRKA mRNA than the AML samples without the t(8;21). The involvement of the NGF/TRKA signaling pathway in human leukemogenesis may represent a new therapeutic target for AML.

## Materials and Methods

**Retroviral Production and CD34 Transduction.** MIGR1, pEQ-PAM3(-E), and pSV-A-MLV-env plasmids were transiently transfected into 293T cells, and the viral supernatant was used to

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Abbreviations: AML, acute myeloid leukemia; CBF, core-binding factor; HSPC, hematopoietic stem/progenitor cell; NGF, nerve growth factor; PBPC, peripheral blood progenitor cell; qPCR, quantitative RT-PCR; CB, cord blood; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; IP, immunoprecipitation; STAT, signal transducers and activators of transcription; TRKA, tyrosine receptor kinase A.

Data deposition: The sequence reported in this paper has been deposited in the AML-1-ETO target genes database (accession no. GSE2049).

<sup>¶</sup>To whom correspondence should be addressed. E-mail: s-nimer@mskcc.org.

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transduce human CD34<sup>+</sup> cells [isolated from mobilized peripheral blood progenitor cells (PBPCs) or cord blood (CB) cells] as described (30). CD34<sup>+</sup> cells were selected by using StemSep CD34 magnetic beads (StemCell Technologies, Vancouver) and Miltenyi MACS CD34 isolation columns (Miltenyi Biotec, Auburn, CA).

Transcript profiling of transduced human CB or peripheral blood cells was conducted by using Affymetrix U95Av2 gene chips. RNA extraction, labeling, and the array processing, image, and data analysis were performed as described (43).

**Primary AML Patient Samples.** After informed consent, bone marrow aspirates or peripheral blood samples were taken at diagnosis from 285 patients with untreated AML enrolled on HOVON protocols. Total RNA was isolated from purified blast cells and normal CD34<sup>+</sup> cells (isolated from three healthy volunteers) as described (44); 10  $\mu$ g total RNA and Affymetrix U133A gene chips were used for expression profiling. Primer sets that span intron–exon junctions and generate  $\approx$ 100-bp cDNA amplicons were chosen for all quantitative RT-PCR (qPCR) amplifications.

Supervised analyses were performed by using significance analysis of microarrays (45), calculating a score for each gene based on the change in gene expression relative to the SD of all 285 measurements.

**Real-Time RT-PCR Analysis.** To quantify the expression of the TRKA mRNA, qPCR amplification was carried out by using the 7700 Sequence detector ABI, and the PCR products were detected using either Sybr green I chemistry or TaqMan methodology (PE Applied Biosystems, Norwalk, CT). For details of the primers and methodologies used, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

**Protein Detection by Using Immunoprecipitation (IP), Immunoblotting, and Flow Cytometry.** The methods used for washing cells and preparing proteins and the antibodies used to perform immunoblotting, IP, and flow cytometry are described in detail in *Supporting Materials and Methods*.

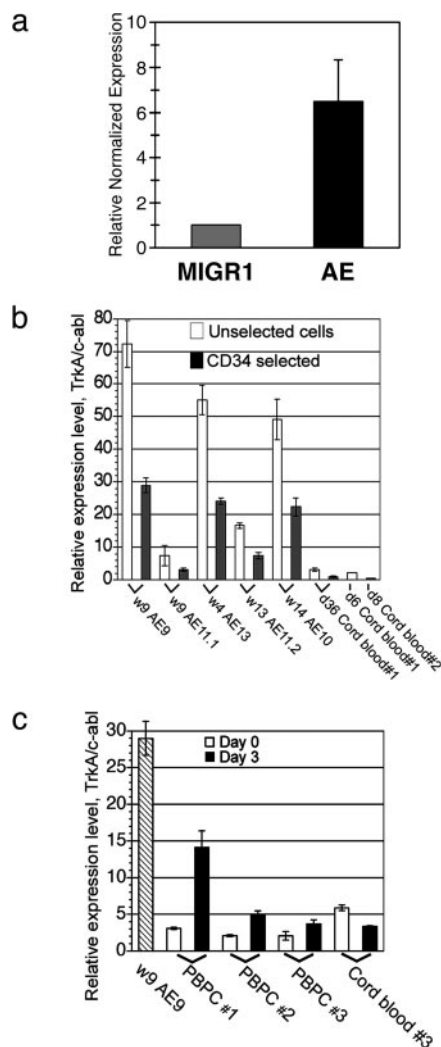
**Cell Proliferation Assay.** Cells were plated into 96-well flat-bottom microtiter plates at  $1\text{--}4 \times 10^4$  cells per well in 0.1 ml of total volume in triplicate. The indicated cytokines were added, cells were cultured for 3–4 days, and 0.01 ml of WST-1 reagent was added as recommended by the manufacturer (Roche Applied Science). After 3–6 h, absorbance was measured at 450 nm by using a microplate reader (Molecular Devices, Sunnyvale CA).

## Results

### The TRKA Oncogene Is Up-Regulated by the AML1-ETO Fusion Protein.

To identify molecular targets of AML1-ETO expression, we compared the transcriptional profiles of human CD34<sup>+</sup> cells transduced with an AML1-ETO-expressing retrovirus or with the control retroviral vector using Affymetrix Hu95A2 oligonucleotide microarrays. We identified the TRKA oncogene as a highly induced mRNA in human CD34<sup>+</sup> cells (2.1- to 9.8-fold) and showed similar induction of TRKA mRNA after the expression of AML1-ETO in the human AML cell line MO91 ( $\approx$ 4- to 5-fold). The early up-regulation of TRKA was confirmed in independent experiments, using qPCR for the CD34<sup>+</sup> CB cells (6.5-fold as shown in Fig. 1*a*) and the MO91 cells (data not shown). Microarray data for the primary human cell experiments can be accessed at [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo) (accession no. GSE2049).

Increased TRKA expression could be detected in AML1-ETO-expressing cells immediately after transduction (Fig. 1*a*) and in cells growing in culture for as short a period of time as 4 weeks (Fig. 1*b* and *c*). We also examined the level of TRKA mRNA in highly purified populations of clonal, cytokine-dependent CD34<sup>+</sup> AML1-ETO-expressing cells. We found 2- to 30-fold more TRKA mRNA in the long-term AML1-ETO-positive cultures (Fig. 1*b*) than in

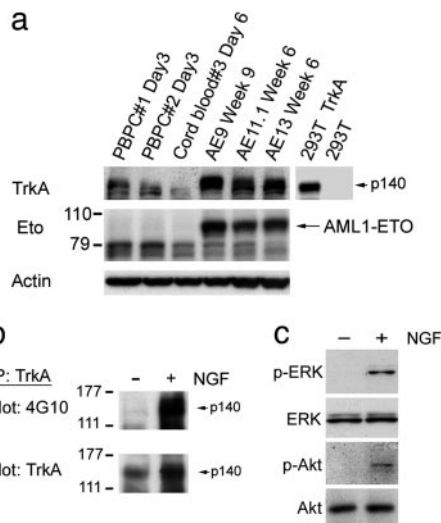


**Fig. 1.** AML1-ETO-positive cells overexpress TRKA mRNA. (a) TRKA expression measured by using qPCR for GFP- and CD34-selected MIGR1 control and AML1-ETO-expressing CB cells. TRKA expression was determined by using TaqMan technology. (b) CD34-selected and unselected cells were analyzed for TRKA expression by qPCR, using Sybr green technology, and expression was normalized using C-ABL staining. w, week; d, day. (c) Quiescent CB cells and mobilized peripheral blood CD34<sup>+</sup> cells, and day 3-stimulated cultures from the same samples were analyzed for TRKA mRNA expression. The week 9 AML1-ETO-expressing cell line AE9 was also included for comparison.

several control CB preparations (in culture for 6–36 days) by qPCR. The CD34<sup>+</sup>-selected cells expressed less TRKA than the total population of AML1-ETO-expressing cells growing in culture, and TRKA expression is substantially lower in normal CD34<sup>+</sup> PBPCs or CB cells than in the AML1-ETO-expressing cells. TRKA was expressed in both noncycling and cycling CD34<sup>+</sup> cells (compare day 0 and day 3 in cytokines; Fig. 1*d*). TRKA mRNA expression is clearly increased by the introduction of AML1-ETO.

### Functional TRKA Protein Expression in AML1-ETO-Positive Hematopoietic Cells.

To examine the levels of TRKA protein, we performed Western blot analysis that showed increased expression of the 140-kDa form of TRKA in three AML1-ETO-positive cultures compared with the three control, CD34<sup>+</sup> cell cultures (Fig. 2*a*). Furthermore, TRKA protein was readily detectable on the surface of the AML1-ETO-expressing cells by flow cytometry, using an antibody specific for a TRKA extracellular epitope (data not shown).

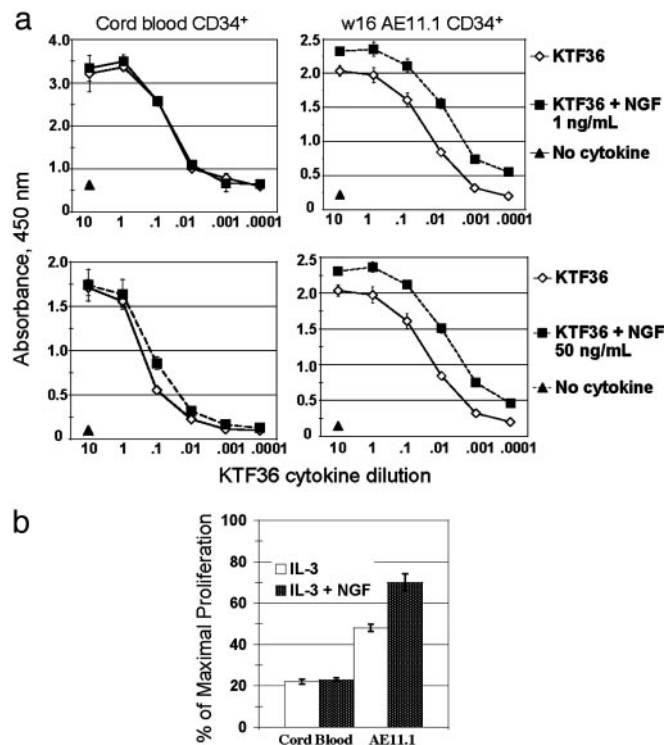


**Fig. 2.** A functional TRKA protein is up-regulated in AML1-ETO-positive cells. (a) Protein lysates from three AML1-ETO-positive cell cultures (lanes 4–6) and three control cultures (lanes 1–3) were analyzed for expression of TRKA. The CB cells and the three AML1-ETO-positive cell cultures were selected for CD34<sup>+</sup> cells. The two PBPC cultures were already predominantly CD34<sup>+</sup>. 293T and 293T-TRKA were included as controls. (b) Cells from an actively growing AE9 culture were rested overnight without cytokines, and then treated with 50 ng/ml NGF. Cell lysates were subjected to IP with an anti-TRKA antibody, and Western blots were probed for anti-phosphotyrosine (4G10) and anti-TRKA. (c) The protein lysates were probed by Western blot for activated ERK and activated AKT, which became phosphorylated in response to NGF.

To determine whether the TRKA receptor was functional in the AML1-ETO-expressing cells, we added NGF for 10 min after overnight cytokine starvation. The 140-kDa TRKA receptor was tyrosine phosphorylated (Fig. 2*b*), as were two downstream kinases, extracellular signal-regulated kinase (ERK) and AKT (Fig. 2*c*), reflecting activation of the mitogen-activated protein kinase (MAPK) and PI3K signaling pathways, respectively. Thus, TRKA signaling pathways are functional in AML1-ETO-positive cells.

**NGF Cooperates with Other Cytokines to Regulate the Proliferation and Survival of AML1-ETO-Expressing Cells.** AML1-ETO-expressing human CD34<sup>+</sup> HSPCs will grow in liquid culture for many months in a five-cytokine mix, maintaining 10–60% CD34 positivity and the capacity to differentiate into various lineages (31). Given the up-regulation of TRKA expression in AML1-ETO-expressing CD34<sup>+</sup> cells, we examined whether NGF had proliferative or survival effects on these cells. Whereas NGF alone had little effect on the proliferation of these cells, the addition of NGF [at 1 ng/ml (Fig. 3*a* Upper) or 50 ng/ml (Fig. 3*a* Lower)] to the five-cytokine mix (stem cell factor, megakaryocyte growth and differentiation factor, FLT3 ligand, IL-3, and IL-6) increased cellular proliferative capacity compared with the five-cytokine mix alone (Fig. 3*a*). This finding implies that NGF may activate signaling pathways in addition to those activated by these five cytokines. No cooperative effect was detected when AML1-ETO was absent, (i.e., in the control, CB samples under the same conditions), although NGF (at 50 but not 1 ng/ml) reproducibly enhanced CB cell proliferation at the 0.1× dilution (Fig. 3*a* Left Lower).

IL-3 alone can temporarily support the survival of AML1-ETO-positive CD34<sup>+</sup> cells in culture, although it does not promote their expansion (Fig. 3*b* and ref. 33). We found that IL-3 and NGF cooperatively promote the proliferation of AML1-ETO-expressing cells ≈70% as effectively as the full-cytokine mix, whereas neither IL-3 alone nor the IL-3/NGF combination had an appreciable



**Fig. 3.** NGF cooperates in the proliferation of AML1-ETO-positive CD34<sup>+</sup> cells but not of normal CD34<sup>+</sup> cells. (a) CD34<sup>+</sup> cells were purified from proliferating cultures by using Miltenyi MACS columns. Cells were grown in 96-well plates at 1–4 × 10<sup>4</sup> cells per well in 100 μl of total volume in 10-fold dilutions of full-cytokine mix (KTF36 = stem cell factor, MDGF, Flt3L, IL-3, and IL-6) ± 1 ng/ml NGF (Upper) or 50 ng/ml NGF (Lower). After 3–4 days, 10 μl of cell proliferation reagent WST-1 was added to each well; the plates were incubated for an additional 4–6 h, and absorbance was measured at 450 nm. Results are the average and SD of triplicate wells. The experiment was repeated twice with similar results. (b) The results of similar experiments where the cells were grown in the presence of 10 ng/ml IL-3 with or without 1 ng/ml NGF.

effect on the control CD34<sup>+</sup> cells (Fig. 3*b*). This response to IL-3 and NGF appears to be unique to the AML1-ETO-expressing cells.

Because the proliferative potential of the AML1-ETO-expressing cells resides predominantly in the CD34<sup>+</sup> subset (31), we examined whether NGF could support the expansion of CD34<sup>+</sup> AML1-ETO-positive cells. Although neither NGF nor IL-3 alone supports the expansion of these cells (Fig. 4*a* and data not shown), CD34<sup>+</sup> cell expansion was observed in response to IL-3 plus NGF (Fig. 4*a*). In contrast, normal control stem/progenitor cells showed only a transient, 1-week expansion of CD34<sup>+</sup> cells under full-cytokine conditions; neither IL-3, NGF, nor their combination stimulated the proliferation of normal CD34<sup>+</sup> CB cells (Fig. 4*a*). We also cultured the AML1-ETO-positive cells under full-cytokine conditions in the presence and absence of NGF (at 20 or 1 ng/ml); we observed a 2- to 8-fold expansion of total cells and CD34<sup>+</sup> cells over a period of 10 weeks (Table 1). Similar experiments performed with normal CD34<sup>+</sup> cells showed no effect of NGF on CD34<sup>+</sup> expansion (data not shown).

To determine whether TRKA overexpression recapitulates the effects of expressing AML1-ETO in CD34<sup>+</sup> cells, we transduced normal CB cells with a retrovirus expressing human TRKA. TRKA expression in the CD34<sup>+</sup> cells did not significantly alter their cellular expansion in liquid culture, compared with the empty MIGR1 vector (Fig. 4*b*), whereas AML1-ETO-expressing cells show significant cellular expansion, as reported. Whereas expression of AML1-ETO clearly leads to the expansion of CD34<sup>+</sup> cells



**Table 1. NGF synergizes with the five-cytokine mix (KTF36) to promote expansion of AML1-ETO-positive cells**

	Total cell expansion × 10 <sup>13</sup>				CD34 <sup>+</sup> cell expansion × 10 <sup>13</sup>			
	KTF36	KTF36 + NGF(20)	KTF36 + NGF(1)	Fold increase	KTF36	KTF36 + NGF(20)	KTF36 + NGF(1)	Fold increase
Week 9 AE9	2.3	17.9		7.8	1.3	10.3		8.2
Week 9 AE11.1	6.5	22.6		3.5	1.9	7.2		3.7
Week 10 AE13	2.5		9.3	3.8	1.1		3.8	3.6
Week 11 AE11.1	6.1		17.6	2.9	2.2		4.6	2.1

The AML1-ETO-positive cells were CD34-selected at the indicated week of growth and expanded in liquid culture using the five-cytokine mixture KTF36 or additionally supplemented with 20 ng/ml NGF or 1 ng/ml NGF. Cells were counted weekly by Trypan blue dye exclusion and stained for expression of CD34. The numbers indicate growth over a 10-week period. Control CB and PBPC CD34<sup>+</sup> cells were cultured under similar conditions, with no effect of NGF on total or CD34<sup>+</sup> cell expansion (data not shown).

significantly higher positive proportions than would be expected. For the 5-fold threshold, only cluster 13 (7 of 21; 33.3%) had significantly higher TRKA mRNA expression. Thus, the level of TRKA expression determined by qPCR (shown in Table 2) confirmed the microarray data with respect to the AML1-ETO-positive clinical samples (cluster 13).

### Discussion

AML is characterized by the accumulation of immature, nonfunctional hematopoietic cells (myeloblasts) that proliferate but are blocked in their differentiation. This behavior is thought to require two or more distinct genetic lesions, because leukemia-associated fusion transcription factors such as AML1-ETO generally are not able to reproducibly generate acute leukemia by themselves in mice (33–37, 46). We have shown that AML1-ETO expression in human CD34<sup>+</sup> hematopoietic cells maintains these cells in culture, allowing their expansion and the generation of factor-dependent, clonal, preleukemic cell cultures (30, 31). The downstream pathways through which AML1-ETO exerts its effects are currently unknown. We have investigated the transcriptional profiles of primary human hematopoietic progenitor cells transduced with AML1-ETO by using Affymetrix oligonucleotide gene arrays, and have demonstrated that the TRKA oncogene is reproducibly up-regulated at both the RNA and protein level. This up-regulation of TRKA results in a proliferative response to NGF that is not apparent by using normal CD34<sup>+</sup> cells. This response is seen even in the presence of the five cytokines typically used to grow these cells, implying that a signaling cascade is initiated by NGF, in the presence of AML1-ETO, which is not provided by saturating

amounts of stem cell factor, megakaryocyte growth and differentiation factor, FLT3 ligand, IL-6, or IL-3.

Whereas several groups have systematically analyzed potential downstream targets of this fusion protein, by using established cell lines for their studies (27–29), we used primary human CD34<sup>+</sup> cells for the identification of AML1-ETO-regulated genes, and identified TRKA as a potentially important component of AML1-ETO function. The retroviral transduction methodology does not allow us to define whether TRKA is a direct or indirect target gene of AML1-ETO, although it did allow us to show its biological relevance. Whereas chromatin IP studies may determine whether the AML1 binding site(s) in the 5'-flanking region of the TRKA gene are occupied by AML1-ETO, other regulatory regions in the gene may be more critical to its regulation, and technical difficulties may also limit interpretation of such studies.

The involvement of TRK family members in acute myeloid leukemia has been well established (47–50). From our data and from previous studies, TRKA mRNA is expressed in both human and murine hematopoietic stem/progenitor cells, and the protein is competent to respond to NGF ligand (42). Our biochemical analyses indicate that NGF activates the MAPK and PI3K signaling pathways in primary human CD34<sup>+</sup> cells, and similarly in AML1-ETO-positive cells. Clearly, the functional consequences of TRKA activation are significantly different in the AML1-ETO-expressing cells. Neither primary CD34<sup>+</sup> cells, nor those expressing AML1-ETO, proliferate in response to NGF alone. Yet, cells expressing AML1-ETO will respond to the combination of NGF and IL-3 with an expansion of CD34<sup>+</sup> cells, but control CD34<sup>+</sup> cells will not.

We analyzed the basal and inducible tyrosine phosphorylation pattern in AML1-ETO-positive cells and in normal human CD34<sup>+</sup>

**Table 2. Real-time qRT-PCR analysis of TRKA mRNA levels in primary AML patient samples**

Cluster	Nos. in cluster	Nos. >2-fold	Proportion >2-fold	P value for		Nos. >5-fold	Proportion >5-fold	P value for	
				2-fold	>5-fold			5-fold	>5-fold
1	12	1	0.083	1	1	1	0.083	1	1
2	17	3	0.176	1	0	0	0.000	1	1
3	18	9	0.500	0.0032	2	0.111	0.9989	1	1
4	15	2	0.133	1	0	0.000	1	1	1
5	42	0	0.000	1	0	0.000	1	1	1
6	8	1	0.125	1	0	0.000	1	1	1
7	18	1	0.056	1	0	0.000	1	1	1
8	12	0	0.000	1	0	0.000	1	1	1
9	21	10	0.476	0.0027	4	0.190	0.2675	1	1
10	23	5	0.217	0.9999	1	0.043	1	1	1
11	9	0	0.000	1	0	0.000	1	1	1
12	19	6	0.316	0.7945	3	0.158	0.5398	1	1
13	21	10	0.476	0.0027	7	0.333	0.0005	1	1
14	10	0	0.000	1	0	0.000	1	1	1
15	8	1	0.125	1	1	0.125	1	1	1
16	11	0	0.000	1	0	0.000	1	1	1

Numbers in cluster are the number of patient samples in each cluster. Numbers >2-fold (or 5-fold) are number of sample with TRKA mRNA levels ≥2-fold (or 5-fold) the level of expression in CD34 selected bone marrow cells. Proportion >2-fold (or 5-fold) is the percentage of samples in that cluster with >2-fold (or 5-fold) TRKA mRNA levels, relative to CD34<sup>+</sup> bone marrow cells. P values are provided for the proportion of samples with a >2-fold (or >5-fold) increase in TRKA mRNA levels.

cells by Western blot using an anti-phosphotyrosine specific antibody. Multiple constitutively tyrosine phosphorylated proteins of  $\approx 45$ , 50–60, and  $>200$  kDa were reproducibly detected in the two AML1-ETO-positive cells tested; they were not seen in normal CD34<sup>+</sup> cells (data not shown). Whether these signaling molecules play a role in the unique response of the AML1-ETO cells to the combination of IL-3 and NGF awaits the identification of these proteins. Whereas the lack of expression of NGF by the AML1-ETO-expressing cells demonstrates that the NGF/TRKA signaling pathway is not essential for the *in vitro* effects of AML1-ETO on human CD34<sup>+</sup> cells, the increased TRKA expression seen in the primary t(8;21)-positive AML patient samples certainly supports the biological relevance of our findings. Furthermore, the inv(16) samples (cluster 9), another subset of the CBF leukemias that contain the CBF $\beta$ -SMMHC fusion protein also show increased TrkA expression ( $\geq 2$ -fold; see Table 2).

From all available data, it is clear that AML1-ETO does not impart a strong proliferative effect on its own, because mouse or human hematopoietic cells expressing the fusion protein strictly depend on cytokines for continued growth (31, 51). However, stromal cells in the bone marrow microenvironment secrete NGF and other cytokines (41); thus, the enhanced expression of the TRKA receptor on AML1-ETO-positive cells could impart a

selective growth advantage. The recent finding that AML1-ETO deregulates genes involved in DNA repair underscores the importance of promoting preleukemic stem cell growth to allow for the acquisition of cooperating mutations that could lead to a fully transformed state (27).

In summary, our data identify a functionally relevant target gene of AML1-ETO in the clinically relevant CD34<sup>+</sup> HSPCs and may be the first example of using primary human cells to identify a downstream target gene of an oncogene. The up-regulation of TRKA by AML1-ETO in human HSPCs and the unique functional response to NGF suggest that TRKA could serve as a potential target for therapeutic intervention in t(8;21) positive leukemias.

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