CCAAT/enhancer binding proteins (C/EBPs) play critical roles in myelopoiesis. Dysregulation of these proteins likely contributes to the pathogenesis of myeloid disorders characterized by a block in granulopoiesis. In one such disease, acute promyelocytic leukemia (APL), a promyelocytic leukemia–retinoic acid receptor α (PML-RARα) fusion protein is expressed as a result of a t(15;17) chromosomal translocation. Treatment of PML-RARα leukemic cells with all-trans retinoic acid (ATRA) causes them to differentiate into mature neutrophils, an effect thought to be mediated by C/EBPs. In this study, we assess the potential for cooperativity between increased C/EBP activity and ATRA therapy. We demonstrate that although both C/EBPα and C/EBPε can significantly prolong survival in a mouse model of APL, they are not functionally equivalent in this capacity. We also show that forced expression of C/EBPα or C/EBPε in combination with ATRA treatment has a synergistic effect on survival of leukemic mice compared with either therapy alone. (Blood. 2006;108:2416-2419)

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Introduction

CCAAT/enhancer binding proteins (C/EBPs) are a family of transcription factors that regulate cell growth and differentiation. Two members of this family, C/EBPα and C/EBPε, are of critical importance in granulopoiesis. Disruption of the C/EBPα gene in mice results in the loss of production of neutrophils and eosinophils, whereas mice that lack C/EBPε generate neutrophils and eosinophils with abnormal function, gene regulation, and morphology.

C/EBPα is the founding member of the bZIP class of DNA-binding proteins. Members of this family contain distinct N-terminal transactivation domains, C-terminal leucine-zipper dimerization domains, and basic DNA-binding regions. C/EBPα’s basic region confers not only its ability to bind DNA but also its inhibition of E2F pathways. Previous studies have shown that the integrity of DNA binding, transactivation, and E2F inhibition is required for C/EBPα-dependent granulocytic differentiation. Although the functional domains required for C/EBP activity have not been well characterized, C/EBP’s role in directing expression of myeloid-specific genes associated with terminal differentiation of granulocytes has been clearly demonstrated.

Because C/EBPα and C/EBPε are required for normal granulocytic differentiation, alterations in expression or function of these proteins likely contribute to the pathogenesis of acute myeloid leukemia (AML), a disease characterized by an early block in granulopoiesis. Prior studies provide evidence that C/EBPα and C/EBPε may play a role in the pathogenesis of acute promyelocytic leukemia (APL), a subtype of AML in which a t(15;17) chromosomal translocation juxtaposes the promyelocytic (PML) gene to the retinoic acid receptor α (RARA) gene, creating an aberrant PML-RARα fusion protein.

A unique characteristic of PML-RARα leukemic cells is their sensitivity to all-trans retinoic acid (ATRA). Treatment with ATRA induces remissions in patients with APL by causing the leukemic cells to differentiate into mature neutrophils. While the mechanism underlying the sensitivity of promyelocytes to ATRA is not completely understood, we and others have suggested that C/EBPs mediate the ATRA-induced maturation of APL cells.

In the present study, we explore the mechanism by which C/EBPs prolong survival in a murine model of APL. We also assess the potential for cooperativity between increased C/EBP activity and ATRA therapy. We demonstrate that both C/EBPα and C/EBPε significantly prolong survival; however, they are not functionally equivalent in this capacity. We also show that forced expression of C/EBPα or C/EBPε in combination with ATRA treatment has a synergistic effect on survival of leukemic mice compared with either therapy alone.

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Study design

Plasmids

A rat C/EBPα cDNA (rC/EBPα) was generated by polymerase chain reaction (PCR) and cloned into the tamoxifen-inducible pBahevpro3.3bbb estrogen receptorα (pBP3:herERα) to generate pBP3:rC/EBPα-ER. For generation of MIG-rC/EBPα-ER, the rC/EBPα-ER fragment was excised from pBP3:rC/EBPα-ER and cloned into the mouse stem cell–

Results and discussion

C/EBPα and C/EBPε play central roles in normal myelopoiesis; therefore, it is likely that altered function of these proteins contributes to the pathogenesis of APL. In a previous study,16 we showed that expression of a tamoxifen-inducible form of C/EBPα, hC/EBPα-ER, in leukemic cells caused them to differentiate into mature neutrophils in vivo. In the present study, we expand this approach to C/EBPα and assess the abilities of both C/EBPα and C/EBPε to prolong survival in a mouse model of APL. To assess the antileukemic effect of C/EBPs in this system, we transduced PML–RARα leukemic cells25 with either C/EBPα-ER or C/EBPε-ER retrovirus and transplanted them into sublethally irradiated histocompatible mice. After leukemias developed in the recipient animals, the mice were treated with either a placebo or 4-HT to induce C/EBP activity. In mice receiving transplants with C/EBPα-ER–transduced leukemias, treatment with 4-HT prolonged mean survival by 7 days compared with animals given a placebo (Figure 1A). Animals that received C/EBPα-ER–transduced leukemias demonstrated a more robust response following treatment with 4-HT (Figure 1B), exhibiting a mean increase in survival of 11 days compared with the placebo group. Therefore, tamoxifen-

Cell culture

The 32Dc13 cell line was modified to express high levels of the ecotropic receptor (32Dc13-eco R), thereby facilitating retroviral transduction. These cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin G, 100 µg/mL streptomycin, and 5% X63Ag8–mouse interleukin-3 (mIL-3)–conditioned media. BOSC23 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. freshly harvested leukemic no. 1111 cells23 from bone marrow and spleen of leukemic mice were cultured in stem cell media (Myelocult M5300; StemCell Technologies, Vancouver, BC, Canada) with 15% FBS, 5% IL-3–conditioned media, 0.4 mM glutamine, 100 U/mL penicillin G, 100 µg/mL streptomycin, 10 ng/mL rIL-3, 10 ng/mL IL-6, and 10 ng/mL stem cell factor.

Retroviral transduction

BOSC23 cells were transfected with retroviral constructs as previously described.24 Retroviral supernatants were collected and used to transduce leukemic bone marrow (#1111; 2 × 106 cells per well) and 32Dc13 cells (100 000 cells per well) as previously described.16 After transduction, leukemic #1111 and 32Dc13 cells were sorted using the fluorescence–

Mice

Mice were bred and maintained at the University of California at San Francisco, and their care was in accordance with Institutional Animal Care and Use Committee guidelines. To examine C/EBPs in vivo, retrovirally transduced GFP-positive leukemic no. 1111 cells (50 000 cells per mouse) were injected into the lateral tail vein of sublethally irradiated (4.5 Gy) 6- to 8-week-old female FVB/N mice. Each group (C/EBPα, C/EBPε, or the mutant version of each) contained 20 mice, with 5 animals in each subgroup (placebo, 4-HT, ATRA, and 4-HT plus ATRA). Treatments (placebo, 25 mg 4-HT, and 10 mg ATRA) were administered to each subgroup as 21-day release, subcutaneous pellets (Innovative Research of America, Sarasota, FL).

Statistical analysis

Statistical analyses were performed in GraphPad Prism using the log-rank test or in Excel 2000 using the Student t test with 2-tailed distribution and unequal variance as appropriate.
the potential for cooperativity between treatment with ATRA and forced expression of C/EBPs. To address this relationship, we transduced PML-RAR
/H9251 leukemic cells with either C/EBP
/H9251-ER or C/EBP
/H9280-ER retrovirus and transplanted them as before. After leukemias developed in recipient animals, 5 mice from each group were given either a placebo or 25 mg 4-HT. Animals that received C/EBP
/ER-transduced leukemias exhibited prolonged survival following treatment with 4-HT (mean survival time of 33 days compared with 26.4 days in the placebo group; P = .002). C/EBP
R211A-ER had no effect on survival of leukemic mice (mean survival time of 37.4 days compared with 36.4 days in the placebo group). In animals receiving transplants with C/EBP
-ER– or C/EBP
R289A-ER–transduced leukemias, treatment with 4-HT significantly prolonged survival (P = .003). Mean survival times for placebo and 4-HT-treated groups were 33.8 days and 46.4 days, respectively, for C/EBP
-ER mice and 31.4 days and 39.8 days, respectively, for C/EBP
R289A-ER mice. (D, F) 32Dc13 cells were plated in 24-well dishes at 100 000 cells per well; transduced with retroviruses expressing C/EBP
, C/EBP
R211A-ER, C/EBP
-ER, or C/EBP
R289A-ER, and cultured in the absence or presence of 20 nM 4-HT. The growth curve represents the number of transduced cells at days 0, 2, 4, and 6. The results are mean ± SD from at least 3 independent experiments. (D) *P < .05 and (F) *P = .01.

Figure 2. Forced expression of C/EBPs in combination with ATRA treatment has a synergistic effect on survival of leukemic mice. Leukemic animals expressing either C/EBP
-ER (A) or C/EBP
-ER (B) were generated as described in Figure 1 and treated with one of the following: a placebo, 25 mg 4-HT, 10 mg ATRA, or a combination of 4-HT and ATRA. The data for each C/EBP group are from 20 animals with 5 mice per treatment. The combined therapy of 4-HT and ATRA resulted in the most profound effect on survival in both groups (P < .001). The mean survival times of placebo, 4-HT, ATRA, and 4-HT plus ATRA groups were 26.4, 33.4, 49.6, and 63 days, respectively, in animals receiving transplants with C/EBP
ER-transduced leukemias and 30.5, 41.8, 69, and 97.8 days, respectively, in C/EBP
ER animals.


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CCAAT/enhancer binding proteins alpha and epsilon cooperate with all-
trans retinoic acid in therapy but differ in their antileukemic activities

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