Granulocyte inducer C/EBPα inactivates the myeloid master regulator PU.1: possible role in lineage commitment decisions

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Several transcription factors have been implicated as playing a role in myelopoiesis. PU.1, an ets-family transcription factor, is required for the development of myeloid and lymphoid lineages, whereas the transcription factor CCAAT–enhancer binding protein family member C/EBPα is essential for granulocyte development. We present here the first evidence that C/EBPα blocks the function of PU.1. PU.1 and C/EBPα interact physically and colocalize in myeloid cells. As a consequence of this interaction, C/EBPα can inhibit the function of PU.1 to activate a minimal promoter containing only PU.1 DNA-binding sites. We further demonstrate that the leucine zipper in the DNA-binding domain of C/EBPα interacts with the β3/β4 region in the DNA-binding domain of PU.1 and as a result displaces the PU.1 coactivator c-Jun. Finally, C/EBPα blocks PU.1-induced dendritic cell development from CD34+ human cord blood cells. The functional blocking of PU.1 by C/EBPα could be the mechanism by which C/EBPα inhibits cell fates specified by PU.1 and directs cell development to the granulocytic lineage. (Blood. 2002;100:483-490)

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Introduction

Hematopoiesis is the regulated development of distinct cellular lineages from a common precursor, the hematopoietic stem cell. Fundamental changes in gene expression result in each cell type expressing a characteristic complement of genes necessary for its function. This is achieved through the action of transcriptional regulators with general and restricted expression patterns in the hematopoietic system.1 The ets domain transcription factor PU.1 is preferentially expressed in myeloid and B cells.2,3 Inactivation of the PU.1 gene in mice causes defects in the development of multiple hematopoietic lineages, including B and T lymphocytes, monocytes, and granulocytes.4,5 PU.1 regulates the expression of almost all characterized myeloid genes, including growth factor receptors. In particular, it directs the monocyte-specific expression of the macrophage colony-stimulating factor receptor.6,7 PU.1 probably plays an important role at several stages in the differentiation process, and there is evidence that it is active at an early stage, mediating commitment of multipotential progenitor cells to the myeloid lineage.8

CCAAT/enhancer-binding protein alpha (C/EBPα) was initially identified in liver and adipose tissue, where it was found to be important for terminal differentiation.9,14 C/EBPα expression is prominent in immature myeloid cells.15-17 C/EBPα-null mice lack the entire granulocyte lineage but develop normal monocytes.18 Recently, we identified dominant-negative mutations of C/EBPα in acute myeloid leukemia19 and a down-regulation of C/EBPα expression by the leukemic fusion protein AML1/ETO,20 suggesting an important role of C/EBPα in leukemogenesis. Ectopic expression of C/EBPα in U937 monocyte leukemia cells induces granulocytic differentiation over a 2-week period and inhibits monocyte differentiation.16 These hematopoietic progenitors require PU.1 to initiate monocyte differentiation and C/EBPα to initiate granulopoiesis. PU.1 has been shown to interact with a C/EBP family member, C/EBPβ.21 However, the interaction of these transcription factors in differentiating to a specific lineage is still unclear.

We propose here that the granulocyte factor C/EBPα interacts with the myeloid master regulator PU.1 and inactivates PU.1. c-Jun belongs to the b-ZIP group of DNA-binding proteins and is a component of AP-1 transcription complexes.22 c-Jun has been shown to be a coactivator of PU.1, resulting in increased macrophage–colony-stimulating factor (M-CSF) receptor expression, and it is involved in the development of the monocyte lineage.23 Here we show that C/EBPα blocks PU.1 function by displacing c-Jun, the coactivator of PU.1. Furthermore, C/EBPα specifies the fate of myeloid progenitor cells to the granulocytic lineage by inactivating PU.1 through protein–protein interactions.

Materials and methods

Cell lines and cell culture

Fibroblast F9 and 293T cells were cultured in Dulbecco Modified Eagle Medium (PAN Biotech GmbH, Karlsruhe, Germany) containing 10% fetal bovine serum (FBS; Gibco BRL, Aidenbach, Germany; catalog no. 10270-106), 1% Penstrep (Gibco BRL; catalog no. 15070-022), and 1% l-glutamine (Gibco BRL; catalog no. 25030-024). Human myeloblast suspension myeloid U937 cells (DSMZ: ACC5; Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), U937 with grant to G.B. (Nv 2042/2-1).

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inducible expression of C/EBPα, and U937 pPC18 cells were grown in RPMI (PAN Biotech GmbH) containing 10% FBS (Gibco BRL; catalog no. 10270-106), 1% Penstrep (Gibco BRL; catalog no. 15070-022), and 1% l-glutamine (Gibco BRL; catalog no. 25305-024).

Reporter constructs and expression plasmids
p(PU.1)4TK is a dimer of both PU.1 sites from the granulocyte colony-stimulating factor receptor promoter from bp +28 to +54 subcloned into pTK811ac, a pXP2-based luciferase construct with a TATA box only as a minimal promoter. 25 pTK with mutated PU.1 sites (p(mutatedPU.1)4TK) is a dimer of both mutated PU.1 sites from the granulocyte colony-stimulating factor receptor promoter (primers, 5′-TCG AGT GTG TGC ACA AAT TTG TGT TGA CGA GAG-3′ and 5′-TCG ACT CTC GTC AAC AAA AGT TGG TGA AAC CAC-3′) subcloned into pTK811ac and was constructed as described for pTK with PU.1 sites. 23,24 As an internal control plasmid for cotransfection assays, the pRL-null construct driving a Renilla luciferase gene (Promega, Madison, WI) was used. 26 Human PU.1 promoter was kindly provided by D.G.T. (Boston, MA). The bacterial GST expression vector pGEX-2TK-PU.1 has been described previously. 27 pGEX-C/EBPα-DNA-binding domain was kindly provided by C. Nerlov (Copenhagen, Denmark). Expression plasmids pMSV C/EBPα (rat), C/EBPβ mutated Basic region (C/EBPβmBR), and C/EBPβ leucine zipper deleted (C/EBPβΔLZ) were kindly provided by Alan Friedman (Baltimore, MD). Human p53 (c-Jun containing wild type c-Jun was kindly provided by Jinmian Tian and Michael Karin (San Diego, CA). pPECE-PU.1 expression plasmid was provided by R. A. Maki (La Jolla Cancer Research Foundation, CA). 28 Gal4 constructs (pGal4-Luc, Gal4-Tel, Gal4-VP16) were kindly provided by S. Bohlander (Munich, Germany).

Transient transfections using lipofectamine plus and reporter assays for firefly and Renilla luciferase
F9 cells and 293T cells were transfected using lipofectamine plus (Life Technologies) as described by the manufacturer. 29 Firefly luciferase activities from the constructs p(PU.1)4TK, 30 p(C/EBP)2TK, and pGal4-DBD and Renilla luciferase activity from the internal control plasmid pRL-null were determined 24 hours after the initiation of the transfection protocols using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activities were normalized to the Renilla luciferase values of pRL-null. Results are given as mean ± SD of at least 6 independent experiments. The following RNA concentrations of the reporter constructs and expression plasmids were used for firefly luciferase plus transfections: 0.1 μg (p(PU.1)4TK, p(mutatedPU.1)4TK, p(C/EBP)2TK, and pGal4-DBD) and 0.05 μg internal control plasmid pRL-null; 0.1 μg expression plasmids for PU.1, C/EBPα, C/EBPβ mutants, c-Jun, Gal4-PU.1 activation domain, Gal4-VP16, and Gal4-Tel; the same concentrations of the empty expression vectors were used as controls, respectively.

Protein interaction assay
Protein interaction assays were performed as described previously. 23,30 c-Jun and C/EBPα were transcribed in vitro and translated using the TNT reticulocyte lysate system (Promega) and labeled with [35S] methionine (NEN Life Science Products, Dreieich, Germany). One microliter labeled in vitro-translated c-Jun or C/EBPα was mixed with 1 μg bacterial GST-PU.1 or with equivalent amounts of GST or glutathione–agarose beads (Pharmacia, Freiburg, Germany) for 1 hour at 4°C in NETN buffer (20 mM Tris, pH 8.0, 200 mM NaCl, 1 mM EDTA, and 0.5% NP40). GST-PU.1 was recovered using glutathione–agarose beads, washed 6 times with NETN buffer, and separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) before autoradiography. The gel was stained with Coomassie brilliant blue (Gibco) to verify that the protein concentrations of GST-PU.1 and GST were the same in all lanes.

Coimmunoprecipitation
293T cells were transfected with expression plasmids of PU.1, C/EBPα, C/EBPβΔLZ, and C/EBPβmBR by using lipofectamine (Gibco BRL). Twenty-four hours after transfection, whole-cell lysates were incubated with primary antibody diluted 1:1000 and bound to protein A agarose beads for 90 to 120 minutes on ice in 2% glycerol-0.5% Nonidet P-40 in 1 mM EDTA–20 mM Tris-HCl, pH 8–100 mM NaCl–10 mM MgCl2–0.1 mM ZnSO4. Beads were washed with prechilled NETN 3 times, and bound proteins were separated on SDS-PAGE gels and transferred to nitrocellulose membranes for Western blotting. For coimmunoprecipitation of PU.1 and C/EBPα, 10 ng PU.1 polyclonal antibody first was coupled to protein A beads. Proteins were detected by enhanced chemiluminescence (Amer sham Pharmacia). Primary antibodies used were rabbit anti-PU.1 (sc-352; Santa Cruz Biotechnology, CA) and rabbit anti-C/EBPα polyclonal antibody (sc-9314; Santa Cruz Biotechnology).

RNA extraction and quantitative real-time polymerase chain reaction
U937 pPC18 and U937 with Zn-inducible expression of C/EBPα cells were stimulated with 100 μM zinc (ZnSO4) for different time points, and total RNA was isolated using RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. One microgram extracted RNA was subsequently transfected in a 20 μL cDNA synthesis reaction using the Omniscript Reverse Transcription protocol (Qiagen).

Real-time polymerase chain reaction (PCR) for PU.1 and for the housekeeping gene glucose-6 phosphate dehydrogenase (G6PD) was performed using the Light Cycler Technology (Roche Diagnostics, Mannheim, Germany). For amplification of G6PD, primers were used according to Emig et al. 31 PU.1 was amplified using the Light Cycler-Primer set for human Spi-1 (Search-LC, Heidelberg, Germany) following the manufacturer’s instructions. G6PD plasmid pGdBXX, kindly provided by A. Hochhaus, was serially diluted to 10 000 fg, 1000 fg, and 100 fg and was used as a standard curve. The concentration of each sample was calculated automatically by reference to this curve. PU.1 concentration in each sample was relatively quantified by calculating the ratio between PU.1 and the housekeeping gene G6PD. PCR for G6PD was performed using 2 μL Mastermix (Light Cycler FastStart DNA Master SYBR Green I; catalog no. 3 003 230; Roche Diagnostics, Mannheim, Germany), 2 μL cDNA (see above), 4 mM MgCl2, 7.5 μM each primer, and water to a final volume of 20 μL. Amplification occurred in a 3-step cycle procedure initiated by 10-minute denaturation at 95°C to activate the polymerase: 95°C for 0 seconds, annealing at 64°C for 10 seconds, and extension at 72°C for 25 seconds for 35 cycles. Fluorescence of SYBR Green I was measured after each extension step at 530 nm in channel F1. The final PCR cycle was followed by a melting curve analysis to confirm PCR product identity and to differentiate it from nonspecific (eg, primer–dimmer) products. For that, the products were denatured at 95°C, annealed at 65°C, and slowly heated up to 95°C with fluorescence measurement at 0.2°C increments. Some amplified products were checked by electrophoresis on 1% ethidium bromide–stained agarose gels. The estimated size of the amplified fragments matched the calculated size; for PU.1 it was 150 bp, and for G6PD it was 343 bp.

Production of retrovirus
Mouse PU.1 DNA followed by internal ribosomal entry site (IRES) nerve growth factor receptor truncated in the cytoplasmic domain (tNGFR) and human C/EBPα cDNA followed by IRES EGFP were subcloned into a retroviral vector, pMSCV, with an LTR derived from MCV (pMSCV-Pu.1-ires-tNGFR and pMSCV-C/EBPα-ires-EGFP, respectively). To produce virus, pMSCV DNA was transfected into 293gp cells (293 cells containing the gag and pol genes but lacking an envelope gene) along with 10A1/env expression plasmid (pCL-10A1) by CaPO4 coprecipitation, and supernatant from the transfected cells was collected to transduce cells.

Transduction of CD34+ cells
Human umbilical cord blood samples were obtained, with informed consent of the parents, from placentas of full-term healthy newborn infants. After the isolation of mononuclear cells from cord blood by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway), CD34+ cells were obtained using magnetic bead separation (MACS CD34+ cell isolation kit; Miltenyi Biotec, Bergisch Gladbach, Germany) according to...
the manufacturer’s instructions. CD34+ cells were prestimulated in Iscoves modified Dulbecco medium (IMDM; Sigma, St Louis, MO) supplemented with 10% FBS, 50 ng/mL stem cell factor, 50 ng/mL thrombopoietin (kindly provided by Kirin, Tokyo, Japan), 50 ng/mL interleukin-6 (IL-6; Peprotech, Rocky Hill, NJ), and 50 ng/mL Fl-3L (Peprotech) for 20 hours. After replating onto recombinant fibronectin fragment-coated culture dishes (Takara Shuzo, Otsu, Japan) containing virus supernatant and 5 μg/mL protamine sulfate (Sigma), cells were centrifuged at 1000g for 30 minutes. Transduction was repeated 3 times with fresh virus supernatant at 12-hour intervals. Sixty hours after the first transduction, NGFR- and EGFP-positive cells were selected by cell sorting on a FACS Vantage (Becton Dickinson, San Jose, CA) and were subjected to subsequent analyses. To detect the expression of NGFR on the cell surface, cells were stained with mouse anti-human NGFR (Chemicon, Temecula, CA) followed by phycoerythrin (PE)-conjugated rabbit anti-mouse immunoglobulin (DAKO A/S, Glostrup, Denmark).

In vitro liquid culture

CD34+ cells transduced with either PU.1 or C/EBPα retroviruses or cotransduced with PU.1 and C/EBPα retroviruses were cultured in IMDM supplemented with 10% heat-inactivated FBS and 50 ng/mL stem cell factor, 50 ng/mL G-CSF, 50 ng/mL GM-CSF, and 50 ng/mL IL-3 (Kirin) at 37°C in 5% CO2 atmosphere. On day 10 of culture, expression of cell surface antigens was analyzed on a FACS Vantage using PE-conjugated anti-human CD1a, CD15, CD14, CD80, CD90, CD86, and HLA-DR (Immunotech, Marseilles, France), CD14, CD80, CD86, and HLA-DR (PharMingen, San Diego, CA). Cells were also cytocentrifuged onto glass slides and were stained with May-Gruenwald-Giemsa solution (Merek, Darmstadt, Germany) followed by Giemsa solution (Kanto Chemical Co, Tokyo, Japan).

Immunolocalization

U937 cells were cytocentrifuged onto glass slides, fixed with ice-cold acetone for 2 minutes, dried, and rehydrated in phosphate-buffered saline (PBS). Slides were blocked in 10% FCS for 30 minutes at room temperature, washed with PBS, and incubated overnight at 4°C in primary antibodies C/EBPα and PU.1 (sc-9314, Santa Cruz Biotechnology; 554268, PharMingen). Cytospins were washed with PBS and incubated with secondary antibodies Texas red and Cy3 (Dianova GmbH, Germany) for 45 minutes. The slides were mounted with antifade solution.

Results

C/EBPα blocks the transcriptional activity of PU.1 on a minimal TK promoter containing PU.1 DNA-binding sites only

Because PU.1 and C/EBPα are present in myeloid progenitor cells, we asked how cells differentiate into a specific lineage and whether there is direct interaction or cross-talk between these 2 major transcription factors. To address this, we used a minimal TK promoter with 4 PU.1 binding sites only. This minimal promoter was transactivated 6-fold on transfection of 293T cells with a reporter construct of a minimal TK promoter with C/EBPα expression plasmid in the same experiment resulted in a 4-fold decrease of PU.1 transactivation capacity (Figure 1A). As a control, cotransfection of PU.1 and C/EBPα did not affect the activity of a minimal TK promoter with mutated PU.1 binding sites. In further control experiments, we transfected 293T cells with a reporter construct of TK promoter with multimerized C/EBPα binding sites and a C/EBPα expression plasmid, transactivating the promoter 11-fold. There was no change in protein expression of PU.1 on cotransfection with the C/EBPα expression plasmid, as observed by Western blot for PU.1 from transfected 293T cells (data not shown). To check whether C/EBPα down-regulates transcription factors in a nonspecific fashion, we transfected 293T cells with expression plasmids of Gal4-VP16, C/EBPα, and the reporter construct pGal4-luc. Met-labeled in vitro-translated C/EBPα (lane 2) was incubated with 1 μg bacterially expressed GST-PU.1 (lanes 5, 6). Equivalent amounts of GST protein or glutathione agarose beads (lanes 3, 4) were incubated with in vitro–translated C/EBPα, and as a control, in vitro–translated c-Jun was incubated with GST-PU.1 in lane 1.

C/EBPα physically interacts with PU.1

To investigate whether there is a direct protein–protein interaction between C/EBPα and PU.1, we used GST-purified GST-PU.1 and incubated it with in vitro–translated C/EBPα. An interaction between PU.1 and C/EBPα was observed. This interaction was resistant to the effect of chaotrope agents such as dithiothreitol and a change in ionic strength during the incubation reaction (Figure 1B). C/EBPα did not bind to GST or beads alone. Given the observed interaction between C/EBPα and PU.1, we examined the intranuclear location of these proteins. U937 cells were cytocentrifuged and labeled with PU.1 and C/EBPα antibodies, respectively.
Secondary antibodies were Texas red for PU.1 and Cy-3 (green) for C/EBPα. We observed diffuse nuclear staining. The overlay shows that both proteins colocalize in the nucleus (yellow) (Figure 2).

C/EBPα inhibits coactivation of PU.1 by c-Jun

Using a minimal TK promoter with multimerized PU.1 binding sites only (p(PU.1)4TK), we observed that PU.1 transactivated p(PU.1)4TK 6-fold in F9 cells (Figure 3A). F9 cells do not express c-Jun (c-Jun expression was determined by reverse transcription [RT]-PCR). When cotransfected with the expression plasmid of c-Jun, we observed a strong synergy of approximately 40-fold, as described before.\(^{23}\) Cotransfection of C/EBPα in the same experiment totally blocked the PU.1 transactivation capacity of the p(PU.1)4TK promoter and the coactivation of PU.1 by c-Jun (Figure 3A). C/EBPα did not down-regulate the transactivation of Gal4-VP16 in a nonspecific fashion under the same conditions.

C/EBPα displaces c-Jun from binding to PU.1 in vitro

As shown before, C/EBPα inhibits the c-Jun coactivation of PU.1. To relate these findings to protein–protein interactions between C/EBPα and PU.1, we performed GST pull-down experiments, and \(^{35}\)S-labeled in vitro-translated c-Jun and C/EBPα were incubated with GST-PU.1. We already demonstrated that c-Jun strongly binds to PU.1,\(^{23}\) and here we show that C/EBPα also binds to PU.1 strongly. When both factors were incubated with PU.1, C/EBPα displaced c-Jun from binding to PU.1 (Figure 3B). We determined that C/EBPα interacted with the β3-β4 region of the DNA-binding domain of PU.1, the same region in which c-Jun interacts with PU.1 (Figure 3C). Incubation of \(^{35}\)S-labeled in vitro-translated PU.1 with the GST-DNA-binding domain of C/EBPα showed that C/EBPα interacts with PU.1 through its DNA-binding domain (Figure 3D).

C/EBPα interacts with PU.1 through the leucine zipper in the DNA-binding domain

To whether both proteins interact in vivo, we transfected 293T cells with expression plasmids of PU.1, C/EBPα, C/EBPαΔLZ, and C/EBPαmBR by using lipofectamine. Whole-cell lysates were immunoprecipitated with either rabbit–immunoglobulin G (IgG) or
C/EBPα does not recruit trichostatin A–sensitive corepressors in down-regulating PU.1 transcriptional activity

To investigate whether C/EBPα recruits corepressors to down-regulate PU.1 transcriptional capacity, we transfected F9 cells with the TK promoter containing PU.1-binding sites and expression plasmids of PU.1 and C/EBPα. We found that C/EBPα blocks the activity of PU.1 to transactivate the minimal TK promoter with PU.1-binding sites. Trichostatin A (TSA) has been shown to be an inhibitor of a class of corepressors. Transcription factor Tel recruits these corepressors and represses the promoter activity of Gal4-luciferase. Addition of TSA releases this repression as it is seen in the transfection of 293T cells with Gal4-Tel. On the addition of TSA to the cells, this repression is lost and the promoter activity is restored. In a similar experiment in which the transactivation block of PU.1 by C/EBPα was seen, the addition of TSA did not release the repression. These data suggest that repression of PU.1 activity by C/EBPα does not occur through the recruitment of TSA-sensitive corepressors (Figure 6).

C/EBPα down-regulates PU.1 expression in myeloid U937 cells

We then investigated whether C/EBPα blocks the expression of PU.1 target genes. Because PU.1 is autoregulatory in its expression, PU.1 itself is a target gene of PU.1. We therefore performed quantitative real-time PCR using real-time Light Cycler technology (Roche) to determine the expression of PU.1 in the U937 cell line with Zn-inducible expression of C/EBPα. To test for variances in the CDNA synthesis step, PU.1 expression was set in relation to the G6PD housekeeping gene by calculating the ratios for PU.1/G6PD. C/EBPα was expressed maximally after 6 hours of zinc induction (data not shown), and 5 time points of zinc induction were included to determine PU.1 expression. PU.1 expression was down-regulated 4-fold after 8 hours. In the control there was only a minimal change in PU.1 expression on induction with zinc in U937 cells carrying the empty vector pPC18 (Figure 7A). The data are consistent with the model that the expression of PU.1 is down-regulated after blocking of PU.1 function by C/EBPα. C/EBPα blocked the transactivation of PU.1 promoter by PU.1. C/EBPα transactivated the promoter alone by 2-fold, and pGL2 was used as a control (Figure 7B).

C/EBPα inhibits PU.1-induced dendritic cell development

We have previously shown that the enforced expression of C/EBPα in a human bipotential myeloid progenitor cell line induces granulocyte differentiation and blocks monocyte differentiation. On the other hand, PU.1 has been demonstrated to instruct transformed chicken multipotent hematopoietic progenitors to differentiate along the myeloid lineage. In human CD34+ hematopoietic progenitor cells, however, enforced expression of PU.1 promotes dendritic cell differentiation with characteristics of Langerhans cells, specific dendritic cells that reside in epidermis (A.I., manuscript in preparation). To investigate the biologic significance of function blocking of PU.1 by C/EBPα, we retrovirally expressed PU.1 and C/EBPα in human CD34+ hematopoietic progenitor cells. In contrast to mock control in which granulocytes
and monocytes differentiated (Figure 8A), single transduction of PU.1 and C/EBPα predominantly promoted the differentiation of CD1a⁺ dendritic cells (Figure 8B,E-F) and granulocytes (Figure 8C), respectively. PU.1-transduced cells were positive for CD1a, HLA-DR, CD80, and CD86 (Figure 8F) suggesting that PU.1 specifically enhanced dendritic cell expression. In the latter case of C/EBPα transduction, terminal differentiation of neutrophils was markedly enhanced compared with mock control. Then we coexpressed PU.1 and C/EBPα in CD34⁺ hematopoietic progenitor cells. In accordance with our mechanistic data of C/EBPα blocking PU.1 transcriptional activity, C/EBPα blocked dendritic cell differentiation by PU.1 and instead induced granulocyte differentiation (Figure 8D-E).

Discussion

Transcription factors PU.1 and C/EBPα play major roles in myelopoiesis. Each factor has been shown to synergize on various promoters, including M-CSF receptor promoter and neutrophil elastase promoter. Each is expressed in a bipotential myeloid cell; therefore, we asked whether there is any protein–protein interaction between these transcription factors and, consequently, whether there is any functional significance of this interaction in lineage commitment.

The present work shows that the transcription factor C/EBPα is capable of functionally blocking the PU.1 protein, and it provides evidence that this interference is mediated through interaction between the β3-β4 region of the PU.1 DNA-binding domain and monocytes differentiated (Figure 8A), single transduction of PU.1 and C/EBPα predominantly promoted the differentiation of CD1a⁺ dendritic cells (Figure 8B,E-F) and granulocytes (Figure 8C), respectively. PU.1-transduced cells were positive for CD1a, HLA-DR, CD80, and CD86 (Figure 8F) suggesting that PU.1 specifically enhanced dendritic cell expression. In the latter case of C/EBPα transduction, terminal differentiation of neutrophils was markedly enhanced compared with mock control. Then we coexpressed PU.1 and C/EBPα in CD34⁺ hematopoietic progenitor cells. In accordance with our mechanistic data of C/EBPα blocking PU.1 transcriptional activity, C/EBPα blocked dendritic cell differentiation by PU.1 and instead induced granulocyte differentiation (Figure 8D-E).

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PU.1-induced dendritic cell differentiation and drives the cells to granulocytes.

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