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Ornithine decarboxylase interferes with macrophage-like differentiation and matrix metalloproteinase-9 expression by tumor necrosis factor alpha via NF-κB

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Abstract

Ornithine decarboxylase (ODC), a tumor promoter, provokes cell proliferation, and inhibits cell death; but the mechanism involved in cell differentiation remains unknown. Herein, we examine whether it functions during macrophage-like differentiation. Previous studies reveal that ODC, a rate-limiting enzyme of polyamine biosynthesis, and polyamines are involved in restraining immune response in activated macrophage. By using 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-differentiated human promyelocytic HL-60 and promonocytic U-937 cells, we discover that polyamines block the expression, secretion and activation of MMP-9. Meanwhile conventional expression of ODC represses tumor necrosis factor-alpha (TNF-α) expression and nuclear factor-kappaB (NF-κB) activation as well as MMP-9 enzyme activity. Following stimulation by TNF-α, the secretion of MMP-9 is restored in ODC-overexpressed cells. In addition, the NF-κB inhibitors (pyrrolidinedithiocarbamate, BAY-11-7082 and lactacystin) suppress the TPA-induced MMP-9 enzyme activity. Concurrently, both the irreversible inhibitor of ODC, α-difluoromethylornithine, and TNF-α could not recover MMP-9 activation following NF-κB inhibitor treatment in parental cells. Furthermore, ODC could directly inhibit and attenuate NF-κB DNA binding and transcriptional activation. Therefore, we suggest that ODC inhibits the TNF-α-elevated MMP-9 activation via NF-κB as TPA-induced macrophage-like differentiation and this interrupting mechanism may provide a new conceivable resolution why leukemia is poorly differentiated besides atypical growth.

Keywords: ODC; MMP-9; TNF-α; NF-κB; Differentiation

1. Introduction

Ornithine decarboxylase (ODC, EC 4.1.1.17) is the initial and rate-limiting enzyme in the biosynthesis of polyamines (putrescine, spermidine and spermine). In mammalian cells, increased ODC activity may be a prime factor in the stimulation of DNA synthesis and cell replication. Our preliminary data revealed that conventional ODC expression prevented extrinsic cytokine (tumor necrosis factor-alpha, TNF-α) and intrinsic anti-tumor drug (methotrexate)-induced apoptosis [1,2]. In terminal differentiation process of hematopoietic cells, the inducing differentiation agents inhibit DNA synthesis and cell growth but increase cell death [3–6]. ODC transcription levels and enzyme activities are down regulated during differentiation of a human promyelocytic leukemia HL-60 cell line [7,8].

Abbreviations: ODC, ornithine decarboxylase; MMP-9, matrix metalloproteinase-9; TNF-α, tumor necrosis factor-alpha.

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Furthermore, α-difluoromethylornithine (DFMO), an irreversible ODC inhibitor, induces differentiation of several cell lines such as Friend's murine erythroleukemia cell [9]. However, the questions of whether it directly functions in blood cell differentiation and how to regulate it remain poorly characterized.

Leukemia is a form of cancer that begins in the blood-forming cells of the bone marrow, the soft inner part of the bones, and spreads through the abnormal proliferation and differentiation of myeloid progenitor cells [10]. Prompted differentiation is the one of the important and possible stratagems for tumor therapy. 1,25-Dihydroxyvitamin D3 and sodium butyrate induce human promyelocyte to differentiate into a monocytic phenotype; while retinoic acid (vitamin A), dimethyl sulfoxide (DMSO) and hypoxanthine encourage a phenotype of granulocytic differentiation. In particular, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), purified from the seed oil (croton oil) of *Croton tiglium* L., a leafy shrub of the *Euphorbiaceae* family, could induce macrophage-like differentiation. The activity of TPA as an extraordinarily potent stimulator of hematopoietic differentiation has been established by studies of HL-60, U-937, NB4, ML-1 and K562 leukemia cell lines as well as the primary human acute myeloid leukemia cells and normal hematopoietic stem cells [5,11–14]. It causes myeloid cells to undergo differentiation into something resembling mature macrophages and treats myelocytic leukemia patients without serious toxicity [15]. TPA also inhibits DNA synthesis or cell replication during cellular differentiation [16].

The cellular proteinases containing leukocyte elastase, cathepsin G and matrix metalloproteinases (MMPs, a major family of zinc-dependent enzymes) are related to extracellular matrix degradation in macrophage [17,18]. Production of these enzymes is frequently correlated with cellular differentiation, maturation and activation. The decreased levels of leukocyte elastase and induction of MMP-9 (EC 3.4.24.35) are found to be associated with macrophage differentiation or in vitro maturation of human monocytes [17,19,20]. MMP-9, also known as 92-kDa type IV collagenase and gelatinase B, cleaves basement membrane collagen types IV and V as well as the different types of gelatin [21]. It activates and degrades the preexisting extracellular matrix (ECM) to disrupt the fibrillar collagen network, allowing inflammatory cells, such as neutrophils and macrophages, to infiltrate the infarct tissue as well as remove the necrotic myocytes [22]. Furthermore, the synthesis and activity of MMP-9 are stimulated by TNF-α during macrophage-like differentiation [23,24]. TNF-α is identical as a differentiation-inducing factor and it is one of the early response genes during the TPA-induced macrophage-like differentiation [25].

The interference of putrescine, spermidine and spermine (polyamines) in cellular differentiation is very divergent [7,26,27]. Moreover, many metabolic enzymes (ODC, S-adenosylmethionine decarboxylase (SAMDC), polyamine synthase, polyamine oxidase, and spermine spermidine *N*-acetyl transferase) involve polyamine homeostasis. In addition, the inhibition of ODC and SAMDC activity could not prevent cellular differentiation [28,29] and overexpression of the human *odc* gene elevates markedly tissue putrescine concentrations but not that of spermidine and spermine [30]. Therefore, whether ODC itself directly participates in or interferes with cellular differentiation remains to be further evaluated. In this study, we investigate whether that the key enzyme of polyamine biosynthesis, ODC, interferes with TPA-induced macrophage-like differentiation. Meanwhile, ODC inhibits MMP-9 enzyme expression and activity during macrophage-like differentiation of HL-60 and U-937 cells. We further explore the molecular mechanism to explain how ODC obstructs the MMP-9 expression on macrophage-like differentiation.

2. Materials and methods

2.1. Cell culture and reagents

The human promyelocytic leukemia HL-60, human promonocytic leukemia U-937 and murine macrophage RAW 264.7 cell lines were grown in suspension culture of RPMI 1640 medium and supplemented with 10% fetal bovine serum (FBS) at a temperature of 37 °C in a humidified and 5% CO_2 atmosphere. Culture cells were passaged with RPMI 1640 medium supplemented with 1% FBS prior to various experiments. DMSO, TPA, putrescine, spermidine, spermine, pyrrolidinedithiocarbamate (PDTC), lactacystin and gelatin were purchased from Sigma–Aldrich (St. Louis, MO, USA), and DFMO and BAY-11-7082 were obtained from Calbiochem (La Jolla, CA, USA). Recombinant human TNF-α (rTNF-α) and anti-TNF-α antibody were purchased from CytoLab (Rehovot, Israel). FITC-conjugated anti-human CD14 antibody was obtained from Serotec (Oxford, UK). Antibodies to ODC, Flag, MMP-9 and actin were supplied by LabVision (Fremont, CA, USA) and Santa Cruz (Santa Cruz, CA, USA), respectively.

2.2. Construction of ODC-expressing cell lines

The plasmid of ODC expression was constructed by inserting the BamHI-EcoRI 1,415 bp coding region fragment into the pCMV-Tag vector (Stratagene, La Jolla, CA, USA). To construct dominant-negative ODC (K69A/C360A ODC; DN-ODC) plasmid, site-directed mutagenesis was performed as described previously [2]. Parental cells were transfected with WT-ODC (overexpressing ODC), m-ODC (frame-shift mutant causing a nonsense stop codon) and DN-ODC (dominant-negative ODC) plasmids according to calcium phosphate-mediated transfections, respectively. Stably transfected cells were selected with the antibiotic G418 (400 μg/ml). After approximately 3 weeks, G418-resistant clones were isolated and analyzed individually for expression of ODC. The individual clones were examined for the