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Regulation of *CYP26A1* expression by selective RAR and RXR agonists in human NB4 promyelocytic leukemia cells

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Abstract

All-*trans* retinoic acid (ATRA) can induce complete remission in acute promyelocytic leukemia (APL), but resistance to this treatment develops rapidly partly due to increased ATRA metabolism. Among the cytochrome P450s (CYPs) involved in ATRA metabolism, the ATRA-inducible cytochrome P450 26A1 (CYP26A1) is particularly active although the molecular mechanisms involved in its regulation are not well defined in the target leukemia cells. To study *CYP26A1* expression and regulation in APL cells, we used the NB4 promyelocytic leukemia cell line. *CYP26A1* constitutive expression was barely detectable in NB4 cells, but ATRA could induce high levels of *CYP26A1* expression, which reached a maximum at 72 h. To further define *CYP26A1* induction mechanisms in the NB4 leukemia cells, we used RARs and RXR selective agonists. The RARα agonist BMS753 could elicit maturation, as expected, but not *CYP26A1* expression. Treatment with the RARβ agonist BMS641, or the RARβ/γ agonist BMS961, could not elicit maturation, as expected, nor induce *CYP26A1* expression. Because *CYP26A1* expression could not be induced by RAR ligands alone, NB4 cells were then co-treated with the RXR agonist BMS649. The RXR agonist alone could not induce *CYP26A1* expression, nor in combination with either the RARβ agonist or the RARβ/γ agonist. However, the combination of the RXR agonist and the RARα agonist could elicit a marked induction of *CYP26A1* expression. In conclusion, we have shown that *CYP26A1* induction is not essential for the granulocytic maturation of NB4 leukemia cells, and that *CYP26A1* induction requires the activation of both RARα and RXR in these cells.

Keywords: *CYP26A1* regulation; NB4 leukemia cells; RAR; RXR; Maturation

1. Introduction

Vitamin A and its derivatives (retinoids) are involved in important physiological processes such as reproduction, cell proliferation, differentiation, apoptosis, and embryonic development [1]. Retinoids also exhibit several pharmacological properties useful in the prevention and treatment of several cancers [2], including acute promyelocytic leukaemia (APL), which is particularly responsive to all-*trans*-retinoic acid (ATRA) [3]. APL is a subtype of myeloid leukemia (M3), characterized by the accumulation of cells blocked at the promyelocytic stage. This leukemia exhibits a specific chromosomal translocation t(15;17) involving the PML locus on chromosome 15 and the RARα locus on chromosome 17, thus generating a chimeric gene PML-RARα, translated into a chimeric nuclear receptor PML-RARα which functions as an aberrant receptor [4–9].

The retinoid signal is transduced by two families of nuclear receptors, the retinoic acid receptor (RAR) family and the retinoid X receptor (RXR) family. These two families comprise three subtypes (α, β, and γ) and include several isoforms [10]. These receptors belong to the superfamily of nuclear hormone receptors and act as ligand-activated transcription factors [10,11]. RARs function as heterodimer together with RXR in vitro and in vivo. The ligand–receptor complexes act as inducible transcription regulators of several genes by binding to specific retinoic acid response elements (RARE). The natural ligands for the RARs are ATRA, its oxidized metabolites, and also its

Abbreviations: APL, acute promyelocytic leukemia; ATRA, all-*trans*-retinoic acid; 9-*cis*-RA, 9-*cis*-retinoic acid; 13-*cis*-RA, 13-*cis*-retinoic acid; CYP, cytochrome P450; CYP26A1, cytochrome P450 26A1; RAR, retinoic acid receptor; RXR, retinoid X receptor

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stereoisomers 9-*cis*-RA and 13-*cis*-RA, whereas RXRs are activated by 9-*cis*-RA [10,12]. The RXR can also act as homodimer on transcription activation in vitro via the retinoid X response element (RXRE), and also as heterodimer with other nuclear receptors, e.g., the thyroid hormone receptor (TR), the peroxisome-proliferator activated receptor (PPAR), and the Vitamin D receptor (VDR), on their specific response elements.

ATRA, which is one of the most active natural retinoid, is metabolized into several oxidized metabolites [13,14] by human cytochrome P450s (CYPs) among which the following ones appear as the most active: CYPs 3A7, 3A5, 2C18, 2C8, 3A4, 2C9, 1A1, and 4A11 [15]. In addition to these identified CYPs, the novel cytochrome P450 26 (CYP26), which is inducible by ATRA and exhibits specific 4-hydroxylase activity, has been identified in zebrafish, mouse and humans [16–18]. Since the discovery of the first human CYP26 (now named CYP26A1), two other CYP26 (CYP26B1 and CYP26C1) have been identified [19–21]. CYPs 26A1 and 26B1 are highly specific for the hydroxylation of ATRA, and are less effective for the hydroxylation of its isomers 9-*cis*-RA and 13-*cis*-RA [22,23], whereas CYP26C1 metabolizes both ATRA and 9-*cis*-RA [20].

Although most patients can achieve clinical remission upon treatment with ATRA in combination with chemotherapy, relapse is observed in about 30% of patients. Patients in relapse frequently become resistant to ATRA due to a selection of non-PML-RARα leukemic clones, to increase cellular levels of cellular retinoic acid-binding proteins (CRABPs), to mutation in the E domain of the mutated PML/RARα, or to increased metabolism and clearance of ATRA due to induction of cytochrome P450s [24–26].

Because *CYP26* induction is involved in ATRA resistance, the purpose of this work was therefore to investigate *CYP26A1* expression and regulation in the target human acute promyelocytic leukemia cells, because *CYP26A1* expression could modulate ATRA levels in cancer cells. To this purpose, we used the human NB4 cell line which is sensitive to ATRA induced-maturation and exhibits the characteristic t(15;17) translocation observed in APL [27]. In the present study, by employing selective RAR and RXR receptor agonists, we show that the induction of *CYP26A1* expression requires the activation of both RARα and RXR nuclear receptors in the NB4 cell line, and that *CYP26A1* induction is not essential for the granulocytic maturation of these leukemia cells.

2. Experimental procedures

2.1. Materials

BMS753, BMS641, BMS961, and BMS649 were kindly provided by Dr. Hinrich Gronemeyer (IGBMC, Illkirch, France). ATRA and TTNPB was purchased from Sigma–Aldrich. α-^{32}P-UTP was purchased from NEN Life Science Products. The rabbit polyclonal antibodies against RAR and RXR receptors, RARα [RPα(F)], RARβ [RPβ(F)2], RARγ [RPγ(mF)], RXRα [RPα(mF)], and RXRβ [RPβ(F)], were kindly provided by Dr. Cécile Rochette-Egly (IGBMC, Illkirch, France). Stock solutions of retinoids (10^{-2} M) were prepared in ethanol and stored protected from light at −80 °C.

2.2. Cell culture

The human NB4 cells [27] were maintained in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% FCS (PAA Laboratories), 2 mM L-glutamine, 50 units/ml penicillin G, 50 μg/ml streptomycin (Life Technologies, Inc.), and were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cell density (10^5 cells/ml) was assessed with an electronic particle counter and size analyzer (Coulter Electronics). Stock solutions of retinoids (at 10^{-2} M in ethanol) were further diluted in culture medium to the desired concentration indicated in each experiment. Final concentration of ethanol in culture medium did not exceed 0.01%. All experimental procedures were light protected.

2.3. RNA isolation

Total RNA was extracted from NB4 cells after the indicated treatment and exposure time, using the Trizol Reagent (Invitrogen) following the manufacturer's instructions, and stored at −80 °C.

2.4. RNAse protection assay

A 332 bp fragment of *CYP26A1* cDNA was generated by RT-PCR using mRNA from NB4 cells treated with ATRA for 48 h at 1 μM (for method description, see RT-PCR assay below). This fragment was cloned in the pGEM-T vector (Promega) and sequenced. This plasmid was used to generate anti-sense mRNA probe for CYP26A1 mRNA detection. The plasmid was linearized by the restriction enzyme SacII (Biolabs, Inc.). As the linearization generated 3′ overhang ends, the linearized plasmid was subjected to the DNA polymerase I large fragment (Klenow) (Promega) for 15 min at 22 °C. Transcription of anti-sense CYP26A1 probes was performed using the RiboProbe In Vitro Transcription System SP6/T7 Kit (Promega) and α-^{32}P-UTP (NEN, Life Science Product, Inc.) according to the manufacturer's instructions. hL32 and hGAPDH probes were also radiolabeled and used as controls. RNAse protection assays were performed using the RiboQuant Ribonuclease Protection Assay Kit (Pharmingen) as described by the manufacturer. Briefly, 20 μg of total RNA were hybridized to the radiolabeled anti-sense probes described above (10^4 cpm/μl for each probe) in 10 μl of