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Integrities of A/B and C domains of RXR are required for rexinoid-induced caspase activations and apoptosis

Suofu Qin[a], Yumiko Okawa[a], Larissa I. Atangan[a], Geoffrey Brown[b], Roshantha A. Chandraratna[a,c], Yi Zhao[a,c,*]

[a] Retinoid Research, Departments of Biology and Chemistry, Allergan Inc., Irvine, CA 92612, United States
[b] Division of Immunity and Infection, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[c] Vitae Pharmaceuticals Inc., 502 West Office Center Drive, Fort Washington, PA 19034, United States

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ABSTRACT

Here we have delineated regions of the retinoid X receptor α (RXRα) that are required for rexinoid (RXR agonist)-induced growth inhibition and apoptosis. Stable over-expression of RXRα in DT40 B lymphoma cells dramatically increased sensitivity to rexinoid-induced growth inhibition. By contrast, DT40 cells that over-expressed RXRα with a deletion of either the A/B or DNA binding domain (C domain) were resistant. We confirmed the importance of C domain integrity by point-mutating Cys^{135} to Ser (C135S) to disrupt zinc-finger formation. Point mutating RXR Lys^{201} to Thr and Arg^{202} to Ala (KTRA) impairs RXR homodimer formation and does not affect RXR heterodimerization. When these mutated RXRs were over-expressed in DT40 cells, they failed to increase sensitivity to rexinoid. Over-expression did sensitize to growth inhibition by RAR and PPARγ agonists. Over-expression of C135S mutated RXRα did not sensitize to RAR and PPARγ agonists. Inhibitors of caspase-3 and/or caspase-9 blocked rexinoid-induced apoptosis, and activations of these caspases correlated with the ability of RXR mutants to induce cell death. These data show that the A/B and C domains of RXR and the ability of RXR to form homodimers are required for rexinoid-driven growth inhibition, caspase activation and subsequent apoptosis.

1. Introduction

The potential of retinoid X receptor (RXR) agonists, termed rexinoids, as anti-cancer agents relates to their abilities to induce growth arrest and apoptosis. Animal studies have shown that agonizing RXR inhibits the growth of rat uterine leiomyoma by driving apoptosis [1]. *Ex vivo* rexinoids induce apoptosis in various malignant cells, including lung and breast carcinomas, lymphomas and leukemias [2–10].

Rexinoids and retinoids provoke changes to cell behaviour by binding to and activating the nuclear RXR (RXRα, β, and γ) and retinoic acid receptors (RARα, β, and γ), respectively, which then regulate the expression of target genes [11,12]. 9-*cis* retinoic acid (9-*cis*-RA) is viewed as the natural ligand for RXRs, but also activates RARs [13–15]. Synthetic rexinoids, such as VTP194204, bind selectively to RXRs with high affinity [16], and these agents have anti-cancer activity.

Some malignant cells are somewhat insensitive to rexinoids, and activity of the protein kinase casein kinase 1α (CK1α) interferes with rexinoid-induced apoptosis [17]. A combination of a RXR agonist and a RAR agonist provokes apoptosis in some rexinoid-insensitive cells, such as P19 embryonal carcinoma cells [18] and the leukemia cell line PLB-985 [19]. Rexinoid sensitivity can be increased over-expression of RXR [17,20,21]. DT40 and Jurkat cells show a significant but limited response in regard to rexinoid (VTP194204)-induced growth arrest that is followed by apoptosis. Stable over-expression of RXRα in both DT40 and Jurkat cells dramatically increased the sensitivity of these cells to VTP194204 [17]. In over-expressing cells, activations of caspase-3 and -9 are VTP194204 dose-dependent. Here we have utilized this sensitization effect to delineate regions of the RXR that are required for agonist-induced growth arrest and apoptosis, and some of the underlying cellular events.

Ligand activation of RXRs, RXR homodimer formation, RXR heterodimerization with many other nuclear receptors, and binding of dimers to DNA are dependent on the integrity of several interactive domains (A to E) [22]. The amino terminal A/B domain contains

Abbreviations: RXR, retinoid X receptor; RAR, retinoic acid receptor; TTNPB, 4-(*E*-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl)benzoic acid; PPAR, peroxisome proliferator-activated receptor; DMSO, dimethylsulphoxide; CTE, C-terminal extension.

* Corresponding author at: Vitae Pharmaceuticals Inc., 502 West Office Center Drive, Fort Washington, PA 19034, United States. Tel.: +1 215 461 2096; fax: +1 215 461 2006.
E-mail address: yzhao@vitaerx.com (Y. Zhao).

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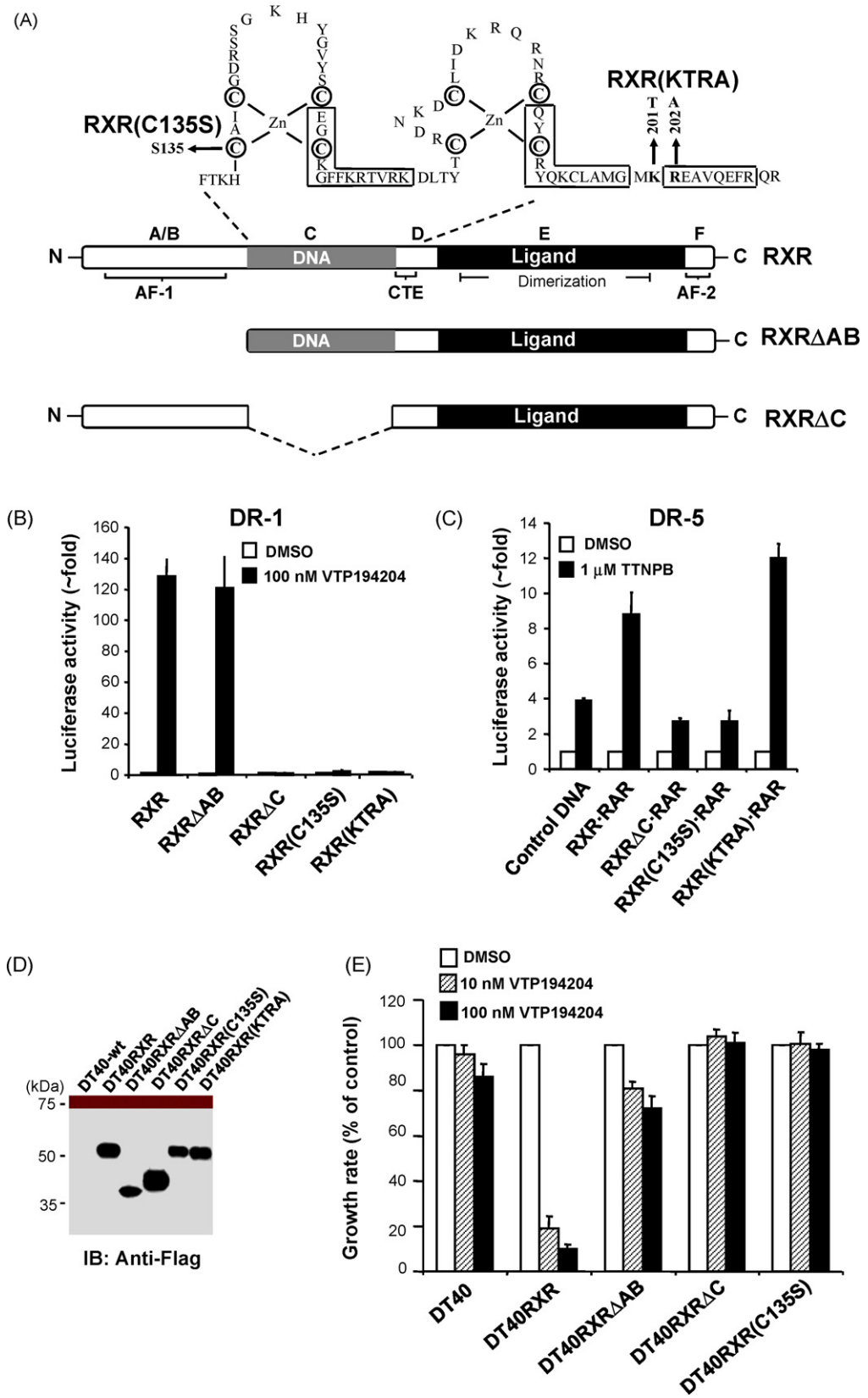

Fig. 1. RXR A/B and integrity of C domain are required for rexinoid-mediated growth inhibition. (A) Schematic representations of RXR protein, deletion mutants (RXRΔAB and RXRΔC), and site-mutated mutants (RXR(C135S) and RXR(KTRA)). (B and C) Effects of mutations of RXR on the transcriptional activity of RXR homodimers and RXR·RAR heterodimers. For transactivation of RXR homodimers (B), CV1 cells were transfected with RXR or its mutants (100 ng), reporter plasmid pRXRE-Luc (100 ng), and β-galactosidase expression vector (100 ng). The pRXRRE-Luc contains five tandem repeats of a 35-base pair sequence (DR-1) from the promoter of the mouse CRBP-II gene [35] inserted immediately upstream of thymidine kinase-luciferase. The transfected cells were treated with DMSO or 100 nM VTP194204 for 16 h and analyzed. For transactivation of RXR·RAR heterodimers (C), CV1 cells were transfected with RXR or its mutants (100 ng), pRAR-V5 (100 ng), reporter plasmid pRARE-Luc (100 ng), and β-galactosidase