Therapeutic Potential of “Rexinoids” in Cancer Prevention and Treatment

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Abstract

Retinoid X receptor (RXR) is a combinatorial partner for one third of the 48 human nuclear receptor superfamily members (1), thus enabling to activate multiple signaling pathways in both ligand dependent and independent manners. RXR forms three different types of dimers; RXR homodimer, permissive heterodimers, and non-permissive heterodimers. RXR homodimers and RXR permissive heterodimers (e.g., PPAR, LXR, and PXR) are activated upon RXR ligand binding because this class of dimer formation triggers a conformational change of the RXR that allows subsequent binding to the RXR. By contrast, nonpermissive heterodimers (e.g., RAR, VDR, TR) cannot be activated by the RXR ligand and RXR serves as a silent binding partner because binding of nonpermissive heterodimers to the RXR does not permit ligand binding to the RXR. These dimeric receptors bind to specific DNA response element target genes (1), and their DNA binding specificity is determined by the number of spacer nucleotides present between the two direct repeats of the canonical binding sequence AGGTCA (2). For example, the RAR/RXR heterodimer binds to the retinoic acid response element (RARE) and the RXR homodimer binds to the retinoid X response element (RXRE), in which the consensus half sites are separated by five (DR5) or a single nucleotide (DR1), respectively. This tight regulatory control of the ligand-dependent transactivation of the nuclear receptor superfamily makes possible a multitude of therapeutic applications including anticancer treatments (3). On the basis of promising anticancer effects of RAR ligands in in vitro and in vivo preclinical studies, the development of RAR selective ligands and their clinical application have been extensively investigated. However, clinical application of RAR ligand has only shown limited efficacy possibly because of multiple adverse effects caused by the requirement of large dosage to reach therapeutic efficacy, (2) frequent loss of, or (3) reduced RAR expression in various cancer types. Conversely, RXR ligands (rexinoid) have shown promising chemopreventive and chemotherapeutic activities with mild toxicity in several cancer types tested, and RXR expression is rarely lost in human tumors.

In an attempt to achieve the activation of multiple pathways, various rexinoids have been developed and some have shown promising antitumor activity in preclinical and clinical studies (4). A specific synthetic rexinoid, bexarotene, was found to induce a 50% overall inhibitory response in patients with refractory or persistent cutaneous T-cell lymphoma when administered either orally or topically with minimal toxicity (5). These clinical results have led to FDA approval of Bexarotene. Another RXR-selective rexinoid, LGD1069, was shown to suppress both estrogen receptor-positive (ER+) tumor development in the methyl nitrosourea (MNU)-induced rat mammary tumor model and ER negative (ER−) tumor development in mouse mammary tumor virus-erbB2 transgenic mice (6). Though the mechanisms underlying rexinoid-induced antitumor effects are not clearly understood yet, growing evidence suggests possible roles of RXR in carcinogenesis. Ablation of RXRα results in hyperplasia in the prostate epithelium and skin (7, 8). In addition, we and others have shown that RXRα overexpression sensitizes tumors to the antigrowth effects of rexinoid both in vitro and in vivo for the induction of cellular differentiation and the control of aberrant cell growth (9). It is evident that RXR plays vital roles in multiple signaling pathways including carcinogenesis via nuclear receptor family members, and the loss of RXR function will likely negatively impact physiological roles of the nuclear receptors.

Multiple pathway activation by a single agent is a highly attractive approach for molecular targeted therapy, however, the molecular mechanisms underlying the gene modulations by ligand activated RXR are highly complex due to a lack of comprehensive understandings of RXR-induced multi-pathway activation. In fact, despite promising results from preclinical studies as well as successful clinical application of rexinoid in the treatment of cutaneous T-cell lymphoma, clinical trials investigating the use of rexinoid for the treatment of lung and breast carcinoma have yielded disappointing results. In a clinical trial conducted with 145 metastatic breast cancer patients, rexinoid (bexarotene) showed limited activity, with a 20% partial response in women with hormone-refractory or chemotherapy-refractory disease (10). In a more recent phase III clinical trial, rexinoid used in combination with first line chemotherapies, such as cisplatin or carboplatin, failed to meet primary endpoints in advanced non small cell lung carcinoma (NSCLC) (ref. 11). However, rexinoid therapy significantly improved the overall survival in certain patient subgroups (i.e., males, smokers, and patients with stage IV disease) compared with the control group (11). The molecular mechanisms underlying this subgroup-dependent response and the molecular basis of rexinoid resistance remains to be understood. In this review,
we will discuss obstacles for rexinoid therapy and possible solutions.

Key Finding

We previously reported two independent mechanisms by which highly invasive breast cancer cells lose their sensitivity to rexinoid, and further addressed an approach to resensitize the unresponsive cells. We have reported that the subcellular localization of RXRα changes whereas the total RXRα protein expression remains the same in highly malignant human breast cancer cells. An altered localization of RXRα to the splicing factor compartments (SFCs), rather than in the nucleoplasm, caused the loss of functional activity of RXRα as a transcription factor (12). The consequence of the loss of RXRα function due to its altered localization is rather severe because many nuclear receptor superfamily members require RXR for their function as a dimerization partner of ligand dependent responses. Interestingly, immunohistochemical analysis of invasive breast cancer tissue revealed that the same RXRα altered localization to the SFCs was also detected in tumor stroma (fibroblast), though not in breast cancer epithelial cells. This altered localization was not due to mutation of RXRα, but rather likely to protein-protein interaction via C-terminus of RXRα. The incubation of the cells with an RXR-terminus peptide corresponding to the RXRα C-terminus allowed for RXR distribution to the nucleoplasm from the SFC and the restoration of responsiveness to RXR ligand. Our preliminary data also showed that this type of altered localization of RXRα may occur frequently in breast carcinoma.3 Our data suggest that loss of RXRα function due to the sequestration, although total expression level remains the same, causes a disruption of RXR-mediated signaling pathways. RXR has three different subtypes and the loss of RXRβ and γ can be compensated by RXRα, however, not the other way round. Therefore, the impact of the loss of RXRα might be severe and the sequestration of RXRα affect the entire RXRα signaling cascades. Another line of studies addressed the molecular mechanism of rexinoid-induced growth inhibition and further highlighted the complex nature of RXR signaling. We showed that ligand-activated RXR upregulates p21 expression at the transcription level via RXR homodimer binding to the two consecutive RXRE in the p21 promoter region, leading to an induction of cell cycle arrest, followed by apoptosis (9, 12). However this rexinoid-induced cell cycle arrest was significantly inhibited by the presence of RAR. Interestingly, we found that both RXREs sequences overlap with a previously reported RAR/RXR heterodimer binding sequence (RARE) (ref. 13), raising the question whether RAR/RXR heterodimer

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binds to the RARE and interferes with the binding of RXR/RXR homodimers to the RXREs, and in turn promotes the loss of RXR ligand-mediated p21 transactivation. We showed that the presence of RAR interferes with RXR ligand-mediated p21 induction owing to RAR binding to the RARE situated between the RXREs. Furthermore, in an RNAi approach to knock down the expression of RARα and RARγ, the RXR ligand upregulated p21 expression concomitant with the reduction in the S phase population of the cells. Together, these data suggested that the level of RXR ligand-mediated growth inhibitory effect could be manipulated by the overall amount of retinoid receptors (RAR and RXR). It might be important to further investigate whether the RAR interference on RXR ligand-mediated cell growth inhibition is RAR isotype specific or not. Overall, our studies further pointed out the complex nature of multiple pathways activation by RXR by demonstrating two independent mechanisms leading to rexinoid resistance: (1) different RXR subcellular localization and (2) an altered balance of RXR and RAR.

**Implications and Questions**

A recent clinical trial with RXR ligand as a single therapeutic regimen has shown a favorable clinical outcome with mild toxicity for a selected subgroup of advanced lung cancer patients (11), suggesting that the downstream target of RXR would provide a mechanistic understanding of the anticancer effect of RXR ligands. One of the affected downstream pathways by liganded RXR is the p21 through RXR homodimer bindings to the two sequential RXREs, which overlap with an RARE (Fig. 1). Because the RAR/RXR heterodimers bind to the RARE with high affinity, RAR/RXR heterodimer prevents RXR homodimer-mediated p21 promoter activation. Histopathological studies have shown that down-regulation of RARα during tumor progression coincides with loss of response to RAR ligand (14), and the loss of RARβ is commonly observed at the early stage of tumorigenesis in multiple cancers (3). Conversely, all RXR isotypes are overexpressed in 66% of breast ductal carcinomas in *in situ* lesions (15), and in particular RXRα upregulation is associated with malignant transformation (16). These aberrant expressions of RAR and RXR during tumorigenesis may change the cellular response to retinoid and rexinoid treatment. The ratio between RXR and RAR is likely one of the key parameters to determine therapeutic outcome of rexinoid therapy, i.e., high RXR to RAR expression level is required for p21 induction (G1 arrest) and high RAR to RXR to prevent p21 expression and lead to apoptosis. Similar to our findings, there have been a few examples that two distinct classes of transcription factors can recognize a common regulatory sequence. Further, it should be noted that the nuclear receptor signaling network can be paradoxical and the divergent responses from multiple cell types may be attributed to the loss of or reduced receptor expression, altered localization, or intricate transcriptional regulation.

In addition to the paradox of RXR signaling pathway, an anti-apoptotic role of p21 has been reported. Although p21 plays a key role in cell growth inhibition by activating p53 upon DNA damage, p21 can also block p53-mediated apoptosis when DNA is damaged at a low degree. Possible anti-apoptotic roles of p21 have been observed mostly in p53 wild-type cells, indicating that p53 activity may be the key determinant factor on whether p21 functions as pro-apoptotic, anti-apoptotic, or as a cell cycle regulator. Similar to this, pathological analysis from non small cell lung cancer (NSCLC) and ovarian cancer patients showed that the 5-year survival ratio of p21 positive and p53 negative patients have the most favorable prognosis over p21 negative and p53 positive patients or over both positive (17). In addition, recent studies revealed a functional disruption of p21 via phosphorylation by Akt in Her2/neu overexpression cancers (18). These findings further suggest that RXR ligand-mediated cell growth inhibition may require a selective molecular signature beyond RAR/RXR ratio. Prescreening of cancer patients by determining the RAR/RXR ratio (Fig. 1), as well as the subcellular localization of RAR and RXR, may be crucial for enhancing the efficacy of p21-targeted RXR ligand therapy via the pleiotropic retinoid response element RXREs/RARE.

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