

Review

Targeting truncated RXR α for cancer therapy

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Abstract

Retinoid X receptor-alpha (RXR α), a unique member of the nuclear receptor superfamily, is a well-established drug target, representing one of the most important targets for pharmacologic interventions and therapeutic applications for cancer. However, how RXR α regulates cancer cell growth and how RXR α modulators suppress tumorigenesis are poorly understood. Altered expression and aberrant function of RXR α are implicated in the development of cancer. Previously, several studies had demonstrated the presence of N-terminally truncated RXR α (tRXR α) proteins resulted from limited proteolysis of RXR α in tumor cells. Recently, we discovered that overexpression of tRXR α can promote tumor growth by interacting with tumor necrosis factor-alpha-induced phosphoinositide 3-kinase and NF- κ B signal transduction pathways. We also identified nonsteroidal anti-inflammatory drug Sulindac and analogs as effective inhibitors of tRXR α activities via a unique binding mechanism. This review discusses the emerging roles of tRXR α and modulators in the regulation of cancer cell survival and death as well as inflammation and our recent understanding of tRXR α regulation by targeting the alternate binding sites on its surface.

Key words: tRXR α , RXR α modulators, nongenomic action, inflammation, PI3K

Introduction

Retinoid X receptor-alpha (RXR α) is a unique and important member of the nuclear receptor superfamily (Fig. 1A). As a master regulator, RXR α acts through homodimerization with itself or via serving as obligatory partner for many other nuclear receptors, including retinoic acid receptor (RAR), thyroid hormone receptor (T3R), vitamin D receptor (VDR), Nur77, peroxisome proliferator-activated receptors (PPARs), liver X receptor (LXR), and farnesoid X receptor [1–9]. Many naturally occurring small molecules have been shown to bind to RXR α and modulate its activities [2–4,10–12]. 9-*cis*-Retinoic acid (9-*cis*-RA) was the first one that was identified as a natural RXR α ligand (Fig. 1B). Subsequently, several dietary fatty acids were found to bind to RXR α and act as natural RXR α ligands. These include docosahexaenoic acid (DHA), oleic acid, and phytanic acid. However, none of these molecules have been proved to be the *bona fide* endogenous ligands of RXR α [13,14].

RXR α possesses a common structural organization that is shared by the nuclear receptor family: a disordered N-terminal A/B region containing activation function 1 (AF-1), a DNA-binding domain

(DBD) containing two zinc fingers, and a C-terminal ligand-binding domain (LBD) composed of 12 α -helices and a short β -turn (Fig. 1A). The LBD consists of a canonical ligand-binding pocket (LBP), an activation function 2 (AF-2), a co-regulator-binding surface groove, and a dimerization surface (Fig. 1A). A well-described mechanism of RXR α action is that RXR α and its partners act as ligand-dependent transcription factors through binding to specific DNA-response elements of the target genes [1–9]. Ligand binding induces a conformational change that triggers a cascade of events such as co-regulator exchanging or binding, leading to positive or negative gene transcription and subsequent biological activities [1–9].

Genetic analysis demonstrates that RXR α is involved in a plethora of developmental and physiological pathways. Knockout of RXR α resulted in embryonic lethality [15]. Tissue-specific inactivation of RXR α has demonstrated a major role of RXR α in hepatocytes [16], skin [17], prostate [18], or adipose tissue [19]. Strong phenotypes observed in most RXR α mutant mice may be related to alternations in pathways regulated by its heterodimerization partners. The role of RXR α homodimer *in vivo* was unraveled recently. Ligand-activated

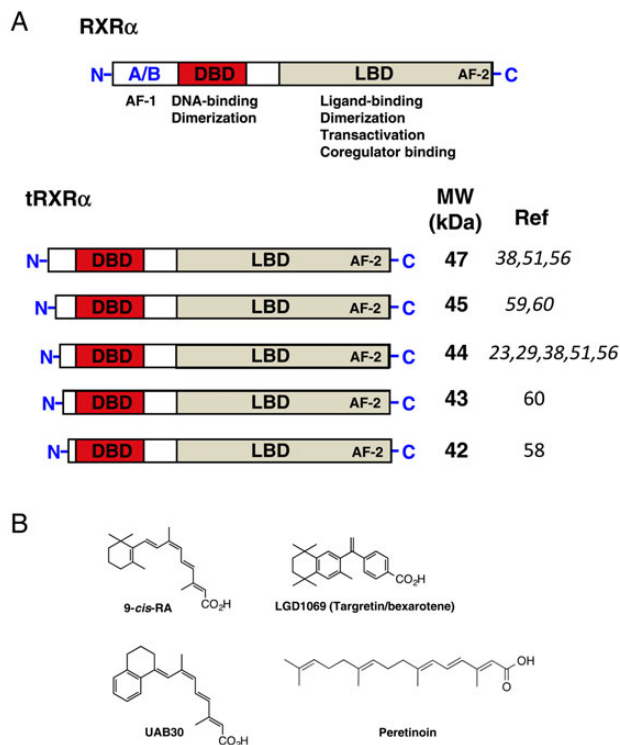


Figure 1. The scheme of RXR α /tRXR α and its ligands (A) Schematic representation of RXR α and tRXR α . DBD, DNA-binding domain; LBD, ligand-binding domain; AF-1, activation function 1; AF-2, activation function 2. (B) Chemical structures of 9-*cis*-RA, Targretin, UAB30 and peretinoin.

RXR α homodimers up-regulate p21 expression through the direct binding of RXR α homodimers to the p21 promoter [20]. Characterization of mice lacking RXR α in myeloid cells reveals an important role of RXR α homodimers in the innate immune response to inflammatory stimuli [21].

Aside from its role in DNA binding and transactivation, RXR α also exerts extranuclear actions through transcription-independent mechanisms [22–26]. RXR α resides in the cytoplasm at different stages during development [27]. In response to differentiation [24], survival [28,29], apoptosis [22], and inflammation [25,26,28,29], RXR α migrates from the nucleus to the cytoplasm. For example, RXR α is translocated from the nucleus to the cytoplasm in response to endotoxin and other inflammatory mediators to inhibit its transactivation function [25,30], while an altered localization of RXR α to the splicing factor compartments occurs in highly malignant human breast cancer cells [31]. These observations revealed the intricacy of RXR α functions and underscored the importance of the RXR α nongenomic signaling.

Post-translational modifications also play a critical role in the regulation of RXR α activities. Phosphorylation of the N-terminal domain of RXR α by mitogen-activated protein kinases occurs in response to several stress agents such as UV radiation, oxidative damage, or ribotoxic agents [32–37], leading to the inhibition of the transcriptional activity of RXR α heterodimers. RXR α could also be phosphorylated at Ser260 in its LBD [33,38]. In human hepatocellular carcinoma (HCC) cells, RXR α is heavily phosphorylated at Ser260, making it resistant to ubiquitination and proteasome-mediated degradation [38,39]. Nuclear export of RXR α in response to inflammatory signaling also involves c-Jun N-terminal kinase (JNK) phosphorylation of

Ser260 [26]. RXR α is also a substrate for modification with small ubiquitin like modifier (SUMO) [40]. SUMOylation of RXR α at Lys108 in its AF-1 domain inhibits its transcriptional activity [40]. Interestingly, a recent study showed that inflammatory mediators increase SUMOylation of RXR α in a JNK-dependent manner [41]. Unlike SUMOylation, acetylation of RXR α by p300 promotes its DNA binding, thereby increasing its transcriptional activity [42].

We recently reported that an N-terminally truncated form of RXR α (tRXR α) (Fig. 1A) produced in cancer cells resides in the cytoplasm to promote the growth of tumor cells [29]. Our investigation of tRXR α action in the cytoplasm revealed an extensive interaction between tRXR α and tumor necrosis factor- α (TNF α) signaling. In this review, we will briefly summarize the role of RXR α in cancer and the cancer therapeutic effect of RXR α ligands (retinoids). We will focus our discussion on the identification of tRXR α in cancer cells and its implication in the regulation of cancer survival and death as well as inflammation through its nongenomic interaction with TNF α -induced phosphoinositide 3-kinase (PI3K)/AKT and NF- κ B signal transduction pathways. Finally, we will summarize recent advances in the discovery of tRXR α modulators with new RXR α -binding mechanisms.

RXR α and Cancer

Altered expression and function of RXR α are implicated in the development of a number of cancers and diseases. Although RXR α -knockout fetus dies at embryonic days [15], targeted disruption of RXR α gene leads to preneoplastic lesions in prostate [18], alopecia, epidermal interfollicular hyperplasia, keratinocyte hyperproliferation, and aberrant terminal differentiation in skin [17], the development of cervical malignant lesions [43], alteration of fatty acid oxidation and hepatocyte lifespan in liver [16], and the resistance to diet-induced obesity due to impaired adipocyte differentiation in adipose tissue [19]. Consistently, diminished RXR α expression is associated with the development of certain malignancies, such as thyroid carcinoma [44,45] and liver cancer [46], and levels of RXR α protein are often reduced in cancer cells and tumor tissues [45,47–51]. In addition to reduced levels of RXR α protein, altered RXR α function by phosphorylation is associated with the development of human HCC [38] and colon cancer [52]. Furthermore, several studies have demonstrated that alteration of the subcellular localization of RXR α is linked to the development of cancer [31]. Recent studies showed that RXR α binding to PML/RAR α is absolutely required for the development of acute promyelocytic leukemia (APL) in transgenic mice [53–55], demonstrating the oncogenic potential of this protein when it acts inappropriately. Several groups have demonstrated that RXR α is proteolytically cleaved in cancer cells [23,45,51,56–60], and our illustration that tRXR α could enhance TNF α activation of PI3K/AKT and NF- κ B pathways revealed that aberration in RXR α signaling by limited proteolysis plays an active role in cancer development [28,29,61–65].

Rexinoids and Cancer Therapy

The pleiotropic actions of RXR α under both physiological and pathological conditions suggest RXR α as an important drug target for pharmacologic interventions and therapeutic applications. This is highlighted by the FDA approval of the RXR-based drug Targretin (bexarotene) for treating cutaneous T-cell lymphoma (CTCL) patients who are refractory to at least one prior systemic therapy [66]. Targretin selectively binds to RXRs and does not have significant RAR binding and transactivation [5,67,68]. Side effects have been reported to be

associated with the Targretin treatment, including hyperlipidemia and hypothyroidism [66,69]. Hyperlipidemia is thought to be associated with the modulation of RXR heterodimers with PPARs and LXRs [70] and hypothyroidism may be due to the inhibition of the TSH production by Targretin through the thyrotrope-restricted RXR γ isoform [71]. To overcome these side effects, efforts to develop novel RXR-based drug for CTCL treatment are ongoing. Recently, it was reported that certain analogs of Targretin possess improved biological properties [72]. The mechanism of Targretin action has yet to be fully elucidated. It has been shown that the drug can induce apoptosis, differentiation and cellular senescence, inhibit metastasis and angiogenesis, and block cell cycle progression, which was reviewed recently [73–76].

Retinoids also show significant effect in non-small-cell lung cancer (NSCLC) [77], in which altered RXR signaling has been observed [78]. Preclinical data showed that Targretin can prevent and overcome acquired paclitaxel resistance in NSCLC [79]. Furthermore, Targretin could act synergistically with standard first-line platinum-based chemotherapy [77,78]. In a randomized Phase III trial comparing Targretin in combination with cisplatin/vinorelbine to cisplatin/vinorelbine alone in a total of 623 patients for over survival as the primary efficacy endpoint, a subgroup (32%) of Targretin-treated patients who developed NCI Grade 3/4 hypertriglyceridemia had longer median survival compared with control patients [80]. In another Phase III trial to determine the effects of addition of Targretin to standard first-line carboplatin and paclitaxel therapy, similar results were obtained that increased survival was correlated with the occurrence of Grade 3/4 hypertriglyceridemia in patients treated with Targretin [81].

Retinoids also induce differentiation in AML patient samples and in various AML cell lines [82,83]. As a differentiation agent, Targretin is being tested for AML treatment [84]. A Phase I dose escalation study in elderly and relapsed AML patients was conducted to investigate whether Targretin could be used in combination with decitabine [84]. It was found that the combination was well tolerated, but produced only modest responses. However, greater AML blast differentiation was observed in patients with clinical response, suggesting a potential of retinoids in AML therapy. Recent studies suggested that Targretin mediates the RXR/LXR-regulated gene expression that was deregulated in AML cells [85].

Other studies have also revealed an emerging role of RXR α in APL [86]. RXR α has been demonstrated to be a binding partner of PML/RAR. Genome-wide epigenetic studies suggested that PML/RAR α /RXR complex acted as a local chromatin modulator [55]. Recruitment of RXR α by the APL fusion protein is crucial for oncogenic transformation and is required for the development of APL in transgenic mice [53–55]. Therefore, retinoids have great potentials for treating APL.

Malfunction of RXR α due to phosphorylation by the Ras-mitogen-activated protein kinase signaling pathway is profoundly associated with the development of HCC and thus may be a critical target for HCC chemoprevention. Acyclic retinoid (also known as peretinoin), a synthetic retinoid that binds to both RXR and RAR, can prevent phosphorylation of RXR α by inhibiting the activities of Ras-Raf-Erk system through an undefined mechanism [87]. Clinical studies have shown that it is effective in suppressing HCC recurrence and improving patient survival rates following curative therapy [87]. A recent gene expression profiling study using liver biopsy from HCC patients underwent therapy revealed that peretinoin not only enhances the expression of retinoid target genes but also regulates various signal transduction pathways involved in hepatocarcinogenesis [23].

Proteolytic Cleavage of RXR α in Cancer Cells

Numerous studies have shown limited proteolytic cleavage of RXR α protein in tumor cells [23,45,51,56–60]. Matsushima-Nishiwaki *et al.* [56] showed a 44 kDa tRXR α in liver cells and found that m-calpain could cleave RXR α into the tRXR α lacking N-terminal A/B region in HuH7 HCC cells (Fig. 1A). A recent study showed that tRXR α accumulates more in several HCC cell lines than in normal immortal human hepatocytes [88]. Cathepsin L-type protease could also cleave RXR α at its N-terminal region, producing a 44 kDa tRXR α in rapid growing hepatocytes, which alters thyroid hormone responsiveness [58], and in HCC cells [60]. In osteosarcoma cells, the production of an aberrant 45 kDa tRXR α is implicated in the resistance to the antiproliferative effects of calcitriol and retinoids [59]. Two tRXR α proteins with 47 and 44 kDa were detected in all seven prostate cancer cell lines, which may have altered subcellular localization [51]. Interestingly, a 44 kDa tRXR α was found in mitochondria [23], suggesting that tRXR α may play a nongenomic role. In our study, we found that tRXR α is produced in many different types of cancer cells [29]. Moreover, tRXR α was detected in primary tumors but not in tumor surrounding tissues or distant normal tissues from the same cancer patients [29], demonstrating a close association of tRXR α production with cancer. We also identified calpain II as a protease that could cleave RXR α protein *in vitro* and *in vivo*. Activation of calpain II by ionomycin enhances the production of tRXR α in cancer cells, which is regulated in a glycogen synthase kinase 3 beta-dependent manner [63].

Limited proteolytic cleavages of RXR α mainly occur at its N-terminus [23,45,51,56–60]. Comparing with its DBD and LBD, the function and regulation of the N-terminal A/B domains of RXR α have not been well studied. The fact that the N-terminal A/B domains of nuclear receptor family members are highly variable suggests that they may mediate specific functions. Phenotypic analysis of mice expressing RXR α with its N-terminal A/B region deleted indicated that the RXR α AF-1 domain is functionally important for efficiently transducing the retinoid signal during embryonic development [89]. An interesting feature of the N-terminal A/B region is that it contains many consensus phosphorylation sites and is therefore the target of multiple kinases, such as JNK [32,34,36,37,90–92]. Hyperphosphorylation of A/B region (Ser61, Ser75, and Thr87) can induce apoptosis [93], while phosphorylation of RXR at Ser260 has been correlated with the unrestrained growth of certain HCC [38]. However, little is known about the mechanisms through which the N-terminal A/B region and its phosphorylation site participate in the regulation of RXR α activity.

Regulated proteolysis is a key step in a number of different signaling pathways that respond to developmental cues or external stimuli [94–100]. Caspase-mediated cleavage of the BH3-only protein Bid generates a truncated protein (tBid), and the subsequent translocation of tBid to mitochondria is implicated in death receptor signaling [99]. Similarly, caspase-3 mediates retinoic acid-induced degradation of the APL PML/RAR α fusion protein [101], and the cleavage product of PML/RAR α contributes to ATRA-mediated differentiation in APL [102]. Proteolytic processing of Notch and nuclear translocation of truncated product is a crucial step in the transduction of the Notch signaling [96]. Cleavage of the androgen receptor by calpain produces a truncated receptor protein that may play a role in the development of androgen-independent prostate cancer [103]. Similarly, cleavage of MET, a membrane-bound receptor tyrosine kinase, results in a truncated nMET, which is localized in the nuclei of malignant cells to promote the growth of castration-resistant prostate cancer cells through

its activation of both SOX9 and β -catenin [104]. Thus, proteolytic cleavage likely represents an important mechanism that regulates the biological function of RXR α .

Nongenomic Action of tRXR α in Cancer

tRXR α and PI3K/AKT survival signaling

PI3K is a heterodimeric protein composed of a catalytic subunit (p110 $\alpha/\beta/\gamma/\delta$) and a regulatory subunit (p85 α/β) that participate in multiple cellular processes, including cell growth, transformation, differentiation, and survival in a number of cell types and human cancers [105–108]. The p85 α /p110 α heterodimer is the major form of PI3Ks, in which the p85 α regulatory subunit binds to the p110 α catalytic subunit to integrate signals from various cellular proteins, providing an integration point for activation of p110 α and downstream molecules such as AKT.

We found that tRXR α but not RXR α could act to mediate TNF α activation of PI3K/AKT in a number of cancer cell lines [29] (Fig. 2). Unlike RXR α that resides in the nucleus, tRXR α is cytoplasmic in response to TNF α treatment, and interacts with the p85 α regulatory subunit, leading to an enhanced activation of the PI3K/AKT survival pathway and anchorage-independent cell growth *in vitro* and cancer cell growth in animals (Fig. 2). Abnormal activation of the PI3K/AKT pathway is often observed in cancer cells, contributing to their growth and survival properties and drug resistance [105–108]. Knocking down of tRXR α could reduce basal AKT activation in some cancer cells, demonstrating that tRXR α may play a critical role in the aberrant activation of PI3K/AKT signaling in cancer cells. Interestingly, the interaction of tRXR α with p85 α and its activation of PI3K/AKT signaling were induced by inflammatory cytokine TNF α but not by some growth factors such as epidermal growth factor, implying that tRXR α may specifically act in inflammatory environment. These results provided another example that a nuclear receptor can act outside of the nucleus to regulate an important biological process and identified tRXR α as a key molecule involved in the aberrant activation of PI3K/AKT pathway in cancer cells. However, many important questions regarding the nongenomic regulation of the PI3K/AKT

pathway by tRXR α and ligands remain to be answered. It is unclear whether the cytoplasmic localization of tRXR α results from its nuclear export or cytoplasmic retention due to its interaction with cytoplasmic proteins such as p85 α . As the wild-type RXR α fails to interact with p85 α , the N-terminal region deleted from RXR α is expected to play a critical role in regulating RXR α activities, which remains to be determined. As RXR α is often phosphorylated in cancer cells, it will be interesting to examine whether phosphorylation or other modifications of RXR α are involved in the regulation of RXR α cytoplasmic localization and its interaction with p85 α . How tRXR α interacts with p85 α is still unknown at present. However, it is worth noting that numerous nuclear receptors including estrogen receptor, androgen receptor, glucocorticoid receptor, and RAR have been shown to interact with p85 α [109–112], implying the existence of a more general mechanism for their interaction with p85 α .

Because of its role in oncogenesis and drug resistance, the PI3K/AKT pathway has therefore been targeted extensively to develop therapeutics against cancer and related diseases, and to overcome drug resistance. However, current targeting strategies that rely on direct inhibition of PI3K/AKT activities have posed profound adverse effects and are thus far confined to the preclinical and clinical evaluation due to toxicity and lack of selectivity. Thus, the identification of tRXR α -mediated activation of PI3K/AKT signaling pathway in cancer cells may provide new strategies to inhibit the activation of PI3K/AKT in cancer cells by targeting tRXR α . Such tRXR α -based PI3K/AKT inhibitors are likely to be more specific and tumor selective than conventional PI3K/AKT inhibitors.

tRXR α and apoptosis

Apoptosis, programmed cell death, is abnormally regulated in cancer cells and the efficacy of chemotherapeutic drugs depends largely on their ability to induce apoptosis [113,114]. Apoptosis often occurs following either triggering of cell surface death receptors (the extrinsic pathway) or perturbation of mitochondria (the intrinsic pathway) [115]. The intrinsic pathway is initiated by the release of apoptogenic factors such as cytochrome *c* from mitochondria, while the extrinsic pathway often involves the activation of the initiator caspase-8 through stimulation of death receptors of the TNF-receptor superfamily. It is likely that tRXR α is involved in the regulation of both intrinsic and extrinsic apoptotic pathways.

Previous studies showed that RXRs and ligands are implicated in the regulation of mitochondria-dependent apoptosis through modulating the Nur77-Bcl-2 apoptotic pathway due to its heterodimerization with Nur77 [116] (Fig. 2). Nur77, also known as TR3 or NGFI-B, is an immediate-early response gene and an orphan member of the nuclear receptor superfamily [117–119]. It mediates apoptosis of numerous types of cancer cells by a variety of apoptotic stimuli including retinoid-related apoptotic molecules, TPA, calcium ionophore, and many chemotherapeutic agents, perhaps being the most potent pro-apoptotic member in the nuclear receptor superfamily [22,120–130]. We discovered that Nur77 could migrate from the nucleus to the cytoplasm where it targets mitochondria through its interaction with Bcl-2, leading to cytochrome *c* release and apoptosis [131,132]. The unique property of the Nur77-Bcl-2 apoptotic pathway is the conversion of Bcl-2 from an anti-apoptotic molecule to a pro-apoptotic one upon binding by Nur77 [132,133]. RXR α cotranslocates with Nur77 from the nucleus to the cytoplasm in response to NGF [24], apoptotic stimuli [22,134], and IGFBP-3 [135]. Interestingly, Casas *et al.* [23] detected a 44 kDa tRXR α in mitochondria. Whether and how cytoplasmic tRXR α modulates the Nur77-Bcl-2 pathway remain to be investigated.

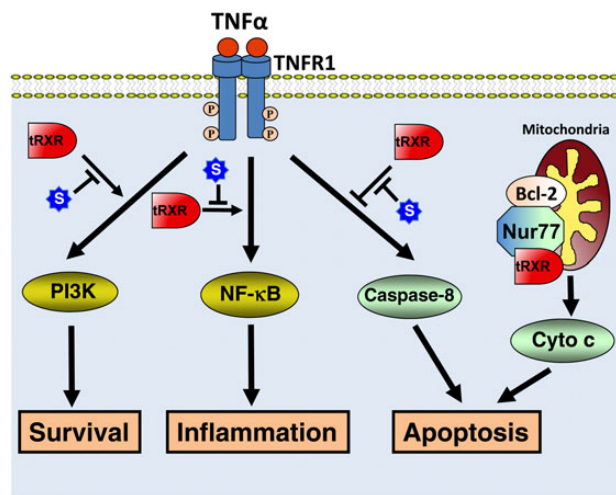


Figure 2. The nongenomic tRXR α actions The cytoplasmic tRXR α acts through its interaction with TNF α -signaling proteins to regulate cell survival, inflammation, and apoptosis. tRXR α effect is negatively regulated by its modulator Sulindac (S). In addition, tRXR α may target mitochondria through heterodimerization with Nur77 to modulate mitochondria-dependent apoptosis.

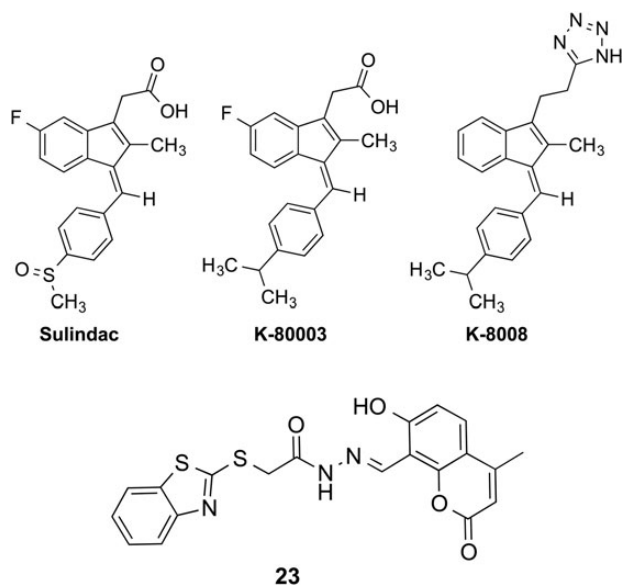


Figure 3. Chemical structures of molecules that act via binding to novel binding regions in RXR α

tRXR α has also been shown to modulate the extrinsic apoptotic pathway upon ligand binding [29]. TNF α is a multifunctional cytokine that controls diverse cellular events such as cell survival and death that control the destiny of cancer cells [136–141]. The diverse cellular effects of TNF α are mediated by TNF α receptor-1 (TNFR1). Upon binding to TNF α , TNFR1 forms a complex that consists of TNF-receptor-associated death domain (TRADD) protein, receptor interacting protein (RIP), and Fas-Associated protein with Death Domain (FADD). When TNFR1 signals apoptosis, FADD binds to pro-caspase-8, resulting in its activation and apoptosis. Alternatively, when TNFR1 signals survival, TNF-receptor-associated factor 2 (TRAF2) is recruited to the complex, leading to activation of AP-1 and NF- κ B pathways [136–141]. TNF α binding to TNFR1 could also result in activation of the PI3K/AKT survival pathway [142]. Although TNF α is capable of inducing apoptosis of cancer cells through death receptor-dependent mechanism, such an effect is often antagonized by its own survival function through its activation of NF- κ B and PI3K/AKT pathways [143–148]. The balance of TNF α -induced survival- and death-signaling is therefore pivotal in determining the fate of TNF α -responding cells [136–142,144,146,149–152]. Since TNF α is produced by malignant or host cells in the tumor microenvironment but not in normal cells, there has been tremendous interest in developing strategies to shift TNF α signaling from survival to death. Although tRXR α is required for TNF α activation of PI3K/AKT to promote the survival of cancer cells, we found that tRXR α binding to nonsteroidal anti-inflammatory drug (NSAID) Sulindac (Fig. 3) or analogs resulted in caspase-8 activation and apoptosis, demonstrating that tRXR α and ligands could regulate the extrinsic apoptotic pathway [29]. The activation of caspase-8-dependent apoptosis by Sulindac and analogs was attributed to their inhibition of tRXR α -dependent activation of the PI3K/AKT survival signaling. This is supported by the observation that Sulindac and analogs inhibit the binding of tRXR α to p85 α [29]. Similarly, inhibition of tRXR α -mediated activation of NF- κ B survival pathway also leads to apoptosis [65]. These results demonstrate that inhibition of tRXR α -mediated activation of PI3K/AKT and NF- κ B pathways could effectively shift TNF α signaling from survival to death. However, currently it cannot

be excluded that tRXR α may be directly involved in the regulation of the death-inducing signaling complex comprised of FADD and pro-caspase-8.

tRXR α and inflammation

Recent reports suggest a strong link between chronic inflammation and the development of cancer [153,154]. Chronic inflammation due to infection, autoimmune disease, malignant and benign tumors, or other pathologies has become a recognized risk factor for epithelial-derived malignancies [155]. The transcription factor NF- κ B is known as a master inflammatory transcriptional regulator, which is highly active in cancer cells. In quiescent cells, NF- κ B proteins are sequestered in the cytoplasm by inhibitory molecules, the inhibitor of NF- κ B (I κ B) proteins [136–141]. NF- κ B requires a signaling pathway for activation. Such NF- κ B-activating pathways are triggered by a variety of extracellular stimuli and lead to the phosphorylation and subsequent proteasome-mediated degradation of I κ B proteins [136–141]. Activated NF- κ B migrates into the nucleus to regulate the expression of multiple target genes associated with cancer cell migration, proliferation, anti-apoptosis, angiogenesis, and metastasis [136–141]. In cancer cells, NF- κ B is often constitutively activated as a result of undergoing inflammation or the consequence of formation of an inflammatory microenvironment during malignant progression.

The role of RXR α in regulating inflammation was suggested by findings that several anti-inflammatory agents act as RXR ligands. Dietary omega-3 fatty acids, such as DHA, exert their beneficial effects primarily through their anti-inflammatory effects. DHA induces growth inhibition and apoptosis by inhibiting NF- κ B activity [156] and suppressing cytokine production in macrophages [157], whereas R-etodolac which is known to bind to RXR α [158] decreases constitutive and RANKL-stimulated NF- κ B activation in macrophages and suppresses TNF α -induced I κ B-kinase (IKK) phosphorylation and subsequent NF- κ B activation in human multiple myeloma cells [159]. LGD1069 down-regulates COX-2 expression in breast cancer cells [160] and inhibits angiogenesis and metastasis in solid tumors [161]. Oral administration of Targretin reduces inflammation in a group of patients with plaque-type psoriasis [162]. Thus, the anti-inflammatory effects of RXR α and its ligands in various cell types underscore its function in the prevention and treatment of cancer and diseases.

Both genomic and nongenomic actions of RXR α could account for the modulation of inflammation in macrophages and cancer cells by RXR α and ligands. For genomic action, interactions between RXR α and proinflammatory transcription factors, particularly NF- κ B and AP-1, have been well described in several reviews [163,164]. RXR α may also employ nongenomic action to negatively regulate the NF- κ B signaling pathway. The role of nongenomic action of RXR α in the regulation of inflammation is also suggested by mounting evidence that subcellular localization of RXR α is altered in response to inflammation [25,30]. Lipopolysaccharide treatment of animals altered the subcellular location of RXR α [25]. RXR α undergoes rapid nuclear export in response to signals initiated by the proinflammatory cytokine IL-1 β in hepatoma cells [26]. Our recent discovery that TNF α could induce cytoplasmic localization of tRXR α underscores the significance of tRXR α cytoplasmic action in the regulation of inflammation (Fig. 2). As discussed above, TNF α is a multiple cytokine that induces not only the extrinsic apoptotic and PI3K/AKT pathways but also the NF- κ B inflammatory pathway through its sequential recruitment of various adaptors including TRADD, RIP1, and TRAF2 to the cytoplasmic membrane [136–141]. This is followed by the

recruitment and activation of the classical IKK complex [136–141]. Once activated, the IKK complex phosphorylates I κ B α , which is subsequently ubiquitinated and degraded via the proteasome pathway. We recently reported that tRXR α could promote TNF α activation of NF- κ B pathway through its interaction with TRAF2 and enhance TNF α -induced RIP1 ubiquitination [65]. A nitrostyrene derivative that binds to tRXR α could inhibit tRXR α interaction with TRAF2 and its induction of NF- κ B activation. These results demonstrate that tRXR α is directly involved in the activation and regulation of the inflammasome. It remains to be clarified whether other RXR α modulators known to have anti-inflammatory effect also target tRXR α -mediated activation of the NF- κ B pathway.

Ligands that Bind to a Novel Site to Regulate the Nongenomic Activities of tRXR α

We identified that NSAID Sulindac (also called CLINORIL[®]) currently used for treating pain and inflammation can inhibit the binding interaction between tRXR α and p85 α , resulting in caspase-8-dependent apoptosis [29]. Furthermore, we synthesized several Sulindac analogs and identified several Sulindac derivatives including K-80003, K-8008, and K-8012 (Fig. 3), which can also bind to tRXR α to inhibit the TNF α -induced interaction of tRXR α with p85 α and activate the TNF α -dependent apoptotic pathway [29]. K-80003, K-8008, and K-8012 are much more effective than Sulindac in inhibiting the growth of various cancer cells, including A549 lung cancer, PC3 prostate cancer, and ZR-75-1 and MB231 breast cancer cells. Both K-80003 and K-8008 inhibit HepG2 tumor growth in animals and do not show apparent toxic effects. Several natural products including CF31 [28] and nitrostyrene derivatives [65] could also activate this death pathway by directly binding to tRXR α .

Our identification of NSAID Sulindac and its analogs as tRXR α binders and modulators prompted us to study their binding mode. The crystal structure of RXR α -LBD in complex with K-8008 or K-8012 [62] demonstrates the existence of different binding site. The complex structures exist as noncrystallographic homotetramer similar to the reported apo-homotetramer [165,166], in which bottoms of two homodimers interface form a tetramer. In a tetramer, two K-8008 molecules were found to bind to one homotetramer in a hydrophobic region that is near the entry and the edge of the cognate LBP [62]. The K-8008 binding region is close to the dimer–dimer interface that does not overlap with the binding region of 9-*cis*-RA (Fig. 4A). With respect to the monomeric and the dimeric RXR α -LBD, the K-8008 binding region is located on the surface of the RXR α molecules. RXR α has been shown to form transcriptionally silent homotetramers in solution, which rapidly dissociate into active homodimers upon binding of agonists or antagonists [165–167]. Therefore, it is intriguing that binding of K-8008, an RXR α antagonist, to a new region does not induce tetramer dissociation, a similar phenomenon observed in the binding of danthron [168]. The structural basis of K-8008 binding suggests that RXR α tetramerization represents a key mechanism for the regulation of RXR nongenomic actions.

Ligands Targeting the Co-regulator-binding Site of tRXR α

Drug discovery and development efforts targeting RXR α have been focused on identifying and optimizing rexinoids that bind to RXR α canonical LBP. However, as mentioned above, there are key limitations of using rexinoids that include rising of plasma triglyceride levels, suppression of the thyroid hormone axis, and induction of hepatomegaly. Therefore, targeting alternate sites on RXR α for regulating

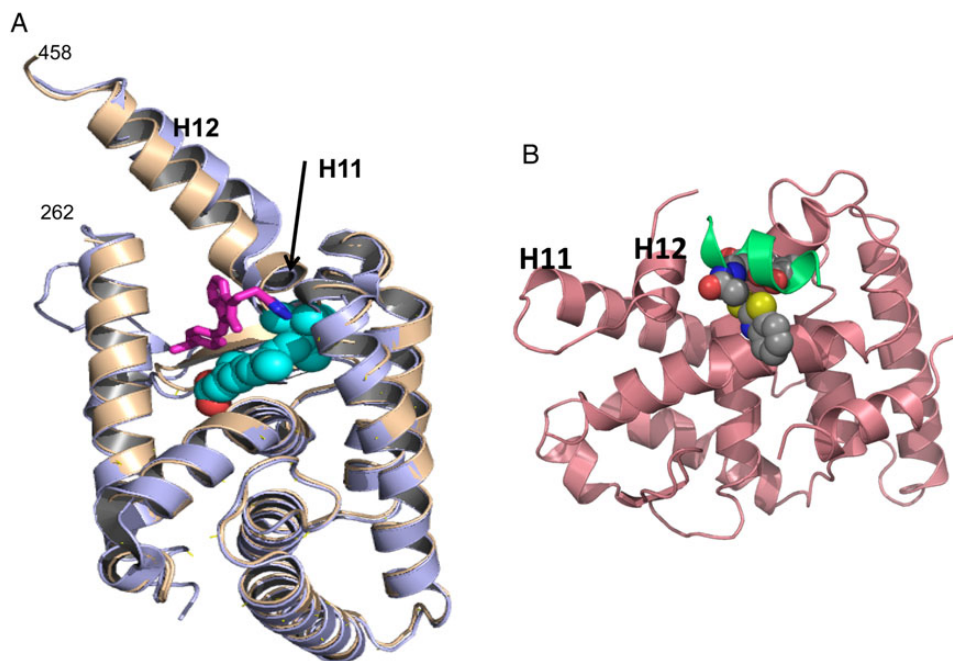


Figure 4. Novel binding regions in RXR α . (A) K-8008 binds to a novel binding region: the K-8008 binding region is away from the 9-*cis*-RA binding area and located on the surface of monomeric RXR α . It shows the superposition of the monomer of RXR α -LBD/K-8008 complex structure (brown) and the apo protein structure (purple, from PDB entry 1G1U). K-8008 is shown as sticks (carbon in magenta and nitrogen in blue). The classic ligand-binding site is indicated by a VDW ball model of 9-*cis*-RA (in cyan/red) taken from a superimposed 1FBY of PDB. (B) The proposed binding region for compound **23**. The compound **23**-binding region overlaps with the coactivator-binding region. Here, compound **23** was docked to the structure 3FUG (in pink) of PDB and the docked conformation (in VDW balls) was displayed with the coactivator peptide (in green) in the structure of 3FUG.

its activities could become a new strategy for RXR α -based drug discovery. Compounds that bind to alternate sites have been identified for other nuclear receptors [169–171], including estrogen receptor, androgen receptor, VDR, and T3R. Among the reported alternate sites on nuclear receptors, the co-regulator binding site is the most studied one. Recently, by employing a docking-based virtual screening approach, we identified some small molecules that bind to the co-regulator-binding surface of RXR α , a region where the binding sites of corepressor and the coactivator overlap (Fig. 4B). One of the identified binder, compound 23 (Fig. 3), could regulate the biological functions of tRXR α . Compound 23 could inhibit the TNF α -induced interaction between tRXR α and p85 α , inhibit AKT activation *in vitro* and in animals, and induce apoptosis [61]. Compound 23 represents the first example of an RXR α modulator that acts via the co-regulator-binding site rather than the classical LBP. Furthermore, compound 23 does not bind to the LBP. Thus, targeting the alternate binding sites on the surface of RXR α for therapeutic intervention may become a new paradigm for nuclear receptor-based drug discovery.

Conclusion and Perspective

RXR α is a unique and important drug target as evidenced by the success of rexinoid Targretin in treating CTCL and the enormous favorable results from testing rexinoids in various cancer models. However, the mechanisms by which RXR α modulate carcinogenesis are complex and remain to be fully defined, which has hampered the exploitation of the therapeutic potential of RXR α . The identification of tRXR α in cancer cells and the illustration of its roles in the control of apoptosis, survival, and inflammation offer new strategies to develop improved therapeutics against cancer by targeting tRXR α .

RXR α -based drug development is also hampered by the side effects associated with targeting its cognate LBP that is highly conserved among many nuclear receptors. Therefore, one of the current challenges in developing RXR α -based drugs is to identify selective RXR α modulators that possess the desired pharmacological activities without unwanted side effects. Many new modulators are being developed. In addition, screening for natural and synthetic RXR α ligand is ongoing. Thus, the findings that RXR α is cleaved in tumor cells and that Sulindac-derived small molecules and others act at the alternate binding sites on the surface of tRXR α will provide new rational for drug design and screening approach. Such an approach may help to identify small molecules specific to tumor- or disease-selective RXR α (i.e. tRXR α or RXR α with abnormal modifications) and may also circumvent side effects associated with binding to the cognate RXR α LBP. However, many questions remain unanswered regarding tRXR α production and function, and the underlying mechanisms of tRXR α actions need to be determined. Binding of Sulindac analogs to the tetrameric form of RXR α LBD is interesting. However, little is known about the biological function of the RXR α tetramer with respect to the regulation of the nongenomic RXR α action. Characterizing the surface-binding sites in tRXR α and developing selective inhibitors targeting the surface-binding sites may support a transition from the traditional paradigm of drugs targeting the LBP to a novel rational approach targeting functionally important surface sites, which may lead to more effective and specific therapeutics.

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