RETINOIDS, REXINOIDS AND THEIR COGNATE NUCLEAR RECEPTORS: CHARACTER AND THEIR ROLE IN CHEMOPREVENTION OF SELECTED MALIGNANT DISEASES

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Background: Retinoids, rexinoids and their biologically active derivatives are involved in a complex arrangement of physiological and developmental responses in many tissues of higher vertebrates. Both retinoids and rexinoids are either natural or synthetic compounds related to retinoic acids that act through interaction with two basic types of nuclear receptors belonging to the nuclear receptor superfamily: All-*trans* retinoic acid receptors (RAR α , RAR β , and RAR γ) and retinoid X receptors (RXR α , RXR β and RXR γ) as retinoid-inducible transcription factors.

Aim: Summarization of selected effects of biologically active natural or synthetic retinoids and rexinoids and their exploitation in chemoprevention of various types of cancer.

Results: Retinoid receptors play a role as ligand-activated, DNA-binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes. They exert both beneficial and detrimental activity; they have tumour-suppressive activity but on the other hand they are teratogenic. A number of nuclear receptor selective retinoids and rexinoids, have been successfully tested using a variety of cell lines or animal models. Retinoids inhibit carcinogenesis, suppress premalignant epithelial lesions and tumour growth and invasion in a variety of tissues.

Conclusions: Natural and synthetic retinoids exert important biological effects due to their antiproliferative and apoptosis-inducing effects. They are also known to cause redifferentiation or to prevent further dedifferentiation of various tumour tissues.

INTRODUCTION

Retinoids and their natural metabolites and synthetic derivatives are polyisoprenoid compounds containing a cyclohexenyl ring. Their parent compound is all-*trans* retinol (vitamin A) and its subsequent oxidation product is all-*trans* retinal (retinaldehyde). From retinaldehyde all-*trans* retinoic acid (ATRA) is formed irreversibly¹. ATRA is considered to be the main biologically active form of vitamin A in a variety of functions in vertebrates. 9-*cis* retinoic acid (9cRA) has a different spectrum of biological activity in comparison with that of all-*trans* retinoic acid. 9cRA is a high affinity ligand for the 9-*cis* retinoic acid (retinoid X or rexinoid) receptors (RXRs), as well as the all-*trans* retinoic acid receptors (RARs)². Retinoids are involved in the complex arrangements of physiological and developmental responses in many tissues of higher vertebrates that include embryonic development, vision, reproduction, bone formation, haematopoiesis, metabolism, growth and differentiation of a variety of cell types, apoptosis and processes of carcinogenesis^{1–3}. It is well known that retinoids are also teratogens and the therapeutic doses of them are contraindicated during pregnancy⁴. This article summarizes selected effects of biologically

active natural or synthetic retinoids and rexinoids, acting through their cognate nuclear receptors, and their use in chemotherapy and chemoprevention of various types of cancer. We would like to apologize to all authors whose original references have not been mentioned in this article due to our intention to limit the references to a reasonable number.

NUCLEAR RECEPTORS – LIGAND INDUCIBLE TRANSCRIPTION FACTORS

A breakthrough in the field of nuclear receptors was discovery of the nuclear receptor superfamily that comprises nuclear receptors for steroid hormones, retinoids, thyroid hormones, vitamin D₃, nuclear receptors for other biologically important compounds, and a number of “orphan” nuclear receptors for which the ligands remain still unknown⁵. It is generally accepted that nuclear receptors represent a superfamily of ligand-inducible transcription factors. Dysfunction of nuclear receptor signalling leads to proliferative, reproductive and metabolic diseases. The nuclear receptor superfamily of 48 identified nuclear receptors in human⁶ is shown in Table 1 and 2.

Proteins of the nuclear receptor superfamily represent single polypeptide chains with several major domains

Table 1. Biologically active ligands and human nuclear receptors.

Receptor (Abbreviation)	Ligand
Thyroid hormone receptor α (TR α)	Thyroid hormone
Thyroid hormone receptor β (TR β)	Thyroid hormone
All- <i>trans</i> retinoic acid receptor α (RAR α)	All- <i>trans</i> retinoic acid, 9- <i>cis</i> retinoic acid, retinoids
All- <i>trans</i> retinoic acid receptor β (RAR β)	All- <i>trans</i> retinoic acid, 9- <i>cis</i> retinoic acid, retinoids
All- <i>trans</i> retinoic acid receptor γ (RAR γ)	All- <i>trans</i> retinoic acid, 9- <i>cis</i> retinoic acid, retinoids
9- <i>cis</i> retinoic acid receptor α (RXR α)	9- <i>cis</i> retinoic acid, rexinoids
9- <i>cis</i> retinoic acid receptor β (RXR β)	9- <i>cis</i> retinoic acid, rexinoids
9- <i>cis</i> retinoic acid receptor γ (RXR γ)	9- <i>cis</i> retinoic acid, rexinoids
Vitamin D ₃ receptor (VDR)	Calcitriol
Peroxisome proliferators-activated receptor α (PPAR α)	Fatty acids, fibrates, leukotriene B ₄
Peroxisome proliferators-activated receptor β (PPAR β)	Fatty acids
Peroxisome proliferators-activated receptor γ (PPAR γ)	Fatty acids, thiazolidinediones
Retinoid-related orphan receptor α (ROR α)	Cholesterol, cholesteryl sulphate
Retinoid-related orphan receptor β (ROR β)	Retinoic acid
Retinoid-related orphan receptor γ (ROR γ)	Retinoic acid
Liver X receptor α (LXR α)	Oxysterols
Liver X receptor β (LXR β)	Oxysterols
Farnesoid X receptor α (FXR α)	Bile acids, fexaramine
Pregnan X receptor (PXR)	Xenobiotics
Constitutive androstane receptor (CAR)	Xenobiotics, phenobarbital
Estrogen receptor α (ER α)	Estradiol-17 β , tamoxifen
Estrogen receptor β (ER β)	Estradiol-17 β
Estrogen receptor-related receptor β (ERR β)	4-OH tamoxifen
Estrogen receptor-related receptor γ (ERR γ)	4-OH tamoxifen
Glucocorticoid receptor (GR)	Cortisol, dexamethasone, RU486
Mineralocorticoid receptor (MR)	Aldosterone, spiro lactone
Progesterone receptor (PR)	Progesterone, RU486
Androgen receptor (AR)	Testosterone, flutamide

(Fig. 1). The amino terminal A/B-domain of the nuclear receptor molecule contains a constitutive activation function independent on ligand (AF-1). The central C-domain is a cysteine-rich DNA-binding region consisting of two highly conserved zinc fingers. The D-domain represents a highly flexible structure, and it plays a role as a hinge of the receptor molecule. The carboxy-terminal E-domain

is responsible for the ligand binding, dimerization, and it contains inducible transactivation function dependent on ligand (AF-2) (ref.⁷⁻⁹).

All nuclear receptors are known to modulate gene transcription, although among them marked differences do exist, predominantly in the mechanisms through which it is accomplished¹⁰⁻¹².

Table 2. Human nuclear orphan receptors.

Receptor	Abbreviation
Reverse erbA α	Rev-erbA α
Reverse erbA β	Rev-erbA β
Human nuclear factor 4 α	HNF4 α
Human nuclear factor 4 γ	HNF4 γ
Testis receptor 2	TR2
Testis receptor 4	TR4
Tailless	TLL
Photoreceptor-specific nuclear receptor	PNR
Chicken ovalbumin upstream promoter-transcription factor I	COUP-TFI
Chicken ovalbumin upstream promoter-transcription factor II	COUP-TFII
ErbA2-related gene-2	EAR2
Estrogen receptor-related receptor α	ERR α
NGF-induced factor B	NGFIB
Nur-related factor 1	NURR1
Neuron-derived orphan receptor 1	NOR1
Steroidogenic factor 1	SF1
Liver receptor homologous protein 1	LRH1
Germ cell nuclear factor	GCNF
DSS-AHC critical region on the chromosome, gene 1	DAX1
Short heterodimeric partner	SHP

All-trans and 9-cis retinoic acid receptors retinoic acid receptors

The diversity of retinoic acid-induced signalling pathway is associated with existence of at least three subtypes for RAR (α , β and γ) and three subtypes for RXR (α , β and γ) with distinct amino- and carboxy-terminal domains. Later on, two major isoforms for RAR α ($\alpha 1$ and $\alpha 2$) and for RAR γ ($\gamma 1$ and $\gamma 2$) and four major isoforms for RAR β ($\beta 1$, $\beta 2$, $\beta 3$ and $\beta 4$) have been found. Similarly,

two major isoforms for RXR α ($\alpha 1$ and $\alpha 2$), RXR β ($\beta 1$ and $\beta 2$), and RXR γ ($\gamma 1$ and $\gamma 2$) have been identified up to now^{5, 13}. All-trans retinoic acid receptors upon all-trans retinoic acid binding act as all-trans retinoic acid-inducible transcription factors by directly interacting as heterodimers with 9-cis retinoic acid receptor. The RXR/RAR heterodimer interacts with specific DNA response elements of target genes and its effect on transcription is mediated also through recruitment of a number of coregulators

Fig. 1. Structural and functional organization of nuclear receptor specific domains

DIRECT REPEATS	TR	RAR	RXR	VDR
DR+1 AGGTCANAGGTCA			+++	
DR+2 AGGTCANNAGGTCA		+++		
DR+3 AGGTCANNNAGGTCA				+++
DR+4 AGGTCANNNNAGGTCA	+++			
DR+5 AGGTCANNNNNAGGTCA		+++		

Fig. 2. RXR/RAR heterodimers binding to specific DNA sequence – retinoic acid response elements (RAREs), characterized by direct repeats of two hexamers AGGTCA separated predominantly by five nucleotides (DR+5) or two nucleotides (DR+2)

Fig. 3. Chemical structure of selected retinoids and rexinoids

(corepressors and coactivators)^{5, 10, 11}. Ligand occupancy of both receptors in RXR/RAR heterodimers was found to synergistically increase transcriptional activity^{14, 15}. RARs bind both all-*trans* retinoic acid and 9-*cis* retinoic acid with similar affinities while RXRs bind only 9-*cis*-retinoic acid. RXR/RAR heterodimers bind to specific DNA sequence – retinoic acid response elements (RAREs), characterized by direct repeats of two hexamers AGGTCA separated predominantly by two nucleotides (DR+2) or five nucleotides (DR+5) (Fig. 2). In the absence of all-*trans* retinoic acid, the RAR/RXR heterodimer recruits nuclear receptor corepressor proteins, e.g. nuclear receptor corepressor (N-CoR) or silencing mediator of retinoid and thyroid hormone receptor (SMRT), and associated enzymes such as histone deacetylases or DNA-methyl transferases that lead to an inactive condensed chromatin structure preventing transcription process. All-*trans* retinoic acid binding leads to the dissociation of corepressor proteins and enables association of coactivator proteins (histone acetyltransferases or histone arginine methyltransferases) with liganded receptor complex yielding in activation of gene transcription^{6, 16–18}. Thus, the retinoid receptors are considered to be ligand-activated, DNA-binding, *trans*-acting, transcription-modulating nuclear proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes⁶.

The retinoids selective for specific binding to RXRs are called rexinoids¹⁹, and RXRs play an crucial role in the nuclear receptor mediated transcription processes for their ability to heterodimerize with many other members of nuclear receptor superfamily, including RARs, thyroid hormone receptors (TRs), vitamin D₃ receptor (VDR), peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXR_s), farnesoid X receptor (FXR), pregnan X receptor (PXR), and constitutively activated receptors

(CARs) (ref.^{6, 20, 21}). RXRs thus behave as promiscuous dimerization partners for a large number of nuclear receptors, and thus play an integrative and crucial role in nuclear receptor mediated pathways^{6, 22, 23}. While RAR ligand (agonist) autonomously activates transcription through RXR/RAR heterodimer, RXRs are unable to respond to RXR ligands (rexinoids) in the absence of RAR ligand. Rexinoid binding to RXR is incapable to induce the dissociation of corepressor molecule from the RXR/RAR heterodimers²⁴ moreover, no interaction was found between RXRs and corepressors NCoR and SMRT²⁵.

Genetic strategies in the mouse in order to determine the function of both RAR and RXR subtypes have shown following results. RAR α null mutant males have been found to be sterile due to degeneration of the seminiferous epithelium causing inhibition of spermatogenesis^{26–28}, RAR β null mice display abnormalities in the vitreous body in eyes and impaired abilities in locomotion and motor coordination, and ablation of RAR γ causes both skeletal and epithelial defects²⁹. The loss of RXR α was found to be lethal during fetal life due to hypoplasia of the myocardium that appears to be the principal cause of animal death. Moreover, fetuses lacking RXR α have ocular malformation. Also, it has been shown that RXR α is involved in the mediation of a teratogenic effect due to administration of exogenous retinoids^{30–32}. The ablation of RXR β led to approximately 50 % in utero lethality. Males lacking RXR β are sterile and exhibit testicular defects and abnormal spermatid maturation, leading to defects of spermatozoa. Moreover, RXR β mutation causes abnormal lipid metabolism in Sertoli cells, suggesting functional interactions of RXR β with other nuclear receptors that control lipid metabolism^{32–34}. RXR γ null mutants are fertile but they have higher serum levels of both L-thyroxine and thyroid-stimulating hormone (TSH), and increased metabolic rate when compared to wild type animals^{32, 35}.

Thus, RXR-RAR heterodimers play an important role in transducing *in vivo* the retinoid signal and that specific heterodimers are involved in many developmental and metabolic processes^{28, 32}.

RETINOID SIGNALLING IN CHEMOPREVENTION OF SELECTED MALIGNANT DISEASES

In animal models, retinoids or rexinoids have been found to suppress carcinogenesis or induce differentiation of malignant cells through their cognate nuclear receptors in a variety of tissues³⁶. Altered expression of all-*trans* or 9-*cis* retinoic acid receptors may be associated with the processes of malignant transformation of animal or human cells in a variety of tissues. Aberrant retinoid signalling mechanisms is oftentimes linked to cancer, and at present, the retinoids are used to a progressively increasing extent in a variety of chemopreventive and chemotherapeutic settings²⁸.

Acute promyelocytic leukaemia and exploitation of retinoids

The most direct implication of RAR in human disease is given by acute promyelocytic leukaemia (APL), which is characterized by selected expansion of immature myeloid precursors or malignant myeloid cells blocked at the promyelocytic stage of hemopoietic development^{36, 37}. Retinoic acids as retinoid inducible transcription factors are involved in myeloid differentiation through nuclear receptor heterodimer (RXR/RAR α) to modulate the expression of target genes. Molecular studies have identified the fusion of the RAR α gene to partner genes as the crucial event in APL pathogenesis³⁸⁻⁴⁰. APL has been found to be associated with reciprocal translocation involving the RAR α gene on chromosome 17 that translocates and fuses to genes including promyelocytic leukemia gene (PML), promyelocytic leukemia zinc finger gene, nucleophosmin gene, nuclear mitotic apparatus gene, and the Stat5b gene^{3, 37, 41}. RAR α usually fuses to the PML gene on chromosome 15 yielding the PML – RAR α chimeric gene, which is expressed in all APL patients with t(15;17)^{39, 42}. Thus, chimeric receptors are responsible for development of leukemias by interfering with the functions of RARs, RXRs and PML expressed by the normal alleles present in the same cell⁴¹. In addition, it has been shown that PML – RAR α is capable to form homodimer through the coiled-coil motif of PML and thus to compete with the RAR α for binding to retinoic acid responsive element of target genes³⁹. The all-*trans* retinoic acid-induced differentiation of cells is based on important findings that it can cause a degradation of PML – RAR α , induce dissociation of corepressors from PML – RAR α ³⁷. All-*trans* retinoic acid (ATRA) effects are also accompanied by cell cycle arrest at G1 phase and inhibition of cell growth. Results based on *in vitro* studies enabled the treatment of APL patients with all-*trans* retinoic acid that caused the malignant cells to differentiate towards mature granulocytes, thus regaining most functions of their normal counterparts³⁹. In the meantime, ATRA (Tretinoin, Vasanoid) was approved by

the FDA for differentiation therapy of patients with acute promyelocytic leukemia and the successful application of all-*trans* retinoic acid for treatment of APL might be considered as a start of the retinoid renaissance period, since it rekindled interest in the use of retinoids or rexinoids for treatment of a variety of established malignancies. In addition, high concentration of ATRA is able to induce postmaturation apoptosis through the induction of the tumour-selective death ligand tumour necrosis factor-related apoptosis-inducing ligand TRAIL, also called Apo2L, a most promising molecule in cancer research^{28, 43, 44}.

Retinoids or rexinoids in breast cancer chemoprevention

Breast cancer incidence, morbidity and mortality remain a major problem in the most of countries. Breast cancer development is associated with deregulation of cell growth and cell death. It has been shown that retinoids are able to inhibit mammary gland cancer in animal models and human breast cancer^{36, 45-47}. They are effective inhibitors of breast cancer cells at the early stages of tumour progression and their effectiveness diminishes as tumours become more aggressive⁴⁸. Retinoid receptors are expressed differently in normal and malignant epithelial cells and are critical for normal development⁴⁹. Recently, we have also shown marked differences between nonlactating and postlactating mammary gland in rat. RAR α , RXR α N-CoR, SMRT in rat were significantly increased in postlactating mammary gland when compared to that of nonlactating mammary tissue. Postlactating mammary glands were found to express all RAR and RXR subtypes studied when compared to nonlactating mammary tissues that express exclusively RAR α and RXR α subtypes. Enhanced expression of a number of nuclear hormone receptors, their coregulators in mammary tissue of postlactating rats in comparison with nonlactating animals identify a potential role for retinoid signalling pathways also after lactation period⁵⁰. Growth inhibition of breast cancer cells by retinoic acid has been associated with induction of the expression of RAR β , which may act as a tumour suppressor and appears to be down-regulated in breast cancer tissue and cell lines, and conversely, up regulated in normal mammary epithelial cells⁵¹. 9-*cis* retinoic acid, a high affinity ligand for RXRs as well as LGD1069 (Targretin, Bexarotene) (Fig. 3), a synthetic RXR-selective ligand have been shown to have efficacy superior to ATRA as a chemopreventive compound in the 1-methyl-1-nitrosourea (MNU)-induced rat mammary gland carcinoma model. Moreover, Targretin was very well tolerated during chronic therapy with no classic signs of “retinoid-associated” toxicities⁵². In animal mammary gland carcinoma models, additional promising retinoids are retinyl acetate or N-(4-hydroxyphenyl)retinamide (Fenretinide)⁵³ (Fig. 3). As mentioned before, lack of RAR β gene expression is a typical feature for human breast cancer and may be one of the major factors responsible for retinoid resistance in those neoplasias. Examination of the expression of all the subtypes of RARs and RXRs in a number of hormone-dependent and hormone-independent breast cancer cells

have shown similar expression of RAR α , RAR γ , RXR α and RXR β . However, all-*trans* retinoic acid was found to be a strong inducer of the RAR β gene expression in several hormone-dependent breast cancer cell lines. Due to up-regulation of RAR β by all-*trans* retinoic acid or other retinoids, the expression of RAR β apparently plays a critical role in increasing retinoid sensitivity. These data indicate that the deficiency of the RAR β expression and a deficient responsiveness of retinoids via RAR β may account for the ineffective treatment with retinoids in patients with advanced breast tumour⁵¹. The potency of retinoids to inhibit proliferation of breast cancer cells was seen when retinoids were administered either alone or in combination with antiestrogens. Tamoxifen and fenretinide combination therapy has been proven to be an active treatment regimen in metastatic breast cancer patients, however, not in estrogen receptor negative metastatic breast cancer or in patients whose disease had progressed on tamoxifen⁵⁴. Retinoids do not require estrogen receptors for their action, they may affect neoplastic transformation in estrogen-negative cells, in contrast to tamoxifen, whose primary mechanism of action is through estrogen receptors. In spite of that, retinoids and rexinoids seem to be more active in estrogen positive than in estrogen negative precancerous tissue⁵⁵. Furthermore, it is known that retinoids can promote apoptosis in breast cancer cells as well as in other types of neoplasias and that induction is predominantly mediated by RAR β ⁵⁶. In spite of a number of important findings achieved recently by many prestigious laboratories, the precise mechanism(s) by which natural or synthetic retinoids inhibit breast cancer cell growth has not been completely elucidated. Great interest has been recently focused on rexinoids, when combined with selective estrogen receptor modulators (SERMs), they dramatically inhibit breast cancer cell growth inducing apoptosis even with intermittent administration⁴⁹.

Retinoids in thyroid gland cancer treatment

Thyroid cancer is a relatively common malignancy. A minority of patients suffers from poorly differentiated thyroid carcinoma that is unresponsive to radioiodine therapy. Redifferentiation agents that "reprogram" these thyroid tumours in order to concentrate radioiodine would be of great value in treating patients with advanced thyroid cancer⁵⁷⁻⁵⁹. The biologically active retinoids or rexinoids are extensively studied from that aspect. In follicular thyroid cancer cells, 13-*cis* retinoic acid has been found to induce radioiodine avidity of cells formerly unable to accumulate radioiodine⁶⁰. In human thyroid carcinoma cell lines, natural or synthetic retinoids were found to induce expression of type I iodothyronine-5'-deiodinase, sodium/iodide-symporter, the thyroid differentiation markers⁶¹⁻⁶³. However, approximately 30 % of the thyroid tumors may start to dedifferentiate and thus finally develop into highly malignant anaplastic thyroid carcinomas⁶⁴. Non-operable thyroid follicular tumors, which due to loss of thyroid specific functions also fail to take up radioiodine, have been treated with 13-*cis* retinoic acid. The beneficial outcome of this treatment was interpreted as partial redifferentiation

of thyroid cancer cells⁶⁴. This effect markedly requires existence of functional nuclear retinoid/rexinoid receptors. The presence of high-affinity and limited capacity of nuclear all-*trans* retinoic acid binding sites has been shown in follicular thyroid carcinoma cell lines⁶⁵. The ability of retinoid receptors in thyroid carcinoma cell lines to bind to their responsive elements has been evaluated by electrophoretic mobility and supershift assays⁶⁶. In clinical pilot studies patients with poorly differentiated, inoperable thyroid carcinoma and lacking or insufficient iodine uptake have been treated with oral doses of 1.0-1.5 mg 13-*cis* retinoic acid per kg body weight and day over 5 weeks. The results revealed an increased radioiodide uptake in 30 %-50 % of patients⁶⁴. In a recent study a fluorine-18 fluorodeoxyglucose positron emission tomography (¹⁸F-FDG PET) was used for the evaluation and monitoring of therapy with 13-*cis* retinoic acid in thyroid cancer. In 6 out of 21 patients, radioiodine accumulation was newly induced after 6-9 months, and in 3 patients a residual iodine accumulation was enhanced⁶⁷. The data correspond well with findings described previously indicating retinoids as promising therapeutic agents for the treatment of thyroid cancer⁵⁹.

Possible use of retinoids or rexinoids in other organs cancer chemoprevention

Retinoids as previously reviewed³⁶ have been implicated in induction of cell death in many tumour-derived cultured cell systems or tumour tissues, e.g., neuroblastoma cells, ovarian cancer, cervical cancer cells, prostate cancer, head and neck squamous cell carcinoma, liver cancer, pancreatic cancer, lung cancer and many other types of carcinomas. Relationship between biologically active retinoids or rexinoids, which can act as effective cancer chemopreventive agents or potential chemotherapeutic compounds and the regulation of the processes such as cell differentiation and apoptosis is very complex and requires identification of new target genes that might be involved in those processes.

CONCLUSION

A variety of biologically active retinoids or rexinoids has been tested on different types of cancer in order to evaluate the precise mechanism of action. In a number of different types of tumours both retinoids or rexinoids are capable to induce both apoptosis and differentiation, and they represent a potentially powerful alternative to present chemotherapeutic treatment of late stages of cancer³⁶.

In conclusion, a number of novel chemical compounds, receptor selective retinoids and rexinoids, have been synthesized up to now and tested *both in vitro* and *in vivo*. In spite of that rapid progress novel synthetic retinoids and rexinoids with greater retinoid receptor selectivity, reduced teratogenic and other side effects are still highly required.

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