In this issue of Clinical Cancer Research, Li et al. investigate the chemopreventive potential of the retinoid, LG100268 (1). The application of retinoids to cancer chemoprevention has a long history which predates the identification of retinoid receptors (2, 3). Early clinical trials evaluated vitamin A derivatives, such as 13-cis-retinoic acid (13-cis-RA), all-trans retinoic acid (ATRA), and 9-cis-retinoic acid (9-cis-RA), in individuals at increased risk of developing cancer. These trials were pivotal in establishing proof of principle for human cancer chemoprevention, and moreover, showed the potential utility of retinoids in this respect. The associated toxicity, however, identified high-dose regimens of naturally occurring retinoids as unsuitable for long-term administration for cancer prevention. Increased understanding of retinoid receptor biology has facilitated the design of synthetic ligands with increased selectivity and hence decreased toxicity. Specifically, ligands selective for the retinoid X receptor subclass have been developed, the so-called rexinoids, which seem to afford equivalent or greater chemopreventive action of the retinoid LG100268 with substantially diminished toxicity, however, identified high-dose regimens of naturally occurring retinoids as unsuitable for long-term administration for cancer prevention. The current state of evolution of this drug class is illustrated by Li et al. (1), in this issue, who describe the chemopreventive action of the retinoid LG100268 with respect to estrogen receptor–negative breast cancer. LG100268 markedly suppresses the formation of both invasive breast cancers and premalignant mammary lesions in rodent breast cancer models, with minimal incidence of common retinoid toxicities (1, 4–6). These studies are paving the way for testing rexinoids for breast cancer prevention.

Retinoid Receptor Biology

Retinoid receptors are nuclear, ligand-regulated transcription factors of the steroid/thyroid hormone receptor superfamily, that are activated in vivo by binding vitamin A–derived retinoids including ATRA, and 9-cis-RA (7, 8). Essential roles for retinoid receptors in growth, reproduction, retinal development, and vision have been inferred from the consequences of vitamin A deficiency, and from the analysis of retinoid receptor–deficient mouse strains.

There are two distinct classes of retinoid receptor, retinoic acid receptors (RAR) and retinoid X receptors (RXR), each of which comprises three isoforms encoded by separate genes, designated α, β and γ, as well as several isoforms generated by alternative splicing or promoter usage (7, 8). RARs and RXRs are distinguished by their differential affinities for naturally occurring ligands. Although 9-cis-RA is a ligand for both groups, only RARs bind ATRA. Furthermore, RXRs and RARs differ in the spectrum of proteins with which they interact, both at the level of nuclear receptors, and in terms of transcriptional corepressors. Functional activity of both RARs and RXRs requires dimerization with a member of the nuclear nonsteroidal receptor family. However, although RXRs dimerize predominantly with RXRs, in marked contrast, RARs can interact with many different factors, indicative of their role as the master heterodimerizing member of the nuclear receptor superfamily. Thus, in addition to RARs, RXRs also dimerize with thyroid hormone receptors (TRs), vitamin D receptor (VDR), peroxisome proliferator–activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptor (FXR), pregnane X receptor (PXR), and constitutively activated receptors. All of these nuclear receptors require RXR as a heterodimerization partner in order to regulate gene transcription.

Retinoid Receptor – Mediated Transcription: Achieving Specificity

Retinoid receptors regulate transcription via interaction with RAR elements and RXR elements in target gene promoters, and subsequent recruitment of transcriptional coactivators. Because RXRs are obligate heterodimerization partners for a multitude of nuclear receptors, retinoids have the potential to regulate the activity of entire regulatory networks. Response specificity is achieved through several levels of control.

Firstly, receptor responsiveness is defined by the nature of the ligand: each retinoid receptor has a unique affinity for individual retinoids. As mentioned above, natural ligands for retinoid receptors are derived from vitamin A and include 13-cis-RA, 9-cis-RA, and ATRA. Of these, ATRA selectively binds to RARs, but 9-cis-RA is a pan-retinoid that binds to both RARs and RXRs, albeit with differing affinities. 13-cis-RA gets isomerized to ATRA, and thus, is functionally equivalent. Comparison of the ligand-binding pockets of RXRs and RARs provides clues as to their differential ligand specificity. Solving the crystal structure of RAR has shown the ligand-binding pocket to be a linear “I” shape, whereas that of RXRα is a shorter and more restrictive “L” shape (7). These crystal structures provide valuable insights for designing...
RXR-selective agents, which are projected to have multiple pharmacologic applications, particularly in light of their reduced toxicity compared with naturally occurring retinoids (discussed later).

The identity of the dimerization partner is another important determinant of specificity because different nuclear receptor combinations bind to distinct sequence motifs in gene promoters. Retinoid response elements consist of direct repeats of polymorphic arrangements of the canonical motif 5'-PuG(T/G)TCA. The number of nucleotides separating each repeat varies between individual promoter elements, with a single nucleotide separator generating a DR-1 element, two bases giving rise to a DR-2 element, and so on. Unique dimer complexes bind to each promoter motif. Thus, RXR-RXR homodimers and RXR-PPAR heterodimers bind to DR-1, RXR-RAR dimers bind to DR-2 and DR-5, RXR-PXR and RXR-LXR complexes bind to DR-3, and RXR-TR and RXR-LXR dimers bind to DR-4 motifs (9). Binding specificity is further regulated by the sequences of the core motif, the spacer, and the flanking nucleotides, as well as by the occurrence of inverted and everted repeats (9).

Additional specificity is provided by the expression patterns of retinoid receptor isoforms, and of other nuclear receptors. For example, whereas RXRβ expression is virtually ubiquitous, RXRα is predominantly expressed in the liver, kidney, epidermis, and intestine, and RXRγ is mostly restricted to muscle, pituitary, and discrete regions of the brain (7).

RXR-containing dimers can be sorted into several discrete classes. RXR homodimerization generates complexes that are solely regulated by RXR ligands (Fig. 1A). Dimerization of RXRs with RARs, or with TR or VDR, generates nonpermissive complexes which cannot be activated by RXR agonists alone, but only by the ligand of the partner receptor, alone or in combination with an RXR ligand (Fig. 1B). In contrast, permissive complexes consisting of RXRs partnered with PPARs, LXR, FXR, or PXR can be activated by RXR ligands and agonists of the partner receptor individually, or in combination, in which case the ligands elicit synergistic activation (Fig. 1C).

The differential responsiveness to RXR ligands of permissive and nonpermissive complexes may be attributable to the distinct interactions of individual nuclear receptors with transcriptional corepressors. Many nuclear receptors, including RARs, bind corepressors including silencing mediators for retinoid and thyroid receptors (SMRT) and nuclear receptor corepressor (N-CoR). In the absence of RAR ligands, DNA-bound RAR-RXR heterodimers form multiprotein complexes with these transcriptional corepressors and histone deacetylases, resulting in the silencing of target genes (Fig. 1B). RXR ligands alone are insufficient to displace this corepressor complex from RAR-RXR heterodimers. RAR agonist binding induces a conformational change, however, displacing the corepressor complex and allowing recruitment of coactivators and communication with the basal transcriptional machinery to initiate target gene expression. In contrast, RXRs and the coreceptors comprising...
permissive complexes can be activated by individual ligation of either component of the complex (Fig. 1C).

The tight regulation offered by nonpermissive heterodimers relative to nonpermissive complexes may be important for minimizing aberrant activation of potentially toxic genes. Consistent with this notion, rexinoids have markedly diminished side effects compared with pan-retinoids and RAR ligands, identifying RAR signaling as the culprit in mediating conventional retinoid toxicities. Thus, rexinoids offer an attractive option for maximizing the clinical potential of retinoids through sidestepping RAR-dependent toxicity.

Retinoids as Anticancer Agents

The exploration of retinoids as anticancer agents has an extensive history that predates the identification of retinoid receptors themselves, the first cloning of which was reported in 1987 (7, 8). Numerous animal studies have evaluated the cancer chemopreventive potential of a range of compounds, including ATRA, 13-cis-RA, and N-([4-hydroxyphenyl]retinamide, and retinoids have been found to be effective in suppressing tumor development in multiple carcinogenesis models, including those of the breast, skin, oral cavity, lung, prostate, bladder, liver, and pancreas (3, 10). Furthermore, retinoids have proven efficacious for suppressing precancerous lesions in humans including cutaneous actinic keratoses, dysplastic nevi, oral leukoplakias, and moderate cervical dysplasia, as well as inhibiting the development of second primary cancers such as head and neck tumors (2).

Encouraging results in clinical trials have led to the use of retinoids as standard therapy for some indications. Of particular note is the application of ATRA as differentiation therapy in acute promyelocytic leukemia, which frequently results from chromosomal translocations involving the RARz and PML genes. The PML-RARα fusion protein binds to the transcriptional corepressors N-CoR and SMRT with greater avidity than RARα, leading to super-repression of RARα signaling, and consequently, the arrest of myelopoesis at the promyelocyte stage. Therapeutic ATRA levels are sufficient to abrogate the blockade and attain complete and lasting remission in some patients with acute promyelocytic leukemia.

Intriguing results with respect to breast cancer have been obtained using the synthetic ATRA derivative N-([4-hydroxyphenyl]retinamide (fenretinide). In premenopausal women, fenretinide showed a durable trend towards reducing second breast malignancies, although an opposite trend was observed in postmenopausal women (11, 12). These data suggest that N-([4-hydroxyphenyl]retinamide could be useful for breast cancer prevention in young women, perhaps in combination with other agents (13). Somewhat ironically, it is unclear whether protective effects are attributable to retinoid receptor modulation because N-([4-hydroxyphenyl]retinamide can modulate the biology of receptor-negative cell lines (14).

Notwithstanding the striking utility of natural vitamin A derivatives for specific applications such as acute promyelocytic leukemia, the early promise of preclinical studies has not translated into the widespread use of these agents for preventing human cancer. Limiting toxicities include teratogenicity, hepatotoxicity, severe headaches, and mucocutaneous toxicity. Thus, the current focus is on the development and validation of RXR-selective ligands, with the goal of maximizing clinical efficacy while minimizing adverse side effects. Compounds of particular interest include LGD1069 (bexarotene, Targretin) and LG100268.

Rexinoids and Breast Cancer Prevention: Animal Models

The rexinoids LGD1069 and LG100268 have been evaluated in animal breast cancer models in both preventive and therapeutic modalities (15). Both drugs have robust chemopreventive efficacy with respect to experimental breast cancer. LGD1069, a synthetic derivative of 9-cis-RA, is protective in transgenic and carcinogen-induced models, indicating efficacy with respect to both estrogen receptor–positive and estrogen receptor–negative breast cancer (16–19). Additionally, potential therapeutic utility is suggested by the ability of LGD1069 to cause regression of established tumors, even after these have become unresponsive to the selective estrogen receptor modulator (SERM) tamoxifen (20–23).

Similar data are available for the rexinoid LG100268, which suppresses mammary tumorigenesis in both carcinogen and transgene-induced models (1, 4–6). Synergistic efficacy has been observed for combinations of LG100268 and SERMs, including both arzoxifene and acolbifene (4–6). Intriguingly, cyclic dosing schedules of the LG100268/arzoxifene combination provide an effective alternative to continuous dosing regimens, leading Sporn and colleagues to propose that chemopreventive agents could be used in an analogous manner to chemotherapeutic agents, with intermittent treatment protocols permitting the use of higher doses than could safely be administered continuously (5). The study from Li et al. reported in the current issue provides further insight into the chemopreventive potential of LG100268, by demonstrating that not only is invasive tumor formation delayed, but that LG100268 also significantly depresses the development of mammary hyperplasia, a frequent precursor of invasive disease (1). These data augur well for the likely utility of LG100268 or similarly selective rexinoids in the clinical setting.

The idea of combination chemoprevention, as exemplified by the LG100268/SERM studies (4–6), has attracted considerable enthusiasm, based on the hypothesis that this approach may offer maximal efficacy with minimal side effects due to the potential to use individual agents at submaximal doses. In line with this model, we conjectured that cyclooxygenase-2 inhibition might provide a useful complement to rexinoids because substantial data support cyclooxygenase-2 as an anti–breast cancer target (24–26). Our recent analyses indicate that the combination of LGD1069 and the cyclooxygenase-2 inhibitor celecoxib is significantly more effective than either agent singly for preventing breast cancer in transgenic mice (manuscript in preparation).

Mechanistically, numerous pathways are known to be modulated by rexinoids which may mediate their antitumor action. The extensive literature on retinoid-mediated regulation of proliferation and apoptosis has recently been reviewed by Simeone and Tari (14). Net effects on cell growth likely reflect the ability of rexinoids to modulate multiple molecular events, including activator protein 1–dependent transcription, cyclooxygenase/prostaglandin signaling, and the cell cycle, as well as directly influencing RXR-regulated target genes (14, 23, 27, 28).
In vivo correlates have been provided with respect to the anti-proliferative effects of retinoids (1, 4, 17–20, 23). Additionally, there is evidence to support a proapoptotic effect of this drug class in mammary tissues (4, 5, 17, 22). Furthermore, retinoids may also target angiogenesis (29). The pleiotropic actions ascribed to this drug class likely reflects the wide range of dancing partners available to RXR proteins in their role as master heterodimerizer of the nonsteroidal nuclear receptor superfamily.

Rexinoids and Breast Cancer Prevention: Clinical Prospects

LGD1069 is currently being tested in numerous clinical trials, and has already been approved by the U.S. Food and Drug Administration for treating refractory advanced stage cutaneous T-cell lymphoma. Based on compelling preclinical data in breast cancer models, two breast cancer–related trials involving LGD1069 have been undertaken. A pilot trial in 148 patients with metastatic breast cancer evaluated both activity and safety (30). Partial responses were observed in 6% of women with hormone- or chemotherapy-refractory disease, and ~20% of patients experienced clinical benefit. Importantly, only two subjects had serious adverse events. However, there were several common adverse events including hypertriglyceridemia (84%), dry skin (34%), asthenia (30%), and headache (27%). Additionally, an ongoing biomarker study led by the Baylor College of Medicine is testing the ability of LGD1069 to modify immunophenotypic markers related to breast cancer progression in women at high genetic risk for breast cancer.1 This trial is now closed to accrual, having enrolled 87 patients. Biomarker analysis is currently under way, with reporting anticipated later this year.

Preliminary clinical safety testing suggested LGD1069 to be significantly less toxic than conventional retinoids, with an absence of characteristic toxicities such as chelitis, headache and myalgias/arthritis (31). Those side effects which were noted (hyperlipidemia, hypothyroidism, and some cutaneous toxicity) may be attributable to the residual RAR-binding activity of LGD1069, and hence, avoidable by designing compounds with no biologically relevant binding to RARs. The achievability of this pharmaceutical goal is suggested by LG100268, which reportedly does not induce hyperglyceridemia. LG100268 seems to be superior to LGD1069 both in terms of improved preclinical efficacy and reduced toxicity, but is not currently under clinical evaluation. Other potential solutions to retinoid toxicity that have been explored in animal models include intermittent drug regimens and combination chemoprevention. Sporn’s group have been instrumental in validating both of these approaches, using retinoids in combination with SERMs (4–6). Extrapolating from these promising preclinical studies, combination drug regimens may offer a viable strategy for the prevention and treatment of human cancer. Thus, trials testing the LGD1069/SERM combination in women at risk for breast cancer are currently being planned. Furthermore, several ongoing clinical trials are testing LGD1069 in combination with other drugs as potential therapy for cancers of multiple organ sites, including lung cancer, acute myeloid leukemia, and cutaneous T-cell lymphoma.

References


