Retinoic acid metabolism

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The tissue distribution of retinoic acid (RA) throughout development is highly restricted, defined by the expression patterns of enzymes involved in RA synthesis and catabolism. Presented is a summary of recent research that examines the role of some of the enzymes involved in RA distribution, particularly those involved in RA catabolism (P450RAI). These latter enzymes protect against premature exposure to RA, and the implications of these findings are discussed. (J Am Acad Dermatol 2001;45:S136-42.)

Retinoic acid (RA) is an important regulator of the proper formation of embryonic axes and limbs and is necessary for the maintenance of epithelial tissues in the adult. RA acts through the regulation of gene expression that is mediated by specific nuclear receptors. The activity of RA in specific tissues is also controlled by the regulation of its availability, balancing the rate of RA synthesis with that of destruction. The consequences of RA excess or deficiency can be severe, particularly during embryogenesis, during which local control over the expression of RA synthetic and catabolic enzymes is tightly controlled.

Therapeutically, RA has been shown to be highly effective in the treatment of skin disorders and has promising anticarcinogenic and antitumor properties. It is important to consider the effects of exogenous RA treatment on normal metabolic processes that regulate RA levels; however, it is only recently that the molecular tools to do so have been made available through the discovery of genes that encode RA synthesizing and catabolizing enzymes.

This review will focus on 1 of these enzymes, P450RAI (CYP26), a cytochrome P450 that plays an important role in the regulation of RA levels in developing tissues during embryogenesis and in adult epithelia. The characterization, activity, and expression of this enzyme will be discussed in context with its role in development, in adult skin, and RA therapy. This overview of recent research will establish the following findings:

- P450RAI is a highly conserved component of retinoid signaling that specifically metabolizes the all-trans isomer of RA.
- P450RAI expression and activity are strongly induced by exogenous RA, forming an autoregulatory feedback loop to control RA levels.
- The expression of P450RAI in embryos and in the adult is consistent with it having a protective role, preventing undue exposure of sensitive cells and tissues to RA.

RA SIGNALING

The activity of RA in a given tissue is determined at a number of levels. First, the effects of RA on gene expression are principally mediated by 2 families of retinoid nuclear receptors comprised of 3 subtypes each, RA receptors (RARs: α, β, and γ) and retinoid X receptors (RXRs: α, β, and γ). RA receptors and RXRs commonly participate together in the form of heterodimers to regulate gene expression. Most tissues, especially during embryonic development, express 1 or more RAR and RXR subtypes in different combinations, possibly giving rise to different responses to RA in different tissues.

On another level of control, the distribution of RA appears to be an important determinant in the patterned regulation of RA responsive genes, especially in the development of tissues. Tight spatial and temporal control over RA synthesis and catabolism may therefore be critical in establishing a regional distribution pattern of RA.

RA SYNTHESIS

Control of RA tissue distribution is established by a balance of the expression of RA-synthesizing and
RA-catabolizing enzymes. There are a number of retinoid-binding components that function in vitamin A metabolism and storage pathways. Retinyl esters and β-carotene are ingested and converted to all-trans-retinol in the intestine, which is then reconverted to retinyl esters for storage, mainly in the liver. Demand for retinol results in the release of retinol that is bound to plasma retinol-binding protein from the liver. Retinol bound to retinol-binding protein is efficiently taken up by many extrahepatic tissues, including eye, skin, adipose tissue, kidney, testes, lung, and bone marrow. Conversion of retinol to the active forms of RA occurs in many different tissues; however, the exact biochemical mechanisms have not been firmly established. The efficient conversion of retinol to retinaldehyde and retinaldehyde to RA by the retinol and retinaldehyde dehydrogenases, respectively, is facilitated by the presence of cellular retinol-binding proteins. Several retinaldehyde dehydrogenases have already been implicated in the irreversible conversion of retinaldehyde to the active RA. RALDH-2 is thought to be a key enzyme in the localized production of RA, especially during development because it exhibits expression patterns that are consistent with those of a retinoid-responsive LacZ reporter transgene. In addition, the fact that RALDH-2 knock-out mice have severe developmental defects and die at mid-gestation supports the notion that localized production of RA is critical for establishing normal morphologic condition in developing tissue.

**RA METABOLISM**

The irreversible conversion of retinaldehyde to RA in tissues in which RALDH-2 is expressed creates a situation in which RA is committed either to activate receptors to regulate RA-responsive genes or is catabolized to inactive forms by the RA metabolic machinery and eliminated (Fig 1). RA catabolism thus governs tissue sensitivity to RA. The metabolism of RA is thought to be initiated by hydroxylation either at the C4- or C18-position of the β-ionone ring of RA. The C4-hydroxylation step is mediated by cytochrome P450 activity, evidenced by the ability of broad spectrum P450 inhibitors (such as ketoconazole and liarozole) to block 4-hydroxylation.

RA metabolism can be induced by RA in certain tissues (including testis, skin, and lung) and in numerous cell lines (such as NIH3T3 fibroblasts, HL60 myelomonocytic leukemic cells, F9 and P19 murine...
embryonal carcinoma cells, MCF7 human breast cancer cells, and HeLa human cervical cancer cells).

P450RAI (CYP26) is a cytochrome P450 enzyme that specifically metabolizes RA and is likely responsible for much of the RA inducible RA metabolism observed in the studies described earlier (Fig 2). In zebrafish, we first cloned and characterized complementary DNAs that encode a cytochrome P450-dependent enzyme (P450RAI), which is induced by RA and metabolizes RA to more polar derivatives that include 4-hydroxy RA and 4-oxo RA. The identification of P450RAI gene is an important step in our understanding of RA signaling, but its presence has been known since Roberts et al.27 first postulated that the catabolism of RA was mediated by a P450 enzyme. More recently, we and others29,30 have isolated cDNAs that encode the full-length human and mouse P450RAI genes whose expression, like that of the fish cytochrome, is highly inducible by RA. Homologs have also been isolated from human, mouse, chick, and xenopus, all of which exhibit a high degree of sequence conservation.31,33 There is extensive identity (68% at the amino acid level) between human and fish P450RAI genes and an even greater similarity (over 90%) between mouse and human (Fig 2). P450RAI/CYP26 metabolizes all-trans-RA but does not appreciably metabolize the 9-cis- or 13-cis-RA isomers, which suggests that it plays a unique role in retinoid biologic factors.

MCF7 has been previously shown to have RA-inducible RA metabolism. The expression of P450RAI in these cells is dependent on the continuous presence of RA. This suggests that P450RAI regulation by RA forms an autoregulatory feedback loop that functions to limit local concentrations of RA (Fig 3) such that, when normal physiologic levels of RA are exceeded, induction of P450RAI acts to normalize RA levels. The inducible expression of P450RAI in mouse embryos also suggests that a similar autoregulatory mechanism may limit exposure to RA-sensitive tissues during development.35

**COORDINATION OF RA SYNTHESIS AND METABOLISM**

Since RA morphogen-like properties were described almost 2 decades ago, several attempts to define gradients of RA in developing tissues have had limited success. Two important approaches gave strong indications that RA levels in tissues were tightly regulated: 1 approach was to directly analyze the RA present in the anterior and posterior portions of a collected pool of 5000 chick wing buds, establishing that there was an A-P graded RA distribution. A second approach used transgenic mice harboring an RARβ-RARE-directed reporter gene to indirectly identify restricted regions of the embryo, possibly synthesizing or sequestering active retinoids.37,38 The recent cloning of RALDH-2 and P450RAI has provided a way to visualize, by whole mount in situ hybridization, both "source" and "sink" of RA in developing tissues. Subdivisions between RA-synthesizing and RA-degrading regions can be seen in early chick embryos in which RALDH-2 expression is localized to presomitic and lateral plate mesoderm, whereas P450RAI is expressed in presumptive mid- and forebrain.35,39 At later stages of development, the complementary patterns of expression of these enzymes can also be observed in the development of the anterior and posterior neural tube, in the developing limb bud, and in the eye.39 At early stages during the development of the mouse retina, P450RAI is expressed in a narrow horizontal strip.
that forms a boundary between 2 dehydrogenases (RALDH-2 ventrally and ALDH-1) capable of converting retinaldehyde to RA dorsally. This creates 3 zones of RA activity that subdivide the retina into territories that promote further eye development.49

**RA RESISTANCE AND CANCER**

Early studies of retinol deficiency indicated a correlation between vitamin A depletion and a higher incidence of cancer and increased susceptibility to chemical carcinogenesis.40 Several animal models have been used to demonstrate the effectiveness of retinoids in the suppression of carcinogenesis in a variety of tissues (including skin, mammary epithelia, oral cavity, aerodigestive tract, liver, bladder, and prostate).41 These studies have led to the preventive use of retinoids to treat premalignant lesions (including actinic keratosis and oral leukoplasia) and in the prevention of secondary tumors of the head and neck and the recurrence of non-small cell lung carcinomas and basal cell carcinomas.8,42 The most dramatic therapeutic use of RA has been in the treatment of acute promyelocytic leukemia (APL).43,44 Studies over the past several years indicate that a high proportion of patients with APL achieve complete remission after a short period of treatment with all-trans-RA. Unfortunately, this high rate of remission is in most cases brief. After relapse, patients are clinically resistant to further treatment with RA.45-47 The nature of this resistance is unknown.

**RA resistance**

Clinical studies suggest that the expression of factors involved in RA metabolism may play a role in the acquired resistance to RA seen in APL. In a limited study, CRABPII expression was elevated in cells that were derived from RA-resistant patients.45 Furthermore, RA-induced RA metabolism has been demonstrated in the APL-derived cell line NB4. Whether these observations are related is unclear but is consistent with increased RA metabolism in patients after RA therapy.48

There is strong evidence that RA metabolism because of P450RAI expression may play an important role in cellular RA resistance: (1) it can be demonstrated in culture that cells that contain a RA-reporter gene coexpressed with P450RAI require 100-fold higher concentrations of RA to achieve the same level of reporter gene activity seen in cells that contain the RA-reporter gene alone;49 (2) similarly, P9 teratocarcinoma cells that constitutively express P450RAI are hypersensitive to the differentiating effects of RA;50 (3) embryonic tissues that express P450RAI have no detectable RA activity, whereas such activity can readily be detected in adjacent tissue in which P450RAI is not expressed;55 and (4) the teratogenic effects of RA in xenopus embryos can be prevented by overexpression of xenopus P450RAI/CYP26.32

Clinical studies also support the possibility that P450RAI presents a barrier to optimal therapeutic activity of RA. For example, leukemic cells taken from patients with APL who exhibit clinical resistance to RA have shown to be sensitive to the differentiating action of RA when the cells are grown in vitro.43,44 This suggests that pharmacokinetic mechanisms may account for the acquired resistance to RA. This possibility is supported by studies that show that peak plasma concentrations of RA were much
higher in patients after the initial administration than in patients who were treated after relapse. This decrease in peak plasma RA concentration was accompanied by a 10-fold increase in urinary 4-oxo-retinoic concentration. In addition, ketoconazole, a broad spectrum inhibitor of cytochrome P450 function was shown to modulate RA pharmacokinetics in vivo.43,44 It is therefore likely that RA increases the rate of its own metabolism, which in turn results in the inability to sustain effective therapeutic doses of RA. P450RAI may be involved because it is the only known RA-inducible cytochrome P450 for which RA is a substrate and because it is expressed both in the liver and in leukemic cells after RA treatment. This presents 2 types of barriers to therapeutic doses of RA: first, liver P450RAI can reduce systemic levels of RA; and second, induced expression of P450RAI in the leukemic cells themselves would reduce their sensitivity to RA.

In summary, factors that directly limit the ability of cells to respond to RA (such as receptor defects) or limit the availability of RA to cells (such as increased RA metabolism) may predispose individuals to cancerous lesions and may reduce the effectiveness of RA therapy.

**BLOCKING P450RAI ACTIVITY**

Because RA synthesis and catabolism are regulated at the tissue level, 1 approach to modulate tissue levels of RA is to block RA metabolism and thus allow RA to accumulate. Although specific inhibitors of RA metabolism have not yet been developed, broad spectrum cytochrome P450 inhibitors (such as the imidazol derivatives ketoconazole and liarozole) can increase cell sensitivity to RA, thus exhibiting retinoid mimetic effects in animal and human subjects.20 This approach has been recently tested in both animal models and clinically for the treatment of cancer and in the treatment of dermatologic disorders.

Liarozole effectively reduced the growth of prostate tumors in the Dunning rat model whether or not the tumors were androgen-dependent or androgen-independent.24 Clinical trials that investigated the effectiveness of liarozole in patients with advanced prostate carcinoma indicated promising objective responses in terms of reduction in prostate specific antigen levels and in patient survival time.49,50 Side effects recorded resembled those of hypervitaminosis A. In the treatment of other non-cancer RA sensitive diseases (such as psoriasis), the inhibition of RA catabolism also appears to be an effective means to increase tissue sensitivity to endogenous RA. These in vivo and clinical studies further support the possibility that induced expression of RA metabolism can decrease responsiveness to RA.

If RA metabolic activity can act as a barrier to effective RA treatment, it is possible that the effectiveness of RA in dermatology could be enhanced by modulating RA metabolic activity. Many studies have also revealed RA-inducible RA metabolic activity in human skin.52,53 The properties of RA-inducible RA metabolic activity in human skin also demonstrate that P450RAI is involved. P450RAI is highly inducible by RA and is selective for all-trans RA; 9-cis- and 13-cis-RA isomers are not substrates for this type of metabolic activity.52 What this implies is that RA metabolism may have differential effects on the distribution of retinoids in skin, the consequences of which are not known. However, the 13-cis and 9-cis isomers of RA may simply be converted to all-trans-RA.51

The inhibition of RA metabolism in skin through topical or systemic means can augment the effectiveness of topically applied RA. This has been demonstrated with liarozole either alone or in conjunction with RA, followed by the analysis of several parameters that include erythema and epidermal thickness; liarozole could significantly augment the activity of low doses of topically applied RA.53 This has important clinical implications because reduced RA levels in combination with a P450RAI inhibitor could reduce the incidence of unwanted side effects of RA. Clinical trials that use liarozole to treat psoriasis have been promising, leading to the reduction of some cutaneous inflammation with certain side effects similar to those observed with RA therapy,54 with efficacy comparable or better than acitretin.55 Although liarozole has some specificity for blocking RA metabolic activity, it can also inhibit the activities of other cytochrome P450s and, as such, may not be useful clinically. However, this approach suggests that new generations of compounds with specific RA metabolism-blocking properties may have a place beside or along with RA derivatives to enhance the therapeutic index of retinoids in the treatment of skin disorders and cancer.

**SUMMARY**

RA metabolism is a critical mechanism in the local control of RA signaling. In developmental processes requiring RA, the balance between RA synthesis and RA catabolism is tightly controlled by the enzymes RALDH-2 and P450RAI, respectively. A knockout of the P450RAI gene will be an important step in the complete developmental role of this enzyme and whether other members of this cytochrome P450 family (CYP26) remain to be characterized. Also, in the adult, the expression of these enzymes in tissues undergoing cell proliferation and differentiation (such as skin or tumor tissues) may have a direct
influence on how they respond to endogenous or therapeutic doses of retinoids. The identification and characterization of P450RAI provide important tools to examine the role that metabolism plays in directing RA signalling and a possible target for the design of novel therapeutics to enhance current retinoid therapies.

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REFERENCES
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