Between Death and Survival: Retinoic Acid in Regulation of Apoptosis

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Abstract

The vitamin A metabolite all-*trans*-retinoic acid (RA) regulates multiple biological processes by virtue of its ability to regulate gene expression. It thus plays critical roles in embryonic development and is involved in regulating growth, remodeling, and metabolic responses in adult tissues. RA can also suppress carcinoma cell growth and is currently used in treatment of some cancers. Growth inhibition by RA may be exerted by induction of differentiation, cell cycle arrest, or apoptosis, or by a combination of these activities. Paradoxically, in the context of some cells, RA not only fails to inhibit growth but, instead, enhances proliferation and survival. This review focuses on the involvement of RA in regulating apoptotic responses. It includes brief overviews of transcriptional signaling by RA and of apoptotic pathways, and then addresses available information on the mechanisms by which RA induces apoptosis or, conversely, inhibits cell death and enhances survival.

Contents

INTRODUCTION

Vitamin A was recognized as an essential factor present in foods almost a century ago (70, 83), and a substantial body of knowledge has since accumulated on its mechanisms of action and biological functions. The vitamin is essential for vision, reproduction, embryonic development, immune function, and tissue homeostasis. These activities are exerted by several types of active metabolites. 11-*cis*-retinal mediates phototransduction and is essential for vision. All-*trans*-retinoic acid (RA) and 9-*cis*-RA (9cRA) can regulate the expression of multiple genes (21, 66) and, consequently, control networks that play critical roles in embryonic development and in tissue maintenance in the adult. As the transcriptional activities of RA can lead to differentiation, cell cycle arrest, and apoptosis, the compound often inhibits cell growth. Such activities underlie the ability of RA to suppress carcinoma cell proliferation and provide a rationale for the use of the compound as a pharmacological agent in cancer therapy. Indeed, RA is clinically used for treatment of malignancies, e.g., promyelocytic leukemia, Kaposi's sarcoma, and neuroblastoma, and premalignancies, such as leukoplakia, actinic keratosis, and xeroderma pigmentosum (3, 103). However, growth inhibition by RA is cell-specific and, in the context of some normal as well as carcinoma cells, the hormone does not suppress growth or it may, paradoxically, enhance proliferation and survival. Hence, despite promising preclinical and clinical results, the use of RA in cancer therapy is hampered by RA resistance in tumors and remains limited. This review specifically focuses on the involvement of all-*trans*-RA in the regulation of apoptosis. It summarizes available information on the molecular mechanisms through which RA triggers programmed cell death and on known pathways through which the compound, conversely, exerts antiapoptotic activities.

RA: all-*trans*-retinoic acid

9cRA: 9-*cis*-retinoic acid

RETINOIDS

Vitamin A and its metabolites, collectively known as retinoids, are composed of three distinct structural domains: they contain a ß-ionone ring, a spacer of a polyunsaturated chain, and a polar end group. The polar end group of naturally occurring retinoids can exist at several oxidation states, varying from the low oxidation state of retinol, to retinal, and to a higher oxidation state in RA (**Figure 1**). Biologically active retinoid metabolites include the 11-*cis* isomer

of retinal, which functions as the visual chromophore and is essential for phototransduction, and RA and 9cRA, which can activate ligand-inducible transcription factors, termed retinoid receptors, and thus regulate the rate of transcription of multiple target genes.

All-*trans*-RA is metabolically generated from the parental vitamin A molecule, all-*trans*-retinol, by two consecutive oxidation steps: retinol is converted to retinal, which is then oxidized into RA (**Figure 1**). These metabolic conversions are mediated respectively by retinol dehydrogenases and retinal dehydrogenases, which catalyze the dehydrogenation of their substrates using the electron acceptors NAD^+ or $NADP^+$. Two classes of enzymes can function as retinol dehydrogenases in vitro: medium-chain alcohol dehydrogenases and members of the family of short-chain dehydrogenenases/reductases (SDRs) that are associated with endoplasmic reticulum membranes in various cells. In contrast with soluble alcohol dehydrogenases, it has been reported that some SDR-type retinol dehydrogenases are able to metabolize retinol when bound to the cellular retinol-binding protein (CRBP)-I (76). As a predominant fraction of retinol in cells is bound to CRBP-I, retinal formation under normal physiological conditions in vivo likely occurs mainly by microsomal SDRs. The first of such enzymes to be cloned were the hepatic all-*trans*-retinol dehydrogenase (20) and the bovine *cis*-retinol dehydrogenase, which is highly expressed in the retinal pigment epithelium of the eye (102). Subsequently, other isozymes have been identified in various tissues. Some retinol dehydrogenases display a dual specificity toward *cis*- and all-*trans*-retinol, while others are more selective for particular isomeric configurations. Several mammalian retinal dehydrogenases that catalyze the NAD^+-dependent oxidation of all-*trans*-retinal to RA have been identified and were shown to play critical roles in RA synthesis in vivo (36, 40, 78).

It has been suggested that retinol- and retinal-dehydrogenases that display selectivity toward *cis*-retinoids may play a role in synthesis of the other transcriptionally active retinoid, 9cRA (63). However, by and large, the metabolic pathway that gives rise to this isomer remains poorly understood. In addition, although 9cRA can effectively activate the nuclear receptors termed retinoid X receptors (RXRs), the compound may not be present in all tissues that express these receptors. Hence, the questions of whether 9cRA is a physiologically meaningful ligand for RXRs and whether other ligands, and perhaps nonretinoid ligands, exist for these receptors remain open.

A wide array of synthetic analogs of retinoids has been developed in efforts to obtain compounds that are specific for particular retinoid receptors or that display a lower toxicity than that of RA. Active synthetic analogs, similarly to naturally occurring retinoids, retain an amphipathic nature typified by a hydrophobic moiety and a polar terminus. Some synthetic retinoids as well as "rexinoids," compounds that are selective toward RXR, are currently being tested for potential clinical uses.

RETINOID NUCLEAR RECEPTORS AND TRANSCRIPTIONAL REGULATION: OVERVIEW

The transcriptional activities of RAs are mediated by ligand-activated transcription factors that are members of the family of nuclear hormone receptors. Forty-eight genes that encode for different nuclear receptors have been identified in the human genomes. Of these, about half are activated by specific small lipophilic biologically active molecules, such as steroid hormones, vitamin D_3, thyroid hormone, oxysterols, and RAs. No physiological ligands have been identified as yet for the other members of the family, and they are thus known as orphan receptors. Receptors in the latter category await the identification of their ligand, but it should be noted that some of these may be true orphans, i.e., their transcriptional activities may not be regulated by any ligand. Nuclear receptors contain several distinct

Retinoids: compounds derived from vitamin A (all-*trans*-retinol). The chemical structure of natural retinoids consists of a β-ionone ring, a polyene side chain, and a polar end group. Synthetic retinoids are defined in terms of their ability to mimic the biological functions of vitamin A

Nuclear receptors: a family of transcription factors whose activities are controlled by small lipophilic compounds, e.g., RA. Of the 48 nuclear receptors found in the human genome, about half are termed "orphan receptors" to denote that the identity of their ligands is unknown

Intracellular lipid-binding proteins: a family of 14 small (~14 KDa) soluble proteins that bind retinoids and long-chain fatty acids

domains (**Figure 2**): a highly variable amino terminal (AF-1) domain, involved in ligand-independent basal transcriptional activity; a DNA-binding domain; a flexible hinge region; and a carboxyl terminal region, termed the ligand-binding domain (LBD) (66). Some receptors contain an additional F domain, the function of which is incompletely understood. The LBD contains the ligand-binding pocket as well as regions that mediate multiple protein-protein interactions, including association with transcriptional coregulators (117), formation of dimers (11, 12, 16, 71), and, in the case of RXR, formation of tetramers (41, 54, 55). The LBD of nuclear receptors thus coordinates their ligand-dependent transcriptional activity. Nuclear receptors regulate transcription through binding to cognate response elements (REs) in regulatory regions of their target genes. These REs are composed of polymorphic arrangements of the motif 5′-PuG(G/T)TCA, usually in the form of two repeats, and most nuclear receptors, including retinoid receptors, bind to their cognate DNA in the form of dimers (21).

Transcriptional regulation by nuclear receptors involves several classes of coregulatory proteins (**Figure 3**) (121). In the absence of ligands, receptors often associate with corepressors, including components that catalyze histone deacetylation, leading to a compact chromatin structure and transcriptional repression. Upon ligand binding, the receptors undergo a conformational change that results in dissociation of corepressors and recruitment of coactivator complexes. In turn, coactivators modify chromatin to loosen its structure, and some of them bridge between the receptors and the general transcription machinery, thereby facilitating transcription (64, 90). Activation and repression of gene expression by nuclear receptors thus involve remodeling of chromatin and stabilization of the general transcription machinery brought about by a ligand-controlled alternate usage of different classes of coregulatory proteins.

The transcriptional activities of RAs are mediated by several nuclear receptors. 9cRA activates RXRs (RXRα, RXRβ, and RXRγ) as well as RA receptors (RARα, RARβ, and RARγ). RXR can regulate transcription as a homodimer, and it also serves as an obligatory heterodimerization partner for multiple other nuclear receptors within subclass 1 of the family, including RARs, peroxisome proliferator-activated receptors (PPARs), and the vitamin D- and thyroid hormone receptors (37, 58, 125). RXR thus serves as a master regulator of multiple transcriptional signaling pathways induced by various lipophilic hormones and nutrients. In the absence of ligand, RXR forms homotetramers that are transcriptionally inactive but that rapidly dissociate into active dimers upon ligand binding (41, 54, 55). RXR tetramers are capable of simultaneously binding two RXR DNA recognition sequences (RXRE), thereby bringing distant genomic regions into close physical proximity (123). As ligand binding induces tetramer dissociation, the ligand-controlled oligomeric switch of this receptor can modulate DNA geometry. Some of the biological activities of RXR may thus be exerted through its unusual function as a DNA architectural factor.

All-*trans*-RA binds and activates all three isotypes of RAR and it also serves as a specific ligand for the PPAR isotype PPARβ/δ. In contrast with RAR, which targets genes involved in inhibition of cell growth, it has been reported that, in addition to its well established activities in regulating lipid and sugar homeostasis (5), PPARβ/δ induces the expression of genes that mediate antiapoptotic and proproliferation functions (108, 109, 115). As RARs and PPARβ/δ regulate the expression of distinct sets of genes, the biological responses of cells to RA differ between cells in which the ligand activates RARs and cells in which it functions through PPARβ/δ. These considerations raise the important question of how the partitioning of RA between these classes of receptors is regulated. Available information indicates that the delivery of RA to

its cognate receptors is controlled by members of the family of intracellular lipid-binding proteins.

RETINOIC ACID–BINDING PROTEINS

RARs and PPARβ/δ are predominantly nuclear even in the absence of their ligands. Activation of these receptors thus requires that RA be mobilized from its sites of synthesis in the cytosol to the nucleus, a process that is hindered by the hydrophobic nature of RA, which limits its ability to freely move across the aqueous space of the cell. Bypassing this limitation, the hormone is transported into the nucleus by specific intracellular lipid-binding proteins (iLBPs), members of a family that includes 14 ∼15 KDa proteins that associate with various retinoids and long-chain fatty acids (81, 105). Among iLBPs, cellular RA-binding protein (CRABP)-II serves to shuttle RA to RAR, and fatty acid-binding protein 5 (FABP5) transports the ligand to PPARβ/δ (**Figure 4**). These proteins, which are cytosolic in the absence of the ligand, move to the nucleus upon association with RA. In the nucleus, these iLBPs specifically interact with a cognate receptor to form a complex through which RA is "channeled" to the receptor. CRABP-II and FABP5 thus facilitate the delivery of RA to RAR and PPARβ/δ, respectively, and they markedly enhance the transcriptional activities of their receptors (17, 18, 35, 110). Indeed, it has been demonstrated that RA signals predominantly through RAR in cells that express a high level of CRABP-II and that it functions through PPARβ/δ in cells in which the CRABP-II/FABP5 ratio is low (7, 94, 95).

Sequence motifs that mediate the nuclear import of proteins, known as nuclear localization signals (NLSs), are usually composed of a series of basic residues in the form K(K/R)X(K/R) (24, 32, 44, 51, 92). Such an NLS is recognized by adapter proteins known as α importins, which, upon binding to their cargoes, mobilize them to the nucleus (24). Interestingly, although CRABP-II and FABP5 are imported into the nucleus in response to binding of RA, the primary sequences of these proteins do not harbor a recognizable NLS. Instead, such a signal was found to occur within the three-dimensional folds of the proteins. Comparison of the electrostatic surface potentials of CRABP-II in the presence and absence of RA revealed a positively charged surface patch, which is present in the holoprotein but not the apoprotein. This patch was mapped to three basic amino acid residues, K20, R29, and K30, that shift their orientation upon ligation to place their side chains in a close alignment with classical NLSs (**Figure 5*a*,*b***). Hence, subtle, ligand-induced shifts in the tertiary structure of CRABP-II result in the appearance of a recognizable functional NLS at the surface of the protein (97). Similarly to CRABP-II, two other iLBPs, FABP4 and FABP5, are known to move to the nucleus in response to cognate ligands and to deliver these ligands to particular nuclear receptors (94, 110). Also similarly to CRABP-II, neither of these iLBPs harbors a recognizable NLS in its primary sequence. Primary sequence alignment (**Figure 5*c***) shows that the three basic residues that comprise the NLS of CRABP-II are present in both FABP4 and FABP5, suggesting that these proteins possess a CRABP-II-like three-dimensional NLS and providing insight into the molecular basis for the ligand-induced nuclear mobilization of these proteins.

iLBPs: intracellular lipid-binding proteins

CRABP: cellular retinoic acid–binding protein

APOPTOTIC PATHWAYS: OVERVIEW

Programmed cell death, also termed apoptosis, is conserved in all multicellular organisms and is characterized by specific features including cell membrane blebbing, cell shrinkage, chromatin condensation, and DNA fragmentation. The process is critical for organogenesis during embryonic development and for normal tissue homeostasis in the adult. Indeed, apoptotic pathways are precisely regulated, and their deregulation results in pathologies such as developmental defects, autoimmune diseases, and cancer. Apoptosis may be

induced through two general routes (**Figure 6**). An intrinsic apoptotic pathway may be triggered by intracellular events such as DNA damage, growth factor deprivation, and oxidative stress. An alternative route, termed the extrinsic pathway, may be activated by cell surface death receptors in response to binding of extracellular ligands such as Fas, tumor necrosis factor α (TNFα), and TRAIL. Both routes utilize caspases, members of a family of cysteine proteases whose activation is a hallmark of apoptosis.

Caspases Mediate the Initiation and Execution of Apoptosis

Caspases are synthesized as inactive precursors containing prodomains that are cleaved upon induction of apoptosis to generate active enzymes. About half of the 14 known mammalian caspases play roles in apoptosis, and these can be classified in two groups. (*a*) Initiator caspases, caspases 2, 8, 9, and 10, contain long prodomains that mediate interactions of the caspase with upstream adaptors and effectors. (*b*) Effector/executioner caspases, caspases 3, 6, and 7, contain short prodomains that are cleaved by initiator caspases. In turn, executioner caspases catalyze downstream steps of apoptosis by cleaving multiple substrates including proteins that mediate and regulate apoptosis, structural proteins, proteins involved in DNA repair, and cell cycle–related proteins (reviewed in 30, 50).

In addition to their activation by proteolytic cleavage, the activity of caspases, and thus the sensitivity of cells to apoptosis, is controlled at other levels including regulation of caspase expression levels, post-translational modifications, and direct association with inhibitory proteins. For example, the expression of caspase 6 and caspase 10 is transcriptionally regulated by the tumor suppressor p53, mouse caspase 9 is phosphorylated and thus inhibited by the survival factor AKT, and some caspases are inhibited by association with inhibitor of apoptosis (IAP) proteins (50).

Intrinsic Apoptotic Pathways Are Controlled Through the Mitochondria and Endoplasmic Reticulum

Intrinsic apoptotic pathways are propagated upon permeabilization of the mitochondrial outer membrane. This process is regulated by members of the Bcl-2 family of proteins and results in release of mitochondrial factors involved in apoptosis and in loss of mitochondrial functions (42). The Bcl-2 family includes antiapoptotic proteins, which promote cell survival, and two groups of proapoptotic members: the multi-BH domain Bax, Bak, and Bok, and the BH3-only proteins, such as Bid, Bad, and Bim. Prosurvival Bcl-2 proteins are localized in the mitochondria, endoplasmic reticulum, and perinuclear membranes (59), where they are involved in maintaining the status of the mitochondrial membrane and in regulation of calcium homeostasis (29). These proteins can inhibit cell death by sequestering proapoptotic Bcl-2 proteins. The sensitivity of a cell to apoptosis is thus largely regulated by the relative expression levels and activities of anti- and proapoptotic Bcl-2 proteins, which, in turn, are controlled at the level of transcription, by post-translational modifications, by modulation of subcellular localization, and by direct interactions with other proteins.

Intracellular death signals trigger the insertion of proapoptotic Bcl2 proteins into the outer mitochondrial membrane, resulting in membrane permeabilization, release of proapoptotic mitochondrial factors such as cytochrome c, and impairment of mitochondria function (29, 77). Upon its cytoplasmic release, cytochrome c binds to Apaf-1 to form an apoptosome, which recruits and activates procaspase 9 and initiates a caspase cascade (62). Mitochondrial membrane permeabilization also releases factors that neutralize the caspase inhibitor IAP proteins as well as proapoptotic proteins with functions unrelated to caspase activation, e.g., proteins that induce DNA fragmentation (reviewed in 50).

Intrinsic apoptotic pathways may also involve cooperation between the endoplasmic

reticulum (ER) and the mitochondria. Protein misfolding, induced by oxidative stress or inhibition of protein glycosylation, can lead to ER stress and to an unfolded protein response. The response includes inhibition of protein synthesis, induction of chaperones, and activation of ER-associated protein degradation. However, prolonged ER stress triggers release of ER Ca^{2+} stores. In turn, Ca^{2+} ions initiate a positive feedback loop between ER and the mitochondria, leading to enhanced activation of caspases and apoptosis (14, 96).

Extrinsic Apoptotic Pathways Are Mediated by Cell Surface Death Receptors

Apoptosis can be activated upon binding of extracellular ligands, e.g., TNFα, Fas, and TRAIL, to cognate death receptors localized at the surface of cells (**Figure 6**). Ligand binding by death receptors leads to recruitment of adaptor proteins that, in turn, bind and activate effectors such as procaspase 8 to induce downstream apoptotic signaling (114). Death receptors may also activate transcription factors, e.g., c-Jun, N-terminal kinase (JNK), and NFκB, which regulate multiple cellular processes including apoptosis (50). The activities of death receptors are subject to regulation at multiple levels. For example, "decoy" TRAIL receptors, i.e., receptors that lack the C-terminal domain that signals to caspase activation, compete with active TRAIL receptors for ligand binding and thus protect cells from TRAIL-induced apoptosis (50).

Notably, extensive crosstalk exists between the extrinsic and the intrinsic apoptotic pathways (**Figure 6**). Hence, extrinsic apoptotic signaling may be amplified by caspase-mediated cleavage of the proapoptotic Bcl2 protein Bid and release of mitochondrial proapoptotic factors (31). Conversely, intrinsic death signaling triggered by DNA damage leads to activation of the transcription factor p53, which targets intrinsic apoptotic effectors, e.g., Bax, and genes involved in the extrinsic pathway genes, such as FasL (53, 120).

PROAPOPTOTIC SIGNALING BY RA

The ability of RA to inhibit proliferation of some cells forms the basis for usage of the compound in oncology. Available information indicates that antiproliferative functions of RA are mediated primarily by RARs and that the pathways by which these receptors inhibit carcinoma cell growth vary between cell types. For example, activation of RAR induces differentiation in embryonic tetratocarcinoma F9 cells (106), G1/G0 growth arrest and myeloid differentiation in HL-60 cells (6, 15), and postmaturation apoptosis in NB4 acute promyelocytic leukemia cells (4). In mammary carcinoma cells, RAR was shown to trigger growth inhibition by inducing cell cycle arrest, or apoptosis, or both (33, 34, 38, 67, 79, 112).

Endoplasmic reticulum (ER) stress response: ER stress is caused by protein misfolding, which can be induced by oxidative stress or inhibition of protein glycosylation. Prolonged ER stress triggers release of ER Ca^{2+} stores and enhances apoptosis

Involvement of RA in Intrinsic Apoptosis Pathways

Precise information on the identity of RAR-controlled genes that mediate proapoptotic activities of RA is surprisingly limited but nevertheless reveals involvement of multiple players in apoptotic cascades. These include caspases, Bcl-2 proteins, transcription factors that regulate apoptosis, and genes involved in DNA fragmentation. It has been reported that caspase 9, the initiator caspase of the intrinsic apoptosis pathway, is a direct target gene for RAR in MCF-7 mammary carcinoma cells. The response element that mediates the ability of RAR to upregulate the expression of caspase 9 was localized to an RAR response element (RARE) that resides within the second intron of the gene (33). The identification of the element in an intron sequence contributes to a growing body of evidence that regulatory elements are not confined to upstream promoter regions of target genes. An interesting question that arises from these observations relates to the temporal occupation of intron response elements following gene activation. Presumably, the transcription factor will be effectively displaced from the element by the traveling polymerase. In line

Intrinsic apoptosis: the process by which cell stress, e.g., DNA damage, can trigger programmed cell death. Intrinsic apoptosis is mediated by permeabilization of the mitochondrial membrane

with the function of CRABP-II in delivering RA to RAR, expression of this binding protein markedly enhances the ability of RAR to upregulate the expression of caspase 9 and to induce apoptosis (33). Interestingly, in addition to enhancing the transcriptional activity of RAR and thus RA-induced apoptosis, CRABP-II appears to display proapoptotic activities that are RA independent. It was reported in regard to this that expression of CRABP-II in the absence of RA upregulates the levels of several proapoptotic factors, including the major protein of the apoptosome Apaf1, and induces cleavage of caspase 9 (33). The mechanism through CRABP-II exerts these activities remains to be clarified.

In MCF-7 cells, RAR also induces the expression of the executioner caspase 7. However, this effect is abolished in the presence of a protein synthesis inhibitor, demonstrating that it is mediated by secondary processes and requires de novo protein synthesis (33).

It has also been reported that RA upregulates the expression of caspase 3, 6, 7, and 9 in keratinocytes (73). Although the activities of caspases are primarily regulated by protease processing, overexpression of these proteases and of other components of the apoptotic response may trigger apoptosis or increase the susceptibility of cells to apoptosis-inducing agents (98–101, 124). Accordingly, in MCF-7 cells, RA induces apoptosis when added as a sole agent (33), whereas in keratinocytes, the hormone does not induce apoptosis by itself, but it sensitizes the cells to apoptosis induced by UVB irradiation and by the DNA-damaging agent doxorubicin (73).

RA Regulates the Expression of Bcl-2 Proteins

In addition to regulating the expression of caspases, RA can also modulate the expression of both proapoptotic and antiapoptotic Bcl-2 proteins and initiate mitochondrial-based death pathways. It was thus reported that RA treatment of MCF-7 cells leads to activation of Bax and to the release of mitochondrial cytochrome c (80), and that, in other breast cancer cells, RA-induced apoptosis is associated with downregulation of Bcl-2 and survivin (89, 91). Similarly, apoptosis induced by RA is accompanied by downregulation of Bcl2, activation of caspase 9 and caspase 3, and cytoplasmic release of cytochrome c in neuroblastoma cells (79), and by downregulation of Bcl-2 in metastatic melanoma (127) and myeloblastic leukemia cells (128). Some of these activities may be mediated by RA-induced upregulation of the tumor suppressor p53. It was reported in regard to this that RA upregulates the expression of p53 in pancreatic cancer cells (61), thymocytes (111), telomerase-immortalized Barrett's cell lines (45), metastatic melanoma cells (127), and myeloblastic leukemia cells (128). The mechanism by which RA regulates p53 expression is poorly understood but may involve the chromatin modifying protein Chmp1A (61) and the basic helix-loop-helix transcription factor stimulated by RA 13 (Stra13) (111).

Involvement of RA in Extrinsic Apoptosis

Several reports suggested that RA may induce apoptosis by targeting death ligands and their receptors. For example, in postmaturation NB4 acute promyelocytic leukemia cells, activation of RAR induces the expression of interferon regulatory factor-1 (IRF-1), which in turn upregulates the death ligand TRAIL (4, 26). Because TRAIL is a secreted ligand, its upregulation by RA can induce apoptosis both in an autocrine and in a paracrine fashion, leading to death signaling in neighboring cells (4).

It was also reported that RA may be involved in extrinsic apoptotic signaling triggered by the death receptor Fas. Upon binding of Fas ligand, Fas aggregates to form a death-inducing signaling complex (DISC), which contains an adaptor protein and procaspase 8 (86). The formation of the DISC triggers the cleavage of caspase 8, initiating a caspase cascade (10). The mechanism by which RA enhances Fas-mediated apoptosis is incompletely understood, but it has been suggested that, in T cells, the effect involves reversal of protein kinase C–induced inhibition

of recruitment of procaspase 8 to the DISC (39).

In human lung cancer and in leukemia cells, RA was found to induce the expression of receptors for the death ligand TNFα, resulting in enhancement of TNFα-induced apoptosis (68, 119).

In addition to targeting death receptors, RA regulates the expression of caspases that are specifically involved in extrinsic apoptosis pathways such as caspase 8, which was reported to be induced by RA in NB4 cells (4), and in neuroblastoma and lung carcinoma cells (49).

Other RA-Regulated Proapoptotic Targets

Other proteins that were suggested to mediate apoptotic activities of RAR include the CCAAT/enhancer-binding protein, a transcription factor that plays important roles in regulating terminal myeloid differentiation and apoptosis (60, 74, 85), the RAR target gene Rig-I, a protein that initiates apoptosis upon sensing viral RNA in infected cells (8), the p38 MAP kinase (45), and the programmed cell death-4 (PDCD4) tumor suppressor protein (2, 84).

RARβ2 Functions as a Tumor Suppressor

The gene that encodes for the RAR isotype RARβ can give rise to four isoforms, which are generated by differential usage of two promoters and alternative splicing (126). Of these, the $RAR\beta_2$ isoform plays a central role in mediating the ability of RA to inhibit carcinoma cell growth and is considered to be a tumor suppressor. Notably, RARβ comprises a direct target gene for the RARα-RXR heterodimer, and its expression is upregulated upon treatment with RA. Hence, RA-induced growth inhibition in some carcinoma cells is mediated, at least in part, specifically by this RA receptor. Epigenetic silencing of the RARβ promoter by histone hypermethylation and deacetylation (13, 28, 75, 116) leads to loss of $RAR\beta_2$ in many malignant tissues (19, 87, 118), and the methylation status of the RARβ promoter has been used as a biomarker for malignancy or to monitor the efficacy of chemoprevention agents in clinical trials (65, 122). The specific mechanisms through which RARβ inhibits carcinoma cell growth and the spectrum of target genes that mediates this activity remain to be clarified.

ANTI-APOPTOTIC SIGNALING BY RA

RA can display potent proapoptotic, antiproliferative activities and, consequently, is currently used in and is being tested as a therapeutic agent in several human cancers (103). However, in the context of some normal as well as carcinoma cells, RA promotes rather than inhibits survival. It was reported in regard to this that RA is critical for neuronal survival (43, 48, 88, 93), inhibits death of basal vomeronasal neurons during late-stage neural circuit formation (46), suppresses radiation-induced apoptosis in keratinocytes (104) and capillary endothelial cells (113), protects hematopoietic cells from activation-induced apoptosis (47, 82), prevents cardiomyocyte apoptosis triggered by angiotensin II and mechanical stretch (25), displays antiapoptotic activities in retinal progenitor cells (56), and protects lung epithelial cells from TNFα-induced apoptosis (9). It was also shown that RA induces hyperproliferation of basal keratinocytes (52, 129) and that it enhances mammary tumor growth in the MMTV-*neu* mouse model of breast cancer (69, 94).

The ability of RA to enhance proliferation and suppress apoptosis is unlikely to be mediated by RAR, whose target genes are usually involved in inhibition of cell growth. In accordance with this notion, it has been demonstrated that, although RA plays critical roles in maintenance of skin integrity, mice lacking RARs display normal keratinocyte proliferation. These observations indicate that RARs are dispensable for keratinocyte renewal (22) and suggest that RA functions in the context of these cells through an alternative pathway. Recent observations demonstrated that at least

Extrinsic apoptosis: the process by which external signals can induce programmed cell death. Extrinsic apoptosis is triggered upon binding of external cytokines, e.g., TRAIL, to specific cell-surface death receptors

some antiapoptotic activities of RA are mediated by the alternative RA receptor PPARβ/δ. Such activities were shown to underlie the ability of RA to enhance survival and proliferation of keratinocytes (94) and to facilitate mammary tumor growth in the MMTV-*neu* mouse model of breast cancer (95). Direct target genes for PPARβ/δ that are responsible for the proliferative and antiapoptotic activities of this receptor include the kinase PDK1, which activates the survival factor Akt1 (108, 109) and the growth factor VEGF (115). Functioning indirectly, PPARβ/δ also inhibits the activity of the tumor suppressor PTEN, leading to activation of PI3 kinase-dependent Rho-GTPase pathways (107).

Available information thus indicates that the ability of RA to trigger apoptosis in some cells and, conversely, evoke antiapoptotic responses in others emanates from shifts in the balance between RA-induced activation of RAR versus PPARβ/δ. As discussed, this balance is largely controlled by the relative expression levels of the two RA-binding proteins: CRABP-II, which shuttles RA to RAR, and FABP5, which transports it to PPARβ/δ. Accordingly, RA induces apoptosis in cells, e.g., MCF-7 mammary carcinoma cells, that express a high CRABP-II/FABP5 ratio and, consequently, respond to RA through RAR-mediated pathways, but it enhances survival in cells, such as keratinocytes, that display a low CRABP-II/FABP5 ratio and in which RA is channeled to PPARβ/δ (94). CRABP-II thus appears to function as a tumor suppressor whereas FABP5 displays distinct oncogenic activities. Indeed, it has been reported that FABP5 overexpression is associated with malignant progression in human cancers, including prostate cancer (1, 72).

It was also demonstrated that tumor development in the MMTV-*neu* mouse model of breast cancer is accompanied by upregulation of FABP5 and suppression of CRABP-II, and thus in dysregulation in RA signaling in these tumors. Consequently, in these tumors, RA induces the expression of PPARβ/δ target genes at the expense of genes controlled by RAR, and it markedly facilitates tumor growth (95).

PERSPECTIVE

The balance between cell apoptosis and survival is critical during development, and in adult tissues, and deregulation of this balance can lead to pathologies such as cancer. RA regulates many facets of apoptotic processes by virtue of its ability to activate cognate nuclear receptors. Proapoptotic activities of the hormone, which are mediated predominantly by RARs, are mediated by genes involved in both intrinsic and extrinsic apoptosis pathways. These activities contribute to the anticarcinogenic potential of the hormone, as exemplified by its remarkable therapeutic efficacy in acute promyelocytic leukemia. In contrast, activation of PPARβ/δ by RA results in induction of genes involved in proliferation and survival, leading to RA resistance and undermining a more general use of the compound in oncology. The findings that the partitioning of RA between its two receptors is controlled by two cognate intracellular lipid-binding proteins suggest a possible strategy for overcoming RA resistance in tumors. For example, inhibition of FABP5 may divert RA from PPARβ/δ to RAR, thereby sensitizing cells to RA-induced growth inhibition and apoptosis. Notably, current knowledge on the nature of RA-controlled genes that mediate the involvement of the hormone in apoptosis and the molecular basis for the cell specificity of anti- and proapoptotic activities of the hormone remains incomplete. Identification of such genes and their associated pathways is bound to provide additional insights into the mechanisms of action of RA as well as point at additional targets for novel strategies in cancer therapy and prevention.

SUMMARY POINTS

1. RA is involved in regulation of apoptosis by controlling gene expression. The transcriptional activities of RA are mediated by two types of ligand-activated transcription factors that are members of the family of nuclear receptors: RA receptors (RARs) and the peroxisome proliferator-activated receptor β/δ (PPARβ/δ).

2. The partitioning of RA between its receptors is regulated by intracellular lipid-binding proteins that transport the compound from the cytosol to specific receptors in the nucleus: CRABP-II delivers RA to RAR and FABP5 shuttles the hormone to PPARβ/δ.
3. The involvement of RA in apoptosis is cell specific. RA induces apoptosis in some cells, whereas in others, it enhances survival and provides protection against apoptotic signals.
4. Apoptosis can be propagated by intrinsic or extrinsic pathways. Intrinsic apoptosis is initiated following cell stress resulting from events such as DNA damage or growth factor deprivation. It is mediated by release of apoptotic signals, most importantly cytochrome C, from the mitochondria, leading to initiation of a caspase cascade and apoptosis. Extrinsic apoptosis is triggered by binding of external ligands to cell surface death receptors, leading to activation of death receptor-associated caspases. The intrinsic apoptosis pathway crosstalks with extrinsic apoptosis as well as with ER stress responses.
5. Proapoptotic activities of RA are mediated predominantly by RAR and its cognate iLBP CRABP-II. RAR target genes include many players in both the intrinsic and the extrinsic apoptosis pathways. Antiapoptotic activities of RA are mediated by PPARβ/δ and its cognate iLBP FABP5. PPARβ/δ target genes include genes that activate survival pathways and genes involved in cell proliferation.
6. Pro- versus antiapoptotic responses to RA are regulated by the relative expression levels of CRABP-II and FABP5. RA induces apoptosis in cells that express a high CRABP-II/FABP5 ratio and thus efficiently activates RAR, and it functions as a survival factor in cells in which the binding protein ratio is low, enabling activation of PPARβ/δ.

FUTURE ISSUES

1. Current knowledge on the nature of RA-controlled genes that mediate the effects of the hormone on apoptosis as well as on the molecular basis for the cell specificity of anti- and proapoptotic activities of the hormone is surprisingly limited. Better understanding of these genes and their associated pathways will provide important insights into the mechanisms through which RA is involved in apoptosis. Such knowledge will allow for identifying new targets for improving existing strategies of usage of RA in cancer therapy and prevention.
2. The observations that CRABP-II and FABP5 control the partitioning of RA between RAR and PPARβ/δ, and thus the switch between proapoptotic and antiapoptotic responses to RA, suggest a novel strategy for overcoming RA resistance in tumors. Development of inhibitor(s) for FABP5 may allow for diverting RA from PPARβ/δ to RAR, thereby sensitizing cells to RA-induced growth inhibition and apoptosis.

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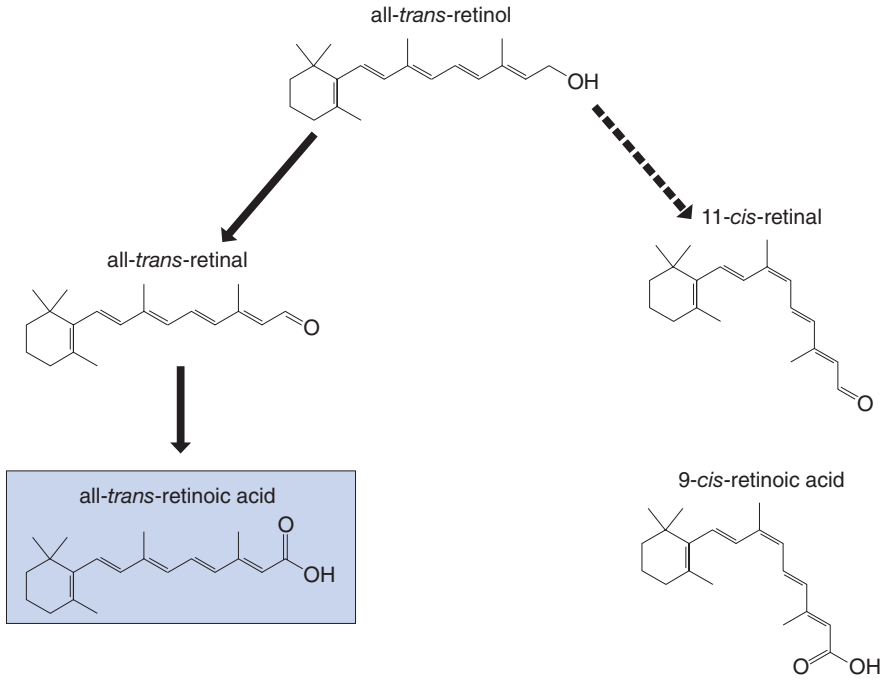

Figure 1

Structures of some retinoids. All-*trans*-retinoic, the focus of this review, is boxed.

Figure 2

Domain structure of nuclear hormone receptors.

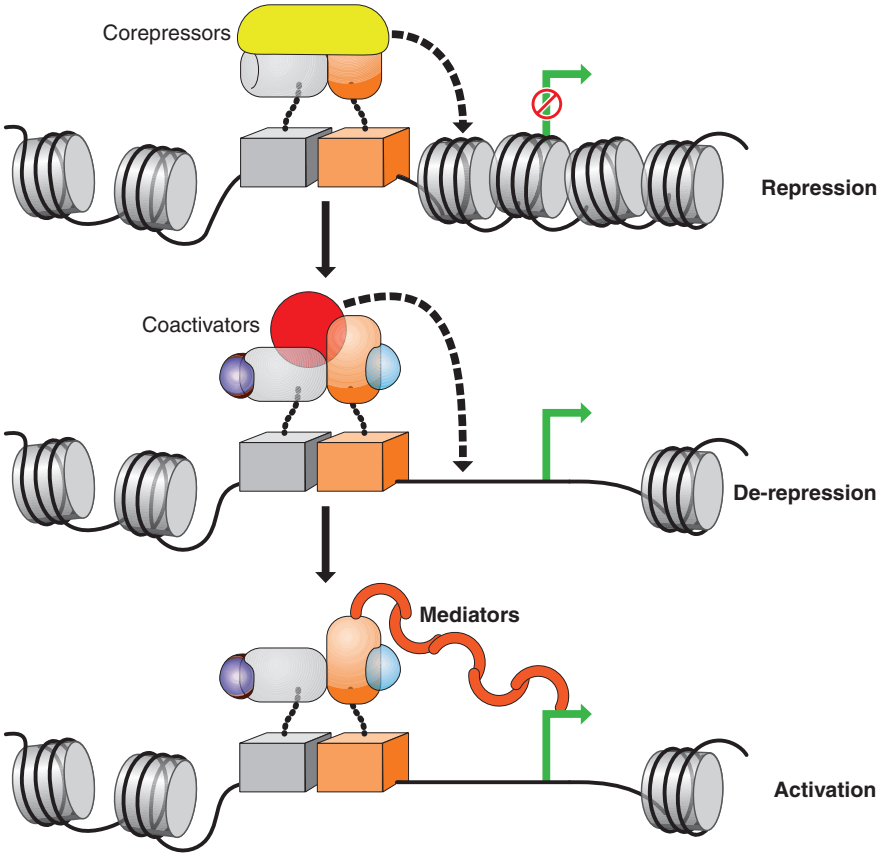

Figure 3

Regulation of gene transcription by nuclear receptors. The scheme depicts the activation of subclass 1 nuclear receptors, which function as heterodimers with RXR. In the absence of ligand, heterodimers, bound to regulatory regions of target genes, associate with a corepressor complex that includes histone deacetylase. Histone deacetylation results in chromatin condensation and repression of transcription. Upon ligand binding, receptors release corepressors and recruit a coactivator complex, which contains components that catalyze histone modification to loosen the structure of the chromatin. Subsequently, a different coactivator complex, termed the mediator, bridges between receptors and the general transcription machinery to stabilize the recruitment of polymerase II and initiate transcription.

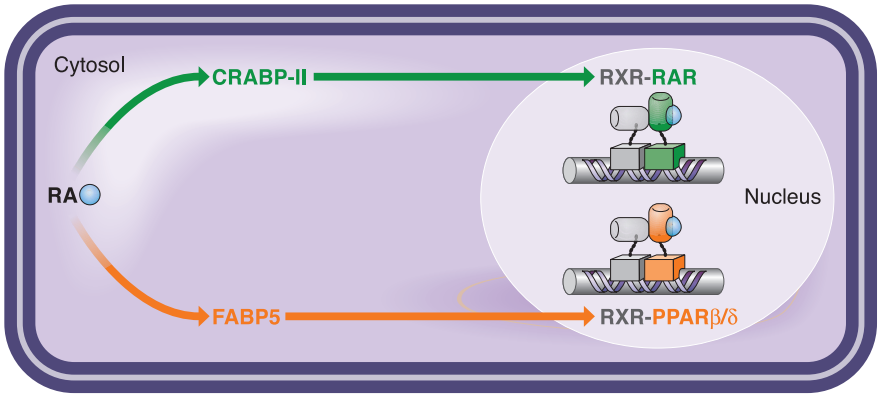

Figure 4

CRABP-II and FABP5 deliver RA to RAR and PPARβ/δ. In the absence of ligand, CRABP-II and FABP5 are present in cytosol. Upon binding of RA, these proteins mobilize to the nucleus, where they associate with RAR and PPARβ/δ, respectively. Complex formation allows for direct channeling of RA from the binding pocket of the binding protein to the ligand-binding site of a cognate receptor. Following ligand transfer, the complex dissociates, and the binding proteins return to the cytosol. CRABP-II, cellular RA binding protein II; FABP5, fatty acid–binding protein 5; PPAR, peroxisome proliferator-activated receptor; RA, all-*trans*-retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor.

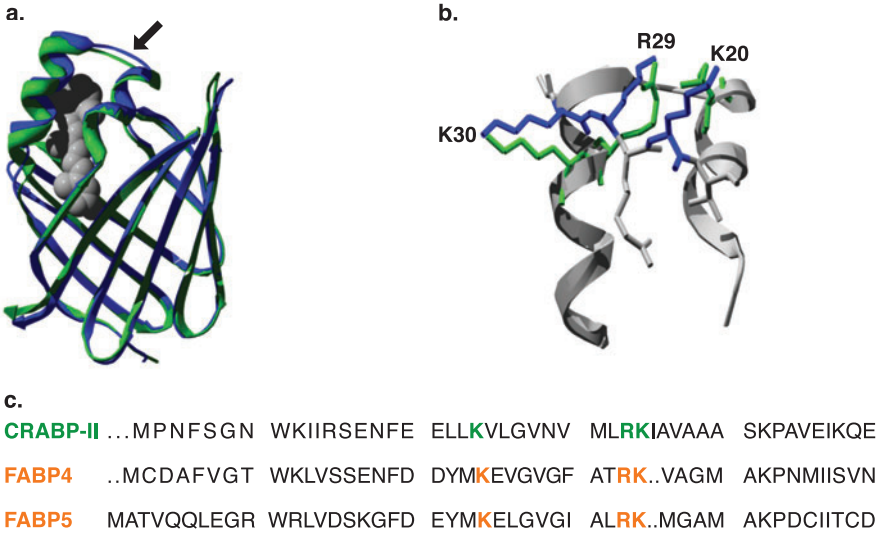

Figure 5

The nuclear localization signal (NLS) of CRABP-II. (*a*) Superposition of the apo- (*blue*) (23) and holo-CRABP-II (*green*) (57). Bound RA is in gray. The arrow points at the protein's helix-loop-helix region, which contains the NLS. (*b*) Superposition of residues K20, R29, and K30 of holo-CRABP-II (*green*) with the classical NLS of the SV 40 antigen (*blue*) (27). (*c*) Sequence alignment of FABP4 and FABP5 with the NLS-containing region of CRABP-II. Amino acids corresponding to the CRABP-II NLS are in red. Alignment was accomplished using accession #s: NP_001869 (hCRABP-II), P15090 (hFABP4), and NP_001435 (hFABP5).

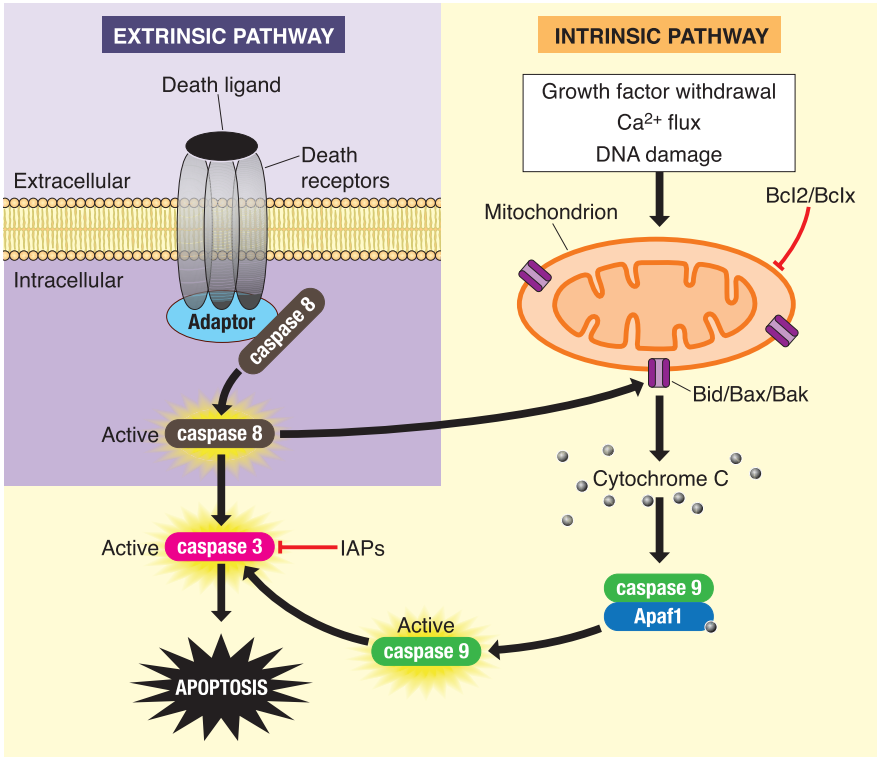

Figure 6

Apoptotic pathways. Intrinsic apoptosis is mediated through the mitochondria, which releases apoptotic factors, including cytochrome C, resulting in assembly of the apoptosome, activation of caspase cascades, and apoptosis. The process is regulated by proapoptotic Bcl2 proteins that enable release of cytochrome c, and antiapoptotic Bcl2 proteins, which inhibit the process. Extrinsic apoptosis is propagated by extracellular death ligands and their receptors. Crosstalk exists between the two pathways.

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Errata

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