EDITORSIAL

N-(4-Hydroxyphenyl)retinamide Activation of a Distinct Pathway Signaling Apoptosis

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The clinical and basic scientific understanding of the role of retinoids in cancer therapy and prevention has seen advances. Retinoids are synthetic and natural analogues of vitamin A [reviewed in (1)], These compounds are ligands for the retinoid receptors, members of the steroid receptor superfamily (2,3). The biologic effects of retinoids are initiated through ligand-dependent activation of nuclear retinoid receptors. This interaction leads, in turn, to activation or repression of “target genes,” which results in growth and differentiation effects. The finding that the retinoid N-(4-hydroxyphenyl)retinamide (4HPR) induces apoptosis in cells resistant to all-trans-retinoic acid, a high-affinity ligand for the retinoid acid receptors (RARs) (2,3), has suggested that 4HPR signals through a mechanism independent of the retinoid receptors (4–7). In this issue of the Journal, an article by Oridate et al. (8) links the generation of reactive oxygen species to 4HPR-induced apoptosis in cervical cancer cells. Confirmation that this pathway is active in other cell contexts would provide important evidence for a novel retinoid mechanism signaling apoptosis in human tumor cells.

Retinoids have reported activity in cancer therapy and prevention. They have been used in the treatment of the premalignant lesion oral leukoplakia (9) and in the prevention of second cancers of the head and neck (10), lung (11), and liver (12). As a single agent, all-trans-retinoic acid induces transient complete remissions in acute promyelocytic leukemia (13–15). When combined with interferon alfa-2a treatment, 13-cis-retinoic acid induces complete and partial remissions in squamous cell carcinoma of the skin and cervix (16,17). This regimen is also reported to be active in the treatment of disseminated renal cancer (18).

Retinoid biologic effects are signaled through two families of structurally related receptors, the RARs and the retinoid X receptors (RXRs) (1,2). The RARs have three subtypes (RAR α, RAR β, and RAR γ), with multiple isoforms. The RXRs also have three family members (RXR α, RXR β, and RXR γ). Orphan nuclear receptors exist for which physiologic ligands are not yet identified. All-trans-retinoic acid activates transcription of the RAR pathway but not the RXR pathway. Retinoid receptor ligands have been engineered to activate or inhibit specific RAR or RXR family members (19). Other retinoid ligands antagonize AP-1 activity (20) to signal biologic effects. While 4HPR mediates its effects through a retinoid receptor-independent mechanism. The finding that 4HPR signals apoptosis through generation of reactive oxygen species (8) is consistent with this interpretation. Since 4HPR activity is seen at concentrations expected for a receptor-dependent pathway, perhaps some receptor-mediated signals contribute to the observed biologic effects.

A convergence exists between clinical and basic scientific findings in the retinoid field. This convergence is illustrated in the link between retinoid-induced RAR expression and clinical responses in oral leukoplakia (22) and in the association between PML/RARα expression and all-trans-retinoic acid-mediated remissions in acute promyelocytic leukemia (24). These examples underscore the importance of understanding which retinoid pathways are engaged to signal biologic and clinical effects. The study by Oridate et al. (8) extends previous work on 4HPR by highlighting a distinct retinoid mechanism signaling apoptosis independent of nuclear receptors. This mechanism is the accumulation of reactive oxygen species induced by 4HPR treatment.

Several novel findings are reported by Oridate et al. 4HPR signals apoptosis in malignant but not in benign cervical epithelial cells. The specificity of the 4HPR pro-oxidant effect is supported by the finding that the major metabolite of 4HPR does not signal apoptosis or generate reactive oxygen species. 4HPR does not appear to alter expression of the Bcl-2 or Bax proteins. These species and p53 are linked to the regulation of apoptosis in vivo (25). Treatment with oxygen radical scavengers reduces reactive oxygen species and inhibits 4HPR-induced apoptosis. A threshold effect is observed, indicating a critical level of reactive oxygen species is needed to generate apoptosis. Notably, while 4HPR is reported to activate transcription by RARγ (21), treatment with an RARγ antagonist did not prevent 4HPR signaling. This finding is consistent with the proposed retinoid receptor-independent mechanism.

The tight link between reactive oxygen species and 4HPR

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apoptotic signals extends previous work in the retinoid field. It has been reported in head and neck cancer (6) and in myeloid leukemia cells (4) that 4HPR, unlike other retinoids tested, preferentially signals apoptosis even when all-trans-retinoic acid does not. An association between 4HPR signals and antioxidant effects was shown through overexpression of Bcl-2 in myeloid leukemia cells (7) antagonizing 4HPR effects. While an analogous experiment was not conducted in cervical cancer cells (8). Oridate et al. state that, in another tumor cell type (i.e., squamous cell carcinoma of the head and neck), transfection of Bcl-2 failed to protect cells from apoptosis induced by 4HPR treatment. It is intriguing to note that other chemoprevention agents, such as oltipraz (26), are reported to increase oxygen radical production. Perhaps other chemoprevention agents engage similar mechanisms as those used by 4HPR to mediate apoptosis. The finding that differential effects are seen between normal cervical epithelial cells and cervical carcinoma cells raises the possibility that 4HPR has selectivity for transformed cells.

Additional studies are needed to confirm and extend the finding that the 4HPR-induced apoptotic pathway is independent of p53 mutational status. Perhaps this signaling pathway is active in diverse tumor cell contexts, including those having frequent p53 mutations. It will be interesting to learn whether 4HPR apoptotic signals already known to occur in other cell contexts (4–7) exhibit a similar dependence on reactive oxygen species. The finding that apoptotic effects are observed in cervical cancer cells negative for human papillomavirus argues against these 4HPR signals depending on the presence of viral sequences.

Because 4HPR preferentially signals apoptosis through distinct mechanisms, it may be useful in combination therapy. While cooperation between 4HPR and other retinoids was not observed by Oridate et al., others have noted cooperative effects (27). Perhaps the lack of cooperation between all-trans-retinoic acid and 4HPR relates to the fact that all-trans-retinoic acid fails to signal a major growth-suppressive or maturation effect in these cells. In other cell contexts, all-trans-retinoic acid cooperates with 4HPR when all-trans-retinoic acid signals biologic effects as a single agent (27). As discussed, clinical cooperation between retinoid and interferon pathways has been reported (16–18). Cooperation between the retinoids and second messenger pathways also occurs in preclinical settings (1). Perhaps ‘‘downstream’’ retinoid-dependent signals, such as regulation of the cell cycle machinery (28), will cooperate with apoptotic signals initiated by 4HPR treatment. It will be interesting to learn whether cooperation between 4HPR and one of these other cellular pathways occurs.

In summary, the study by Oridate et al. (8) describes a distinct effect of 4HPR during the signaling of apoptosis, i.e., the generation of reactive oxygen species. The accumulation of reactive oxygen species is tightly linked to the observed biologic effects of 4HPR in cervical cancer cells. Perhaps examination of patient-derived biopsy specimens will reveal whether 4HPR signals reactive oxygen species accumulation in vivo. Should the accumulation of reactive oxygen species become recognized as a common mechanism responsible for 4HPR-mediated apoptotic signals, it must be learned how 4HPR generates reactive oxygen species. The challenge will be to rapidly translate these in vitro findings into the clinical setting using 4HPR as a single agent or as part of combination therapies. Such treatment may benefit patients who already have cancer or prevent cancers from arising in ‘‘high-risk’’ individuals.

References

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Note

PML/RAR α is a fusion gene caused by the chromosomal translocation t(15;17), as reviewed in (23). Variant translocations exist that also rearrange RAR α. Paradoxically, in cases of acute promyelocytic leukemia with the t(15;17) rearrangement, the induction of leukemia cell differentiation in vivo and complete remissions are linked to the expression of PML/RAR α, which, when not bound to ligand, acts as a dominant-negative receptor.