Editorials

Fenretinide Activates a Distinct Apoptotic Pathway

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This is an exciting period in molecular oncology. Molecular pharmacologic pathways are being discovered that regulate tumor cell proliferation, differentiation, and apoptosis. In this regard, retinoids are worthy of study because these natural and synthetic derivatives of vitamin A can inhibit proliferation, promote differentiation, trigger apoptosis, and affect other signaling pathways. Moreover, retinoids are worthy of study because the two major types of retinoids—classical (such as all-trans-retinoic acid) and nonclassical (such as fenretinide)—exert distinct biologic effects. Understanding how fenretinide preferentially engages the apoptotic pathway is the subject of the work by Lovat et al. (1) in this issue of the Journal.

Classical retinoids activate the family of nuclear retinoic acid receptors (RARs) of which there are three members (RARα, RARβ, and RARγ), along with multiple isoforms. By contrast, some nonclassical retinoids, such as targarin (bexarotene), transcriptionally activate the family of retinoid X receptors (RXRs), or retinoid pathway, which also has three family members (RXRa, RXRβ, and RXRγ). Other nonclassical retinoids, including fenretinide, engage other signaling pathways. Fenretinide is particularly intriguing because it has been shown to act through both retinoid receptor–dependent and –independent mechanisms (2–7). Unlike classical retinoids that often induce differentiation, fenretinide triggers distinct biologic effects, such as the generation of reactive oxygen species (ROS) and the promotion of apoptosis (3,5–7).

Clinical interest in understanding the mechanisms of fenretinide action is the result of several observations. First, in premenopausal women with early-stage breast cancer, fenretinide can function as a chemopreventive agent against second breast malignancies (8). Second, the classical retinoid 13-cis-retinoic acid was used successfully to treat minimal residual disease in patients with neuroblastoma, indicating that retinoids have clinical activity in this setting (9). Fenretinide may exert greater therapeutic activity than a classical retinoid because it promotes an apoptotic rather than a differentiation response (2–7). Third, the treatment of some solid tumors, including neuroblastoma, with fenretinide and modulators of ceramide metabolism induced synergistic cytotoxicity (10). Fourth, fenretinide increases intracellular ceramide levels, generates ROS, and promotes apoptosis in neuroblastoma cells (11). These and other findings led to a phase I clinical trial of fenretinide in children with neuroblastoma that had encouraging preliminary results (12). Preclinical (3–7,10,11,13) and clinical (8,12) studies highlight the need to understand precisely how fenretinide generates ROS or promotes apoptosis in neuroblastoma and in other tumors. Such a study was undertaken by Lovat et al. (1).

Lovat et al. (1) offer a mechanistic basis for how fenretinide generates ROS and triggers apoptosis. Their findings reveal that, in neuroblastoma cells, gangliosides link acidic sphingomyelinase–mediated ceramide induction to 12-lipoxygenase-dependent apoptosis following fenretinide treatment. The authors used a comprehensive approach to uncover mechanisms responsible for fenretinide actions that included using pharmacologic inhibitors and genetic knockdown with small interfering RNA (siRNA). Enzymatic targets of fenretinide included acidic sphingomyelinase, glucosylceramide synthase, and GD3 synthase. The authors found that pharmacologic inhibitors of acidic sphingomyelinase, but not neutral sphingomyelinase or ceramide synthase, blocked fenretinide-induced ROS generation and apoptosis. Targeting acidic sphingomyelinase by siRNA inhibited fenretinide-dependent increases in ceramide, ROS, and apoptosis. Glucosylceramide synthase activity was also increased by fenretinide treatment. The resulting apoptosis was antagonized by an inhibitor of this enzyme.

Glucosylceramide synthase is important in ceramide metabolism and in the regulation of apoptosis (14). Lovat et al. (1) found that fenretinide increased ganglioside D3 (GD3) levels, whereas siRNA-mediated knockdown of GD3 synthase prevented fenretinide-dependent induction of GD3, ROS, and apoptosis. They confirmed these findings independently through the addition of exogenous GD3, which also induced ROS and apoptosis. These effects were prevented by use of a 12-lipoxygenase inhibitor, implicating 12-lipoxygenase as a downstream mediator or pharmacologic target in this response. Even though GD2 was also increased by fenretinide treatment, exogenous GD2 did not confer effects similar to those conferred by exogenous GD3, demonstrating that different biologic effects were associated with these distinct gangliosides. These findings indicate that a specific fenretinide-mediated apoptotic pathway exists. Furthermore, the results illustrate the usefulness of fenretinide or other retinoids as pharmacologic tools to delineate therapeutic pathways.

What are the consequences of uncovering mechanisms for this fenretinide-dependent apoptotic pathway? Fenretinide is known to augment intracellular levels of ceramide (10,11,15–17), a lipid second messenger that signals diverse biologic effects, including the regulation of apoptosis and cytotoxicity (18). Fenretinide-induced generation of GD3 from the actions of glucosylceramide synthase and GD3 synthase implicates the increase in ceramide leading to 12-lipoxygenase activation as a key signal that mediates this apoptotic response. Although GD3 is a neuronal marker of differentiation induced in response to classical retinoids in human embryonal carcinoma cells (19), the
findings presented by Lovat et al. (1) indicate that this ganglioside plays a biologic role beyond that of a differentiation marker. Furthermore, fenretinide promotes apoptosis in human embryonal carcinoma cells that are resistant to classical retinoids or cisplatin or that have alterations in the p53 pathway (3,20). The work of Lovat et al. (15) indicates that cooperation between fenretinide and chemotherapeutic agents might be clinically beneficial by targeting independent therapeutic pathways. The clinical relevance of fenretinide-based combination therapy in neuroblastoma is now under study using GD2 as an immunotherapy target in a clinical setting (21).

These (1,21) and other findings (22) provide the rationale for continued exploration of combination therapy with fenretinide and anti-GD2 antibodies, as well as fenretinide and cytotoxic chemotherapy and differentiation-based therapy in neuroblastoma. Perhaps other retinoid analogs would prove superior to fenretinide in triggering apoptosis in neuroblastoma or other tumor cells (23). Additional studies are warranted to determine whether such is the case and to assess the role of targeted combination therapy in neuroblastoma.

The work of Lovat et al. (1) has advanced the field of fenretinide research by uncovering a mechanistic basis for how this agent generates ROS and signals apoptosis in neuroblastoma cells. They have used a nonclassical retinoid as a pharmacologic tool to learn that gangliosides link acidic sphingomyelinase-mediated ceramide induction to 12-lipoxigenase-dependent apoptosis. The rate-limiting enzymatic steps affected by fenretinide to generate ROS and augment apoptosis need to be established, because these enzymatic steps (or their regulators) might represent novel molecular therapeutic targets. Future studies should also determine whether there are therapeutic effects in diseases other than neuroblastoma, accounting for apoptotic responses observed with fenretinide in other tumor cell contexts. Such studies would broaden the impact of the work by Lovat et al. (1) by identifying other therapeutic indications for fenretinide.

The general outline of the fenretinide-dependent apoptotic pathway is now delineated. Yet interesting questions remain to be answered, such as How do gangliosides affect lipooxygenase activity or trigger the apoptotic response? Are these highlighted in vitro pathways also activated in vivo? The first question can be answered by in vitro studies, and the second, during the course of clinical trials. A translational research approach is needed to extend the findings of Lovat et al. (1) from the bench to the bedside. This approach seems most appropriate for improving treatment of those diagnosed with neuroblastoma or other cancers. Perhaps fenretinide would even inhibit carcinogenesis in individuals at high risk of developing neuroblastoma or other cancers. Future work should pursue these important clinical possibilities.

**REFERENCES**


