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Biochemical and Biophysical Research Communications 331 (2005) 810–815
BBRC
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Review

Fenretinide: A p53-independent way to kill cancer cells

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Received 25 March 2005
Available online 5 April 2005

Abstract

The synthetic retinoid fenretinide [*N*-(4 hydroxyphenyl)retinamide] induces apoptosis of cancer cells and acts synergistically with chemotherapeutic drugs, thus providing opportunities for novel approaches to cancer therapy. The upstream signaling events induced by fenretinide include an increase in intracellular levels of ceramide, which is subsequently metabolized to GD3. This ganglioside triggers the activation of 12-Lox (12-lipoxygenase) leading to oxidative stress and apoptosis via the induction of the transcription factor Gadd153 and the Bcl-2-family member protein Bak. Increased evidence suggests that the apoptotic pathway activated by fenretinide is p53-independent and this may represent a novel way to treat tumors resistant to DNA-damaging chemotherapeutic agents. Therefore, fenretinide offers increased clinical benefit as a novel agent for cancer therapy, able to complement the action of existing chemotherapeutic treatment regimes. Furthermore, synergy between fenretinide and chemotherapeutic drugs may facilitate the use of chemotherapeutic drugs at lower concentrations, with possible reduction in treatment-associated morbidity.

Keywords: Ceramide; Gadd153; Glucosylceramide synthase; GD3; Retinoids

Programmed cell death is the physiological process used by multicellular organisms to eliminate cells during normal development and tissue homeostasis. It is a highly regulated process, also termed apoptosis, that counters cell division and ensures proper tissue function [1]. This process is regulated by several complex biochemical pathways requiring the action of a large number of protein families. Different death signals may trigger the apoptotic process such as cytokines, growth factor withdrawal, radiation, TNFα, CD95, pharmacological agents, etc. These signals are interpreted by cells that decide for their future: to live or to die [2]. This complex scenario gives an idea of the power of the apoptotic process and explains why deregulation of the apoptosis signal control can result in a variety of diseases. One example and possibly the most frequent disease associated with the loss of apoptosis regulation is cancer. In fact, among the changes occurring during the transformation process an important role is played by the lack of proper apoptotic regulation. Cancer is thus considered as a disease characterized by an imbalance between cell division and cell death [3]. Using drugs to restore or enhance the capacity of cells to undergo apoptosis in response to biological signaling or DNA damage may be the most effective way to counteract tumor growth.

More than 50% of tumors are defective in the transcription factor p53, as a result of mutation [4].

The p53 tumor suppressor protein has been defined "the guardian of the genome," since as a consequence of DNA damage, p53 can either trigger cell cycle arrest

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.184

to permit adequate DNA repair, or trigger apoptosis to maintain the intrepidity of the tissue in which the cell resides. Inheriting a mutated allele of p53 increases the susceptibility to cancer and reduces sensitivity to chemotherapy by DNA-damaging agents [5].

Mutations in the p53 gene can indeed result in abrogated function of the protein and it is this dysfunction that is linked to tumor progression and genetic instability. Since the functions of p53 target genes are diverse, this may be the basis by which p53 acts as a multifunctional protein, and may explain why loss of p53 activity has been found to result in the development of human tumors. Unlike p53 knockout mice, mice lacking p21 do not develop tumors [6,7]. Therefore, it would appear that it is the ability of p53 to induce apoptosis rather than growth arrest that is central to its role as a tumor suppressor.

Since cells with defective p53 signaling are relatively resistant to apoptosis, it may be appropriate to seek cytotoxic agents that induce apoptosis independently of p53. Recent work on retinoids suggests that members of this class of compounds have the ability to induce apoptosis of tumor cells through cell signaling pathways that are not p53 dependent.

Retinoids are a group of natural and synthetic analogs of vitamin A that can inhibit proliferation, promote differentiation, trigger apoptosis, and affect other signaling pathways [8–11]. Retinoic acid can induce differentiation rather than apoptosis and may render tumor cells resistant to chemotherapy [12]. On the contrary, some synthetic derivatives of retinoic acid, such as fenretinide, are able to induce apoptosis rather than differentiation [13,14], and, unlike retinoic acid, show synergistic responses with chemotherapeutic drugs in a wide range of tumor cell types [15–20]. Therefore, fenretinide or similar compounds may provide opportunities for novel approaches to cancer therapy. To fully exploit the potential of fenretinide for cancer therapy, it is essential to know the molecular mechanisms by which it induces apoptosis. Although unanswered questions still remain, there has been substantial progress in the last few years in elucidating the mechanism of action of fenretinide, and novel therapeutic targets have emerged from these studies [17,19–23].

Fenretinide, caspases, and ROS

Apoptosis is mediated by three known pathways: an "extrinsic" or cell-surface death receptor pathway and two "intrinsic" pathways involving damage or stress within mitochondria or the endoplasmic reticulum, respectively [24]. Activation of one or more of these pathways results in downstream caspase activation and cell death [25]. In broad terms, the cellular pathways controlling apoptosis [26] can be regulated at three levels: (i) receptors (transducers) receiving the initiation signal; and (ii) effector mechanisms linking these transducers to (iii) downstream components of apoptotic pathways which culminate in nuclear condensation, cell shrinkage, and death.

The effects of fenretinide are likely to be mediated by itself rather than metabolism, since neuroblastoma cells do not metabolize fenretinide appreciably [27] and in other cell types the main metabolite, *N*-(4-methoxyphenyl)retinamide, has little biological activity [28].

Apoptosis in response to fenretinide has been demonstrated in neuroblastoma cells using a variety of techniques [19]. However, it has also been reported in some human cancer cell lines that fenretinide at high doses will induce necrosis as well as apoptosis [14]. In neuroectodermal tumor cells, fenretinide-induced apoptosis mediated by caspase-dependent pathways activated by the release of cytochrome *c* from mitochondria may be due to mitochondrial membrane permeabilization (MMP) triggering involving the proapoptotic Bcl-2 family members Bax and Bak [29,30,19] (Fig. 1). It has been

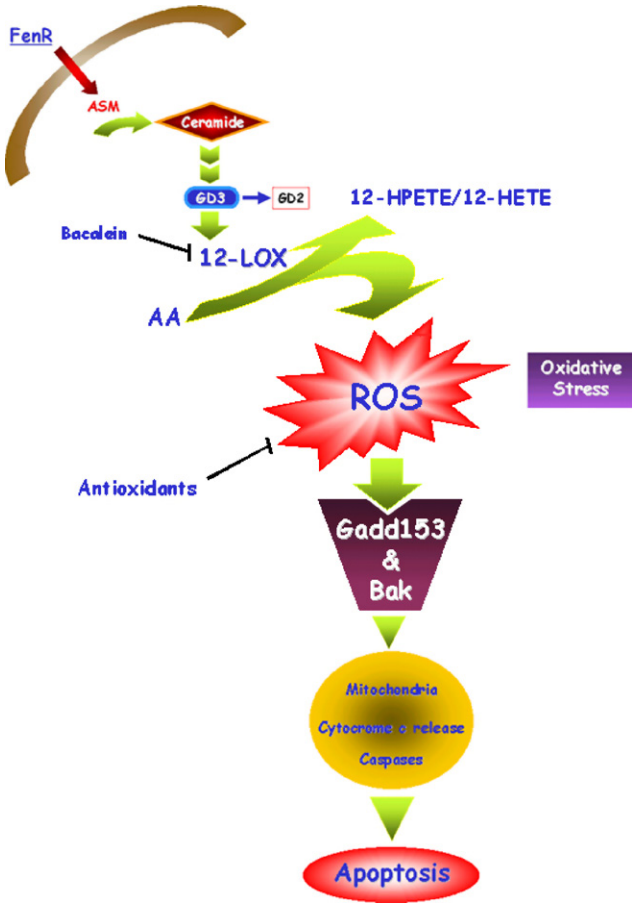

Fig. 1. Fenretinide-induced apoptotic pathway. Fenretinide increases ASM activity resulting in ceramide production, subsequently converted to GD3. 12-Lox-increased activity by GD3 mediates ROS generation and consequent oxidative stress status. The downstream fenretinide-induced apoptotic pathway is mediated by Gadd153 and Bak upregulation, and caspase-activation due to the release of cytochrome *c* from mitochondria.