BH3-only proteins in tumorigenesis and malignant melanoma

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Abstract. BH3-only proteins are a subset of the Bcl-2 family of apoptotic regulators. BH3-only proteins function as ‘damage sensors’ in the cell; they are activated in response to cellular stress or DNA damage, whereupon they initiate apoptosis. Apoptosis is the primary mechanism by which the body rids itself of genetically defective cells and is critical for preventing the accumulation of cells with tumorigenic potential. Therefore, dysregulation of BH3-only proteins may promote tumorigenesis. Furthermore, functional apoptosis pathways are required for the success of most cancer treatments, including chemotherapy. Resistance to chemotherapy, as seen with malignant melanoma, often reflects an inability of tumor cells to undergo apoptosis. By deciphering the roles of BH3-only proteins in tumorigenesis, we may learn how to manipulate cell death pathways to overcome apoptotic resistance. This review summarizes the current knowledge of BH3-only proteins and how they contribute to tumorigenesis, with particular attention given to studies involving melanoma.

Keywords. BH3-only protein, Bcl-2 family, apoptosis, tumorigenesis, melanoma.

Introduction

It is now recognized that cancer is not simply a disease of excessive cell proliferation. More accurately, tumor development reflects an imbalance between cell production and cell elimination [1]. Over-production may result from the mutation of cell cycle control genes or the hyperactivation of cell proliferation pathways. Ineffective cell elimination, on the other hand, is thought to arise from the deregulation of cell death pathways, such as apoptosis. Apoptosis (programmed cell death) is the primary mechanism by which the body rids itself of damaged, genetically defective, or superfluous cells [2], and is therefore critical for preventing the accumulation of cells with tumorigenic potential. Cancer cells often acquire defects in genes regulating apoptosis, allowing them to evade cell death. Furthermore, conventional cancer treatments such as chemotherapy and radiation work primarily by inducing apoptosis in tumor cells and thus require functional apoptotic pathways [3]. Chemo-therapeutic resistance, in many cases, may actually reflect an underlying resistance to apoptosis. Malignant melanoma is a notoriously apoptotic-resistant tumor type that responds poorly to both chemotherapy and radiation treatment [4]. Melano-ma tumors have been shown to exhibit low rates of spontaneous apoptosis compared with other tumor types [5]. It is generally thought that melanocytes acquire a resistance to apoptosis during their transformation from normal to melanoma cells [6]. In accordance with this notion, melanocytic nevus cells show greater resistance to apoptosis than melanocytes...
when grown in collagen gels [7]. Furthermore, melanoma cell lines are usually resistant to drug-induced apoptosis [8]. Melanoma patients with advanced disease have essentially no treatment options and face a dismal prognosis: only 16% of metastatic melanoma cases survive for 5 years [9]. There is an urgent need to develop effective therapies for this disease. Part of the solution lies in understanding why melanoma cells are so resistant to DNA damage-induced apoptosis and how we can manipulate apoptotic pathways to overcome this resistance.

Apoptosis is a complex process, controlled by many different genes and proteins. A particular subset of proteins, known as BH3-only proteins, have recently emerged as critical effectors of apoptosis in mammalian cells. Deciphering the roles of BH3-only proteins in melanoma cell death is critical to understanding how melanoma cells can be induced to undergo cell death. In this review we summarize the current knowledge of BH3-only proteins and how they contribute to tumorigenesis, with particular attention given to studies involving melanoma.

### Mechanisms of BH3-only protein-induced cell death

BH3-only proteins function as the “damage sensors” of the cell. They are activated in response to cellular stress or DNA damage, whereupon they initiate apoptosis (Fig. 1) [10]. BH3-only proteins are activated to induce apoptosis by a diverse range of stimuli including cytokine withdrawal, loss of adhesion to the extracellular matrix, DNA damage (by chemotherapeutic drugs or radiation), and oncogene activation [11]. Distinct BH3-only proteins are induced depending on the nature of the cytotoxic stimulus and the tissue type involved.

BH3-only proteins are a subgroup of the Bcl-2 protein family, all of whose members either promote or inhibit apoptosis. The Bcl-2 family can be divided into three functional groups: (i) Pro-survival Bcl-2-like members (Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and A1), (ii) pro-apoptotic Bax-like members (Bax, Bak and Bok), and (iii) Pro-apoptotic BH3-only members (Puma, Noxa, Bid, Bim, Bmf, Bad, Bik, and Hrk) [2]. The cell controls apoptosis by finely balancing the expression and activities of pro-survival proteins with those of pro-apoptotic proteins. In terms of protein structure, all Bcl-2 family proteins possess one or more Bcl-2 homology (BH) domains, BH1, BH2, BH3, and BH4. Pro-survival members share all four BH domains (except Mcl-1, which does not contain BH4), Bax-like members have three BH domains (BH1, BH2, BH3), and BH3-only members share just the BH3 domain, as their name suggests (Fig. 2) [11].

There are two other pro-apoptotic Bcl-2 family proteins, Bcl-XL and Bcl-XAK, that do not fall into the aforementioned categories. Bcl-XL contains a BH3 and BH4 domain, while Bcl-XAK contains a BH2 and BH4 domain. Both proteins are alternative splice products of the \( bcl-x \) gene that codes for Bcl-xl. The abilities of both Bcl-XL and Bcl-XAK to induce apoptosis have been described in melanoma cells [12, 13], but their mechanisms of action so far remain unclear.

Multidomain Bax-like proteins appear to possess intrinsic cell death-inducing ability while BH3-only proteins act more indirectly, by engaging other Bcl-2 family members to either suppress pro-survival proteins (Bcl-2, Bcl-XL) or to activate pro-apoptotic multidomain proteins (Bax, Bak) [14]. BH3-only protein activity is tightly controlled via transcriptional upregulation (Puma, Noxa, Bim, Hrk) or posttranslational modification (Bid, Bim, Bmf, Bad, Bik). Activated BH3-only proteins interact with Bcl-2-like proteins at the mitochondrial membrane, neutralizing their pro-survival function and “priming” the cell for apoptosis. Activated BH3-only proteins are also thought to activate pro-apoptotic Bax and Bak proteins. Activated Bax/Bak form homo-oligomers that lead to permeabilization of the mitochondrial outer membrane, release of cytochrome c, caspase activation, and, ultimately, apoptotic cell death.