Bcl-2 Inhibition of Autophagy: A New Route to Cancer?

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Abstract

Bcl-2 was the first identified cellular protein that functions as an oncogene by blocking apoptotic cell death. Beclin 1, the first identified mammalian autophagy gene product, is a haploinsufficient tumor suppressor that was originally isolated as a Bcl-2-interacting protein. We recently showed that Bcl-2 negatively regulates Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death. These findings raise the possibility that Bcl-2 family members may function as oncogenes not only by blocking apoptosis but also by blocking autophagy (Cancer Res 2006; 66(6): 2885-8).

Background

Bcl-2 was initially cloned from the breakpoint of the t(14:18) chromosomal translocation found in the majority of patients with follicular lymphoma (1–3). Its oncogenic activity was established when transgenic mice bearing a Bcl-2 immunoglobulin minigene fusion that recapitulates the t(14:18) translocation were found to develop follicular hyperplasia and lymphoma (4, 5). The identification of Bcl-2 established a new class of oncogenes; unlike previously characterized oncogenes that shared the ability to increase cellular proliferation, Bcl-2 inhibited programmed cell death (4, 6). For almost two decades, Bcl-2 has been thought to act by inhibiting one specific form of programmed cell death, apoptosis. Nearly all of the effects of Bcl-2 in cancer have been attributed to its effects on the apoptotic pathway, although it is known that Bcl-2 family members are multifunctional proteins that can influence other cellular processes, including cell cycle progression, calcineurin signaling, glucose homeostasis, and transcriptional repression by p53 (reviewed in refs. 7, 8).

In 1998, Liang et al. identified a new binding partner for Bcl-2, termed Beclin 1, which was isolated from a mouse brain library in a yeast two-hybrid screen (9). Beclin 1 mapped to a tumor susceptibility locus on chromosome 17q21 that is monoallelically deleted in 40% to 75% of cases of sporadic breast, ovarian, and prostate cancer (reviewed in ref. 10). Beclin 1 was shown to have tumor suppressor function in breast cancer cells; it reduced human breast carcinoma cell proliferation, clonogenicity in soft agar, and tumorigenicity in nude mice (11). Although biallelic mutations of beclin 1 have not yet been identified in human cancer, beclin 1 is a haploinsufficient tumor suppressor gene in mice. Mice lacking one allele of beclin 1 develop mammary neoplastic lesions and display an increased incidence of spontaneous malignancies, including B cell lymphomas, lung adenocarcinoma, and hepatocellular carcinoma (12, 13).

Beclin 1 is the first identified tumor suppressor protein that functions in the lysosomal degradation pathway of autophagy. In contrast to the ubiquitin-proteasome system which is the major cellular route for the degradation of short-lived proteins, autophagy is the major cellular route for the degradation of long-lived proteins and cytoplasmic organelles (reviewed in refs. 14, 15). Autophagy is a dynamic process involving the rearrangement of subcellular membranes to sequester cytoplasm and organelles for delivery to the lysosome where the sequestered cargo is degraded and recycled. Autophagy is mediated by a set of evolutionarily conserved gene products (termed the Atg proteins) originally discovered in yeast (16). The mammalian autophagy protein, Beclin 1, is an orthologue of yeast Atg6, and is part of a complex with class III phosphoinositide-3-kinase, Vps34, that is responsible for autophagosome formation (17). Gene deletion studies in yeast, Caenorhabditis elegans, plants, Dictyostelium, Drosophila, and mice have revealed a conserved function of Atg6/Beclin 1 proteins in autophagy (12, 13, 18–22).

Defects in autophagy were first implicated in the development of cancer nearly 30 years ago (23). At that time, normal cell growth was viewed as balance between the cell's biosynthetic machinery (e.g., protein synthesis and organelle biogenesis) and its degradative processes (e.g., protein degradation and organelle turnover); autophagy represented the major form of protein degradation that contributed to this balance (23–25). Indeed, an inverse relationship has often been observed between autophagic activity and transformation; malignant cells frequently display lower levels of basal autophagic activity than their normal counterparts and fail to increase autophagic activity in response to serum deprivation or high cell density (two potent inducers of autophagy; refs. 23, 26–29). In recent years, new biochemical and genetic data has led to a resurgence in interest in the role of autophagy in tumor suppression. In addition to the discovery that an autophagy execution protein, Beclin 1, is a tumor suppressor protein, oncogenic signaling molecules suppress autophagy (e.g., class I phosphoinositide-3-kinase, Akt, and mTOR) and tumor suppressors (e.g., PTEN, p53, and DAPK) stimulate autophagy (30–34). Furthermore, certain chemotherapeutic agents (e.g., rapamycin and tamoxifen) are potent inducers of autophagy (35, 36). The proposed mechanisms by which autophagy functions in tumor suppression include (a) the degradation of specific organelles and long-lived proteins essential for regulating cell growth, (b) the removal of damaged organelles that generate reactive oxygen species and increase genotoxic stress, and (c) the induction of a non apoptotic form of programmed cell death, known as “autophagic cell death.”

The emerging role of autophagy in tumor suppression has led scientists to wonder: what is the functional significance of the interaction between Bcl-2, a cellular oncoprotein, and Beclin 1, an autophagy and tumor suppressor protein? We have recently begun to address this question by defining a novel role of Bcl-2 antia apoptotic proteins in the inhibition of Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death (36).
Key Findings

**Bcl-2 inhibits Beclin 1-dependent autophagy.** We used yeast and mammalian model systems to evaluate the effects of wild-type Bcl-2, viral Bcl-2 encoded by Kaposi’s sarcoma herpesvirus (KSHV v-Bcl-2), and Beclin 1-binding defective mutants of Bcl-2 on the ability of Beclin 1 to rescue starvation-induced autophagy in autophagy-defective cells. We found that cellular Bcl-2 and KSHV v-Bcl-2 (but not Beclin 1-binding defective mutants of Bcl-2) inhibited the ability of wild-type Beclin 1 (but not Bcl-2-binding defective mutants of Beclin 1) to rescue starvation-induced autophagy in yeast disrupted of the **beclin 1** orthologue, **atg6**. Similarly, in MCF7 human breast carcinoma cells that express low or undetectable levels of Beclin 1, both Bcl-2 (but not Beclin 1-binding defective mutants of Bcl-2) and KSHV v-Bcl-2 blocked the ability of Beclin 1 to rescue starvation-induced autophagy. Together, these findings showed that cellular and viral Bcl-2 proteins inhibit autophagy through a mechanism that most likely involves their interaction with Beclin 1.

To explore the mechanism by which Bcl-2 inhibits starvation-induced autophagy, we used an autophagy-competent cell line, HT-29 colon carcinoma cells, that expresses endogenous Beclin 1. In HT-29 cells, stable transfection of Bcl-2 inhibits starvation-induced autophagy, decreases the association of Beclin 1 with Vps34, and decreases the magnitude of Beclin 1-associated class III phosphoinositide-3-kinase activity. This finding suggests that Bcl-2 overexpression blocks the formation of the autophagy-promoting Beclin 1-Vps34 complex. Whereas the Beclin 1-Vps34 complex localizes to the trans-Golgi network (which is not a site of subcellular localization of Bcl-2), we found that endogenous Beclin 1 also localizes to the two major sites in the cell where Bcl-2 is found, the mitochondria and the endoplasmic reticulum. Using mutant constructs of Bcl-2 that are selectively targeted to each of these organelles, we found that endoplasmic reticulum–targeted Bcl-2, but not mitochondrial-targeted Bcl-2, inhibits autophagy. These findings are most consistent with the hypothesis that the interaction between Bcl-2 and Beclin 1 in the endoplasmic reticulum blocks a signal that is essential for the formation of the Beclin 1-Vps34 autophagy-promoting complex.

We confirmed that endogenous Bcl-2 down-regulates starvation-induced autophagy. In HeLa cells (an autophagy-competent cell line that expresses both Bcl-2 and Beclin 1), we found that Bcl-2 gene silencing with a hairpin small interfering RNA significantly increases the magnitude of starvation-induced autophagy. Thus, the basal levels of Bcl-2 do not prevent starvation-induced autophagy, but they do down-regulate the magnitude of increase induced by the potent autophagy-inducing stimulus, starvation. Furthermore, the ability of normal cells to increase their autophagic activity in response to starvation seems to be regulated by the dissociation of the Bcl-2-Beclin 1 complex. In HeLa cells, we observed minimal levels of Bcl-2 communo-precipitate with Beclin 1 during autophagy-inducing conditions (i.e., starvation), high levels of Bcl-2 commounprecipitate with Beclin 1 during autophagy-inhibitory conditions (i.e., growth in nutrient-rich medium), and intermediate levels of Bcl-2 communo-precipitate with Beclin 1 during growth in normal medium. When Bcl-2 is overexpressed, as occurs in certain cancer cells, the balance of Bcl-2 and Beclin 1 may be tipped in favor of continuous, rather than nutritionally regulated, inhibition of Beclin 1-dependent autophagy.

We also confirmed that enforced Bcl-2 expression could inhibit starvation-induced autophagy in mice. We crossed mice that transgenically express Bcl-2 under the control the cardiomyocyte-specific promoter, MHC, with mice that express a fluorescent autophagy protein marker, GFP-LC3, and found that Bcl-2 transgene expression significantly reduces the magnitude of starvation-induced autophagy in cardiac muscle. Thus, Bcl-2 inhibits starvation-induced autophagy in several different cell types and both in vivo and in vitro, suggesting that it is a physiologically important negative regulator of autophagy.

**Bcl-2 inhibits Beclin 1-dependent autophagic cell death.** The term autophagic cell death describes a form of programmed cell death morphologically distinct from apoptosis which is presumed to result from excessive autophagy (37). The consensus view has been that autophagic cell death occurs primarily when the developmental program (e.g., insect metamorphosis) or homeostatic processes in adulthood (involution of hormonally responsive tissues) require massive cell elimination. Autophagic cell death has also been described in diseased mammalian tissues and in tumor cell lines treated with chemotherapeutic agents (reviewed in ref. 38).

It is controversial whether the visualization of autophagy in dying cells indicates a causative role for autophagy in the death execution process, or alternatively, a cellular survival strategy. In most studies, autophagic cell death has been defined by morphologic criteria which fails to distinguish between these possibilities. However, there is now genetic evidence that autophagy can function both as a pro-survival and as a pro-death mechanism, depending on the context. When intracellular nutrients are limited, as in the case of starvation or growth factor deprivation, autophagy genes are essential to maintain cellular ATP energy production, macromolecular synthesis, and cell survival (39). The absence of autophagy genes results in death in nitrogen-starved yeast, starvation-induced chlorosis in plants, death of starved Dictostelium, defective dauer development in *C. elegans*, early neonatal lethality in mice following interruption of the placental food supply, accelerated death of growth factor–deprived, apoptosis-resistant fibroblasts, and apoptosis of starved HeLa cells (15, 20, 40–42). In contrast, in other settings, the knockdown of autophagy genes suppresses death, including in L929 cells treated with caspase inhibitor, ZVAD, in *bax*−/−, *bak*−/− murine embryonic fibroblasts treated with etoposide or staurosporine, or in HeLa cells treated with IFN-γ (43–45).

Our recent findings indicate that the Bcl-2/Beclin 1 complex may function as a rheostat that controls not only the levels of cellular autophagy, but also autophagy gene–dependent cell death (36). When we expressed mutant forms of Beclin 1 that cannot bind to Bcl-2 in MCF7 cells, we found that Bcl-2-binding defective mutants of Beclin 1 resulted in “supernormal” levels of autophagy. The levels of basal autophagy were nearly doubled in MCF7 cells expressing Bcl-2-binding defective mutants of Beclin 1 as compared with MCF7 cells that lack Beclin 1 expression or MCF7 cells that express wild-type Beclin 1. This increased basal autophagy is associated with cell death that is caspase-independent and inhibitable by small interfering RNA against a downstream autophagy execution gene, **atg5**. Thus, in the absence of binding to Bcl-2, Beclin 1 triggers excessive levels of autophagy that result in autophagic cell death. These results suggest that Bcl-2 blocks autophagic cell death by binding to Beclin 1 and thereby, down-regulating levels of cellular autophagy.
Implications

Our findings show a novel function of the Bcl-2 oncoprotein, i.e., inhibition of Beclin 1-mediated autophagy and autophagic cell death. Since Beclin 1 has tumor suppressor activity, it is reasonable to speculate that inhibition of Beclin 1 function by Bcl-2 may contribute to the oncogenic potential of Bcl-2 (Fig. 1). As noted earlier, Bcl-2 was originally discovered in the context of its role in human follicular lymphoma (1–3). Moreover, deletion of one allele of beclin 1 decreases autophagy in B lymphocytes and predisposes mice to developing spontaneous B cell lymphomas, suggesting that levels of Beclin 1-mediated autophagy influence the risk of malignancies arising from B cells (12, 13). Thus, in addition to inhibitory effects on the apoptosis pathway, the inhibitory effects of Bcl-2 on Beclin 1-dependent autophagy may contribute to the development of B cell lymphomas. Because viral Bcl-2 encoded by KSHV also inhibits Beclin 1-dependent autophagy (36), viral Bcl-2-Beclin 1 interactions may also play a role in oncogenesis mediated by KSHV and other γ-herpesviruses.

It has not been definitively established that the autophagy execution function of Beclin 1 is responsible for its tumor suppressor effects, but this seems likely based on mutational analyses of Beclin 1 (46, 47) and observations that other tumor suppressors stimulate autophagy (reviewed in ref. 48). Furthermore, it has not been definitively established that autophagy is a bona fide tumor suppressor mechanism or exactly how it functions in tumor suppression. Despite these uncertainties, the recent findings that Bcl-2, an oncoprotein, inhibits the autophagy function of Beclin 1, a tumor suppressor protein, raises an important question for cancer biology. Does Bcl-2-mediated inhibition of autophagy represent a novel mechanism of oncogenesis?

If so, this would have implications both for understanding the molecular pathogenesis of cancer, as well as for the development of cancer therapeutics. Our findings suggest that the relative amount of Bcl-2 and Beclin 1 complexed to each other within a cell determines the levels of autophagy and autophagic cell death. Thus, compounds that disrupt the binding of Bcl-2 to Beclin 1 or compounds that increase the levels of Beclin 1 expression would be expected to increase cellular autophagy and inhibit tumorigenesis. Tamoxifen represents an example of a chemotherapeutic agent that has the latter activity, significantly up-regulating levels of Beclin 1 expression and inducing autophagic cell death in human breast carcinoma cells (49, 50). It will be of interest to determine whether more specific inducers of Beclin 1 expression also have antitumor

Figure 1. A speculative model of the interrelationship between Bcl-2, Beclin 1, autophagy, and cancer. Beclin 1 is known to be an autophagy and tumor suppressor protein (11–13). Bcl-2 binding to Beclin 1 is known to inhibit autophagy (36). According to this model, Beclin 1 stimulation of autophagy inhibits oncogenesis, and the inhibition of Beclin 1-dependent autophagy by Bcl-2 stimulates oncogenesis. The oncogenic effects of Bcl-2 inhibition of autophagy may relate to the blockade of autophagic cell death and/or cell death–independent functions of autophagy.
effects. Compounds may also exist in clinical development that disrupt the binding of Bcl-2 to Beclin 1 (reviewed in ref. 51). Because an identical mutation in the BH1 domain of Bcl-2 that disrupts the binding of Bcl-2 to Beclin 1 (reviewed in ref. 51).

Further studies are needed to examine the role of Bcl-2-mediated inhibition of autophagy in oncogenesis and the utility of blocking this potential function of Bcl-2 in cancer chemotherapy. Until then—based on the clues provided by recent studies on Beclin 1, autophagy, and cancer—it may no longer be safe to assume that the oncogenic effects of Bcl-2 relate solely to apoptosis inhibition.

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References


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