Ubiquitination, localization, and stability of an anti-apoptotic BCL2-like protein, BCL2L10/BCLb, are regulated by Ubiquilin1

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AUTHOR SUMMARY

Alterations in the processes that affect homeostatic cell death mechanisms are found in nearly all malignancies (1). One of the most well studied families of proteins that regulate cell death is the BCL2 family of proteins. BCL2 proteins can be either the guardians of the stability of mitochondrial outer membrane permeability (anti-apoptotic BCL2 family members) or the executioners of cell death caused by induction of increased mitochondrial membrane permeability (proapoptotic BCL2 family members) (2). An imbalance in the levels of anti- vs. proapoptotic BCL2 family members is frequently observed during cancer progression, and the imbalance can determine the outcome of cancer therapy. The imbalance can be either quantitative, determined by the total amount of a particular anti- or proapoptotic signal, or qualitative, as determined by the particular signal that is disrupted (3).

We have been studying the activities of the six human anti-apoptotic BCL2 proteins. We previously reported that each of the six BCL2 genes could cooperate with MYC (myelocytomatosis oncogene) in a mouse model of leukemia (4). However, we documented differences in the potency with which individual members could cooperate: BCL2, BCLd, and BCLw were more potent oncogenes than BCLb, BFL1, and MCL1.

In an effort to elucidate the differences in cooperative potential, we have begun to examine a number of properties of the six anti-apoptotic BCL2 proteins. Most importantly, as reported here, we have found that the three less potent oncogenic BCL2 proteins are less stable proteins, because they disappear rapidly when expressed in cells treated with cycloheximide, an inhibitor of translation. This finding dictates its ability to participate in oncogenic transformation. We further support this observation by demonstrating that a stabilized version of the BCLb protein, from which all of its lysine residues have been removed (BCLbK0) to prevent addition to ubiquitin and subsequent degradation, is fully stabilized and acts as a more potent oncogenic protein in our model of leukemia (Fig. P1). Having shown that BCLb is less oncogenic than other BCL2 proteins and that stabilization of BCLb could increase its oncogenic potential, we set out to identify proteins that interact with BCLb and affect its stability. To this end, we performed immunoprecipitation followed by mass spectrometry to identify BCLb-interacting proteins. One protein that we identified as a BCLb-interacting protein was Ubiquilin1, a protein with ubiquitin-like and ubiquitin-associated domains. Previous work had identified Ubiquilin1 as a protein capable of interacting with the proteasome and with ubiquitinated proteins, acting as a bridge to bring substrates to the proteasome for proteolytic degradation (5). In addition to this role of Ubiquilin1, it has also been suggested that Ubiquilin1 can play roles in protein trafficking and ER-associated protein degradation. We therefore attempted to determine how the interaction with Ubiquilin1 affects BCLb function.

We observed that Ubiquilin1 interacts specifically with BCLb and not with any of the other five BCL2 proteins. Following interaction with Ubiquilin1, BCLb is altered in multiple ways. Most obviously, the amount of BCLb that is ubiquitinated on multiple lysine residues is increased.

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**Fig. P1.** The regulation of concentrations of BCLb by Ubiquilin1 and ubiquitination. Treatment of cells expressing BCLbwt or BCLbK0 with cycloheximide leads to varied outcomes of the BCLb proteins depending on whether BCLb contains lysine residues or if Ubiquilin1 is present. Treatment of cells expressing BCLbwt with cycloheximide leads to rapid degradation of BCLb proteins (top), whereas, there is no affect on BCLbK0 levels following treatment with cycloheximide (bottom). When Ubqln is expressed with either BCLbwt or BCLbK0, BCLb proteins remain stable during prolonged treatment with cycloheximide (middle).
Importantly, following interaction with Ubiquilin1, BCLb protein is more resistant to degradation as shown by treatment of cells with cycloheximide (Fig. P1). Coincident with these alterations, BCLb moves from membranes into the cytosol, and the complex containing Ubiquilin1 and ubiquitinated BCLb is exclusively in the cytosolic fraction.

How does the interaction with Ubiquilin1 relate to human cancer? The answer to this question is likely complex and context dependent, but we offer some insight into a potential role of Ubiquilin1 in human lung adenocarcinoma. Examining the expression of Ubiquilin1 from biopsies of primary lung adenocarcinomas and adjacent normal lung tissues from the same patient, we show that Ubiquilin1 mRNA is elevated in cancer samples. Also, if patients are grouped according to the level of Ubiquilin1 mRNA expression in the tumor samples, tumors with higher Ubiquilin1 mRNA levels are associated with shorter survival times than those with relatively lower Ubiquilin1 mRNA levels.

In the future, we hope to examine in more detail the relationship between Ubiquilin1 and BCLb in mouse models of cancer and human cancer. We propose that high expression of Ubiquilin1 in some human cancers may directly regulate the protein levels of BCLb (or other oncogenic proteins). In this situation, inhibition of Ubiquilin1 activity or expression may reduce the levels of oncogenic proteins, resulting in cancer cell death or susceptibility to cytotoxic drugs.