

BH3-only Members of the Bcl-2 Family Are Critical Inducers of Programmed Cell Death

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Apoptosis is an evolutionarily conserved mechanism for removing unwanted cells. Genetic and biochemical studies have shown that 3 classes of proteins are required for cell killing: cysteine proteases, their adaptor molecules and pro-apoptotic members of the Bcl-2 family. Anti-apoptotic Bcl-2 family members are essential for cell survival. We have cloned Bim and Bmf, two novel pro-apoptotic Bcl-2 family member which have only a BH3 domain but no other region of similarity to this family. In healthy cells Bim is sequestered to the microtubule-associated dynein motor complex by binding to DLC1/LC8 dynein light chain and Bmf is sequestered to the myosin V motor complex on the actin cytoskeleton. Different apoptotic stimuli cause release of Bim or Bmf, liberating them to relocate to Bcl-2 and its homologs and block their anti-apoptotic activity. Bim-deficient lymphocytes, myeloid cells and neurons are resistant to certain, but not all, apoptotic stimuli that can be blocked by Bcl-2 or its homologs. These results demonstrate that distinct death stimuli activate different BH3-only proteins by freeing them to interact with anti-apoptotic family members. Bim-deficient mice have 2- to 5-fold increased numbers of all types of leukocytes, develop progressive plasmacytosis and most succumb to SLE-like autoimmune kidney disease. We have used several model systems to study thymocyte and B cell negative selection (TCR or Ig transgenic mice, super-antigens, anti-CD3 or anti-Ig antibody treatment) and found that Bim is essential for apoptotic cell death of autoreactive thymocytes and immature as well as mature B lymphocytes. These results demonstrate that the pro-apoptotic BH3-only protein Bim is essential for programmed cell death during hematopoiesis, for leukocyte homeostasis and imposes a vital barrier against autoimmunity. We are currently generating mice lacking the other BH3-only proteins to identify their essential functions in development.

Suggested reading:

- (1) Bouillet, P., Metcalf, D., Huang, D. C. S., Tarlinton, D. M., Kay, T. W. H., Köntgen, F., Adams, J. M., and Strasser, A. (1999). Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286, 1735-1738.
- (2) Puthalakath, H., Villunger, A., O'Reilly, L. A., Beaumont, J. G., Coultas, L., Cheney, R. E., Huang, D. C. S., and Strasser, A. (2001). Bmf: a pro-apoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science* 293, 1829-1832.
- (3) Bouillet, P., Purton, J. F., Godfrey, D. I., Zhang, L.-C., Coultas, L., Puthalakath, H., Pellegrini, M., Cory, S., Adams, J. M., and Strasser, A. (2002). BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 415, 922-926.
- (4) Marsden, V., O'Connor, L., O'Reilly, L. A., Silke, J., Metcalf, D., Ekert, P., Huang, D. C. S., Cecconi, F., Kuida, K., Tomaselli, K. J., Roy, S., Nicholson, D. W., Vaux, D. L., Bouillet, P., Adams, J. M., and Strasser, A. (in press). Bcl-2 regulated apoptosis via caspase activation upstream of cytochrome c /Apaf-1/caspase-9. *Nature*.