Emetine and the Alternative Splicing of Bcl-X: Where to next?

Jacqueline C. Shultz[1] and Charles E. Chalfant[1,2,3,*]
[1]Department of Biochemistry, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0614, USA
[2]Hunter Holmes McGuire Veterans Administration Medical Center, Richmond, VA 23249, USA
[3]The Massey Cancer Center, Richmond, VA 23298, USA
*Correspondence: cechalfant@vcu.edu
DOI 10.1016/j.chembiol.2007.12.002

Interference with the alternative splicing of apoptotic factors offers an innovative and specific mechanism to target malignant cells. In this issue of *Chemistry & Biology*, Zhou et al. [1] report on the regulation of the alternative splicing of Bcl-x pre-mRNA in response to emetine, a potent protein synthesis inhibitor, as well as define a major player in the signaling mechanism.

Apoptosis is a mechanism by which cells execute endogenous programs of cell death, often in response to adverse external or internal signals. It is now well known that the inactivation or deregulation of apoptotic pathways can lead to tumor development and chemotherapy resistance. The relationship between apoptosis and cancer was originally established in 1988 when David Vaux and colleagues demonstrated that the *bcl-2* gene specifically inhibits the death of B cells in follicular lymphoma. Several years later, Stanley Korsmeyer, David Hockenbery, and colleagues characterized *bcl-2* as a "brake" gene, acting to prevent apoptosis. Since then, a number of proteins related to Bcl-2 have been grouped into a collective Bcl-2 family, which has been widely implicated in regulating apoptotic machinery.

One member of the Bcl-2 family, Bcl-x, is a well-established modulator of apoptosis induced by a multitude of stimuli [2–4]. The regulation of Bcl-x expression is a complex mechanism involving both transcriptional and posttranscriptional processes [2, 5]. In regards to posttranscriptional processing, the *bcl-x* gene produces two main isoforms via alternative splicing: the antiapoptotic Bcl-x_L protein and the proapoptotic Bcl-x_S variant [5]. Bcl-x_S is produced via activation of an alternative upstream 5′ splice site in exon 2 of the Bcl-x pre-mRNA transcript, whereas Bcl-x_L is produced when the entirety of exon 2 is included in the mature mRNA transcript. In a biological context, this mechanism of alternative splicing has consequences in cell signaling. For example, numerous studies have shown that overexpression of the Bcl-x_L isoform in cells elicits resistance to apoptotic stimuli and cooperates with oncogenic factors (e.g., c-Myc) in tumorigenesis [6–8]. In addition, many cell types spontaneously resistant to chemotherapeutic agents exhibit increased levels of Bcl-x_L [9, 10]. In contrast, the scientific literature has shown that expression of the antagonistic splice variant, Bcl-x_S, will induce apoptosis, alleviate multidrug resistance, and overcome Bcl-x_L overexpression to confer apoptotic sensitivity [5, 11, 12]. Importantly, relatively low Bcl-x_S expression can overcome Bcl-x_L blockage of apoptosis [3, 4]. The mechanism by which Bcl-x_S confers sensitivity to apoptotic stimuli is largely unstudied as Bcl-x_S has been shown not to compete with Bcl-x_L for binding to other apoptotic proteins [3, 4].

In recent years, the alternative pre-mRNA splicing of apoptotic factors has been given greater attention in cancer research. For example, both Sharp and colleagues and Krainer and colleagues have demonstrated that RNA *trans*-factors can act as oncogenes [13, 14]. It has also been discovered that splice variants of a variety of specific signaling factors have an opposite/dominant-negative function, and dysregulation of alternative splicing is also a common characteristic of human cancer. Since the normal control of the apoptotic process is often disturbed in transformed cells, scientists such as Zhou and colleagues are now focused on understanding how the production of proapoptotic and antiapoptotic splice variants are regulated, and uncovering new ways to interfere with splicing control, specifically the alternative splicing of Bcl-x pre-mRNA.

Several previous reports have demonstrated that the alternative splicing of Bcl-x pre-mRNA can be regulated by small molecules such as ceramide, as well as receptor agonists such as Il-6 and Il-1α [5, 15, 16]. In this issue of *Chemistry & Biology*, Zhou and colleagues report on the role of emetine on the alternative pre-mRNA splicing of Bcl-x [1]. In efforts to identify additional small molecules responsible for regulating Bcl-x splicing, Zhou and colleagues screened 1040 FDA approved drugs and compounds using RT-PCR analysis in C33A cells, a cervical cancer cell line. Emetine, a potent protein synthesis inhibitor, was demonstrated to downregulate Bcl-x_L mRNA with a concomitant increase in the level of Bcl-x_S mRNA in a dose- and time-dependent manner. Interestingly, cycloheximide also regulated alternative splicing of exon 2 in the *bcl-x* gene. After confirming the emetine and cycloheximide-mediated effect on Bcl-x splicing, the authors proceeded to perform cell-based experiments to determine phosphorylation dependency. C33A and PC3 cells were treated with calyculin A and okadaic acid, two phosphatase inhibitors. Results demonstrated that treatment of C33A and PC3 cells with calyculin A, an inhibitor of both protein phosphatase-1 (PP1) and protein phosphatase-2A

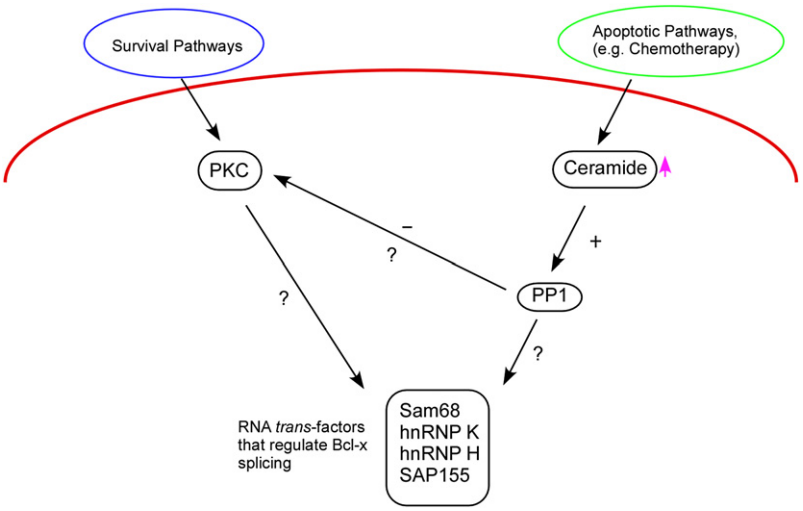

Figure 1. Known Signal Transduction Pathways and RNA *Trans*-Factors That Regulate the Alternative Splicing Bcl-X Pre-mRNA

(PP2A), completely blocked the emetine effects on Bcl-x splicing in contrast to okadaic acid, a selective PP2A inhibitor. Thus, Zhou and colleagues concluded that emetine exerts its effect on Bcl-x splicing via protein phosphatase-1. This method of action is similar to what has been described for ceramide and the chemotherapeutic agent gemcitabine. Specifically, treatment of A549 lung adenocarcinoma cells with cell-permeable ceramide or gemcitabine downregulated Bcl-x_L mRNA levels with a concomitant increase in the levels of Bcl-x_S mRNA in a dose- and time-dependent manner [5]. Similarly, PP1 was also demonstrated to mediate the ceramide-induced effect on Bcl-x splicing [5]. The studies by Zhou and colleagues not only extend these findings, but also demonstrate the translatability of this system to cervical and prostate cancer.

These studies by Zhou and colleagues have largely defined the signal transduction pathway leading to the activation of the Bcl-x_S 5′ splice site in response to protein synthesis inhibitors such as emetine. These are exciting studies, and future studies by Zhou and colleagues as well as other laboratories will hopefully pursue the identification of both the apoptotic and prosurvival pathways of signal transduction that regulate the fate of a cell, and thus, a whole organism. In this regard, two recent studies by Sette and colleagues [17] and Chabot and colleagues [18] are also beginning to shed light on possible survival pathways, which regulate this RNA splicing mechanism. All of these studies now present an intriguing question for the scientific community to answer: Does ceramide, emetine, and gemcitabine affect the 5′ splice site selection of Bcl-x pre-mRNA via a direct apoptotic signaling mechanism or indirectly by "shutting down" constitutive survival pathways (Figure 1)?

REFERENCES

1. Boon-Unge, K., Yu, Q., Zou, T., Zhou, A., Govitrapong, P., and Zhou, J. (2007). Chem. Biol. *14*, this issue, 1386–1392.
2. Boise, L.H., Gottschalk, A.R., Quintans, J., and Thompson, C.B. (1995). Curr. Top. Microbiol. Immunol. *200*, 107–121.
3. Minn, A.J., Boise, L.H., and Thompson, C.B. (1996). Genes Dev. *10*, 2621–2631.
4. Sumantran, V.N., Ealovega, M.W., Nunez, G., Clarke, M.F., and Wicha, M.S. (1995). Cancer Res. *55*, 2507–2510.
5. Chalfant, C.E., Rathman, K., Pinkerman, R.L., Wood, R.E., Obeid, L.M., Ogretmen, B., and Hannun, Y.A. (2002). J. Biol. Chem. *277*, 12587–12595.
6. Pena, J.C., Rudin, C.M., and Thompson, C.B. (1998). Cancer Res. *58*, 2111–2116.
7. Pena, J.C., Thompson, C.B., Recant, W., Vokes, E.E., and Rudin, C.M. (1999). Cancer *85*, 164–170.
8. Naik, P., Karrim, J., and Hanahan, D. (1996). Genes Dev. *10*, 2105–2116.
9. Gardner, C.R. (2004). Expert Rev. Anticancer Ther. *4*, 1157–1177.
10. Kim, R. (2005). Biochem. Biophys. Res. Commun. *333*, 336–343.
11. Sumantran, V.N., Lee, D.S., Woods Ignatoski, K.M., Ethier, S.P., and Wicha, M.S. (2000). Neoplasia *2*, 251–260.
12. Massiello, A., Salas, A., Pinkerman, R.L., Roddy, P., Roesser, J.R., and Chalfant, C.E. (2004). J. Biol. Chem. *279*, 15799–15804.
13. Cheng, C., and Sharp, P.A. (2006). Mol. Cell. Biol. *26*, 362–370.
14. Karni, R., de Stanchina, E., Lowe, S.W., Sinha, R., Mu, D., and Krainer, A.R. (2007). Nat. Struct. Mol. Biol. *14*, 185–193.
15. Li, C.Y., Chu, J.Y., Yu, J.K., Huang, X.Q., Liu, X.J., Shi, L., Che, Y.C., and Xie, J.Y. (2004). Cell Res. *14*, 473–479.
16. Pollock, A.S., Turck, J., and Lovett, D.H. (2003). FASEB J. *17*, 203–213.
17. Paronetto, M.P., Achsel, T., Massiello, A., Chalfant, C.E., and Sette, C. (2007). J. Cell Biol. *176*, 929–939.
18. Revil, T., Toutant, J., Shkreta, L., Garneau, D., Cloutier, P., and Chabot, B. (2007). Mol. Cell Biol. *27*, 8431–8441.

