Bcl-2 targeting ryanodine receptors: more than apoptosis?

Tim Vervliet¹, Elke Decrock², Jordi Molgó³, Vincenzo Sorrentino⁴, Ludwig Missiaen¹, Luc Leybaert², Humbert De Smedt¹, Nael Nadif Kasri⁵, Jan B. Parys¹ and Geert Bultynck¹

¹KULeuven, Leuven, Belgium
²University of Ghent, Ghent, Belgium
³Institut de Neurobiologie Alfred Fessard, Gif sur Yvette cedex, France
⁴University of Siena, Siena, Italy
⁵Donders Institute for Brain, Radboud University, Nijmegen, Netherlands

Anti-apoptotic B-cell lymphoma 2 (Bcl-2) proteins counteract apoptosis at the mitochondria by scaffolding pro-apoptotic Bcl-2-family members, but also act at the endoplasmic reticulum, controlling intracellular Ca²⁺ signalling. Bcl-2 suppresses Ca²⁺ release by targeting the inositol 1,4,5-trisphosphate receptor (IP₃R). The Bcl-2-binding site on the IP₃R shows striking similarities to a site present in all ryanodine receptor (RyR) isoforms. We now show that Bcl-2 interacts with RyRs in ectopic expression systems and in rat hippocampus. Detailed molecular studies (including SPR) revealed that Bcl-2, via its BH4, binds to purified RyR domains containing the putative binding site. Bcl-2 overexpression inhibited caffeine-induced Ca²⁺ release in RyR-expressing HEK293 cells. Consistent with the ability of the biotinylated BH4 domain to bind RyRs, a BH4-Bcl-2 peptide was sufficient to suppress RyR-mediated Ca²⁺ release in HEK293 cells and dissociated rat hippocampal neurons. Hence, these data indicate that besides IP₃Rs Bcl-2 targets RyR channels. Yet, while the BH4 domain of Bcl-XL fails to bind to and inhibit IP₃Rs, due to a critical conserved amino acid difference with BH4-Bcl-2 (i.e. Asp11 in Bcl-XL versus Lys17 in Bcl-2), BH4-Bcl-XL could target RyR channels, indicating that the binding determinants for complex formation with Bcl-2/Bcl-XL are similar for IP₃Rs and RyRs, but not identical. These data now set the stage for discovering novel biological functions for anti-apoptotic Bcl-2 proteins by targeting RyR channels in different cell types.