Oral presentation

Structural analysis of Bak in complexed with Pxt1, a noncanonical BH3-only protein

Dahwan Lim

gbori28@gmail.com

Peroxisomal testis-specific 1 (Pxt1) is exclusively expressed in the mammalian testis and induces germ cell apoptosis upon overexpression. However, how Pxt1 regulates cell death has been poorly defined. In this study, we investigated direct binding between Bak and the BH3 domain of Pxt1 by a combination of structural, biochemical and cellular studies. Isothermal titration calorimetry (ITC) measurements showed that the Pxt1 BH3 peptide interacted with recombinant Bak with 15.4 □M. The Bak-Pxt1 BH3 complex crystals was obtained in 0.1 M sodium citrate (pH 4.8) and 17% (w/v) polyethylene glycol 3000. Using the crystals, we determined the structure of Bak bound to Pxt1 BH3 to the 2.2 Å resolution, and analyzed their interactions at the atomic level. In the crystal structure, Pxt1 directly interacts with Bak in the BH3 consensus motif-dependent manner. Binding between Bak and Pxt1 BH3 mainly depends on the four consensus hydrophobic residues and one aspartate residue of Pxt1 that are accommodated into the hydrophobic binding groove. Moreover, additional intermolecular hydrophobic and electrostatic interactions are shown in the complex structure. Alanine substitution of Leu82 and Leu86 of Pxt1 was expected to significantly impair this complex formation that was detected by ITC and immunoprecipitation analysis. Liposome assay showed that Pxt1 BH3 activated Bak and induced the release of dye entrapped in mitochondria-mimicking liposome. Consistently, Pxt1 disrupted the mitochondrial outer membrane and caused the release of cytochrome c to provoke apoptotic cell death in a Bak-dependent manner in HeLa cells. Our results provide the mechanism and atomic details of the molecular association between Bak and Pxt1.