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Role of Bcl-rambo in Apoptosis and Mitophagy

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Short Communication

Bcl-rambo, also known as Bcl-2 Like protein 13 (Bcl-2-L13), with homology to Bcl-2 protein and might contain all of the four conserved Bcl-2 homology (BH) domains, BH1, BH2, BH3 and BH4 (Figure 1) [1,2].

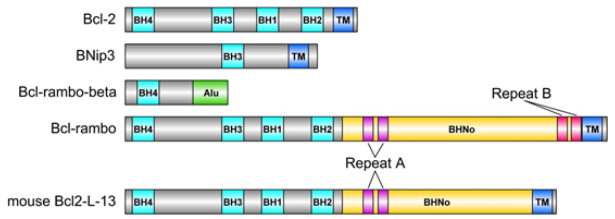

Figure 1: Schematic of the secondary structure of human Bcl-rambo and mouse Bcl-2-L13 compared with some other Bcl-2 family proteins. Human Bcl-rambo includes all four BH domains, and a BHNo domain. Repeat A and Repeat B are included within the BHNo domain, while mouse Bcl2-L-13 does not have Repeat B.

It was first reported in 2001 and was classified as pro-apoptotic member of the Bcl-2 protein family [2]. Its NH2-terminal is stretching into the cytoplasm and a few amino acids on the COOH-terminal are remained in the mitochondrial inter-membrane space (IMS). Bcl-rambo gene is located on the human chromosome 22q11.21 [3,4] with its mRNA expressed in many tissues and cell lines, such as lymph node, human heart and Hela cells.

While previous studies have shown that mitochondrial oriented Bcl-xL requires the COOH-terminal transmembrane (TM) domain to be flanked by at least two basic amino acids at both ends to maintain localization stability [5,6], this fashion is also found in the other Bcl-2 family members, including Bcl-rambo. Besides BH domains, Bcl-rambo has a 250 amino acids fragment in front of the TM domain termed BHNo, which contains two distinct repeat sequences, Repeat A and Repeat B [2]. The presence of BHNo domain significantly enhances the apparent molecular weight of Bcl-rambo showing on SDS-PAGE, and more importantly, it confers a new manner for Bcl-rambo to performance. Apoptosis promoting activity of Bcl-rambo was first reported along with its finding. In many mammalian cell types, Bcl-rambo is located at the mitochondrial outer membrane (MOM) and its overexpression can cause apoptosis [7,8]. The pro-apoptotic activity could be specifically inhibited by the expression of inhibitor of apoptosis proteins (IAPs) [9], while it was not influenced when the inhibitors acting on upstream death receptor pathway or mitochondrial apoptotic pathway were added [10,11]. The pro-apoptotic function of Bcl-rambo is mediated by the BHNo domain and TM domain rather than the BH domains. While Bcl-2 family proteins generally display anti-apoptotic or pro-apoptotic functions through forming hetero- or homodimers [12,13], however, Bcl-rambo does not interact with any of the other Bcl-2 family proteins, nor does it form homodimers [14], even using the BH domains only.

Overexpression of Bcl-rambo also causes apoptosis in HEK293T cells, Hela cells, PC-3 prostate cancer cells and S2 cells, which might through its interaction with adenine nucleotide translocase (ANT) to open the mitochondria membrane permeability transition pore (MPTP), and in turn inducing a cytochrome c (Cyt c) release [14,15]. ANT, together with cyclophilin-D (Cyp D) and voltage-dependent anion channel (VDAC), form the complex of MPTP [16-18]. Different from the pore formed by Bak or Bax to induce MOMP (mitochondrial outer membrane permeabilization), MPTP is a more selective channel for small molecules inside mitochondrial inner membrane (MIM), such as glutathione, Ca^{2+}, etc. However, the open of MPTP sometimes lead to Cyt c release and apoptosis because of the induction of Bak/Bax activation. ANT is localized at the MIM, which plays a role in the cellular oxidative phosphorylation process by exchanging ATP in the mitochondrial matrix with ADP in the cytosol [19]. When interacted with Bcl-rambo, ANT was inactivated (Figure 2).

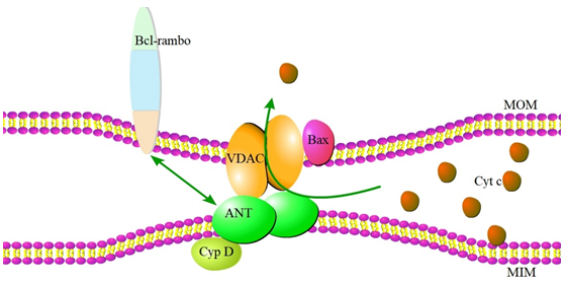

Figure 2: Mechanisms of pro-apoptotic function of Bcl-rambo. Components of MPTP are formed in the closely contact regions of MOM and MIM. The ADP/ATP transposition is inhibited when Bcl-rambo impinges on ANT, and Cyt c escaped into the cytoplasm for the damaged MPTP, and in turn apoptosis may be triggered.

In this case, MOM localization is essential for the pro-apoptotic function of Bcl-rambo, rather than the specificity of the amino acid sequence of TM domain. Bcl-rambo-beta is a splicing variant of Bcl-rambo in human with BH4 domain only, which is generated by a stop

codon within the inserted Alu sequence. It is distributed in the cytoplasm because of lacking the COOH-terminal TM domain [20]. It is surprising that it preserves the pro-apoptotic activity, which might due to its Alu element [21]. On the other hand, Bcl-rambo is highly expressed in a variety of tumors, including childhood acute lymphoblastic leukemia (ALL), gastric cancer, liposarcoma and glioblastoma [22]. However, incompatible to its pro-apoptotic function, elevated expression of Bcl-rambo is strongly associated with poor prognosis in cancer [23]. In some studies, Bcl-rambo overexpressing could inhibit Bax oligomerization and showed an anti-apoptotic function. Bcl-rambo bound to ceramide synthase 2 (CerS2) and ceramide synthase 6 (CerS6) through the BHNo domain, preventing the formation of CerS2/6 complex, therefore inhibiting its pro-apoptotic function (Figure 3) [24-26].

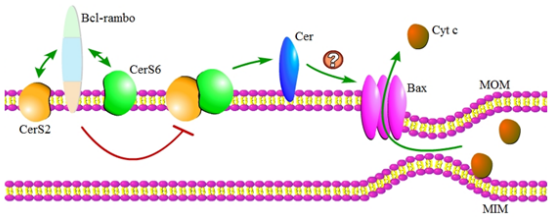

Figure 3: Mechanisms of anti-apoptotic function of Bcl-rambo. In general, CerS2 and CerS6 can form heterodimers Cer, which could promote Bax oligomerization with a not yet fully elucidated mechanism. Bax can form channels after oligomerization and consequently release Cyt c into the cytosol. Bcl-rambo inhibits this process by binding to CerS2 and CerS6 respectively, and exhibits an anti-apoptotic function.

More studies are needed to shed light on that why Bcl-rambo showed opposite functions under different situations? Perhaps we can get some inspiration from the physiological function studies. After ectopically expressing Bcl-rambo in Drosophila, induction of caspase activity was detected in the eye imaging disc of the third instar larvae. The chrysalis showed abnormal phenotype with chest fissure, wing atrophy and rough eye, while there was no significant change in the pupillary retina at the same time [22], suggesting that Bcl-rambo has a stronger pro-apoptotic role in the active cells than in the quiescent cells [26]. Analysis of embryos obtained from in vitro fertilization (IVF) indicates that Bcl-rambo is constitutively expressed during early human embryonic development; however, the cellular localization alteration during its physiological activities is a curious phenomenon [27,28].

Bax is an effector pro-apoptotic Bcl-2 family protein, sharing structural homology with bacterial toxins [29,30], which could permeabilize membranes by forming membrane channels. While Bcl-rambo was involved in regulating Bax mediated MOM permeabilization, however, its mouse homolog was also found to induce mitophagy. Interestingly, another mitophagy receptor, Parkin, was also involved in regulating Bax mediated apoptosis. Parkin directly inhibits the translocation of Bax from cytosol to mitochondria, therefore inhibits Bax mediated apoptosis [31]. PINK1-Parkin mediated mitophagy pathway is the major pathway for the quality control of damaged mitochondria [32,33], in which the degradation of PINK1 protects mitochondria from the toxic effects [34]. The mitochondrial damage still occurs during Bcl-rambo induced apoptosis, so it is still uncertain whether Bcl-rambo can play a role in inducing mitophagy to avoid inadvertent activation of apoptosis [2]. At least it could act as a sensor of the physiological changes within the mitochondria theoretically.

As mentioned above, mouse Bcl2-L-13 could induce mitophagy, which is a homolog of yeast Atg32, and also the homolog of human Bcl-rambo. The significant difference between human Bcl-rambo and mouse Bcl2-L-13 is that the latter has a truncated BHNo domain but does not have the Repeat B domain. Heterologous expression of mouse Bcl2-L-13 in HEK293A cells and Hela cells promotes mitophagy rather than apoptosis [35]. This activity is independent of Parkin, which induces mitophagy through an ubiquitination based pathway [36,37]. Mouse Bcl2-L-13 binds to LC3 via the LC3 interacting region (LIR), the WXXI motif, inducing mitochondrial fragmentation and mitophagy (Figure 4).

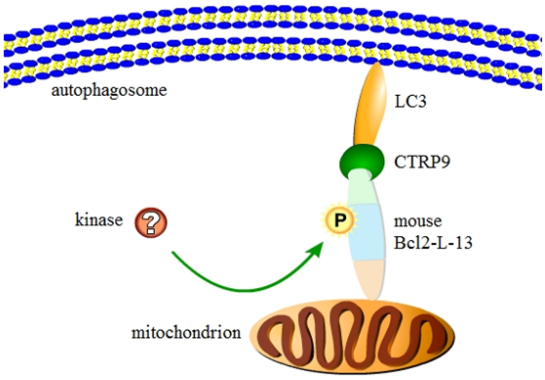

Figure 4: Schematic of mouse Bcl2-L-13-mediated mitophagy. Mouse Bcl2-L-13 contains LIR motif, it interacts with LC3 with the help of CTRP9 to initiate mitophagy. The phosphorylation level of mouse Bcl2-L-13 is positively correlated with LC3 binding, however, the mechanism of which remains to be further studied.

The adipocytokine CTRP9 plays as adaptor protein during the interaction of LC3 and mouse Bcl2-L-13. After mitochondrial fragmentation mediated by the BH domains [38,39], mouse Bcl2-L-13 may require a latent kinase to further activate and attach to LC3. Because human Bcl-rambo has all the domains of mouse Bcl-2-L13 (Figure 1), it will not surprise that human Bcl-rambo also has the function of inducing mitophagy under some situations.

Conclusion

In general, Bcl-rambo can categorize into pro-apoptotic group of the Bcl-2 family, and the BHNo domain confers it a profound change in mode of action compare to canonical fashion. However, sometimes it exhibits anti-apoptotic activity in cancer cells. This paradox may be due to the fact that Bcl-rambo plays a different role in different cell growth states, or some different splicing variants of Bcl-rambo may exert opposite functions. The physiological role of Bcl-rambo is a fascinated field, but the mechanisms have not yet been elucidated. Studies carried out on the Drosophila, human IVF embryos, yeast, and mice have deepened our understanding of the function of Bcl-rambo. The fact that mouse Bcl2-L-13 has a different domain structure might count for its mitophagy inducing function. Although Bcl-rambo behaves differently from other Bcl-2 family proteins, the most similar

protein might be BNip3 [40,41], which also has pro-apoptotic and mitophagy inducing functions. Further studies on Bcl-rambo might get some clues by examining the functions of BNip3.

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References

1. Birkinshaw RW, Czabotar PE (2017) The BCL-2 family of proteins and mitochondrial outer membrane permeabilisation. Semin Cell Dev Biol 72: 152-162.
2. Kataoka T, Holler N, Micheau O, Martinon F, Tinel A, et al. (2001) Bcl-rambo, a novel Bcl-2 homologue that induces apoptosis via its unique C-terminal extension. J Biol Chem 276: 19548-19554.
3. Collins JE, Wright CL, Edwards CA, Davis MP, Grinham JA, et al. (2004) A genome annotation-driven approach to cloning the human ORFeome. Genome Biol 5: 84.
4. Dunham I, Shimizu N, Roe BA, Chissoe S, Hunt AR, et al. (1999) The DNA sequence of human chromosome 22. Nature 402: 489-495.
5. Bertini I, Chevance S, Del Conte R, Lalli D, Turano P (2011) The anti-apoptotic Bcl-x(L) protein, a new piece in the puzzle of cytochrome c interactome. PLoS One 6: e18329.
6. Kaufmann T, Schlipf S, Sanz J, Neubert K, Stein R, et al. (2003) Characterization of the signal that directs Bcl-x(L), but not Bcl-2, to the mitochondrial outer membrane. J Cell Biol 160: 53-64.
7. Schouten M, Fratantoni SA, Hubens CJ, Piersma SR, Pham TV, et al. (2015) MicroRNA-124 and-137 cooperativity controls caspase-3 activity through BCL2L13 in hippocampal neural stem cells. Sci Rep 5: 12448.
8. Banga S, Gao P, Shen X, Fiscus V, Zong WX, et al. (2007) Legionella pneumophila inhibits macrophage apoptosis by targeting pro-death members of the Bcl2 protein family. Proc Natl Acad Sci USA 104: 5121-5126.
9. Ali R, Singh S, Haq W (2018) IAP proteins antagonist: an introduction and chemistry of smac mimetics under clinical development. Curr Med Chem 12. Doi: 10.2174/0929867325666180313112229.
10. Booth LA, Tavallai S, Hamed HA, Cruickshanks N, Dent P (2014) the role of cell signalling in the crosstalk between autophagy and apoptosis. Cell Signal 26: 549-555.
11. Correia C, Lee SH, Meng XW, Vincelette ND, Knorr KL, et al. (2015) Emerging understanding of Bcl-2 biology: Implications for neoplastic progression and treatment. Biochim Biophys Acta. 1853: 1658-1671.
12. Antonsson B (2001) Bax and other pro-apoptotic Bcl-2 family "killer-proteins" and their victim the mitochondrion. Cell Tissue Res 306: 347-361.
13. Suzuki M, Youle RJ, Tjandra N (2000) Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 103: 645-654.
14. Kim JY, So KJ, Lee S, Park JH (2012) Bcl-rambo induces apoptosis via interaction with the adenine nucleotide translocator. FEBS Lett. 586: 3142-3149.
15. Halestrap AP, McStay GP, Clarke SJ (2002) the permeability transition pore complex: another view. Biochem 84: 153-166.
16. Maldonado EN, DeHart DN, Patnaik J, Klatt SC, Gooz MB, et al. (2017) ATP/ADP turnover and import of glycolytic ATP into mitochondria in cancer cells is independent of the adenine nucleotide translocator. J Biol Chem 292: 16969.
17. Parks RJ, Menazza S, Holmström KM, Amanakis G, Fergusson M, et al. (2018) Cyclophilin D-mediated regulation of the permeability transition pore is altered in mice lacking the mitochondrial calcium uniporter. Cardiovasc Res.
18. Springer JE, Prajapati P, Sullivan PG (2018) Targeting the mitochondrial permeability transition pore in traumatic central nervous system injury. Neural Regen Res 13: 1338-1341.
19. Marzo I, Brenner C, Zamzami N, Jürgensmeier JM, Susin SA, et al. (1998) Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 281: 2027-2031.
20. Yi P, Zhang W, Zhai Z, Miao L, Wang Y, et al. (2003) Bcl-rambo beta, a special splicing variant with an insertion of an Alu-like cassette, promotes etoposide - and Taxol-induced cell death. FEBS Lett 534: 61-68.
21. Morales ME, White TB, Streva VA, DeFreece CB, Hedges DJ, et al. (2015) The contribution of alu elements to mutagenic DNA double-strand break repair. PLoS Genet 11: e1005016.
22. Nakazawa M, Matsubara H, Matsushita Y, Watanabe M, Vo N, et al. (2016) The human Bcl-2 family member Bcl-rambo localizes to mitochondria and induces apoptosis and morphological aberrations in Drosophila. PLoS ONE 11: e157823.
23. Yang YL, Lin SR, Chen JS, Lin SW, Yu SL, et al. (2010) Expression and prognostic significance of the apoptotic genes BCL2L13, Livin, and CASP8AP2 in childhood acute lymphoblastic leukemia. Leuk Res 34: 18-23.
24. Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, et al. (1997) Inhibition of Bax channel-forming activity by Bcl-2. Science 277: 370-372.
25. Stiban J, Caputo L, Colombini M (2008) Ceramide synthesis in the endoplasmic reticulum can permeabilize mitochondria to proapoptotic proteins. J Lipid Res 49: 625-634.
26. Jensen SA, Calvert AE, Volpert G, Kouri FM, Hurley LA, et al. (2014) Bcl2L13 is a ceramide synthase inhibitor in glioblastoma. Proc Natl Acad Sci USA 111: 5682-5687.
27. Boumela I, Assou S, Haouzi D, Déchaud H, Aït-Ahmed O, et al. (2014) Developmental regulated expression of anti-and pro-apoptotic BCL-2 family genes during human early embryonic development. Curr Med Chem 21: 1361-1369.
28. Fabian D, Koppel J, Maddox-Hyttel P (2005) Apoptotic processes during mammalian preimplantation development. Theriogenology 64: 221-231.
29. Youle RJ, Strasser A (2008) The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 9: 47-59.
30. Westphal D, Kluck RM, Dewson G (2014) Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. Cell Death Differ 21: 196-205.
31. Johnson BN, Berger AK, Cortese GP, Lavoie MJ (2012) The ubiquitin E3 ligase parkin regulates the proapoptotic function of Bax. Proc Natl Acad Sci USA 109: 6283-6288.
32. Sekine S, Youle RJ (2018) PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. BMC Biol 16: 2.
33. Lazarou M, Jin SM, Kane LA, Youle RJ (2012) Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. Dev Cell 22: 320-333.
34. Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, et al. (2011) PINK1 cleavage at position A103 by the mitochondrial protease PARL. Hum Mol Genet. 20: 867-879.
35. Tomokazu M, Osamu Y, Ayako H, Shungo H, Toshihiro T, et al. (2015) Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. Nat Commun 6: 7527.
36. Hu L, Dai H (2018) Advances in the Study of Mitophagy. Chinese Journal of Cell Biology 40: 594-601.
37. Yamano K, Wang C, Sarraf SA, Münch C, Kikuchi R, et al. (2018) Endosomal Rab cycles regulate Parkin-mediated mitophagy. Elife 7. pii: e31326.
38. Weidberg H, Shvets E, Shpilka T, Shimron F, Shinder V, et al. (2010) LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J 29: 1792-1802.
39. Otsu K, Murakawa T, Yamaguchi O (2015) BCL2L13 is a mammalian homolog of the yeast mitophagy receptor Atg32. Autophagy 11: 1932-1933.

40. Ray R, Chen G, Vande Velde C, Cizeau J, Park JH, et al. (2000) BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. J Biol Chem 275: 1439-1448.

41. Ney PA (2015) mitochondrial autophagy: Origins, significance, and role of BNIP3 and NIX. Biochim Biophys Acta 1853: 2775-2783.