REGIONAL CENTRE FOR BIOTECHNOLOGY
Seminar series

Targeting γ-herpesvirus Bcl-2 inhibition of autophagy and apoptosis.

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Abstract

γ-Herpesviruses (γHVs) infect 95% of humans. γHVs encode homologs of anti-apoptotic cellular Bcl-2s, critical to viral reactivation and oncogenic transformation. Cellular Bcl-2s like Bcl-X_L inhibit apoptosis by binding to the BH3 domain (BH3D) of pro-apoptotic proteins. We have shown that the key autophagy effector Beclin1 contains a large intrinsically disordered region that includes a BH3D, allowing Bcl-2s to also bind Beclin1 and inhibit autophagy. Our structure of the γHV Bcl-2-Beclin1 BH3D complex, shows that the Beclin1 BH3D binds to a hydrophobic groove on the Bcl-2 surface and has helped to identify residues key for binding to Bcl-2s. Further the Beclin 1 BH3D undergoes a disorder to helix transition upon binding. The Beclin1 BH3D binds with a K_d of ~1 micromolar to both γHV Bcl-2 and Bcl-X_L, and involves the same Beclin1 residues, yet there are subtle differences in residues lining the groove of γHV Bcl-2 and Bcl-X_L, dictating varying affinities for other BH3D-containing proteins. To delineate these differences, we used isothermal titration calorimetry to identify Beclin1 BH3D mutants that bind to γHV Bcl-2, but not to Bcl-X_L. Further, the effect of these mutants on binding to γHV Bcl-2 and Bcl-X_L inside cells was evaluated using co-immunoprecipitation assays and autophagy levels were quantified by counting GFP-LC3-labeled autophagosomes. These data demonstrate that BH3D mutations that knockout Bcl-2 binding, also prevent autophagy inhibition by Bcl-2s and have also allowed us to identify a mutant BH3D that binds to γHV Bcl-2, but not Bcl-X_L. We have now determined the structure of this mutant BH3D in complex with γHV Bcl-2. We have exploited these differences to develop a peptide inhibitor that inhibits γHV Bcl-2, but not Bcl-X_L. Further, we have tested the ability of Bcl-X_L inhibitory drugs like ABT-737, to abrogate autophagy and apoptosis inhibition by γHV Bcl-2. In the future, we will design small-molecule inhibitors that bind selectively to γHV Bcl-2 and prevent γHV Bcl-2-mediated autophagy and apoptosis down-regulation.