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DETERMINING THE ESSENTIAL FUNCTIONS OF DISTINCT PRO-SURVIVAL BCL-2 FAMILY MEMBERS BY USING GENE-SWAP MICE

GUEST LECTURE by

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MC1.F.05.016 (SR Pathology 01, MED Campus, tract F, 5th floor), Med Uni Graz

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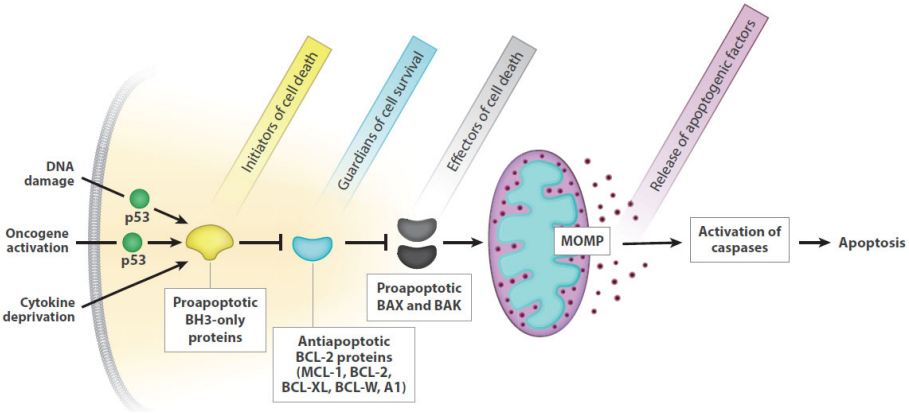
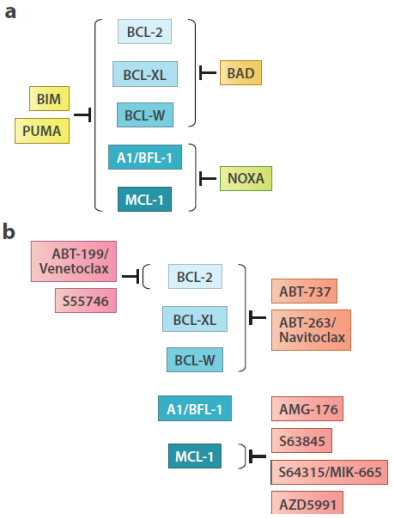
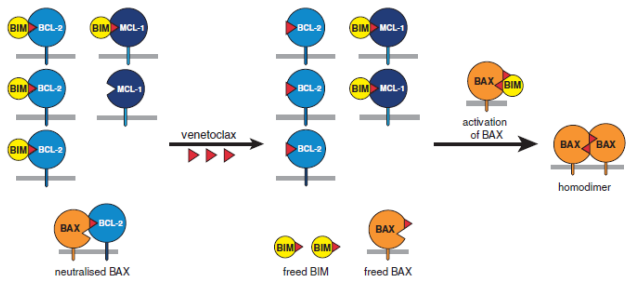


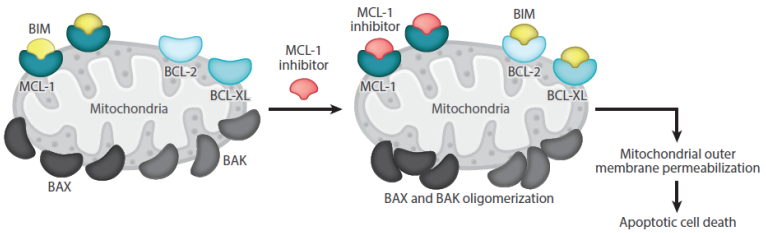
The intrinsic apoptotic pathway is controlled by three groups of proteins of the BCL-2 family of proteins.
Kelly & Strasser (2020) Annu Rev Cancer Biol. 4: 299-313



Specificities of BH3-only proteins (a) and BH3-mimetic drugs (b) for the antiapoptotic BCL-2 family proteins.
Kelly & Strasser (2020) Annu Rev Cancer Biol. 4: 299-313



Killing of 'primed' cancer cells by BH3 mimetics.
Adams & Cory (2018) Cell Death Differ. 25: 27-36



Mode of action of an MCL-1-specific BH3-mimetic drug to kill cells.
Kelly & Strasser (2020) Annu Rev Cancer Biol. 4: 299-313

Determining the essential functions of distinct pro-survival BCL-2 family members by using gene-swap mice

Proper control of programmed cell death, apoptosis, is critical for normal embryonic development and tissue homeostasis in adulthood. Accordingly, defects in apoptosis, for instance caused by the overexpression of pro-survival members of the BCL-2 protein family, are implicated in a broad range of diseases, particularly cancer and autoimmunity[1]. The pro-survival BCL-2 family member *Mcl-1* is among the top 10 oncogenes that is amplified in human cancers[2]. Inhibitors of MCL-1 have recently entered clinical trials, however despite promising pre-clinical results, certain concerning side effects were observed[2]. In order to apply BH3-mimetic drugs that target MCL-1 safely and effectively in the clinic there is an urgent need to better understand the properties of this protein. Amongst the 5 mammalian pro-survival BCL-2 family members, that inhibit the mitochondrial apoptotic pathway, MCL-1 is unique as it is essential for early embryonic development and the survival of many cell types that are not impacted by the loss of any of the other pro-survival BCL-2 proteins. To date it remains unclear what characteristics of MCL-1 make it so unique; is it the protein structure or short half-life, binding affinity to the different pro-apoptotic proteins, or its purported non-apoptotic functions in mitochondrial structure and energy production that have been proposed? For the clinical testing and hopefully implementation of MCL-1 inhibiting drugs, it is important to know whether apoptosis-unrelated functions of MCL-1 do actually exist and, if so, whether they contribute to the toxicity to normal tissues - and/or efficacy against malignant cells - of MCL-1 inhibitors.

To identify the unique functions of MCL-1 we generated “gene-swap” mice by replacing its coding region with that for BCL-XL, BCL-2 or A1. As control, the MCL-1 coding region was replaced with an *Mcl-1* cDNA. We found that in contrast to the E3.5 embryonic lethality of *Mcl-1*$^{-/-}$ mice, homozygous *Bcl-X>Mcl-1* and *Bcl-2>Mcl-1* gene-swap mice developed until E12 and beyond. This reveals that there is no unique apoptosis-unrelated functions of MCL-1 that is essential for early embryogenesis. Flow cytometric analysis demonstrated that adult heterozygous *Bcl-X>Mcl-1* and *Bcl-2>Mcl-1* gene-swap mice have increased numbers of certain haematopoietic cell subsets. This reveals that replacement of short-lived MCL-1 with long-lived BCL-XL or BCL-2 causes increased survival and accumulation of cells. Thus, the different pro-survival BCL-2 family members with their distinct biochemical properties (protein lifespan, binding to pro-apoptotic BCL-2 family members) must have evolved to provide adequate control of cell survival in a cell type specific, developmental checkpoint specific and stress condition specific manner.

1. Adams, J. M. & Cory, S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell death and differentiation* **25**, 27-36, doi:10.1038/cdd.2017.161 (2018).
2. Kelly, G. L. & Strasser, A. Toward Targeting Antiapoptotic MCL-1 for Cancer Therapy. *Annual Review of Cancer Biology* **4**, 299-313, doi:10.1146/annurev-cancerbio-030419-033510 (2020).