U-CLL (Unmutated-Chronic Lymphocytic Leukaemia) and M-CLL (Mutated-Chronic Lymphocytic Leukaemia), suggesting a possible correlation between the I卜k expression levels and aggressiveness of disease. Recently, we provided the evidence of I卜k as a prognostic marker of CLL progression.

**Material and methods** I卜k*−/−* mice were bred with congenic Em-myc transgenic mice, a pre-clinical mouse model of Non-Hodgkin’s Lymphoma, to generate I卜k*−/−*Em-myc mice, which were monitored for survival and tumour development. We used flow-cytometry for B cells immunophenotyping. Primary murine B cells were compared using different approaches: proliferation, cell viability and cell death assays.

**Results and discussions** Our data provide the evidence that I卜k gene increases survival and delays tumour onset in Em–myc mice. I卜k*−/−*Em-myc mice mostly developed pre-B lymphoma and to a lesser extent mature B lymphoma, which was consistent with the tumour phenotype of Em-myc mice. Loss of I卜k substantially reduced the number of premalignant B-lymphoid cells without affecting their proliferation rate. In particular, pre-cancerous immature B cells were reduced in bone marrow and spleen of I卜k*−/−*Em-myc compared to I卜k*+/−* Em-myc mice. In Em-myc mice, the pre-cancerous state is characterised by aberrant proliferation of B-lymphoid cells, which is initially offset by pro-apoptotic action of c-Myc. We have previously shown that the enhanced expression of I卜k in CLL cells down regulate the expression of pro-apoptotic genes, thus counteracting apoptosis. According to this hypothesis, we found an increased spontaneous apoptosis of pre-cancerous I卜k*−/−*Em-myc B cells *ex vivo* and *in vitro* without added cytokines.

**Conclusion** c-Myc is abnormally expressed in a great majority of human cancers. The evidence that I卜k promotes the survival of c-Myc–driven premalignant B cells could have general implications for oncogenesis. Our findings support a synergistic role of I卜k in Myc-driven B-lymphomagenesis conferring resistance to apoptosis. In summary, this study provides the rationale for novel therapeutic approaches of B-lymphoma.

**Cell Death – Autophagy**

**PO-060** ERBB2/HER2-DEPENDENT DOWNREGULATION OF A TRANSCRIPTION FACTOR IRF6 IN BREAST CANCER CELLS IS REQUIRED FOR THEIR THREE-DIMENSIONAL GROWTH

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**Introduction** The ability to resist anoikis, a form of apoptosis triggered by detachment of non-malignant epithelial cells from the extracellular matrix (ECM), is thought to be critical for breast tumour invasion and metastasis. ErbB2/Her2, an oncprotein often overproduced by breast cancer cells, blocks anoikis of breast cancer cells via mechanisms that are not well understood. Our goal is to understand these mechanisms.

**Material and methods** We studied the expression of a transcription factor IrF6, an important mediator of the mammary gland homeostasis, in non-malignant breast epithelial cells MCF-10A and non-malignant primary human mammary epithelial cells (HMEC) before and after their detachment from the ECM. We also examined IrF6 expression in detached ErbB2-overproducing breast cancer cells. We used small interfering RNAs (siRNAs) to knock down IrF6 in the non-malignant cells, infected breast tumour cells with an IrF6-encoding retrovirus to overexpress IrF6 and measured detachment-induced apoptosis of the cells before and after the indicated changes in IrF6 expression.

**Results and discussions** We found that detachment of MCF-10A or HMEC cells upregulates IrF6 and that IrF6 upregulation promotes their anoikis. We established that ErbB2 downregulates IrF6 in detached breast cancer cells. We also found that an anti-ErbB2 antibody trastuzumab and a small molecule ErbB2 inhibitor lapatinib used for ErbB2-positive breast cancer treatment upregulate IrF6 in ErbB2-overproducing detached human breast cancer cells but not in the isogenic variants of these cells selected for, respectively, trastuzumab and lapatinib resistance. Moreover, we demonstrated that ectopic IrF6 causes anoikis of ErbB2-overproducing breast cancer cells and blocks their anchorage-independent growth. We found that the effect of ErbB2 on IrF6 requires the activity of a protein kinase Mek, a mediator of ErbB2 signalling. We also observed that detachment-induced IrF6 upregulation in MCF10A cells is mediated by a transcription factor deltaNp63 and that deltaNp63 expression is blocked by ErbB2 or an activated Mek mutant in detached ErbB2-overexpressing breast cancer cells.

**Conclusion** Our data indicate that deltaNp63-dependent IrF6 upregulation causes anoikis of non-malignant breast epithelial cells, and that ErbB2/Mek-driven deltaNp63 downregulation and the resulting IrF6 loss blocks this anoikis. Thus, we have identified a novel mechanism by which ErbB2, a major oncoprotein, promotes anchorage-independent growth of breast cancer cells.

**PO-061** BCL-2 FAMILY OF PROTEINS, BCL-XL AND MCL-1, REGULATE APOPTOSIS AND CANCER CELL SURVIVAL BY DIFFERENT MECHANISMS

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**Introduction** Abnormal cell survival through resistance to apoptosis is a cardinal feature of most malignancies and plays a key role in chemoresistance. As the major regulators of the intrinsic mitochondrial pathway of apoptosis, the pro-survival BCL-2 family proteins (BCL-2, BCL-XL and MCL-1) are attractive targets for novel cancer therapeutics. The concept of employing BH3 mimetics (small molecule inhibitors of the BCL-2 family) as anticancer agents has been substantiated by the efficacy of selective drugs, such as navitoclax and venetoclax, in treating BCL-2-dependent haematological malignancies. However, most solid tumours depend for survival on both BCL-XL and MCL-1, which are highly amplified in multiple cancers. Several MCL-1 inhibitors that have been generated so far and demonstrate early promise *in vivo* but most fail to exhibit specificity and potency in a cellular context.

**Material and methods** All cell lines and primary cells, BH3 mimetics and antibodies, unless received as gift from our collaborators, were purchased from commercial vendors. Differential scanning fluorimetry (DSF) was performed using a
StepOnePlus Real-Time PCR machine. The extent of cytochrome c release was quantitated using immunofluorescence and apoptosis assessed using an Attune NxT flow cytometer. Gel filtration and immunoprecipitation experiments were performed according to standard protocols. Statistical analysis was performed using a two-way ANOVA with p values: * for p≤0.05, ** for p≤0.005 and *** for p≤0.001.

Results and discussions Employing a rapid DSF-based assay, we screened a panel of BH3 mimetics to identify that only S63845 and to a smaller extent, A-1210477, demonstrated enhanced binding to MCL-1 that correlated with a rapid, concentration-dependent apoptosis in relevant cell lines. At higher concentrations, S63845 also appeared to weakly bind BCL-2. Furthermore, S63845 synergized with other BH3 mimetics to induce apoptosis in several cancer cell lines. However, in the colorectal HCT-116 cells, BCL-XL-regulated apoptosis required all known BH3-only members, whereas apoptosis and cellular proliferation regulated by MCL-1 appeared to occur independently of all known BH3-only proteins.

Conclusion The anti-apoptotic and cell survival roles of BCL-XL and MCL-1 could be distinct, as antagonising BCL-XL induced BH3-dependent apoptosis, whereas MCL-1 appeared to regulate apoptosis even in the absence of all known BH3-only proteins.

BAX AND BAK INTERACTION WITH THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP) IS REQUIRED FOR TAXOL-MEDIATED APOPTOSIS

Introduction Microtubule Interfering Agents (MIA’s), such as Taxol (Paclitaxel) are used in the treatment of cancers such as breast, ovarian and non-small cell lung cancer. MIA’s arrest cells in mitosis by activating the mitotic checkpoint. Prolonged mitotic arrest results in apoptotic cell death although the precise mechanism of Taxol-induced cell death is unclear. Previous studies have shown that Taxol activates the pro-apoptotic effector proteins, Bak and Bax, which accumulate at the mitochondrial outer membrane. At the mitochondrial Bak and Bax are thought to oligomerize and/or interact with the Mitochondrial Permeability Transition Pore (MPTP) to increase the permeability of the mitochondrial outer membrane which then leads to cytochrome c release and apoptosis. In this study we have examined the requirement for Bak and Bax in Taxol-induced apoptosis and their possible interaction with components of the MPTP.

Material and methods Human cervical carcinoma (HeLa) cells were treated with either Bax, Bak or both Bax and Bak siRNA, synchronised and treated with Taxol (60 nM) for varying times (0–24 hour). Apoptosis was assessed using either cytochrome c 18 cleavage (M30 antibody), poly (ADP-ribose) polymerase (PARP) cleavage or activation of pro-caspases (3 and 9). Bak and Bax were immunoprecipitated using anti-active Bak (N-20) and anti-active Bax (6A7) antibodies in CHAPS lysis buffer. To identify interacting proteins, Bak and Bax IPs were subjected to peptide mass fingerprint analysis by mass spectrometry. Confocal microscopy was used to examine the intracellular localization of Bax and Bak following Taxol treatment.

Results and discussions The results of our siRNA studies indicate that although Bax and Bak can form homo-oligomers in the absence of each other, both proteins are required for Taxol-induced apoptosis. Our immunofluorescence study shows that Bak and Bax co-localise at mitochondria in the Taxol-arrested cells. Our proteomic and co-IP analyses indicate that Bak and Bax form a complex specifically in the Taxol-arrested cells that also includes components of the MPTP such as the voltage dependent anion channel (VDAC), the adenine nucleotide translocator (ANT2) and Bcl-2.

Conclusion We conclude that the oligomerisation of Bax and Bak is insufficient for Taxol-mediated apoptosis. However, the interaction of activated Bax and Bak with the MPTP appears to be necessary for Taxol-induced apoptosis.

TRITERPENOIDS ISOLATED FROM NATURAL PRODUCT REGULATES TNF(TUMOUR NECROSIS FACTOR)-MEDIATED RIP(RECEPTOR-INTERACTING SERINE/THREONINE-PROTEIN KINASE)1-DEPENDENT APOPTOSIS

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Introduction TNF(Tumour necrosis factor) have important role in NF-kB(Nuclear factor kappa-light-chain-enhancer of activated B cells)-mediated inflammatory response and is a pharmacological target of cancer. Activation of TNF signalling can result in cell survival and also death pathway. However, in most cell types including cancer cells, TNF stimulation does not induces cell death. This fact reflects that TNF-mediated cell death pathway is disrupted in cancer cells, and provides TNF is unsuccessful in anti-tumour therapy as a single agent. Nevertheless, recent studies demonstrated that TNF-mediated cell death sensitises RIP1-dependent apoptosis or necroptosis in a certain conditions, thereby suggesting that regulating TNF-mediated cell death can be new therapeutic target for cancer.

Material and methods 1. Cell culture

MEF(Mouse embryonic fibroblast) cells were cultured in DMEM(Dulbecco’s modified eagle’s medium) supplemented with 10% heat-inactivated FBS(Fetal bovine serum), 2 mM glutamine, antibiotics (100 U/mL penicillinG and 100 µg/mL streptomycin), and were maintained at 37°C in a humidified incubator containing 5% CO2.

2. Immunoblot analysis

Cells were collected and lysed in M2 buffer and 20 µg of the cell lysates were fractionated by 10% SDS polyacrylamide gel and blotted onto PVDF membrane. After blocking with 5% skim milk in PBS/T, the membrane was proved with the relevant antibody and visualised by ECL(Enhanced chemiluminescence).

Results and discussions To identify chemical compound inducing TNF-mediated cell death, we screened triterpenoids isolated from natural product, it is already known that have anticancer effect.

Here, we propose that triterpenoids isolated from natural product induces TNF-mediated RIP1-dependent apoptosis or