**PO-057** GENISTEIN, A MAJOR ISOFLAVONE COMPONENT, SUPPRESSES SRC-INDUCED PROLIFERATIVE ACTIVITY BY ARRESTING CELL CYCLE AT G2/M THROUGH INCREASING THE P53 AND P21 LEVELS

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10.1136/esmoopen-2018-EACR25.101

**Introduction** Src oncogene have been strongly implicated in the development, growth, progression, and metastasis of a variety of human cancers. Although soy isoflavones have been shown to have potential anticancer activity, the role of isoflavones in the oncogenic activity of Src remains unknown. Using HAG-1 human adenocarcinoma cells transfected with v-src, we investigated the functional role of Src in anti-proliferative activity of isoflavones such as genistein, daidzein, glycitin and equol.

**Material and methods** The growth inhibitory activities of those isoflavones against Src- and vehicle-transfected cells (HAG/src and HAG/neo) were investigated using WST-1 cell proliferation assay. Effects of those isoflavones on apoptosis and cell cycle perturbation were evaluated by FACS analyses.

**Results and discussions** The growth of HAG/neo cells was inhibited potently by genistein and equol, but modestly by daidzein and glycitin. In contrast, activated Src conferred resistance to either daidzein, glycitin or equol, but rendered the cells more sensitive to genistein, compared to HAG/neo; Genistein strongly inhibited the growth of HAG/src cells in a dose-dependent manner with IC50 value of 25 μM, whereas in other three isoflavones, the inhibitory effects were minimal without reaching an IC50 even at a dose of 100 μM. Upon treatment with 50 μM genistein for 72 hour, HAG/src cells were significantly arrested at the G2/M compared to HAG/neo cells (37.7% versus 7.0%). By contrast, the same concentration of either daidzein, glycitin or equol could not arrest HAG/src cells at any checkpoint of the cell cycle. The sub-G0/G1 apoptotic cell populations were not increased following 72 hour exposure with either isoflavones. Therefore, it appears that growth inhibition by genistein in Src-activated cells would be mediated mainly by the G2/M arrest of cell cycle rather than apoptosis induction. Genistein increased the expression levels of p53 and p21 with decreased phosphorylated p21. The levels of other main cell cycle-related proteins such as cyclin B, cyclin E, CDK2, and cc2d were not altered. These data suggest that genistein would be the only isoflavone component that may potentially suppress oncogenic activity driven by Src through increasing the p53 and p21 levels.

**Conclusion** These data suggest that genistein would be the only isoflavone component that may potentially suppress oncogenic activity driven by Src, providing a mechanistic rationale for the potential use of genistein in the prevention and treatment of human cancers with activated Src.

**PO-058** UNRAVELLING THE PROTECTIVE ROLE OF ANDROGENS/ANDROGEN RECEPTOR IN BREAST CANCER: WHEN BAD GOES GOOD

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10.1136/esmoopen-2018-EACR25.102

**Introduction** Androgen receptor (AR) expression in breast cancer growth and progression appears to be clinically relevant and disease context specific. In oestrogen receptor (ER)α-positive primary breast cancers, AR positivity correlates with lower tumour grade and a better clinical outcome. These clinical-pathological findings mirror the capability of androgens to counteract ERα-dependent proliferation in both normal mammary epithelium and ERα-positive breast cancer preclinical models in which androgen/AR-dependent pro-apoptotic effects have been also evidenced.

Here we report a novel additional mechanism underlining the protective, anti-proliferative role exerted by AR signalling. This mechanism involves modulation of the expression, cellular distribution and function of BAD, a pro-apoptotic member of the Bcl-2 family proteins, whose expression is related to a significantly better disease free survival in (ER)α-positive human breast cancers.

**Material and methods** MCF-7, TD47D, ZR-75 breast cancer cells; qReal Time PCR; western blotting (WB); immunofluorescence analysis (IF); immunoprecipitation assay (IP); DNA affinity precipitation assay; Chromatin Immunoprecipitation Assay.

**Results and discussions** The expression of a panel of pro/anti-apoptotic proteins was investigated in cellular protein lysates from ERα/AR-positive MCF-7 cells cultured for 1, 3 and 6 days under androgen treatment. The expression of the anti-apoptotic Bcl-2 protein, or the pro-apoptotic BID and BAX remained unchanged, while a sustained increase in the expression of the pro-apoptotic BAD could be observed, reducing the Bcl-2/BAD ratio and, thus, shifting the delicate balance between inhibitors and inducers of cell death. Interestingly, androgens induced a marked BAD levels increase into the nuclear compartment in ERα/AR-positive MCF-7, T47D and ZR-75 as well as in ERα negative/AR-positive SKBR3 cells. The androgen-regulated intracellular localization of BAD involved an AR/BAD physical interaction, suggesting a nuclear role for BAD upon androgen stimulation. Indeed, androgens induced both AR and BAD recruitment at a AP-1 and at a ARE site within the cyclin D1 promoter region, contributing to explain the anti-proliferative effect of androgens in breast cancer cells.

**Conclusion** We defined a novel mechanism by which androgens modulate BAD expression and force its ability to act as a cell cycle inhibitor through modulation of cyclin D1 gene transcriptional activity, strengthening the protective role of androgen signalling in estrogen-responsive breast cancer.

**PO-059** IBTK PROMOTES B CELL LYMPHOMAGENESIS IN Eμ-MYC TRANSGENIC MICE CONFERRING RESISTANCE TO APOPTOSIS

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10.1136/esmoopen-2018-EACR25.103

**Introduction** Ibtk acts as substrate receptor of a Cullin 3-dependent E3 ligase, and promotes proteasomal degradation of Pdcd4, a translation inhibitor. There are evidences that Ibtk is involved in cell survival. RNA interference of Ibtk reduced viability of Ras-positive colorectal cancer cells and mouse embryonic fibroblasts under reticulum stress. Differential methylation of the Ibtk genomic region was reported for
U-CLL (Unmutated-Chronic Lymphocytic Leukaemia) and M-CLL (Mutated-Chronic Lymphocytic Leukaemia), suggesting a possible correlation between the IBtk expression levels and aggressiveness of disease. Recently, we provided the evidence of IBtk as a prognostic marker of CLL progression.

**Material and methods** *Ibtk*-/- mice were bred with congenic *Em-myC* transgenic mice, a pre-clinical mouse model of Non-Hodgkin’s Lymphoma, to generate *Ibtk*-/-*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 *Ibtk* gene increases survival and delays tumour onset in *Em-myC* mice.*Ibtk*-/-*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 *Ibtk* 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 *Ibtk*-/-*Em-myC* compared to *Ibtk*+/+ *Em-myC* mice. In *Em-myC* mice, the pre-cancerous stage 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 IBtk 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 *Ibtk*-/-*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 IBtk promotes the survival of c-Myc-driven premalignant B cells could have general implications for oncogenesis. Our findings support a synergistic role of IBtk 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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10.1136/esmoopen-2018-EACR25.104

**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 oncoprotein 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 upregulated 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.