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 cdc2 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 RECEPTORIN 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