Cell Proliferation – Cell Cycle

PROLACTIN ACTIVATION OF JAK2/STAT3 SIGNALLING PATHWAY THROUGH GHR IN NSCLC

**PO-183**

**INTRODUCTION**

Milk secretion is stimulated by prolactin, a hormone secreted from anterior pituitary gland. It is known that prolactin could lead breast and prostate cancer progression. Recent reports point out the concentration of serum prolactin in non small cell lung cancer (NSCLC) patients were significantly higher than health people. The patients with higher concentration of serum prolactin have lower survival rate than normal level on serum prolactin patients. PRL receptor (PRLR) expression level, however, was not related to patients’ survival rate. It is unclear whether prolactin promoted lung cancer progression and the action mechanism of prolactin in lung cancer.

**MATERIAL AND METHODS**

Cells were seeded in 96 well plate with complete medium. After 24 hour of plating, cells were serum starved for 16 hour and then treatment with PRL. MTT assay was applied for cell proliferation. The protein level of JAK2/STAT3 and VEGF were determined by western blot. The mRNA level was measured by quantitative real time polymerase chain reaction (q-PCR).

**RESULTS AND DISCUSSIONS**

Our data show that prolactin promoted cell proliferation in NSCLC cells. Expressed growth hormone receptor (GHR), not prolactin receptor (PRLR), was observed in all NSCLC cell lines. Increased expression of p-JAK2 and p-STAT3 were found in cells treated with prolactin. Treatment with prolactin also increased STAT3-regulated downstream gene VEGF mRNA and protein expressions. In contrast, the protein expression of p-JAK2 was decreased after inhibition of GHR.

**CONCLUSION**

Prolactin binds to and activates GHR downstream signalling pathway JAK2/STAT3. Activated STAT3 translocates to nucleus and increases downstream gene VEGF transcription activity. Also, prolactin promotes cell proliferation and VEGF expression in NSCLC cells.

Receptors and Signal Transduction

**PO-184**

**PROTEOMIC PROFILING TO PREDICT RESPONSE TOWARDS THERAPEUTIC MONOCLONAL ANTIBODIES IN HER2 LOW BREAST CANCER.**

**INTRODUCTION**

ERBB family of receptors tyrosine kinases (RTK) are potent drivers of tumorigenesis and metastasis. In breast tumours, 70%-80% of patients, although clinically not classified as HER2 positive, show low or moderate expression of HER2 along with EGFR and ERBB3. Concomitant blockade with specific monoclonal antibodies based on proteomic profile determined by Reverse Phase Protein Array (RPPA), appears as a beneficial approach to improve treatment strategies.

**MATERIAL AND METHODS**

Cancer cells lines from different breast cancer subtypes were selected based on their ERBB expression levels. Cell number was quantified upon exposure to different ligands (HRG1β, EGF, AREG, TGF) and to combinations of monoclonal antibodies targeting EGFR, ERBB2 and ERBB3 (cetuximab, trastuzumab/pertuzumab and lumretuzumab respectively). Using RPPA and over 100 specific antibodies, abundance and phosphorylation states of downstream signalling molecules in an extended MAPK/AKT network were quantified.

**RESULTS AND DISCUSSIONS**

Short time stimulation with ERBB ligands, allowed to determine phosphorylation state of the ERBB receptors and their immediate downstream signalling effectors in different cell lines, and thus, to elucidate which combination therapy would be more effective in reverting such activation. For luminal A cell lines T47D and MCF7, stimulation with HRG1β led to a high phosphorylation levels of ERBB3 in both cell lines, but not for ERBB2, with higher phosphorylation in the T47D cell line. Proteomic activation profiles correlated with efficacy of inhibition after combination treatment with pertuzumab/lumretuzumab. For the triple negative cell line MDA-MB468 and MDA-MB231, response to ligands stimulation did not identify a common activation pattern and thus only blockade of EGFR receptor showed minimal effects in MDA-MB468. Phosphorylation status of downstream signalling effectors in the AKT/MAPK network may help to identify putative targets responsible for incomplete unresponsiveness to ERBB blockade.

**CONCLUSION**

Targeting ERBB members in a HER2 moderate/low scenario seems to be a promising approach when combining targeted therapies. Phenotypic characterisation together with mathematical modelling based on the proteomic activation profile, aroused as a way to better predict response to drug combinations. Better characterisation of ERBB network activation profile in different tumour cell lines and in further tumour tissues, should help to pave the way to improve personalization treatment in HER2 low breast cancers.

**PO-185**

**NON-CANONICAL S727 STAT3 PTM ACTIVATION GOVERNS ITS DIMERIZATION AND DOWNSTREAM FUNCTION IN TRIPLE NEGATIVE BREAST CANCER**

**INTRODUCTION**

Over-expressed proteins controlling signal transduction pathways can be attractive therapeutic targets for triple negative breast cancers (TNBCs). Signal transducer and activator of transcription 3 (STAT3) is a key oncoprogenic molecule which over-expresses in more than 60% of breast cancer cases. So far, activation of STAT3 via pY705 is well established in many cancers, but the role of non-canonical pathway (pS727 and AcK685) in controlling STAT3 function is poorly understood.
Material and methods Immunohistochemical scoring for total-STAT3, pY705 and pS727 was done in 75 paired core-biopsy and post-NACT TNBC samples. Bioinformatics resonance energy transfer based sensor (PhosphoBRET) was developed by N-terminal fusion of STAT3 with Nanoluc (donor) and TurboFP635 (acceptor) and validated on multiple cancer cell lines (MCF7, A549, HT1080, PC3) or drug inhibitors (Niclosamide, Static). We also engineered 3'UTR STAT3shRNA cells overexpressing Nanoluc-STAT3 variants i.e. Wild type [wt], Y705F, S727A, K685R and performed biochemical as well as phenotypic assays to study biological role of STAT3 pathway.

Results and discussions Over 90% TNBC cases showed positive staining for pS727 as compared to pY705 STAT3 indicating hyperactivation of non-canonical pathway. 3'UTR STAT3 shRNA cells over-expressing S27A PTM mutant, showed decreased pY705 expression along with abrogation of K685 acetylation. The level of downstream STAT3 targets like Myc, cyclin D1 etc. remained intact while some of the novel target genes (Her2, E-Cadherin and ERα) showed elevated expression in S727A and K685R mutants. Additionally, S727A or K685R mutant also exhibited 2-fold decrease in overall cell proliferation and survival potential. The designed STAT3-PhosphoBRET sensor demonstrated up to 3-fold gain in BRET ratio (p<0.01) using IL6 or EGF ligand. Further, PhosphoBRET sensor expressing either Y705F or S727A or K685R showed increased BRET ratio (p<0.001) implicating higher STAT3 dimerization and activation. Additionally, the PhosphoBRET platform was also extended to test various STAT3 inhibitors, of which Niclosamide was identified as a potent dual blocker (pS727 and pY705) of STAT3 activation for the first time.

Conclusion Predominant expression of pS727 STAT3 in TNBC cases along with in vitro data implicate crucial role of non canonical (pS727 and acK685) pathway in controlling STAT3 dimerization and downstream function independent of pY705 activation. Hence, future drug design strategy should aim both arms of STAT3 pathway to completely abrogate its oncogenic function.

PO-186 GENERATION OF THE GLI1, GLI2 AND GLI3 KNOCK-OUT OVARIAN CANCER CELL LINES USING THE CRISPR/CAS9

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Introduction Hedgehog signalling pathway is a developmental signalling pathway which is dormant in most adult differentiated tissues, but aberrantly activated in various tumours. In ovarian tumours it can be activated in a canonical way, by the SHH ligand, or the non-canonical way, by upregulation of the GLI transcription factors. Expression of GLI1 is usually associated with tumour progression in a clinical setting, but GL2 and GL3 also play a role by modifying the activity of GLI1 and transcription of their common transcriptional targets. In ovarian cancer, GLI3 protein is expressed in the full-length activator form, and not the shortened repressor form which is the predominant form for GLI3 protein.

Material and methods To study the roles of the three GLI proteins in detail, we used the CRISPR/Cas9 guided gene editing to generate knock-out lines for each of the three GLI proteins. The sgRNA were designed using the online tool at crispr.mit.edu web site, and cloned into the pX330 vector expressing the sgRNA and the Cas9 protein. The sgRNAs were designed to target the region close to the ATG site of each of the three GLI proteins, and after Cas9 nuclease activity the double stranded DNA break should be repaired by non-homologous end joining, which generates indels and leads to frameshift. Frameshift close to the ATG often leads to early termination of the protein, generating a knock-out. The SKOV3 ovarian cancer cell line was transfected with each of the vectors, the cells were split to the density of a single cell/ well in a 96-well plate, and cell lines were expanded from single cells to obtain homogenous lines.

Results and discussions SKOV3 ovarian cancer cell lines with knock outs in each of the three GLI proteins, GLI1, GLI2 and GLI3 were generated. The knock-out was confirmed by qRT-PCR and Western blot, and DNA was sequenced to show the exact genetic modification of the lines leading to the knock-out. The modified cell lines showed differences in cell morphology in relation to the original SKOV3 cell line immediately after expansion from the single cell.

Conclusion The Hedgehog signalling pathway plays an important role in ovarian cancer. Cells harbouring knock out for the effector proteins of the Hedgehog signalling pathway, GLI1, GLI2 and GLI3 show distinct changes in cell morphology and viability. The Hedgehog signalling pathway, and more specifically GLI proteins, may be a good potential therapeutic target in management of ovarian cancer.

PO-187 DEFECTIVE LIVER REGENERATION ABILITY OF SOS1-KO AND SOS1/2-DKO, BUT NOT SOS2-KO MICE

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Introduction To ascertain specific functional role(s) of the Sos1 and Sos2 Ras-GEF activators we investigated phenotypic effects of single or combined disruption of Sos1 and/or Sos2 in adult mice by using a tamoxif tin-inducible Sos1-KO system. Upon TAM induction, the resulting Sos1/2-DKO animals die precipitously (in about 2 weeks) whereas single Sos1-KO or Sos2-KO adult mice are perfectly viable. Hence, future drug design strategy should aim both arms of STAT3 pathway to completely abrogate its oncogenic function.

Material and methods To determine cause(s) of the quick death of Sos1/2-DKO mice, we analysed blood and GI tissues from 6–8 week-old mice of 4 relevant Sos genotypes (WT, Sos1-KO, Sos2-KO and Sos1/2-DKO) similarly treated with TAM for 13 days. Different biochemical parameters were quantitated in blood serum and liver samples, and various IHC assays were also performed on different organs of the GI tract. Liver regeneration was characterised by means of partial hepatectomy studies of WT, Sos1-KO and Sos2-KO animals previously treated with TAM for 10 days.

Results and discussions Combined loss of Sos1 and Sos2 in DKO mice resulted in markedly reduced levels of total serum protein and increased serum levels of lactate dehydrogenase, creatinine kinase and other liver enzymes, suggesting the occurrence of substantial liver failure in these animals. Histological analysis of the DKO animals showed a quick overall segue.