knockdown or over-expression influences expression or activity of FOXA1 and HER3 at mRNA and protein level, and DNA-protein binding assay to show FOXA1/HER3 promoter gene interaction.

Results and discussions Results from our study showed that stable overexpression of JAM-A in MCF7 breast cancer cells (MCF7-JAM) increased both mRNA and protein expression of HER3. Correspondingly, transient, genetic silencing of JAM-A reduced the mRNA and protein expression of HER3 in MCF7 and SKBR3 cells. In MCF7 cells, JAM-A gene silencing phenocopied that of HER3 gene silencing by reducing protein expression of the HER downstream effectors phospho-AKT and phospho-ERK, in parallel with significant reductions in cell viability (measured by Alamar Blue assay). To begin exploring the mechanism whereby JAM-A regulates HER3 expression, we focused on the HER3 transcription factor FOXA1 following a bioinformatic search. JAM-A gene silencing reduced FOXA1 expression in all cell lines tested; while JAM-A overexpression had the opposite effect in increasing FOXA1 expression. FOXA1 gene silencing was sufficient to reduce HER3 expression in the same cells. Furthermore FOXA1 in nuclear extracts of breast cancer cells bound specifically to an oligonucleotide sequence corresponding to the HER3 gene promoter, and gene silencing of JAM-A reduced FOXA1 binding activity to this sequence. Taken together, our data provide novel evidence of a direct relationship between levels of JAM-A, FOXA1 and HER3 in breast cancer cells.

Conclusion In conclusion, we suggest that JAM-A merits investigation as a novel target to inhibit HER3-dependent tumorigenic signalling in breast cancer.

PO-164 IDENTIFICATION OF THE HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K/J (HNRP K/J) AS A REPRESSOR OF THE INHIBITOR KAPPA B ALPHA (IkBα) GENE IN AGGRESSIVE BREAST CANCER CELLS

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Introduction Advanced breast cancers do not respond well to therapies and represent a relevant focus for studying molecular mechanisms involved in the tumour progression and drug resistance. The transcription factor NF-κB is often activated constitutively in aggressive breast cancer cells and plays a significant role by inducing many target genes involved in tumour progression and drug resistance. Mechanisms controlling constitutive NF-κB activation are not all clearly understood. Among them, repression of the gene encoding the NF-κB inhibitor, IκBα is not well known. This protein controls NF-κB activation by sequestering it in the cytoplasmic compartment. The present study reports the identification of the hnRNP K/J protein, which is initially known for its role in mRNA splicing and translation, as a repressor of the IκBα gene expression.

Material and methods Identification of hnRNP K/J protein on the IκBα promoter was carried out by DNA pull-down coupled with a mass spectrometry analysis, and chromatin immunoprecipitation (ChIP) using a specific polyclonal antibodies. The hnRNP K/J protein was overexpressed in breast cancer cell lines by transient transfection, and consequence on the IκBα expression and the proximal IκBα promoter activity was evaluated by RT-qPCR and gene reporter assay, respectively. The hnRNP K/J protein localization was visualised in cells by Western blotting using nuclear and cytoplasmic extracts.

Results and discussions The IκBα gene is expressed higher in nonaggressive compared to aggressive breast cancer cells. We used previous data showing the importance of the proximal promoter at position −495 from the transcription site for DNA pull-down with nuclear protein extract from MCF-7 cells. Mass spectrometry analysis led to identify hnRNP K/J protein, whose the binding to this region of the proximal IκBα promoter was confirmed by ChIP. The IκBα expression at mRNA level and proximal IκBα promoter activity was strongly decreased in hnRNP K/J-overexpressing breast cancer cells, in contrast to the respective parental cells, suggesting the role of hnRNP K/J protein as a gene repressor.

Conclusion The identification of hnRNP K/J as a repressor of IκBα gene expression depicts a new molecular mechanism, which may contribute to the high constitutive NF-κB activation in aggressive breast cancer cells and suggest to take it into account in the development of new therapies targeting NF-κB pathway in advanced breast cancers.

PO-165 EFFECT OF NOTCH PATHWAY AND SKP2 INHIBITION IN CELL VIABILITY/PROLIFERATION OF MEDULLOBLASTOMA CELLS

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Introduction Medulloblastoma (MB) is an embryonic neuroepithelial tumour and important embryonic development pathways, such as WNT, Sonic Hedgehog (SHH) and Notch, are dysregulated on this cancer. The Notch pathway is important to cell differentiation and proliferation and it has a role in initiation and progression of MB regulating downstream effectors. Skp2 gene is a transcriptional target of Notch pathway and it has been associated with tumorigenic properties in some cancers, however, there is no study in MB. Therefore, the aim of this study was to investigate the biological role of Notch/Skp2 axis in MB.

Material and methods NOTCH1, 2, 3 and 4, as well as, their ligands, DLL1, 3 and 4, JAG1 and JAG2, and SKP2 gene expression levels were analysed in MB samples, subdivided in the molecular subgroups, WTN, SHH, group 3 and 4, comparing with cerebellum samples, from public databases (Robin-son et al. 2012; Kool et al. 2008; Pfister et al. 2000 and Roth et al. 2006) using R2: Genomics Analysis and Visualisation Platform. NOTCH 1 and SKP2 gene expression level were analysed in Uw402, Uw473, ONS-76 and DAoY MB cell lines. DAoY, ONS-76 and Uw2282 cell lines were
submitted to the Notch pathway inhibitor (gamma secretase inhibitor, DAPT), and to the SKP2 inhibitor, SZL-P1-41, at 2, 4, 8, 16 uM concentrations, and after 48 hour the cell viability was analysed. The cell cycle was also analysed after treatment with DAPT in DAOY cell line after 48 hour.

Results and discussions SKP2 was overexpressed in the WNT and SHH when compared to other groups and the same pattern was found for NOTCH2 and DLL3 in the database analysis. All MB cell lines expressed SKP2 and NOTCH1 genes and in the general the SKP2 expression was higher than NOTCH1 in all cell lines. After DAPT treatment the cell viability was reduced in DAOY, ONS-76 and Uw2282 cell lines after 48 hour. In the cell cycle analysis there was a decrease of cells on S-phase after Notch pathway inhibition in the DAOY cell line.

Conclusion As conclusions from these results, we can suggest that the Notch pathway/SKP2 axis activation occur mainly in WNT and SHH MB subgroups through interactions among NOTCH2 receptor, DLL3 ligand and SKP2 and that Notch pathway and SKP2 inhibition cause a decrease in MB cell viability and/or proliferation, with Notch pathway inhibition also decreasing the percentage of cells on S-phase cell cycle which may explain the decrease in cell proliferation. These are preliminary results but may suggest Notch/SKP2 axis as a possible new molecular targeted therapy for MB.

Omics Technologies

**PO-166 THE EXPRESSION OF NICOTINATE NUCLEOTIDE PYROPHOSPHORYLASE IS ELEVATED IN COLORECTAL CANCER TISSUES**

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Introduction Colorectal cancer (CRC) is one of the most prevalent cancer worldwide including Korea and has poor prognosis. Recent improvement in methods for proteome analysis has offered the possibility of identifying disease-associated protein markers to assist in diagnosis or prognosis, and for selecting potential targets for specific drug therapy.

Material and methods Proteomic analysis was performed with proteins from colorectal cancer (CRC) and the corresponding normal colorectal tissues, and analysed a number of proteins to isolate and identify tumor-specific proteins in CRC by two dimensional gel electrophoresis (2-DE), tryptic digestion, and MALDI-TOF/mass spectrometry. To further investigate the targeted protein with altered expression level, western blot analysis, real-time PCR, and immunohistochemistry were performed.

Results and discussions In current study, the proteome of colorectal adenocarcinoma and the corresponding normal tissues was visualised and analysed a number of proteins for CRC. Ten proteins were dominantly expressed and five proteins were largely repressed in CRC by the analysis of 2-DE and MALDI-TOF/MS. Nicotinate nucleotide pyrophosphorylase was focused to be increased in CRC tissues compared with normal colorectal tissues. The elevated expression of nicotinate nucleotide pyrophosphorylase in CRC was confirmed by western blot analysis and real time PCR. Immunohistochemistry also verified largely up-regulation of nicotinate nucleotide pyrophosphorylase in 85 CRC patients.

Conclusion These findings suggest that nicotinate nucleotide pyrophosphorylase could be possible tumour biomarker candidates in CRC tissues.

**PO-167 METABOLOMICS BIOMARKERS IN BREAST CANCER TUMOURS TREATED WITH NEOADJUVANT THERAPY**

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Introduction Breast cancer is one of the most prevalent cancers in the world. Traditionally, early breast cancer treatment is based on surgery and, after surgery, hormone treatment or chemotherapy. However, the neoadjuvant treatment is increasingly used. Metabolomics is the most recent ‘omics’ which allows quantify metabolites into blood patient samples. Coupled with computational analyses it could be possible to study differential metabolomics patterns and associate them with neoadjuvant response.

Material and methods Blood plasma samples from patients with breast cancer treated with neoadjuvant chemotherapy were used to perform metabolomics experiments. One sample before the treatment (basal) and one sample after the chemotherapy (post-treatment) were analysed and clinical data regarding response (complete response or partial response) was also collected. Metabolomics experiments were performed using liquid chromatography coupled with mass-spectrometry.). Bayesian network and class comparison analyses were used to establish differential metabolic patterns between conditions. Additionally, a response prediction model based on logistic regression was build using metabolomics data from basal samples.

Results and discussions A network showing the relationships between metabolites was build. Comparing metabolite measurements between complete response and partial response tumours in basal samples, 19 metabolites showed a differential quantification between both types of responses. Moreover, one of these metabolites is linoleic acid, previously described as a biomarker of complete response in neoadjuvant treatment in breast cancer. Using these 19 differential metabolites, a response predictive model was build. According to this model, it is possible to predict response to neoadjuvant treatment based on the amount of one metabolite, still only identified by its mass and charge. On the other hand, comparing basal and post-treatment samples, the network showed differential metabolomics patterns. These differential metabolites could be used as predictive biomarkers of response.

Conclusion This study is a proof of concept that using a new ‘omics’ technique such as metabolomics in blood samples, coupled with computational analyses, it is possible to identify differential metabolomics patterns between complete and partial response or basal and post-treatment samples and design predictive models of response. These results could facilitate in the future the implementation of blood-based tests into the clinical routine.