

BRCA1-interacting proteins, and the possible existence of SUMO interacting sites both in BRCA1 and partners point to a intricate network encompassing several pathways related to stress response, disruption of which may contribute to carcinogenesis.

**[720] Simultaneous HER2/neu and PTEN deregulation correlates with aggressive phenotype in hepatocellular carcinoma: a tissue microarray analysis**

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**Background:** Hepatocellular carcinoma (HCC) is a highly aggressive and chemo resistant type of cancer. Although novel anti-HER2/neu targeted therapeutic strategies have been developed and applied in some types of malignancies, specific mechanisms of deregulation in HER2/neu (17q21) depended – signaling transduction pathway remain under investigation in HCC. Our aim was to investigate the potential role of simultaneous HER2/neu and PTEN (10q21-suppressor gene) dysregulation in HCCs.

**Materials and Methods:** Using tissue microarray technology, fifty-two (n = 52) formalin fixed and paraffin embedded tissue samples of histologically confirmed primary HCCs were cored and re embedded in the final paraffin block (core diam 1.5 mm). Immunohistochemistry (IHC) was performed by applying anti-HER2/neu and anti-PTEN antibodies. Fluorescence in situ hybridization (FISH) analysis was also performed regarding those genes.

**Results:** Protein over expression was observed in 12/52 (23%) cases regarding HER2/neu, whereas PTEN decreased or loss of expression in 22/52 (43%) cases. HER2/neu gene amplification was confirmed in 7/52 (13%) cases, whereas no one of the examined cases demonstrated PTEN gene numerical imbalances. Combined HER2/neu and PTEN aberrant expression was observed in 9/52 cases associated to the grade of the examined tumours (p = 0.01).

**Conclusions:** HER2/neu up-regulation combined to PTEN down-regulation is a relatively frequent and critical genetic event in HCC correlated also with an aggressive phenotype. PTEN decreased expression maybe is a negative prognostic factor for applying anti-HER2/neu targeted monoclonal antibody therapy (high chemo-resistance levels) in patients with HCC, based on breast adenocarcinoma management experience.

**[721] Aromatase inhibitor resistance; a role for estrogen receptor and AIB1 in differential gene regulation**

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**Background:** Aromatase inhibitors (AIs) are fast becoming the first line treatment for post menopausal breast cancer patients. However, it is evident that AIs do not remove all estrogen and molecular studies suggest that this can result in adaptive estrogen hypersensitivity of the estrogen receptor- $\alpha$  (ER $\alpha$ ) with consequent resistance to therapy. We hypothesised that, in the AI-resistant setting, ER $\alpha$  may have the capacity to recruit its coactivator protein AIB1 to drive transcription of ER sensitive genes and induce tumour proliferation.

**Materials and Methods:** MCF7 breast cancer cells were stably transfected with the aromatase enzyme to generate an AI-sensitive cell line (MCF7aro). To acquire an AI-resistant cell model, MCF7aro was treated long-term with the AI, letrozole, until it lost sensitivity to letrozole (MCF7aroR-Let).

Cellular proliferation was measured by crystal violet staining; ER target gene expression levels were analysed by PCR, real-time PCR and Western blotting; chromatin immunoprecipitation was used to determine recruitment of ER and AIB1 to the promoters of target genes; the expression of, and interactions between, ER and AIB1, were analyzed by co-immunoprecipitation and quantitative coassociation immunofluorescent microscopy, using cell lines.

**Results:** In the AI-sensitive cell line, increased proliferation and expression of ER target genes pS2, c-myc and cyclinD1 was observed in response to aromatase substrate androstenedione; this effect was inhibited by letrozole. In the AI-resistant cell line, letrozole failed to inhibit proliferation induced by androstenedione, nor expression of pS2 and c-myc. However, cyclinD1 expression remained sensitive to letrozole treatment.

Chromatin immunoprecipitation studies in these cells demonstrated that treatment with letrozole induced recruitment of both ER $\alpha$  and AIB1 to the promoter of ER target genes pS2 and c-myc in the AI-resistant cell line but not to the promoter of cyclinD1.

Co-immunoprecipitation and co-localisation of ER- $\alpha$  and AIB1 were increased in the AI-resistant cell line following treatment with letrozole in comparison with AI-sensitive MCF7aro.

**Conclusions:** These data suggest that in the AI-resistant setting ER $\alpha$  can utilise AIB1 to drive tumour progression in the presence of an AI, and that this occurs in a target gene-specific context. An alternative signalling network may be involved in regulating cyclinD1 gene expression and allowing AI-resistant cells to retain some sensitivity to AI treatment.

**[722] Characterisation of gene expression profiles in HeLa cells expressing BRCA1 missense variants**

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**Background:** Most BRCA1 mutations originate non functional truncated proteins that predispose women to early-onset breast and ovarian cancer. A number of missense mutations whose role in the disease is often difficult to ascertain, however, have also been detected in hereditary breast cancer patients. To investigate the molecular mechanisms that may underlie a pathogenetic role for two missense variants located within the BRCT domain of BRCA1, the M1775R and the A1789T, we compared the expression profiles of HeLa cells transfected with these two BRCA1 variants and HeLa cells transfected with BRCA1 wild type. The M1775R variant has widely been described as deleterious by functional assays, but to date a characterization of its effects on gene expression in human cells has never been reported. The A1789T variant has never been studied before by other groups.

**Materials and Methods:** The gene expression profiles of five clones of HeLa cells transfected with plasmids expressing each of the two BRCA1 missense variants were compared by microarrays to those of five clones transfected with plasmids expressing BRCA1 wild-type. A reference design was adopted and the reference sample was obtained by pooling the mRNAs from the wild-type clones. Gene expression was investigated by two-colour microarray analysis, using the Whole Human Genome 4x44k Microarray G4112F (Agilent Technologies, Palo Alto, CA, USA).

**Results:** Compared to BRCA1 wild-type, the M1775R variant showed 159 differentially expressed genes, 108 down-regulated and 51 up-regulated, while the A1789T variant showed 188 differentially expressed genes, 77 down-regulated and 111 up-regulated. Out of these genes 15 were differentially expressed with the same fold-change direction by both the mutations. Pathway analyses mapped 33 out of the 159 and 33 out of the 188 differentially expressed genes in 54 and 55 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways respectively. For both variants many of the pathways with the highest values of impact factor were involved in cancer, including pathways implicated in cancer general network and those describing the co-regulation mechanisms which underlie different type of cancers.

**Conclusion:** Our findings indicate that the M1775R and the A1789T variants of BRCA1 gene affect the expression of many genes associated with known mechanisms of cancerogenesis and thus contribute to sustain the hypothesis that these two mutations have a role in the pathogenesis of familial breast cancer.

**[723] Expression of HDAC1, 2, 3 and 7 as a prognostic markers in hepatocellular carcinoma**

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**Background:** Histone deacetylases (HDAC) are enzymes that are responsible for the transcriptional control of genes through modifications of histone proteins. Among others, they play a factor in the control of tumour suppressor genes. Hypoacetylated histone proteins have been associated with precancerous and malignant lesions and for some tumour entities, such as prostate cancer and colon cancer HDAC expression has been identified as an independent prognostic factor. Since inhibitors of histone deacetylases (HDACi) emerge as promising therapeutics in the management of solid tumours, including hepatocellular carcinoma, we analyze the importance of the expression of 4 HDAC isoenzymes as a prognostic marker in hepatocellular carcinoma.

**Method:** Tissue micro arrays of primary HCCs and adjacent normal tissue of 170 patients (male n=145, 85.3%; female n=25, 14.7%; mean age 61.9±11.0 years) were evaluated immunohistochemically for the expression of HDAC isoenzymes 1, 2, 3, 7 and ki-67 antigen. Intensity and extensity of expression were evaluated by two independent blinded observers, a product score calculated (IRS, immunoreactivity score) and the data was correlated with histopathological and clinical criteria. Based on mean HDAC expression for each isoenzyme, patients were stratified into high and low expression groups and the groups compared in terms of clinical data.