Of these, 8 had inoperable or metastatic disease at presentation and a further 17 developed locally advanced or metastatic disease.

16 received an aromatase inhibitor. (9 anastrazole, 5 letrozole, and 2 exemestane).

13 patients received tamoxifen prior to an aromatase inhibitor. 15 patients received AI as either a first or second line therapy.

Duration of AI therapy was assessable in 14 patients. The median duration of aromatase inhibitor therapy was 17 weeks (1−90 weeks) and 6 patients (43%) remained on therapy beyond 24 weeks.

7 patients (47%) on AI had no documented clinical or radiological benefit and had therapy discontinued at the first review.

When comparing those who had a documented response to AI compared with those who never responded, responders were older (median age of 72.5 years (95% CI 69.9–75.1) vs. 64 years (95% CI 59.3–68.7)) and more likely to have recived prior anthracycline based chemotherapy (37.5% vs 14%).

Conclusions: Response rates to aromatase inhibitors in men are lower than would be expected in a similar population of women. Women receiving anastrazole after tamoxifen had a median time to progression is reported as 21 weeks compared to 17 weeks in our cohort (Buzdar 2001). 43% of patients were felt never to have had a clinical or radiological response to AI.

More clinical studies are required to establish why some male breast cancer patients respond to AI but the differences between responders and non-responders suggest testicular function may play a role.

Testicular function is known to decrease with age and patients responding to AI were older on average and a greater percentage of patients responding to AI had prior anthracycline chemotherapy. This case series supports the use of aromatase inhibitors in selected male breast cancer patients but there is still a need for further research into the cellular mechanisms of male breast cancer and the role of testicular steroid hormone production in AI resistance.

References

Buzdar, et al 2001 Phase III, Multicenter, Double-Blind, Randomized Study of Letrozole, an Aromatase Inhibitor, for Advanced Breast Cancer Versus Megestrol Acetate. Journal of Clinical Oncology 19(14): 3357−66.

Thursday, 22 March 2012 12:30–13:30

POSTER SESSION

Molecular Biology, Tumour Biology and Immunology

262 Poster discussion

MMP11 Expression Increases During Progression of Breast Cancer

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Background: The ductal carcinoma *in situ* (DCIS) of the breast is considered to be the pre-invasive form of the invasive duct carcinoma (IDC). The aim of this project is (1) the identification and validation of potential progression markers and (2) to identify markers for high risk DCIS with aggressive potential. MMP11 (matrix-metalloproteinase 11) is a marker for the transition from DCIS to IDC. It is associated with tumour cell invasion and a poor clinical outcome.

Material and Methods: 15 formalin fixed and in paraffin embedded (FFPE) tissue samples with a 'pure' DCIS without IDC component (patients were at least five years free of cancer), and 15 paraffin tissue samples with DCIS/IDC tumours were selected. Tissue sections were prepared, stained with hematoxylin-eosin and the epithelial cells were isolated by laser capture microdissection (LCM). 200 ng RNA were extracted, hybridized to the Whole Genome DASL Array (Illumina) and bioinformatically evaluated. The RNA was linear amplified using the Ribo-SPIA® technology (WT-Ovation™ FFPE System, NuGen™) and the validation was done by qRT-PCR using the LightCycler® 480 System (Roche).

Results: We were able to identify 993 transcripts that are differentially expressed between DCIS and IDC of the same tumour and 1138 transcripts which are differentially expressed between 'pure' DCIS and DCIS/IDC tumours. Differential expression was validated for 9 transcripts using two sample sets, the technical validation sample set (15 DCIS/IDC tumours, 15 'pure' DCIS) and an independent validation sample set (26 DCIS/IDC tumours, 17 'pure' DCIS). MMP11 is highly expressed in IDC and moderately expressed in DCIS with IDC component. In 'pure' DCIS less or no expression of MMP11 was determined.

Conclusions: We identified progression-specific candidate transcripts using LCM and microarray analysis from FFPE breast cancer tissues. MMP11 is a progression marker which differentiates between high and low risk DCIS.

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Computational Prioritization of NsSNP's Involved in Causing Breast Cancer in Human

A. Kumar[1]. [1]*VIT University, School of Biosciences and Technology, Vellore, India*

Breast cancer is counted among the most common invasive and fatal cancer's in women. It has been reported to cause 458,503 deaths worldwide in year 2008. Therefore the in-depth scientific research is important to gain complete information regarding the molecular pathway related to this disease and to discover the effective pharmacological treatment. We used computational approach to identify the SNPs involved in causing breast cancer. In order to identify the possible locus of tumorigenic mutations we analyzed 535 nsSNPs in 20 candidate genes (BRCA1, BRCA2, CDH1, CHEK2, DIRAS3, ERBB2, MYC, CCND1, TRIM37, APPBP2, TRAP240, RAD51C, BCAS3, PTEN, STK11, TP53, AR, ATM, RB1CC1, AKT1, BARD1, PALB2, RAD51L1, NQO1, NQO2, RAF1, ZNF217, TGFB1, TOX3, CYP1A1, CASP8, HMMR, LSP1, RAD51 and MAP3K1) taken from journals and publications based on the case control studies. Using evolutionary conservation analysis and statistical potential energy function evaluation algorithm we prioritized 171 SNPs that were predicted to be damaging. Further using Support Vector Machine based classifier we selected 63 nsSNP's that were reported to be extremely deleterious and could be the possible cause of inducing cancers in human breast region. Among these 63 variants 12 were reported to distrupt the lignad binding site and 7 lead to the overpacking at the burried regions. Molecular Dynamic Simulation of native and mutant proteins were carried out to analyze the structural dependency of the mutants tumorigenic property. The clear variation in the RMSD (Root Mean Square Deviation) values were observed in all the 19 variants which accounts for the loss of proper signal transdution in the cellular pathways which may induce the oncogenicity leading to Cancer. To study the pathway based dependencies of these mutations we used Ordinary Differential Equation and Boolean Algebra to understand the mutation induced relative variation in the rate of activation of phosphorylases and kinases mediated cell divisons. Genetic algorithm is used to predict the unknown concentration of the involved proteins, ligands and enzymes in the native and mutant pathway conditions. These findings will facilitate the understanding of the involvement of nsSNP's in causing breast cancer in human.

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Mitochondrial DNa Mutations and Copy Number Alteration in Breast Cancer Patients From Romania

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Background: Breast carcinoma is one of the most common types of malignancy worldwide and the leading cause of mortality from cancer among Romanian women. Mutations in mitochondrial DNA (mtDNA) as well as alterations in mtDNA content have been reported in numerous cancers examined. However, it still remains unclear whether the alterations in mtDNA are related to the clinicopathological features and/or the prognosis in breast cancer.

Material and Methods: Total DNA (nuclear and mitochondrial) was isolated (High Pure PCR Template, Roche Diagnostics) from breast cancer and paired normal breast tissues originating from 40 Romanian patients. Somatic mutations in the D-loop region (4,977-bp deletions) were investigated using Mutector mtDNA kit (TrimGen Corporation). MtDNA copy number was quantified using a one-step quantitative multiplex real-time PCR. A FAM labeled probe and primers were used to amplify the mtDNA sequence of the ATP 8 gene, and a VIC labeled probe and primers were designed to amplify the beta-globine gene.

Results: MtDNA copy number in stage I breast cancer patients was significantly lower than in other stages ($P = 0.0015$). A reduced mtDNA copy number was found often in post menopausal cancer group ($P = 0.024$). The study revealed no difference in mtDNA content related to age ($p = 0.255$) or lymph node involvement ($p = 0.173$).

We failed to detect any mtDNA mutations in normal breast tissue specimens. 16.66% of stage I breast cancer patients presented mutations in D-loop region whereas 28.57% of stage II cases showed mutations in mtDNA. 4,977-bp deletions were detected in 66.66% cases of stage III cancer cases.

Conclusion: These studies provide strong evidence that instability in the D-loop region of mtDNA may be involved in breast malignancy. We suggested that mtDNA mutations may play a role in breast cancer development in Romanian patients but their role in the mechanism of carcinogenesis remains to be solved. The use of mtDNA copy number may have a diagnostic value but further studies on a larger cohort of patients are necessary in order to validate it as a potential biomarker for early detection of breast carcinoma.

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Dual Color in Situ Hybridization and Mutational Analysis of Triple Negative Breast Cancer with EGFR Protein Overexpression

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Background: It has been well known that triple negative breast cancer (TNBC) is a heterogeneous tumor and highly express epidermal growth factor receptor (EGFR). Although TNBC with EGFR protein overexpression has been extensively studied, systematic studies of EGFR protein overexpression and gene amplification are rare. We studied correlation of EGFR protein overexpression with EGFR gene amplification, EGFR activating mutation, and expression of components of Akt pathway.

Materials and Methods: Tumor tissues were obtained from 84 patients with TNBC. We analyzed the status of EGFR, phosphorylated Akt (pAkt), phosphorylated mammalian target of rapamycin (p-mTOR), and other markers using immunohistochemistry (IHC). We also analyzed EGFR gene and chromosome7 copy numbers by DISH. Tumors with $\geqslant$15 copies in $\geqslant$10% of cells or EGFR gene cluster ($\geqslant$4 spots) in $\geqslant$10% of cells or EGFR signal/chromosome7 signal ratio $\geqslant$2 were interpreted as positive for gene amplification in this analysis. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples. Analysis of EGFR gene activating mutations were performed for these samples using the peptide nucleic acid (PNA)-clamp smart amplification process version 2 (SmartAmp2).

Results: Characteristic histopathological types of TNBCs with EGFR overexpression are typical medullary carcinoma, apocrine carcinoma, small cell carcinoma, metaplastic carcinomas, and adenoid cystic carcinoma. EGFR protein overexpression was found for 28/84 (33.3%), but EGFR gene amplification was not detected. Chromosome7 polysomy expression (positive score $\geqslant$4%) and high polysomy expression ($\geqslant$4 copies in $\geqslant$ 40% of cells) were found in 46/84 (54.7%) and 6/84 (7.1%) samples, respectively. There were significant correlations between EGFR protein overexpression and each of chromosome7 polysomy and high polysomy expression. But we could not find any relation between EGFR protein overexpression and each of pAkt and p-mTOR and the other clinicopathlogical factors. Although we were able to analyze EGFR gene activating mutations in 55 (65.4%) of 84 FFPE samples, we found no evidence of EGFR gene activating mutations in these 55 samples.

Conclusions: Several clinical trials have been performed to test the role of anti-EGFR directed therapy for TNBC with EGFR protein overexpression. The present study indicates that chromosome7 polysomy expression might be a candidate biomarker for selection of targeted therapy.

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MiRNA-218 Regulation of Human D-glucuronyl C5-epimerase Expression in Breast and Prostate Cancer

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Human D-glucuronyl C5 epimerase (GLCE) is one of the key enzymes of the biosynthesis of heparan sulfate proteoglycans (HSPG) involved in cell-cell and cell-matrix interaction and signaling. GLCE catalyses epimerization of D-glucuronyc acid into L-iduronic acid residues in the heparan sulfate polysaccharide chains which gives the flexibility to the HS chains and facilitates their interaction with numerous extracellular ligands including growth factors. We have recently demonstrated that GLCE expression is significantly decreased both in breast and prostate tumours and its ectopic expression inhibits cancer cell proliferation *in vitro* and tumour growth *in vivo* suggesting a potential tumour-suppressor function of GLCE. However the mechanism of the down-regulation of GLCE in cancer is still unclear.

In the study, molecular mechanisms of GLCE inactivation were investigated with a special attention on the possible miRNA involvement in GLCE regulation in cancer. Design of the study included the measurement of GLCE and miRNA-218 expression in the same breast and prostate tumours by Real Time RT-PCR analysis, Western blot and TaqMan Small RNA Assay. Correlation of GLCE and miRNA expression levels was calculated with OriginPro 8 software. To investigate the effect of miRNA-218 on GLCE expression *in vitro*, miRNA-218 and anti-miRNA-218 were transfected into breast and prostate cancer cells (MCF7, PC3, DU145) and GLCE expression level was determined by Real Time RT-PCR and Western blot.

It was shown that promoter methylation is not involved in the regulation of GLCE expression in cancer cells while a chromatin structure affects it both directly and indirectly (through the activation of some positive GLCE regulators). miRNA-218 is able to regulate GLCE protein level in breast and prostate cancer cells *in vitro* with GLCE mRNA level being unaffected. However, no significant correlation between GLCE and miRNA-218 in breast and prostate tumours was shown.

The obtained data revealed a complex regulation of GLCE expression in cancer cells and breast and prostate tumours. Different molecular mechanisms contribute to the regulation including chromatin structure and miRNA-218 which affects GLCE expression *in vitro* but not *in vivo*. Possibly the miRNA-218 influence on GLCE expression *in vivo* is more complicated due to an involvement of some other miRNA-218-regulated proteins.

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Correlation Between Expression of NF-kB and ODC with Other Molecular Markers in Tumors of Patients with Breast Cancer

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Background: The aim of research is to study NF-kB (p50/p65), ornithine decarboxylase (ODC), c-erbB2, ER, PR, Ki-67, p53 expression in breast tumors and analyze correlation between these markers.

Methods: We selected 69 patients with breast cancer before treating with radio- or chemotherapy. The I stage was diagnosed for 5, II − 44, III − 8, IV − 2 patients, 10 patients had 'X' stage at that moment. All tumors belong to invasive ductal carcinoma with different Grade (G). Expression of molecular markers was determined by immunohistochemistry on paraffin-embedded tissue sections.

Results: The correlation between G and ER ($r = -0.995$, $p < 0.05$); G and Ki-67 ($r = 0.995$, $p < 0.05$); ER and p53 expression ($r = -0.987$, $p = 0.052$) was defined.

We grouped the patients with high levels of p50 and p65; high p50 and low p65; low levels of subunits. The correlation between expression of NF-kB and hormone receptors (ER: $r = -0.999$, $p < 0.01$; PR: $r = -0.999$, $p < 0.05$) was evaluated. Then we divided the patients with high level of p50 into two subgroups: first − with high ODC level, and second − with low ODC level. It was shown the decrease of ER expression ($r = 0.991$, $p < 0.01$) and increase of p53 accumulation ($r = 0.997$, $p < 0.01$) in this direction: p65−/p50− − p65−/p50+/ODC+ − p65−/p50+/ODC− − p65+/p50+.

Also, NF-kB, ODC, Ki-67 and p53 expression in different molecular types of breast tumors was analyzed. Breast tumors are divided on Luminal (ER+/c-erbB2−), HER2 (ER−/c-erbB2+), Luminal-HER2 hybrid (ER+/c-erbB2+), and basal-like (ER−/c-erbB2−) types. HER2 type had the highest expression of p65 ($r = 0.878$, $p = 0.061$), p50 ($r = 0.908$, $p < 0.05$), p53 ($r = 0.970$, $p < 0.05$) and basal-like type had the greatest level of proliferation (Ki-67: $r = 0.989$, $p < 0.01$).

Conclusion: The correlation between G and molecular markers (ER and Ki-67) was defined. ER and p53 levels were found to be changed in dependence of NF-kB/ODC expression profile. Also, correlation between NF-kB, Ki-67 and p53 expression and tumor type was shown. So, our findings are the reason for further research in this field, because the results of such investigations may be useful for prognosis of disease flowing.

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Analysis of Large Genomic Rearrangements of BRCA1/2 Genes in Korean Breast Cancer Patients

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Background: The BRCA1 and BRCA2 genes are associated with inherited susceptibility to breast and ovarian cancer. Most of disease-causing