**538** POSTER

**Expression profiling of specific microRNAs in human breast tissue using real-time quantitative PCR**

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**Background:** MicroRNAs, which regulate the expression of specific mRNA targets at the post-transcriptional level, have been shown to be aberrantly expressed in several cancers including breast cancer. This study aimed to identify suitable microRNA endogenous control genes and to use these genes to normalise real-time quantitative PCR (RQ-PCR) data for two specific microRNAs, miR-26b and miR-30a-3p, in human breast tissue. Significant upregulation of miR-26b has been shown in estrogen receptor (ER) positive versus ER negative breast tumours. Transcripts targeted by miR-30a-3p include angiogenesis-related mRNAs.
**Materials and Methods:** Following informed consent, malignant (n = 33), benign (n = 5) and normal (n = 5) breast tissues were retrieved from patients at the time of surgery at University College Hospital, Galway. The breast cancer tissues were grouped according to the patient's metastatic status five years from first diagnosis into metastasis-free (MF, n = 13), bone-metastases positive (BM, n = 11) and visceral and bone-metastases positive (VBM, n = 9) groups. Stem-loop gene-specific primers were used for cDNA synthesis and gene expression was measured using RQ-PCR. PCR amplification efficiencies were determined using standard procedures. Candidate endogenous control genes were identified from a previous experiment (unpublished data). Following relative quantification using qBase software (v1.3.5), ANOVA and Tukey multiple comparison post-hoc tests (Minitab v.15) were used to compare the expression of the target genes between groups.
**Results:** Let-7a and miR-16 were validated as endogenous control genes. There was a significant upregulation of miR-26b in the BM versus MF groups ($P < 0.01$). There was no significant difference found in miR-26b expression in relation to ER status. Expression of miR-30a-3p was significantly downregulated in the VBM versus BM groups ($P < 0.05$).
**Conclusions:** This study further implicates miR-26b in the process of breast cancer progression. This study, which reports deregulation of miR-30a-3p for the first time in human breast cancer, has implications for therapeutic targeting.

**539** POSTER

**Genetic pathways of breast carcinomas with 17q12q21 amplification**

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Breast cancer is the most common malignancy in western women and is a particularly heterogeneous disease. One breast cancer subtype is characterized by ERBB2 amplification and its identification is essential for therapy planning.
We analysed 211 breast carcinomas by Comparative Genomic Hybridization (CGH), 46 of which (21.8%) presented 17q12q21 gain or amplification (encompassing the ERBB2 locus). In order to assess the genetic alterations associated with ERRB2 amplification, we evaluated the chromosomal copy number changes by unsupervised hierarchical clustering, time of occurrence and principal component analyses.
Hierarchical cluster analysis revealed 3 groups of genomic imbalances: one characterized by 1q and 17q gains, another by 8q and 20q gains and 8p, 11q and 17p losses, and a third by 13q and 16q losses and 16p and 8p gains. Time occurrence and principal component analyses gave an idea of the temporal orders by which the various genomic alterations arise during breast carcinogenesis.
This work gave some insights on the genetic pathway of ERBB2-positive breast cancer and may help explain the mechanisms of resistance to target therapy.

**540** POSTER

**Grp78 is over-expressed in head neck cancer and is a potential molecular target for the inhibition of oncogenesis**

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Grp78 (Glucose-regulated protein 78) is one of the best-characterized endoplasmic reticulum (ER) chaperone protein. Recently, elevation of Grp78 has been reported associated with a variety of cancer, but the exact function is obscure. To identify whether Grp78 associated with HNC, we examined the protein expressions between paired cancer and grossly normal mucosa tissues. Of 56 patients assayed, 34 (61%) had two fold of over-expression, suggesting that this molecule participates in carcinogenesis of HNC. To further characterize the role, the effects of Grp78 knockdown by RNAi was examined in six HNC cell lines, including 2 nasopharyngeal cancers, 2 oral cancers, and 2 pharyngolaryngeal cancers. Consistent with the clinical findings, inhibition of Grp78 significantly reduced cell growth and colony formation to 53% ~ 11% in six HNC cell lines. Use of an in vitro wound healing and Matrigel invasion assays, we found that cell migration and invasive ability were also inhibited to 18% ~ 42% in these cell line tested. Two lines of in vivo xenograft studies showed that administration of Grp78-RNAi plasmid significantly inhibited HNC tumor growth for 2 months in BALB/C nude mice. In conclusion, Grp78 is identified over-expressed in HNC. Inhibition of Grp78 significantly suppresses carcinogenic potential in cellular and in vivo animal studies. These findings suggest that GRP78 is a potential molecular target in the development of adjuvant therapy for HNC.

**541** POSTER

**Evaluation of the prognostic and predictive value of EGFR protein levels in primary tumors of high-risk breast cancer patients**

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**Background:** To assess the prognostic and predictive significance of EGFR protein levels in high-risk patients with breast cancer treated with dose-dense sequential adjuvant chemotherapy.
**Materials and Methods:** 595 high-risk breast cancer patients were treated with adjuvant anthracycline-based dose-dense sequential chemotherapy (E-CMF vs. E-T-CMF). Disease free survival (DFS) was the primary endpoint. EGFR was assessed by immunohistochemistry (IHC) in 312 patients, using the 31G7 clone of the mouse monoclonal antibody (Zymed). Slides were considered positive for EGFR expression when $\geqslant$1% of the tumor cells had membranous staining of various intensities (1+, 2+, 3+). In addition, HER-2 and p53 were assessed by IHC as well, using standard methods. HER-2 scores of 2+ were further assessed by FISH.
**Results:** EGFR expression was detected in 54 out of 312 patients (17%). Positive expression of EGFR was significantly associated with negative receptor status (52% vs. 17%, $p < 0.001$), worse histological grade (70% vs. 45%, $p = 0.001$), HER-2 over-expression (46% vs. 27%, $p = 0.01$), and positive p53 expression (48% vs. 19%, $p < 0.001$). With a median follow-up of 7 years, the total number of events (disease relapses) was 105/312 (34%), and the total number of deaths 69/312 (22%). The analysis for DFS provides significant evidence that the EGFR effect on the hazard of disease progression was different according to treatment (interaction $p = 0.02$). More specifically, in the subgroup of patients treated with E-CMF the hazard of disease progression was significantly higher among patients with EGFR over-expression [hazard ratio (HR) = 2.09, $p = 0.01$], while no such effect was present in the subgroup of patients treated with E-T-CMF (HR=0.59, $p = 0.26$). In the multivariate model additional factors found to be related to poorer DFS was positive p53 expression ($p = 0.001$) and more than three positive nodes ($p = 0.02$). Regarding overall survival (OS), a trend towards significance for an interaction of EGFR and treatment was found ($p = 0.07$). In the subgroup of patients who did not receive Taxol an increased hazard of death was detected for those with positive EGFR levels (HR = 2.70, $p = 0.004$).
**Conclusions:** The present study demonstrated a differential effect of positive EGFR expression in the two treatment groups with EGFR over-expression being a negative prognostic marker in the absence of Taxol.

**542** POSTER

**Abnormal coagulation as prognostic factor to impact on efficacy of immunotherapy in metastatic renal cell carcinoma patients**

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**Background:** In experimental systems, interference with coagulation can affect tumor biology. We revealed that hypercoagulation is a frequent symptom in metastatic renal cell carcinoma (MRCC) patients (pts) and clinically correlates with progression of the disease. It has been suggested that hypercoagulation is a possible negative predictor for response to therapy in MRCC pts.
**Methods:** From January 2004 to September 2006, 72 patients were enrolled in the study prospectively. One group of pts (n = 28) had high

level of fibrinogen, D-dimer or fibrin, antithrombin III and prothrombin time (hypercoagulation group). Second group (n = 28) had normal values of coagulation (control group). All pts had received at least 2 cycles low-dose immunotherapy (IL-2, 1 MIU, i.v, 3 tiw + IFN, 5 MU, s.c, 3 tiw – 3 weeks on, 3 weeks off). Tumor response was assessed radiographically every 2 cycles using RECIST criteria. Median overall survival (OS) was estimated according to Kaplan-Meier method.
**Results:** Hypercoagulation was present at study entry in 38.8% of MRCC pts. 71.4 and 75% of pts were male, median age at on-study was 62 and 60.1 years in hypercoagulation and control group, respectively. 46.4% of pts had poor prognosis by MSKCC score in both groups (13 = 13 pts), and 53.6% of pts had good or intermediate prognosis. 25 (89.3%) pts of control group and 26 (92.9%) pts of hypercoagulation group had clear-cell histology. Pts with normal coagulation and treated with IL-2+IFN had a statistically longer survival and higher response rate than those who had abnormal coagulation (Table). Pts with hypercoagulation had predisposition to disease progression after 2 cycles of immunotherapy.

| | Hypercoagulation group | Control group |
|---|---|---|
| CR | – | 1 (3.6%) |
| PR | 1 (3.6%( | 5 (17.9%) |
| OR | 3.6% | 21.4% |
| SD | 11 (39.3%) | 14 (50.0%) |
| PD | 16 (57.1%) | 8 (28.6%) |
| Median OS*, months | 7.1 | 14.5 |
| 95% CI | 6.0−8.2 | 10.4−18.6 |

*Cancer-related survival; logrank p < 0.001.

**Conclusions:** These early results demonstrate that abnormal coagulation can be an independent prognostic factor for survival and efficacy to therapy in pts with MRCC.

**543** POSTER

**Soluble E-selectin levels and CEA expressing blood-borne cells in colorectal cancer patients. A causal relationship?**

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**Purpose:** The recognition of E-selectin by colorectal cancer (CRC) cells is an essential step for adhesion to activated endothelium and metastatization. Increased expression of E-selectin has been found in small vessels surrounding lesions in CRC and elevated levels of soluble (s) E-selectin have been found in metastatic compared with non-metastatic CRC patients. One of the newer areas being explored in the management of CRC is the use of reverse transcription-PCR (RT-PCR) to analyze the blood of cancer patients for the detection of mRNA expressed in tumor cells. Thus, this study was aimed to verify whether CEA mRNA levels in blood-borne cells correlate with cytokines and adhesion molecules involved in the haematogenous spread of CRC cells.
**Methods:** CEA mRNA (by RT-PCR), proinflammatory cytokines (IL-6, IL-1beta, TNF-alpha) and sE-selectin levels (all by R&D immunoassays) were analyzed in blood samples obtained from 64 CRC patients, treated at "Tor Vergata" Clinical Center, 40 patients with benign CR diseases and 59 control subjects. Patients were histologically diagnosed with primary [Dukes' Stage A (n = 4), Stage B (n = 27), Stage C (n = 17) and Stage D (n = 2, with a single resectable liver metastasis)] or relapsing (metastasis to the liver: n = 8, peritoneum: n = 2, lung: n = 2 and multiple metastasis: n = 2) CRC. The study was performed under the appropriate institutional ethics approvals, and informed consent was obtained from each patient.
**Results:** Median sE-selectin levels were higher in patients with CRC (44 ng/ml) compared to controls (34 ng/ml) or patients with benign CR diseases (31 ng/ml, H = 18.5, p = 0.0001). Increased levels of sE-selectin were significantly associated with CEA mRNA positivity by RT-PCR (p
**Conclusions:** The findings obtained suggest that circulating cancer cells, or their released products, might be responsible, through cytokine release, for the elevation of circulating adhesion molecules in CRC patients.

**544** POSTER

**Searching for susceptibility alleles: emphasis on bilateral breast cancer**

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**Background:** Polygenic inheritance plays an essential role in cancer susceptibility, however identification of at-risk alleles is compromised by poor reproducibility of case-control studies. We and others suggested earlier that the use of subjects with "extreme" characteristics of cancer risk, e.g. patients with multiple tumors, may provide highly demonstrative results of molecular epidemiological analysis.
**Materials and Methods:** Literature has been searched for case-control gene-association studies which analyzed both bilateral and unilateral breast cancer (BC) cases. 8 relevant reports have been identified, including 6 papers involving our contribution and 2 articles published by independent groups. The results of these investigations were compared against reference studies (i.e. meta- or pooled analyses) for each at-risk allele.
**Results:** Good concordance has been observed between the data obtained on limited number of bilateral BC and the larger data sets involving unilateral BC cases: all at-risk alleles (e.g., BRCA1 5382insC, CHEK2 1100delC, NBS1 657del5, ATM Ser49Cys) demonstrated some degree of overrepresentation both in women with a single tumor and in those with multiple cancers, while the "negative" studies (e.g., those for p53Arg72Pro, CYP17 −34 T/C polymorphisms) failed to reveal an effect in either of the patient groups. Most importantly, in all instances where a gene-disease interaction has been firmly established, the odds ratio observed for bilateral BC patients evidently exceeded the one calculated for unilateral series. Furthermore, the results of the analysis of bilateral BC corresponded well with the published reference studies.
**Conclusions:** For truly at-risk alleles, comparison of bilateral BC against controls always provides higher odds ratio estimates than the traditional analysis of non-selected BC cases; therefore, use of bilateral BC relaxes the requirements for the study size. Emphasis on bilateral form of breast cancer may significantly facilitate the search for genetic determinants of BC predisposition.

**545** POSTER

**Possible participation of fragile sites in her2/neu gene amplification on 17q12−21 chromosome in breast cancer**

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**Background:** Overexpression of HER2/neu protein which plays a significant role in breast cancer development and progression was strongly associated with amplification of its coding gene her2/neu. A number of genes located around her2/neu were shown to be co-amplified with it in breast cancer. However initial events and mechanism of amplification in this locus is not clear until now. The aim of this study was to investigate 17q12-q21 chromosome region for potential fragile sites that could play a major role in amplification of her2/neu in a group of patients with breast cancer.
**Materials and Methods:** We examined genomic DNA from fresh frozen breast cancer tumor samples of 130 patients for amplification of HER2/neu by Real Time PCR with TaqMan technology. The sequence of the region around her2/neu was investigated by TwistFlex software to reveal loci with high level of flexibility.
**Results:** 35 out of 130 cases (27%) had increased her2/neu gene dosage. Among these 35 cases we analyzed amplification level of genes located in 17q12−21 chromosome region around HER2/neu: LASP1, MLN64, PPARBP, CASC3, TOP2A. Gene dosage of genes located in HER2/neu-TOP2A region was higher as compared with normal tissue in 14 out of 35 cases. PARBP-HER2-GSDML region had high level of amplification in 21 out of 35 cases.
Recent publications described involvement of fragile sites in various chromosomal rearrangements. One of the basic features of fragile sites is sequence flexibility. We analyzed the region around her2/neu gene for the presence of flexible sequences. Two loci with high flexibility were detected. First locus located within intron sequence of ZNFN1A3 gene, which situated 36 kb telomeric to her2/neu, the second locus was found within FBXO40 gene, which located on 720 kb centromeric to her2/neu gene. Interestingly, both genes were described as tumor suppressor genes in different tumor types.
**Conclusion:** We proposed that these two sites with high level of flexibility might play a critical role in amplification of this region consistent with the