convenient. The aim of this study was to evaluate whether the detection of DEC in either PB or BM predicts overall survival.

Methods: PB and BM samples were collected from 148 patients with BC with stage I to IV disease prior to the initiation of any local or systemic treatment. PB of healthy volunteers and BM of patients without any malignancy served as the control group. DEC was detected by measuring relative gene expression (RGE) for CK-19 and MAM using a quantitative RT-PCR detection method. The mean follow-up time was 786 days (±487). Kaplan Meier analysis was used for predicting overall survival (OS).

Results: Taking the 95 percentile of the RGE of CK-19 (BM: 26.3 and PB: 58.7) of the control group as cut-off, elevated CK-19 expression was detected in 42 (28%) BM samples and in 22 (15%) PB samples. MAM expression was elevated in 20% (both PB and BM) of the patients witch BC. There was a 68% (CK-19) and 75% (MAM) concordance between PB and BM samples when classifying the results as either positive or negative. Patients with an elevated CK-19 or MAM expression in the BM had a worse prognosis than patients without elevated expression levels (OS: log-rank test, $p = 0.0045$ (CK-19) and $p = 0.025$ (MAM)). For PB survival analysis no statistical significant difference was observed between patients with or without elevated CK-19 or MAM expression (OS: log-rank test, $p = 0.551$ (CK-19) and $p = 0.329$ (MAM)).

Discussion and Conclusion: DEC, measured as elevated CK-19 or MAM mRNA expression, could be detected in both PB and BM of patients with breast cancer. Only the presence of DEC in BM was highly predictive for OS. The occurrence of DEC in the BM is probably less time-dependent and may act as a filter for circulating breast cancer cells. The use of either larger volumes of PB or performing an enrichment step for circulating tumour in blood cells, might improve these results.

316 Poster

Potentiation of estrogen receptor-mediated transcription by steroid and xenobiotic receptor (SXR) in breast cancer cells

N. Rokutanda[1], T. Iwasaki[2], R. Nagaoka[1], W. Miyazaki[2], A. Takeshita[3], Y. Koibuchi[1], J. Horiguchi[1], Y. Iino[4], Y. Morishita[1], N. Koibuchi[2]. *[1]Gunma university graduate school of medicine, Department of thoracic and visceral organ surgery, Maebashi, Gunma, Japan; [2]Gunma university graduate school of medicine, Department of Integrative Physiology, Maebashi, Gunma, Japan; [3]Toranomon Hospital, Okinaka Memorial Institute for Medical Research, Division of Endocrinology and Metabolism, Tokyo, Japan; [4]Gunma university graduate school of medicine, Department of Emergency Medicine, Maebashi, Gunma, Japan*

Estrogen receptor (ER) is a key regulator of proliferation and differentiation in normal mammary gland and breast cancer cells. ER activity can be modulated by other nuclear receptors. On the other hand, steroid and xenobiotic receptor (SXR), an adapted orphan nuclear receptor, has been shown to mediate the genomic effects of steroid hormones, including estrogen and xenobiotics. This receptor regulates the expression of the cytochrome P-450 3A (CYP3A) gene family, which plays important roles in the metabolism of endogenous steroids and xenobiotics. It has been reported that SXR is expressed mainly in liver and small intestine, however, recent study showed that SXR is also expressed in both normal and neoplastic breast tissue. To study whether ER activity is altered by SXR, we investigated the effect of SXR on Estrogen(E_2)-induced transcription through ER using transient transfection-based reporter assays. SXR potentiated ER-mediated transcriptional activity of the estrogen responsive element (ERE)-containing promoter in the presence of E_2 in MCF-7 breast cancer cells. On the other hand, SXR alone did not affect ERE-containing promoter activity in ER negative CV-1 cells. In semi-quantitative RT-PCR studies, SXR up-regulates a classic E_2-dependent gene such as pS2. To study further the mechanism of SXR potentiation of ER-mediated transcription, we performed a series of experiments. Using GST pull down, mammalian two hybrid, and electropholetic mobility shift assays, we showed that (i) SXR did not interact with ER, (ii) SXR did not bind to ERE, and (iii) SXR did not alter the binding between ER and steroid receptor coactivator (SRC)-1. Thus we focus on the effect of SXR on the binding between ER and corepressors. It has been reported that corepressors nuclear receptor corepressor (N-CoR) and silencing mediator for retinoid and thyroid receptors (SMRT) are expressed in breast cancer, and may be recruited by ER in the presence of E_2 and tamoxifen. In reporter assays, increasing amounts of SMRT reversed the potentiation of ER activity by SXR. The binding of ER with SMRT was decreased by SXR in GST-pull down assay and mammalian two-hybrid assay. These results suggest that SXR induced ER-mediated transcriptional activity by squelching limiting amounts of SMRT corepressor. In conclusion, we demonstrate that SXR induces ER signaling, which may play crucial role for cell growth, cell differentiation, and xenobiotic metabolism in breast cancer cells.

317 Poster

Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) modifies Epidermal Growth Factor (EGF)-related breast cancer growth depending upon the extracellular-matrix (ECM)

Z. Winters, G.J. Dennison, C.M.M.J. Perks, J.M.P. Holly. *IGFs and Metabolic Endocrinology Group, Clinical Sciences North Bristol, The medical School, Bristol, United Kingdom*

Introduction: Insulin-like growth factor binding protein-3 (IGFBP-3) is the most abundant IGFBP in serum and is able to modulate cell proliferation independently of its ability to bind IGF. Tumour-associated increases in IGFBP-3 levels relate to up-regulation of EGFR and HER-2 with increasing oestrogen-independence. Remodelling of the extracellular matrix with increased fibronectin expression in poor prognostic tumours further enhances EGFR levels and signaling. We have explored the potential interaction of these pathways using the EGFR/HER-2 tyrosine kinase inhibitor, Iressa.

Aims: We have examined the effects of IGFBP-3 on EGF-mediated growth in both normal breast and breast cancer cells in the presence and absence of fibronectin.

Material and Methods: Normal breast epithelial cells (MCF-10A) and breast cancer cells (T47D) were dosed with EGF (5 ng/ml & 10 ng/ml) or IGFBP-3 (100 ng/ml) or SPD (an IGFBP-3 peptide that mimics IGF-independent actions of IGFBP-3) or Iressa (0.25 μm) either alone or combinations of each, on plastic and on fibronectin (0.25 μg/ml). Cellular proliferation was evaluated by cell counting and thymidine incorporation (TLI).

Results: In MCF10A cells, EGF and IGFBP-3 each increased cell proliferation on their own (by 55.2% and 31.7%, respectively) and together synergistically enhanced cell growth relative to EGF alone (by 123%). We found that the proliferative effect of IGFBP-3 alone, like that of EGF, was completely abrogated in the presence of an effective dose of Iressa.

In T47D cells, EGF increased cell proliferation (by 204%), IGFBP-3 alone had no effect, but in combination, in contrast to the normal cells, IGFBP-3 markedly inhibited EGF-mediated cell proliferation (by 85% relative to EGF alone). The IGF-independent effects of IGFBP-3 were corroborated by SPD with the same results for both cell lines. MCF10A cells on fibronectin responded significantly to EGF (increased TLI by 285%), with IGFBP-3 suppressing EGF in contrast to plastic. On fibronectin, EGF increased cell growth (by 86%) of T47D, with IGFBP-3 enhancing EGF-induced growth (additional 27%) compared to its inhibitory effect on plastic.

Summary and Conclusion: IGFBP-3 has differential effects on EGF-mediated proliferation in normal and breast cancer epithelial cells that are switched when the cells are plated onto fibronectin, which is indicative of a more invasive phenotype. Future characterisation of breast tumours, in addition to EGFR/HER-2, may also include fibronectin and IGFBP-3 production to predict clinical responses to agents targeting the EGFR pathway.

'Iressa' kindly donated by Astra Zeneca.

318 Poster

The age of women at which bilateral breast cancer was diagnosed; reference to the presence of germline mutations in BRCA1, BRCA2 and CHEK2 genes and their family history of neoplasm

E. Skasko[1], A. Niwinska[2], Z. Paszko[1], E. Kwiatkowska[1], A. Balabas[1], A. Kluska[1], M. Piatkowska[1], D. Nowakowska[1], T. Pienkowski[3]. *[1]Sklodowska-Curie Memorial Cancer Center, Endocrinology Department, Warsaw, Poland; [2]Sklodowska-Curie Memorial Cancer Center, Breast Cancer and Reconstructive Surgery Department, Warsaw, Poland; [3]Sklodowska-Curie Memorial Cancer Center, Breast Cancer and Reconstructive Surgery Department, Warsaw, Poland*

Background: 163 women with bilateral breast cancer were examined. 30.1% of women had synchronous and 69.9% metachronous cancer.

Material and Methods: The DNA of peripheral blood lymphocytes of patients was examined for the presence of selected germline mutations in BRCA1, BRCA2 and CHEK2 genes using molecular biology techniques. Patients' family history of neoplasm was also analysed.

Results: The following mutations in BRCA1 gene were identified: 185delAG in 1 patient, T300G in 2 patients, 5382insC in 17 patients and 3875del11ins7, C5370T, IVS20+60ins12 and IVS2−16G>A in 1 patient each. In BRCA2 gene, 9631delC mutation was found in 1 patient and IVS16−116ins3 mutation in another one. In CHEK2 gene, 430T>C mutation was identified in 10 patients and 1100delC in 2. BRCA1/2 mutations were identified in 16% of patients (26/163) and CHEK2 mutations in 7.4% (12/163). It was carried out that the presence of the mutations in BRCA1/2 genes among patients with bilateral breast cancer is associated with an earlier occurrence of the first and the second breast cancer than in patients without germline mutations in these genes (a difference of

7.2 to 8.5 years, p = 0.0001–0.0008). In patients with CHEK2 mutations, breast cancer occurred 2.1 to 3.8 years earlier than in patients without mutations in CHEK2 gene. However, the identified differences were found not significant (p = 0.4802 and 0.2060). Among remaining 125 patients with bilateral breast cancer, who had not germline mutations in BRCA1, BRCA2 and CHEK2 genes, 85 had family history of neoplasm and 40 had not. The women with bilateral breast cancer and family history of breast cancer only were not diagnosed for earlier occurrence of bilateral breast cancer. However, the age of women with metachronous breast cancer and with family history of breast and ovarian cancer or ovarian cancer only was different from the age of women with no such family history (a difference of 7.7 years, p = 0.0608).

The age of women with family history of neoplasm other than breast and ovarian, at which bilateral breast cancer was diagnosed, was significantly lower than the age of patients with no such family history (a difference of 5.2 years, p = 0.0169).

319 Poster

The role of steroid sulfatase (STS) and organic anion transporter polypeptide B(OATP B) mRNA expression in predicting the clinical outcome in human breast cancer

W. Al Sarakbi[1], M. Reed[3], M. Salhab[1], W. Jiang[4], K. Mokbel[1,2]. *[1]St.George's Hospital and Medical School, Breast Unit, London, United Kingdom; [2]Brunel University, Institute of Cancer Genetics and Pharmacogenomics, London, United Kingdom; [3]Imperial College, St.Mary's Hospital, Faculty of Medicine, London, United Kingdom; [4]Wales College of Medicine, University of Cardiff, Department of Academic Surgery, Cardiff, United Kingdom*

Introduction: Steroid sulfatase (STS) is the enzyme responsible for hydrolysing biologically inactive estrogen sulfates to active estrogens. Therefore it plays a significant role in supporting the growth of hormone-dependent tumours of the breast, endometrium, and prostate. There is little evidence as to what controls its expression in vivo. OATP-B is a member of family of membrane transporter proteins that regulates the uptake of steroid sulfates through cell membranes.

This study examines mRNA expression of STS and OATP-B genes located on chromosome X (location Xp22.32) and chromosome 11 (location 11q13) respectively. Our objective was to determine, using quantitative PCR, whether the mRNA expression levels of these genes were positively correlated with clinical outcome in human breast cancer.

Methods: A total of 153 samples (120 tumour tissues and 33 normal tissues) were analysed. The levels of transcription of STS and OATP-B were determined using real-time quantitative PCR. The mRNA expression was normalized against CK19. The levels of expression were analyzed against tumour's stage; grade, nodal status, local relapse, distant metastasis and survival over a 10 year follow up period. The levels were also analysed against hormone receptors status including ERα, ERβ, and HER1–4.

Results: The levels of STS mRNA were significantly higher in malignant samples compared with normal breast tissue samples (p = 0.031). They were also higher in node positive disease (p = 0.0222). STS mRNA expression increased with increasing tumour grade but this did not reach statistical significance. We also noted an increase in levels correlating with tumour stage using TNM classification. This became statistically significant when we compared stages TNM1 and TNM2, TNM2 and TNM3, and TNM3 and TNM4 ($p \leqslant 0.00001$, 0.0017, and 0.02 respectively). Furthermore, STS expression levels positively correlated with progression of disease as levels were significantly higher in samples of patients who developed metastasis, local recurrence, or died of breast cancer when comparing to those who were disease free for > 10 years (p = 0.0036).

We found no significant correlation between levels of STS expression and ERα/ERβ status. The levels positively correlated with HER1 and HER3 receptors.

The levels of mRNA expression of OATP-B were higher in malignant tissue compared to normal tissue, this however did not reach statistical significance (p = 0.4045). Levels were also higher in node positive disease (p = 0.0672). Expression levels increased with increasing tumour grade, this became statistically significant when comparing grade 1 to 2, and grade 2 to 3 (p = 0.0271, 0.0289 respectively). We also observed an increase in levels correlating with TNM tumour staging, this however did not reach statistical significance. There was no significant correlation between OATP-B expression levels and clinical progression of breast cancer. We found no correlation between STS and OATP-B expression levels.

Discussion: This study demonstrates a compelling trend for STS transcription levels to be higher in cancerous tissues. These levels were even higher in patients who developed progressive disease. OATP-B expression levels correlated with the grade and stage of the disease but not with clinical outcome. These results suggest that STS mRNA has a significant potential as an independent predictor of clinical outcome in patients with breast cancer.

320 Poster

p21 as a target for breast cancer therapy: the role of p53 status in its efficacy

P. Lin[1,2], R. Weiss[1]. *[1]University of California, Davis, Division of Nephrology, Internal Medicine, Davis, CA, USA; [2]University of California, Davis, Immunology Graduate Group, Davis, CA, USA*

$p21^{waf1/cip1}$ has emerged as an important but pleiotropic regulator of differentiation, cell cycle progression, senescence, and apoptosis, and this molecule has been suggested by us and others as a molecular target in breast cancer treatment. Recent reports have shown that doxorubicin differentially activates Akt in some breast cancer cell lines, including p53-mutant T47D cells, and that activated Akt increases cytosolic expression of p21. We undertook this study to investigate whether p21 is conveying anti-apoptotic effects in breast cancer as a function of p53 status. We find that doxorubicin treatment of T47D, a p53-mutant human breast cancer cell line, results in a dose-dependent decrease in both p21 levels and the anti-apoptotic protein, XIAP, with a concomitant increase in PARP cleavage (indicating apoptosis). No changes in the apoptosis-related proteins CAS (cell apoptosis susceptibility) and Apaf-1 were seen with doxorubicin treatment. Similar results were found in a p53 mutant renal cell carcinoma cell line, 786-O. To determine whether p21 is conferring the anti-apoptotic effect seen with lower dosage of doxorubicin treatment, we used an RNAi approach. Down regulation of p21 with siRNA did not change PARP cleavage or expression levels of XIAP, CAS, and Apaf-1 as compared to cells treated with doxorubicin alone. However, p21 down regulation enhanced apoptosis induced by lower dosage of doxorubicin in the p53-wt renal cell carcinoma cell line, ACHN. We are currently investigating this effect in p53-wt breast cancer cell lines. Due to the fact that p21 is a downstream target of p53 in the DNA repair pathway, our results suggest the anti-apoptotic function of p21 is dependent on p53 status and are consistent with previously published data showing that p21 accumulation after doxorubicin treatment only occurs in p53 wild-type breast cancers. More importantly, these results suggest caution when choosing p21 as a therapeutic target in breast cancer therapy. The genetic composition of tumors, such as p53 status, should be carefully considered in selecting the therapeutic regimen.

321 Poster

Analysis of genetic alterations in plasma DNA from breast cancer patients: a possible molecular biomarker in early detection and prognosis of breast cancer

C. Shou-Tung[1], T. Kuei-Wen[2], C. Yi-Chih[2]. *[1]Changhua Christian Hospital, Comprehensive Breast Cancer Center, Changhua, Taiwan; [2]National Changhua University of Education, Department of Biology, Changhua, Taiwan*

Introduction: Genetic alterations are associated with the development of breast cancers, which are the most common malignancy in women. Tumor metastasis is the major cause of death in cancer patients. In the process of metastasis, tumor cells disseminate from the original site through the circulatory system and establish the secondary tumors in distant organs. Therefore, increasing levels of circulating tumor DNA was found in the bloodstream of cancer patients and apoptotic and necrotic cells are a major source for plasma DNA. Plasma DNA may be an indicator for cancer. Early diagnosis and identification of molecular tumor markers are the main topics of clinical cancer research. Molecular tumor markers described in plasma include oncogene amplifications, and microsatellite alterations, such as loss of heterozygosity (LOH) and microsatellite instability (MSI). It was postulated that identifying the genetic alterations in plasma may play an important role in the cancer diagnosis and prediction of cancers.

Patients and Methods: A total of 116 cases were analyzed in our study, including 34 non-metastatic patients with breast cancer, 41 metastatic patients with breast cancer originally, and 41 anonymous individuals without tumor, with previously identified breast cancer-specific microsatellite grade I markers, such as LPL, TP53, and D16S413, and grade II marker, D17S855, which located in the intron of the BRCA1 gene, using ABI 3100 capillary genetic analyzer. Additional 42 plasma samples, including 13 non-metastatic patients with breast cancer, 16 metastatic patients with breast cancer originally, and 13 anonymous individuals without tumor, were also used to analyze MYCN oncogene amplification with real time quantitative PCR using LightCycler instrument.

Results: LOH/MSI was detected in 6 of 41(14.6%) anonymous individuals with non-tumor disease, in 14 of 34(41.2%) non-metastatic patients, and in 18 of 41 (43.9%) metastatic patients. The frequency of LOH/MSI of plasma DNA was significantly lower in anonymous individuals