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.

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

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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.

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

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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.

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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

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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