(qRT-PCR) analysis of hormone receptor positive (HR+) tumors from patients with advanced breast cancer resistant to first-line tamoxifen therapy. The aim of this study was (a) to correlate SIAH2 expression with disease outcome including patients treated with other therapy strategies and (b) to determine the role of SIAH2 in endocrine therapy resistance using in vitro cell line models.
Materials and Methods: In 1321 retrospectively collected primary breast tumor specimens SIAH2 levels were measured with qRT-PCR and related with disease outcome in different patient subsets. Human breast cancer cell lines ZR-75−1, EGFR transfected ZR-75−1 (ZR/HERc), and MCF7 were treated with estrogen (E2), epidermal growth factor (EGF) and ICI164.384 (a selective estrogen receptor modulator). ZR/HERc is resistant to ICI whereas ZR-75−1 and MCF7 are sensitive. Furthermore, SIAH2 expression was down regulated in MCF7 with siRNAs and subsequently treated with ICI. SIAH2 levels were determined with qRT-PCR and western blotting. Cell number counts were determined as a measure of therapy resistance.
Results: Low SIAH2 levels in tumors from lymph node positive patients with HR+ tumors associated significantly with a worse disease free survival (DFS) after adjuvant tamoxifen therapy (N = 145; HR = 0.76; P = 0.003) or chemotherapy (N = 231; HR = 0.77; P = 0.003). Multivariate analysis of SIAH2, as continuous variable, showed an independent and significant association with DFS (N = 145; HR = 0.80; P = 0.048) in the adjuvant tamoxifen setting and with progression-free survival in the advanced tamoxifen setting (N = 298; HR = 0.81; P = 0.010).
Our cell line studies confirmed the regulation of SIAH2 expression by the estrogen receptor since it was induced by E2 and repressed by ICI. Interestingly, EGF treatment of ZR/HERc decreased SIAH2 levels. Mock silenced MCF7 remained sensitive to ICI and had significant less cell counts after 96hrs ICI treatment compared to ICI untreated cells (23% decrease; P < 0.001; N = 3). In contrast, SIAH2 silencing resulted in a modest decrease in cell number after 96hrs ICI treatment (2%; P = 0.57), indicating that SIAH2 is involved in therapy resistance.
Conclusions: Low SIAH2 levels in breast tumors are associated with resistance to endocrine therapy in adjuvant as well as in advanced setting and in vitro studies demonstrated ICI resistance after SIAH2 gene silencing.

2003 ORAL

Gefitinib enhances response to chemotherapy in triple-negative Breast Cancer (BrCa)

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Background: Triple-negative BrCa lacks expression of hormone receptors and HER-2 but frequently expresses EGFR. Triple-negative BrCa is associated with early relapse and poor survival. There is currently no specific targeted therapy for triple-negative BrCa. The aim of this study is to assess the potential role of EGFR inhibition in the treatment of triple negative BrCa.
Methods: EGFR expression and downstream signalling was examined in triple-negative BrCa cell lines grown in the presence and absence of serum (BT20, HCC1937, and MDA-MB-231), by western blot. IC50 assays were determined using the acid phosphatase assay. Three EGFR inhibitors, gefitinib (G) and erlotinib (T), which are small-molecule tyrosine kinase inhibitors, and cetuximab (E) which is a monoclonal antibody against EGFR, and chemotherapy (CRx) drugs docetaxel (D), carboplatin (P) and doxorubicin (A) were tested. The controls were HER2+ BrCa cell lines, BT474 and SKBR3 which express low levels of EGFR.

HCC1937	% inhibition single agent	% inhibition combination
G (5 μM)	21.7±7.6	-
P (2.5 μM)	17.7±3.4	39.3±4.1
P (5.0 μM)	37.7±8.8	52.4±5.8
P (10.0 μM)	52.0±10.2	62.5±6.9
D (0.75 nM)	25.9±5.3	51.6±5.7
D (1.5 nM)	52.8±3.4	70.0±2.5
D (3.0 nM)	68.8±0.6	77.7±1.8
A (8.75 nM)	25.9±10.9	50.3±11.0
A (17.5 nM)	41.5±9.9	61.7±8.1
A (35 nM)	53.0±8.1	74.6±7.6

Results: The three triple-negative cell lines express high levels of EGFR. EGFR and downstream signalling molecules, Akt and MAPK, were constitutively phosphorylated in the serum-free medium, that is, in the absence of exogenous ligand. IC50 values for G and T were significantly higher in the triple-negative than in the HER2+ cell lines. E did not cause significant inhibition in any cell line (max inhibition 20% at 100 μg/ml E). IC50 values for G were lower than for T in the triple-negative cell lines (IC50s for HCC1937: G – 8.4±1.5 μM; T – 26.2±9.3 μM). Combined EGFR inhibition with CRx was tested in HCC1937 cells. G combined with P, D or A for 5 days showed an additive effect on inhibition of proliferation (Table). Alternate scheduling of the drugs did not significantly influence response.
Conclusions: Our results suggest that EGFR signalling is constitutively activated in triple-negative BrCa cells. Although they are not as sensitive to EGFR inhibition as HER2+ BrCa cells, the addition of gefitinib appears to enhance response to CRx in triple negative BrCa cells.

2004 ORAL

Novel breast cancer susceptibility loci identified in west Swedish families and candidate gene analysis

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Background: The two major breast cancer susceptibility genes BRCA1 and BRCA2 were identified more than ten years ago. Depending on population studied, mutations in these genes are responsible for a varying percentage of familial breast cancer. However, the increase in risk of developing breast cancer cannot be explained by mutations in BRCA1/2 in a majority of familial cases. In this study, we attempted to identify chromosome regions harboring cancer predisposing genes and subsequently analyze selected candidate genes.
Methodology: One large family and 13 small to medium-sized families with multiple cases of breast cancer were analyzed by genome-wide linkage analysis. In order to reduce genetic heterogeneity all families were selected within a relatively isolated geographic region (western Sweden). The genome scan was performed by genotype analysis of 10,000 SNP markers on microarrays (Affymetrix). Candidate genes SAFB1, SAFB2, TP53, XRCC1, CYP17, ERCC2 were analyzed by direct DNA sequencing in patient germline DNA.
Results: The strongest evidence of linkage (HLOD 2.34) was obtained on chromosome region 10q23.32-q25.3. A further two regions were identified, with HLOD scores above 2.10 on 12q14-q21 and 19p13.3-q12. The large family in the study exceeded LOD 1.5 in three regions: 10q23.32-q25.3, 19q13.12-q13.32, and 17p13. Mutation analysis of SAFB1 and SAFB2 revealed three silent polymorphisms in coding sequence and further two in intronic sequence. Breast cancer associated low risk alleles of TP53, XRCC1, CYP17 and ERCC2 were present in various numbers in affected women.
Conclusion: Our results indicate that one or more of the suggested regions may harbor genes that are involved in the development of breast cancer. Possible polygenic effect due to multiple, incompletely penetrant susceptibility genes may explain why multiple regions were identified. Fine mapping of identified chromosome regions is warranted in order to narrow down the candidate regions as well as the analyses of further candidate genes.

2005 ORAL

Breast cancer incidence in relation to oestrogen hormone receptor status in Denmark 1994–2005

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Objective: Breast cancer is the most common cancer among women in Denmark. Within the last 40 years the incidence has been increasing 2−3% per year. The rise in incidence has not been investigated in relation to oestrogen hormone receptor status (ER) on larger population-based material. We investigated the increase in breast cancer within age groups of premenopausal and postmenopausal women in a 12 year period selected due to the stringent use of immune histochemistry for ER definition.
Material: Register data was obtained from the Danish Breast Cancer Group database, which contains close to all Danish women registered with a histologically verified diagnose of invasive breast cancer, between 1 January 1994 and 31 December 2005. Oestrogen receptor (ER) status was defined as positive if more than 10% of the tumour cells were positive using uniform immune histochemistry technique. In all, 36,482 women were included.