cell-cycle inhibitory protein p27, uncontrolled cell proliferation and poor overall survival in breast cancer. The mammalian target of rapamycin (mTOR) is a downstream effector of the PI3K/Akt signaling pathway that mediates cell proliferation. By targeting mTOR, rapamycin induces cell-cycle arrest in the G_1 phase and upregulates p27 expression. The effects of rapamycin on Skp2 expression and the mechanism of p27 proteolysis, however, are unknown.

Materials and Methods: Rapamycin-treated and untreated T47D and MDA-MB-231 breast cancer cell lines were subjected to real time RT-PCR and western blot analysis to determine p27 and Skp2 mRNA and protein levels, respectively. Cells were also transiently transfected with a plasmid containing a Skp2 insert to determine the effect of rapamycin on translational regulation. Skp2 degradation rate was assessed in cycloheximide-treated cells and by pulse-chase analysis.

Results: Skp2 levels were downregulated in rapamycin-treated cells in a dose and time-dependent fashion, which was more prominent in cells expressing higher basal Skp2 levels. The decrease in Skp2 levels was followed by a reciprocal decrease in p27 ubiquitination, an increase in nuclear p27 levels and cell-cycle arrest at G_1. Rapamycin significantly inhibited Skp2mRNA transcription and rate of protein degradation, but not protein translation.

Conclusions: Rapamycin inhibits Skp2 expression and ubiquitin-mediated degradation of p27, resulting in upregulation of p27 and cell-cycle arrest. These results suggest that mTOR inhibition may be a novel target for the treatment of Skp2 overexpressing breast cancers.

326 Poster

Tumour aromatase as measured by immunohistochemistry in patients treated neoadjuvantly with either letrozole or tamoxifen in the P024 randomised trial – correlations with other biomarkers

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Aromatase, the key enzyme responsible for oestrogen biosynthesis, is a major therapeutic target of endocrine therapy in patients with breast cancer and is present in about 70% of tumours.

The aim of this study was to measure aromatase in tumours before and after 4 months of treatment with letrozole or tamoxifen by immunohistochemistry using a novel monoclonal antibody (677), which has recently been developed to detect aromatase in archival material. IHC staining was performed in 185 cases from the P024 neoadjuvant trial randomising patients to tamoxifen or letrozole. Scoring was measured by assessing the proportion of immuno-positive cells and their intensity of reactivity in malignant epithelial, stromal, adipose and normal/benign compartments in each case.

Of 185 patients, sufficient tumour material was available for meaningful analysis in paired pre- and post-treatment samples from 171 cases (81 on letrozole and 90 on tamoxifen). Staining was detected in all tumour compartments but the highest scores were generally observed in malignant epithelial cells. Although there were highly significant correlations in scores between the malignant, stromal, adipose and normal compartments, patterns of staining differed between individual cases. Aromatase staining was not related to oestrogen receptor score, progesterone receptor status, Ki67 score or tumour size. Whilst median tumour aromatase values did not change significantly with treatment, marked changes in score (either increases or decreases) did occur in individual cases; effects were more noticeable for patients treated with letrozole. Correlation of these changes with clinical response is ongoing.

It is concluded that the 677 monoclonal antibody is a useful tool by which to assess and immunolocalise aromatase protein in breast cancers. Staining occurs in both malignant and non-malignant compartments and expression may be influenced by treatment with endocrine therapy.

327 Poster

c-kit: identification of coregulated genes in breast cancer patients by gene expression analysis

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Expression of the proto-oncogene c-kit has been found in malignant tissue including a subset of breast cancers. c-Kit is also expressed in normal breast tissue and several authors found a loss of c-kit expression in breast carcinoma suggesting it might be involved in the growth control of mammary epithelium. Until now, only a few markers were described to be co-regulated with c-kit as e.g. CK-5/-17, Her-1 and PDGFR. To elucidate the possible role of c-kit in malignant transformation, we analyzed gene expression data of breast cancer patients.

Experimental procedures: Tumor tissue of n = 171 breast cancer patients were analyzed by gene expression profiling using Affymetrix Hg U133 Arrays (22,500 genes) and bioinformatic analyses. Tumor samples with high stromal and low epithelial cell content by gene expression profiling were excluded for further analysis.

Summary: A total of 10.5% of the tumors showed strong c-kit expression (2.5 fold above median). Comparing gene expression profiles of 10 samples with highest c-kit vs. those 10 with lowest expression did not reveal genes with a good correlation to c-kit. However, when samples were first subdivided into molecular tumor subtypes using the intrinsic gene set according to Sorlie et al., most of the tumors with highest c-kit expression clustered in the normal like subgroup. Interestingly, analyzing samples from the different molecular tumor subtypes separately revealed strong correlations of c-kit with the expression of a large cluster of genes containing several for whom c-kit coexpression was already described (HER1, CK-5/-17, PDGFR) as well as several members of the wnt signalling pathway, providing a possible novel link to mammary epithelial differentiation.

Conclusions: Gene expression analysis of mammary tumor subgroups according to Sorlie et al. provides new insight in the biological function of c-kit. This may help to identify patients who could have a benefit from the treatment with STI571 as a new therapeutic option.

328 Poster

Insulin-like growth factor-1 expression and its prognostic significance in breast cancer

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Introduction: It is established that Insulin-like Growth Factor-1 (IGF-1) has pro-mitogenic, anti-apoptotic and growth stimulatory properties on breast cancer cells. Within the area of breast cancer, the main source of IGF-1 production, whether it is by tumour or normal breast tissue has yet to be confirmed. Also, the prognostic significance of high levels of local IGF-1 production and IGF-1 receptor (IGF-1R) expression in breast cancers remains to be validated. We examine the expression IGF-1 and IGF-1receptor expression in breast cancer and adjacent normal tissue (ANT) and the effects of IGF-1 on established prognostic factors used in breast cancer management.

Methods: Using real-time quantitative PCR, we measured the IGF-1 and IGF-1R expression in 88 oestrogen receptor (ER) positive and 40 ER negative pairs of breast cancer tissue samples and their corresponding ANT. CK-19 was used as a housekeeping gene for comparison of mRNA levels. The level expressed in these tissues were correlated with patient's age at surgery and prognostic factors: grade, lymph node status, tumour size and presence of lymphovascular invasion.

Results: In ER negative breast tissue, there was a significantly higher expression of IGF-1 in ANT compared to tumour tissue ($p = 0.000$) but this was not the case for ER positive breast cancers ($p = 0.887$). There was no difference in mean IGF-1 expression in ER positive and negative breast cancers. In general, there was also no difference in IGF-1R expression in tumour or ANT.

In ER positive cancers, there was a positive correlation between the number of positive metastatic lymph nodes and IGF-1 expression in normal tissue ($p = 0.034$) but not by IGF-1 expression in tumour tissue ($p = 0.564$). There was a marginally significant higher occurrence of lymphvascular invasion in samples with high tumour IGF-1 expression in ER positive tumours($p = 0.080$). In ER negative tissues, IGF-1 expression in tumour and ANT did not have any correlation with age or any of the prognostic factors.

In all cases, IGF-1R expression did not have any effects on prognostic factors.

Conclusion: We conclude that IGF-1 expression is higher in ANT which supports a paracrine production IGF-1. This data suggests that this relationship is mainly present in ER negative breast cancers. Increased IGF-1 expression by ANT and tumour tissue is associated with an increase in some prognostic factors but not IGF-1R. Future treatments of breast cancers involving a reduction IGF-1 production in normal and cancerous breast tissue should be considered.