at levels as low as 1%. DNA samples containing various proportions of mutant KRAS were analyzed by StripAssay hybridization and compared to results from real-time PCR, dideoxy sequencing and pyrosequencing. While all methods correctly identified samples containing 25% mutant DNA, dideoxy sequencing and pyrosequencing failed to detect levels of 12.5% or lower. Both StripAssay hybridization and real-time PCR, however, unambiguously identified 10%, 5% and 1% of mutant KRAS in the presence of excess wild-type DNA.
Conclusions: The existing StripAssay is currently being extended to contain additional mutations, such as KRAS codon 61 variants. The simultaneous detectability of multiple mutations in a single experimental set up with excellent sensitivity will make the StripAssay a very useful tool for the KRAS/BRAF mutation assessment on tumour samples.

190 Breast fluid elevations of progesterone independent of serum concentrations in postmenopausal women

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Background: Progesterone has been implicated as a risk factor by inference from data in the Women's Health Initiative and other studies of postmenopausal hormone replacement (HR). The combined treatment of equine estrogens and medroxyprogesterone acetate, in particular, has been associated with a higher incidence of breast cancer than estrogen treatment alone, and maximum proliferation is seen in the breast in the mid-luteal phase of the menstrual cycle when serum progesterone levels are highest. In the present study progesterone concentrations were measured in serum and nipple aspirate fluid (NAF) of premenopausal women during the mid-luteal phase of the menstrual cycle and in postmenopausal women.
Materials and Methods: NAF was collected 3 times within a month from 13 postmenopausal women for assessment of hormone levels in serum and NAF. The age range was 43 to 55, median 50; Gail scores ranged from 0.6 to 1.6, median 0.9; BMI, ranged from 18.6 to 41.3, median, 26.2. The breasts were warmed with towels, massaged, and a vacuum device was applied to the nipple. Droplets of NAF were collected in calibrated capillary tubes. NAF was diluted, and progesterone was analyzed by an immunoassay after extraction and separation of progesterone from phenolic steroids by partition between 0.4 M NaOH and isooctane. The comparison group was 99 premenopausal women, age 20−40 yr.
Results: Mean serum and NAF progesterone concentrations were 0.38 and 2.10 ng/ml, respectively. The correlation between serum and NAF was 0.068. The regression between right and left breasts was not significant, $p = 0.83$; correlation, 0.139. Four subjects had concentrations of >100 ng/ml in one or more breasts one or more times during the sampling period. The mean serum concentration in these four subjects was 1.96 ng/ml. By comparison, premenopausal women had serum and NAF progesterone concentrations of 14.1 and 41.5 ng/ml, respectively. They had a positive regression between right and left breasts, $p = 0.001$; correlation, 0.741.
Conclusions: Breast fluid concentrations of progesterone in postmenopausal women were sporadically elevated non-coordinately in right and left breasts by as much as 50-fold over the average for the group while serum progesterone was elevated by only 5-fold. This is different from the more coordinate levels found in premenopausal women and is evidence for local production of progesterone in the breast of postmenopausal women under some circumstances.

191 Variation of intrinsic cetuximab sensitivity in head and neck squamous cell carcinomas

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Background: Cetuximab is a monoclonal antibody directed against the epidermal growth factor receptor (EGFR). It has proven a sufficient treatment in combination with radiotherapy in head and neck squamous cell carcinoma (HNSCC). However, far from all patients benefit from this therapy and predictive biomarkers of response to cetuximab are therefore required.
Materials and Methods: We evaluated the intrinsic cetuximab sensitivity (ICeS) in 35 cell lines (established by Professor Grénman, University of Turku, Finland) by a crystal violet assay, and results were expressed as survival compared to control cells. EGFR expression was measured with an ELISA assay and correlation analysis was performed.
Results: The mean ICeS was 0.76, and the variation was between 0.16 and 1.4. Cell lines with survival exceeding 0.95 were considered resistant, and survival below 0.5 regarded as sensitive. Interestingly, two cell lines proliferated significantly under cetuximab treatment. Twelve cell lines (34%) were resistant to cetuximab, whereas five (14%) were sensitive. The EGFR expression varied greatly among the cell lines. However, there was no correlation between cetuximab sensitivity (ICeS) and EGFR expression ($r^2 = 0.11$). In order to reveal novel predictive markers of cetuximab sensitivity, a number of resistant and sensitive cell lines were selected for analysis on Affymetrix SNP 6.0 chips in order to detect copy number variations between the two groups. Common copy number variations will be detected with fluorescent in situ hybridization in order to evaluate the presence in patient material.
Conclusions: Our results show a great divergence in the cellular response to cetuximab treatment. Since the expression of the receptor itself is not an adequate predictive marker, other factors must be uncovered. The possibility of using gene copy number variation as a predictive marker is being evaluated at present.

192 Constitutive expression of carbonic anhydrase IX in hypoxic and normoxic non-small cell lung cancer fragments

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Background: Hypoxia is typically present in solid tumours like lung cancer and is known to enhance tumour progression and resistance to therapy. Surrogate markers like carbonic anhydrase IX (CA IX) are often used instead of direct oxygen measurements to assess tumour hypoxia. The aim of the study was to analyze the value of CA IX expression as hypoxia marker in non-small cell lung cancer (NSCLC) fragments.
Materials and Methods: A novel model using fragmented NSCLC surgery explants cultured *ex vivo* in normoxia or hypoxia was developed. The viability of cultured fragments was confirmed by histomorphology, apoptosis measurements, and a formazan-based viability assay.
Results: CA IX mRNA was significantly upregulated in NSCLC fragments ($P = 0.032$) and NSCLC cell lines cultured in hypoxia (1% O_2) for three days compared to normoxic conditions. However, CA IX mRNA expression and immunostaining at baseline (in normoxic fragments) displayed considerable variation. CA IX mRNA levels and mRNA levels of the upstream transcription factor hypoxia-inducible factor (HIF)-1alpha were significantly elevated in NSCLC samples compared to unaffected normal lung tissue ($P < 0.001$ each).
Conclusion: Both, hypoxia dependent and hypoxia independent expression of CA IX, are present in NSCLC. The hypoxia pathway leading to CA IX expression thus seems to be constitutively active in NSCLC cells. From our data we conclude that CA IX might be a tumour marker, rather than a marker for tumour hypoxia in NSCLC.

193 Expression and significance of Epidermal Growth Factor Receptor (EGFR) in carcinoma breast – a study from the cancer centre in India

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Background: Abnormalities in oncogene expression probably influence the breast cancer cell through specific growth factors or growth factor receptors. These include estrogen and progesterone and other growth factors such as TGFα and β, IGF I and II, EGFR, HER-2, somatostatin receptors and retinoid acid receptors. Epidermal Growth Factor Receptor (EGFR) is a member of Class I tyrosine kinase receptor family. This study was performed on Indian patients to investigate the expression and significance of EGFR in Breast Cancer.
Materials and Methods: The expression of EGFR in 210 specimens of breast cancer was detected by immunohistochemistry. The correlation of EGFR expression to clinico-pathological features by breast cancer was analyzed.
Results: EGFR expression was positive in 41.4% of breast cancer. The expression of EGFR was positively correlated to pathological tumour size ($p = 0.001$), Her-2 neu ($p = 0.000$), Visceral metastasis ($p = 0.000$) and Disease Free Survival ($p = 0.002$) but inversely correlated to ER ($p = 0.000$), and Overall survival ($p = 0.001$). There was no correlation between EGFR and age, menopausal status, histology or lymph node status. In multivariate analysis, EGFR ($p = 0.000$; hazard ratio 0.357, 95% CI 0.208–0.616) and Pathological Nodal stage ($p = 0.002$; hazard ratio 0.409, 95% CI 0.231–0.725) were found to be significant.
Conclusions: The expression of EGFR in Breast Cancer is an adverse prognostic marker and related to poor survival. Future trials should aim at incorporating EGFR targeted therapies in order to improve the outcome.