technique into clinical routine. A stiffening of the tip of the optical probe seems needed to improve the live imaging of laryngeal structures.

186 Study of zinc-dependent aggregation of metallothionein from human prostatic cancer cell lines

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Background: Metallothioneins (MTs) are heavy-metal binding cysteine-rich proteins. MTs are thought to play a role in tumour disease. They can be used for studying of course, prognosis and metastating of a tumour. The enhanced MTs expression leads to the formation of cytostatics resistance in tumour cells. One the current accepted opinion is that these mechanisms are closely connected to ability of MT to bind heavy metal ions and form aggregates.
The aim of this work was to study the changes in aggregation of MT under various concentrations of zinc and different redox conditions in human prostatic cell lines.
Material and Methods: Reduced apo-MT was oxidized with 0.75 and 3.5 % H_2O_2, 0.5 and 1 μM $K_2Cr_2O_7$, and 0.5 and 1 μM $KMnO_4$ for 2 hours at 37ºC. To the reduced and oxidized apo-MT $ZnCl_2$ was added. Reaction was measured spectrometrically for 1 hour at 37ºC. MT oxidation and interaction with zinc was evaluated by measuring of spectra within the range from 200 to 300 nm. Human cancer prostatic cell line (PC-3) and control cell line (PNT1A) were used.
Results: In *in vitro* experiments we observed Zn binding and aggregation of both (reduced and oxidized) forms of MT. The oxidized observed a peak with maximum at 250 nm. The height of signals of both forms increased with time of the interaction and $ZnCl_2$ concentration. Comparing reduced and oxidized MT, the oxidized molecules had higher binding capacity. The increased formation of MT aggregates in molecular weight higher than 75 kDa was observed in dependence on $ZnCl_2$ concentration and degree of oxidation as a proportion of the most intense signal at 25 kDa measured by Experion. In cancer cell lines increased viability was observed compared to controls and the same MT aggregates were observed as at standards in dependence on $ZnCl_2$ concentration.
Conclusion: It can be concluded, that zinc supports MT expression and growth in human prostatic cell lines and that MT forms aggregates in dependence on redox conditions and zinc concentration both in *in vitro* and in *in vivo* conditions.
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187 Expression of multidrug resistance gene and DNA ploidy in locally advanced gastric and gastroesophageal junction adenocarcinoma

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Background: We intended to assess the expression of multidrug resistance (MDR-1) gene & DNA ploidy and correlate these with disease outcome in patients with locally advanced gastric/gastroesophageal junction adenocarcinoma (LAGC) receiving neoadjuvant chemotherapy (NACT), surgery and postoperative chemoradiation (POCRT).
Materials and Methods: We enrolled 14 patients (pts) of LAGC (stage II-IV, age $\leqslant$70 years, KPS $\geqslant$70) in a single arm phase-II trial. After 2 cycles of NACT (cisplatin 80 mg/m^2 D1, capecitabine 2 g/m^2/day D1−14 q3 weeks), response was assessed by upper GI endoscopy & CECT abdomen. Pts with resectable tumours underwent radical total/subtotal gastrectomy with D2 lymphadenectomy & POCRT (45 Gy/25#/5 weeks, concomitant capecitabine 1.5g/m^2/day). Inoperable pts received salvage chemoradiation (SCRT) or best supportive care (BSC). MDR-1 gene expression was evaluated by flowcytometric assay of P-glycoprotein positivity. DNA ploidy of tumour was assessed by DNA flowcytometry.
Results: Median age was 50 years. Male to female ratio was 9:5. Tumour locations included GE junction/proximal stomach (4), distal stomach (8) & diffuse (2). Radiology showed N+ & T4 disease in 11 & 6 pts respectively. In 50%, tumours were initially unresectable. At a mean follow-up of 7.36 months, only 3 pts completed assigned treatment & all 3 had complete response (CR). Among others, locoregional progression, distant metastasis & noncompliance were noted in 4 (28.6%), 3 (21.4%) & 4 (28.6%) pts, respectively. After NACT, response rate, disease control rate & symptomatic benefit were 28.6%, 57.2% & 78.6%, respectively. MDR-1 expression was monotonously low (mean 5.13%) & was not turned on after NACT (mean 3.2%). Difference in MDR-1 gene expression in-between tumour (mean 5.13%) & control tissue from normal gastroesophageal mucosa (mean 4.43%) at baseline was unremarkable. 66.6% of the analyzed patients had pure diploid tumours & 33.3% had mixed (diploid & aneuploid) tumours at baseline. Multiploidy (>1 aneuploid stemline) was noted in 1 patient (11.1%). All 3 patients who completed the assigned treatment & had CR, had diploid tumours at baseline. All 3 patients who had distant failure (2 peritoneal dissemination & 1 liver metastasis) had a sizable aneuploid cell population at baseline.
Conclusions: NACT followed by POCRT is a novel & safe approach in LAGC. Still, only 21.42% pts completed assigned treatment & had CR, possibly owing to high noncompliance (28.6%) & adverse patient characteristics. MDR-1 gene pathway is probably not the major mechanism of chemoresistance in LAGC in our patients. DNA ploidy might be a useful prognostic marker in LAGC with distant failures being associated with aneuploidy.

188 A novel mechanism for lung cancer migration and invasion through LPA-CARMA3-NF-kappaB signaling axis

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Lung cancer is one of the most common cancers in the world. It is a leading cause of cancer death in men and women in the United States and throughout the world. Lung cancer can be triggerd by many factors, such as lysophosphatidic acid (LPA). LPA is a type of G protein-coupled receptor ligand, and a bioactive mediator that promotes cancer cell proliferation and motility through activation of cell surface G protein-coupled receptors. LPA activates NF-kappaB, which is an important transcription factor and plays critical roles in tumourigenesis, such as tumour migration and invasion. We have previously reported that CARMA3 (CARD and MAGUK domain-containing protein 3) is indispensable in LPA-induced nuclear factor kappa B (NF-kappaB) activation in mouse embryonic fibroblast cells. However, it remains unknown whether the CARMA3 plays an important role in LPA-induced lung cancer cell migration and invasion. In the present study, using CARMA3 shRNA, we knockdowned its protein expression level in lung cancer cell lines. Consistent with previous reports, we found that down-regulation of CARMA3 strikingly impaired LPA-induced IKK activity and NF-kappaB activation in lung cancer cells. In addition, *in vitro* transwell migration and matrigel invasion assays demonstrated that CARMA3 shRNA significantly attenuated LPA-induced lung cancer cell motility and invasiveness. Together, our results provide the evidence that CARMA3 serve as a critical regulator in LPA-induced, NF-kappaB-mediated lung cancer cell migration and invasion. Therefore, we speculate that CARMA3 may represent an attractive therapeutic target for lung cancer cell and many other alignancies.

189 Sensitive detection of KRAS and BRAF mutations using mutant-enriched PCR and reverse-hybridization teststrips

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Background: KRAS and BRAF are key players in growth factor receptor induced signalling pathways. Somatic mutations in the two genes are known to play a role in oncogenesis and are found in various types of tumours, including colorectal, pancreatic, thyroid, lung and skin cancer. The most critical region of the KRAS gene for oncogenic activation are mutations in codons 12 and 13. Among BRAF mutations, V600E is by far the most frequently observed. KRAS and BRAF mutations are also known to be predictive for the response to cancer therapy with certain anti-EGFR monoclonal antibodies.
Materials and Methods: We have developed a reverse-hybridization StripAssay targeting ten KRAS codon 12/13 mutations as well as BRAF V600E. The test is based on PCR in the presence of KRAS/BRAF wild-type suppressors (mutant-enriched PCR), followed by hybridization of PCR products to teststrips presenting a parallel array of allele-specific oligonucleotide probes. The hybridization and detection steps can be carried out fully automated using commercially available instrumentation. StripAssay performance was evaluated on genomic DNA obtained from cultured cell lines, formalin-fixed paraffin-embedded (FFPE) tissue and stool.
Results: Using serial dilutions of DNA from various KRAS- and BRAF-mutant tumour cell lines into normal DNA, each mutation was shown to be detectable

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.