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