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Modulation of hepatocyte growth factor plasma levels in relation to the dose of exogenous heparin administered: an experimental study in rats

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Introduction: Partial liver transplantation has been consolidated to be a valid treatment option. We sought to understand the factors that modulate and may be harnessed to accelerate hepatocyte regeneration. We sought to determine the impact of heparin on m-hepatocyte growth factor (HGF) plasma concentrations.
Materials and Methods: Sixteen rats were assigned to four groups of four animals each: group A, without heparin; group B, 600 IU/kg; group C, 1000 IU/kg, group D, 1400 IU/kg. Blood samples (0.5 mL) were obtained from each rat at baseline and at 30, 60, 120, and 240 minutes. After the samples were centrifuged to separate supernates from the cell phase they were stored at −20 degrees C in the m-HGF reagent and subsequently tested using enzyme-linked immunosorbent assay. Results were analyzed using SPSS 11.5 statistical software.
Results: Among the 16 rats, one died at 110 minutes, just prior to the last extraction. The remaining rats were sacrificed. Mean weight was: 466±64.24 g with no intergroup differences ($P = 0.149$). The comparative results (using Student t test) were: baseline A(1−4) versus A(1−4) 30 minutes: $P < 0.05$; baseline A(1−4) versus A(1−4) 60 minutes: $P < 0.05$; baseline A(1−4) versus A(1−4) 120 minutes: $P = 0.10$ (NS); baseline A(1−4) versus A(1−4) 240 minutes: $P = 0.15$ (NS). No significant differences were found among group B: baseline C(1−4) versus C(1−4) 30 minutes and 60 minutes: NS; baseline C(1−4) versus C(1−4) 120 minutes: $P < 0.001$; baseline C(1−4) versus C(1−4) 240 minutes: $P < 0.10$ (NS). Finally, the results in group D were: baseline D(1−4) versus D(1−4) 30 minutes: NS; baseline D(1−4) versus D(1−4) 60 minutes and 120 minutes: $P < 0.05$; baseline D(1−4) versus D(1−4) 240 minutes: $P < 0.0005$. When we compared group A to C and D, we detected differences (albeit not when compared to B) with P values = 0.01. Peak values were obtained at 120 and 240 minutes (225.21 pg/mL and 221.78 pg/mL) among groups C and D.
Conclusion: Heparin has a positive effect to increase serum HGF concentrations among rats. The effect was dependent on the administered dose and the time elapsed.

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Preliminary evidences for recruitment of innate responses to rectal cancer cell death elicited by neo-adjuvant radio-chemotherapy

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Background: Colorectal cancer is the fourth cancer in the world with 1.023.000 new cases and 529.000 death. Rectal cancer patients with a cT3N+M0 tumor stage responds to the neo-adjuvant therapy, which causes necrosis and inflammation in situ. We cannot predict which patients will response. We focused our attention on macrophages, which represent specialized sensors of injury in the midst of living tissues; in particular we assessed the expression of Heme Oxygenase (HO-1), CD68, CD163, CD206, Tie2, RAGE. Moreover, we assessed inflammatory molecules and soluble pattern recognition receptors.
Methods: We collected blood and tissue samples at three time points: at diagnosis, at the end of the first CT cycle and at 8 week after the end of the therapy (coinciding with the surgical resection time of the tumour). At each time point we characterized circulating monocytes by flow cytometry, infiltrating macrophages by immunohistochemistry and selected inflammatory molecules in serum and plasma.
Results: We recruited 28 pts, with so far five complete pathological remission, five partial responses and five no responses. No substantial changes were detectable in the number of circulating monocytes. In contrast we observed a clear expansion of CD14/CD86 and CD14/CD163 double positive subsets. This event was transient; it abated at the later time point suggesting a causal relationship to the treatment. It correlated with sensitivity to the treatment. In fact we observed that in the responder patients the expansion of the CD14/86 subset was clear in the first weeks of treatment and decreased there after. In contrast in non-responder patients it was already expanded before the neo-adjuvant therapy. All the patients had an initial expansion of the CD14/163 subset. In the responder patients this population was still present at the time of surgery. The immunohistochemical study revealed a massive tumoral infiltration by macrophages that displayed clear features of alternative M2 polarization.
Conclusion: These data suggest that neo-adjuvant therapy modulates the cellular components of innate immune responses that could represent valuable predictive factor.

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Direct cell entry of gold/iron-oxide magnetic nanoparticles in adenovirus mediated gene delivery

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Background: Gold/iron-oxide MAgnetic Nanoparticles (GoldMAN) are composite nanoparticles comprising magnetic nanoparticles with gold nanoparticles immobilized on their surface. Because GoldMAN strongly interacts with biomolecules containing thiol via an Au-thiol interaction, it imparts useful magnetic properties to various biomolecules. Here, we show that GoldMAN enhances the gene transduction efficiency of the adenovirus vector (Ad), which is widely used for *in vitro*/*in vivo* gene transfer. Ad-mediated gene transfer strongly depends on the expression level of the coxsackievirus and adenovirus receptor (CAR) on the target cell surface. Therefore, its application is limited in low CAR-expressing cells, including some important immune cells, cancer cells, and stem cells. To overcome this problem, we conjugated Ad with GoldMAN and facilitated the penetration of the target cells by applying a magnetic field.
Material and Method: Ad and GoldMAN solutions were mixed at room temperature. The formation of Ad/Goldman complex was confirmed by transmission electron microscopy (TEM). To examine the efficiency of GoldMAN, Ad/GoldMAN complex was incubated on the B16/BL6 CAR(−) cells in the presence of magnetic field from the bottom of the plate. The enhancement of gene transduction efficiency was assessed by Luciferase and Green fluorescent protein (GFP) gene expression. Cell entry mechanism of Ad/GoldMAN was examined by Luciferase gene expression under the 4ºC or in the presence of anti-CAR antibody. Intracellular distribution of fluorescence-labeled GoldMAN was also analyzed by the confocal laser scanning microscopy.
Results: TEM observations of Ad/GoldMAN indicated that Ad was easily immobilized on GoldMAN's surface. The Ad/GoldMAN complex was introduced into the cell using a magnetic field, which increased gene expression over 1000 times that of Ad alone. Analysis of the cell entry mechanism indicated that GoldMAN directly penetrated the plasma membrane, independent of the cell-surface CAR and endocytosis pathway. Similar results were observed with confocal laser scanning microscopy. This mechanism of entry into the cell may improve the gene expression efficiency of Ad.
Conclusion: This technology provides a useful tool for extending Ad tropism and enhancing transduction efficiency. Due to its unique cell-entry mechanism, GoldMAN also makes possible the effective introduction of various biomolecules within the cell.

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Significance of the neurotensin – Na+/H+-exchanger axis for the metastatic potential of pancreatic carcinoma cell lines

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Background: Pancreatic cancer is a highly metastatic disease. Production of neurotensin (NT) and expression of neurotensin receptors (NTR), mediating effects not fully characterized, is frequently found in this tumor entity.
Material and Methods: Intracellular Ca^{2+}- and pH-responses were measured by spectrofluorimetry (fura-2, BCECF), proliferation in MTT-assays, NTR1 surface expression using B-N6 antibody, and production of IL-8 in an ELISA assay. Phosphoproteins were assessed in a Kinase Array (R&D) and genome-wide gene expression in microarrays (Human Genome Survey Microarray V2.0, Applied Biosystems).
Results. Stimulation of NTR1 in BxPC-3 and PANC-1 pancreatic cancer cells by the stable analog lys-ψ-lys-NT(8−13) revealed a marked increase in intracellular Ca^{2+} and intracellular alkalinization of 0.15 – 0.2 pH-units. Both effects were abrogated following application of the NTR inhibitor SR142948. In contrast, MIAPaCa-2 pancreatic cancer cells, exhibited a minor intracellular acidification despite a detectable Ca^{2+} response. The

NT analog was shown to exert minor effects on proliferation of the cancer cell lines in MTT assays. The NT analog stimulated Na^+- and amiloride-sensitive proton flux of the Na^+/H^+-exchanger 1 (NHE1). Activity of NHE1 is regulated by phosphorylation and, ERK1/2, p38α MAPK and mitogen- and stress-activated kinase1/2 (MSK1/2) were identified as responsible kinases in phosphoprotein arrays. Functional involvement of these kinases was proved with inhibitors PD 98059, SC68376 and dimethyl fumarate (DMF), respectively. Downstream targets of are MSK1/2 are CREB and NFκB and DMF was reported to inhibit metastasis of melanoma cells in experimental animals. In BxPC-3 and PANC-1 cells, lys-ψ-lys-NT(8−13) enhanced the production of IL-8, an important inducer of tumor cell dissemination, and these cells were acquired the ability to evade from an extracellular matrix gels. The NT analog upregulated expression of genes encoding cytoskeletal and adhesion proteins, glycolytic enzymes and metalloproteinases.

Conclusion. In conclusion, NT stimulated the aggressiveness of pancreatic cancer cells by induction of intracellular alkalinisation/extracellular acidosis and increased production of IL-8, in addition to its minor growth promoting effects.

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Pharmacogenetics of peripheral neuropathy in elderly patients (>65years) with advanced gastric cancer receiving oxaliplatin based chemotherapy within a randomized phase II study

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Background: Peripheral neuropathy (PNP) is a dose-limiting side effect of oxaliplatin based chemotherapy. High grade PNP may compromise quality of life especially in elderly patients (pts). A randomized multicenter phase II study was conducted to compare fluorouracil, leucovorin, oxaliplatin with or without docetaxel (FLO vs. FLOT, respectively) in elderly pts with advanced gastric cancer (AGC). Our purpose was to identify pharmacogenetic markers as predictors of high grade PNP within this study.
Methods: 143 pts were enrolled in this study. Pts. were numerically >65 years or numerically >59 years but classified biologically >65 years as defined by an *Instrumental Activities of Daily Living* score of <8. PNP was classified according to an oxaliplatin specific scale. Genotyping was performed using PCR-based RFLP or TaqMan®-based allelic discrimination. 20 polymorphisms in 13 genes being part of the metabolism of the applied drugs or DNA repair were analyzed. Statistical analyses were based on stepwise multivariate cox regression models and included genotypes and clinical parameters.
Results: Median age was 71 years (range 60−83). Pts received in median 6 cycles of treatment (range 1−12). 130 pts were evaluable for PN at time of analyses. Of these, 68 received FLO and 62 received FLOT. Cumulative grade 3 PNP occurred in 49% of pts without a significant difference between FLO and FLOT receiving pts (44% and 53%, respectively, $p = 0.4$). Genotypes of TS and MTHFR could be identified as independent risk factors for grade 3 PNP by multivariate analyses. Pts carrying a TS promoter genotype known to be associated with low TS expression (2R/2R, 2R/3RC, 3RC/3RC) were at higher risk for developing grade 3 PNP compared to pts without one of these genotypes (OR 3.0 [95%CI 1.27; 7.06], $p = 0.01$). Pts carrying MTHFR1298AC or CC genotypes were also at higher risk for experiencing grade 3 PNP compared to pts with the wildtype MTHFR-1298AA genotype (OR 3.1 [95%CI 1.26; 7.60], $p = 0.01$). In fact, 89% of pts that experienced grade 3 PNP were carriers of at least one of these risk genotypes.
Conclusion: Polymorphisms of TS and MTHFR might be associated with grade 3 PNP in AGC pts receiving oxaliplatin based chemotherapy.

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The effect of adjuvant chemotherapy with a taxane and a bisphosphonate on bone mass and bone strength in an animal model

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Background: Taxane-containing chemotherapy is becoming a standard in the treatment for many different tumors such as breast cancer. The purpose of the present animal study was to investigate the direct effects of docetaxel as a modern chemotherapy agent on bone strength and bone imaging parameters and wether a bisphosphonate in an osteoporosis equivalent dose mitigates the putative effects of the chemotherapy on bone.
Materials and Methods: 45 female Sprague-Dawley rats were randomized to three experimental groups. All groups underwent a sham ovariectomy. The first group received a placebo treatment with saline injections subcutaneous while the second and third group (each n = 15) were treated with 6 cycles of docetaxel in a 3 week term. One chemotherapy group received in addition daily subcutaneous application of 1 μg/kg ibandronate while the second group received a placebo treatment.
Following methods were used in order to characterize the effects of the different treatments on bone mass and strength: Peripheral Quantitative Computer Tomography (pQCT) bone density scans and structural analysis (μCT) scans were performed at the center of the femoral neck and shaft. After bone density and structural analysis the right femora were tested in 3 point-bending, while the left femora were tested in compression mode of the femoral neck. For both tests the load displacement curve was analyzed for stiffness and ultimate load.
Analysis of the vertebral bodies included μCT and a compression test of LVB 5.
Results: 6 cycles of taxane-containing chemotherapy caused a significant decrease in almost all parameters determining bone mass and bone strength. The effects followed the same pattern in all used methods. The treatment with ibandronate was able to preserve those parameters significantly compared to the negative effects in the group treated with chemotherapy only.
Conclusion: Since hypogonadism is not the result of the treatment with docetaxel it is likely that a direct negative effect on bone is the reason for decreased bone mass and bone strength. Estrogen might even protect the bone from more devastating destruction due to chemotherapy. Further experimental investigations are underway to clarify the protective role of estrogen in cancer treatment. Furthermore, we were able to show that an osteoporosis equivalent dose of the bisphosphonate ibandronate is able to mitigate the negative effects of chemotherapy in the animal model used.

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Correlation of Sodium Iodide Symporter (NIS) and Retinoic Acid Receptor Alpha (RARA) expression in breast cancer

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Background: Sodium Iodide Symporter (NIS) expression in the thyroid gland supports imaging and treatment of thyroid disease using radioactive iodide. NIS expression also occurs in malignant breast tissue suggesting potential for radioiodide in breast cancer imaging and therapy. Both in vitro and in vivo animal studies have shown that NIS expression in breast cancer is regulated by retinoic acid.
Aim: The aim of this study was to quantify NIS and retinoic acid receptor-a (RARA) gene expression in normal, benign and malignant breast tissue using RQ-PCR, and to correlate levels with clinicopathological details.
Method: Breast tissue specimens (n = 92) harvested at surgery were homogenised and RNA extracted using the Qiagen RNeasy Mini Kit. Following Nanodrop RNA quantification and reverse transcription, the corresponding cDNA was interrogated for NIS and RARA expression using RQ-PCR. To determine relative quantification (RQ) values, levels of NIS and RARA expression were normalised using the average levels of endogenous control genes PPIA and MRPL19, and expressed relative to the lowest detectable level for correlation of data. To compare individual breast cancer subtypes, results were expressed relative to levels detected in normal breast tissue. Statistical significance was analysed using the Student t test and correlation between NIS and RARA was determined using the Pearson Correlation Coefficient.
Results: NIS expression was detected in 74/76 breast cancer tissues analysed (Mean ±SEM, 1.17 ± 0.06 $\log_{10}$ RQ). There was a significant positive correlation between NIS and RARA expression in all breast tissues samples (Pearson correlation coefficient = 0.215, $p < 0.05$). The highest