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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

J.C. Meneu-Diaz[1], A. Moreno-Elola[1], V. Barra-Valencia[1], B. Perez-Saborido[1], Y. Fundora-Suarez[1], M. Abradelo[1], A. Gimeno[1], I. Aleman[1], I. Justo[1], E. Moreno Gonzalez[1]. *[1]University Hospital 12 de Octubre, Surgery, Madrid, Spain*

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

A. Tamburini[1], A. Castiglioni[2], K. Bencardino[3], E. Orsenigo[1], M. Salandini[1], L. Albarello[4], M. Ronzoni[3], A. Manfredi[2], C. Staudacher[1]. *[1]University Vita-Salute San Raffaele, Gastrointestinal Surgery, Milan, Italy; [2]University Vita-Salute San Raffaele, Cancer Immunotherapy and Gene Therapy Program (CIGTP), Milan, Italy; [3]San Raffaele Scientific Institute, Oncology, Milan, Italy; [4]University Vita-Salute San Raffaele, Pathology, Milan, Italy*

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

Y. Mukai[1], H. Kojima[1], T. Yoshikawa[1], K. Kamei[1], M. Yoshikawa[1], T.A. Yamamoto[2], Y. Yoshioka[3], N. Okada[1], S. Seino[2], S. Nakagawa[1]. *[1]Osaka University, Graduate School of Pharmaceutical Sciences, Suita Osaka, Japan; [2]Osaka University, Graduate School of Engineering, Suita Osaka, Japan; [3]Osaka University, The Center of Advanced Medical Engineering and Informatics, Suita Osaka, Japan*

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

U. Olszewski[1], E. Ulsperger[2], K. Geissler[2], G. Hamilton[1]. *[1]Medical University of Vienna, LBC Translational Oncology, Vienna, Austria; [2]KH Hietzing, LBC Translational Oncology, Vienna, Austria*

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