observed in tumours. In particular, we demonstrated that patients at the stage $T_3N_0M_0$ were characterized by highly elevated levels of expression of LMP7 (β5i), LMP2 (β1i) and 19S activator (up to 4 times in last two cases) in the tumours in contrast to normal tissues and tumour samples at stage $T_2N_0M_0$. We have also identified a population of immune cells penetrating the tumour, which were also characterized by increased expression of LMP2 (β1i) and LMP7 (β5i), but not 19S activator. Low expression of 19S activator was revealed in stromal cells.
Conclusions: Taken together on the basis of obtained data we can conclude that the accumulation of 19S activator in tumour cells is associated with tumour progression. In this regard we assume that the utilization of 19S activator as a possible antitumour drug target could become a promising approach in thyroid cancer therapy.

1080 POSTER

VEGF/VEGF-R Blockade Modulates Tumour-induced Immunosuppression in Colorectal Cancer

M. Terme[1], S. Pernot[1], E. Marcheteau[1], F. Sandoval[1], E. Tartour[1], J. Taieb[2]. *[1]INSERM U970, PARCC- Hôpital Européen Georges Pompidou − Université Paris-Descartes, Paris, [2]INSERM U970 and Service d'Hépatogastroentérologie et d'oncologie digestive, PARCC- Hôpital Européen Georges Pompidou − Université Paris-Descartes, Paris, France*

Background: Anti-angiogenic molecules targeting Vascular endothelial growth factor (VEGF)-A (bevacizumab) or its receptors (sunitinib, axitinib, sorafenib. . .) are routinely used as first or second line treatment of cancer patients. Anti-angiogenic molecules act not only on the vascular/endothelial system, but also seem to have an impact on immune escape mechanisms. We and others have recently shown that regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) decrease after Sunitinib administration in mice and metastatic renal cancer patients (Adotevi et al., J Immunother 2010; Ko et al., Clin Cancer Res). However, Sunitinib is a multi-target inhibitor specific of VEGF, KIT, PDGF, SCF, Flt3-L receptors, and signalization pathway(s) involved in immunomodulation induced by anti-angiogenic molecules is/are unidentified.
Methods: To better understand the role of the VEGF blockade in these immune phenomenon, we administered sunitinib or the mouse ortholog of bevacizumab (anti-VEGF antibody) to colorectal tumour-bearing mice (CT26 tumour model). The CT26 tumour cell line was chosen because of the known efficacy of sunitinib and anti-VEGF in this model and the use of anti-VEGF therapy in colorectal cancer patients. We analyzed tumour-induced immunosuppressive cells such as Treg, MDSC and PD-1 expressing T cells.
Results: In CT26 tumour model, Treg, MDSC, and PD-1 expressing T cells were significantly decreased after Sunitinib treatment in spleen and tumours, but also after anti-VEGF antibody administration. This decrease was not correlated with tumour size suggesting an immunomodulatory effect independent of a direct anti-tumour effect. Though Treg numbers were decreased after anti-VEGF treatment their regulatory functions were not altered by any of the treatment used. Moreover the use of masitinib, a tyrosine kinase inhibitor acting on KIT, PDGFR and FAK but not on the VEGF/VEGF-R pathway, was not able to modulate these different immunosuppressive cell populations. Finally, Treg, MDSC and $PD1^+$ T cells were also reduced in the peripheral blood of colorectal cancer patients after bevacizumab therapy.
Conclusion: Our results suggest that the blockade of the VEGF/VEGF-R pathway is sufficient to inhibit the induction of immunosuppressive cells by the tumour in mice and humans with colorectal cancer. This new property of anti-VEGF antibody opens perspectives for the use of such a molecule in association with anti-tumoral vaccination strategies in the future.

1081 POSTER

A Pilot Study on the Efficacy of RGTA OTR4120, a Family of Regenerating Agents, on the Restoration of Bone Microarchitecture of the Mandible and Nasomaxilla in a Murine Model

U. Djasim[1], L. Spiegelberg[1], J.W. van Neck[2], E.B. Wolvius[1], K.G.H. van der Wal[1]. *[1]Erasmus MC, Oral and Maxillofacial Surgery, Rotterdam, [2]Erasmus MC, Plastic and Reconstructive Surgery, Rotterdam, The Netherlands*

Background: RGTA (ReGeneraTing Agent) comprises a family of heparan sulphate mimetics, and are considered to be able to stimulate repair and regeneration in various tissues, such as skin, muscle, and bone. The purpose of this pilot study was to investigate the effects of RGTA OTR4120 on the bone microarchitecture in the irradiated murine mandible and nasomaxilla.
Materials and Methods: Mice received either radiotherapy only or radiotherapy followed by weekly RGTA injection until sacrifice at 2, 6 and 10 weeks after radiotherapy. Mandibles and nasomaxillas were harvested for microcomputed tomographic analysis. Bone volume, trabecular pattern factor, trabecular thickness, trabecular separation, and trabecular thickness were quantified and compared.
Results: Generally, there seemed to be no effect of RGTA-treatment compared to the RT-only group, although incidental positive effects were observed in trabecular separation and trabecular number.
Conclusion: RGTA has been reported to be a promising healing agent that can be effective in tissue repair and regeneration in various tissue defects. However, based on the current results, no positive effects of RGTA OTR4120 on repair and regeneration of irradiated bone tissue could be identified, although additional research is needed to further explore and determine the role that the relatively new healing agents of the RGTA-family can play in the repair and regeneration of irradiated bone tissue.

1082 POSTER

Nigella Sativa Oil Ameliorates Methotrexate-induced Liver Toxicity

R. Labib[1], H. Hafez[1], O. Badary[2]. *[1]National Cancer Institute, Pharmacology, Cairo, [2]Faculty of Pharmacy, Pharmacology, Cairo, Egypt*

Backgound: Methotrexate (MTX), a chemotherapeutic agent, is used to treat many types of cancer. However its use is limited by its side effects. We studied the use of Nigella sativa L (N. sativa) oil, a natural antioxidant, as a protective agent against MTX-induced liver toxicity.
Materials and Methods: Twenty-four male albino rats were divided into four groups: saline, N. sativa oil (10 ml/kg), saline plus MTX (20 mg/kg, ip single dose) or N. sativa oil plus MTX. Blood samples were collected for hematological assessment of hemoglobin (Hb%), RBCs, WBCs and platelets, and also to determine serum MTX levels of the two groups receiving MTX. All rats were then sacrificed; a section from liver was removed for pathological examination and another was homogenized for analysis of liver enzymes.
Results: Body weight loss in N. sativa oil plus MTX treated group compared to MTX group was (12.7% versus 29.4%, $P < 0.05$). N. sativa oil showed significant decrease in SOD content which was elevated in case of MTX ($P < 0.05$). GSH was significantly decreased by 53.75% ($P < 0.05$) in MTX group compared to combination group. Furthermore histologically, severe degeneration of the liver parenchyma which was observed in MTX-treated group was improved by N. sativa oil. There were alterations in MTX-treated rat group including dilated congested portal vein and central veins, marked lymphocytic infilteration in the portal area. Furthermore, many binucleated hepatocytes were seen. Degeneration of hepatocytes in the form of vacuolation of cytoplasm, pyknosis of nuclei and fatty degeneration of some cells were also observed. As well as, intrahepatic haemorrhage, areas of necrosis and marked periportal and porto-portal fibrosis were also observed. Addition of N. sativa oil, caused improvement in the lymphocytic infiltration, no porto-portal fibrosis, some binucleation, some vacuolation of cytoplasm, less congestion in the portal vein and less extent periportal fibrosis were all observed. However, there was still degeneration of hepatocytes in the form of vacuolation of cytoplasm and few pyknosis of nuclei. Moreover, addition of N. sativa oil did not significantly affect the therapeutic level of MTX ($P > 0.05$).
Conclusion: Administration of N. sativa oil before and after MTX injection ameliorated MTX-induced liver toxicity and maintained its structure through anti-oxidant activity. These results can lead to further clinical applications for prevention of MTX-induced liver toxicities.

1083 POSTER

The Effects of Heparan Sulfate Mimetic RGTA-OTR4120 on Radiation-induced Salivary Gland Dysfunction in Mice

L. Spiegelberg[1], U.M. Djasim[1], J.W. van Neck[2], E.B. Wolvius[1], K.G.H. van der Wal[1]. *[1]Erasmus MC, Oral and Maxillofacial Surgery, Rotterdam, [2]Erasmus MC, Plastic and Reconstructive Surgery, Rotterdam, The Netherlands*

Background: To study the effects of the heparan sulfate mimetic RGTA-OTR4120 on the salivary glands of mice that have been irradiated in the head and neck region.
Methods and Materials: Female C3H mice were irradiated with a single dose of 15 Gy in the head and neck region. RGTA-OTR4120 was injected 24 hours after radiotherapy, followed by weekly injections. At 2, 6 and 10 weeks after radiotherapy, salivary flow rates were measured and animals were sacrificed to obtain parotid and submandibular glands for histology. Periodic acid Schiff stain was performed to visualize mucins that are produced by acinar cells. Amylase and total protein content were measured in saliva samples.
Results: Salivary flow rates were increased at 2 and 6, but not at 10 weeks after radiotherapy with RGTA-OTR4120 administration, compared to irradiated controls. Two and ten weeks after radiotherapy, the mucin production activity of acinar cells was increased under influence of RGTA

administration. RGTA-OTR4120 did not have an effect on amylase or total protein secretion.
Conclusion: RGTA-OTR4120 administration has a positive effect on salivary flow rates in irradiated mice on the short term. The effect was absent 10 weeks after radiotherapy, while at that time point, mucin producing activity of acinar cells was elevated by RGTA-OTR4120 administration. Given these results and the advantages of RGTA use in irradiated patients, further investigation on the potential of this drug to treat radiation-induced xerostomia, alone or in combination with other drugs, such as amifostine, is suggested.

1084 POSTER

Claudin-1 Acts as a Tumour Suppressor in Hepatoma Cell Lines

A. Somorácz[1], P. Tátrai[1], G. Lendvai[1], K. Baghy[2], A. Kiss[1], I. Kovalszky[2], Z. Schaff[1]. *[1]Semmelweis University, 2nd Department of Pathology, Budapest, [2]Semmelweis University, 1st Department of Pathology and Experimental Cancer Research, Budapest, Hungary*

Background: Altered expression of tight junction proteins such as occludin and claudins is widely implicated in carcinogenesis. In our previous study, we detected an overexpression of claudin-1 in a subset of HCCs. Furthermore, in an immunohistochemical study by Higashi et al., correlation was found between decreased claudin-1 expression and dedifferentiation, as well as portal invasion of HCCs. Our aim was to investigate how claudin-1 influences cell proliferation in hepatoma cell lines and to determine whether it acts as a tumour suppressor.
Methods: To establish a cell line that stably overexpresses claudin-1, low-expressing HepG2 cells were transfected with pCI-neo vector containing full-length claudin 1 cDNA and selected with geneticin. The control line was transfected with empty pCI-neo vector. Downregulation of claudin-1 expression was carried out by siRNA targeted to claudin-1 in Hep3B cells that exhibit high basal claudin-1 levels. Down- and upregulation of claudin-1 expression was confirmed by quantitative RT-PCR and Western blotting. Cell proliferation was investigated with sulforhodamine-B test. HepG2 cells (20 million/animal) were injected subcutaneously into nude mice (5−5 for CLDN1-overexpressing and control cells) in order to investigate the tumour formation.
Results: Stably transfected HepG2 cells showed an overexpression of claudin-1 (10-fold at mRNA, and 2-fold at protein level) as compared with control cells. On the other hand, siRNAs decreased claudin-1 expression by 67%. According to the sulforhodamine-B test, downregulation of claudin-1 expression resulted in an accelerated cell proliferation of Hep3B cells (1.35-fold; $p < 0.01$), whereas increased claudin-1 production minimally decreased the proliferation index of HepG2 cells (0.97-fold; $p < 0.01$). Tumour formation of HepG2 controll cells was observed in 3/5 mice, whereas no subcutan nodules could be detected in animals with HepG2-CLDN1 cells ten weeks after the injection.
Conclusions: The minimally reduced cell proliferation together with the inhibited tumour formation of HepG2 cells due to claudin-1 overexpression, as well as the accelerated cell division by siRNA silencing in Hep3B cells indicate that claudin-1 acts as tumour suppressor in HepG2 an Hep3B hepatoma cell lines.
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1085 POSTER

The Longitudinal Trajectory of Post-Traumatic Growth: a Longitudinal Study

M. Scrignaro[1], M.E. Magrin[1], S. Barni[2], M.L. Bonetti[2]. *[1]University of Study of Milano-Bicocca, Psychology, Milan, [2]UO Medical Onchology Hospital of Treviglio, Psychology, Treviglio, Italy*

Background: The aim of the study is to investigate longitudinally the trajectory of post-traumatic growth (PTG; Tedeschi & Calhoun, 2006) in cancer patients. Secondly the study aims to study the relationship between PTG and intrusion vs. avoidance symptoms. Recently, empirical evidence highlights the presence of PTG (i.e. changes in the perceptions of oneself, one's social relationships, and one's life priorities) in 50% to 90% of patients. However less is known about the temporally trajectory of PTG and its correlates, because off the cross-sectional design of studies.
Material and Methods: A longitudinal study was conducted with a group of 53 cancer patients currently in the treatment and management phase of their illness. Data were collected by means of a written questionnaire, at two time points (T1 and T2) that were 24 months apart.
Post-traumatic growth was assessed by the Post-traumatic Growth Inventory. Intrusion and avoidance symptoms was measured by the Impact of Events Scale.
Results: Analysis showed that neither PTG levels neither avoidance symptoms change during the 24 months. On the contrary, intrusion symptoms increased significantly ($t = -2{,}02$, $df = 52$, $p < 0.05$). Further, both intrusion and avoidance symptoms was strongly related with PTG at t2 (respectively, $r = 0.37$; $r = 0.47$).
Conclusions: Data highlighted the temporal stability of the growth process that seems to be related to a cognitive engagement processes. From a clinical point of view data suggest the crucial role of meaning making process in fostering psychological adjustment to cancer illness and treatment.

1086 POSTER

Identification of the Receptor Tyrosine Kinase AXL as a New Target for Prostate Cancer Therapy

J.D. Paccez[1], G. Vasquez[1], R.E. Tamura[1], K. Duncan[1], L.F. Campesato[1], J. Vasconcellos[2], K. Chibale[3], T. Liberman[2], L.F. Zerbini[1]. *[1]International Centre for Genetic Engineering and Biotechnology (ICGEB), Cancer Genomics Group, Cape Town, South Africa; [2]Beth Israel Deaconess Medical Center (BIDMC), Harvard University, Boston, USA; [3]Department of Chemistry, University of Cape Town, Cape Town, South Africa*

Axl is a receptor tyrosine kinase of the family of TAM receptors (which includes TYRO3 and MER) and plays roles in different types of cancer. It is highly expressed in sarcoma, metastatic colon carcinoma, gastric and certain types of breast cancer. As Axl is upregulated in several metastatic cell types it may play a role during invasion and metastasis. Tyrosine kinases (TKs) represent a major class of proto-oncogenes and are involved in tumour growth, progression and metastasis of cancer cells. TKs are being actively studied as targets for therapeutic intervention and several of them have shown efficacy in clinical trials. Prostate cancer (PCa) is the most common solid cancer in older men and is one of the most frequent causes of deaths. Although androgen ablation therapy, surgery and radiation therapy are effective for the treatment of local PCa, there is no effective treatment available for patients with the metastatic androgen-independent disease. In this work we demonstrated the role of Axl in PCa progression and identified Axl as a potential target for PCa therapy. Using real time PCR to assess the level of tyrosine kinase receptors' expression in PCa cell lines and human tissue, we observed that Axl has consistent over-expression across cell lines and human prostate tumour tissue, providing a model for testing the targeting of Axl. Our data shows a significant increase in Axl expression in metastatic PCa cells and clinical samples (48% of adenocarcinomas of prostate compared with normal prostate tissue). Blockage of Axl gene expression using lentivirus encoding siRNA against Axl inhibits proliferation, migration and invasion of PCa cells. Our pilot studies in a xenograft subcutaneous model demonstrate that inhibition of Axl reduces tumour formation by 50%. Moreover, microarray analysis in addition to pathway analysis of Axl knockdown cells show that some survival pathways are inhibited, but strikingly all members of the NF-kB pathway are down regulated. To establish an alternative for PCa treatment we tested different inhibitors of the NF-kB pathway. Treatment of PCa cells with these drugs reduce proliferation and induce apoptosis. Furthermore, treatment of Axl knockdown cell lines with these drugs enhances their effects. Finally, in order to develop a specific inhibitor for Axl, we are evaluating a library of natural compounds from the African and Asian continents. We have found a compound that reduces proliferation and induces apoptosis in PCa cell lines and reduces Axl levels, thus representing a good candidate for future tests. Taken together our data demonstrates that Axl plays a role in migration, invasion and tumour development and can be used as a marker for invasive and metastatic tumours highlighting it as a target for drug therapies.

1087 POSTER

The Synergic Effect of CKD-516 to Conventional Chemotherapy

S. Kim[1], S.A. Yoon[1], D.H. Lee[1], H.J. Shin[1], I.T. Lim[1], H.S. Yu[1], D.H. Kim[1], S.K. Kim[1], S.S. Lee[1]. *[1]CKD Research Institute, Pharmacology, Yongin, Korea*

Background: Tubulin polymerization inhibitors had emerged as one of promising anticancer therapeutics because of their dual mechanism of action, i.e. apoptosis by cell-cycle arrest and VDA, vascular disrupting agent. VDAs are believed to be more efficient, less toxic, and several of them are currently undergoing clinical trials.
CKD-516 is a vascular disrupting agent (VDA) that attacks only tumour vessels inhibiting microtubule assembly. CKD Pharmaceuticals is conducting a phase I study of CKD-516 in patients having refractory solid cancers in Korea.
The clinical success of VDA inhibitors depends on their combination with other conventional therapeutic agents. In search for new therapeutic modalities to target NSCLC, we investigated the effect of CKD-516 as a single treatment or in combination with the established therapeutic agents such as carboplatin and paclitaxel.