processes. Overexpression of this protein in tumour in relation to normal tissue was earlier reported in human breast ductal carcinoma. The aim of this work was to elucidate whether the intensity and location (cytoplasmatic *vs*. membranous) of calreticulin overexpression in tumour cells are related to the elevated humoral immunity to calreticulin in patients with benign or malignant breast disease.
Material and Methods: This study involved 27 patients with benign and 58 patients with malignant breast tumours prior to surgical resection of the tumour. The control group consisted of 38 healthy volunteers. Determination of the cytoplasmatic or membranous calreticulin overexpression in malignant or benign cells in paraffin embedded tissues was done using immunohistochemistry (IHC). Determination of the levels of the serum anti-calreticulin autoantibodies was done by ELISA.
Results: Analysis of the localization of calreticulin overexpression in malignant or benign tumour tissues reveals that in some of examined tissues calreticulin could also be (co)localized membranously besides its cytoplasmatic position. Statistically significant differences between serum levels of IgA of anti-calreticulin Ab in controls and patients with breast tumour, ($P < 0.01$) and controls and patients with non-malignant breast diseases, ($P < 0.05$) were found, but not between levels of serum IgG anti-calreticulin antibodies.
Conclusions: This study confirmed that calreticulin is overexpressed in lobular breast carcinoma in lower extent than in ductal breast carcinoma. It was shown that the frequency of patients with membranously located calreticulin is higher in benign than in malignant tumours. It needs to be mentioned that elevated anti-calreticulin IgA antibodies are present more frequently in patients with locoregional lymph nodes (9/17), in comparison to the only one out from 6 patients with elevated anti-calreticulin IgG antibodies who had positive locoregional lymph nodes. Otherwise, data showed that intensity and location of calreticulin cellular overexpression are not useful for the discrimination of malignant from benign tumour tissues. Also humoral immunity to calreticulin developed against cytoplasmatic calreticulin was not correlated to the intensity of its overexpression and was present even in the absence of its membranous localization.

1077 POSTER

Serum Activity of DPPIV and Its Expression on Lymphocytes in Patients With Melanoma

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Background: Dipeptidyl peptidase IV (DPPIV or CD26) is a multifunctional serine protease involved in regulation of immune, inflammatory and neuroendocrine processes. Decreased expression of CD26 on melanoma cells and even the absence of this molecule on metastatic melanoma cells is already proved, but there are no data on the extent of the expression of this molecule on immunocompetent cells and its serum activity in melanoma patients.
The aim of this research was to determine CD26 expression on total white blood cells and on lymphocytes and to determine serum DPPIV activity in the groups of patients with melanoma, and in healthy controls.
Material and Methods: The research involved 36 patients with melanoma, before surgical resection of the tumour. Obtained tissue samples were cytologically and pathohistologically examined. The presence of metastases in the regional lymph nodes was found in 19 out from 36 patients with melanoma. Control group consisted of 24 healthy volunteers. Flow cytometry was performed for analysis of CD26 expression on total white blood cells. The activity of DPPIV in serum was determined by colorimetric test.
Results: For the first time, results from this research show statistically significant decline in the percentage of CD26+ total white blood cells and in the percentage of lymphocytes as well, in the melanoma patients in comparison to the group of healthy control people ($p < 0.001$, $p < 0.001$ respectively). Furthermore, there is a statistically significant decrease in the serum DPPIV activity between groups of patients with melanoma and healthy controls ($p < 0.05$). It is important to note that 15 out from 36 patients with melanoma which had decreased serum DPPIV activity also had lower percentage of CD26+ white blood cells. Among mentioned melanoma patients 14 also were with decreased percentage of lymphocytes.
Conclusions: This study indicate the need for exploring the cause and the importance of the disturbancies in the CD26 expression on white blood cells and in the serum DPPIV activity in melanoma.

1078 POSTER

Anti-melanin Immunity in Patients With Melanoma

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The importance of the antitumour immune response in the control of malignant diseases is already proven, especially in HER-2 positive breast cancers where monoclonal antibody Herceptin, though antibody dependent cell-mediated cytotoxicity (ADCC), suppresses malignant process.
The aim of this work was to learn on humoral immunity to melanin antigens and to immune cells included in possible ADCC action in patients with melanoma and in healthy control people.
Material and Methods: The study involved 35 patients with melanoma. The presence of metastases in the regional lymph nodes was found in 19 out from 35 patients with melanoma. Control group consisted of 19 healthy volunteers. The levels of serum anti-melanin IgA, IgG and IgM antibodies were determined by ELISA. Synthetic melanin (SIGMA) was used as the antigen. Concentrations of serum anti-melanin antibodies were expressed in AU/ml; sera with the high anti-melanin immunity were used for calibration. Cut-off values for each immunoglobulin were (Xav$\pm$SD) AU/ml, obtained analyzing anti-melanin immunity in healthy people. Flow cytometry was performed for analysis of CD89 and CD16 expression on granulocytes and lymphocytes. Two-tailed Student's T test was used testing of statistical analysis of experimental data.
Results: Enhanced IgA levels of immunity to melanin were found in 10/19 healthy people, and in 14/35 melanin patients (6 of these 14 were with metastatic disease). Enhanced levels anti-melanin IgG levels were found in 13/19 healthy people, and in 12/35 melanoma patients (6 of them were with metastatic disease). Enhanced anti-melanoma IgM levels of immunity to melanin was found in 4/19 healthy people, and in 4/35 melanoma patients (none of them was with metastatic disease).
The percentage of CD89+ granulocytes was statistically significantly higher ($P < 0002$) in melanoma patients than in controls, while the percentage of CD16+ lymphocytes was significantly decreased ($P < 0.0007$) in melanoma patients in comparison to controls.
There was no statistical difference between the percentage of CD16+ granulocytes between melanoma patients and controls.
Conclusion: Humoral antimelanin immunity is expressed in some of healthy controls, and in lower number of patients with melanoma. This set a question is there any possibility to create some new IgG or IgM antibody (like Herceptin) for the treatment of melanoma (similarly to that already used in breast cancer).

1079 POSTER

Changes in Proteasome Pool in Human Papillary Thyroid Carcinoma Development

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Background: Proteasomes, multisubunit multiproteinase complexes, are the main sites of intracellular protein hydrolysis in mammalian cells. Due to their function and involvement in antigen presentation immune proteasomes are of major interest when carcinogenesis is concerned. In this regard a novel impulse for antitumour drug development based on proteasomal targets may arise. In this study, changes in the proteasome pool in the development of human papillary thyroid carcinoma were investigated.
Materials and Methods: Samples of tumours and adjacent and distant tissues were obtained from thyroid gland parts surgically removed from patients (16 totally) with verified papillary thyroid carcinoma at the stage $T_2N_0M_0$ and at the stage $T_3N_0M_0$. The chymotrypsin-like (ChTL) and caspase-like (CL) proteasome activities were determined by hydrolysis of fluorogenic peptides. Changes in the expression of the total proteasome pool, proteasome 19S activator subunit, proteolytic constitutive subunits X(β5), Y(β1) and immune subunits LMP7 (β5i) and LMP2 (β1i) were investigated by Western blotting. The distribution of the proteasome subunits in thyroid gland and tumour cells was detected by immunofluorescence and confocal microscopy.
Results: It was shown that the ChTL and CL activities were slightly increased in the tumour at the stage $T_2N_0M_0$. However in the tumour (stage $T_3N_0M_0$) the ChTL activity increased by 4 times and the CL activity by 5 times, compared to those in the distant tissue. The increased expression of the total proteasome pool, 19S activator and immune subunits was

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

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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

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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

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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

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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