Material and Methods: Quantitative Real Time PCR (qPCR) and Tissue Microarray Immunohistochemistry (TMA-IHC) were performed to validate the expression of DREV1. Silencing of DREV1 was carried out using DHARMACON® SmartPooled Small Interfering RNA (Thermo Scientific) on the A549 lung adenocarcinoma cell line. Cell viability and apoptosis were measured using CellTiter-Glo® Luminescent Cell Viability Assay (Promega) and Caspase-Glo® 3/7 Assay (Promega) respectively. The effect of DREV1 silencing on cell invasion was studied using QCM® 24-well Collagen-Based Cell Invasion Assay − Colorimetric (Chemicon). The downstream genes and signal cascades were interrogated using Illumina HumanRef-8 v3.0 Expression BeadChips. Data analysis was performed using Genespring version 10.0.
Results: qRT-PCR confirmed that the expression of DREV1 was significantly higher in the long survival group (n = 8) compared to the short survival group (n = 8). TMA-IHC showed the DREV1 expression was reduced in advanced stages (Stage III and IV) compared to the early stages (Stage I and II) of NSCLC. Silencing of DREV1 significantly increased cell proliferation, reduced apoptosis and increased cell invasion. Microarray gene expression analysis revealed that silencing of DREV1 activated SRC, GNAQ and PIK3R, mediators of PAR1 and PKY2/ERK/MAPK pathway which are associated with increased cell proliferation, migration and cell invasion.
Conclusion: Reduced expression of DREV1 may contribute to poor survival in NSCLCs through increased cancer cell proliferation and cell invasion, and reduced apoptosis.

1073 POSTER

17AAG Inhibits TGF-beta1-induced Cell Migration in Mv1Lu Cells

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Background: TGF-beta is well known to induce cell migration in various cell types. Recently, Heat shock protein 90 (HSP90) has also been reported to be associated with cancer cell invasion and metastasis. However, it is unknown if they share the common mechanism to increase cell motility. In the present study, we investigated the relationship between TGF-beta1 and HSP90 on cell migration using the specific HSP90 inhibitor, 17-allylamino-demethoxy-geldanamycin (17AAG) in Mv1Lu cells.
Materials and Methods: Mv1Lu cells were treated with 17AAG and/or TGF-beta1. We investigated the differences of TGF-beta1 signaling and cell migration by using western blot analysis and trans-well migration assay. Truncated form of HSP90 (ΔHSP90) and active Smad2/3 constructs were also used for verification.
Results: TGF-beta1 increased cell migration in Mv1Lu cells. However, we observed significant reduction of cell migration in Mv1Lu cells, pretreated with 17AAG or transfected with ΔHSP90 regardless of TGF-beta1 treatment. We also examined whether the inhibition of HSP90 by 17AAG or ΔHSP90 attenuate TGF-beta1 signaling through inactivation of Smads. Regardless of TGF-beta1 stimulation, Mv1Lu cells pretreated with 17AAG or transfected with ΔHSP90 showed the attenuation of phospho-Smad2 and phospho-Smad3. The attenuated Smads signaling was also confirmed by localization of Smad4. Additionally, transfection with constitutively active Smad2 (Smad2-EE) or Smad3 (Smad3-EE) significantly increased cell migration. Although Smads signaling was activated by Smad2-EE or Smad3-EE, cell migration was reduced upon HSP90 inhibition by 17AAG or ΔHSP90.
Conclusions: Thus, our data strongly suggest that HSP90 modulates TGF-beta1-induced cell migration through the regulation of Smads signaling.

1074 POSTER

Effects of Zoledronic Acid and Denosumab on Human Vγ9Vδ2 T-cell-Mediated Cell Death of RANK-Expressing Breast Cancer Cells

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Background: Zoledronic acid (ZOL) inhibits osteoclast (OC)-mediated osteolysis by blocking farnesyl pyrophosphate synthase (FPPS), leading to accumulation of isopentenyl pyrophosphate (IPP), a phosphoantigen for anticancer gamma-delta T cells (Vγ9Vδ2). Indeed, interleukin 2 (IL-2) + ZOL stimulates expansion of Vγ9Vδ2 T cells from human peripheral blood mononuclear cells (hPBMCs), and ZOL induces IPP accumulation and secretion by breast cancer (BC) cells, resulting in activation and chemotaxis of Vγ9Vδ2 T cells to BC tumours. Denosumab (Dmab), a fully human monoclonal antibody against RANKL, inhibits osteolysis by blocking RANKL rather than FPPS. As RANKL may also play a role in activating BC cells, the effects of Dmab on BC are unknown.
Material and Methods: Effects of ZOL and Dmab on (1) the expansion of Vγ9Vδ2 T cells and (2) Vγ9Vδ2 T-cell cytotoxicity toward RANK-expressing BC cells were evaluated in vitro. hPBMCs were obtained from healthy donors. Expansion of Vγ9Vδ2 T cells in hPBMCs and RANK expression by BC cells were evaluated by flow cytometry, and IPP accumulation was measured by mass spectrometry. BC cell lines studied had high (T47D) or low (MDA-MB-231/B02; B02) FPPS activity.
Results: IL-2 + ZOL (1−10 μM) but not + Dmab (0.001−0.1 mg/mL) caused expansion of Vγ9Vδ2 T cells. Adding Dmab to IL-2 + ZOL did not block Vγ9Vδ2 T-cell expansion. This lack of Vγ9Vδ2 T-cell modulation was observed despite substantial in vitro activity of Dmab to inhibit RANKL- and macrophage colony-stimulating factor-induced OC differentiation from hPBMCs (⩾2.5× inhibition by 0.001−0.1 mg/mL Dmab treatment). ZOL (1−10 μM, 1h) caused high IPP accumulation in T47D but not B02 cells. RANKL-stimulated T47D BC cells were targeted for IPP-dependent cytotoxicity by Vγ9Vδ2 T cells after ZOL, but not Dmab, pretreatment. B02 cells were not targeted under any of these conditions. Moreover, Dmab pretreatment (0.01 or 0.1 mg/mL) neither induced nor blocked Vγ9Vδ2 T-cell cytotoxicity against RANKL-stimulated T47D cells induced by ZOL pretreatment.
Conclusions: These data suggest that BC cells producing high IPP levels after ZOL treatment are most likely to respond to Vγ9Vδ2 T-cell-mediated immunotherapy. Dmab had no immunomodulatory effects at concentrations that inhibit OC differentiation.

1075 POSTER

The Clinical Significance of RCAS1 Expression in Primary Lung Neoplasms

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Background: RCAS1 (Receptor-binding Cancer Antigen expressed on SiSo cells) is a membrane protein that is expressed in different types of cancer. It halts the cell cycle and/or induces the apoptosis of the immune system cells within the tumour microenvironment. Hence, it is possible that this molecule is involved in the mechanism of the tumour cells' escape from the immune system surveillance (immunoescape).
Material and Methods: Patients with primary lung cancer, eligible for surgical treatment, were included in the study. The tissue samples (paraffin cubes) were processed using the Tissue Micro-arrays Method. Then, an immunohistochemical study followed, specific for the RCAS1 and the Ki-67 (a cell proliferation marker). The image analysis was feasible due to a special program. In addition, a database was created that included the clinical and pathological characteristics of the patients.
Results: In total, 108 patients were examined (81 men and 27 women), mean age 62 years old. Almost 44% of the cases were adenocarcinoma, 31% squamous cell, 9% large cell, and 16% other types of lung cancer. Associations between variables were analyzed by the application of Univariate Analysis Of Variance with SPSS v15.0 software (SPSS Inc., Chicago, IL, v.15.0). Two tailed p values ⩽0.05 were considered to be statistically significant. Statistical significance was identified correlating RCAS1 overall expression to grade III of the tumours (p-value 0.006) and in a positive correlation between RCAS1 and Ki-67 (p-value 0.005). Moreover, there is a trend of RCAS1 over-expression in advanced or metastatic stages. In contrast, protein expression was not strongly associated to tumour size, to histological type, to patient age or to gender.
Conclusions: The most important conclusions of this study are that there is an over-expression of RCAS1 protein mainly in grade III lung cancers and that there is a positive correlation between RCAS1 and Ki-67 expression which means that when the Ki-67 increases the expression of RCAS1 is higher.

1076 POSTER

Overexpression of Calreticulin in Malignant and Benign Breast Tumours: Relationship to the Humoral Immunity

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Introduction: Calreticulin is a multicompartmental protein which regulates a wide array of cellular responses in physiological and pathological

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