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.

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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.

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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.

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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.

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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