172 Expression of Apoptosis and Cell-cycle Regulators in Rat Prostate Overexpressing Regucalcin

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Background: Regucalcin (RGN) is a calcium (Ca^{2+})-binding protein, also known as Senescence marker protein-30 (SMP30) by its characteristic down-regulated expression along with aging process. RGN plays a role as Ca^{2+} homeostasis regulator and has been shown to catalyse an important step in L-ascorbic acid biosynthesis being also associated with protection against oxidative stress. In addition, *in vitro* overexpression studies have been indicating that RGN has suppressive effects on cell proliferation and apoptosis. Moreover, hepatocytes from SMP30 knockout mice are highly susceptible to tumor necrosis factor-α and actinomicyn D induced-apoptosis. Recently, we reported a diminished expression of RGN in human prostate cancer cases which correlates with cellular differentiation of prostate adenocarcinoma. In the present study we analyzed the expression of cell-cycle and apoptosis regulators in the prostate of transgenic rats overexpressing RGN in comparison with their wild-type counterparts.
Material and Methods: *Sprague Dawley* rats overexpressing RGN (Tg-RGN) were obtained from Japan SLC, Inc. Whole prostates (n = 7 for each group) were collected from Tg-RGN and wild-type 3 month-old animals, and longitudinally divided for mRNA and protein extraction. Expression of cell-cycle and apoptosis regulators was determined by means of real-time PCR and Western Blot.
Results and Discussion: Tg-RGN rats showed altered prostatic expression of Bcl-2, BAX, caspase 9 and caspase 3, when compared with wild-type animals. Also, the expression of oncogenes, H-ras and c-myc, and that of tumor suppressor gene p53 was modified in Tg-RGN rats. Chk2 and p21 expression was not significantly changed in the prostate of Tg-RGN rats. Cellular homeostasis is maintained by the establishment of a tight equilibrium between cell death, survival and proliferation. The altered patterns of above mentioned proteins in the prostate of animals overexpressing RGN, suggest it may play a role in the control of apoptosis and proliferation of prostate cells.
Conclusion: RGN seems to play a role in cell death/survival balance of prostate cells and therefore may be involved in prostate tumor development and/or progression.

173 A Novel MUC16 (CA125) Monoclonal Antibody

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Introduction: The MUC16 mucin was identified as the serum antigen detected in the CA125 biomarker assay used to monitor patients with ovarian cancer. A number of monoclonal antibodies (MAbs) including OC125 and M11 are available to detect MUC16. Despite considerable efforts the epitopes detected by these MAbs have remained elusive and glycosylation has been proposed to play a role for the epitopes. Existing MAbs react with MUC16 expressed in both normal cells and in cancer and hence detect enhanced levels of MUC16 derived from both benign and malignant cells.
Material and Methods: In this study, we used an *E. coli* expressed MUC16 fragment glycosylated in vitro with Tn for immunization of mice and selected a novel MAb (5E11) with a cancer reactivity. Overlapping peptides covering the tandem repeat domain were used for epitope mapping in ELISA and microarrays assays.
Results and Discussion: Comprehensive analysis of the fine specificities of existing MUC16 MAbs and the new MAb have been undertaken and we found that existing MAbs react with a conformational epitope of the 156 amino acid tandem repeat sequence of MUC16 without dependence of O-glycosylation. The novel MAb in contrast reacts with a linear peptide epitope, which expression is dependent on glycosylation.
Conclusion: We characterized existing MAbs for MUC16 (M11 and OC125) and produced a new MUC16 MAb (5E11) that recognizes a glycosylation dependent epitope on the tandem repeat region of MUC16.

174 Quantification of 3D Tumor Vessel Networks by Vessel Caliber, Amount and Distribution, Allows Comparison Among Effects of Vascular-targeted Therapies

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Background: Assessment of the efficiency of anti-tumor, vascular-targeted therapies is hampered by difficulties in objective quantification. In this respect, we speculated that maps of very thin capillaries might behave as fingerprints of a given vascular tree. To test this hypothesis, we analysed whether amount and distribution of capillaries classified by caliber might be a characterizing feature in tumors treated with antiangiogenic or antivascular drugs.
Material and Methods: In vivo experiments were performed in NOD/SCID mice bearing subcutaneous KMS-11 human tumors treated, or not, with sorafenib, sunitinib and combretastatin-4-phosphate (CA4P). In vivo biotinylation of endothelial cells with sulfo-NHS-LC-biotin allowed imaging of functional vessel networks at high magnification (40x), leading to 3D reconstruction of tumor vasculature. Custom ImageJ routines were used to classify vessels, and to quantify signal amounts and distribution, extending to 3D a planar space-filling approach (Righi et al., Lab. Invest. 89, 1063–1070, 2009).
Results: Treatment-specific changes were observed after vessel classification according to approxymated cross-section (up to 10 μm in diameter). These results highlighted, in all samples, an unexpected relationship between microvessel amounts and distribution. Vessel skeletonization minimized the artefacts due to drug-specific wall swelling and allowed unbiased comparisons. All treatments reduced vessel sprouts up to calibers of 2.5 μm but only antiangiogenic drugs markedly reduced the signal from larger vessels up to 2–4 folds. In addition, CA4P did not alter the spatial distribution of vessels, whereas antiangiogenic drugs caused an increasing clusterization, according to vessel diameter. Finally, we analyzed the relationship among the spatial distributions of microvessels from contiguous classes as an indirect indicator of vessel branching. Again, CA4P gave results similar to untreated tumors whereas results from both antiangiogenic drugs diverged significantly, suggesting a drop in branching in the analyzed networks. Thus, our data appear in accordance whith what is known about the effects of the analyzed drugs.
Conclusions: In this work we report that quantification of parameters of very thin microvessels can provide a way to characterize pathological vascular trees. We propose this approach as an integrated, yet multifaceted, analysis to quantitate the effects of vascular-targeted therapies.

175 MYC Directs Transcription of MCL1 and EIF4E Genes to Control Sensitivity of Gastric Cancer Cells Towards HDAC Inhibitors

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Introduction: Histone deacetylases (HDACs) are providing fine tuned epigenetic regulation of numerous essential processes in cells. Deregulated expression of HDACs is observed in many cancers and therefore HDAC inhibitors (HDACi) are currently investigated in clinical trials. However, mechanisms controlling the responsiveness of cancer cells towards HDACi are incompletely understood.
Material and Method: In order to investigate resistance toward HDACi we used eight human and murine gastric cancer cell lines and treated them with suberoylanilide hydroxamic acid (SAHA). IC_{50} values were calculated from MTT viability assays and apoptosis levels were determined using PI/Annexin V staining and fluorescence activated cell sorting (FACS). Protein and mRNA levels were investigated by western blot and qPCR, respectively. Molecules conferring HDACi resistance to gastric cancer cells were targeted pharmacologically and by RNA interference. Promoter analysis was conducted by quantitative ChIP assays.
Results and Discussion: Gastric cancer cell lines resistant towards SAHA treatment had higher protein levels of anti-apoptotic Bcl2 family members, Bcl_{XL} and Mcl1. siRNA mediated knockdown of Mcl1 and Bcl_{XL} increased sensitivity of gastric cancer cell lines for SAHA. It is known that c-myc overexpression is associated with therapeutic resistance of certain tumors. Consistently, pharmacological as well as genetic inhibition of c-myc increased sensitivity towards SAHA. Interestingly, c-myc drives the expression of both anti-apoptotic Bcl2 family members, Bcl_{XL} and Mcl1. Whereas the Mcl1 gene promoter is directly regulated by c-myc in gastric cancer cells, Bcl_{XL} is indirectly controlled by c-myc. We observed that c-myc binds to the promoter of the eIF4E gene, a rate-limiting factor of eukaryotic translation. Knockdown of eIF4E decreased expression of Bcl_{XL}, arguing that the c-myc-eIF4E axis controls translation of Bcl_{XL}.
Conclusion: Our data reveal a new molecular mechanism for how c-myc controls SAHA responsiveness of gastric cancer cells and provide a rationale for a concerted inhibition of HDACs and c-myc in gastric cancer.