in the myoepithelium, while intense cytoplasmic p63 expression in the associated normal or hyperplastic epithelium.
Materials and Methods: Our current study attempted to further define these epithelial structures using immunohistochemistry with a panel of aggressiveness and invasiveness related markers and comparative genomic hybridization (array-CGH) with over 30,000 DNA probes.
Results: Our study revealed a number of unique alterations in these structures, including: (1) significantly reduced nuclear p63 expression in the myoepithelium of terminal ducts, (2) immunohistochemical and morphological resemblance to adjacent invasive cancer cells, (3) significant gain in the copy number of DNA coding genes for morphogenesis, angiogenesis, and metastasis, and (4) significant loss in the copy number of DNA coding genes for tumor suppressors, cell adhesion, and macromolecular complex assembly or intra-cellular trafficking. Detected array-CGH alterations correlated well with in vivo expression of a number of corresponding proteins tested.
Conclusion: Our findings suggest that reduced p63 expression in the myoepithelium may result in increased invasiveness in the associated epithelium, and that normal or hyperplastic epithelial cells with cytoplasmic p63 expression may represent a biologically more aggressive population that may progress to invasive lesions without undergoing through the stage of in situ cancer.
Supported by grant 2006CB910505 from the Ministry of Chinese Science and Technology Department, grants DAMD17-01-1-0129, DAMD17-01-1-0130, PC051308 from Congressionally Directed Medical Research Programs, grant BCTR0706983 from The Susan G. Komen Breast Cancer Foundation, and grant 2008-02 from US Military Cancer Institute and Henry M. Jackson Foundation.

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A promising method for visualization of immune responses in immunoproteomics

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Background: Breast cancer is the most common diagnosed cancer type in women worldwide. The early detection of this disease is a key factor for its successful treatment. The immunogenicity of cancer has been decribed in the last decade. Although the importance of the biomarkers CEA and CA 15-3 has been showed, the new specific protein biomarkers for the early detection of breast cancer are still missing. A new tool for visualisation of humoral responses and the following de novo sequencing of the involved proteins would be of great benefit.
Materials and Methods: Different protein extracts which were obtained from a healthy breast tissue or from a carcinoma were separated via sodium dodecylsulfate polyacrylamide gel electrophoresis (1D SDS-PAGE). The proteins were then cut out of the gel, digested with trypsin and spotted on nitrocellulose microarray slides. Each subarray was incubated either with sera of breast cancer patient or with control sera and afterwards labelled with a cyanine 5-labelled human anti-immunoglobulin G antibody (IgG).
Results: Ater the incubation of digested proteins from the breast tissue with different sera and labelling with anti-IgG antibody we have detected the antibody profiles as a part of the immune response.
Conclusion: This method enables the visualization of antibody profiles in the presence of breast cancer. Further subsequent de novo screening of the involved proteins could help to understand their role in the emergence, development and pathogenesis of this complex disease.

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Green tea catechins mixture (Polyphenon E) is an equally potent proteasome-inhibitor as purified EGCG

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Background: Among the constituents of green tea catechins, Epigallocatechin gallate (EGCG) found in green tea is the most potent chemopreventive agent that appears to affect a number of molecular processes including to potently and selectively inhibit the proteasome activity in intact human prostate cancer cells and consequently accumulates IkBα and p27 proteins, leading to growth arrest. Constitutive activation of NFkB has been reported in many tumors, associating it with progression of epithelial cells, including prostate, toward malignancy. However, since EGCG has poor bioavailability and stability to be used in chemoprevention trials, the purpose of this study is to see if a mixture of green tea polyphenols is equally potent inhibiting the proteasome activity as purified EGCG.
Materials and Methods: The effects of a mixture of green tea catechins (Polyphenon-E) on the PGPH-like and trypsin-like activities using a cell-free proteasome assay with a purified rabbit 20S proteasome was determined. To observe change in the levels of proteasome target proteins, human multiple myeloma U266 and prostate cancer LNCaP cells were treated with different concentrations of Polyphenon-E for 24 hours, followed by measurement of levels of the cyclin-dependent kinase inhibitor p27Kip1, a well known target protein of the proteasome.
Results: Similar to purified EGCG, Polyphenon E significantly inhibits the chymotrypsin-like activity of the purified rabbit 20S proteasome with an IC50 value of 0.88 μM. Polyphenon-E inhibited PGPH-like activity of the purified rabbit 20S proteasome with an IC50 value of 7 μM. The IC50 value for trypsin-like activity was above 100 μM, thus demonstrating that Polyphenon-E preferentially inhibits the proteasomal chymotrypsin-like activities over other activities. Polyphenon-E inhibits proteasome activity in intact cells in a concentration-dependent manner and treatment of Polyphenon-E, at all used concentrations in both in human multiple myeloma and prostate cancer cells lines increased accumulation of the proteasome target Protein p27Kip1. Levels of actin were found to be relatively unchanged during the Polyphenon E treatment, which was used as a loading control
Conclusion: The proteasome is a prostate cancer-related molecular target of a green tea catechin mixture, Polyphenon E similar to observations with purified EGCG and has significant potential to be validated in tissue biomarkers obtained in Phase II chemoprevention trials.

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Vascular endothelial growth factor (VEGF) inhibition and erythropoiesis − a missing link?

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Background: Sustained angiogenesis is one of six proposed hallmark characteristics acquired by normal cells to attain a malignant phenotype. Among the plethora of intricately regulated factors involved in maintaining angiogenic homeostasis, the pro-angiogenic factor VEGF, has been identified as a key protein in enabling and sustaining angiogenesis, thereby promoting tumor progression. AZD2171, a small molecule tyrosine kinase inhibitor of VEGF receptors, is being evaluated in clinical trials. Some patients who were treated with this agent at our center, were noted to develop erythrocytosis − a peculiar finding in a population that is otherwise prone to anemia. The objective of our project was to look for similarities amongst this cluster of patients and arrive at a hypothesis regarding the cause for this effect.
Materials and Methods: The charts of four patients consulted consecutively to the hematology department at the Juravinski Cancer Center for unexplained erythrocytosis on AZD2171, were reviewed. Detailed histories and physical examinations were performed to rule out secondary causes and complications of erythrocytosis. Erythropoietin levels, RBC scans and CT scans were some of the investigations done to rule out secondary causes of absolute erythrocytosis as well as the entity of relative polycythemia. JAK2 mutation analyses via Polymerase Chain Reaction (PCR) technology were performed to rule out primary Polycythemia. A literature search was conducted to evaluate a plausible biologic rationale for this phenomenon.
Results: Three of the four patients included in the review showed evidence of inappropriately elevated erythropoietin levels in the absence of anemia during the course of treatment with AZD2171. One patient showed inappropriately elevated erythropoietin in the presence of erythrocytosis. One patient was noted to have inappropriately normal erythropoietin in the presence of erythrocytosis suggesting a defect in the homeostatic function of erythrocytosis induced suppression of erythropoietin. A review of the literature confirmed the occurrence of this phenomenon in pre-clinical models. Of note, VEGF inhibition seemed to be selective for erythrocytosis. There seemed to be a differential effect amongst the different routes of VEGF inhibition.
Conclusion: VEGF inhibition may trigger erythrocytosis via an EPO dependant mechanism. This effect needs to be validated through prospective trials. Clinical implications of this effect need to be further evaluated.

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Response of non small cell lung cancer xenografts to targeted therapies is not related to epithelial-to-mesenchymal transition

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Background: Epithelial-to-mesenchymal transition may play a crucial role in the sensitivity of established non small cell lung cancer (NSCLC) cell lines to epidermal growth factor receptor (EGFR) inhibitors, such as erlotinib and cetuximab. It has been described that cell lines with epithelial