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.
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P75

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.

P115

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.

P71

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.

P39

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

phenotype showed better response rates to EGFR inhibitors than cell lines with mesenchymal characteristics. Cancer cells, that have undergone transition, are less dependent on EGFR, because of the activation of alternative growth pathways. Loss of E-Cadherin, is the first indication in the EMT. Usually, E-Cadherin is located in the cell membrane but it was described recently that cytoplasmatic location is associated with a mesenchymal phenotype for NSCLC, and even nuclear translocation occurred for other cancer types.
Materials and Methods: The expression of epithelial and mesenchymal markers, and of proteins of the EGFR downstream signal transduction pathways of 26 patient-derived xenografts was determined via Western analysis. The E-Cadherin localization within the tumor cells was investigated with immunofluorescence.
Results: One goal of this study was to find out whether EMT plays a role in the sensitivity of 26 patient derived NSCLC xenograft models to erlotinib and cetuximab. Each tumor model showed a unique expression pattern. However, a distinct classification to either an epithelial or a mesenchymal phenotype was not possible, as many xenografts expressed both, epithelial and mesenchymal markers.
In our xenografts E-Cadherin was not only localized in the membrane, but also in the cytoplasm. Therefore, cytoplasmatic translocalization of E-Cadherin could not be shown clearly. Constitutive nuclear E-Cadherin localization among the 26 tumor models was not observed. A nuclear expression was only found, when the tumor cells were damaged due to apoptosis or necrosis after therapy.
Conclusion: Opposite to literature reports a correlation between the expression of epithelial or mesenchymal, and signal transduction markers to the response of the xenografts to the EGFR inhibitors erlotinib and cetuximab could not be found.

P134

A sensitive tamoxifen response profile in patients with metastatic breast cancer indicates that an Interferon-gamma (IFN-γ) centered cellular immune response is involved in tamoxifen resistance

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Background: Only half of the patients with advanced ER-positive breast cancer respond to tamoxifen while the other half is resistant. The aim of this study was to develop a gene signature that is predictive for response to tamoxifen treatment and to study molecular mechanisms that contribute to tamoxifen resistance.
Materials and Methods: The study was performed on 101 estrogen receptor-positive breast carcinomas from patients with metastatic disease treated with tamoxifen in first-line. Main clinical end point was the effect of tamoxifen on time until tumor progression. RNA was isolated from the primary tumor samples collected at two independent cancer clinics in the Netherlands, and hybridized to Agilent whole genome 4x44K microarrays. A training set of 68 tumors were randomly divided into training and test groups. By using a t-test and 1000 rounds of 10-fold cross validation, the best discriminating genes were identified.
Results: A 26-gene signature was developed that can identify tamoxifen non-responders with a sensitivity of 77% and specificity of 79% in the training set. The signature was validated on an independent set of 33 tumors (sensitivity 75% and specificity 71%). The difference in median TTP for the predicted groups was 9.7 months (14.6 months for responders versus 4.9 for non-responders) (log-rank P = 0.0115). The analysis of biological function and canonical pathways indicated that genes involved in immune response were enriched in the signature. Gene network analysis revealed that an Interferon-gamma (IFN-γ) centered immune response network is upregulated in tamoxifen resistant patients.
Conclusion: A 26-gene signature was developed that can predict the response to tamoxifen treatment for patients with metastatic disease. Cellular immune response may play a critical role in tamoxifen resistance.

P124

MicroRNAs as circulating tumor cells biomarkers in gastrointestinal cancer

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Background: MicroRNAs (miRNA) are small noncoding RNAs with relevant posttranscriptional regulatory functions. miRNAs have potential as diagnostic biomarkers and therapeutic targets in cancer.
Objective: To identify miRNAs with diagnostic value for circulating tumor cells (CTC) detection in peripheral blood (PB) from patients with gastrointestinal (GI) cancer.
Materials and Methods: Phase I preclinical study was carried on by means of bioinformatic tools for miRNAs profiling including miRGator, miRBase, SmiRNAdb, Gene-Hub Gepis, microRNA.org and miRNAMap, highly expressed in GI cancer, but absent in hematopoietic-derived sources. qRT-PCR mature miRNA Detection Kit (Ambion) was used to profile the expression of selected miRNAs. U6 and 5S were used as internal controls.
Results: In silico analysis showed a set of miRNAs highly expressed in GI cancer in relation to hematopoietic samples, including miR-141 and miR-200 family, miR-31, miR-32, miR-192 and miR-375. In order to validate the uselfulness of miRNAs as molecular markers for circulating tumor cells detection, qPCR experiments were performed in GI cell lines and a cohort healthy donors and GI patients. Preliminary analysis of GI cancer PB showed higher relative expression levels for selected miRNAs comparing with age-matched controls' PB.
Conclusion: Our results indicate that miRNA bioinformatic approach is an useful method to select GI cancer-associated miRNAs. This miRNA profiling in PB should be further validated as markers for CTC detection.
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P136

KRAS mutation is highly correlated with EGFR alterations in patients with non-small cell lung cancer

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Background: The activation of epidermal growth factor receptor (EGFR) pathways that mediate a variety of cellular responses, including cell division, invasion, and cellular repair, are very important to the carcinogenesis of non-small cell lung cancer (NSCLC). A major downstream signaling route of the EGFR is via the RAS–RAF–MAP (mitogen-activated protein) kinase pathway. Activation of RAS initiates a multistep phosphorylation cascade that leads to the activation of MAPKs in cancer cells. Up to the present date, it remains controversial about the co-existing mutations of KRAS and EGFR, and the co-existence of KRAS mutations and EGFR overexpression in NSCLC.
Materials and Methods: In order to elucidate KRAS and EGFR gene alterations, and their association among NSCLC patients in Taiwan, we analyzed for mutations of KRAS and EGFR genes in surgically NSCLC to determine the prevalence of these mutations in Taiwanese lung cancer patients. In addition, we examined the relationship between the mutations and clinicopathologic features of NSCLC patients by direct sequencing and Northern blotting.
Results: Of 72 cancerous tissues, EGFR mutation was present in 17 samples (24%) and EGFR overexpression was detected in 26 samples (36%). The KRAS mutation was found in 39% (28/72) of the tissue samples. The co-existing mutations of KRAS and EGFR were found in only 1.4% (1/72) of the samples; the co-existence of EGFR mutation and overexpression was found in 16 samples (22%).
Conclusion: These results described above showed that the mutations of KRAS and EGFR would not co-exist regardless of which clinical stage or tumor size the patients experienced or whether the presence of metastasis. Also, nearly no co-existence was found between KRAS mutation and EGFR overexpression. These outcomes greatly assist the prediction on the efficacy of the current anti-EGFR therapeutic targeted drugs for cancer patients.

P54

Irradiation-enhanced mammalian target of rapamycin (mTOR)-targeted glioblastoma therapy with CCI-779 (temsirolimus)

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Background: The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway plays a critical role in oncogenesis, and dysregulation of this pathway is particularly common in human malignant gliomas. In these tumors, activation of PI3K/AKT/mTOR signaling leads to cell cycle progression, neovascularization, escape from apoptosis and inhibition of autophagy, and is associated with poor prognosis. CCI-779 (temsirolimus), a soluble ester analogue of rapamycin, is a small-molecule inhibitor of the mTOR kinase that has been demonstrated to have some antiglioma activity. Given that postoperative radiochemotherapy is the standard of care in the first-line