expression status of Cyr61. Correlations of Cyr61 over-expression with various clinicopathologic factors were also determined. Statistical analysis was performed to explore the links between expression of the Cyr61 and clinicopathological parameters.
Results: On Western blot analysis Cyr61 up-regulation was observed in colorectal cancer tissues (17/21,80.9%). In 234 colorectal cancers, tumour tissue microarray revealed significantly up-regulated Cyr61 protein expression in colorectal cancer tissues versus normal tissues adjacent to tumour. Cyr61 expression was high in 136 of 234 cases of colorectal carcinomas (58.1%). Cyr61 over-expression was significantly associated with TNM stage ($P = 0.012$) and regional lymph node involvement ($P = 0.018$). Kaplan–Meier survival analysis showed that over-expression of Cyr61 was related to poor survival of colorectal cancer patients ($P = 0.031$). But significant associations were not found between Cyr61 expression versus tumour grade, age and gender.
Conclusions: Our results suggest that Cyr61 is highly expressed in colorectal carcinomas and Cyr61 may play a role in the progression of colorectal cancers. Also, Cyr61 might be a new molecular marker to predict the prognosis and serve as valuable targets for therapeutic intervention of patients with colorectal carcinoma.

1147 POSTER

Inter-reader Agreement in Response to Therapy Evaluation of Advanced Lung Cancer: Benefits of a Volume-derived Imaging Biomarker

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Background: Imaging-based endpoints are used to assess cancer response to therapy with lesion size-based criteria such as RECIST. Recent initiatives from interdisciplinary communities investigate other imaging biomarkers such as lesion volume. As discordance in the response evaluation is a critical issue, the expected benefit of a novel biomarker should be an improvement of the inter-reader agreement. The goal of this study is to evaluate the impacts of a volume-based measurement on the inter-reader variability.
Material and Methods: A retrospective study was performed on 10 patients having at least one Non-Small Cell Lung Cancer (NSCLC) lesion. These patients were followed over time with an average of 7 Computed Tomography (CT) studies. 3 readers delineated the volume of each lesion at each time point. Volume was automatically computed after a semi-automatic segmentation completed slice-by-slice with help of a manual tool. From the volume delineation, Longest Axial Diameter (LAD) and Spherical Equivalent Diameter (SED) were extracted. For each patient 2 response evaluations were performed according to RECIST thresholds based on LAD and SED. Quantitative inter-reader variability was analyzed relying on non-parametric statistics of Bland-Altman limit of agreement. Inter-reader agreement of the Best Overall Response was analyzed using Kappa coefficient.
Results: The variability in the measure was reduced from 26% (LAD) to 21% (SED). This benefit in measurement brought an improvement in the inter-reader agreement from Kappa = 0.15 (LAD) to 0.55 (SED).
Conclusions: We measured a reduction of quantitative variability using SED instead of LAD and an improvement of the inter-reader agreement.

1148 POSTER

DAB2 Interactive Protein (DAB2IP) Methylation in Serum DNA of Non-Small-Cell Lung Cancer (NSCLC) Patients (p) With Epidermal Growth Factor Receptor (EGFR) Mutations

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Background: DAB2IP loss promotes primary tumour growth by activating Ras and drives metastasis through NFkB, serving as a signaling scaffold to coordinately regulate these pathways. DAB2IP is frequently methylated in lung cancer, and methylation in the m2a region is a key regulatory factor for DAB2IP expression in prostate cancer. We examined DAB2IP methylation in cell lines and in serum from erlotinib-treated NSCLC p with EGFR mutations.
Material and Methods: In human lung, breast and colorectal cancer cell lines, we analyzed DAB2IP promoter methylation in regions m2a and m2b by methylation-specific PCR (MSP) and bisulfite genomic sequencing. In circulating serum DNA from 152 erlotinib-treated NSCLC p with EGFR mutations, we analyzed methylation in the m2a and m2b promoter regions of DAB2IP by MSP. Methylation status was correlated with clinical outcome.
Results: Methylation was detected in the m2a region of 42 (27.63%) p, and in the m2b region in 51 (33.55%) p. There were no major differences in clinical characteristics (age, gender, smoking history, EGFR mutation type, metastatic sites) between p with methylation in the m2a region and p with methylation in the m2b region. Overall progression-free survival (PFS) was 15 months (m), and median survival (MS) 28 m for all 152 p. For the 41 p with bone metastases (mets), PFS was 14 m for 30 p without methylation in the m2a region vs 8 m for 11 p with methylation in the m2a region (P = 0.01), and MS was 23 m vs 10 m, respectively (P = 0.19). For the 57 p with distant mets but no lung mets, PFS was 18 m for 36 p without methylation in the m2a region vs 10 m for 21 p with methylation in the m2a region (P = 0.01), and MS was 24 m vs 16 m, respectively (P = 0.03). No differences in either PFS or MS were observed according to the methylation status of the m2b region.
Conclusions: Methylation in the m2a region of DAB2IP in serum DNA correlates with PFS and MS to erlotinib in NSCLC p with EGFR mutations with non-lung mets. Surveillance of DAB2IP methylation status in circulating DNA could be a useful tool to predict outcome to erlotinib in EGFR-mutated NSCLC p with non-lung mets.

1149 POSTER

Patient-derived Tumourgrafts − Models for a Systemic Cancer Biology Research

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Background: Cancer is a complex genetic disease leading to a high variety of phenotypes among the different individuals. Each tumour presents a very specific pattern of molecular changes and responses to drugs. The correct identification of predictive biomarkers selecting the most appropriate therapies and avoiding unnecessary treatments for an individual patient is still a challenge. Patient-derived tumourgrafts allow preclinical investigations in a clinically relevant way. We performed investigations to improve the understanding of cancer complexity and to draw rational conclusions for therapy decisions.
Methods: The tumour models were established by direct transplantation of surgical specimens to immunodeficient mice and were maintained in early passages. A high congruence between original patient sample and xenograft could be proven both at gene and protein level. The following tumourgrafts are available: 10 breast, 28 colo-rectal, 25 lung, 6 ovarian, 10 sarcomas, 25 ALL, 5 AML. We will show examples for which purposes the models are appropriate and focus on non-small cell lung (NSCLC) and colon cancer.
Results: The xenografts were characterized for response towards clinically used cytotoxic and novel targeted drugs. The analysis for mutations revealed that all NSCLC models were *EGFRwt*, 5/25 were *KRASmut* and 12/25 were *P53mut*. None of the mutations correlated with response to therapy. In the colon cancer xenografts *KRAS, BRAF* and *PIK3CA* mutations predicted resistance to Cetuximab.
Using Affymetrix based gene profiling we identified a potential set of 20 genes which were differentially expressed between Oxaliplatin responder and non-responder.
In a preclinical Phase II study, the response of 22 NSCLC xenografts to a novel Epothilone was evaluated; *P53* mutations and overexpression of a cytochrome P450 enzyme were identified as potential biomarkers for the stratification of patients. The individual comparison of responses of colon cancer patients and their derived xenografts resulted in a congruence in 5 out of 5 patients included.
Conclusions: Patient-derived xenografts are a valuable model system to address clinically relevant questions in a standardized and strictly controlled fashion. They show a high concordance with the clinical specimens concerning marker expression and response to therapy.

1150 POSTER

Analysis of Biological Markers, Tumoral Predictors and Clinical Features as Prognosis Factors to Cnemotherapy Response in Metastatic Carcinomas of Unknown Primary Site

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Background: The authors investigated prognosis factors to chemotherapy response such as clinicopathological features: age, gender, performance

status (PS), metastatic site number, tumour location, impact on survival, histology, differentiation grade; and biological markers: DHL, Albumin, CA125, PSA, CEA, CA19-9, AFP in metastatic carcinomas of unknown primary (CUP).
Material and Methods: One hundred forty-nine patients with metastatic CUP were retrospectively identified in our institution (Oncology Hospital, National Medical Center "Siglo XXI", IMSS) from January 2002 to December 2009. All data were collected from digital clinical files and analyzed in a computer data base to evaluate survival and response rate using Kaplan Meier method, multivariate and univariate analysis and proportional hazards regression.
Results: Median age was 56.9 years, gender proportion was 51.6% males; 48.4% females; percentage distribution of PS scale was: 65.7% to level 1, 32.8% to level 2 and 1.3% to level 3. Number of metastatic involved organ distribution was: 0.6% to one organ, 53.6% to 2−3 organs and 13.4% to >3 organs. The most frequent site involved was: 33.5% Liver, 30.2% Neck and 24.8% Lung. Histopathological diagnostic was: 48.3% Adenocarcinoma, 38.2% Undifferentiated carcinoma, 12% Squamous carcinoma and 1.3% Neuroendocrine carcinoma. Tumoral differentiation grade was: 74.4% poorly differentiated, 22.8% moderately differentiated, 2.6% well differentiated. Serum levels of biological parameters were: 41.6% to DHL elevated and 12.1% to low Albumin level. About tumoral markers, 34.2% showed an elevation in serum levels, and distribution of these was: 16.7% to Ca125, 15.4% to CEA, 4% CA19-9, 2.6% AFP and 1.3% PSA. Median overall survival was 14.2 and progression free survival was 7.1 months. PS level 1 was an independent survival prognosis factor (25 vs 7 months). A significant correlation between Chemotherapy response rate and PS level was 91.7% to PS level 1 with complete response ($p = 0.004$). No significant correlation was found in others clinicopathological features.
Conclusions: This study validated PS level 1 as prognosis factor to chemotherapy response rate, progression free survival and overall survival. Additional clinicopathological and tumoral markers variables had not an impact on response rate or survival. Only high levels of DHL were correlated in a univariated analysis with better chemotherapy response rate ($p = 0.03$).

1151 POSTER

Hyperthermia Triggers Down-regulation of Estrogen Receptor α Isoforms and Its Co-activators DEAD-box5 and DEAD-box17 in Breast Cancer Cells

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Background: Hyperthermia is used concomitant to chemotherapy or radiotherapy, that might improves the effect of those classical anti-cancer treatments. RNA helicases p68 (DEAD-box5, DDX5) and p72 (DEAD-box17, DDX17) act as transcriptional co-activators of several tumour-relevant genes, e.g. estrogen receptor α (ERα). Both factors regulate ERα-activity in breast cancer. We investigated potential regulatory effects of hyperthermia on the expression of these breast cancer-related factors.
Materials and Methods: Various ERα-positive breast cancer cell lines (MCF-7, ZR-75−1, T47D, BT-474) were cultured under hyperthermia (42ºC, 2 hrs) followed by maintenance under regular culture conditions (37ºC, 4 hrs). As a negative control the same cell lines were cultivated under regular temperature conditions permanently. mRNA and protein expression levels of ESRα isoforms, DDX5 and DDX17 were analyzed by RT-PCR, Western blot and immunocytochemistry.
Results: The analyses revealed markedly decreased mRNA and protein levels of ERα isoforms, as well as of DDX5 and DDX17 in cells exposed to hyperthermia compared to cells cultured under regular conditions.
Conclusion: Our results clearly indicate regulatory effects of hyperthermia on both, the mRNA and protein expression of the breast cancer-relevant gene ERα and its co-activators DDX5 and DDX17. Thus, hyperthermia may represent a method improving classical anti-cancer therapies by down-regulating the activity of important factors in breast cancer biology. We hypothesize that hyperthermia inhibits the expression of ERα isoforms and its co-activators, thereby probably leading to a suppression of tumour progression. However, the molecular background of hyperthermia-dependent alterations and its concomitant effects on tumour biology still need to be investigated in more detail.

1152 POSTER

CYP3A4*1B Polymorphism − a Prognostic Value in Ovarian Cancer?

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Background: Ovarian cancer (OC) is the sixth most common cancer and the seventh cause of death from cancer in women, representing the most lethal gynecological cancer. The survival of patients with OC stands at 46% at 5 years despite advances in surgery and chemotherapy.
Given that exposure to estrogen is associated with OC it is plausible that enzymes involved in metabolism of these hormones might influence the development and progression of this disease. CYP3A4 encodes a critical enzyme for oxidation of estrogens and its inhibition results in higher circulating estrogen levels. Furthermore, CYP3A4 is one of the most important metabolizing enzymes being involved in metabolism of clinic drugs.
The *CYP3A4*1B*studies evaluated the prognostic value of this polymorphism in patients with OC.
The aim of this study was to evaluate the influence of *CYP3A4*1B* polymorphism as prognostic factor in patients with OC.
Material and Methods: DNA was extracted from peripheral blood of 206 patients diagnosed with OC submitted to a platinum based chemotherapy (Platin and Paclitaxel). Patients were first divided by histologic subtype and then by FIGO stage in: stage I/II, stage III and stage IV. The characterization of *CYP3A4*1B* genotypes was performed by RFLP-PCR.
Results: The frequencies obtained for the AA, AG and GG genotypes were 88%, 11% and 1%, respectively. The *CYP3A4*1B* polymorphism genotypes were grouped as AA genotype and G carrier genotypes. The polymorphism was significantly associated with overall survival in patients with Papillary serous tumours (PST): patients with genotypes carrying G allele (GG/GA) had significantly diminished survival when compared with patients with AA genotype (103.93 months and 122.56, respectively, $P = 0.019$). When stratified by FIGO stage, patients with PST and in stage III subgroup with genotypes carrying G allele had significantly diminished overall survival when compared with AA genotypes (95.74 months and 120.30 months, respectively, $P = 0.050$).
Conclusion: CYP3A4 shows great importance in a metabolic level and is greatly studied in the field of translational research. Our results exhibit an association between *CYP3A4*1B* and a diminished survival of patients with OC. Due to this prognostic value, these results could help in the monitorization of patients with OC and to define the role of this genetic variant in the pharmacogenomic profile of OC.

1153 POSTER

Uncovering the Unknown Relation Between the Alternative Pathway of NF-kB and NSCLC

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Background: Many signalling pathways are implicated in lung carcinogenesis but the role of the alternative pathway of NF-kB in lung cancer pathogenesis and progression has not been investigated. The aim of our study was to investigate the role of this pathway in patients with non small cell lung cancer (NSCLC).
Methods: NF-kB2 and RelB protein expression was retrospectively assessed by immunohistochemistry in tissue samples from 109 NSCLC patients. Cases with staining in >10% of cells were considered positive. Immunohistochemical reactivity was graded on a scale of 0−3 according to the intensity of the staining and the percentage of immunopositive cells. NFkB2 and RelB expression was categorized in three groups (high vs medium vs low) using as a cut-off point the 33rd and 66th percentiles. The total score for each slide was the sum of the intensity and distribution of expression.
Results: Cytoplasmic NF-kB2 and RelB protein levels were higher in tumour cells than in non-neoplastic adjacent tissue ($p < 0.001$ for both). Higher RelB levels were noted in the nucleus of tumour cells compared to normal tissue ($p = 0.003$). Cytoplasmic immunoreactivity of NF-kB2 and RelB was correlated with stage. In addition, cytoplasmic NF-kB2 levels significantly differed between low and high grade tumours ($p = 0.046$). Expression of RelB in the cytoplasm was type-specific, with squamous cell carcinomas having the highest protein levels. Furthermore, a significant association of RelB cytoplasmic expression with patients' two-year survival outcome was observed ($p = 0.038$). No correlation was found with age, sex, maximum tumour diameter, relapse rate or smoking.
Conclusions: The deregulation of the alternative NF-kB pathway in NSCLC could play a crucial role in the development and progression of the disease. Further studies to elucidate this role now appear warranted.