calculated as a measure of the association between CYP3A5*3 genotypes and NSCLC progression.
Results: The frequency of the GG genotype was 79.3% in LC patients and 86% in controls. The frequency of the heterozygous genotype A/G was of 20.3% in patients and 13.3% in controls. Homozygous individuals for the A allele where rare: 0.1% in LC patients and 0.6% in controls. The analysis of the genotypic frequencies of the CYP3A5*3 polymorphism indicates that individuals with GG genotype present a 38% protection for the development NSCLC (P = 0.020; OR = 0.621; 95% CI = 0.415−0.931).
Conclusions: Individual differences in the metabolism of carcinogens may influence the susceptibility to cancer development and behaviour. Our results suggest that that individuals with GG genotype present a lower risk of developing NSCLC than individuals with genotypes carrying the A allele (OR = 0.621). This is probably due to a decreased activation of procarcinogens present in tobacco smoke in result of the lack of CYP3A5 in individuals with genotypes carrying the CYP3A5*3 allele.

6537 POSTER

Selenite mediated cytotoxcicity in human lung cancer and the role of Thioredoxin reductase 1

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Background: The human selenoenzyme thioredoxin reductase 1 (TrxR1) is a very important enzyme for cell growth, differentiation, and the defense against oxidative stress. Several studies have shown that TrxR1 is upregulated in tumor cells and it is a target for many anti-cancer drugs. The regulation of TrxR1 is very complex and involves the expression of different transcript forms of mRNA.
Materials and Methods: We have, by quantitative polymerase chain reaction, investigated the total expression of TrxR1 mRNA and quantified the expression of alternative mRNA forms in five different human lung cancer cell lines. IC50 values for selenite were determined for the different cell lines and compared to the sensitivity towards doxorubicin.
Results: The results indicated an inverse relationship between resistance towards doxorubicin and selenite induced cytotoxicity. In addition, inhibition of TrxR resulted in enhanced selenite cytotoxicity. Selenium treatment resulted in increased expression of almost all TrxR1 mRNA variants while the TrxR protein activity decreased. Total TrxR1 and the less abundant forms were detected in human tissue samples from both squamous and adenocarcinoma from lung, using specific peptide antibodies. Expression of TrxR1_v.2, 3, 5 isoforms and Trx1 in the tumor correlated with degree of differentiation.
Conclusions: Our results show that TrxR1 is involved in selenite mediated cytotoxicity and investigation of alternative transcript variants of TrxR1 could further be a valuable tool in the diagnostics and characterization of tumors.

6538 POSTER

Preclinical studies on the antitumor activity induced by novel modified steroidal alkylating esters of propenoic acid against murine Lewis lung carcinoma (LLC)

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Background: The sensitivity of some neoplasms to hormonal intervention provides a rational basis for utilizing steroid hormones as a biological “platform” for cytotoxic agents in cancer therapy. The purpose of this study is to investigate the relationship between structure activity and antineoplastic effect of in vivo biological system, treated by five newly synthesized modified steroidal derivatives of p-bis(2-chloroethyl)aminophenylpropenoic acid (PK 11–PK 15).
Materials and Methods: The acute toxicity of the compounds was determined fol-lowing a single i.p. injection into C57BL mice in groups of 10 mice/dose. C57BL mice were used for the evaluation of the antitumor activity. Experiments were initiated by implanting the LLC cells. These were injected subcutaneously [0.2 ml tumor brei of 13 (w:v)]. Each treated group consisted of 6 mice and 8 mice comprised the control group, treated with saline only.
Results: The antitumor activity was assessed from the inhibition of tumor size (I) and from the oncostatic parameter (T/C).
Conclusions: The antitumor activity of compound PK 11 is distinctly superior to that of compounds PK 12, PK 14, PK 15, whose activity is marginal. Compound PK 13 is less effective than PK 11. Most likely, the highest effect of compound PK 11 is due to the presence of double bond in the homoazasteroidal nucleus (ring B) and the 3β-(cis) configuration.

Acute toxicity and antitumor activity of PK 11–PK 15 on LLC.

Compound	LD10 (mg/kg)	LD10 dose (mg/kg)	Treatment schedule	Growth inhibition (%)	T/C (%)
Control	–	Saline	–	0	100
PK 11	500	500	Day 1	57	194
PK 12	400	400	Day 1	28	127
PK 13	600	600	Day 1	42	171
PK 14	200	200	Day 1	21	124
PK 15	100	100	Day 1	19	117

6539 POSTER

Malignant mesothelioma of pleura: potentialities of immunocytochemistry

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Considering that surgical treatment is extremely traumatic, pathologist must be sure in the diagnosis. It is very important to use supplementary methods of diagnostics.
An objective of this work was to study potentialities of cytological diagnostics of pleural mesothelioma using immunocytochemistry. The data of 90 patients with malignant mesothelioma of pleura was investigated in Altai Oncological Hospital during 5 years including females 48 (53.3%) and males 42 (46.7%). Immunocytochemistry was used from 65 (72.2%) patients. Specimens were prepared using Centrifuge and Streptavidin-biotin system with a set of markers (11 antibodies).
Epithelioid mesothelioma was diagnosed in 82 cases (91.1%). The sarcomatoid variant of pleural mesothelioma was determined in 2 (2.2%) and biphasic in 6 (6.7%) cases. Both cases of sarcomatoid mesothelioma resemble fibrosarcoma. For differential diagnostics immunocytochemistry was used. The reactions with Cytokeratins (C MNF 116, C AE1/AE3) were most important. Biphasic mesothelioma contains both epithelial and sarcomatoid cells.
The cells of mesothelioma were positive with Keratins (C MNF 116, C AE1/AE3), also positive cytoplasmatic reactions with Vimentin were noticed in all cases of mesothelioma. The cells of tumours were immunonegative with mono- and polyclonal Carcinoembrionin antigen (CEA). Tumour cells had weak reactivity with polyclonal Carcinoembrionin antigen in 3.1% of cases. Immunonegative reaction of mesothelioma cells was noticed with Epithelial antigen (Ber-EP4).
All cases of mesothelioma (100%) showed positive reactions with Methotelial Cell (HBME-1). Calretinin and Thrombomodulin were studied only in 12 cases of mesothelioma (18.5%). Cells of mesothelioma with Calretinin had nuclear and cytoplasmaticstaining.It wasn’t noticed in cells of carcinoma excluding serous papillary of ovarian carcinoma. Metastases of carcinoma had immunonegative reactions with Thrombomodulin. Cells of mesothelioma showed negative reactions with CD-15. Immunoreactivity of Epithelial Membrane Antigen (EMA) was noticed in the membranes of cells.
The data show that the cells obtained for cytological examination have the same characteristics as those in biopsy materials. Sarcomatoid mesothelioma may be limited of Cytokeratins. Reactions with Calretinin, Mesothelin and Thrombomodulin are the most important for Epithelial Mesothelioma with positive reactions with Vimentin, Keratins. Immunonegative reactions with CEA, Ber-EP4 and CD-15 are typical.

6540 POSTER

p53 gene mutation, mrna expression, aberrant protein expression and clinicopathological features in resected non-small cell lung cancer

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Background: Functional abnormality of p53 plays a pivotal role in occurrence of malignant tumors including lung cancer. Aberrant expression of the p53 protein using immunohistochemistry has been investigated in many cancers. However, immunohistochemical detection cannot distinguish expression of wild type p53 protein from mutant one, so that clinical significance of p53 aberrant expression should be analyzed with regard to the presence or absence of p53 gene mutations. In this study, we investigated relationships among gene mutation, mRNA expression and aberrant protein expression of the p53, and analyzed their clinical

significance as well as prognostic impact in resected non-small cell lung cancer (NSCLC) patients.
Patients and Methods: A total of 112 patients with p-stage I-IIIB NSCLC without any preoperative therapy were included in this study. 76 patients (67.9%) received postoperative adjuvant chemotherapy, 64 with oral administration, 4 with systemic chemotherapy, and 8 with both. p53 gene mutations within exon 5, 6, 7 and 8 were screened using PCR single-strand conformational polymorphism method, and were determined with direct sequencing. The expression level of p53 mRNA was measured using quantitative real-time RT-PCR. Aberrant expression of p53 protein was evaluated with immunohistochemical staining. The clinicopathological parameters and p53 status were integrated to statistical analyses including overall survival and disease free interval.
Results: p53 gene mutation was observed in 33 cases (29.5%) including 3 cases with multiple mutations. Aberrant expression of p53 protein was demonstrated in 50 cases (47.1%). p53 mRNA expression was higher in cases with p53 aberrant expression than in cases without aberrant expression ($p = 0.005$). In wild-type p53 adenocarcinoma cases, mRNA expression decreased in order of differentiation status (well > moderate > poor), and was higher in node negative cases than in node positive cases ($p = 0.036$), although that of mutant p53 adenocarcinoma or other histological types did not show such tendency. There was no prognostic impact in any of single parameter such as gene mutation, mRNA expression and aberrant protein expression in multivariate analysis.
Conclusions: The wild-type p53 mRNA expression level is associated with tumor differentiation and nodal status in lung adenocarcinoma patients.

6541 POSTER

Genetic polymorphism of the epidermal growth factor gene – value for the treatment of non-small cell lung cancer (NSCLC)

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Background: In western world, lung cancer is the third type of cancer and non-small-cell lung cancer (NSCLC) accounts for 80% of all lung cancers representing the leading cause of death from cancer. The epidermal growth factor (EGF) has an established important role in lung carcinogenesis. EGF+61G/A is a biallelic G/A functional polymorphism, located in the 5′-UTR, which leads to increased EGF expression. The aim of our study was to evaluate the genetic influence of this polymorphism in NSCLC development.
Material and Methods: DNA samples extracted from peripheral blood cells of 171 patients (pts) with NSCLC, with an accurate stage and a 3 month minimum of follow-up, were analyzed. From 171 pts, with a mean age 62.7 years (median 64.0), 136 were males, 131 had a smoking history, and 85 had adenocarcinoma. The EGF genotypes were determined using the PCR-RFLP methodology.
Results: Regarding the frequency of the EGF+61G/A polymorphism genotypes, 63.2% of patients showed genotypes carrying the G allele and 36.8% presented the homozygous genotype AA. Among G carrier genotypes, 16.7% corresponded to NSCLC patients with stages I/II and 83.3% to advanced stages of the disease (III/IV). Regarding AA genotype, 30.2% of the patients were diagnosed with early stage NSCLC (I/II) and 69.8% presented advanced stages of NSCLC (III/IV). These differences were statistically significant and suggest that individuals with genotypes carrying the G allele present a 2.16-fold higher risk for the progression from early stages of NSCLC (I/II) to clinically more advanced stages of the disease (III/IV) (OR = 2.16; 95% CI: 1.03−4.52; $P = 0.039$).
Conclusions: These preliminary results indicate that the EGF+61G/A is involved in NSCLC progression, which is in agreement with previous findings that suggest that EGF overexpression is associated with worst prognosis of the disease. This makes EGF polymorphism an attractive factor for prognosis in NSCLC.

6542 POSTER

FISH and immunohistochemical analysis of PTEN in human mesothelioma cell lines

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Background: Pleural Malignant Mesothelioma (MM) is a highly aggressive and rapidly fatal tumour that is resistant to conventional chemotherapy. New molecular signalling pathways in MM are being explored, aimed at new, more effective treatment strategies. PTEN (phosphatase and tensin analog), a tumour suppressor, has been implicated in a large number of human tumours. PTEN is a phosphatase that can modulate signal-transduction pathways. At least part of its role is to regulate the activity of the serine/threonine kinase AKT and thus influence cell survival signalling. A recent study has shown elevated AKT activity in 65% of human MM specimens and in a human MM cell line exhibiting loss of PTEN. In the present in-vitro study, a possible role of PTEN in MM tumourigenesis is investigated.
Materials and Methods: PTEN protein expression was investigated by an immunocytochemical analysis using a commercial MAb (clone 28H6; Lab Vision Co.) in 12 human MM cell lines established from pleural effusions of histologically confirmed MM patients. Dual colour FISH using DNA probes for cytoband 10q23.3 (PTEN locus) and region 10p11.1-q11.1 (centromere of chromosome 10) (LSI PTEN/CEP10; Vysis Inc.) was performed to assess the PTEN gene status.
Results: A predominantly nuclear PTEN staining was observed in 7 of 12 (58.3%) MM cell lines. In the other 5 MM cell lines no PTEN expression was detected. Of these 5 PTEN negative cell lines, 2 showed the loss of a PTEN gene allele.
Conclusions: These data show that the loss of functional PTEN occurs in 41.7% of MMs and the down-regulation of PTEN protein can be related in a minority of cases (2 of 5) to loss of heterozigosity (LOH). One copy of PTEN may be haploinsufficient and the 50% reduction of gene function due to loss of one allele results in an abnormal phenotype. Since LOH can rarely be detected in MM, different mechanisms may be responsible for PTEN protein deregulation, such as inactivating mutations, protein instability, promoter hypermethylation and unknown epigenetic mechanisms. These findings are an important consideration for novel therapeutic trials in MM in which biological efficacy is influenced by the activity level of PTEN.

6543 POSTER

Molecular markers expression in mediastinal nodes from resected stage I non-small cell lung cancer (NSCLC): prognostic impact and potential role as markers of occult micrometastasis

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Background: 5-year survival in surgically resected Stage I NSCLC is 60−70% whereas in cases with affected lymph nodes (LN) is <50%. The main risk factor for recurrence is nodal disease. Current histopathological analysis can miss occult micrometastases in nodal tissues at initial diagnosis. Detection of micrometastases with a more sensitive technique would be useful to define a high-risk population selecting patients (p) for postoperatory treatment. We assessed the role of several genes mRNA expression in pathological negative LN from resected Stage I NSCLC p as markers of occult micrometastases and correlated the results with relapse, and survival
Materials and Methods: Paired tumor and histological negative LN ($n = 344$) obtained by systematic mediastinal lymphadenectomy from 38 surgically resected Stage I NSCLC p were analyzed for the presence of 12 genes mRNA expression using RT-Q-PCR in an ABIPRISM 7500. RNA was extracted using ABIPRISM 6100. Specifically designed primers and probes were purchased from Applied Biosystems as Assay-on-demand; GADPH was used as an endogenous control. Samples were also analyzed by ICH for LN staging.
Results: 38 NSCLC p; 12 adenocarcinoma, 16 squamous cell, 10 undifferentiated. From the 12 tested genes CEA and PLUNC were found with high expression in lung tissue and low or null expression in normal LN. We consider molecular positive LN those in which expression of CEA or PLUNC was detected. In the 344 pathological negative LN, 13% (44/344) were positive for CEA, 16% (54/344) for PLUNC. The expression patterns were similar for both markers. At a median follow-up of 24 months (9−46) 11 p had died from NSCLC and 1 had died without recurrence. None of the living p had tumor recurrence. For the prognostic assessment, molecular positive LN were classified as N1 and N2. Median disease free survival was 15 ± 11.74 months in p with N2 molecular positive nodes and has not yet reached in cases of molecular negative LN ($p = 0.028$). Median survival of p with N2 molecular positive nodes was 17.3 ± 5.7 months and has not yet reached ($p = 0.0083$) for molecular negative LN.
Conclusions: CEA and PLUNC mRNA expression could be used as molecular markers of occult micrometastases in mediastinal LN showing a