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.

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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