118. New aspects of lung cancer biology

P1213

Immunohistochemical expression of Hsp27 and alphaB-crystallin in different histological subtypes of lung cancer

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Aim: To evaluate the immunohistochemical expression of heat shock protein 27 (Hsp27) and alphaB-crystallin (Cryab) in resected lung cancers.

Methods: Surgically resected specimen from 105 patients (men n=72; women n=33, age 57±12) with lung cancer were studied: carcinoid tumors(CT)-35, small cell lung carcinoma(SCLC)-20, large cell neuroendocrine carcinoma(LCNEC)-10, adenocarcinoma(AC)-20, and squamous cell carcinoma(SCC)-20. The histological subtype, pTNM stage, and the immunohistochemical expression of Hsp27 and Cryab were evaluated.

Results: The distribution in stages was as follows: CT-I stage-25(71%),II-7(20%),IIIA-3(9%); SCLC-I-7(35%), II-4(20%), IIIA-8(40%), IV-1(5%); LCNEC-I-4(40%),II-5(50%),III-10(10%); AC-I-8(40%),II-2(10%),III-10(50%); SCC-I-8(40%),II-1(5%),III-11(55%) ($p<0.001$). Hsp27 cytoplasmic expression was observed in 97% of tumors (71% of all cases express Hsp27 in >75% ot tumor cells),and in combination with nuclear expression in 46%. Nuclear expression was in: 100% of LCNEC,50%-SCLC and SCC,35%-AC, 29%-CT ($p<0.001$). Significant correlation between citoplasmic expression of Hsp27 and pTNM stage was observed ($p<0.05$).Nuclear expression of Cryab was observed in 99%,and in combination with cytoplasmic-in 23% (65%-AC,17%-CT,10%-SCC,10%-LCNEC,5%-SCLC;p=0.001). Significant correlations between nuclear expression of Cryab and N-status($p<0.001$),pTNM stage(p=0.02),tumor size(p=0.04); and between citoplasmic expression of alphaB-crystallin and pTNM stage ($p<0.001$) were observed.

Conclusion: Low weight heat shock proteins play a major role in cancerogenesis of lung cancer, eventually has a prognostic value and may be used as targets for therapy.

P1214

The effect of SDF-1/CXCR4 on expression of VEGF and MMP-9 in small cell lung cancer

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Objective: To explore the effect of SDF-1, AMD3100, and LY294002 on expression of VEGF and MMP-9 in NCI-H446 cell, and to explore the mechanism of small cell lung cancer invasion by SDF-1.

Methods: There were 5 groups in the experiment: control group (SDF-1 negative group); SDF-1 50ng/ml group; SDF-1 100ng/ml group; SDF-1 (100ng/ml) + AMD3100 group; SDF-1 (100ng/ml) + LY294002 group. With serum-free medium for 24 hours, to observe the VEGF and MMP-9 expression with different treatment conditions using RT-PCR to detect the expression of VEGF & MMP-9, and ELISA to measure the expression of VEGF and MMP-9 in cell culture supernatant.

Results: VEGF and MMP-9 expression in supernatants were increased in SDF-1 treated group and could be inhibited by AMD3100 and LY294002. Compared with the control group, 100ng/ml SDF-1 treated group, the concentration of VEGF and MMP-9 was significantly increased, [(826±102) pg/ml VS (360±21) pg/ml], [(105±4) pg/ml VS (30±9) pg/ml] ($P<0.05$); VEGF concentration of SDF-1 (100ng/ml) + AMD3100 treated, SDF-1 (100ng/ml) + LY294002 treated group decreased, [(224±55) pg/ml VS (826±102) pg/ml], [(379±203) pg/ml VS (826±102) pg/ml] ($P<0.05$). So as to MMP-9 levels, [(31±2) pg/ml VS (105±4) pg/ml], [(25±4) pg/ml VS (105±4) pg/ml] ($P<0.05$).

Conclusion: After treated with SDF-1, the expression of VEGF and MMP-9 was significantly increased, and could be inhibited by AMD3100 and LY294002, which suggested that SDF-1/CXCR4 participate in tumor invasiveness and metastasis in small lung cancer by promoting secretion of VEGF and MMP-9.

P1215

Profile of WT1 methylation in human lung cancer

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Background: CpG island hypermethylation of the promoters of the genes is a well-known mechanism of genetic alteration involved in carcinogenesis. Wilms tumor gene (WT1) is involved in the regulation of human cell growth and differentiation and it is a modulator of oncogenic K Ras signaling in lung cancer; methylation of WT1 was never studied in human lung cancer. The primary aim of this study is to compare the methylation profile of WT1 promoter in samples of neoplastic and non-neoplastic lung tissue taken from the same patient.

Methods: Lung tissue obtained from 16 patients with non small cell lung cancer (NSCLC) and 6 patients with small cell lung cancer (SCLC). The methylation status of 29 CpG islands in the *5'* region of WT1 by means of pyrosequencing was investigated.

Results: The mean percentage of methylation, considering all CpG islands of WT1 in the neoplastic tissues of all NSCLC patients, was 16.2±3.4, whereas in the control tissue from the same patients it was 5.6±1.7 ($p<0.001$), adenocarcinomas present grater methylation vs squamous cell carcinomas ($p<0,001$). In SCLC was not statistically significant difference between neoplastic tissue and control tissue of same patients.

Conclusions: Although, WT1 methylation does not seem to play a role in the pathogenesis of SCLC, hypermethylation of WT1 seems to be involved in the carcinogenesis of NSCLC. CHT, histotype, exposure to cigarette smoke are all factors influencing the gene CpG islands which become hypermethylated in NSCLC.

P1216

Global histone H3 lysine 4 (H3K4) dimethylation is an important prognostic factor in lung cancer

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Introduction: Epigenetic modifications may contribute to the development and progression of cancer. This study seeks to elucidate the role of global histone modifications as a clinicopathological factor in lung cancer.
Methods: A series of 92 surgical specimens from patients with lung cancer were utilized from the surgical files of Kanazawa Medical University Hospital between 2001 and 2008. The 92 tumor specimens were examined by immunohistochemistry. Dimethylated histone 3 lysine 4 (H3K4diMe), Acetylated histone 3 lysine 9 (H3K9Ac), acetylated histone 3 lysine 18 (H3K18Ac), trimethylated histone 3 lysine 27 (H3K27trime), himethylated histone 4 arginine 3 (H4R3diMe) was assessed in paraffin-embedded tumor samples. They were dichotomized into two categories as low expression and high expression. The association between staining intensity and clinicopathological factor was analyzed.
Results: Lung cancers of various histologic subtypes showed that dimethylated histone 3 lysine 4 (H3K4diMe), acetylated histone 3 lysine 9 (H3K9Ac), acetylated histone 3 lysine 18 (H3K18Ac), trimethylated histone 3 lysine 27 (H3K27trime), dimethylated histone 4 arginine 3 (H4R3diMe) were high expressed in tumor cells of 40, 10, 9, 9, 10%. Expression of dimethylated histone 3 lysine 4 (H3K4diMe) correlated positively with recouurence (p=0.039) and stage (p=0.005) and cell differentiation (p=0.002). Univariate analysis showed that high expression of dimethylated histone 3 lysine 4 (H3K4diMe) correlated with recouurenc
Conclusion: We hypothesize that expression of H3K4diMe may be considered as a significatnt factor for patients with lung cancer.

P1217

Expression of RASGRF2 in non-small cell lung cancer and its effect of transfection on biological behavior of human NSCLC lines H1299

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For RASGRF2 participating in H-ras signaling pathway, it has been identified as one of potential tumor suppressor genes. There are few reports on whether RASGRF2 plays a role in the pathogenesis of NSCLC. Our study was designed to explore the expression of RASGRF2 in NSCLC, and its effectiveness on clinical and pathological features. Furthermore, the effectiveness of RASGRF2 on the biological characteristics of NSCLC lines H1299 was considered. The expression of RASGRF2 was detected by SP in cancerous tissues and adjacent non-neoplastic tissues of 48 patients with NSCLC. Of 18 cases, mRNA were detected by RT-PCR.H1299 were transfected with RASGRF2-GFP. The effectiveness of RASGRF2-GFP on mitotic cycle and apoptosis of stable expression ones were evaluated by Flow Cytometry, and its proliferation were examined by MTS.The loss expression of RASGRF2 in cancerous tissues was 54%,but it was 88% in normal tissues($P<0.05$); The mRNA of RASGRF2 in normal tissues and cancerous tissues were 0.7834 ± 0.35490 and 0.2236 ± 0.12173, respectively($P<0.018$).There were no significant differences between the expression of RASGRF2 and gender, age, smoking status, histological types, classifications, lymph nodule metastasis and stages($P>0.05$). The mitotic cycle of transfected H1299, which had stable expression of RASGRF2-GFP, were found blocked at S stage, but the apoptosis and proliferation had no significant changes. The inactivation of RASGRF2 is an initial issue in the pathogenesis of lung cancer. Detecting RASGRF2 will help make a definite diagnosis of lung cancer in some extent. RASGRF2 may suppress the development of carcinoma by interfering mitotic cycle.

P1218

Regulator and effector T lymphocytes in late stages of non small cell lung cancer and chronic obstructive pulmonary disease

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The outcome of lung caner may be determined by anticancer immunresponse. There are only few data available on how DCs (dentritic cell), effector T, NK cells and various regulatory subsets like Treg-s (foxp3+), or NK T-s respond to lung cancer (LC).
Blood was drawn from 50 pts (age 36 to 77) suffering from non-small cell lung cancer in either III/B or IV stages. Since 80% of LC suffered also from chronic obstructive pulmonary disease (COPD), one control group was formed from COPD (n=25) and another one from healthy individuals (n=25). Peripheral blood mononuclear cells were separated and analyzed by FACS using labeled monoclonal antibodies. Activated effector T cells were determined by CD4CD45RO, the naive T cells were specified by CD4CD45RA, the regulatory T lymphocytes by CD4highFoxP3 and dendritic cells by Lin1- HLA DR positivity. The NKT cells were defined by Va24Vb11 (iNKT) and CD161CD3 (NKT) markers.
In lung cancer, the ratio of naiv CD4+ lymphocytes was increased as compared to COPD ($p<0.001$). The ratio of dentritic cells was decreased in LC as compared to healthy control ($p<0.001$). The ratio of regulatory T lymphocytes (CD4+FoxP3+) was significantly elevated in lung cancer when compared with COPD ($p<0.001$ and healthy groups ($p<0.01$). The ratios of iNKT and NKT cells were elevated in LC when compared to COPD ($p<0.01$).
The antigen specific immune responses diminished, regarding the decreased level of dentritic cells and increased level of naiv CD4 cells. The elevation of NKT cells represents the propagation of an antigen independent immune response. The rise of regulatory T cells facilitates tumor progression.

P1219

Reciprocal imbalance or coexistence? The distribution of Th17 and Treg cells in peripheral blood lymphocytes in patients with non-small-cell lung cancer and its significance

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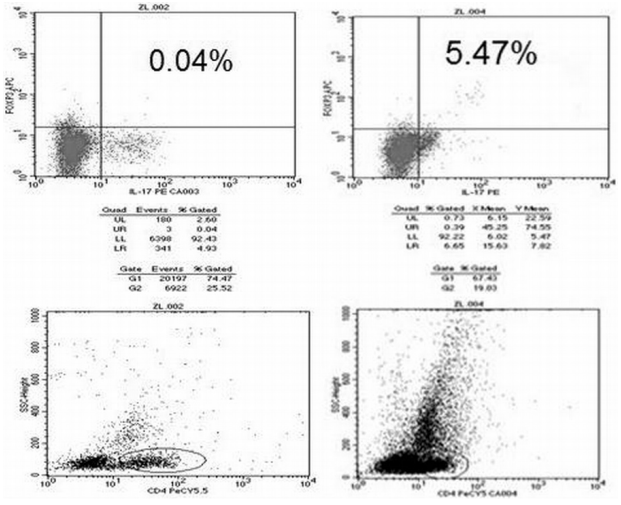
Objective: To investigate the the distribution of Th17 in relation to Treg in PBMC in patients with non-small cell lung cancer and its significance.
Methods: We analyzed T cells in the peripheral blood of 22 patients with Stage IV, 18 patients with Stage III, 8 patients with Stage I~II and 20 healthy volunteers.The proportions of Th17 and Treg cells in peripheral blood were determined by flow cytometry.



The plasma level of IL-17 and TGF-β were measured by ELISA and the mRNA expressions of RORγt and FOXP3 were detected by realtime PCR.
Results: The frequency of blood Th17 cells and IL-17A levels in plasma were increased in all patients with non-small cell lung cancer. The ratio of Th17 and Treg cells in patients with Stage IV are much higher than that in early stage. Particulary, we detected a small portion of CD4+IL17+Foxp3+T cells in the peripheral blood. Patients with Stage IV have much this kind of double positive helper T cells than other groups.
Conclusions: Reciprocal imbalance of Th17/Treg were found in patients with early disease. The elevated Th17 cell and Treg cell responses were associated with advanced disease, which indicated coexistence of Th17 cell and Treg cell instead of reciprocal imbalance was the major part in the antitumor immunity with the progression of non-small cell lung cancer.

P1220

Glycolytic phenotype mapping in lung cancer cell lines

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Background: It is well established that cancer cell can activate their glycolysis pathway in order to survive in hypoxia micro-environment. Tumors cells show a stinking rate of glycolysis and lactate production, even in the presence of oxygen. Importantly, some cancer cells demonstrate impaired mitochondria respiration and high glycolysis, namely Warburg effect. It is known that most types of cancers fit the "Warburg hypothesis" because a decreased expression of mitochondrial ATP synthase, which is a bottleneck of mitochondrial oxidative phosphorylation.
Objective: Our overall goal is to profile the lactic acid production in lung cancer cell lines (NSCLC vs SCLC) in order to prototype the glycolytic phenotype that might be used as a diagnostic tool for the early detection of lung cancer.
Study design and preliminary data: Our main steps are: (1) quantify lactic acid production of representatives of NSCLC and SCLC cell lines in order to select lung cancer cell lines which demonstrate the highest glycolytic phenotype; we choose to study representative of adenocarcinoma (e.g. H2030, A549, H1563, H1650), squamous carcinoma (e.g. H226) and SCLC cell line (e.g. SHP77) (2) measure the glycolysis (e.g.GAPDH and pyruvate kinase) and oxidative phosphorylation

enzymes (e.g. ATP synthase) level in order to study the glycolytic phenotype of lung cancer cell lines. Our preliminary results indicate that the adenocarcinoma cell lines, namely A549 and H2030 secreted lactic acid to the medium in concentration of 9.5nmol/ul and 11nmol/ul, respectively.
Impact: Our study could serve as a basis for understanding the glycolytic phenotype mechanism *in vitro* and therefore for developing diagnosis test for early detection of lung cancer.

P1221
Induced expression of B7-H3 on the lung cancer cells and macrophages suppresses tumor-specific T cell immunity
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Macrophages are prominent components of solid tumors and have complex dual functions in their interaction with cancer cells. Strong evidence suggests that TAM is part of inflammatory circuits that promote tumor progression. B7-homologue 3 (B7-H3), a recently identified homologue of B7.1/2 (CD80/86), has been described to exert co-stimulatory and immune regulatory functions. Here, we showed that a fraction of macrophages in tumoral stroma expresses surface B7-H3 molecules in lung cancer carcinoma model. Normal macrophages activated by tumor cell strongly express B7-H3 proteins. Although a lung cancer cell line constitutively expressed B7-H3 mRNA and protein in plasma, primary tumor cell isolated from the transplanted lung carcinoma model expressed B7-H3 on the surface. Interestingly, in transplanted lung carcinoma model, the expression of membrane-bound B7-H3 in tumor cells was increased as prolonging of tumor transformation. Interleukin 10 released from TAM could stimulate cancer cell expression of membrane bound B7-H3. Furthermore, Lung cancer and TAM-related B7-H3 was identified as a strong inhibitor of T-cell effect. B7-H3 expression may significantly influence the outcome of T cell immune response and TAM-tumor cell interactions induced membrane-bound B7-H3 represents a novel immune escape mechanism which links the proinflammatory response to immune tolerance in the tumor milieu.

P1222
The ability of SDF-1/CXCR4 axis to proliferation adhesion and invasion of small lung cancer cell
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Objective: To investigate the effect on tumor invasion and the role of PI3K signaling pathway after the binding of SDF-1 and CXCR4 receptor.
Methods: Flow cytometry (FCM) and RT-PCR were utilized to detect the expression of CXCR4 in NCI-H446. CCK-8 assay was utilized to detect the proliferation of tumor cells treated by SDF-1,CXCR4 antagonist AMD3100 and the PI3K inhibitor LY294002.Chemotaxis and transwell invasion experiments were made in different groups to observe the changes of adhesion and invasion ability.
Results: NCI-H446 with high expression of CXCR4(91.6±2.1%). Compare with the control group,SDF-1,AMD3100 did not affect the proliferation of NCI-H446. LY294002(20μmol/L) could inhibit the proliferation of NCI-H446. 24h, 48h, 72h inhibition rates were 19.4%, 31.3%, 18.4%. Compared with the normal control group,100ng/ml of SDF-1 increased the adhesion ability of NCI-H446,OD values were 1.253±0.107 VS 0.783±0.071(P <0.05); it also increased invasion capacity, the penetrating number were 83.2±15.7 VS 30.8±6.5(P <0.001). Compared with 100ng/ml of SDF-1 treatment group, AMD3100 and LY294002 could inhibit the adhesion and invasion, OD values were 0.759±0.088 VS 1.253±0.107,0.652±0.076 VS 1.253±0.107 (P<0.05) respectively, the penetrating number were 37.8±5.1 VS 83.2±15.7,37.6±7.2 VS 83.2±15.7 (P <0.001).
Conclusion: Small cell lung cancer cell line NCI-H446 has a high expression of the chemokine CXCR4, its ligand SDF-1 can promote the adhesion and invasion of the tumor cells. PI3K signaling pathway involved in the proliferation works in the adhesion and invasion of NCI-H446 by CXCR4 activation.

P1223
The clinical effect and expression of alpha-methylacyl-CoA racemace (AMACR) in the differential diagnosis of malign mesothelioma and adenocarcinoma of lung
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Background: Alpha-methylacyl-CoA racemace (AMACR) which is an intracellular enzyme involving in the beta-oxidation of branched fatty acids has emerged as an immunohistochemical marker for many types of cancer. This study is conducted to investigate AMACR expression in the differential diagnosis of malign pleural mesothelioma (MPM) and lung adenocarcinoma, and its correlation with clinical characteristics and survival.
Methods: The clinicopathologic characteristics of 144 patients (73 adenocarcinoma, 71 MPM) were reviewed retrospectively. The resection materials were evaluated by immunohistochemical method. The patients who were given adjuvant chemotherapy and/or radiotherapy, with an evidence of residual tumor and who died due to postoperative mortality and due to reasons not related to lung cancer were excluded for survival analysis. Data from remaining 77 patients (37 adenocarcinoma, 40 MPM) were used for survival analysis.
Results: AMACR expression was more frequent in adenocarcinoma group than MPM group (p=0,046). The specificity and sensitivity of AMACR immunostaining in detecting adenocarcinoma were %58,9, and %57,7 respectively. AMACR positive and negative groups were similar for age, sex, smoking history, tumor diameter, lymph node involvement, tumoral differentiation, T-N factor, and stage. Overall survival was not significantly different between the groups, either.
Conclusion: The specificity and sensitivity of AMACR immunostaining was not high enough to use it as a diagnostic tool in differential diagnosis of MPM and lung adenocarcinoma. AMACR expression did not have a prognostic value in MPM or in adenocarcinoma.

P1224
Aberrant promoter methylation of CDH13 and MGMT genes is associated with clinicopathological characteristics of primary non small cell lung carcinoma
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Introduction: Systemic methylation changes may be a diagnostic marker for tumor development or prognosis. We investigate the relationship between gene methylation in lung tumors relative to normal lung tissue, and whether DNA methylation changes can be detected in paired blood samples.
Material and methods: 65 patients were enrolled in a surgical case series of non-small cell lung cancer (NSCLC) at a single institution. Using bisulfite pyrosequencing, CpG methylation was quantified at five genes (RASSF1A, CDH13, MGMT, ESR1 and DAPK) in lung tumor, pathologically normal lung tissue, and circulating blood from enrolled cases.
Results: The analyses of methylation in tumors compared to normal lung identified higher methylation of CDH13, RASSF1A and DAPK, while ESR1 and MGMT methylation did not differ significantly between these tissue types. We examined whether the three aberrantly methylated genes could be detected in blood. The difference in methylation observed in tumors was not reflected in methylation status of matching blood samples, indicating a low feasibility of detecting lung cancer by analyzing these genes in a blood-based test. Lastly we probed whether tumor methylation was associated with clinical and demographic characteristics. Histology and gender were associated with methylation at the CDH13, while stage was associated with methylation at MGMT.
Conclusion: Our results show higher methylation of RASSF1A, CDH13 and DAPK genes in lung tumors compared to normal lung. The lack of reflection of these methylation changes in blood samples from patients with NSCLC indicate their poorly suitability for a screening test.

P1225
Expression of vascular endothelial-cadherin and epithelial cadherin in non-small cell lung cancer and their clinical significances
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To assess the expression of vascular endothelial cadherin (VE-cadherin) and epithelial cadherin (E-cadherin) in human non-small cell lung cancer (NSCLC) tissues and to correlate their expression with the clinicopathological characteristics of NSCLC. The expression of them were examined by immunohistochemistry in NSCLC tissues from 97 patients and their adjacent non-neoplastic tissues.The mRNA of them were detected by RT-PCR in 18 specimens.The positive rates of them were 51.5% and 42.3%, respectively. The positive rate of VE-cadherin was higher than that in adjacent non-neoplastic tissues 0% (P < 0.05), The positive rate of E-cadherin was lower than that in adjacent non-neoplastic tissues 67.0% (P < 0.05). The VE-cadherin expression level correlated with lymph node metastasis (P < 0.05), while the E-cadherin expression level inversely correlated with lymph node metastasis (P <0.05). There were no significant differences between the expression of VE-cadherin, E-cadherin and sex,age,smoking,histological type,differentiation of tumor and TNM stage.The relative expression intensity of VE-cadherin mRNA in NSCLC tissues and adjacent non-neoplastic tissues were 0.626±0.192 and 0.209±0.062(P< 0.05). The relative expression intensity of E-cadherin mRNA were 0.700±0.123 and 1.050±0.254,respectively(P< 0.05). We demonstrated the aberrant expression of VE-cadherin in NSCLC and the down-regulation of E-cadherin expression. Both of them are associated with lymph node metastasis. These results indicate that both of them may take part in the growth and metastasis of NSCLC and thus may be therapeutic targets for the treatment of NSCLC.

P1226

Expression of vascular endothelial-cadherin and vascular endothelial growth factor in non-small cell lung cancer and their clinical significances

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To investigate the expression of Vascular Endothelial-cadherin(VE-cadherin) and Vascular Endothelial Growth Factor(VEGF) with the association of clinicopathological characteristics and their correlation in non-small cell lung cancer(NSCLC). Expression of them were examined by immunohistochemical staining in 72 cases of NSCLC patients and their adjacent non-neoplastic tissues, 45 of them were measured by RT-PCR.The positive rates of them in cancer cells were 69.4% and 84.7% respectively,which were higher than the normal tissues(0% and 16.7%) (P<0.01); they were 52.8% and 62.5% in the vascular endothelial cells,which were higher than the normal tissues(20.8% and 29.2%) (P<0.01).In cases with lymph node metastasis,the expression of them in cancer cells and vascular endothelial cells were respectively higher than those in cases without lymph node metastasis(P<0.05),The positive rate of them in III-IV stage were higher than that in I-IIstage (P<0.05);There were no significant differences between the expression of them and sex,age,smoking,histological type,differentiation of tumor(P>0.05). The relative expression intensity of VE-cadherin mRNA in NSCLC tissues and adjacent non-neoplastic tissues were 0.621±0.182 and 0.445±0.052(P<0.05). The relative expression intensity of VEGF mRNA in NSCLC tissues and adjacent non-neoplastic tissues were 0.275±0.103 and 0.050±0.154(P<0.05). The expression of them in lymph node metastasis were higher compared to that in cases without lymph node metastasis(P<0.05).Our results suggest that VE-cadherin may play an important role in angiogenesis and act in a synergistic manner in NSCLC.

P1227

Increased incidence of Merkel cell polyoma virus in non-small-cell lung cancer

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Introduction and aim of the study: Polyoma viruses such as BK virus (BKV), JC virus (JCV) and Merkel Cell Polyoma virus (McPyV) are typically non-oncogenic. The evidence for their role in human cancer remains controversial although they have been detected in a variety of human neoplasms. The aim of this study is to determine the incidence of the most common polyoma viruses in adults McPyV, BKV and JCV in a large non-small cell lung cancer (NSCLC) patient population.
Methods: Real Time PCR and nested PCR were employed to assess the presence of BKV, MCPyV and JCV DNA in tissue biopsies from 100 patients with primary NSCLC as well as lung tissue specimens from macroscopically healthy sites of their lung.
Results: BKV and JCV DNA were not detected in lung tissues biopsies or control specimens. However, ten specimens from lung cancer tissue were found positive for the presence of MCPyV DNA (10/100, 10%), whereas no control sample was tested positive for the virus. The MCPyV positive samples were obtained from male patients in 90% (9/10) of the cases, with a mean age of 64 years and the following histological types: adenocarcinoma 5/10 cases (50%), squamous cell carcinoma 3/10 cases (30%), bronchoalveolar carcinoma 1/10 cases (10%) and undifferentiated large cell lung carcinoma 1/10 cases (10%).
Conclusion: The detected MCPyV prevalence in NSCLC may suggest a pathogenetic role of this virus in NSCLC of the lung. These results may implicate MCPyV mainly with lung adenocarcinoma, while providing evidence of the potential oncogenic role of this virus in NSCLC.

P1228

The long non-coding MALAT-1 stimulates cellular growth, migration and proliferation

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Introduction: In non small cell lung cancer, the expression levels of the large non-coding RNA (lncRNA) MALAT-1 are associated both with patient survival and with tumor promoting effects. Here, we focus on the molecular genetic impact of MALAT-1 on cellular gene regulation.
Methods: Gene expression was studied in murine fibroblasts (NIH 3T3) either transduced with PINCO::MALAT-1 expression vector or with empty control vector using Next Generation Sequencing (Mouse Gene 1.0 ST array, Affymetrix, CA). Differentially expressed genes were analyzed by the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Inc. Redwood City, CA).
Results: Out of 29.000 genes, 250 genes were significantly (at least twofold) up-regulation or down-regulation. Differentially expressed genes were categorized into “Top Bio Functions” groups. The three most important groups were “cellular growth and proliferation” (n=90), “cellular movement” (n=75), and “inflammatory response” (n=65). The observation of a positive association of MALAT-1 gene expression with cellular growth, migration and proliferation was confirmed in vitro with migration assays, colony assays, and scratch assays (p<0.01, all comparisons).
Conclusions: These data demonstrate that enhanced MALAT-1 expression levels stimulate cellular migration, colony formation and wound healing and contribute to the idea of multidimensional effects of MALAT-1 ncRNA on important cellular functions in malignant lung cells.

P1229

The effect of siRNA on invasion capability of small cell lung cancer

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Objective: To study the inhibitory effect of CXCR4-targeted small interference RNA on invasion capability of NCI-H446 in vitro.
Methods: To design chemical synthesis of CXCR4-specific siRNA based on the target sequence for CXCR4 cDNA with NCI-H446 cells transfected with siRNA. To detect the expression of CXCR4 by RT-PCR and Western Blot. Invasion capability of NCI-H446 cells in vitro was evaluated by transwell chamber model and the proliferation capability was determined by CCK-8 assay.
Results: After transfected with CXCR4-siRNA, the expression of CXCR4 mRNA and protein was down-regulated significantly. The invasion capability of cells in vitro decreased compared with the empty liposome group. The penetrating number in CXCR4-siRNA transfected group was 32.3±3.8 VS 62.1±8.2(P<0.001).There was no effect on cell proliferation after transfection of CXCR4 siRNA on NCI-H446 cells.
Conclusion: CXCR4 siRNA effectively down-regulated the expression of CXCR4 gene and decreased invasion capability of NCI-H446 cells in vitro.

P1230

microRNA-155 negatively regulates Apaf-1 and enhance sensitivity of A549 to cisplatin

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MicroRNA-155(miR-155) overexpression is often found in malignancies including lung cancer. The objective of this study is to verify the hypothesis that miR-155 is involved in development and progress of lung cancer by modulating cell apoptosis and DNA damage through regulation on Apaf-1, which is postulated according to the bioinformatics analysis. Firstly, the expression of miR-155 and Apaf-1 protein in the lung cancer tissues were measured. The results showed that expression of miR-155 is significantly higher in lung cancer tissues compared with the paracancerous tissues and normal tissues, while Apaf-1 protein expression level decreased in lung cancer tissues. Then the miR-155 silenced and Apaf-1 overexpressed A549 cell models were established through transfection with pMAGic2.0-BIC-siRNA and pcDNA3.1-Apaf-1, respectively. The cell apoptosis and DNA damage of different cell models under treatment with cisplatin were assessed, and the untranfected A549 cells were used as negative control. The results showed that silence of miR-155 resulted in elevated expression of Apaf-1 protein, but the Apaf-1 mRNA level had no significant difference compared with the control group. Both miR-155 silencing and Apaf-1 overexpression in A549 cells seemed greatly increase the cellular sensitivity to cisplatin treatment as evidenced by elevated apoptosis rate and DNA damage. Further, dual-transfection with both miR-155 siRNA and Apaf-1 siRNA in A549 cells resulted in attenuation and alleviation of cell apoptosis and DNA damage. In conclusion, inhibition of miR-155 can enhance the sensitivity of A549 cells to cisplatin treatment by regulation on cell apoptosis and DNA damage through Apaf-1 mediated pathway.

P1231

The effect of methylene blue in the photodynamic therapy on A549 lung cancer cell lines

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Background: Methylene blue (MB) is a safe and cheap chemical widely used in the medical fields for a long time. MB was recently known to act as a photosensitizer and some clinical trials of using it in the photodynamic therapy on the tumor cell lines were reported. In this study, we investigated the effects of MB as a photosensitizer on the A549 lung cancer cell lines.
Methods: After pretreatment of A549 cells with MB in the concentrations of 1 μ/ml and 2 μ/ml, respectively, Diode LASER of 650 nm wavelength was used with the energy of 30, 60 and 120 J/cm^2 respectively. Cytotoxicity was evaluated

with MTT, and the formation of ROS was checked with DCFH. To evaluate the mechanism of apoptosis, the activation of Caspase family was checked, and the fragmentation of PARP-1 was studied by the western blotting method.
Results: By the increases of the concentration of MB and the energy of Diode LASER, the level of apoptosis of cell lines and ROS synthesis were significantly increased. Pretreatment with antioxidant suppressed these findings. In the induction of apoptosis, the activation of Caspase 3, 8 and consequent fragmentation of PARP-1 was observed.
Conclusion: MB induced apoptosis in the A549 lung cancer cell lines as a photosensitizer, and its effect was increased by the concentration of MB and the energy of Diode LASER.

P1232
Tumor size and inflammatory cytokines in exhaled breath condensate in patients with non small cell lung cancer (NSCLC)
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Background: Both local and systemic inflammation play a key role in the genesis and progression of lung cancer; vascular endothelial growth factor (VEGF) has been related to progression and local extension of many tumors. Involvement of T helper 17 lymphocytes (Th17) and their cytokines (IL17, IL6) in cancer has been recently postulated. Lung inflammation may be non-invasively assessed by cytokine assay in exhaled breath condensate (EBC).
Aim and objectives: To assess the possible correlations between systemic and local Th17 related cytokines, VEGF and tumor size evaluated by CT-scan in patients with NSCLS.
Methods: Fifteen consecutive patients (12 males; mean age 64 years) with NSCLC classified in stage IA-IB-IIA, were enrolled. Lung CT-scan, EBC and serum samples were obtained in each patient. IL-6, IL-17 and VEGF were measured by ELISA.
Results: Tumor mean diameter was 3.28 cm (SD 2.33). Mean cytokines values in serum and EBC are shown in table 1.

Table 1

Cytokine	Serum	EBC
IL-17	0.17 (±0.43)	2.85 (±1.22)
IL-6	1.05 (±1.27)	0.29 (±0.09)
VEGF	19.27 (±29.04)	78.45 (±29.65)

Mean (±SD) serum and EBC cytokines levels (ng/ml).

EBC level of VEGF was significantly correlated with EBC IL-6 (r=0.314, p=0.030) and IL-17 (r=0.697, $p<0.001$). A significant correlation between tumor diameter and IL-6, IL-17 and VEGF in EBC was observed (r=0.440 p=0.013, r=0.444 p=0.013, r=0.332 p=0.039 respectively). No correlation was found between serum cytokine and tumor size.
Conclusion: This is the first observation reporting Th17 cytokines in EBC in NSCLC. The correlation between Th17 cytokines and tumor size suggests the involvement of Th17 cells in the progression of neoplasia.