42. Lung cancer biology

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Cytokine effects on the cell death of the A549 carcinoma cells

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Background: we investigated the effects of TNFa, IL1β, IL13 and IFNγ on the Fas/CD95-induced cell death of the A549 lung non-small cell carcinoma cells.
Methods: A549 cells with suppression of the canonical or the non-canonical NF-κB pathway were generated by silencing with RNA interference of the two kinase subunits of the IKKβ kinase (IKK) complex, IKKβ (canonical NF-κB pathway) or IKKα (non-canonical NF-κB pathway). A549 cells without suppression of the NF-κB pathway were also treated with TNFa or IL1β or IL13 or IFNγ and Fas/CD95 antibody (clone CH11). Cell cycle and cell death were analyzed by flow cytometry. Protein expression was analyzed by Western blot.
Results: In A549 cells without NF-κB suppression a) TNFa or IL1β or IL13 pretreatment decreased and IFNγ pretreatment increased CH11-induced cell death, b) TNFa and IL1β anti-cell death effects on CH11-induced cell death were attenuated by BAY-117082 [NF-kB inhibitor] pretreatment and c) IL-13 anti-cell death effects on CH11-induced cell death were attenuated by LY-294002 [inhibitor Phoshatidylinositole-3 kinase (PI3-K)] pretreatment. In A549 cells with suppression of the NF-κB pathway, TNFa or IL1β or IL13 pretreatment did not induce significant alterations in CH11-induced cell death. By Western blot the cleaved form of PARP1 protein was detected in CH11-treated cells and the TRAF1 protein was detected in TNFa-treated cells without NF-κB suppression.
Conclusion: TNFa, IL1β and IL-13 attenuated the pro-cell death effects of Fas/CD95 on A549 cells. TNFa and IL1β anti-cell death effects were mediated, at least partially, by the NF-kB signaling pathway. IL13 anti-cell death effects were mediated, at least partially, by the PI3-K signaling pathway.

E283

Clinical significance of hypoxia inducible factor-1α and VEGF expression in lung cancer

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Background: Hypoxia inducible factor 1 (HIF-1) controls the expression of genes involved in angiogenesis, cell metabolism, apoptosis, invasion or metastasis. Although increased expression of its alpha subunit HIF-1α has been observed in non-small-cell lung cancer (NSCLC), little is known about its expression in small-cell lung cancer (SCLC).
Objective: To evaluate the expression of HIF-1α and the correlation between HIF-1α and VEGF expression in different histological types of lung cancer.
Methods: The expression of HIF-1α and VEGF was evaluated by immunohistochemistry in bronchial biopsies from 55 patients with advanced SCLC (n=22) and NSCLC [n=33; 17 squamous carcinoma (SC), 16 adenocarcinoma (AC)]. The patients underwent platinum-based chemotherapy and were in follow up. The association of HIF-1α and VEGF expression with tumor stage, clinical response to chemotherapy and survival was estimated.
Results: A significant difference was shown in the expression of nuclear HIF-1α among NSCLC and SCLC (75.8% vs 45.5%, p=0.022). HIF-1α was expressed in 88.2% of SCs, 62.5% of ACs and 45.5% of SCLCs. There was a significant correlation between HIF-1α and VEGF expression (p=0.001). In subgroup analysis, significant correlation between HIF-1α and VEGF expression was observed only in SC (p=0.022) but not in AC (p=0.118) or SCLC (p=0.119). No correlation was found between VEGF expression and the cancer histological type. The expression pattern of HIF-1α and VEGF in all groups was not correlated with tumor stage, clinical response to chemotherapy or survival.
Conclusion: HIF-1α expression may differ according to histological type in advanced lung cancer, implicating new aspects in therapeutic approaches.

E284

Association of polymorphisms in the genes of the urokinase plasminogen activation system with susceptibility to and severity of nonsmall cell lung cancer

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Background: Extracellular matrix degradation, mediated by the urokinase plasminogen activation (uPA) system, is a critical step in tumor invasion and metastasis. We examined the single nucleotide polymorphisms (SNPs) with a potential effect on expression of genes in the uPA system for their role in non-small cell lung cancer susceptibility and severity.
Patients and methods: Using a case-control study design, we compared the allele frequencies and genotype distributions of each single nucleotide polymorphism in the promoter regions of uPA rs4065 T/C and uPAR rs344781-516 A/G in 375 NSCLC cases and 325 sex-matched controls recruited from the health check-up unit using polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) analysis.
Results: Overall, the distribution of the genotype frequencies of uPA rs4065 T/C and uPAR rs344781-516 A/G were significantly different between the lung cancer patients and the healthy controls, and also different between patients with lung cancers of various stages. Logistic regression analysis revealed that higher odds ratios (ORs) for lung cancer were seen for individuals with uPA TT genotype against CC/CT genotypes (an OR of 12.3, 95% CI 4.41-34.6, $p < 0.0001$), and lower odds ratios (ORs) for lung cancer were seen for individuals with uPAR AA genotype against GG/GA genotypes (an OR of 0.52, 95% CI 0.37-0.73, $p < 0.0001$).
Conclusions: A significant association between the uPA rs4065 T/C and uPAR rs344781-516 A/G genetic polymorphisms and the susceptibility to lung cancer was demonstrated. Also, these two polymorphisms were associated with the severity of lung cancer.

E285

Expression of caveolin-1 and ttf-1 in lung adenocarcinomas with various histological subtypes

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Lung adenocarcinomas comprise several histological subtypes. Aims of this study were to analyze histological subtypes in lung adenocarcinoma patients, to evaluate immunohistochemical expression of caveolin-1 and TTF-1, and to correlate quantity of caveolin-1 and TTF-1 with amount of different histological subtypes, cell differentiation i.e. gradus, age, sex and smoking history. Study material included 140 patients with lung adenocarcinoma. 99 patients underwent surgical operation. Quantity of subtypes was scored and extent and intensity of immunohistochemical expression of TTF-1 and caveolin-1 were evaluated. The ultrastructural localization of caveolin-1 and TTF-1 were analyzed by immunoelectron microscopy in selected cases. Histological subtypes and immunohistochemical findings for caveolin-1 and TFF-1 were correlated with clinical data. 40% of lung adenocarcinomas were made up of one, 36% of two and 24% of three or more histological subtype. The most common subtypes were acinar, solid and papillary. Solid subtype correlated with high gradus and papillary subtype with low gradus of tumor. TTF-1 and caveolin-1 were positive in 84% and 55% of the cases, respectively. Expression of TTF-1 correlated with acinar component of tumor, whereas expression of caveolin-1 correlated with solid component. Cigarette smoking correlated with high gradus. We concluded that lung adenocarcinomas commonly constituted more than one histological subtype. Solid and papillary subtypes had a positive correlation with the gradus of tumor cells and moreover, caveolin-1 and TTF-1 expression correlated with solid and acinar subtypes.

E286

Lung cancer and metabolism: a role for glucagon?

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There is an association of lung cancer with various paraneoplastic metabolic

syndromes. A possible involvement of glucagon in the development of the major histological types, namely Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC) were studied by using iodinated glucagon binding to isolated plasma membranes from these tumors and analyzing the binding kinetics.
Methods: Membranes were obtained by differential centrifugation on a discontinuous sucrose gradient. The binding was measured following incubation of membrane aliquots in 0,1 M tris-HCl buffer, ph 7,4 at 37° C. Separation of bound from free was done by centrifugation of the incubation mixture aliquots in twenty fold excess of ice cold buffer at 12600g.
Results: Glucagon binding to SCLC membranes was 6,2±0,8 fml/100μgr protein, binding to NSCLC was 4,2±0,4 and to normal lung (control) 3,1±0,3. The binding kinetics were different between groups.
Conclusion: These data may support a possible role of glucagon/receptors in the development of lung cancer particularly SCLC. This needs further investigation.
Clinical Implication: Targeting inhibition of the glucagon receptor might be of therapeutic value in the future.

E287
Epigenetic inactivation of *checkpoint kinase 2* gene in nonsmall cell lung cancer and its relationship with clinicopathological features
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Lung cancer is the leading cause of cancer deaths worldwide and is usually associated with late diagnosis and poor prognosis. Tumor-acquired methylation of the promoter CpG islands is an important mechanism for silencing tumor suppressor genes. The checkpoint kinase 2 (CHK2) is a tumor suppressor that plays a crucial role in regulating cell-cycle checkpoints and apoptosis following DNA damage. The methylation status of the promoter region of human *CHK2* gene in 139 non-small cell lung cancers (NSCLCs) and their corresponding non-malignant lung tissues were examined using a nested methylation-specific PCR and the results were correlated with clinicopathological features. *CHK2* methylation was found in 39 (28.1%) of the 139 NSCLCs. *CHK2* methylation was more frequent in squamous cell carcinomas than in adenocarcinomas (40.0% vs 19.0%, p=0.006) and its frequency was higher in ever-smokers than in never-smokers with a borderline significance (31.7% vs 17.1%, p = 0.071). However, *CHK2* methylation was not significantly related to other clinicopathological factors such as age, gender, pathological stage, or *EGFR* mutation status in adenocarcinomas. RT-PCR analysis showed that *CHK2* methylation correlated with its expression. These results suggest that the down-regulation of *CHK2* gene via promoter methylation may play a role in the pathogenesis of NSCLC, particularly squamous cell carcinoma. However, further studies with large numbers of patients are needed to confirm our findings.

E288
C/EBP-β expression in mesothelioma cells is regulated by mitogen activated protein kinase p38-β and -γ
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Mesothelioma is induced by asbestos inhalation leading to constant pleura irritation. Resident mesothelial cells exposed to asbestos activated mitogen activated protein kinases (MAPK) which signal through the transcription factor family of C/EBPs. We investigated p38MAPK isoform and C/EBP-beta expression in isolated human mesothelial and mesothelioma cells. Total, cytosolic and nuclear proteins were isolated from cells stimulated with either PDGF-BB or TGF-beta1 and protein expression was tested by immuno-blotting as described earlier. Inhibitory small RNA (siRNA) for p38MAPK isoforms were used to block p38 expression. Mesothelial and mesothelioma cells expressed and activated Erk1/2MAPK after stimulation with PDGF-BB, but not TGF-beta1. Both cell types expressed p38-alpha, -beta and –gamma, but not –delta MAPK. In mesothelial cells all p38MAPK-isoforms were located in the cytosol and p38-alpha and –gamma were translocated into the nucleus after stimulation. In mesothelioma cells p38-alpha was only cytosolar, while p38-beta and –gamma were located in the nucleus, none of the p38MAPK isoforms was affected by any stimulus. In mesothelial cells down-regulation of Erk1/2MAPK by siRNA blocked C/EBP-beta expression and proliferation, while in mesothelioma cells both Erk1/2 and p38MAPK had to be blocked to inhibit C/EBP-beta expression and proliferation. Our data confirmed the constitutive activation of p38MAPK and further specifies p38MAPK isoforms. We show that p38MAPK has to interact with Erk1/2MAPK to stimulate mesothelioma cell proliferation via C/EBP-beta. The findings may provide the basis of a novel therapeutic strategy for mesothelioma.

E289
Methylation for cystic fibrosis transmembrane regulator gene in non-small cell lung cancer
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Introduction: Cystic fibrosis transmembrane regulator (PFTR) is known to be related to cell apoptosis and regulation of the glutathione concentration as an antioxidant. CFTR was worthy to be studied further as possible candidates of tumor suppressor genes by epigenetic mechanism for non-small cell lung cancer (NSCLC).
Objectives: In this study, we investigated quantitative evaluation of methylation for CFTR using pyrosequencing method and relationship of promoter meththylation and CFTR expression. We examined the functional study of CFTR.
Methods: Total of 28 patients with primary NSCLC who underwent lung surgeries from 2006 to 2008 in Konyang university hospital were included. Cancer tissue & adjacent normal tissue were acquired. We evaluated methylation for promotor lesion of CFTR using pyrosequencing method and CFTR expression using real time PCR. We examined the function of CFTR using small interfering RNA (siRNA).
Result: The methylation index was 7.53 for normal tissue and 22.80 for cancer tissue. Inverse correlation between endogenous mRNA levels of CFTR and methylation index of CFTR DNA determined by qRT-PCR. Short-interfering RNA (siRNA) mediated silencing of CFTR also leads to an increased cell population and decreased apoptosis in lung cancer cell.
Conclusion: We observed that CFTR was highly methylated in lung cancer cells and negatively correlated with CFTR expression. In Short-interfering RNA (siRNA)-mediated silencing of CFTR, CFTR is implicated in apoptosis pathway. It is indicate novel functions of CFTR in Lung cancer biology.

E290
Globo h tumor antigen expression is associated with blood group a1 and negative prognosis in NSCLC
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Blood group related antigens are reported both to initiate and to maintain malignant transformation processes. Globo H antigen, a cell-surface glycosphingolipid, resembles the H antigen of the ABH system residing on type 4 chain and is found on a range of cancer cell lines. Despite being regarded as an attractive target for immunotherapy, its role in NSCLC is yet not known.
Eighty-six paraffin-embedded tissue sections of non-small cell lung cancer (NSCLC) patients in stage I–IV, who underwent surgical resection of their primary tumor (72% male; median 65 years; 38% squamous cell cancer, 35% adenocarcinoma) were stained with a new, highly specific monoclonal antibody against Globo H (clone A69-A/E8).
A positive antigenetic expression was observed in 21% of patients. Unlike in peripheral blood, Globo H expression is almost exclusively associated with blood group antigen A1 ($p<0.0001$). Univariate analysis identified Globo H expression on tumor cells as a marker of reduced overall survival in A1 positive patients ($p=0.024$). Multivariate analysis confirmed this observation: Globo H negativity, non squamous cell lung cancer and stage I disease were independent markers of favourable prognosis ($p<0.01$, all comparisons).
We conclude that blood group related carbohydrate antigens on tumor cells and in peripheral blood may have a distinct function for tumor progression in resected NSCLC.

E291
Exhaled aldehydes as markers of disease in lung cancer patients
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Oxygenated compounds such as aldehydes and ketones in breath have been described as biomarkers of metabolism, oxidative stress and cancer. Conventional analysis of these compounds in the low ppb range is hampered by their reactivity and poor stability. This study was intended to determine exhaled aldehydes as disease markers in cancer patients by means of a smart and rapid combination of sample preparation and analysis.
35 patients and volunteers (12 cancer patients, 11 smokers and 12 healthy volunteers) were investigated. 10 mL of alveolar gas were taken under control of expired CO2. C1-C9 aldehyde concentrations were assessed quantitatively by means of solid phase micro extraction, on fibre derivatization (SPME-OFD) using PFBHA

and analyzed by GC/MS. Results were corrected for inspired concentrations and analysed by means of ANOVA on ranks.
Expired concentrations of C1-C9 aldehydes ranged from 0.02 to 8.23 nmol/L. Lung cancer patients exhaled significantly ($p<0.001$) higher concentrations of pentanal than healthy controls and healthy smokers (0.418 (0.287–0.717), 0.0394 (0.000-0.249), 0.000 (0.000–0.124))
Exhaled hexanal and octanal concentrations were significantly higher ($p=0,008$; $p= 0,016$) in lung cancer patients than in smokers but were not different from concentrations in healthy volunteers. Exhaled concentrations of C1–C4, C7, C9 aldehydes showed no significant differences between the groups.
Because of its high selectivity and sensitivity the SPME-OFD-GC/MS assay enables reliable detection of volatile C1–C9 aldehydes in trace amounts. Analysis of exhaled C1–C9 aldehydes bears the potential of non invasive monitoring and recognition of pathological processes and diseased states.

E292
Autophagic cell death of the nutrient deprivation augmented by cytotoxic drugs in lung cancer cell
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Autophagy is known for its role in cellular homeostasis, development, cell survival, aging, im-munity, and cancer. Autophagy has emerged as another major'programmed' mechanism to control life and death much like "programmed cell death" is for apoptosis in several types of cancer. To be elusive that autophgic cell death on nutrient starvation in combination with cytotoxic drugs, we investigated whether its increase synergistically in two mixed conditions. When cancer cells were subjected to extreme nutrient starvation by culturing in a medium without serum and amino acids or with 2-deoxyl-D-glucose, a chemical inhibitor of glucose metabolism, cells death occurred within early time. At nutrient deprived media with cisplatin or gemcitabine treatment, Cell survival revealed a markedly decrease in percentage of living cells undergoing nutrient starved medium with each of two cytotoxic drugs compared with those drugs respectively. The staining of cells in normal media with acridine orange disp-layed green fluorescence with cytoplasmic and nuclear components in normal media but showed considerable red fluorescence in combined medium or cytotoxic drugs in each treated cells, suggesting formation of numerous acidic autophagolysosomal vacuoles. During autophagy is advanced, LC3 type II increased by conversion from LC3 type I. We figured out that the autophagosome-incorporated LC3 II protein expression more increased in cell contained nutrient-deprived medium with cytotoxic drugs compare with cisplatin or gemcitabine alone. The autophagic cell death potentially increased in nutrient-deprived conditions combined with cytotoxic drugs in human lung cancer cell lines.

E293
Endoplasmic reticulum Ca^{2+}-homeostasis is altered in lung cancer cell lines and contributes to cisplatin-resistance
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Calcium is a ubiquitous signal molecule that is involved in the control of proliferation and apoptosis. We aimed to investigate if the ER Ca^{2+}-homeostasis is different in lung cancer and normal human bronchial epithelial (NHBE) cells and if alteration of the ER Ca^{2+}-homeostasis contributes to cisplatin-resistance.
The intracellular Ca^{2+}-signaling was investigated using fluorescence-microscopy. The expression of Ca^{2+}-regulating proteins was assessed using Western Blot analysis and manipulated using siRNA techniques.
In a small cell lung cancer (H1339) and an adeno carcinoma lung cancer (HCC) cell line the ER Ca^{2+}-content was reduced compared to NHBE cells. The reduced Ca^{2+}-content correlated with a reduced expression of SERCA pumping calcium into the ER, an increased expression of IP_3R releasing calcium from the ER, and a reduced expression of calreticulin buffering calcium within the ER. H1339 and squamous cell carcinoma cells (EPLC M1) cells were exposed to 4 cycles cisplatin analogue to the *in vivo* kinetics, which resulted in a partial cisplatin-resistance. In the resistant clones, the ER Ca^{2+}-content was reduced compared to cisplatin-naïve cells. This correlated with a reduced expression of SERCA in resistant H1339 cells and an increased expression of IP_3R in resistant EPLC M1 cells. Altering the expression of SERCA and IP_3R in the respective cells, the essential role of the Ca^{2+}-regulating proteins in the cisplatin-resistance could be confirmed.
We conclude that the ER Ca^{2+}-homeostasis may play a central role in carcinogenesis as well as cisplatin-resistance of lung cancer.
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E294
The expression and biological significance of PD-L1 on lung cancer cell lines
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Background and objective: Tumor-associated B7-H1 expression was recently shown to promote T-cell apoptosis and proposed as a potential mechanism of immune evasion by tumors. On the basis of the ability of tumor-associated B7-H1 to mediate activated T-cell death, it is likely that manipulation of the B7-H1 pathway at defined time points during the development of the T-cell antitumor immune response can enhance the efficacy of T-cell-based immunotherapy. Here, the expression of PD-L1 on lung cancer cell lines and its role in interaction of CTL and target cells was investigated.
Methods: Human PBMC derived DCs were loaded with apoptotic tumor cells and stimulated by CD40 mAb (5C11). Tumor specific CTL was generated in vitro by autologous T cells co-cultured with mature DCs. Expression of PD-L1 on lung cancer cell lines H1299 and A549 were analyzed by FCM. JAM assay was used to detect the cytolytic activity of CTL with or without blocking PD-L1 by CD40 mAb respectively. The concentrations of IFN-γ in supernatants from distinct groups were analyzed by ELISA.
Results: Tumor cells-loaded mature DCs could induce the generation of the tumor specific CTL. Expression of PD-L1 was low on A549 cell, but high.on H1299 cell. Blockade of PD-L1 on A549 could not improve cytolytic effect of CTL on target cells and IFN-γ production, but fragmentation of H1299 cells and IFN-γ production were significantly enhanced by the combination of PD-L1 mAb and CTL.
Conclusion: Gene expression of PD-L1 on lung cancer cell line can decrease the cytolytic effect of CTL on target cells.

E295
A novel *ex vivo* model for the evaluation of hypoxic adaptation in nonsmall cell lung cancer using surgery explants
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Hypoxia is common in solid cancers, such as lung cancer, and promotes aggressive tumor growth and chemotherapy resistance. The molecular mechanisms of adaptation to hypoxia have been largely investigated in tumor cell-lines. They include proteomic changes resulting in loss of apoptotic potential, change of cellular metabolism, and release of angiogenic growth factors. These changes are observed already under moderately hypoxic conditions with O_2 concentrations of 1-2% (7-14 mmHg). We developed a novel *ex vivo* model using fragmented surgery explants in order to investigate mechanisms of hypoxic adaptation in non-small cell lung cancer (NSCLC). The results of our study show that NSCLC fragments can be maintained in culture for four days, both in ambient oxygen and moderate hypoxia (1% O_2), without loss of viability, as demonstrated by formazan-based viability assay and histomorphologic evaluation of fragments. Apoptosis rates of tumor cells were investigated in fragments maintained under normoxia or chronic hypoxia for four days using antibodies against cleaved caspase 3 and showed no significant differences between normoxia (5.3±2.6%) or hypoxia (5.7±2.7%, P=0.92). Cisplatin treated fragments served as a positive control. As a conclusion the novel *ex vivo* NSCLC fragment model allows the investigation of hypoxic adaptation in short time culture. It combines the advantage of a 3D culture and the use of primary NSCLC tissue instead of cell lines.

E296
Expression of claudins 1, 2, 3, 4, 5 and 7 in lung cancer
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Claudins are tight junction proteins localized on the apical side of epithelial cells. Changes in their expression cause dysfunction in cell junctions which can lead to uncontrolled proliferation, invasiveness and loss of cohesion in cancer. The expression of claudins 1, 2, 3, 4, 5 and 7 was investigated by immunohistochemistry in 313 patients with different histologic types of lung cancer, carcinoid tumors and in pulmonary metastases of other cancers. The expression was graded from 0 (negative) to 4 (very strong).
The immunoreactivity of claudin 7 was strong or very strong in all types of carcinoma. The expression of claudins 3 and 4 was stronger in adenocarcinoma than in squamous cell carcinoma ($p<0.001$ for both). Likewise, the expression of claudins 3 and 4 was stronger in small cell carcinoma compared to large cell carcinoma ($p=0.001$ and $p= 0.035$, respectively) whereas the expression of claudin 2 was weaker in small cell carcinoma compared to large cell carcinoma ($p=0.031$). The immunoreactivity of claudin 1 was negative in carcinoid tumors and weak in pulmonary metastases; in all other lung cancer types it was moderate or strong. Claudin 5 immunoreactivity was weak or negative in all lung cancers. Cases with low claudin 4 expression tended to have positive pN-status ($p=0.032$) whereas claudin 7 expression associated with a small size of tumors ($p=0.027$). Claudin 1, 4 and 7 expression showed a weak association with pack-years.

Claudins are variably expressed in various types of lung tumors and may, in a restricted sense, be used as differentiation markers. Low claudin 4 expression was associated with a positive pN status suggesting that its downregulation might associate with a greater metastatic tendency of the tumors.

E297

Importance of clusterin expression at tissue level for the prognosis of nonsmall cells lung cancer

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Lung cancer is the leading cause of death from malignancy. Although TNM stage is the most significant prognostic parameter, the variability of survival within staging groups require additional parameters predicting outcomes. Clusterin is a protein widely distributed in mammalian tissues, which causes growth inhibition and death. Eighty three patients with definitive diagnosis of primitive adenocarcinoma of the lung were considered for this study. Cytoplasmatic clusterin immunostaining was present in 63 patients (75,9%). In order to compare two homogeneous groups, we consider a clusterin staining index of at least 40 as definitively positive. A significant association was noted between clusterin Staining Index and gender (p=0.024), histological grading (p=0.002), presence of bronchoalveolar carcinoma (BAC) component (p=0.001) and pathologic stage (p=0.016). Clusterin Staining Index showed a highly significant linear correlation with the percentage of BAC component (Pearson's coefficient 0.726; R^2=0.527; p=0.001). The clusterin Staining Index resulted a significant prognostic factor affecting either Overall Survival and Disease-Free Survival, as well as pathologic stage and tumor grading. We also stratified the analysis of survival by pathologic stage and the clusterin Staining Index remained a significant prognostic factor even at early stages (IA+IB). Furthermore, at the multivariate analysis, Clusterin Staining Index resulted the only one independent factor affecting recurrence (p = 0.048) and had an association with an improved overall survival that approached statistical significance (p= 0.052), suggesting that clusterin may be a biomarker for prognosis in patients with NSCLC.

E298

Proteomic analysis of human small cell lung cancer tissues

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Introduction: There has been few published proteomic data about human small cell lung cancer.
The goal of this study was to isolate and identify differentially expressed proteins between small cell lung cancer and normal respiratory epithelial tissues by two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).
Methods: A total of 6 small cell lung cancer samples and 6 normal respiratory epitheliums were analyzed by 2-DE. 2-DE gels were silver stained and analyzed using the PDQuest analysis software (BioRad). Tumor specific spots were detected and identified by consecutive MALDI-TOF-MS and database search.
Results: In total, 64 spots of 2-DE showed significant quantitative differences in protein expression. From analyzing the 64 spots, we were able to identify a total of 7 proteins and protein isoforms, significantly up-regulated in small cell lung cancer tissues. Laminin B1, Ubiquitin-conjugating enzyme E2-25K, Ubiquitin carboxyl-terminal esterase L1, gamma-actin, Tubulysin A-1B, Coactosin-like protein, and Carbonic anhydrase 1 were upregulated in human small cell lung cancer tissue.
Conclusions: We report the valuable proteomic data for identification of differentially expressed proteins involved in the pathogenesis of small cell lung cancer, establishing human small cell lung cancer proteome database and screening molecular markers to further studies.

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p53 mutations do not predict response to paclitaxel in metastatic nonsmall cell lung carcinoma

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Introduction: The authors previously demonstrated thatresponse to paclitaxel and concurrent radiation was not affected by p53 mutations in nonsmall cell lung carcinoma (NSCLC). We sought to determine whether p53 mutations affect response to paclitaxel alone in patients with metastatic NSCLC.
Methods: Twenty-nine patients with metastatic NSCLC who participated in Clinical Center Kragujevac- Oncology Group protocols utilizing single-agent weekly paclitaxel had tumor tissue that was adequate for p53 analysis. Tumor tissue was evaluated for p53 gene mutations in exone 5 through 8 by single-strand conformation polymorphism analysis. Mutations were confirmed by direct sequencing af altered mobility polimerase chain reaction products.
Results: Mutations in p53 were found in 8 of 29 patients (27,6%). The response rates of 67% for patients with tumors with p53 mutations and 49% for patients with wild-type p53 do not differ significantly (p=0,283). The 1-year survival rates for patients with and without p53 mutation after treatment with weekly paclitaxel were 65% (95% confidence interval [CI], 34-100%) and 51% (95% CI, 31-89%), respectively.
Conclusions: p53 mutations do not adversely affect response to paclitaxel as a single agent in metastatic NSCLC. These results provide clinical support for in vitro observations that paclitaxel can bypass mutant p53 and lead to tumor cell death by alternate pathway(s).

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Changes and significance of $CD4^+CD25^+$ T cells in patients with lung cancer

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Objective: To investigate the changes and significance of $CD4^+CD25^+$ T cells (regulatory T cells) in the peripheral blood of patients with lung cancer.
Methods: sixtynewly inpatients with definite lung cancer were enrolled as the study group, twentypatients with benign pulmonary diseases and twenty healthy volunteers were selected for control groups. flow cytometry was used to detect the percentage of $CD4^+CD25^+$ T lymphocytes present in total $CD4^+$ T cells in the peripheral blood, and electrochemiluminescence immune assay (ECLIA) was used to detect the levels of CEA, CYFRA21-1 and NSE in serum of all subjects.
Results: The percentages of $CD4^+CD25^+$ T cells in the peripheral blood of patients with lung cancer (20.93%±4.47%) was significantly higher than that in benign pulmonary diseases group (15.14%±4.79%) and healthy control group (15.42%±3.44%). There was no significant difference in the change of the percentage of $CD4^+CD25^+$ T cells in the peripheral blood in patients with early stage of lung cancer compared to the late stage patients (P=0.348), and also no significant difference between the adenocarcinoma and squamous cell carcinoma patients (P=0.582). The patients who had lower the percentage of $CD4^+CD25^+$ T cells in the peripheral blood seem to have better prognosis (P=0.02). The diagnostic value as a tumor marker of the percentage of $CD4^+CD25^+$ T cells in the peripheral blood was similar to CEA and CYFRA21-1.
Conclusion: There are an increased proportion of regulatory T cells in patients with lung cancer, and manipulation of this subpopulation could be an important component of cancer immunotherapy.

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Does expression of excision repair cross-complementation group 1 correlate between primary nonsmall cells lung cancer tumor and metastases?

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Background: Chemotherapy is the cornerstone of the treatment of metastatic lung cancer. Excision repair cross-complementation group 1 (ERCC1) is a protein involved in the DNA repairing process. It is responsible of a resistance to platinium-based chemotherapy while removing DNA-adducts. Over-expression of ERCC1 is associated with resistance to Cisplatin. Little is known about the biology of this protein. In particular there is no data about the conservation of its expression during the metastatic process.
Aims and objectives: The aim of this study was to determinate if expression of ERCC 1 is correlate between primary non small cells lung cancer (NSCLC) tumors and matched metastasis.
Methods: Twenty-eight patients with metastatic NSCLC were included. Primary tumor and metastases were operated or biopsied for each patient. The expression of ERCC 1 in each tumor was evaluated with immunohistochemistry.
Results: ERCC 1 was over-expressed in 9 (39%) primary tumors and 16 (64%) metastases. Expression ERCC 1 was discordant between primary tumors and metastases in 9 (39%) pairs of tumors. Expression ERCC 1 is neither correlated (Spearman's correlation coefficient = 0.34, p = 0.116) nor concordant (Kendall's tau B test = 0.34 p = 0.06) between primary tumor and metastases in NSCLC.
Conclusions: ERCC 1 expression is not correlated between primary tumor and metastases in NSCLC. Decision of using Cisplatin, or not, can't be based only on the expression of ERCC1 in the primary tumor.