## 160. Progress in pathology of lung cancer

**P1477**
**LSC 2011 Abstract: Molecular interplay between inflammation and occurrence of proliferation: Role of cadmium**
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**Introduction:** Cadmium is one of the inflammation-related xenobiotics with potent carcinogenicity. The mechanism between inflammation and cell proliferation due to chronic cadmium exposure has not been studied yet (Lau AT, *et al*., *Toxicol Appl Pharmacol* 2006).
**Objectives:** The present study was undertaken to determine molecular mechanism of inflammation linked cell proliferation due to cadmium exposure in mice and lung cancer cell line.
**Methods:** Swiss albino mice and A549 cell line were chosen for experiments. Levels of different cytokines, expression level of cell cycle regulatory proteins estimated by ELISA, western blot and immunoprecipitation. Other techniques used scanning electron microscopy, histopathology and Cytotoxic assay, Cell cycle analysis, DNA fragmentation assay and RT-PCR experiments.
**Results:** Prolonged exposure of low concentration of cadmium resulted in up regulation of proinflammatory cytokines and cell cycle regulatory molecules both in vivo and in vitro (Waalkes MP *et al*., *Toxicol Sci* 1999). We found that cadmium induced upregulation of epidermal growth factor receptor (EGFR) along with different proinflammatory cytokines. The major distinct feature of EGFR expression is promoting inflammatory responses along with cell proliferation.
**Conclusions:** These data provide a new insight into the relation between chronic inflammation and cell proliferation in vivo due to cadmium toxicity.

**P1478**
**LSC 2011 Abstract: Cigarette smoke-induced inflammation promotes melanoma cell metastasis in lung parenchyma**
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It is only during the last decade that clear evidence has been obtained that inflammation plays a critical role in different stages of tumor development, including initiation, promotion, malignant conversion, invasion and metastasis. There is increasing evidence that an inflammatory microenvironment is an essential component of all tumors (Psaila and Lyden, Nat Rev Cancer, 2009 Apr;9(4):285-93). In the present study, we assessed *in vivo* the impact of cigarette smoke (CS) on the tumor cell extravasation in lungs after tail vein injection of B16F10 melanoma cells. We first characterized airway inflammation obtained after smoke exposure (reference cigarettes 3R4F) for varied time periods (1, 2, 4, 8 and 12 weeks). Smoke exposure was performed 5 days a week. Neutrophils, alveolar macrophages, interstitial macrophages, dendritic cells, T cells and natural killer T (NKT) cells, were characterized in lung tissues of mice exposed to CS and AIR using flow cytometry. *In Vitro*, the direct effect of cigarette smoke extract (CSE) on proliferation of B16F10 melanoma cells was determined for 1 to 5 days. *In vivo,* mice exposed for 2 weeks to cigarette smoke or air were injected with B16F10 melanoma cells in the tail vein. After 3 weeks, hematoxylin-eosin staining allowed quantification of lung metastasis (tumor area/total lung area). An increase of metastasis and implantation site in lungs was observed in CS exposed group. Conceivably, CS constituents significantly promote extravasation of melanoma cells in lung tissues. The mechanism or signaling pathway responsible for this dissemination needs to be further investigated.

**P1479**
**SK-216, an inhibitor of plasminogen activator inhibitor-1, limits tumor growth and lung metastasis formation probably through the reduction of tumor angiogenesis**
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**Introduction:** Plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of plasminogen activators, is known to be involved in tumor progression.
**Objectives:** To investigate whether a PAI-1 inhibitor, SK-216, can limit tumor growth and lung metastasis formation.
**Methods:** C57BL/6 mice were subcutaneously inoculated with Lewis lung carcinoma (LLC) cells and tumor volume ($mm^3$) was measured twice a week until 2 weeks after the inoculation. The numbers of lung tumors were also counted 21 days after the injection of LLC cells through the tail vein. The mice were given either water or SK-216 (500 p.p.m.) in drinking water. In addition, the sections of tumor were stained with CD31 antibody, and the number of CD31-positive vessels was counted in three random microscopic fields per section.
**Results:** The volumes of subcutaneous tumors 14 days after the inoculation of LLC cells were significantly smaller in SK-216-treated group than those in control group (mean ± SD; 1566±515.9 and 2354.3±559.3, respectively; p=0.018). The numbers of lung surface tumors were significantly lower in SK-216-treated group than those in control group (3.3±4.1 and 12.1±7.3, respectively; p=0.02). The numbers of CD31-positive vessels in subcutaneous or lung tumor sections were significantly lower in SK-216-treated group than those in control group (subcutaneous tumor; 60.5±15.9/field and 78.7±21.8/field, respectively, p=0.004: lung tumor; 24.0±7.9/field and 44.8±4.4/field, respectively, p=0.002).
**Conclusion:** These results suggest that SK-216 limits tumor growth and lung metastasis formation probably through the reduction of tumor angiogenesis.

**P1480**
**The long non-coding MALAT-1 RNA indicates a poor prognosis in NSCLC and induces migration and tumor growth**
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**Introduction:** The functions of large non-coding RNAs (ncRNA) have remained elusive in many cases. MALAT-1 (Metastasis-Associated-in-Lung-Adenocarcinoma-Transcript-1) is a ncRNA, that is highly expressed in several tumor types.
**Methods:** Overexpression and RNA interference (RNAi) approaches were used for the analysis of the biological functions of MALAT-1 RNA. Tumor growth was studied in nude mice. For prognostic analysis MALAT-1 RNA was detected on paraffin embedded lung cancer tissue probes (n=352) using in-situ hybridization.
**Results:** MALAT-1 was highly expressed in several human non-small cell lung cancer cell lines. MALAT-1 expression was regulated by an endogenous negative feedback loop. In A549 NSCLC cells, RNAi mediated suppression of MALAT-1 RNA suppressed migration and clonogenic growth. Forced expression of MALAT-1 in NIH 3T3 cells significantly increased migration. Upon injection into nude mice, NSCLC xenografts with decreased MALAT-1 expression were impaired in tumor formation and growth. In-situ hybridization on paraffin embedded lung cancer tissue probes revealed that high MALAT-1 RNA expression in squamous cell carcinoma of the lung was associated with a poor prognosis.
**Conclusions:** These data indicate that MALAT-1 expression levels are associated with patient survival and identify tumor promoting functions of MALAT-1.

**P1481**
**Expression of miR-126 and miR-126* in primary tumors and metastasis of adenocarcinoma and squamous carcinoma of the lung**
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MicroRNAs are a family of small-non coding RNAs that negatively regulate gene expression at post-transcriptional level. Their expression has been proved to be associated to cancer but several aspects of this association remain elusive.

miR-126 and miR-126* are processed from the same precursor microRNA and are down-regulated in non-small cell lung cancer (NSCLC). However, information is lacking regarding their involvement in metastatic potential and differential expression in adenocarcinoma and squamous carcinoma of the lung. In this study, these questions were approached by comparing microRNA levels in 37 samples of matched adjacent lung parenchyma, primary tumour and lymph node metastasis (when present). Each kind of tissue was isolated by laser microdissection and, after RNA extraction, the two microRNAs were quantified by RT-qPCR. Both miR-126 and miR-126* were found to be down-regulated in primary tumour cells comparatively to matched normal tissue (p-value<0.001). On the contrary, no significant differences were found between primary tumour and lymph node metastasis cells or between tumor cells of metastatic and non-metastatic samples. Although overall expression was similar in adenocarcinoma and squamous carcinoma, in non-metastatic tumors miR-126 and miR-126* expression was lower in squamous carcinoma (p-value=0.037 and p-value=0.035, respectively). These results suggest that these microRNAs might act as tumor suppressors but are not involved in the metastatic process. In addition, their expression seems to relate to the type of NSCLC in non-metastatic tumors and might therefore be useful for their characterization.

**P1482**

**Expression and significance of VE-cadherin and E-cadherin in non-small cell lung cancer**

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**Objective:** The aims of our study were 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 these expression levels with the cliniopathological characteristics of NSCLC.
**Methods:** The expression levels of VE-cadherin and E-cadherin were examined by immunohistochemistry in NSCLC tissues from 39 patients and in 30 adjacent non-neoplastic tissues that were at least 5 cm away from the tumor tissues.
**Results:** The positive rates of VE-cadherin and E-cadherin in NSCLC were 51.3% (20/39) and 43.6% (17/39),respectively. The positive rate of E-cadherin in NSCLC was lower than in adjacent non-neoplastic tissues (73.3,22/30, 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).
**Conclusions:** We demonstrated the aberrant expression of VE-cadherin in NSCLC and the downregulation of E-cadherin expression. Both of these proteins are associated with lymph node metastasis. These results indicate that both of these proteins may take part in the growth and metastasis of NSCLC and thus may be therapeutic targets for the treatment of NSCLC.

**P1483**

**Transcriptional regulation of the human osteopontin promoter in non-small cell lung cancer**

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**Background:** Elevated osteopontin (OPN) transcription often correlates with increased metastatic potential of transformed cells, and OPN has been shown to enhance metastatic ability. We hypothesized that transcription determines OPN expression, tried to find out response element resion in the DNA sequence element and suppression molecules.
**Methods:** We investigated the transcriptional regulation of OPN in non-small celll lung cancer cell lines. These elements were treated with pp2 (src tyrosine kinase inhibitor), FTI (Farnesyl transferase inhibitor), and 2BP (2 bromo palmitate) in order that confirm suppress transcriptional expression.
**Results:** Deletion and mutagenesis analyses of the OPN promoter region identified a proximal promoter element (-123 to -89 relative to the transcription initiation site) that is essential for maintaining high level of OPN expression in the tumor cells. Proximal promoter elements (-123 and -89) were treated with pp2, FTI and 2BP, whether if transcriptional expression could be suppressed by these molecules. In case of FTI, OPN expression was suppressed, but statistical significance wasn't meaningful.
**Conclusion:** The proximal promoter element (-123 to -89) was essential for maintaining high level of OPN expression in non-small cell lung cancer cell lines. Further investigation for searching material to suppress OPN expression is needed.

**P1484**

**PD-L1 is a potential negative prognostic factor for non-small cell lung cancer: A 5-year-follow-up study**

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**Objectives:** We found programmed death-1-ligand 1 (PD-L1) was a negative prognosis factor for patients with non-small cell lung cancer (NSCLC) for 3 years follow-up in our previous study. In order to further confirm our study result, we followed up 5 years and picked up some different patients with NSCLC underwent surgical resection in the same period in our hospital.
**Methods:** PD-L1 expression in 120 NSCLC tissue specimens and 10 benign control samples embedded with wax were retrospectively detected by immunohistochemistry.
**Results:** No PD-L1 expression was found in 10 benign controls, while 57.5% tissue specimens of NSCLC had PD-L1 expression. There was no relationship between PD-L1 expression and patient age, gender, histopathological type. However, PD-L1 expression was significantly correlated to the degree of tumor cell differentiation, local lymph node metastasis, stage of tumor-node-metastasis (TNM) and survival time of patients. Poor tumor cell differentiation, local lymph node metastasis, and advanced stage of TNM were related with higher PD-L1 expression. PD-L1 negative NSCLC patients had longer overall 5-year survival time compared with PD-L1 positive ones ($P<0.0001$). PD-L1 status was a significant independent prognostic factor of NSCLC ($\chi^2 = 18.153$, RR = 2.946, $P<0.001$).
**Conclusion:** Up-regulated PD-L1 expression in NSCLC is related with the degree of tumor cell differentiation, local lymph node metastasis and stage of TNM. PD-L1 expression is an important risk factor associated with poor prognosis of NSCLC.

**P1485**

**Expression of survivin and p53 protein in pulmonary tumours**

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Survivin is a member of the inhibitor of apoptotic gene family, which has been implicated in both the inhibition of apoptosis and mitosis regulation. p53 is one of the tumour suppressor genes; prevents tumour formation through cell cycle blocking and eliminates damaged cells via activation of apoptosis. To investigate the possible regulation of survivin by p 53 we examined the expression of both proteins in 67 patients with diagnosed lung cancer using immohistochemical visualisation. Survivin was predominantly expressed in both nucleus and cytoplasm, whereas p53 was expressed in nucleus. There was negative correlation between survivin and p53 expression. Decreased intensity of expression and less number of positive cells for survivin in small cell lung cancer in comparison to other lung cancer types was detected. There was no significant difference in intensity of expression and in number of positive cells for p53 between small cell and non-small cell lung cancer types.
This work was supported by project "Center of excellency for research in personalized therapy (CEVYPET) co-financed from EC sources and European Regional Development Fund.

**P1486**

**Inhibitor of DNA-binding (Id) – 1 and 3 proteins overexpression has impact on prognosis of lung adenocarcinoma**

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The inhibitor of DNA–binding (Id) are involved in cell cycle regulation, apoptosis and angiogenesis. Many authors suggest participation of Id genes in development and progression of large number of human cancer, but their role in lung cancer has not been evaluated.
**Objectives:** To evaluate Id 1, 2 and 3 expressions in lung adenocarcinoma and relation to stage and survival.
**Methods:** IDs were quantified in tumoral and non-tumoral tissues from 43 patients who underwent lobectomy for lung adenocarcinoma. Their preoperative clinical stages were $T_{1-3}N_{0-1}M0$ and the mean follow-up was 26.7 months. Immunohistochemistry was applied to analyze the intensity of Ids expression in nucleus and cytoplasm (quantitative scores performed in 500 cells). The impact of these markers was tested on follow-up until death from recurrent lung cancer.
**Results:** Distinct profile of Ids expression was observed between tumoral and the matched adjacent nonmalignant tissue. Ids were significantly more expressed in tumoral cells. Patients whose tumor cells expressed lower scores of cytoplasm Id3 tended to present better long-term survival (p=0.05). The Cox model controlled for

lymph node stage showed up Id 1 and 3 were associated with survival. A median score for nuclear Id1 in adjacent non-tumoral cells (> 49.7) and for tumoral cytoplasmic Id3 (> 394.9) identified patients with worse prognosis and higher risk of death (2.02 and 1.97, respectively).
**Conclusion:** These findings suggest Ids 1 and 3 may provide additional information about tumor behavior. Further studies are required to determine whether Ids 1 and 3 expressions might be useful prognostic markers in non small cells lung cancer.
Support: FAPESP

**P1487**
**Adenovirus-mediated AP-2α down-regulates MnSOD expression in lung cancer A549 cells and its molecular mechanism**
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**Objective:** MnSOD is potential therapy target for lung cancer and it is important to control MnSOD aberrant expression in lung cancer cells. In this study, we investigate the effect of AP-2α activation on MnSOD expression in lung cancer cell line and provide experimental evidence on the applications of AP-2 for lung cancer therapy.
**Methods:** A549 cells were transfected with the Adenovirus-AP-2α construct (Ad-AP-2α) at multiplicities of infection (MOI) of 0, 10, 30, 50 MOI or with the adenovirus-lacZ reporter gene construct (Ad-lacZ) at 50 MOI. A549 cells were transfected for 0, 6, 12, 24, 48 hrs with Ad-AP-2α at 50 MOI and also were transfected for 24hrs with the dominant-negative mutant AP-2α construct with liposome-mediated method. The time- and dose-dependent effects of AP-2 on MnSOD expression were detected by RT-PCR and Western blot.
**Results:** (1) With increasing Ad-AP-2 titers, the expression of MnSOD is decreased. (2) Transfected with 50 MOI Ad-AP-2α and measured expression of MnSOD at 0, 6, 12, 24 and 48 hr, we observed a concomitant decreasing in MnSOD expression. (3) Compared with the controls, the expression of MnSOD was declined after transferring the dominant-negative mutant AP-2α construct into lung cancer (A549) cells.
**Conclusions:** The results suggest that AP-2 represses MnSOD expression in lung cancer cells and down-regulation of MnSOD may be related to DNA binding domain of AP-2α.

**P1488**
**Telomerase (h-TERT) and targeting EGFR in non small cell lung carcinoma: A combined immunohistochemistry and chromogenic in situ hybridization study based on tissue microarrays**
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**Purpose:** Our aim was the evaluation of EGFR gene and protein alterations in NSCLC and the potential role of telomerase in the regulation of its expression.
**Methods:** Using tissue microarray technology, forty (n=40) paraffin embedded histologically confirmed primary NSCLCs were cored twice at a diameter of 1mm and re-embedded into a recipient block. Immunohistochemistry was performed by the use of monoclonal antibodies anti-EGFR (31G7), and anti-telomerase/h-TERT (44F12). Also, a Chromogenic in situ hybridization protocol was applied based on the use of EGFR gene and chromosome 7 centromeric probes. Computerized Image Analysis was performed for the evaluation of immunohistochemistry results.
**Results:** EGFR overexpression was observed in 23/40 (57.5%) cases correlating to stage (p=0.001) and histological type (p=0.04). Telomerase was overexpressed in all examined cases (high and moderate levels) correlating to stage (p=0.001). A significant value of concordance (kappa=0.686, 0.677-0.695) was assessed comparing telomerase and EGFR protein expression. EGFR gene amplification was identified in 2/40 (5%) cases associating to histological type (p=0.027) and chromosome 7 aneuploidy in 7/40 (17.5%) cases.
**Conclusions:** NSCLC is characterized by rare cases of EGFR gene amplification and this genetic event maybe affect the efficacy of targeted therapeutic strategies based on monoclonal antibodies. Also, the strong concordance between EGFR and telomerase overexpression demonstrates that the enzyme is potentially involved in the growth-controlling gene expression.

**P1489**
**Inhibition of B7-H4 gene expression by RNA interference (RNAi) in lung cancer A549 cell line**
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**Background:** B7-H4, a member of the B7 family, is involved in the regulation of antigen-specific immune responses. Here we addressed its expression in non-small-cell lung cancer (NSCLC) pathology and correlation to the number of $CD3^+$ tumor infiltrating T-lymphocytes in invasive carcinomas. We also observe the effects of B7-H4 gene expression on cell proliferation and migration in the human NSCLC cell line.
**Methods:** B7-H4 expression was evaluated by immunohistochemistry in 102 patients with NSCLC who underwent surgical tumor resection. Expression data was correlated with clinicopathologic features and with the number of tumor-infiltrating T cells. B7-H4 shRNA was cloned into pGCsi-U6/Neo plasmid and the product was transfected into A549 cells with Lipofectamine 2000.
**Results:** B7-H4 is transcripted in three cell lines. In tumor tissues, expression of B7-H4 is found both in the cell membrane and in the cytoplasm. B7-H4 transfection vector-pGCshB7-H4 was successfully constructed. After transfected with pGC shB7-H4, the expression of B7-H4mRNA in A549 cells was obviously decreased with an increased cell proliferation observably. Compare to cell lines without treatment, the proliferation of cell lines cultivated with decreased B7-H4 gene was increased, the cell cycle was blocked in the $G_2$ as well, invasion and mobility abilities of cells in vitro were added.
**Conclusion:** Our observations also suggest that the B7-H4 gene is associated with A549 cell proliferation, migration and cell cycle distribution. RNAi recombinent of B7-H4 gene could effectively inhibit the expression of B7-H4 mRNA in A549 cells.

**P1490**
**Novel functions of stanniocalcin-1 (STC1) through uncoupling protein 2 (UCP2) up-regulation; promoting survival of cancer cells under oxidative stress and inducing the uncoupling respiration (Warburg effect)**
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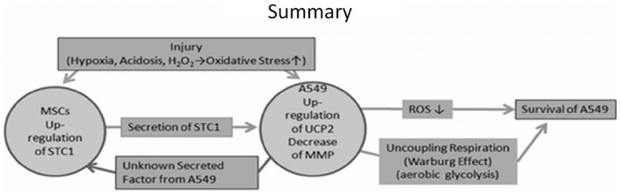
We have demonstrated that multipotent stromal cells (MSCs) promote cell survival though upregulation and secretion of stanniocalcin-1 (STC1) (Stem Cells 2009; 27: 670-81). This study demonstrates that MSC derived STC1 promotes survival of lung cancer A549 cells through the uncoupling of oxidative phosphorylation, the reduction of intracellular reactive oxygen species (ROS) and concomitant shift towards a more glycolytic and more oxygen consumptive metabolic profile (known as the Uncoupling Respiration). MSC-derived STC1 upregulated uncoupling protein 2 (UCP2) in injured A549s in an STC1 dependent manner. Knock down of UCP2 reduced the ability of MSCs to reduce cell death in the A549 population.




Our data suggest that MSCs promote cell survival by regulating the uncoupling respiration in an STC1 dependent manner. Furthermore, STC1 may provide promising avenues for treatment of reactive oxygen species and metabolic disorders.

**P1491**
**TNF neutralization ameliorates urethane-induced lung carcinogenesis**
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**Background:** Previous work has identified protumorigenic functions of tumor necrosis factor (TNF) in the lungs.
**Aim:** To preclinically investigate the therapeutic potential of TNF blockade against lung carcinogenesis.
**Methods:** Long-term studies: Balb/c mice received four weekly doses of intraperitoneal urethane (1g/kg), followed by twice-weekly sTNFR:Fc (etanercept; Amgen-Wyeth; 10 mg/kg) during weeks 0-4 (early), 20-24 (late), or 0-32 (continuous) post-urethane. End-points were lung tumor number (ν) and diameter (δ) at 32 weeks. Short-term studies: Balb/c mice received urethane or saline followed by twice-weekly sTNFR:Fc or saline. End-point was BAL inflammatory cells and mediators at 7 days.
**Results:** Compared with controls ($\nu=16.2\pm1.6$; $\delta=1.01\pm0.07$mm), early sTNFR:Fc-treated mice had fewer lung tumors of equal size ($\nu=10.0\pm1.3$, $P<0.05$; $\delta=1.07\pm0.03$mm, $P>0.05$), but late sTNFR:Fc-treated mice had equal numbers of smaller lung tumors ($\nu=14.1\pm1.2$, $P>0.05$; $\delta=0.71\pm0.07$mm, $P<0.01$). Continuous sTNFR:Fc resulted in reduced lung tumor number and size ($\nu=9.3\pm1.0$, $P<0.05$; $\delta=0.76\pm0.03$mm, $P<0.05$). sTNFR:Fc-treated mice had fewer macrophages, but higher IFN-γ and IL-10 levels in BAL; tumors from these mice showed slower proliferation and angiogenesis. In short-term studies, sTNFR:Fc inhibited urethane-induced macrophage influx and TNF expression, but enhanced IFN-γ and IL-10 expression in BAL. A possible source of IFN-γ and IL-10 in sTNFR:Fc-treated mice were macrophages, which responded to sTNFR:Fc by enhanced IFN-γ and IL-10 expression.

**Conclusion:** TNF blockade halts lung tumorigenesis in mice, and may be useful in lung cancer chemoprevention.
Financial support: Hellenic Thoracic Society, Thorax Foundation.

**P1492**

**Krebs von den Lungen-6 (KL-6) is a prognostic biomarker in patients with surgically resected non-small cell lung cancer**

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**Background:** By immunizing mice with a lung adenocarcinoma cell line, we previously established a murine IgG1 monoclonal antibody that recognizes a sialylated sugar chain designated Krebs von den Lungen-6 (KL-6). KL-6 is a high–molecular-weight glycoprotein classified as a human MUC1 mucin. The aim of this study was to determine whether KL-6 expression in tumors correlates with circulating KL-6 levels and whether circulating KL-6 has any prognostic value in patients with surgically resected non-small cell lung cancer (NSCLC).
**Method:** Immunohistochemical analysis of KL-6 expression was performed on 103 NSCLC tissues, and its associations with serum KL-6 levels and survival were examined. We also evaluated whether KL-6 expression patterns and/or serum KL-6 levels could predict prognosis in these NSCLC patients.
**Result:** Immunohistochemical analysis of KL-6 in NSCLC tissues showed that a depolarized KL-6 expression pattern was associated with a high level of circulating KL-6 and a poor prognosis in NSCLC patients who underwent curative surgery. Furthermore, a high circulating KL-6 level was associated with both poorer progression-free survival (PFS) and overall survival (OS), and multivariate analyses confirmed its independent prognostic value for both PFS and OS ($p$=0.041 and 0.023, respectively).
**Conclusion:** Our data suggest that preoperative serum KL-6 level reflects KL-6 expression patterns in NSCLC tissue, and can serve as a useful prognostic biomarker in NSCLC patients who undergo curative surgery.

**P1493**

**Potential therapeutic significance of CIK cells in gefitinib resistant NSCLC with EGFR mutations**

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The gefitinib resistant limits its efficacy in the treatment of non-small cell lung cancer (NSCLC), thus, we tried to evaluate the drug-resistance reversal to gefitinb by the cytokine induced killer (CIK) cells. The gefitinib resistant cell line PC-9/GR was developed from a NSCLC cell line PC-9 harboring EGFR E746-A750 deletion by gefitinib selection. The effect of CIK cells alone or combination with gefitinib on PC-9/GR was determined by the cytotoxicity in vitro as well as growth inhibition of tumors in the nude mice. The immune cell subsets and cytokines expression before and after CIK cells infusion in six patients with tumors were also analyzed. An additional EGFR T790M mutation was obtained in the PC-9/GR with a resistance index of 100.16 to gefitinib. The induction of G0/G1 arrest and apoptosis and inhibition of activation of p-EGFR, p-ERK and p-AKT signaling were significantly attenuated in PC-9/GR following treated with gefitinib. Interestingly, an evident cytolytic activity was presented in the PC-9 and PC-9/GR treated with CIK cells in vitro and vivo, however, there was no significant difference in the cytolytic activity of CIK cells against PC-9 and PC-9/GR targets. In addition, a synergistic cytotoxicity was obtained by the combination CIK cells and geifitinb in PC-9/GR through the inhibition of p-ERK and p-AKT activity by CIK cells. Finally, CIK cells infusion could significantly increase the frequency and function of NK cells and decrease the frequency of Treg cells and level of TGF-β in patients with tumors. In conclusion, CIK cells have a strong cytotoxicity to PC-9/GR and a clinical immune modulation, thus it maybe a favorable treatment of NSCLC with EGFR mutations.

**P1494**

**Expression of immunohistochemical markers chromogranin A, Ki-67, CD99, EGFR in resected pulmonary neuroendocrine tumors**

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**Aim:** To evaluate the immunohistochemical expression of the markers Chromogranin A, Ki-67, CD99, EGFR in resected pulmonary neuroendocrine tumors (pNET).
**Methods:** Surgically resected specimen from 49 patients (men-31, 63.3%, women–18, 36.7%) with pNETs were studied: typical carcinod (TC)-20 (40.8%), atypical carcinoid (AC)-7 (14.3%), small cell lung carcinoma (SCLC)-20 (40.8%), and large cell neuroendocrine carcinoma (LCNEC)-2 (4.1%). The histological type, pTNM stage and the immunohistochemical expression of Chromogranin A, Ki-67, CD99 and EGFR was evaluated.
**Results:** The distribution in stages was as follows: TC & AC-I stage-20 (74.1%), II-5 (18.5%), IIIA-2 (7.4%); SCLC-IB-3 (15%), II-4 (20%), IIIA-9 (45%), IIIB & IV-4 (20%); LCNEC were in II stage. Neuroendocrine differentiation proved in all tumors by *Chromogranin A*. The mean values of *Ki-67* were: TC-1.7%, AC–11.7%, SCLC–75.8%, LCNEC–63.5% (p=0.001). *CD99* expression was proved in 22 (44.9%) of the cases (in 9 – it was expressed in 100% of the tumor cells). 11 (50%) of them were carcinoid tumors, 9 (40%) – SCLC, 2– LCNEC. 40% of all TC&AC express CD99. 45% of SCLC express CD99. In AC CD99 positive cases, Ki-67 was 22.3%, and in AC CD99 negative cases Ki-67 was 3.75% (p=0.008). From 49 cases in 10 (19.7%) an *EGFR* expression was observed – TC&AC-4 (20.4% of all carcinoids), SCLC-5 (25% of all SCLC), and LCNEC-1. In 3 of the carcinoid tumors and in 4 of the SCLC 100% of the tumor cells express the marker while in the remaining tumors it was clonal.
**Conclusion:** The CD99 expression correlates statisticaly significant with high Ki-67 index in AC. EGFR expressing NETs may be candidates for treatment with tyrosine – kinase inhibitors.