

MONDAY, SEPTEMBER 17TH 2007

---

## 195. Biology of thoracic malignancies

---

**E1810****Formation of tumor fragment spheroids (TFS) from lung cancer, *in vitro* model as like *in vivo***

K.-U. Kim<sup>1</sup>, Y.-K. Kim<sup>1</sup>, Y.-M. Lee<sup>1</sup>, D.-J. Na<sup>2</sup>, S. Uh<sup>1</sup>. <sup>1</sup>*Internal Medicine, Division of Respiratory and Allergy, Soonchunhyang University Hospital, Seoul, Korea, Republic of;* <sup>2</sup>*Internal Medicine, Division of Respiratory and Allergy, Eulji University Hospital, Daejeon, Korea, Republic of*

**Introduction:** Tumor fragment spheroids, which got from parental tissue, have more similar to *in vivo* than cell line research, because they have well preserved cancer cells, fibroblasts, even tumor associated macrophages. Our objective is making spheroids similar to *in vivo* tissue at the *in vitro* condition. We got 15 tissues from lung cancer patients. The tissue which had been cultured got sample serially and compared their characteristics to original tissue.

**Methods:** Fifteen freshly resected tumors which were obtained during surgery were diced finely and cultured agar coated plate with culture in DME media. Tissues from culture were got serially, fixed to formalin and embedded in paraffin. Sectioned tissue were stained hematoxylin and eosin, and masson-trichrome. To detect of tumor characteristics, including tumor cells and stroma, immunohistochemical stain with primary antibody for cytokeratin, CEA, TTF-1, and CD-68 were done. Proliferation of tumor and stromal cell were identified by double immunohistochemical stain of Ki67 and cytokeratin.

**Results:** 11 cases of lung cancer tissue had been formed spheroids well. Adenocarcinoma had been noticed round shapes within 3 days after culture, and made spheroids during 7 to 10 days. After 2 weeks, most of the fragments from adenocarcinoma had been made tumor fragment spheroids. Their shapes were maintained 4 weeks. Immunohistochemical stain for cytokeratin, CD-68, and TTF-1, tumor fragment spheroids from serial culture and original tissue had not different. Ki67 had been stained both tumor fragment spheroids and original tissue.

**Conclusion:** Tumor Fragment Spheroids will be another promise to study of tumor researches *in vitro* as like *in vivo*.

**E1811****Clone and initial identification of differentially expressed genes of human small-cell lung cancer multi-drug resistance cell line**

K. Li<sup>1,2</sup>, G. Wu<sup>2</sup>, F. Ji<sup>2</sup>, G. Huang<sup>2</sup>. <sup>1</sup>*Department of Respiration, Daping Hospital, Third Military Medical University, Chongqing, China;* <sup>2</sup>*Institute of Respiratory Disease, Xinqiao Hospital, Third Military Medical University, Chongqing, China*

**Objectives:** This research aims to build the subtracted cDNA library of differentially expressed genes of SCLC Multidrug resistance (MDR) cell and to detect the expression of some differentially expressed cDNA fragments in SCLC MDR cell and various other tumour cells, using a cell line created in the preliminary research.

**Methods:** 1. The tester is SCLC MDR cell H446/CDDP cDNA; the driver is SCLC cell H446 cDNA; SHH and T/A cloning technology were done to build the subtracted cDNA library in this research. 2. The dot blot hybridization and sequencing and homology analysis were used to obtain differentially expressed cDNA fragments. 3. Semi-quantitative RT-PCR and Northern blot were used to detect the expressions of PDE2A and I-2<sup>PP2A</sup> in seven tumour cells including H446, H446/CDDP, A549, A549/CDDP, SK-HEP-1, SGC7901.

**Results:**

1. The study successfully built the subtracted cDNA library of differentially expressed genes of H446/CDDP and obtained 21 differentially expressed cDNA fragments of cell H446/CDDP.

MONDAY, SEPTEMBER 17TH 2007

- Sequencing and homology analysis shows that the 21 fragments respectively represents 6 known genes.
- Semi-quantitative RT-PCR and Northern blot shows that PDE2A only has expression in H446/CDDP; I-2<sup>PP2A</sup> has expression in H446, H446/CDDP, and Namalwa.
- Further image analysis and statistical treatment show that I-2<sup>PP2A</sup> expression in H446/CDDP is significantly higher than that in H446 ( $P < 0.01$ ) and that I-2<sup>PP2A</sup> expression in H446/CDDP is not significantly different from that in Namalwa ( $P > 0.05$ ).

**Conclusion:** PDE2A and I-2<sup>PP2A</sup> have differential expression in H446/CDDP; the two genes might participate in the formation of the MDR in H446/CDDP.

#### E1812

##### Distinction in immunoprofile of unusual lung tumours

J. Stojic<sup>1</sup>, B. Milenkovic<sup>1</sup>, M. Percinkovski<sup>1</sup>, J. Radojicic<sup>1</sup>. <sup>1</sup>Institute for Lung Diseases and Tuberculosis, Clinical Centre of Serbia, Belgrade, Serbia

**Introduction:** Alveolar adenoma (AA), sclerosing hemangioma (SH), lympho-angiomyomatosis (LMM) and clear cell or "sugar" tumour (ST) are rare lung tumours with unusual morphology containing 2 types of proliferated cells.

**Aim:** To point that despite of tumour cell appearance their origin determinate their immunophenotype.

**Patients:** All 4 tumours were detected in 49 to 69-years-old females, as "coin-like", accidentally detected lesions on radiological examinations. On surgery, gross pattern of AA and LMM was multicystic and of SC and ST was solid and yellowish. Cytoplasmatic or nuclear staining were labeled as immunopositivity. Spindle, stromal cells of AA and LMM originated from mesenchymal, smooth muscle cells, positive to vimentin and smooth-muscle actin. Flattened epithelial cells originated from pneumocytes are immunoreactive to thyroid transcriptive factor-1 (TTF-1) in AA and LMM and both proliferated cuboidal and ovoid in SH. ST contained clear and eosinophilic cells originated from perithelial cells as well as stromal cells in LMM, immunoreactive as focal, aberrant expression of S-100 protein and HBM-45. Broad spectrum cytokeratin (CK) was positive in epithelial cell of AA and SH.

**Conclusion:** Rare lung tumours with similar clinical course, radiological finding, gross pattern and unusual morphology could be distinguished by different immunoprofile.

##### Immunoprofile of rare lung tumours

Tumour	Cell type	Monoclonal antibody					
		actin	vimentin	CK	TTF-1	S-100	HBM-45
AA	Epithelial	-	-	+	+	-	-
	Stromal	+	+	-	-	-	-
LMM	Epithelial	-	-	-	+	-	-
	Stromal	+	+	-	-	-	+
SH	Oval	-	-	+	+	-	-
	Round	-	-	+	+	-	-
ST	Clear	-	-	-	-	+	+
	Eosinophilic	-	-	-	-	+	+

#### E1813

##### Expression of MIC molecules in non-neoplastic tissue of patients with primary lung neoplasm: evidence for a whole organ immunostimulatory signal

M. Orozco-Levi<sup>1,2,3</sup>, A. Sanchez-Font<sup>1</sup>, A. Ramirez-Sarmiento<sup>1</sup>, L. Pijuan<sup>2</sup>, A. Gayete<sup>3</sup>, J. Gea<sup>1,2,3</sup>, V. Curull<sup>1,4</sup>. <sup>1</sup>Servei de Pneumologia, Hospital del Mar, Barcelona, Spain; <sup>2</sup>CEXS, Universitat Pompeu Fabra, Barcelona, Spain; <sup>3</sup>URMAR, Municipal Institute of Medical Research, Barcelona, Spain; <sup>4</sup>Servei de Patologia, Hospital del Mar, Barcelona, Spain; <sup>5</sup>Servei de Radiologia-CRC Mar, Hospital del Mar, Barcelona, Spain; <sup>6</sup>Facultat de Medicina, Universitat Autònoma, Barcelona, Spain

MHC class I related chains (MIC) serve as ligands for the NKG2D-DAP10 receptor complex, which activates NK cells and costimulates effector T cell subsets, and are frequently associated with epithelial tumors.

**Aim:** This study was aimed at investigating the potential expression of MIC molecules in non-neoplastic epithelia of humans with and without a primary pulmonary neoplasm.

**Methods:** 82 patients (65±10 yrs; FEV<sub>1</sub> range: 21–118 % pred.) have been studied. Never smokers (n=11), former smokers (n=29) and current smokers (n=42) were included. 61 (74%) patients accomplished criteria for COPD. Bronchial biopsies from anatomical places far or contralateral from the primary lung neoplasm were obtained during fiberbronchoscopy and processed for immunohistochemistry using monoclonal anti-MIC-A as primary antibodies. A primary lung neoplasm was histopathologically confirmed in 56 (68%) cases.

**Results:** MICA was not expressed in samples from never-smokers. However, epithelial MICA expression was evident in contralateral and non-neoplastic tissue.

This MICA expression significantly associated with the presence of pulmonary lung neoplasm as assessed by risk estimations (OR: 2.9; CI<sub>95%</sub>: 1.1–8.0;  $p = 0.038$ ) even when adjusting for current cigarette smoking.

**Conclusions:** This study shows that MICA proteins (1) are expressed in non-neoplastic human bronchial epithelium and (2) are associated with the presence of pulmonary lung neoplasm. These evidences suggest that MIC ligands are aberrantly expressed in non-neoplastic epithelium of patients with lung cancer. Grants: BAE-2006–2007, ARMAR, Red Respira RTIC-C03/011, and ENIGMA-QLTR-2002–02285, FIS-01/1324.

#### E1814

##### The role of human papilloma virus in the development of lung cancer

G. Ucar, I. Hanta, S. Kuleci, S. Hasturk, E. Guveloglu, H. Zeren. *Chest Diseases, Cukurova University, Adana, Turkey; Chest Diseases, Cukurova University, Adana, Turkey; Chest Diseases, Cukurova University, Adana, Turkey; Chest Diseases, Cukurova University, Adana, Turkey; Pathology, Cukurova University, Adana, Turkey; Pathology, Cukurova University, Adana, Turkey*

**Background:** In recent years, there are several studies about the role of Human Papilloma Virus (HPV) in the carcinogenesis of lung cancer in several Asian countries. However this association has not been proved in European studies. In Turkey, as a bridge country, between European and Asia, we planned to evaluate HPV expression among lung cancer patients.

**Method:** In this study, seventy adult patients who were diagnosed as lung cancer by bronchoscopic biopsy between June 2004 and January 2006 at Department of Chest Diseases were included. As a control group, thirty-three adult patients diagnosed as benign lung disease by bronchoscopy were also included. Biopsy specimens were stained with HPV-NCL-HPV 18 Novocastra Mouse Monoclonal Antibody by using immuno-histochemical method at the Pathology Department. Specimens with nuclear staining were accepted as positive for HPV, while in specimens without nuclear staining were accepted as negative.

**Results:** Of seventy lung cancer patients, 64 (91.4%) were male, 6 (8.6%) were female; median age was 60 (39–80) years. Of 33 patients with benign lung disease, 14 (42.4%) were male, 19 (57.6%) were female and median age was 47 (15–78) years. The most common histological cell type among lung cancer group was epidemoid carcinoma (n=29, 41.4%). Among lung cancer group no HPV positivity were detected. However, in control group only 3 (9%) patients were positive for HPV. This difference was found to be significant between two groups for HPV ( $p = 0.03$ ).

**Discussion:** In this study, none of the lung cancer patients were found positive for HPV. Thus we were unable to support the hypothesis for the role of HPV in the carcinogenesis of lung cancer.

#### E1815

##### Characterisation of dendritic cells generated from peripheral blood monocytes of NSCLC patients

K. Wojas-Krawczyk<sup>1</sup>, P. Krawczyk<sup>1</sup>, J. Milanowski<sup>1</sup>, J. Rolinski<sup>2</sup>. <sup>1</sup>Department of Pneumology, Oncology and Allergology, Medical University of Lublin, Lublin, Poland; <sup>2</sup>Clinical Immunology Department, Medical University of Lublin, Lublin, Poland

Dendritic cell-based immunotherapy is a novel approach in therapy of cancer. Specific *in vitro* culture condition is one of the factor responsible for properly dendritic cells generation. On the other hand, it is postulated that DCs in cancer-bearing host show several functional defects.

The aim of our study was to generate fully competent dendritic cells from NSCLC patients. Here, we generated DC-like cells (Mo-DCs) from PBMC of NSCLC-bearing patients in the presence of rhGM-CSF and rhIL-4 in medium containing: 10% autologous serum; 10% allogenic serum or 2% human albumin. We checked:

- loss of CD14 expression and presence of CD1a during the culture;
- apoptosis during culture period;
- phagocytic activity;
- expression of specific mature-DC markers;
- level of tumour-specific factors (IL-6, IL-10, VEGF and TGF- $\beta$ ) in patient's serum.

During the culture we observed progressively loss of CD14 and occurrence of CD1a markers in all carried cultures. Moreover, examination of apoptosis level shown gradually increase of apoptosis in the culture supplemented with human albumin when compared to the autologous or allogenic serum. DCs generated in human albumin displayed the weakest phagocytic activity. Induction of maturation resulted in high expression of CD83, CD80, CD86 and co-expression of HLA-DR markers in culture supplemented with autologous serum. Our results demonstrate that kind of culture supplementation has a great impact on functional and phenotypical properties of dendritic cells. These would also conductive to improve the methods of generation autologous dendritic cells and facilitate the decision of what source of medium supplementation would be the best for future immunotherapy.

MONDAY, SEPTEMBER 17TH 2007

**E1816****The expression of PD-L1 on the lung cancer cell and its role in T cell energy**

J.A. Huang<sup>1</sup>, C. Chen<sup>1</sup>, C.Y. Mu<sup>1</sup>, Q.X. Qu<sup>2</sup>, X.G. Zhang<sup>2</sup>. <sup>1</sup>*Respiratory Department, The First Affiliated Hospital of Soochow University, Suzhou, China;* <sup>2</sup>*Key Laboratory of Medicine and Clinical Immunology of Province of Jiangsu, The First Affiliated Hospital of Soochow University, Suzhou, China*

**Objective:** To study the expression of program death-1 ligand (PD-L1) on lung cancer cell lines and its role in interaction of cytotoxic T lymphocyte (CTL) and target cells.

**Methods:** Human PBMC derived dendritic cells (DCs) were loaded with apoptotic tumor cells and stimulated by CD40 mAb (5c11) for additional 48 hours to maturation. 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 was analyzed by FCM. JAM assay was used to detect the cytolytic activity of CTL with or without blocking PD-L1 by mAb respectively.

**Results:** Tumor cells-loaded mature DCs could induce the generation of the tumor specific CTL. Expression of PD-L1 on A549 cell line was low (19.4%±5.2%), but H1299 cell line expressed high level of PD-L1(90.3%±4.2%). Blockade of PD-L1 on A549 could not improve cytolytic effect of CTL on target cells, but fragmentation of H1299 cells was significantly enhanced by the combination of PD-L1 mAb and CTL.

**Conclusion:** Lung cancer cell line highly expressed PD-L1, which could decrease the cytolytic effect of CTL on target cells.

**E1817****Expression of ERCC1 in normal and tumor tissues in non-small cell lung cancer**

M.-H. Jung<sup>1</sup>, K.-R. Park<sup>1</sup>, C.-H. Oak<sup>1</sup>, T.-W. Jang<sup>1</sup>, C.-H. Sohn<sup>2</sup>, S.-D. Park<sup>3</sup>, H.-K. Chang<sup>4</sup>. <sup>1</sup>*Internal Medicine, Gospel Hospital, Kosin University, Busan, Korea, Republic of;* <sup>2</sup>*Internal Medicine, Dong-A University Hospital, Busan, Korea, Republic of;* <sup>3</sup>*Chest Surgery, Gospel Hospital, Kosin University, Busan, Korea, Republic of;* <sup>4</sup>*Pathology, Gospel Hospital, Kosin University, Busan, Korea, Republic of*

**Purpose:** In tumor cells, excision repair cross complement 1 (ERCC1) blocks the effect of cisplatin by repairing the cisplatin-DNA adduct. Thus the expression of ERCC1 in the tumor cells could be a predictor of chemotherapy, but the significance in the normal lung tissue is not well known. In this study, the levels of ERCC1 in the tumor tissue and normal tissue were compared.

**Materials and Methods:** The level of ERCC1 was measured in the tumor tissue from the surgical specimens of 28 patients with non-small cell lung cancer, by the real-time RT-PCR. In 13 patients, the expression of ERCC1 was measured in both the normal and the tumor tissues simultaneously. The level of ERCC1 was expressed as a percentage value compared to that of the A549 lung cancer cell line.

**Result:** The mean level of ERCC1 in 28 tumor tissues was significantly higher than that in the 13 normal tissues (192.9 % (0-1460.1 %) vs. 8.2 % (0 to 28.2 %)). In the 13 cases, in which ERCC1 was measured simultaneously, ERCC1 was increased in the 11 tumor tissues, but not significant (p=0.233). When the upper limit of ERCC1 expression in the normal tissues of 30 % was used as the cut-off level, 13 cases (46%) expressed more than that level. Some tendency of increased expression of ERCC1 was observed in the groups with higher T stages and adenocarcinoma.

**Conclusion:** The expression of ERCC1 was higher in the tumor tissue than the normal tissue. In 46 % of the tumor tissues, ERCC1 was expressed above the highest of the normal tissues. Comparison of the therapeutic response to cisplatin by the quantitative expression of ERCC1 would be warranted.

**E1818**

**Prevalence of angiogenic squamous dysplasia in bronchial biopsies from patients with bronchogenic carcinoma underwent whitelight bronchoscopy**  
S. Karimi<sup>1</sup>, F. Mohammadi<sup>1</sup>, P. Mihan<sup>1</sup>, L. Seyfollahi<sup>1</sup>, B. Moslem<sup>1</sup>. <sup>1</sup>*Pathology, National Research Institute of Tuberculosis & Lung Disease, Tehran, Iran, Islamic Republic of*

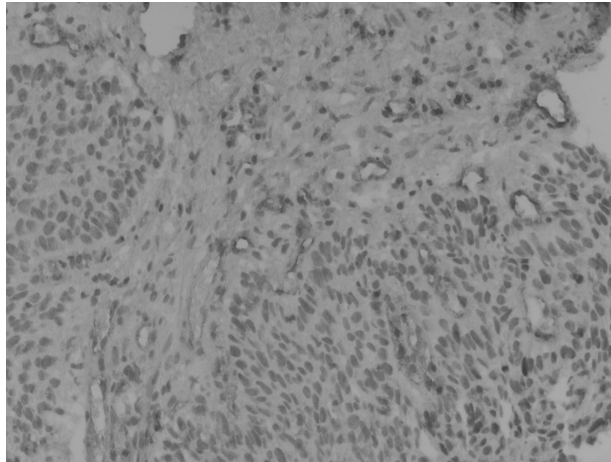
The use of Fluorescence Bronchoscopy has recognized a new morphological entity, Angiogenic Squamous Dysplasia (ASD). It is a unique lesion consists of capillary blood vessels projecting into dysplastic or metaplastic squamous bronchialepithelium.

The aim was better understanding of characteristic morphology of ASD & its association with bronchogenic carcinomas using White Light Bronchoscopy (WLB).

In a case control study, we studied 177 formalin fixed paraffin embedded blocks of bronchial specimens from archives of Dept. Pathology of our center. Our cases were underwent WLB in 2004-05. 127 case with bronchogenic carcinoma & 50 case with non-neoplastic lesions were included. The H&E slides & CD31 immunohistochemistry stainings were examined for histologic diagnosis by pathologists.

ASD occurred at high frequency in patients with neoplastic lesions compared to non-neoplastic (28/136vs1/50). It was present in 23/56 patients with SCC, as comparing to Small Cell Carcinoma & AdenoCarcinoma, that only were 4/32 and 1/39 in order. 78.5% (22/29) of our ASD cases were smoked cigarette. Among the cases of bronchogenic carcinoma with or without ASD, there were no significant differences in age & sex. Morphology of ASD in H&E and CD31 was prominent

microvasculature & capillary projections closely juxtaposed to variable degrees of dysplasia or metaplasia.



ASD as a unique morphologic entity should be considered by pathologists even in biopsies using WLB. It is highly associated with SCC and may represent a potential biomarker of risk for it.

**E1819****Prognostic impact of histologic demonstration of chromogranin A and neuron specific enolase in pulmonary adenocarcinoma**

M. Petrovic<sup>1</sup>, I. Tomic<sup>2</sup>, S. Ilic<sup>2</sup>, N. Ilic<sup>3</sup>, I. Cekerevac<sup>1</sup>. <sup>1</sup>*Pulmonary Department, Clinical Centre, Kragujevac, Serbia;* <sup>2</sup>*Lung Clinic, Military Medical Academy, Belgrade, Serbia;* <sup>3</sup>*Private Practice, Cika Jova Zmaj, Kragujevac, Serbia*

Several studies have suggested that biochemical or molecular markers examined in non-small cell lung cancer carry prognostic or treatment response information.

**Method:** Seventy six patients with inoperable adenocarcinoma of the lung were evaluated by immunohistochemistry with monoclonal antibodies against Neuron Specific Enolase (NSE) and Chromogranin A (Chr A) in order to determine the frequency and prognostic impact of such antigen expression. All patients previously untreated and received chemotherapy according to a prospective randomized trial.

**Results:** The tumors of 10 patients (13%) had more than 10% positive cells stained with anti-NSE, 15 (20%) had 1-10% positive cells and those of 51 patients (67%) contained no NSE-positive cells. The corresponding figures for Chr A were: 13 patients(17%), 16 patients (21%) and 47 patients (62%), respectively. Sixty per cent of the patients with more than 10% positive NSE cells responded to chemotherapy (either complete or partial remissions) compared to 20% of the patients with fewer than 10% positive cells (p < 0.01). The corresponding values for Chr A were 40% responders versus 13% responders (p < 0.01). The median survival for patients with more than 10%, 1-10% or no NSE-positive cells was 18.7 months, 13.5 months and 9.2 months, while for Chr A it was 17.9 months, 14.1 months and 9.4 months. The survival curves for both NSE and Chr A according to the various levels of positivity were significantly different.

**Conclusion:** The presence of neuroendocrine marker in pulmonary adenocarcinoma seems to be associated with increased sensitivity to chemotherapy and better survival.

**E1820****Polymorphism in the length of CA repeat in intron 1 of EGFR of NSCLC in Japan**

N. Morikawa<sup>1</sup>, T. Fukuhara<sup>1</sup>, A. Inoue<sup>1</sup>, T. Sakakibara<sup>1</sup>, Y. Saijo<sup>2</sup>, T. Nukiwa<sup>1</sup>. <sup>1</sup>*Department of Respiratory Oncology and Molecular Medicine, Institute of Development, Aging, and Cancer, Tohoku University, Sendai, Miyagi, Japan;* <sup>2</sup>*Department of Molecular Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan*

Epidermal growth factor signaling has an important role for lung cancer development and progression. Recent studies revealed that the EGFR gene mutation is closely correlated with sensitivity to EGFR tyrosine kinase inhibitors in non-small cell lung cancer. Higher rates of EGFR mutations were observed in non-smoking, oriental, and adenocarcinoma patients. Polymorphisms in the length of a CA simple sequence repeat (CA-SSR) in intron 1 of EGFR gene has been reported to be associated with EGFR expression, and to vary among races. Therefore, we hypothesized that CA-SSR polymorphism could be associated with EGFR mutation status in NSCLC patients. First, the length of CA-SSR of EGFR were measured in total 36 tumor samples consisting both 18 samples with EGFR mutation and 18 with wild type from Japanese patients. Mean length of CA-SSR were 18.5±1.1 at EGFR mutation group and 18.5±1.7 at wild type, showing no significant difference. We are analyzing CA-SSR of 118 tumor samples (44 with mutation and 74 with wild type) using fragment analysis which can precisely

MONDAY, SEPTEMBER 17TH 2007

analyze the length of CA-SSR. We will present these data and association with clinico-pathological status.

**E1821****Tumor-derived monocyte chemoattractant protein-1 promotes malignant pleural effusion**

G.T. Stathopoulos<sup>1</sup>, D. Graf<sup>2</sup>, A. Moustaki<sup>2</sup>, C. Moschos<sup>1</sup>, A. Kollintza<sup>1</sup>, I. Psallidas<sup>1</sup>, H. Petrocheilou<sup>1</sup>, P. Theodoropoulou<sup>1</sup>, S.A. Papis<sup>3</sup>, M. Joo<sup>4</sup>, T.S. Blackwell<sup>4</sup>, C. Roussos<sup>1</sup>, I. Kalomenidis<sup>1,3</sup>, <sup>1</sup>Applied Biomedical Research and Training Center "Marianthi Simou", Department of Pulmonary and Critical Care Services, General Hospital "Evangelismos", School of Medicine, University of Athens, Athens, Greece; <sup>2</sup>Institute of Immunology, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece; <sup>3</sup>2nd Department of Pulmonary Medicine, University Hospital "Attikon", School of Medicine, University of Athens, Athens, Greece; <sup>4</sup>Division of Allergy, Pulmonary & Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, TN, United States

**Background:** Tumor-derived monocyte chemoattractant protein (MCP)-1 is present in human malignant pleural effusion (MPE) (Anthony VB et al, *J Immunol* 1993;151:7216-23), but its role is unknown.

**Aim:** To determine the role of tumor-derived MCP-1 in the pathogenesis of MPE. **Methods:** MPE was generated in C57BL/6 mice via delivery of  $1.5 \times 10^5$  intrapleural Lewis lung cancer (LLC) cells. MCP-1 expression by LLC cells was knocked down by small interfering (si)RNA. For this, two short hairpin RNAs targeting the MCP-1 mRNA (NM011333) (*si1* & *si3*) or a scramble sequence (*si0*) were cloned into the pSUPER.retro.puro vector (Oligoengine, Seattle, WA) and stably expressed in LLC cells.

**Results:** LLC cells produced abundant MCP-1 *in vitro* and *in vivo*. MCP-1 was correlated with mononuclear cells ( $P=.002$ ), TNF $\alpha$  ( $P=.002$ ), and protein ( $P=.004$ ) in mouse MPE, and promoted vascular permeability in the mouse skin ( $P<.001$ ). Compared with *si0* LLC cells, *si1* and *si3* LLC cells exhibited 90-95% MCP-1 knockdown, 3-fold reduced *in vitro* chemotaxis ( $P<.001$ ), but intact proliferation and expression of other mediators. Mice that received intrapleural *si1* and *si3* LLC cells were protected from cachexia and survived longer ( $P<.01$ ), developed fewer and smaller MPEs ( $P<.001$ ) and fewer pleural tumors ( $P=.002$ ), had reduced mononuclear cells in MPE ( $P=.002$ ) and blood ( $P<.001$ ), and 5-fold lower levels of MCP-1 in MPE fluid ( $P=.001$ ), compared with *si0* LLC cells.

**Conclusion:** Tumor-derived MCP-1 drives MPE formation and intrapleural cancer dissemination, promoting mononuclear recruitment, vascular permeability, tumor-related cachexia and death.

**Acknowledgements:** These studies were supported by the Thorax Foundation, Athens, Greece.

**E1822****The equilibrium of oxidant and anti-oxidant in lung cancer**

B. Erturk<sup>1</sup>, C. Ones<sup>1</sup>, D. Marasli<sup>1</sup>, A. Soyhan<sup>1</sup>, A. Eren<sup>1</sup>, A. Hazer. <sup>1</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey; <sup>2</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey; <sup>3</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey; <sup>4</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey; <sup>5</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey; <sup>6</sup>Chest Diseases, Sureyyapasa Chest Diseases and Chest Surgery Hospital, Istanbul, Turkey

In our study we aimed to investigate the role of equilibrium of oxidant and anti-oxidant in lung cancer which is one of the most seen cancers of all. In an organism when there is an increase of a free radical, superoxide dismutase, oxidative stress upon DNA, proteins, lipids and other components also increase. And if anti-oxidant system cannot overcome this oxidative stress various mutations can be formed which can lead to cancer formation. Between September 2005 and February 2006 sixty male patients that were diagnosed lung cancer were included in the study. Four patients with additional diseases were excluded from the study. Three patients died during study. The rest of 53 male patients were assigned for study group. Thirty six healthy subjects were taken as control group. The age ratio of patients with cancer was  $61 \pm 10$ . Thirty-seven of the cancer patients had non-small cell carcinoma while 16 had small cell carcinoma. We measured the level of aldehyde structured malonaldehyde (MDA) that is released by lipid peroxidation and the level of total antioxidant capacity (TAOC) which shows the total anti-oxidant capacity of enzymes in study and control group. The level of MDA in study group was found significantly higher than the control group and the level of TAOC was found significantly lower than the control group. In lung cancer patients we found that there is a decrease in antioxidant activity against increased oxidant stress. The loss of oxidant and anti-oxidant equilibrium can be the probable cause of cancer development but it might be the result of neoplastic process as well. We believe that in order to explore the role of oxidant and anti-oxidant in development of malignancy further investigations are needed.

**E1823****Vascular endothelial growth factor and oxidative stress in patients with primary lung cancer**

A. Katsabeki<sup>1</sup>, T. Kerenidi<sup>1</sup>, K. Kostikas<sup>1</sup>, T. Kiroopoulos<sup>1</sup>, E. Dalaveris<sup>1</sup>, K. Gourgouliani<sup>1</sup>. <sup>1</sup>Department of Respiratory Diseases, University Hospital of Larissa, Larissa, Greece

**Background:** Vascular endothelial growth factor (VEGF) is known to play crucial role in tumor angiogenesis. It is demonstrated that VEGF can be up regulated by oxidative stress.

**Aim:** To determine the serum levels of VEGF and oxidative stress in patients with primary Lung Cancer and to investigate their association with clinicopathologic factors.

**Materials and Methods:** We measured serum levels of VEGF and oxidative stress in 63 patients (median age 63 years) with primary Lung Cancer before any treatment (39 NSCLC and 24 SCLC, 6 patients stage I, 3 stage II, 25 stage III and 29 stage IV) and 25 normal subjects. The serum levels of VEGF were analyzed by using an enzyme linked immunosorbent assay. Serum oxidative stress levels were detected by D-ROMS method.

**Results:** The levels of oxidative stress and VEGF in patients ( $555.3 \pm 30.35$  and  $428.1 \pm 38.42$ ) were higher than those in normal subjects ( $360 \pm 17.46$  and  $298.8 \pm 19.89$ ) with  $p=0.0002$  and  $p<0.05$  respectively. Serum VEGF level is significantly associated with the clinical staging (N-stage) of the patients ( $p=0.0232$ ), performance status ( $p=0.0045$ ) and age ( $p=0.0045$ ). Additionally in patients, oxidative stress was significantly correlated with VEGF ( $p=0.0002$ ).

**Conclusions:** Oxidative stress and VEGF are significantly increased in patients with Lung Cancer. They could be valuable diagnostic and prognostic markers. However, the correlation between them might implicate new aspects of the mechanisms controlling angiogenesis and appears to be of clinical interest in the future.

**E1824****Immunohistochemical study of intratumoral micro vessels in resected non-small cell lung carcinomas, N-status, pTNM-stage and survival period of the patients**

Y. Slavova<sup>1</sup>, D. Petrov<sup>5</sup>, V. Dzambazov<sup>5</sup>, W. Olszewski<sup>3</sup>, S. Nachev<sup>2</sup>, D. Marinova<sup>4</sup>. <sup>1</sup>Pathology, University Hospital of Lung Diseases "St.Sofia", Sofia, Bulgaria; <sup>2</sup>Clinical Pathology Center, University Alexander's Hospital, Sofia, Bulgaria; <sup>3</sup>Pathology, Oncological Institute, Warsaw, Poland; <sup>4</sup>Medical University, Sofia, Bulgaria; <sup>5</sup>Thoracic Surgery, Hospital of Lung Diseases "St.Sofia", Sofia, Bulgaria

**Goal:** Study of intratumoral microvessels in resected non-small cell carcinomas (NSCLC), N-status, pTNM-stage and survival period.

**Material and Method:** Resected material from 54 patients radically operated for NSCLC is observed. 21 cases concern N0-status, 33 - N1.2 - status, 48 cases concern I, II and IIIA, and 6 -IIIB and IV pTNM stage. The number of the intratumoral microvessels (NITMV) is determined through application of CD31. There is an account of high (NITMV =>75), and a low (NITMV <75) degree of vascularisation. Intratumoral vessel invasion is determined. Statistical Methods: t - test, chi-square, survival according to Kaplan-Meier, logistic regression analyses.

**Results:** The average survival period in low vascularisation is 1731 days, and in high vascularisation - 1158 days (a 573 days difference,  $p=1067$ ). NITMV has a statistically significant influence on the N-status: chi-square -  $p=0.041$ , logistic regression analyses -  $p=0.045$ . A significant dependency between the average NITMV and pTNM stage ( $p=0.029$ ) has been proven. In vessel invasion (in 27.4% of the cases) the survival period is shorter with 478 days. In 28 cases (54.9%) intratumoral vessels immediately bordering tumor cells are observed, while in 5 NSCLC there are intratumoral vessels, in part of whose walls endothelial cells are not found.

**Conclusion:** NITMV has a statistically significant influence on the N-status. The survival period is longer in NSCLC with low vascularisation.

**E1825****K-RAS oncogenic mutations in patients with nonsmall cell lung cancer**

G. Cvetkovic, G. Plavec, I. Tomic. Lung Clinic, Military Medical Academy, Belgrade, Serbia; Lung Clinic, Military Medical Academy, Belgrade, Serbia; Lung Clinic, Military Medical Academy, Belgrade, Serbia

**Preface:** It has been established cytogenetically that mutations in onkogens and tumor suppressor gens are among the key moments in genesis and developing of lung cancer. It has been also pointed that K-RAS oncogenic mutation is of possible significant indicator of survival rate and drag response [1]. We review initial results of K-RAS gen mutation in bronchial aspirate in NSCLC subgroups.

**Patients and Methods:** In 40 patients with NSCLC bronchial aspirate were taken during bronchoscopy and DNA was isolated by fenolic extraction. By PCR-SSCP method K-RAS gen mutations were detected.

**Results:** Mutations in 12th and 13th K-RAS gen codon were detected in 16 of 40 patients. Mutations were detected in 11 (47.8%) of 23 patients with squamocellular carcinoma, in 3 (27.2%) of 11 with adenocarcinoma and in 2 (66.6%) of 3 with adenosquamos carcinoma. Of 29 aspirate samples with cytology positiveness on malignancy K-RAS positive findings were 12 (41%), and in 4 (36.6) of 11 negative aspirate samples. In the moment of diagnosis of carcinoma only 2 (5%) patients

MONDAY, SEPTEMBER 17TH 2007

had I and II, while 9 (22.5%) IIIa, 21 (52.5%) IIIb and 8 (20%) IV clinical stadium of disease.

**Conclusion:** We detected K-RAS mutations frequently in squamocellular than in case of adenocarcinoma, that is not in concordance with previous series [2]. We also find that presence of malignant cells in aspirate sample is not of influence on K-RAS mutation findings.

#### Reference(s)

- [1] Wiest JS, Franklin WA, et al. Genetic markers for early detection of lung cancer and outcome measures for response to chemoprevention. *J Cell Biochem Suppl* 1997; 28–29: 64–73.
- [2] Fleishhacker M, Beinert T, Possinger K. Molecular genetic characteristic of lung cancer useful as “real tumor markers”? *Lung Cancer* 1999; 25: 7–24.

#### E1826

**IGF-1 and IGFBP-3 concentrations in the blood serum and lung cancer risk**  
T. Izycki<sup>1</sup>, W. Naumnik<sup>1</sup>, M. Ossolinska<sup>1</sup>, E. Chyczewska<sup>1</sup>. <sup>1</sup>Department of Lung Diseases and Tuberculosis, Medical University, Bialystok, Poland

**Aim of study:** The aim of our study was to check whether there is a relation between the concentrations of IGF-1 and IGFBP-3 in the blood serum and lung cancer risk.

**Material and Methods:** The study included 84 patients diagnosed histologically with lung cancer in the Department of Lung Diseases and Tuberculosis in Bialystok from 2004 to 2006 year. The control group consisted of 84 people (group II).

**Results:** A positive relation between the level of IGF-1 ( $p=0.0001$ ) was found in the analysis of the risk. OR for the highest quartile equaled 2.8 compared to the lowest quartile (OR=0.08). A decreased level of IGFBP-3 was also associated with the higher risk of lung cancer incidence; OR for the lowest quartile was 3.1 ( $p=0.0001$ ), and for the highest quartile 0.28. Village inhabitants had a significantly higher risk of lung cancer than city dwellers (OR=1.38 and OR=0.72  $p=0.004$ , respectively). Current smokers presented the higher risk of lung cancer than non-smokers and former smokers (OR=2.12 OR=0.65 and OR=0.44, respectively).

**Conclusion:** Determination of IGF-1 and IGFBP-3 levels may be significant in defining lung cancer risk.

#### E1827

**Utility of serum soluble mesothelin as a follow-up marker in patients with malignant pleural mesothelioma**  
B.-D. Grigoriu<sup>1,6</sup>, B. Chahine<sup>2</sup>, M. Conti<sup>3</sup>, L. Kedziora<sup>4</sup>, T. Gey<sup>1</sup>, M.-C. Copin<sup>5</sup>, P. Lassalle<sup>1</sup>, G. Marchandise<sup>1</sup>, H. Porte<sup>3</sup>, A. Scherpereel<sup>1,2</sup>. <sup>1</sup>INSERM U774, Institut Pasteur de Lille, Lille, France; <sup>2</sup>Thoracic Oncology, University Hospital of Lille, Lille, France; <sup>3</sup>Thoracic Surgery, University Hospital of Lille, Lille, France; <sup>4</sup>Pulmonary Disease, Hospital of Denain, Denain, France; <sup>5</sup>Pathology, University Hospital of Lille, Lille, France; <sup>6</sup>Pulmonary Disease, University of Medicine, Iasi, Romania

**Introduction:** Previous data suggested that serum level of soluble mesothelin (SM) may be related to tumor size and may have prognostic significance. We tested the hypothesis that SM could also be useful for monitoring response to treatment.

**Methods:** Serum SM values were determined at diagnosis, and every four months thereafter in 29 patients diagnosed with MPM.

**Results:** Five patients with epithelioid MPM have been subjected to extrapleural pneumonectomy. The mean values of SM before and one year after surgery were 1.8 nM and 0.74 nM respectively. Seven patients had initial SM values below 1 nM (mean 0.57 nM) which slightly increased over time to 0.65 nM at 4 months, 1.24 at 8 months and 1.18 nM at one year after the diagnosis. In the follow-up (median 9 months) of the 17 remaining patients, we observed 6 deaths which had a mean increase of serum SM of 4.64 nM (median 1.87 nM) between the first and the last available samples while the surviving patients had a mean SM elevation of only 0.08 nM (median 0.05 nM). In this group of 17 patients, those having a progressing disease ( $n=12$ ) on the CT scan evaluation had a mean increase of serum SM of 2.79 nM (median 1.24 nM) while the patients who were considered having a objective response (3 cases) or being stable ( $n=2$ ) had a mean decrease of serum SM of 0.82 nM (median 0.99 nM).

**Conclusions:** Increasing serum levels of soluble mesothelin is associated with disease progression and worse outcome while stable or decreasing values suggest response to treatment. If confirmed in larger series soluble mesothelin could be used for monitoring the MPM patients under treatment as recently proposed in the USA by the Food and Drug Administration.

#### E1828

**Pretreatment levels of TNF- $\alpha$  in serum and exhaled breath condensate in patients with lung cancer**  
E. Dalaveris<sup>1</sup>, T. Kerenidi<sup>1</sup>, K. Kostikas<sup>1</sup>, T. Kiroopoulos<sup>1</sup>, A. Katsabeki<sup>1</sup>, K. Gourgoulis<sup>1</sup>. <sup>1</sup>Department of Respiratory Diseases, University Hospital of Larissa, Larissa, Greece

**aim:** TNF- $\alpha$  has emerged from recent studies as one of the mediators that seem to interfere with both antiproliferative and tumorigenic effects. The aim of this study

was to measure pre-therapeutic TNF- $\alpha$  levels in serum and in exhaled breath condensate of lung cancer patients and correlate them with clinicopathological parameters.

**Material and Methods:** We enrolled 30 patients with histological evidence of lung cancer (23 males and 7 females, with a mean age of  $65.2\pm 10.5$  years), 22 with Non Small Cell Lung Cancer, 8 with Small Cell Lung Cancer and according TNM stage were classified: 9/ I, 1/ II, 13/ III and 7/ IV. The control group consisted of 15 healthy individuals. Exhaled breath condensate (EBC) was collected with the Ecoscreen device (Jaeger, Germany). We measured TNF- $\alpha$  exhaled and serum levels by enzyme linked immunosorbent methods.

**Results:** TNF- $\alpha$  was detected in the integral number of samples we have collected. A statistically significant difference was observed between lung cancer patients and the control group, regarding the values of TNF- $\alpha$  measured both in EBC ( $52.93\pm 5.007$  pg/ml vs  $19.48\pm 3.970$  pg/ml,  $p<0.0001$ ) and serum ( $44.59\pm 6.308$  pg/ml vs  $22.25\pm 4.311$  pg/ml,  $p=0.02$ ). There were not found significant correlations between TNF- $\alpha$  levels and other clinicopathological parameters.

**Conclusions:** These findings suggest that EBC provides a simple, non invasive method of obtaining samples from the airways. Moreover our results demonstrate that the analysis of TNF- $\alpha$  in serum and EBC might be of use in the diagnosis and monitoring of patients with lung cancer.

#### E1829

**Association between protein expression of p63 and final cause of death in non small cell lung cancer**

R. Moreno Zabaleta<sup>1</sup>, P. Benavides Mañas<sup>1</sup>, A. Lopez Encuentra<sup>1</sup>, R. Garcia Lujan<sup>1</sup>, E. Conde Gallego<sup>2</sup>, F. Lopez-Rios<sup>2</sup>. <sup>1</sup>Pneumology, Hospital Universitario 12 de Octubre, Madrid, Spain; <sup>2</sup>Pathology, Hospital Universitario 12 de Octubre, Madrid, Spain

**Aim:** To know the association between protein expression p63 protein and final cause of death in a group of patients with non small cell lung cancer (NSCLC) in initial pI-II stage who underwent surgical resection.

**Population:** Inclusion criteria: 1)Patients with NSCLC and pI-II stage who underwent surgical resection at our hospital between 1–1-94 and 30–9-97. 2)Perioperative mortality excluded. 3)Final cause of death knew in National Statistics Institute data asked before 2003. 4)Protein expression of p63 positive or negative by immunohistochemistry (IHC) after the agreement of 2 observers. All tumours were reclassified by 2 pathologists according to the 2004 WHO classification. Population was divided into 2 groups according to the cause of death: NSCLC or Other cause.

**Results:** Population included was 50 and 45 (90%) were males. The mean age was 67 (SD $\pm 8.41$ ). The histopathology types were: Squamous cell: 30 (60%), adenocarcinoma 12 (24%), large cell 7 (14%), mixed 1 (2%). In 14 (27.5%) patients the final cause of death was different than lung cancer. IHC of p63 was positive in 14 (28%) cases, all of them squamous. Positive and valuable expression in IHC of p63 according to final cause of death in total population ( $n=50$ ) and in squamous group ( $n=30$ ) are summarized in the table.

**Conclusions:** In this group of patients with NSCLC in pI-II stage with surgical resection, p63 was higher expressed in those with non NSCLC cause of death in total population and with a higher difference in squamous group.

p63 expression

	Total population			Squamous group		
	NSCLC	Other cause	p	NSCLC	Other cause	p
n	36	14		21	9	
p63+	7 (19%)	7 (50%)	0.03	7 (33%)	7 (78%)	0,02