## 405. Lung development and neoplasia

**P3800**

WITHDRAWN

**P3801**

**Developmental profile of cellular pathways regulating protein breakdown in the fetal and postnatal diaphragm**

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Studies on diaphragm weakness and atrophy focus on adult muscle, but the preterm diaphragm might be more susceptible to injury. Characterization of the ontogeny of protein degradation pathways responsible for muscle atrophy would help further understanding of the altered signaling pathways under pathologic conditions in preterm babies. Here, we performed the baseline study of major proteolytic pathways and antioxidant capacity in lambs from 75 d to 200 d postconceptual age. The diaphragm tissues were collected and analysed for gene expression and/or protein abundance in a set of key pathway components in conjunction with proteolysis/antioxidant activity. Our results showed that calpain and caspase 3 in gene and protein expressions exhibited a similar profile with advancing gestation, increasing from 75 d to 100 d/128 d and subsequently decreasing gradually toward full term. In contrast, ubiquitin conjugating and ligase genes did not change during gestation. All proteolytic genes examined (except Ubiquitin) were up-regulated rapidly after delivery, with a similar developmental trend observed in protease activities. Unlike proteolytic pathway pattern, antioxidant gene expression demonstrated a steady increase from 75 d gestation until 24 h after birth, followed by a significant reduction at 7 w of postnatal age ($p \leq 0.002$). The proteolytic signaling and antioxidant capacity patterns reflected the adaptive process to metabolic change and muscle maturity with development. The data from this study may partly explain the susceptibility of preterm infants to respiratory failure in response to injurious ventilation and/or clinical stressors such as inflammation and hypoxia.

**P3802**

**PECAM-1 single nucleotide polymorphisms and idiopathic pulmonary fibrosis**

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**Background:** Idiopathic pulmonary fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias and carries a prognosis worse than many cancers. However, the pathogenesis of this disease remains incompletely understood.
Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) is a 130 kDa glycoprotein that has been implicated in pulmonary fibrosis in a strain of knockout mice. We assess the prevalence of the PECAM-1 single gene polymorphisms and the serum concentration of PECAM-1 in IPF patients versus controls.
**Methods:** Samples of full blood were collected from 37 patients with IPF and 41 controls. DNA was extracted from whole blood using QIAmp DNA Blood Midi Kit (Qiagen, Valencia, CA). PCR was performed using primers specific to exon 3, exon 8 and exon 12 of the PECAM-1 gene. Purified PCR products were sent to Eurofins MWG Operon for sequencing. The serum concentration of soluble PECAM-1 was assessed using ELISA.
**Results and discussion:** No significant difference was observed in the prevalence of single gene polymorphisms in exon 3, 8 or 12 of the PECAM-1 gene. The serum PECAM-1 concentration was significantly higher in the IPF group versus controls (106.177ng/ml vs 84.327ng/ml respectively, p=0.0398) with elevated sPECAM-1 concentrations associated with better lung function (p=0.038).
**Conclusion:** No significant difference in the prevalence of single gene polymorphisms in the PECAM-1 gene was observed between IPF and control groups. Serum PECAM-1 concentration was elevated in IPF patients versus controls however it is not possible to conclude whether this is important in the pathogenesis of the disease or a secondary event resulting from cell injury and aberrant tissue repair.

**P3803**

**Up-regulation of collagen type V mRNA in a model of systemic sclerosis**

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**Background:** We have described an animal model of systemic sclerosis (SSc) induced by type V collagen (COLV) that resembles the human disease. The aim of this study was to investigate the early disease in the lung of this model with special emphasis on collagen deposition and mRNA collagen synthesis.
**Methods:** Female rabbits from New Zealand lineage were immunized with COLV plus Freund's adjuvant (FA). Animals immunized only with FA were used as controls. The animals were sacrificed at day-7, day-75 and day-210; and the lungs submitted to immunofluorescence, real-time qPCR and biochemical examination.
**Results:** The immunolabeling for the COL I, III and V by immunofluorescence showed an intense expression of COLV in the bronchovascular interstitium near the inflammatory infiltrate of COLV-rabbits at day-210. Additionally, morphologic analysis demonstrated that the progressive remodeling of the extracellular matrix observed in this group of animals was characterized by thickened COLV deposition with distorted fibrils. In accordance with these observations, the real-time qPCR revealed markedly up-regulation of COLV mRNA of the COLV-group at day-210 comparing with controls ($p<0.001$). The content of hydroxyproline was compared for COLV-group and controls at day-7, day-75 and day-210, suggesting that the day-210 group represents an early disease without established fibrosis.

**Conclusions:** We found an early up-regulation of COLV mRNA in the lung tissue of this experimental model emphasizing the role of this protein in the pathogenesis of SSc. In addition, our results reinforce the importance of this model, emerging promising to study the early stages of pulmonary fibrosis in this severe disease.

**P3804**

**Anti-fibrotic effects of liver X receptor agonists in human fetal lung fibroblasts**

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**Introduction:** Idiopathic pulmonary fibrosis is a progressive life-threatening disease for which anti-inflammatory agents such as glucocorticoids have no therapeutic effect. Liver X receptors (LXRs), which are nuclear receptors activated by oxysterols as natural ligands, show similar tissue-distribution patterns with glucocorticoid receptor and peroxisome proliferator-activated receptor (PPAR)-γ. LXRs play important roles not only in cholesterol metabolism and triglyceride synthesis, but also in inflammation and innate immunity. Previous studies showed anti-fibrotic effects of PPARγ agonists, but it remains unknown whether LXR agonists have anti-fibrotic abilities.
**Aims:** The aim of our current study is to analyze anti-fibrotic effects of LXR agonists on human lung fibroblast.
**Methods:** Serum-deprived MRC5 cells (human fetal lung fibroblast cell line) were pre-incubated with synthetic LXR agonist T0901317 and natural agonist 22(R)-hydroxycholesterol, and were differentiated into myofibroblasts by TGF-β1 for 48 hours. Changes in α-smooth muscle actin (α-SMA) and phosphorylated-Smad2/3 were analyzed by western blot. The inhibitory effects of irreversible PPARγ antagonist GW-9662 on anti-fibrotic abilities of LXR agonists were also examined.
**Results:** LXR agonist T0901317 and 22(R)-hydroxycholesterol inhibited TGF-β1-driven myofibroblast-differentiation as determined by α-SMA by western blot. T0901317 did not inhibit Smad2/3 phosphorylation. An irreversible PPARγ antagonist GW-9662 did not reverse the inhibition of myofibroblast-differentiation by T0901317.
**Conclusion:** LXR agonists have novel and potent anti-fibrotic effects in human lung fibroblasts.

**P3805**

**Identification of stable housekeeping genes for real-time PCR in human pulmonary fibroblasts**

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**Background:** Quantitative real time PCR is an important tool in investigating gene transcription levels under different biological conditions. Accurate results rely on controlling for differences in mRNA quantity and quality between samples, commonly achieved by normalisation to expression of housekeeping genes (HKGs). Expression stability of the HKGs used is critical, but to date no systematic study has been performed in pulmonary fibroblasts.
**Methods:** Microarray data of gene expression in explanted fibroblasts from SSc-ILD (systemic sclerosis lung biopsies, n=8) and control (normal periphery of resected tumors, n=10) lung tissue was used to assess variation in expression of commonly used HKGs; *ACTB*, *GAPDH*, *HPRT1*, *RPL32*, *TBP*, and *YWHAZ* (HUGO nomenclature). The four most stable genes (<15% variability) were tested by qRT-PCR in an independent experiment in which SSc-ILD (n=3) and control (n=3) fibroblasts were cultured in 0.1% BSA for 24hrs, and a further 24hrs with or without IFNγ (10ng/mL). A measure of expression stability (M), the average pairwise variation with each of the other studied genes (with a recommended maximum value of 1.5) for each HKG, was calculated using the program geNorm (Vandesompele, J. *et al.* Genome Biology 2002;3:34.1-12).
**Results:** While ACTB and GAPDH were relatively unstable, the four HKGs tested further in geNorm all had an expression stability of M<1.0; the most stable gene was *YWHAZ* (M=0.56), followed by *HPRT1* (M=0.60), *RPL32* (M=0.67), and *TBP* (M=0.70).
**Conclusion:** We have identified four HKGs suitable for normalisation of mRNA expression levels in human lung fibroblasts under the conditions tested, and show the necessity for empirical identification of HKGs.

**P3806**

**Molecular screening – A search for potential drugs for idiopathic pulmonary fibrosis**

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**Background:** Idiopathic pulmonary fibrosis (IPF) is a fibrotic lung disease with a poor prognosis and very few therapeutic options. On a molecular level, patients with IPF have increased amounts of BMP inhibitor gremlin in their lungs (BMP, bone morphogenetic protein). Gremlin decreases BMP signalling and, as a consequence, TGF-β signalling is increased (TGF-β, transforming growth factor-beta). The balance between BMP and TGF-β signalling activities in the lung is of crucial importance during e.g. regenerative processes. Thus, severe alterations in these signalling activities are likely to contribute to the pathogenesis of IPF.
**Aim:** To use high-throughput chemical screening as an approach to find molecules which could normalize the TGF-β/BMP balance in the fibrotic lung.
**Methods:** The screen was performed with a commercial chemical library with ca. 2000 compounds. The set-up is based on two reporter cell lines that contain either a BMP- or a TGF-β-responsive element linked to a luciferase gene. The effect of the chemicals is evaluated by measuring the changes in the expression of the luciferase gene.
**Results:** Using this approach we have been able to identify compounds which modulate the BMP/TGF-β signalling balance in the reporter cell lines. One compound was also shown to modulate endogenous BMP/TGF-β signalling activities in lung epithelial cells.
**Conclusions & future directions:** Using chemical compound screening it is possible to identify small-molecular weight compounds that enhance BMP signalling in lung epithelial cells. These compounds are potential drugs for the prevention of progression of IPF. Their effects will be evaluated in preclinical *in vivo* studies using a mouse model of pulmonary fibrosis.

**P3807**

**Serum amyloid A as a potential biomarker of sarcoidosis**

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At the beginning of our studies on proteomics we compared serum protein profiles in interstitial lung diseases and we found increased expression of certain acute phase proteins, such as serum amyloid A (SAA), in sarcoidosis serum samples.
An increased expression of SAA1 was detected by a proteomic approach (two-dimensional electrophoresis) in serum of sarcoidosis patients, therefore we decided to validate this finding evaluating its concentrations by a quantitative assay (ELISA) and to further investigate SAA patterns in patients with sarcoidosis.
Our population consisted of 48 patients affected by sarcoidosis and 17 healthy controls. Patients were divided according to phenotype activity score SCAC. Proteomic analysis of SAA in serum revealed that the protein resolved in 2 different spots with isoelectric point/molecular weight of 5.69/11000 KDa and 6.15/11000 Kda. SAA1 spots were never observed in samples of healthy subjects but only in sarcoidosis patients. ELISA assay reported significantly higher concentrations of SAA in patients than controls (p=0.002). Protein concentrations inversely correlated with FEV1 percentages in sarcoidosis patients and were significantly higher in SCAC group 6 than in SCAC group 4 (p<0.001) and SCAC group 5 (p<0.05). Interestingly the molecular weight of the two isoforms of SAA identified in our study corresponded to two unidentified serum proteins, proposed as sarcoidosis biomarker, in a previous proteomic study performed by Bons et al.
In conclusion SAA could be a useful biomarker of sarcoidosis since its levels are significantly higher in patients than controls, especially in those with lung function impairment and requiring prolonged steroid treatment.

**P3808**

**Screening for alpha-1 antitrypsin deficiency in Polish lung or liver disease patients**

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**Background:** In Poland, the overwhelming majority of individuals with alpha-1 antitrypsin (AAT) deficiency still remains undiagnosed. We estimated the AAT gene frequency and prevalence in a large cohort of Polish chronic lung or liver disease patients eligible for AAT testing.
**Methods:** Blood samples were collected prospectively from 419 respiratory patients (COPD, emphysema, bronchiectasis, asthma) and 281 patients referred for liver transplantation due to cirrhotic and non-cirrhotic chronic disease. AAT serum

concentration was measured by turbidimetry and PI-phenotype identified by isoelectrofocusing. The PI*S and PI*Z alleles were confirmed by real-time PCR; rare phenotypes were identified by sequencing.
**Results:** 53 (12.6%) lung disease patients and 18 (6.4%) liver disease patients demonstrated AAT deficiency phenotypes. Calculated frequencies expressed per 1000 were for PI*Z 46.6 (95% CI: 32.3-60.8), PI*S 20,3 (95% CI: 10.8-29.8) in respiratory patients and PI*Z 19.6 (95% CI: 8.1-31), PI*S 10.7 (95% CI: 2.2-19.2) in liver disease patients. The AAT gene prevalence calculated by Hardy-Weinberg equilibrium were: 1/1.16 for MM, 1/26 for MS, 1/2429 for SS, 1/11 for MZ, 1/530 for SZ and 1/462 for ZZ in respiratory patients and 1/1.07 for MM, 1/48 for MS, 1/8773 for SS, 1/26 for MZ, 1/2393 for SZ and 1/2610 for ZZ in liver disease patients.
**Conclusion:** Our results show relatively high frequency of AAT deficiency among Polish patients with chronic obstructive respiratory disorders. Estimated frequency for PI*Z and PI*S allele in respiratory group was about two-fold higher than in liver disease patients and four-fold higher than estimated prevalence in healthy Polish population.

**P3809**
**Proteomic analysis of antioxidant proteins in bronchoalveolar lavage of patients with pulmonary Langerhans-cell histiocytosis**
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Pulmonary Langerhans-cell histiocytosis (PLCH) is a tabacco smoke-related diffuse lung disease of unknown etiopathogenesis. Oxidative-mediated lung injury has been documented in patients with PLCH. In this study we analyzed by 2-dimensional electrophoresis, BAL protein expression of antioxidant proteins in a group of patients with PLCH, a group of healthy smokers and a group of no-smoker controls in order to better understand the potential role of oxidant/antioxidant balance in the disease pathogenesis. Among the differentially expressed proteins identified by us, the proteins implicated in the antioxidant defense where thioredoxin (THIO), ALBU, ceruloplasmin (CERU) and glutathione peroxidase 3 (GPX3). THIO was a protein identified from a spot down-regulated in PLCH versus smoker controls ($p<0.01$). It plays a protective role against cigarette smoke-induced lung oxidative damage and against Th2-driven airway inflammation. CERU isoform 1 and 2 were significantly upregulated in PLCH patients compared to no-smoker controls ($p<0.01$) while CERU isoform 3 was upregulated in patients than smoker controls ($p<0.05$). GPX3 was absent in 2-DE gels of patients with PLCH and it was significantly over-expressed in smokers than no-smoker controls. In conclusion antioxidant defense could be involved in PLCH pathogenesis being the expression of antioxidant proteins significantly different in BAL of patients with respect to smoker or no smoker controls.

**P3810**
**Endothelin-1 (ET-1) is a useful biomarker for early detection of ventilator-assotiated pneumonia (VAP) in mehanical ventilation children**
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**Objectives:** VAP is a severe complication limiting survival children. ET-1 is a peptide produced by pulmonary vascular endothelial cells that play a role in the pathophysiology of lung dysfunction. Whether ET-1 could predict VAP development is unknown.
**Methods:** Transbronchial biopsy specimens and serum and bronchoalveolar lavage were obtained from 23 children with VAP and 24 without VAP. The serum and bronchoalveolar lavage ET-1 concentrations were measured by enzyme-linked immunosorbent assay, and the ET-1 mRNA expression in the transbronchial biopsy specimens was examined using real-time polymerase chain reaction.
**Results:** ET-1 serum concentrations were greater in the patients with VAP (P=0.02); and ET-1 mRNA was significantly in the lung of those with versus those without VAP at 3 and 12 day after start mehanical ventilation (P=0.01). At 3 and 12 days after start mehanical ventilation, the ET-1 concentrations were significantly elevated in the serum ($P <0.01$ and $P < 0.0001$, respectively) and bronchoalveolar lavage ($P <0.01$ and $P =0.01$, respectively) of children with compared with those without VAp. On logistic regression analysis, 3-day after start mehanical ventilation serum ET-1 level predicted for VAP (odds ratio, 1.01; 95% confidence interval, 1.003-1.027; $P <.007$; odds ratio, 2.8; 95,3% confidence interval, 1.01 - 8.4; $P < 0.001$). The serum ET-1 level at 12 days was diagnostic for VAP (odds ratio, 4.1; 95% confidence interval, 1.4- 11.3; $P =0.009$).
**Conclusions:** Elevated serum ET-1 concentrations were predictive of VAP, and the assessment of circulating ET-1 might be beneficial in diagnosing and monitoring VAP.

**P3811**
**Does beta2 adrenoceptor (ADRβ2) density vary in circulating lymphyocytes compared with monocytes?**
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Circulating lymphocytes are increasingly used as a surrogate cell type to reflect changes in ADRβ2 density elsewhere in the body, particularly the respiratory system. However, ADRβ2 density is non-uniform among lymphocyte subsets and potentially varies between individuals.
**Aim:** To assess the extent of variability in ADRβ2 density on human peripheral blood mononuclear cells (PBMC) including lymphocytes and monocytes.
**Method:** PBMC were isolated from 10 healthy subjects by density gradient centrifugation with Ficoll-Paque. Cell surface & total ADRβ2 of lymphocytes (CD14+) and monocytes (CD3+) measured using FACS. Geometric mean fluorescence (GMF) was used as the indices for ADRβ2 density per cell.
**Result:** *Surface ADRβ2* - GMF increased by 3.54 and 4.62 folds over negative controls for lymphocytes and monocytes respectively (p=0.16). However, distribution of GMF between samples suggests greater variability in ADRβ2 density in lymphocytes vs monocytes (p=0.06). Proportion of ADRβ2-positive cells was higher in monocytes vs lymphocytes (71.9% vs 36.7, p=0.02). *Total ADRβ2* – GMF increased by 12.4 and 9.61 folds for lymphocytes and monocytes respectively ($p>0.05$). Proportion of ADRβ2-positive cells were similar between samples (lymphocytes 80%, monocytes 86%, $p>0.05$) but greater variability was observed for lymphocytes (range 27–99%) vs monocytes (range 66–100%).
**Conclusions:** Despite similarities in surface and total ADRβ2 density, lymphocytes display greater inter-subject variability compared with monocytes. This has implication in experimental designs & interpretation of changes in ADRβ2 density in studies using human PBMC as an alternative to primary cells from organ of interest.

**P3812**
**Apocynin augments IL-6, IL-8 and TNF expression in A549 cells**
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Reactive oxygen species (ROS) play an important role in many pathways and processes. An enzyme responsible for ROS production - NADPH oxidase represents an attractive therapeutic target. Apocynin is a small molecule that reversibly blocks NADPH oxidase activation and therefore inhibits ROS formation.
Apocynin, in our previous studies, reduced some ROS concentrations in exhaled breath condensate of asthmatics, COPD patients, and healthy subjects. Therefore we decided to investigate apocynin influence on expression of IL-6, IL-8 and TNF *in vitro*. A549 cells were incubated with apocynin in concentrations: 0.25, 0.5, 1, 2 and 3 mg/ml. After 1h, 2h and 4h of incubation with each concentration RNA was isolated and reverse transcription was made. mRNA expression was assessed using real-time PCR.
Time-response analysis revealed apocynin most effective influence after 2 and 4 hours of incubation. It caused increased expression of IL-6 (expression level (RQ) 0.64 – 2h and 0.62 – 4h *vs.* 0.007 – base), and IL-8 (RQ 25.18 – 2h and 25.12 – 4h *vs.* 1.74 – base) in A549. Moreover, apocynin caused TNF expression increase in A549 after one and four hours of incubation (RQ 0.037 – 1h and 0.036 – 4h *vs.* 0.017 – base). Dose-response analysis showed apocynin to be most effective in concentration of 0.5 mg/ml. This dose caused increase of IL-6 expression (RQ 2 *vs.* 0.02 - control sample (CS)) and IL-8 expression (RQ 101 *vs.* 2 - CS, but also 1 mg/ml – RQ 30 *vs.* 2 - CS). TNF expression was increased in samples incubated with 0.25 mg/ml (RQ 0,037 *vs.* 0.02 - CS)
These findings may provide a novel therapeutic strategy and might be of importance in relation to inflammatory diseases.

**P3813**
**Comparison the frequency of human T-lymphocyte type 1 (HTLV 1) infection in tuberculosis patients with control group without tuberculosis**
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**Introduction:** Regarding to recognize the predisposing factors in Tuberculosis infection which is common in Khorasan, Northeastern province of IRAN,this study investigate whether HumanT-lymphocyte type 1 (HTLV 1) as an immunosuppresive factor increase the risk of tuberculosis.
**Methods and materials:** A case –control study was conducted on 278 pulmonary and extrapulmonary tuberculosis during 2007-2010, in city of mashad, capital of khorasan.Tuberculosis has been documented by goldstandard tests like sputumculture, BAL culture or cytology.For detection of HTLV1 antibody, ELISA method and as a confirming test western Blot were performed. Healthy sex and age matched control group were 276 persons.

**Results:** The mean age of tuberculosis patients was 49.6721.36 and 48.3620.74 in controls.In pateints group 114 (41.6%)were male and 160 (58.4%)were female and in controls 123 (44.6)were male and 153 (55.4)were female.Pulmonary tuberculosis was seen in 84.2% in patients. The overall frequency of HTLV1 in TB patients was2.9% and 3.3% in controls.HTLV1 frequency was higher in male cases and increased with ageing.
**Conclusion:** Regarding to this study HTLV 1 infection alone,is not sufficient to increase the risk of tuberculosis.

**P3814**
**Application of GenoTypeCM and GenoTypeAS to species identification of nontuberculous mycobacteria as a routine method in microbiology lab of CTRI, Moscow**
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There is a acute problem with diagnostics of mycobacteriosis in Russia. Species identification of mycobacterial cultures is not performed in bacteriology labs over Russia. There are no any statistical data for the incidence of NTM detection.
We aimed to evaluate the level of NTM detection at examination of cultures from BACTEC MGIT 960 (BD, USA) in the course of 2010.
1520 positive MGIT tubes (obtained in 2010) were studied. Primary screening of cultures was performed using standard microbiological techniques – blood agar culture and Ziehl-Neelsen stain. In addition real-time PCR IS6110 was done for all 1520 cultures. For suspected samples species identification using GenoTypeCM/AS (HAIN Lifescience, Germany) were carried out.
Of 1520 cultures, 75 cultures (4,9%) belonged to NTM. Using GenoTypeCM/AS we performed species identification for 72 strains from 75. Following spectra of NTM strains were detected: M.fortuitum (15 strains), M.peregrinum (2 strains), M.chelonae (5 strains), M.abscessus (6 strains), M.xenopi (3 strains), M.gordonae (5 strains), M.simiae (7 strains), M.lentiflavum (4 strains), M.kansasii (9 strains), M.avium (13 strains), M.intracellulare (3 strains). One of 3 detected by Hain test-system as a Mycobacterium ssp. strains belonged to M.nonchromogenicum according to sequence of 16S rRNA.Data allowed to conclude that 4,9% of 1520 cultures obtained from BACTEC MGIT 960 were NTM. Usage of exact and rapid molecular-genetic method - GenoTypeCM/AS helps to perform accuracy species identification of NTM, it helps physicians to ensure accurate diagnosis and prescribe adequate treatment.

**P3815**
**The molecular mechanisms of lymphocyte apoptosis disregulation at Th1- and Th2- cytokine disbalance**
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Much of the literature elevates the Th1/Th2 balance concept to the level of paradigm. Although Th1 and Th2 cells are now virtually anointed with the responsibility for coordinating the immune system, critical investigators are finding discrepancies in the hypothesis (Dent, L.A. J Reprod Immunol 2002; 57: 255-272). By the other hand apoptosis is one of the fundamental mechanisms of immune reactions regulation. So the purpose of our research was to establish the molecular mechanisms of cytokine-mediated lymphocyte apoptosis disregulaion at polarization of the immune answer on Th1- or Th2-ways.
We assessed the concentration of IL-2, IL-4, IL-10, IL-12 and TNFα in supernatant fluid of mononuclear leukocytes of 44 people (21 man and 23 women from 18 to 50) with acute and chronic tick-borne encephalitis and 23 healthy donors by dint of enzyme-linked immunosorbent assay. Secondly we analyzed the level of spontaneous and IL-2-, IL-4- and TNFα-induced apoptosis, the quantity of complementary receptors by flow cytometry. In addition the assessment of Bax, Bad, Bcl-2, Bcl-xl, p53 and NF-kB level was done due to immunoblotting.
It was established that influence of cytokines on the apoptosis realization has dose-dependent character. Features of apoptosis realization revealed in experimentally created cytokine imbalance conditions by means of recombinant IL-2, IL-4 and TNFα due to change of a transcriptional factors level (p53, NF-kB) and pro-/antiapoptotic proteins correlation (Bax, Bad, Bcl-2, Bcl-xl). Molecular mechanisms of cytokine-mediated apoptosis disregulation can form a basis for development of immune cell modulation methodology at the immune answer polarization for Th1-or Th2-ways.

**P3816**
**Diverse effects of PAI-1 proteins on lung and prostate cancer cell invasion**
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Acquisition of ability to uncontrolled migration is one of the fundamental properties of cancer cells, enabling them to infiltrate tissues and metastasize. PAI-1 is the major physiological inhibitor of urokinase (uPA), which plays a key role in migration and invasion of tumor cells. The aim of present study was to analyze the impact of increasing concentrations of PAI-1 mutated forms: VLHL PAI-1 with very long half-life time, Vn neg PAI-1 - devoid of affinity towards vitronectin and wPAI -1 on lung (A549, H1299) and prostate (LNCaP, DU145) cancer cells invasive activity. Selected cell lines are characterized by different (normal and high) urokinase production.
No effect of PAI-1 proteins on invasiveness of lung cancer cells was observed, while dose-dependent significant inhibition was demonstrated in both prostate cancer lines (DU145 and LNCaP) cultured with VLHL PAI-1 (respectively $p<0,05$ and $p<0,01$) and Vn PAI-1 neg ($p<0,05$). Not surprisingly wPAI-1 significantly stimulated prostate cancer cells invasiveness in all concentrations.
PAI-1 inhibitory effect on prostate cancer invasive activity is associated with anti-proteinase activity. Lung cancer cells invasiveness regulation seems not to be PAI-1-urokinase regulated.

**P3817**
**Role of bronchoscopy in lung cancer translational research: Tumor harvest for the lung cancer mutation consortium (LCMC) studies**
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**Introduction:** The LCMC is a multi-institution collaboration to collect 1,000 cases of advanced (stage IIIB/IV) Adenocarcinoma for rapid analysis of multiple genes felt important in LC pathogenesis, to accelerate biomarker validation & discovery, to develop novel therapeutics.
**Aim:** To address the need to harvest sufficient tumor from subjects who are often not suitable surgical candidates. To advance bronchoscopic harvest of tumor sufficient for multigene studies.
**Method:** 68 lung AdenocarCA pts consented (3-10 and 2-11) for sending tumor tissue to Univ Colorado for multiple molecular analysis – FISH analysis for MET gene & ALK protein fusion, gene mutations by SNaPshot testing: APC, AKT1, BRAF,CTNNB1, EGFR, FLT3, JAK2, KIT, KRAS, MAP2K1, NOTCH1, NRAS, PIK3CA, PTEN, TP53. One pathologist (P.I.) review slides for tumor cellularity and adequacy for 20 extra slides.
**Results:** 2 subjects ineligible (non AC, withdrew), 15 QNS, 33 with tissue successfully sent, 18 awaiting review of slides. Material deemed insufficient - 4 effusion cell block, 2 excised nodes, 9 bronch samples but 3/9 had sufficient tissue for in-house testing KRAS,EGFR,ALK, hence likely exhausted.
Of 33 successful send-out: 17 lung surgery, 5 brain mets, 2 pleural met biopsies, 2 CT core bx. 7/33 (21%) from bronchs: 3 endo & 2 each transbronchial forceps and TBNA.
**Conclusion:** Of 16 bronch specimens evaluated for multiplex tumor pathway testing after local site clinical testing, 7 (44%) adequate, 6 (37%) QNS, 3 (19%) likely used up for local biomarker testing. With additional effort at tumor collection at time of diagnosis, large panel testing for tumor biomarkers should be possible in a majority of cases.

**P3818**
**GSTT11 and CYP1A1 polymorphisms, tobacco smoking and lung cancer in northern India**
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**Background:** Susceptibility to lung cancer has been shown to be modulated by inheritance of polymorphic genes encoding cytochrome P450 1A1 (CYP1A1) and glutathione S transferases (GSTT1), which are involved in the bioactivation and detoxification of environmental toxins.
**Aim:** To investigated the role of *GSTT1* and *CYP1A1 -6235T>C* polymorphisms in the susceptibility to lung cancer patients in northern India.
**Methodology:** We have recruited 456 study subjects, 218 cases of lung cancer and 238 healthy controls were recruited from northern India. healthy Controls, age sex matched, and Sociodemographic information by interviewed. This study was conducted to investigate putative interactions of environmental toxicants mainly PAH with genetic susceptibility. Extraction of genomic DNA and genotyping of variants in *-6235 T>C* (rs4646903) and *GSTT1* were conducted to investigated the role of polymorphisms in the susceptibility to lung cancer patients in northern India. subjects were genotyped through polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).
**Results:** The frequencies of *GSTT1* Null, alleles were significantly differ between patients and controls, with odds ratio [OR] =0.52; 95% confidence interval [CI] = 0.35-0.79; p= 0.002, but *-6235 T>C* genotype were not associate with lung cancer patients with healthy controls.GSTT1 null genotype were significant associated

with the smoker lung cancer patients, with odds ratio [OR] = 1.76; 95% [CI] = 1.17-2.66; p= 0.007.
**Conclusion:** In this northern Indian population, 86.7% of male and 13.3% of female lung cancer incidence were explained by tobacco smoking. Deletion polymorphisms of the *GSTT1* had an effect on the risk of lung cancer.

**P3819**
**Comparing LYVE-1 and D2-40 expression in small cell lung carcinomas (SCLC); association with clinical parameters and lymphatic invasion**
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**Introduction:** Lymphangiogenesis is actively facilitating tumor metastasis.LYVE-1 and D2-40 are two new lymphatic endothelial markers;their expression and prognostic impact in SCLC still remain vague.
**Aim:** To investigate the lymphangiogenetic expression in SCLC we measured the intratumoral lymphatic microvessel density (ILMVD), the lymphatic invasion (L.I) and their correlation with clinical parameters.
**Materials and methods:** Retrospective study of 55 SCLC patients (mean age:68.1 years,range 40-89). Histological specimens from all patients were immunohistochemically stained for D2-40 (epitope of podoplanin) and LYVE-1 (CD44 homolog). Calculation of ILMVD and assessment of L.I were performed and correlation with clinical data followed.
**Results:** D2-40 and LYVE-1 were expressed in all specimens whilst L.I in 59.6%. D2-40 and LYVE-1 ILMVD were associated with the stage at diagnosis (p=0.017 and p=0.03 respectively) and L.I (p=0.00 and p=0.02 respectively). D2-40 ILMVD was particularly associated with certain target organs for metastasis (liver:p=0.001,adrenals: p=0.046,brain: p=0.007). L.I was associated with stage (p=0.00) and all target organs for metastasis ($p<0.05$). ILMVD D2-40 and LYVE-1 weren't associated with L.I and survival.
**Conclusions:** D2-40 and LYVE-1 and L.I were highly expressed in SCLC and independently associated with poor patient outcome, thus presenting new insights in prognosis. D2-40 presented a more potent role by associating with certain target organs for metastases. Defining subgroups of patients with poorer prognosis at the time of diagnosis could reinforce the development and application of new targeted therapeutic strategies.