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Reliability of EGFR mutations detection in NSCLC brain metastases by two different allele-specific PCR methods

Michal Skronski[1], Pawel Krawczyk[2], Kamila Wojas-Krawczyk[2], Bozena Jarosz[3], Tomasz Kucharczyk[2,5], Paulina Jagus[1], Marek Sawicki[4], Barbara Wilczynska[4], Tomasz Trojanowski[3], Janusz Milanowski[2,6], Joanna Chorostowska-Wynimko[1]. *[1]Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; [2]Department of Pneumonology Oncology and Allergology, Medical University of Lublin, Lublin, Poland; [3]Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Lublin, Poland; [4]Department of Thoracic Surgery, Medical University of Lublin, Lublin, Poland; [5]Postgraduate School of Molecular Medicine, SMM, Warsaw, Poland; [6]Unit of Fibroproliferative Diseases, Institute of Agricultural Medicine, Lublin, Poland*

Reliable detection of EGFR mutations in lung cancer metastases requires highly sensitive and robust molecular method.
Our aim was to assess effectiveness of two highly sensitive PCR methods in detection of EGFR activating mutations in FFPE samples of brain metastases from 142 NSCLC patients.
Isolated DNA was analyzed for EGFR exon 19 deletions and exon 21 L858R mutations by real-time PCR PNA-LNA PCR clamp and allele-specific PCR (ASP-PCR). If discrepant, results were re-evaluated by TaqMan genotyping. Direct sequencing analyses are ongoing.
All samples were successfully analyzed. In 37 samples (26%) DNA was of low quality and PCR pre-amplification was performed prior to PNA-LNA PCR clamp analysis. In total 11 out of 142 samples (8%) proved positive for EGFR activating mutations. 6 samples (55% of detected) were positive for exon 19 deletions (n=3) and L858R substitution (n=3) as assessed by both PNA-LNA PCR clamp and ASP-PCR. ASP-PCR, but not PNA-LNA PCR clamp, detected 3 further L858R mutations, whereas 1 L858R substitution and 1 rare A859T mutation were detected by PNA-LNA PCR clamp only. None of discrepant L858R substitutions were confirmed by TaqMan genotyping.
Different approach to molecular diagnostics represented by two highly sensitive methods employed in our study might be responsible for observed discrepancies. ASP-PCR utilizes allele-specific primers while in PNA-LNA PCR clamp method PNA probe inhibits wild type allele amplification. In FFPE samples with low tumor cells content, where direct sequencing is not applicable, it might be advisable to re-evaluate diagnostic results by another equally sensitive, but methodologically different diagnostic technique.

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The role of the regulated retrotransposon transcriptome in asthma

N. Miglino, M. Roth, Michael Tamm, P. Borger. *Department Biomedicine, University Hospital, Basel, Switzerland*

Rationale: Genetic studies identified over 200 asthma susceptibility genes that associate with the asthmatic pathology. In the light of the novel functions for mobile DNA elements, we propose that complex diseases, such as asthma, may result from transposed and transposable elements (TEs) which integrate in asthma susceptibility genes or in their regulatory elements.
Objectives: To investigate whether TEs may cause asthma. Therefore, we performed (i) in-silico analysis of TEs in selected asthma susceptibility genes which could potentially function as transcription modulators; (ii) establish CAGE libraries (cap analysis of gene expression) using human lung tissue of asthma patients and of healthy controls; and (iii) analyze the transcriptome of the lung tissue CAGE libraries.
Methods: The gene sequence of twelve asthma susceptibility genes (DPP10, CYFP2, HLAG, GPRA, SFRS8, PHF11, ADAM33, PCDH1, CH13L1, ORMDL3, PDE4D, DENN1B) were analyzed in silico for the presence of TEs. In addition, we analyzed the 100'000 5'-upstream bp region in order to localize TEs that can potentially act as alternative promoters/enhancers.
Results: In silico analysis showed that the TE content in the 12 analyzed gene

ranged from 8% (ADAM33) to 49% (GPRA). Within the 12 genes we observed a significant gene-specific distribution of the TE types (SINE, LINE, LTR). The analysis of the upstream 5'-regions showed that the TE content ranged from 28% to 58%.
Outlook: DNA sequencing of the named genes will reveal differences in TE content in asthma vs. control. CAGE analysis will enable us to compare the retrotransposon transcriptome of asthma and controls.

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Quantitative proteomics on nasal lavage fluid from asthma phenotypes
Linda Ekerljung[1], Serena O'Neil[1], Sara Hansson[2], Carina Sihlbom[2], Jan Lötvall[1], Bo Lundbäck[1]. *[1]Krefting Reseach Centre, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [2]Proteomics Core Facility, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

Asthma is a complex disease composed of many phenotypes with different underlying mechanisms. In line with the united airways concept, identification of proteins in nasal lavage fluid (NLF) could reflect the proteome of the lung. This study aimed to explore differences in mechanisms between asthma phenotypes using quantitative proteomics on NLF.
NLF was collected from 3 groups of asthmatics; aspirin intolerant asthma (AIA, n=9), multi-symptom asthma (MSA, n=9), and MSA with chronic rhinosinusitis (CRS, n=9) and a group of healthy subjects (n=9). Proteins extracted from NLF were quantified using tandem mass tag technology and a nano-LC MS/MS instrument. Pathway analysis was used to identify global proteome differences.
In all, 474 proteins could be identified and quantified in at least 3 samples per group. In total, 93 proteins in AIA, 113 proteins in CRS and 75 proteins in MSA met the fold change cut off of 1.3 compared to healthy. Of these, 21 proteins were found in all asthma phenotypes, which could reflect common processes in asthma, while 33 proteins were unique to AIA, 56 to CRS and 26 to MSA. Analysis of the global proteomes revealed mechanistic differences between the phenotypes. CRS was more associated with epithelial associated conditions than AIA and MSA. AIA was more associated with carbohydrate metabolism than CRS and MSA, which had more proteins associated with cellular movement and signalling.
Evidence of inflammatory processes can be found in different asthma phenotypes compared to healthy individuals. A comparison between asthma phenotypes revealed mechanistic differences. Quantitative proteomic analysis of NLF was thus capable of detecting relevant disease mechanisms in different asthma phenotypes.

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Regulation of microRNA-mRNA target pairs in a model of bronchopulmonary dysplasia
Christopher Collura[1], William Carey[1], Jie Dong[2], Y.S. Prakash[3], Dennis Wigle[2]. *[1]Division of Neonatal Medicine, Mayo Clinic, Rochester, MN, United States; [2]Division of General Thoracic Surgery, Mayo Clinic, Rochester, MN, United States; [3]Department of Anesthesia, Mayo Clinic, Rochester, MN, United States*

Background: Bronchopulmonary dysplasia (BPD) is a chronic lung disease of premature neonates characterized by arrested pulmonary alveolar development.
Objective: Because microRNAs (miRNAs) may regulate the translation of messenger RNAs (mRNAs) during normal lung organogenesis, we hypothesized that an experimental model of BPD would be characterized by the altered expression of miRNAs and their mRNA targets.
Methods: Neonatal mice were exposed to 80% oxygen (O_2) or room air (RA) for either 14 or 29 days. Lung histology was assessed using standard techniques. Comprehensive miRNA and mRNA profiling was performed using lung tissue from each group. Potential direct mRNA targets of miRNAs were systematically predicted through miRNA-mRNA correlations and computational mapping in miRBase. Functional significance was investigated using Gene Ontology (GO) term enrichment analysis for miRNA regulatory networks using the DAVID and MetaCore databases.
Results: At both 14 and 29 days, the lungs of O_2 mice displayed histological changes consistent with BPD. Between the two time points we identified 2,714 mRNAs and 66 miRNAs that were dynamically regulated by O_2 exposure. All but one of the miRNAs were up-regulated. We identified 581 dynamically regulated, direct mRNA targets of these miRNAs by computational mapping in miRBase. Gene ontology enrichment and pathway analysis revealed that hyperoxia modulated genes involved in a variety of lung developmental processes, including cell cycle, cell adhesion, inflammation and angiogenesis.
Conclusion: A murine model of BPD is characterized by dynamic regulation of miRNAs and their computationally predicted, developmentally relevant mRNA targets.

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Krüppel-like zinc finger proteins in end-stage COPD lungs with and without severe alpha1-antitrypsin deficiency
Rembert Koczulla[2], Jonigk Danny[3], Thomas Wolf[8,6], Christian Herr[7], Sarah Noeske[2], Walter Klepetko[5], Sabine Wrenger[1], Claus Vogelmeier[3,2], Nils von Neuhoff[8], Heiko Golpon[1], Robert Vosswinkel[4], Tobias Welte[1], Sabina Janciauskiene[1]. *[1]Department of Respiratory Medicine, Hanover Medical School, Hanover, Germany;[2]Division of Pulmonary Diseases, Philipps University Marburg, Germany; [3]Institute for Pathology, Hanover Medical School, Hanover, Germany; [4]University of Giessen Lung Center, University Hospital, Giessen, Germany; [5]Division of Thoracic Surgery, Medical University of Vienna, Austria; [6]Department of Theoretical Bioinformatics, German Cancer Research Center DKFZ, Heidelberg, Germany; [7]Department of Internal Medicine V, Pulmonology, Allergology, Respiratory and Environmental Medicine, Saarland University, Homburg/Saar, Germany; [8]Institute of Molecular Pathology, Hanover Medical School, Hanover, Germany*

Chronic obstructive pulmonary disease (COPD) is influenced by environmental and genetic factors. An important fraction of COPD cases harbor a major genetic determinant, inherited ZZ (Glu342Lys) α1-antitrypsin deficiency (AATD). Severe, ZZ AATD is associated with a predisposition to early onset, rapidly progressive COPD where emphysema is a major component. We hypothesized that gene expression pattern differs in end-stage COPD with and without AATD. Tissues from explanted lungs of end-stage AATD-related (ZZ, n=3, never treated with AAT augmentation therapy) and "normal" (MM, n=3) COPD were used for microarray gene expression analysis. A total of 162 genes were found to be differentially expressed (p-value $\leq$ 0.05 and $|FC| \geq 2$) between MM and ZZ COPD patients. Of those, 134 gene sets were up-regulated and 28 were down-regulated in ZZ relative to MM lung tissue. A subgroup of genes, zinc finger protein 165, snail homolog 1 (Drosophila), and Krüppel-like transcription factors (KLFs) 4 (gut), 9 and 10, perfectly segregated ZZ and MM COPD patients. The relative expression of KLF 9 and 10 was higher in lung and in liver cirrhosis tissue from ZZ (n=6) compared to MM (n=6) as verified by RT-PCR. Genes associated with COPD or lung function decline generally come from three groups: protease-antiprotease, oxidant/antioxidant and immune/inflammatory mediators. In this small cohort, we show that end-stage COPD patients with and without AATD can be perfectly grouped by the cluster of the zinc-finger family of transcriptional regulators. Our data provide new insight into the putative difference in the mechanisms involved in COPD development in subjects with and without inherited AATD.

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Decreased Wnt/β-catenin signaling in COPD: Possible involvement of Wnt antagonists
Ling-li Guo[1], Tao Wang[1], Lian Liu[1], Chun Wan[2], Fu-qiang Wen[1,2]. *[1]Division of Pulmonary Diseases, State Key Laboratory of Biotherapy of China, West China Hospital of Sichuan University, Chengdu, Sichuan, China; [2]Department of Respiratory Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China*

Background: COPD is a devastating disease characterized by chronic bronchitis and emphysema. The Wnt/β-catenin signaling has been implicated in emphysema, but its role in the pathogenesis of chronic bronchitis remains unknown.
Methods: Human lung tissues were obtained from 4 groups of patients: healthy nosmokers (n=7), healthy smokers (n=7), COPD nosmokers (n=10) and COPD smokers (n=18). Human airway epithelial cells (*16HBE*) were treated with cigarette smoke extract (CSE; 0, 2%, 4% or 8%) for 24h. Mice were treated with cigarette smoke (CS) exposure (200mg TPM/m^3, 4h/d, 5d/wk) and SB-216763 injection (a GSK3β inhibitor, 3mg/kg/d, *i.p.*) for 1 month. The activation of Wnt/β-catenin signaling was analyzed by RT-PCR and western blot.
Results: We observed a significant increase in mRNA levels of Wnt antagonist Wif-1 (10.5-fold) and a decrease in Wnt receptor Fzd4 expression in COPD smokers as compared to COPD nonsmokers. Expression of β-catenin/Tcf4 was also reduced in COPD smokers compared with healthy smokers, indicating impaired Wnt activity in smoking-related COPD. In 16HBE cells, CSE induced the expression of Wnt antagonists Wif-1 and Dkk1, while reduced β-catenin and Tcf4/Lef1 expression. In vivo, the mRNA levels of Wnt antagonists Wif-1 and Sfrp1 were elevated 2.5-fold and 1.6-fold respectively in CS-exposed mice. SB-216763 treatment attenuated CS–induced BALF total leukocyte and macrophage accumulation, airway inflammation and β-catenin downregulation.
Conclusion: Decreased Wnt/β-catenin signaling may be involved in airway inflammation and airway epithelium injury. Overexpression of Wnt antagonists may contribute to this process, implicating potential therapeutic targets for the treatment of COPD.

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ADAM33 protein found in bronchial brushings and biopsies is increased in bronchial carcinomas
Romana Mikes[1], Bernd Lamprecht[1], Allison-Lynn Andrews[2], Stephen T. Holgate[2], Donna E. Davies[2], Michael Studnicka[1], Hans Michael Haitchi[2]. *[1]Department of Pulmonary Medicine, Paracelsus Medical University, Salzburg, Austria; [2]Roger Brooke Laboratory, Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, United Kingdom*

Background: The asthma and COPD susceptibility gene, *ADAM33*, is selectively expressed in mesenchymal cells and the activity of soluble ADAM33 has been linked to angiogenesis and airway remodeling.
Aims: We hypothesized that *ADAM33* mRNA & protein are differentially expressed in bronchial biopsies from healthy airways and bronchial carcinomas.
Methods: Paired primary bronchial fibroblasts (n=4) from healthy and tumor tissue were grown +/– TGFβ2 to induce myofibroblast differentiation. Fibroblasts,

bronchial biopsies (n=12) and brushings (n=12) were analysed for ADAM33 expression using quantitative RT-PCR and western blotting. Immunohistochemistry for ADAM33 was performed on bronchial biopsies.
Results: TGFβ2 caused induction of α-SMA and suppression of *ADAM33* mRNA expression in normal and tumor fibroblasts. *ADAM33* mRNA expression tended to be decreased in tumor biopsies whereas ADAM33 protein expression was significantly increased (bands of 45 and 75 kDa). In bronchial brushings *ADAM33* mRNA was not detectable. However, there was a single band at ~75kDa for ADAM33 and also specific staining for ADAM33 in the epithelium of bronchial biopsies.
Conclusions: Similar to cells from healthy and asthmatic volunteers TGFβ suppressed expression of *ADAM33* mRNA in normal and tumor fibroblasts. ADAM33 protein was increased in bronchial tumor biopsies suggesting potential roles in tumorigenesis and growth. The presence of ADAM33 protein in bronchial brushings and biopsies in the absence of ADAM33 mRNA expression in brushings suggests that the mesenchyme is the source for ADAM33 protein in the epithelium.
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Independent validation of prognostic value of 22 microRNAs (miRs) in stage I-II lung adenocarcinoma (AC)

Marcin Skrzypski[1], Krzysztof Goryca[2,3], Amelia Szymanowska-Narloch[4], Ewa Jassem[4], Piotr Czapiewski[5], Wojciech Biernat[5], Ryszard Pawlowski[6], Witold Rzyman[7], Jacek Jassem[1]. *[1]Oncology and Radiotherapy, Medical University of Gdansk, Pomorskie, Poland; [2]Department of Oncological Genetics, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Mazowieckie, Poland; [3]Laboratory of Bioinformatics and Systems Biology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Mazowieckie, Poland; [4]Allergology and Pulmonology, Medical University of Gdansk, Pomorskie, Poland; [5]Pathology, Medical University of Gdansk, Pomorskie, Poland; [6]Forensic Medicine, Medical University of Gdansk, Pomorskie, Poland; [7]Thoracic Surgery, Medical University of Gdansk, Pomorskie, Poland*

Background: About 50% of NSCLC patients (pts) will develop distant metastases following pulmonary resection. Currently, apart from clinical stage at diagnosis, there are no reliable factors to select the high risk pts for adjuvant chemotherapy. We previously demonstrated prognostic value of 22 miRs in frozen tissue samples of early stage SqCLC, and the feasibility of their expression assessment in formalin fixed paraffin embedded (FFPE) samples (Skrzypski et. al. J Clin Oncol 2010; 28;15s). In this study, we validated the prognostic value of these miRs in an independent cohort of early AC pts.
Methods: FFPE tumor samples were obtained from 82 stage I-II AC pts who underwent radical pulmonary resection, 44% of whom developed distant metastases. Median follow-up of pts who did not develop metastases was 5.53 years (range, 3,01-8.9 years). miRs were isolated from tumor tissue with RecoverAll kit (Ambion). Expression of 22 miRs previously found to be related to the risk of metastases was analyzed by RT-PCR assay (Appliedbiosystems). Raw data were normalized vs. the expression of U6. Individual miRs were correlated with distant metastases-free survival (MFS).
Results: MiR-222* (p=0,0003) and miR-222 (p=0,002) were significantly related to MFS. Using the median of the miR-222* expression as a cut-off value, the median MFS was 2.12 years in the high risk group, and not reached in the low risk group (HR=1.95). Using the median of the miR-222 expression as a cut-off value, the median MFS was 2.56 years in the high risk group, and not reached in the low risk group (HR=1.78).
Conclusions: MiR-222* and miR-222 are strong predictors of distant relapse in operable early AC.