sion varies depending on the histological type of NSCLC. Identification of tumor immunophenotype makes possible to obtain more data about metastasis potential, malignancy rate and prognosis. This molecular markers panel can be used for early detection of LC recurring and metastasis.

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P55. IN SITU MOLECULAR ASSESSMENT OF CD8+ T CELL REACTIVITY IN NSCLC

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Background: While in cancer from colon and ovary number and reactivity of tumor infiltrating lymphocytes (TILs) were associated with survival, in NSCLC, the clinical significance of TILs is unknown. While TILs demonstrated antigen specific Interferon (IFN)-γ secretion and cytotoxicity in vitro, their in situ activation status remains difficult to determine. Here, we examine molecular assessment of CD8+ T cell reactivity in NSCLC.
Methods: We determined CD8+ T cell counts in 19 patients with NSCLC by immunohisto-chemistry and assessed T-cell immune reactivity by measuring mRNA IFN-γ levels by quantitative RT-PCR (TaqMan) from corresponding tissue. CD8+ T cells were classified into two groups: Intra-tumoral (distributed in cancer cell nests and stroma) and peri-tumoral (presented along the invasive margin). Five hpf were evaluated per each localization (IT and PT, respectively). Distribution of CD8+ T cells was semi-quantitatively classified into: 0-none or mild; I-moderate; II-severe. The relationship between the median CD8+ T cell density/hpf and the CD8-normalized IFN-γ mRNA expression was tested (Spearman's correlation).
Results: Semi-quantitative analysis revealed significantly higher CD8+ T cell counts within the tumor compared to the invasive margin ($p < 0.001$). However, immune activation status of TILs represented as IFN-γ/CD8 ratio was higher in the peri-tumoral than in the intra-tumoral compartments ($p = .022$).
Conclusion: In human NSCLC, IFN-γ reactivity of CD8+ T cells is mostly attributed to the tumor–host interface and indicates an inadequate activation of TILs within the tumor. This methodology can be applied to a variety of experimental trials if tumor tissue is available.

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P56. UPREGULATION OF COX-2 EXPRESSION OCCURS ALREADY IN GASTROESOPHAGEAL REFLUX DISEASE BUT IS FURTHER INCREASED IN BARRETT'S ESOPHAGUS AND BARRETT'S CANCER

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Background: Cyclooxygenase-2 (COX-2) mRNA expression is known to be progressively increased in the metaplasia–dysplasia–adenocarcinoma (MDA) sequence of Barrett's cancer (BC) development. Much less however, is known about COX-2 mRNA expression in patients with gastroesophageal reflux disease (GERD).
Methods: Endoscopic biopsies from 3 patient groups were analyzed: 43 patients undergoing evaluation for GERD (20 GERD positive/23 GERD negative) by 24-h pH-monitoring, 20 patients with Barrett's esophagus (BE) without dysplasia and 47 with BC. COX-2 mRNA expression was determined by quantitative real-time RT-PCR (TaqMan™) assays. The Demeester composite score (DS > 14.72) was used to match COX-2 mRNA expression levels with the degree of acid exposure.
Results: Median COX-2 mRNA expression was significantly upregulated in Barrett's metaplastic epithelium compared to matched normal squamous epithelium (NE) in the BE ($p = 0.03$) and BC group ($p = 0.001$). COX-2 mRNA expression levels in NE did not differ significantly between the 3 study groups however, within the group of patients evaluated for GERD, specimens obtained from patients with a mean Deemester score >14.72 showed significantly upregulated COX-2 mRNA levels in the distal acid-exposed esophagus ($p = 0.01$).
Conclusion: Our findings suggest that the induction of increased COX-2 mRNA expression occurs already in GERD without the presence of Barrett's metaplasia. A field effect as shown for other genes could not be detected for COX-2 expression in squamous epithelium in GERD positive, BE or BC patient groups. Chemoprevention strategies using selective or non-selective COX-2 inhibitors might be useful in patients with GERD to potentially prevent the development of Barrett's metaplasia.

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P57. ASSOCIATION OF CYCLOOXYGENASE-2 EXPRESSION WITH DEVELOPMENT AND PROGRESSION OF BARRETT'S METAPLASIA–DYSPLASIA–CARCINOMA SEQUENCE AND THE ENVIRONMENTAL INFLAMMATORY REACTION

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Background: Epidemiological data assume a reduction of risk for developing an adenocarcinoma of the esophagus for individuals taking non-steroidal anti-inflammatory drugs. One of the inhibited enzymes, cyclooxygenase-2, is supposed to be involved in the pathogenesis of Barrett's cancer. We examined a possible association between COX-2 protein expression and the progression of Barrett's sequence and the type and degree of the environmental inflammatory reaction.
Methods: Squamous epithelium, metaplastic, low-grade and high-grade dysplastic lesions and tumor tissue of 49 resection specimens from patients with Barrett's adenocarcinoma were analyzed. Immunohistochemical staining was performed with a

monoclonal anti-COX-2-antibody, active and chronic inflammatory reactions were classified according to the Updated-Sydney-System.

Results: Within Barrett's sequence, a significant progressive increase in COX-2 expression was identified ($p < 0.0001$). The most significant differences were detected between squamous epithelium and Barrett's metaplasia ($p < 0.001$) and from low- to high-grade dysplasia ($p < 0.0001$). Active and chronic inflammation were significantly different between squamous epithelium and Barrett's metaplasia ($p < 0.0001$) but not during further progression in the MDC sequence.

Conclusion: There is a significant association between increasing COX-2 protein levels and the development and progression of Barrett's MDC sequence. Increasing COX-2 expression is associated with a change in type or degree of the associated morphological inflammation in Barrett's metaplasia but not during progression of MDC. Chemoprevention strategies aiming at a reduction of COX-2 expression can likely not be monitored by morphological analysis of the inflammatory reaction.

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P58. HIF-1α mRNA IS SIGNIFICANTLY OVEREXPRESSED IN ESOPHAGEAL SQUAMOUS CELL CANCER BUT NOT ASSOCIATED WITH HISTOPATHOLOGIC REGRESSION FOLLOWING NEOADJUVANT CHEMORADIATION

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Background: Hypoxia-inducible factor-1α (HIF-1α) expression was reported to be associated with tumor growth, progression and resistance to radio- and chemotherapy. We analyzed if HIF-1α mRNA or protein expression is associated with histomorphologic response or prognosis following neoadjuvant chemoradiation and surgery in locally advanced esophageal cancers.

Methods: Fifty-three patients with resectable, locally advanced esophageal cancers (cT2-4, N_x, M_0) received neoadjuvant chemoradiation (cisplatin, 5-FU, 36 Gy) followed by transthoracic en bloc esophagectomy. RNA was isolated from endoscopic biopsies (paired tumor and normal tissue) prior to neoadjuvant treatment and quantitative real-time reverse transcriptase PCR (RT-PCR, TaqMan™) assays were performed to determine HIF-1α mRNA expression. HIF-1α protein expression in pretreatment biopsies and posttherapeutic resection specimens was analyzed by immunostaining of tumor cells.

Results: In squamous cell cancer, HIF-1α mRNA expression was significantly higher in tumor tissue as in paired normal epithelium (Wilcoxon test: $p < 0.001$). Normal squamous epithelium showed significant elevated expression in adenocarcinomas suggesting a field effect (Mann–Whitney test: $p < 0.04$). HIF-1α protein expression showed a significant downregulation after chemoradiation.

Conclusion: However, neither HIF-1α mRNA nor protein expression was associated with histomorphologic regression or prognosis following neoadjuvant chemoradiation and surgery in locally advanced esophageal cancers. HIF-1α mRNA expression is differentially upregulated in esophageal squamous cell cancer compared to adenocarcinomas however does not predict tumor regression or prognosis.

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P59. TOWARDS THE MOLECULAR CHARACTERIZATION OF DISEASE: COMPARISON OF MOLECULAR AND HISTOLOGICAL ANALYSIS OF ESOPHAGEAL EPITHELIA

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Background: Reliable quantification of gene expression offers the possibility of more accurate and prognostically relevant characterization of tissues than potentially subjective interpretations of histopathologists. The aim of this study was to evaluate the feasibility of molecular characterization of normal and pathologic esophageal epithelia. Therefore we measured the expression of 18 selected genes and compared them to histological features in a spectrum of esophageal disease.

Methods: Esophageal tissue biopsies from patients with foregut symptoms were laser-capture microdissected and the expression levels of 18 selected genes were measured by QRT-PCR (Taqman®). Linear discriminant analysis, which uses combinations of genes to distinguish between histological groups, was performed to compare gene expression and the following 5 histological groups: (1) normal squamous epithelium, ($n = 32$); (2) reflux-esophagitis, ($n = 13$); (3) non-dysplastic Barrett's, ($n = 17$); (4) dysplastic Barrett's, ($n = 10$); (5) adenocarcinoma, ($n = 22$).

Results: A panel of 7 genes had 90–94% predictive power to distinguish non-dysplastic and dysplastic Barrett's esophagus. Clustering analysis revealed structure in gene expression values even in the absence of histology. Expression levels in 17 genes differed significantly across histological groups. Classification based on gene expression agreed with histopathological assessment in the following percentage of cases: normal squamous epithelium = 53%, reflux-esophagitis = 31%, non-dysplastic Barrett's = 76%, dysplastic Barrett's=40% and adenocarcinoma = 59%. Interestingly, predictive power improved markedly when inflammatory and dysplastic tissues were removed (77–94%).

Conclusion: Gene expression classification agrees well with histopathological examination. When differences occur, it is unclear whether this effect is due to intra-observer variability in pathological diagnosis or to a genuine difference between gene expression and histopathology.

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