HABILITATIONSSCHRIFT

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„Molecular markers in primary neoplasms of the lung – Implications for diagnosis and outcome“

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Sechs ausgewählte Arbeiten zum gemeinsamen Thema mit Angabe von Impact Factor (IF) und Ranking nach ISI 2008:

1. Schmid K, Variu S, Herberger B, Schuhmacher U.

Expression of ubiquitin C-terminal hydrolase L1 in neuroendocrine tumours of the lung. Lung Cancer, submitted on Oct. 3Ist, 2009. *IF: 2,790 (Ranking: Oncology Standard)*

2. Schmid K, Angerstein N, Geleff S, Gschwendtner A.

Quantitative nuclear texture features analysis confirms WHO classification 2004 for lung carcinomas. Mod Pathol. 2006;19:453-9. *IF 4,678 (Ranking: Pathology Top)*

3. Schmid K, Birner P, Gravenhorst V, End A, Geleff S.

Prognostic value of lymphatic and blood vessel invasion in neuroendocrine tumors of the lung. Am J Surg Pathol. 2005;29:324-8. *IF: 4,020 (Ranking: Pathology Top)*

4. Filipits M, Haddad V, Schmid K, Huynh A, Dunant A, André F, Brambilla E, Stahel R, Pignon JP, Soria JC, Popper HH, Le Chevalier T, Pirker R.

Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program. Clin Cancer Res. 2007;13:3892-8. *IF: 6,488 (Ranking: Oncology Top)*

5. Filipits M, Pirker R, Dunant A, Lantuejoul S, Schmid K, Huynh A, Haddad V, André F, Stahel R, Pignon JP, Soria JC, Popper HH, Le Chevalier T, Brambilla E.

Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program. J Clin Oncol. 2007;25;2735-40. *IF: 17,157 (Ranking: Oncology Top)*

6. Schmid K, Oehl Natalie, Wrba F, Pirker R, Pirker C, Filipits.

EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding loco-regional lymph node metastases. Clin Cancer Res. 2009;15:4554-60, *IF: 6,488 (Ranking: Oncology Top)*

EXPOSÉ

„ Molecular markers in primary neoplasms of the lung –

Implications for diagnosis and outcome “

Katharina Schmid

Lung carcinomas comprise a heterogeneous group of different histological subtypes with different clinical properties. The presently most widely applied classification system is the World Health Organization (WHO) classification system[1], with adenocarcinoma, squamous cell carcinoma, and large cell carcinoma being the most common subtypes. These three subtypes are classified as non-small cell lung carcinoma (NSCLC), which needs to be carefully discriminated from small cell lung carcinoma (SCLC). SCLC is the most aggressive malignant lung tumour, and requires a different therapeutic approach than NSCLC. In spite of intial sensitivity to chemotherapy, the clinical outcome is extremely poor, with a five-year survival of less than 50% even in early stages[2]. Apart from SCLC, also typcial (TC) and atypical carcinoid (AC) as well as large cell neuroendocrine carinoma (LCNEC) derive from the diffuse neuroendocrine system of the lung and are summarised as neuroendocrine lung tumours; typical and atypcial carcinoid are of low and intermediate malignancy, respectively, whereas LCNEC is also a highly malignant tumour[3].

Currently there are only few diagnostic markers to discriminate between NSCLC and SCLC; moreover, reliable prognostic markers to predict response to therapy and overall clinical outcome of malignant lung tumours are still rare. With the onset of targeted and thus individualized therapies in patients with lung tumours, molecular markers for potential

assessment of the clinical course of individual patients would greatly facilitate decision making. During the past decades, various molecular markers and techiques have shown promising diagnostic and prognostic value in different human tumour entities. In view of this, we have assessed the value of various molecular markers in pulmonary tumours, as outlined in the following paragraphs.

Discrimination between non-small cell lung carcinoma und small cell lung carcinoma

It was reported that UCH-L1 (PGP9.5), a deubiquitinating enzyme widely expressed in neuronal tissues and used as a neuroendocrine marker, has prognostic significance in various human tumour's entities[4,5]. Our study "**Expression of ubiquitin C-terminal hydrolase L1 in neuroendocrine tumours of the lung"** was initiated to assess the diagnostic and prognostic impact of UCHL1 in neuroendocrine lung tumours. In our series, we could demonstrate immunohistochemical expression in 76% of 147 investigated cases of TC, AC, SCLC, and LCNEC, suggesting that UCH-L1 might be a potentially valuable additional diagnostic marker for neuroendocrine lung tumours. In addition to the diagnostic value of UCH-L1, we could also demonstrate that UCHL-1 immunoreactivity was positively associated with the presence of vessel invasion and lymph node metastases. UCHL-1 expression, however, did not appear to have prognostic value in NE lung tumours as demonstrated both on uni- and multivariate analysis

In addition to immunohistochemistry, also nuclear texture feature analysis has repeatedly been used to discriminate between various malignant tumors of the lung[6,7]. Modern image analysing systems have the advantage of being easy to handle and they are specifically designed for automatic, objective, and reproducible quantification of images of tissue

specimens. The objective of our study "**Quantitative nuclear texture features analysis confirms WHO classification 2004 for lung carcinomas"** was therefore to discriminate the main subsets of lung carcinomas as described in the 2004 WHO classification by nuclear chromatin texture feature analysis. By applying a classification rule based on granularity of the nuclear chromatin (as defined by four different parameters), SCLC and NSCLC could correctly be discriminated by this automated method in 93% of cases. No significant discrimination, however, was possible within the various subtypes of large cell carcinomas (including LCNEC). When using compactness of chromatin (defined by four texture parameters) as a means of discrimination, carcinoids and NSCLC were correctly distinguished in 92%. No significant discrimination within the group of neuroendocrine tumors was achieved. This is in line with the 2004 WHO classification, which discriminates neuroendocrine lung tumours according to their mitotic counts and the presence of necroses but not based on their morphology.

Prognostic significance of vessel invasion in neuroendocrine lung tumors

Various reports have suggested that vessel invasion might play a crucial role the progression of different tumours. However, only limited data on the influence of vessel invasion on the progression of neuroendocrine lung tumors are available in the current literature[8]. Due to the lack of specific markers, previous studies could not reliably discriminate between lymphatic vessels and blood vessels. In our study "**Prognostic value of lymphatic and blood vessel invasion in neuroendocrine tumors of the lung",** we have evaluated lymphatic as well as blood vessel invasion in 120 neuroendocrine lung tumour specimens by immunostaining for podoplanin[9], which is specific for the lymphatic endothelium, and CD34 antigen. Lymphatic vessel invasion could be demonstrated in patients with high-grade neuroendocrine tumors and

advanced tumor stages, and appeared to be significantly associated with lymph node metastases. Lymphatic vessel invasion correlated with a decreased disease free survival (DFS) in univariate analysis, whereas blood vessel invasion had no impact of DFS. Upon multivariate analysis, only tumor grade and lymph node status remained statistically significant prognostic factors. Our results suggest that the evaluation of lymphatic vessel invasion in neuroendocrine lung tumors might serve as a prognostic parameter for disease free survival.

Impact of multidrug resistance proteins and cell cycle regulators on the outcome of adjuvante chemotherapy in non-small cell lung carcinoma

In our study "**Multidrug resistance proteins do not predict benefit of adjuvant chemotherapy in patients with completely resected non-small cell lung cancer: International Adjuvant Lung Cancer Trial Biologic Program**" we have evaluated whether multidrug resistance proteins are of prognostic and/or predictive value in patients who were enrolled into the International Adjuvant Lung Cancer Trial (IALT)[10]. As opposed to many retrospective trials on prognostic markers, this approach had the advantage of using a strictly defined and uniformly treated large patient collective. Expression of MRP1 and MRP2[11] was immunohistochemically assessed in 782 NSCLC tumor specimens. We could detect positive MRP1 expression in 47% and MRP2 expression in 40% of patients analyzed. MRP2-positive patients had a significantly shorter overall survival than MRP2-negative patients (adjusted hazard ratio for death, 1.37; 95% confidence interval, 1.09-1.72; P = 0.007). As opposed to this, there was no significant association between MRP1 expression and overall survival. Neither MRP1 nor MRP2 expression however, predicted response to adjuvant cisplatin-based chemotherapy. Therefore, MRP2 expression appears to be an independent prognostic factor in

patients with completely resected NSCLC, but neither MRP1 nor MRP2 were of predictive value for the activity of adjuvant chemotherapy.

In extension of this evaluation, our study **"Cell cycle regulators and outcome of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer: the International Adjuvant Lung Cancer Trial Biologic Program**", was performed to assess whether cell cycle regulators had prognostic and/or predictive value in 778 NSCLC IALT patients[12]. Expression of $p27^{Kip1}$, $p16^{INK4A}$, cyclin D1, cyclin D3, cyclin E, and Ki-67 was immunohistochemically assessed in tumor specimens, and a relationship between $p27^{Kip1}$ status and benefit of cisplatin-based chemotherapy could clearly be demonstrated (test for interaction, P = 0.02). In patients with $p27^{Kip1}$-negative tumours, cisplatin-based chemotherapy resulted in a significantly longer overall survival as compared to patients randomized to the control-arm (adjusted hazard ratio for death = 0.66; 95% CI, 0.50 to 0.88; P = 0.006). In patients with $p27^{Kip1}$-positive tumors, the overall survival was not different between patients treated with cisplatin-based chemotherapy and controls. As opposed to $p27^{Kip1}$ none of the other cell cycle regulators evaluated was predictive for the benefit of adjuvant cisplatin-based chemotherapy and none of these biomarkers was significantly associated with overall survival.

Significance of EGFR-Mutationstatus in lung adenocarcinomas

The advent of drugs targeting tyrosine kinase pathways has spurred interest in markers predictive of response to these agents. Initial reports have suggested that the mutational status of the epidermal growth factor receptor (EGFR) could predict clinical response to therapy with EGFR/tyrosin kinase inhibitors[13,14]. In our study "**EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding loco-regional lymph node metastases"** we have compared the mutational status of EGFR (exon 18-21), KRAS (codons

12/13 and 61-68), and BRAF (exon 15) in 96 primary lung adenocarcinomas and the corresponding loco-regional lymph node metastases. Mutations in EGFR, KRAS, and BRAF were observed in 7 (7%), 36 (38%), and 2 (2%) patients, respectively. Interestingly, KRAS mutations were observed in two patients with an EGFR mutation. Mutations in primary tumors and lymph node metastases were identical in 1 of 7 (14%) patients for EGFR and 11 of 36 (31%) patients for KRAS. One patient harbored different KRAS mutations in primary and corresponding metastatic tumors. Comparative genomic hybridization analysis revealed similar patterns of chromosomal changes, strongly supporting a common clonal origin of primary tumors and metastases. It thus can be concluded that the possibility of differences in the mutational status of EGFR, KRAS, BRAF between primary tumors and corresponding lymph node metastases should be considered whenever these mutations are used for the selection of patients for EGFR-directed tyrosine kinase inhibitor therapy.

Summary

In our studies, we have assessed various molecular markers for their potential use as prognostic parameters in pulmonary routine pathology. We have analyzed UCHL1, an immunohistochemical marker, as well as the technique of nuclear texture feature analysis to discriminate between NSCLC and SCLC in routine pulmonary pathology. Our results could show that both - additional immunohistochemialcal staining with UCHL1 as well as nuclear chromatin texture feature analysis - are highly reliable methods and could therefore be included into routine lung tumour pathology in case of unclear/borderline cases. Moreover, we have demonstrated that the evaluation of lymphatic vessel invasion in neuroendocrine lung tumours might be predictive for the development of lymph node metastases and decreased disease free survival. To predict the outcome of NSCLC patients with completely resected

tumors following adjuvant chemotherapy, we have investigated multidrug resistance proteins 1 and 2 as well as cell cycle regulators and found MRP2 expression as an independent prognostic factor. Furthermore, we have disclosed a relationship between $p27^{Kip1}$ status and the benefit of cisplatin-based chemotherapy suggesting that immunohistochemical assessment of $p27^{Kipl}$ status in patients with NSCLC might be suitable for selecting patients most likely to benefit from adjuvant cisplatin-based chemotherapy. In addition, we have tested the concordance of the EGFR/KRAS/BRAF mutational status in samples of lung adenocarcinomas and corresponding loco-regional lymph node metataseses and found that in the majority of cases the mutational status of primary and metastatic tumour did not correlate; therefore the assessment of the EGFR-status to predict the responsiveness to anti-EGFR therapy should be reconsidered.

Finally, all our studies are the result of national and international collaborations and provide the basis for the further development of a multidisciplinary research program on pathology, diagnosis, and treatment of lung carcinomas at the Medical University of Vienna.

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