Pulmonary Carcinoids

Impact

In this thesis, 1) the reliability of the histopathological diagnosis and prognosis of patients suffering from pulmonary carcinoid is examined using a unique population-based cohort, 2) the molecular mechanisms underlying carcinoid tumorigenesis are investigated, 3) molecular biomarkers to improve the prognostication of pulmonary carcinoid patients are identified and verified, and 4) the first patient-derived tumor organoids for pulmonary neuroendocrine neoplasms to uncover therapeutic vulnerabilities are reported. In this impact chapter, we will place our findings into both a scientific and social perspective.

Pulmonary carcinoid is an orphan disease with an incidence ranging from 0.2/2 per 100,000 persons per year in both the United States and Europe.[1,2] Nevertheless, its occurrence has increased rapidly over the past decades mostly due to improved diagnostic techniques and increased awareness amongst clinicians. Moreover, pulmonary carcinoids are more often found by incident because people undergo scans for all kinds of diseases and conditions nowadays. Pulmonary carcinoids occur predominately at the fifth or sixth decade of life and can be morphologically (e.g., mitotic index and presence of necrosis) subdivided into low-grade typical carcinoids and intermediate grade atypical carcinoids.[3] The ratio between typical and atypical carcinoids is about 8-10:1. The post-operative median 10-year overall survival (OS) in patients with typical carcinoids is about 89% (range 83-100) and the disease-free survival (DFS) is 90% (range 73-95) [4]. In patients with atypical carcinoids the 10-year OS is 51% (range 38-74) and the DFS is 45% (range 24-71).[4] While most pulmonary carcinoids can be curatively treated by means of surgical resection, distant disease relapse occurs in 1-6% of patients with typical carcinoids and 14-29% of patients with atypical carcinoids.[5-9] Known predictors of distant relapse are atypical carcinoid, lymphatic involvement, and incomplete resection status. However, none of them can be reliably used, alone or in combination, to exclude patients from long-term follow-up. As a result, all patients require a rather intensive follow-up of 10-15 years dependent on the guideline used, while only a proportion of patients are at high-risk for relapse.[10-12] The extensive follow-up may cause morbidity, high anxiety levels and radiation exposure (due to (yearly) CT-scans) to patients, as well as substantial economic burden. The rising incidence together with the aging population, and the absence of reliable prognostic markers to safely exclude patients from long-term follow-up, could result in a continued increase of both disease and economic burden.

The requirement of additional reliable prognostic markers is illustrated by several treatment dilemmas within the management of pulmonary carcinoids. Findings in this

thesis show that the preoperative biopsy specimen diagnosis is unreliable in daily clinical practice resulting in discordance between the preoperative biopsy and paired resection diagnosis in 57% of patients (**Chapter 3**). The World Health Organization (WHO) criteria therefore state that definitive diagnosis is only feasible postoperatively.[3] However, in current clinical practice, treatment decisions are based on the pathological diagnosis of the preoperative biopsy. The preferred treatment for carcinoid disease is surgical resection, but in case of typical carcinoid parenchymal sparing strategies may be considered. Consequently, an imprecise preoperative diagnosis may result in over/under treatment regarding extent of surgery. In addition to imprecise preoperative biopsy diagnosis, our research group has previously shown that the WHO classification criteria (e.g., mitotic index and presence of necrosis) are subject to high-interobserver variation on postoperative resection specimen, affecting particularly atypical carcinoids.[13] This results in over-, and underestimation of the relapse risk as atypical carcinoid is a poor prognostic factor. Another poor prognostic factor for relapse risk is lymph node involvement as also reported in this thesis (**Chapter 2**). For this purpose, the European Society of Thoracic Surgeons guideline for intraoperative lymph node staging in non-small cell lung cancer recommends complete lymphadenectomy, but this is rarely (7%) performed in routine care of pulmonary carcinoid patients. Together these clinical dilemmas argue for the identification and implementation of additional markers to improve both diagnostic and prognostic carcinoid classification. In addition, clinicians are advised to interpret the preoperative biopsy diagnosis with caution in deciding the extent of surgery, and to always include a dedicated lymph node dissection as it provides prognostic information on disease relapse.

Despite the prognostic value of and research performed on molecular markers in pulmonary carcinoids, to date, none have been incorporated as a criterion in the WHO 2021 classification. Assessment of immunohistochemical expression of the nuclear protein MIB-1 (Ki-67) may improve current histopathological subclassification of pulmonary carcinoids (**Chapter 4**). Nevertheless, current studies evaluating the prognostic value of Ki-67 are contradictory and the absence of clear cut-off values separating typical from atypical carcinoids hampers diagnostic implementation into the WHO 2021 criteria. By expression profiling, we previously identified highly sensitive molecular markers to identify patients at risk for disease progression, i.e., orthopedia homeobox (*OTP*) alone or in combination with *CD44.*[14] The prognostic value of *OTP* has since been evaluated in larger series, confirming that loss of expression is associated with a poor prognosis (**Chapter 5**). Since these studies all contained selected institutional patient cohorts with incomplete follow-up, we validated the prognostic significance of *OTP*, *CD44,* and *Ki-67* in a large, unselected population-based cohort (**Chapter 6**). Results showed that the negative predictive value (NPV) of the marker panel was 95.9%,

indicating that our IHC marker panel can, regardless of WHO classification, reliably predict which patients will most likely not relapse over time. As a result, patients characterised with a low-risk IHC profile on the resection specimen may be excluded from long-term follow-up, which will benefit both patient and economic burden. When this high NPV withstands future prospective studies, the predictive marker panel may empower a biomarker driven post-surgery follow-up management.

To contribute to the implementation of reliable OTP immunostaining in routine diagnostics, new monoclonal OTP specific antibodies have been developed and verified, on two automated staining platforms (**Chapter 8**). Cross-platform assessment showed excellent agreement and good reproducibility on FFPE material. In addition, intratumor heterogeneity analysis showed that OTP is homogeneously expressed throughout the tumor resulting in a homogeneous staining pattern, indicating the applicability of OTP immunohistochemical staining on biopsy specimen. All together, these findings further encourage the implementation of OTP in routine diagnostics in both a pre- and postoperative setting to assist the pathologist in diagnostic decision making.

Recently, multi-omics analysis revealed three prognostically relevant molecular clusters of pulmonary carcinoids.[15] The existence of the three molecular clusters with distinct clinical features was further confirmed by a study of Laddha *et al.*.[16] Integrative genomic analysis of both studies revealed that expression of *OTP*, *HNF1a*, and *ASCL1* messenger RNA (mRNA) expression enabled sufficient separation of the molecular clusters. Subsequently, it could be shown that clustering based on mRNA expression strongly correlated with clustering based on protein expression of the three markers. Results revealed strong prognostic relevance and unique clinical features, independent of typical/atypical histology, for the different clusters (**data not shown in this thesis**).

The development of pulmonary neuroendocrine patient-derived tumor organoids (PDTO) facilitates studies investigating the molecular biology of neuroendocrine neoplasms to gather insights into the tumorigenesis. In addition, the model enables drug testing which may unravel therapeutic sensitivities for pulmonary neuroendocrine tumors (**Chapter 9**). Furthermore, we showed that differential *OTP* expression is associated with changes in DNA methylation levels (**Chapter 7**). Together these findings raise the question whether new potential therapies might be unravelled for pulmonary carcinoid patients and whether epigenetic therapies might play a role in the future.

The results and perspectives of this thesis contribute to the refinement of current clinical care by analyzing prognostic and diagnostic molecular markers, identify a prognostic marker panel that may allow a biomarker driven follow-up management for pulmonary

carcinoid patients in the future, provide insights in the management of follow-up after treatment, and reports a new primary culture model to derive insights into the biology of pulmonary carcinoids as well as possible new therapeutic targets. This has both scientific and economic impact, as it encourages the implementation of clinically relevant molecular biomarkers in current pulmonary carcinoid patient care. The clinical applicability of these molecular markers will eventually result in prognostically relevant patient subgroups. Hence, patients who have a low risk for relapse may benefit from shorter follow-up thereby reducing both health- and economic burden while high-risk patients are aided by a more dedicated follow-up.

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