Summary & Discussion
SUMMARY AND DISCUSSION

In this thesis parameters for predicting the clinical outcome of a cohort of individuals harboring pre-invasive endobronchial lesions have been studied. More specifically, we evaluated molecular biomarkers to identify lesions that ultimately progress to invasive cancer, thereby allowing more tailored preventive measures. In this chapter, the main findings of the studies are summarized and put into clinical perspective.

Clinical outcome of subjects harboring pre-invasive endobronchial lesions

In Chapter 2 we analyzed the long-term clinical outcome of 164 individuals who had one or more pre-invasive endobronchial squamous lesions as detected by autofluorescence imaging (so-called AFB-visualized lesions) at first bronchoscopic examination. These subjects underwent close surveillance with early lung cancer detection techniques (i.e., AFB and/or LDCT) that allowed us to assess lung cancer incidence and identify potential risk determinants. Lung cancers were observed in 34% (55/164) of individuals, both at pre-invasive lesion sites and at distant sites in the lungs. Whereas none of the evaluated clinical parameters (i.e., smoking status, cancer history and COPD-status) demonstrated the ability to predict cancer occurrence, histopathology to a certain degree could. Individuals bearing high-grade pre-invasive endobronchial lesions were more likely to develop invasive cancer at any site within the lungs as compared to those with low-grade lesions (p=0.03). These data corroborate previous findings indicating that presence of pre-invasive lesions, especially high-grade lesions, may be used as a marker of lung cancer risk1-3. On the other hand, histological classification was not able to predict site-specific progression of pre-invasive lesions to an invasive phenotype at the individual level4,5. Furthermore, it suffers from considerable inter- and intra-observer variation6,7. Therefore, our studies continued at the molecular level aiming to identify biomarkers for risk assessment of individual AFB-visualized pre-invasive endobronchial lesions.

Copy number aberrations in pre-invasive endobronchial lesions

Chromosomal aberrations are known to be involved in lung carcinogenesis. However, previous studies investigating these anomalies in pre-invasive endobronchial lesions have largely been restricted by methodological issues, such as limited sample size or low-resolution analyses8-11. In Chapters 3 and 4, we exploited high-resolution array comparative genomic hybridization (arrayCGH) to investigate DNA copy number aberrations (CNAs) in pre-invasive endobronchial squamous lesions with known biological behavior during follow-up. These molecular studies were performed in a case-control design nested within our cohort of individuals harboring AFB-visualized pre-invasive lesions (as described in Chapter 2).

In Chapter 3, we showed that the mean number of CNAs in AFB-visualized squamous metaplastic (SQM) lesions with carcinoma (in situ) outcome (i.e., cases) was significantly higher as compared to SQM lesions with cancer-free outcome (i.e., controls) (p<0.01). The presence of specific CNAs at 3p26.3-p11.1 (loss), 3q26.2-q29 (gain) and 6p25.3-24.3 (loss) was able to predict endobronchial cancer with 97% accuracy. These CNAs were observed solely at the site of future cancer thereby suggesting their potential use as highly specific markers of cancer progression. The abovementioned loci are significantly associated with lung SqCC12, and importantly were identified early in cancer development, from the stage of squamous metaplasia onwards, in our study.

In Chapter 4, an independent series of pre-invasive endobronchial lesions of various histological grades was subjected to arrayCGH with the aim to validate the abovementioned CNA-based classifier. In this set of squamous metaplastic and dysplastic lesions the classifier demonstrated 92% accuracy for endobronchial carcinoma (in situ) prediction (75% sensitivity, 100% specificity) thereby further substantiating the potential of the CNA-based classifier as an objective, molecular determinant of lung cancer risk in individuals harboring pre-invasive endobronchial lesions.

Chromosomal gain/amplification of the 3q26.2-q29 region was the most uniform finding in progressive lesions, thereby pinpointing to the key importance of this chromosomal aberration in early squamous cell lung carcinogenesis, as described before13,14. Based on our findings, the validated CNA-based classifier, or specific detection of 3q gain, holds great promise for improving the clinical management of individuals diagnosed with endobronchial squamous metaplastic and dysplastic lesions by providing guidance to clinicians on follow-up surveillance and (early) treatment strategies. Future prospective studies are warranted to proof the effectiveness of the CNA-based classifier in clinical decision making. A large-scale, multicenter randomized controlled prospective trial may answer the question whether treatment of biomarker-positive lesions has a favorable impact on patient outcome.

Circulating plasma DNA levels in subjects with pre-invasive endobronchial lesions

Detection of molecular biomarkers in non- or low-invasively collected biomaterials, such as blood or sputum, might serve as a prerequisite for AFB surveillance by identifying individuals at highest risk of lung cancer. Quantification of free circulating plasma DNA (cpDNA) levels is a non-invasive tool that has shown potential to detect patients with invasive lung cancer, independently of tumor stage. In Chapter 5, we evaluated whether analysis of cpDNA levels could be used to identify individuals harboring high-grade pre-invasive endobronchial lesions. Our results showed that, whereas cpDNA levels of patients with invasive lung SqCC were significantly higher as compared to healthy control individuals (p<0.01), cpDNA levels in subjects with high-grade pre-invasive lesions were highly comparable to those diagnosed with low-grade pre-invasive lesions, and could not discriminate subjects with pre-invasive lesions from healthy controls. Therefore, quantification of free circulating DNA in plasma may not be a useful parameter for identifying subjects at highest risk of lung cancer. Future research may include more specific markers, such as gain of 3q analysis, to evaluate whether individuals with progressive premalignant endobronchial lesions could be identified by blood-based screening.
The association between human papillomavirus and lung cancer

Since the association and therefore a potential etiological link between high-risk human papillomavirus (hrHPV) infection and lung cancer remained a matter of debate, we evaluated the presence of hrHPV in lung cancer in Chapter 6 using multiple molecular techniques. All hrHPV-positive lung tumors that were rarely identified in lung cancers in this study shared a clonal relationship with a previously diagnosed primary, hrHPV-positive cancer elsewhere in the body. As such, hrHPV presence in the lungs in fact points to the occurrence of pulmonary metastasis from an hrHPV-associated primary carcinoma originating elsewhere, rather than being an hrHPV-positive primary lung cancer. Thus, no support was found for an attribution of hrHPV infection to the development of primary lung cancer.

CONCLUSIONS AND FUTURE PERSPECTIVES

Lung cancer is the leading cause of cancer-related death in the Western world. Despite advantages made in diagnosis and treatment strategies, the overall survival has remained disappointing over the last decades.\(^{13,14}\) The major reason for this is the diagnosis of the disease at a late stage, thereby limiting treatment options and potential cure. Thus, primary and secondary prevention and early detection strategies are warranted to reduce mortality of this highly lethal disease. Molecular biomarker tests may improve effectiveness of current early detection approaches, such as LDCT and AFB. These tests may be used to pre-select individuals that are at the highest risk of lung cancer development, thereby reducing the number of participants that have to undergo AFB and/or LDCT. In this context, biomarker testing should be performed on easily accessible patient materials such as sputum or blood with high sensitivity, and only in those with a positive biomarker test, AFB and/or LDCT imaging will be applied. Alternatively, these tests may be used to assess cancer progression-risk of premalignant lesions, thereby preventing over-treatment and guiding clinical follow-up policies. In this case, biomarker testing should preferably be performed on biopsy samples of respective lesions. Identifying biomarkers for abovementioned purposes is an exciting, though challenging field of research. Many of the previously identified (epi)genetic changes in the respiratory epithelium are indicative of smoking-related tissue damage per se (airway field of injury) and therefore may not directly point to an increased risk of lung cancer, but actually reflect a host response to the acquired damage.\(^{13,15}\) Currently, the identification of CNAs at 3p26.3-p11.1 (loss), 3q26.2-q29 (gain) and 6p25.3-24.3 (loss), or more specifically the 3q amplification\(^{15,16}\), during the evolution of pre-invasive disease seems to be the most promising biomarker of squamous cell lung cancer risk, and has shown herein to be well applicable to biopsies obtained from AFB-visualized lesions for risk stratification. However, additional discovery, validation and standardization studies are required to further improve diagnostic accuracy for early stages of lung cancer.

For screening purposes, combining LDCT imaging with adequate sampling\(^{17}\) and biomarker testing of sputum samples might serve as an interesting approach, given the direct relation of this biospecimen to the respiratory epithelium. Another readily available biospecimen for biomarker testing is peripheral blood. Quantification of the cpDNA fraction in blood has been found to detect patients with clinically overt lung cancer\(^{20,21}\) and to help selecting subjects who are best suited for LDCT screening.\(^{22}\) Since the cpDNA fraction in blood of cancer patients is partly composed of tumor DNA originating from necrotic and apoptotic cancer cells\(^{23}\), specific tumor-associated alterations including point mutations, small insertions and deletions, and CNAs can also be identified within this fraction\(^{24,25}\). This opens up possibilities for clinical application of (serial) cpDNA analysis in cancer patients to be able to guide the selection of targeted therapies, to monitor treatment response and resistance, and to detect disease recurrence and progression\(^{26,27}\). Further topic of research is the improvement of assay sensitivity while retaining high specificity given the fact that cpDNA levels are generally low. This may well be accomplished with the advent of novel highly sensitive and specific detection techniques for tumor-associated (epi)genetic alterations, and the potential use of alternative blood components, such as exosomes\(^{28}\) and tumor-educated platelets\(^{29,30}\).

Given the above, high-throughput molecular profiling of sputum and/or blood from individuals with pre-invasive endobronchial lesions in relation to lesion outcome (progression or regression over time) is warranted to reveal novel biomarkers for screening and early detection purposes.

Therapeutic opportunities

Apart from the identification of early detection biomarkers, the molecular profiling studies suggested above may also identify novel targets for chemopreventive agents and therapeutic strategies. Currently, there are no established chemopreventive agents for lung cancer. This is, at least in part, likely due to the genetic complexity of the disease already observed in progressive premalignant lesions, which makes chemoprevention with an agent targeted to a single driver mutation unlikely to be broadly effective. Therefore, targeting altered cancer-related pathways seems to be a more promising strategy\(^{31,32}\). Since the use of histological changes (regression/progression) in pre-invasive disease as surrogate endpoint in most trials is highly questionable because most pre-invasive lesions regress spontaneously, incorporation of molecular biomarkers such as phosphatidylinositol 3-kinase (PI3K) pathway activation as intermediate endpoint in future chemoprevention trials is warranted\(^{33,34}\).
REFERENCES


