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The natural history of human papillomavirus infection in the head and neck squamous epithelium with particular respect to the significance of p16^{INK4a} expression as a marker of human papillomavirus-induced transformation

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Up to 70% of head and neck squamous cell carcinomas (HNSCC) in the oropharynx and up to 30% in the non-oropharynx are induced by high-risk human papillomaviruses (HR-HPV). Patients with HPV-associated HNSCC have an overall better prognosis compared to HPV-negative HNSCC patients. Consequently, therapy de-escalation is currently discussed for patients with HPV-associated HNSCC, highlighting the importance of reliable markers to diagnose HPV-induced transformation in those carcinomas. The cellular protein p16^{INK4a} is regularly overexpressed in HPV-transformed cancers. However, heterogeneous definitions of a positive test result and sporadic detection in non-transformed cells on immunohistochemistry (IHC) limit its diagnostic use in the head and neck. At the same time, little is known on the stages that precede HPV-induced carcinomas in the head and neck, impeding a potential early diagnosis of HPV-induced pre-malignant lesions in this location.

In the first part of this thesis, indications for distinct HPV infection stages were assessed in 50 non-dysplastic, 34 dysplastic and 147 HNSCC samples from the oropharynx and non-oropharynx. A protocol allowing for the detection of HPV oncogene transcription (HR-HPV 16 E6*I mRNA) from microdissected FFPE tissue was successfully established. HR-HPV DNA was detected in 8% of non-dysplastic, 41.2% of dysplastic and 13.6% of HNSCC samples by a highly sensitive amplification and hybridization-based assay. In the HPV DNA-positive non-dysplastic and dysplastic samples, no viral oncogene transcription or signs of a permissive, non-transforming HPV infection stage (HPV L1 IHC expression, koilocytes, viral load) were observed. While those observations will require confirmation in future prospective studies they do not support the existence of a classical permissive HPV infection stage in the head and neck as it is known from the uterine cervix. A transforming relevance of HPV was detected in 17.2% of oropharyngeal and 1.2% of non-oropharyngeal carcinomas using the algorithm of "E6*I mRNA detection on HR-HPV 16 DNA-positive samples".

The second part of the thesis focussed on the potential diagnostic value of sole p16^{INK4a} and combined p16^{INK4a}/Ki-67 expression patterns for HPV-induced transformation in the oropharynx and non-oropharynx. A comprehensive panel of p16^{INK4a} IHC expression parameters was conceptualized to identify HPV-induced transformation (as assessed by HR-HPV E6*I mRNA) in those locations. A diffuse p16^{INK4a} expression pattern in the oropharynx and ≥50% of p16^{INK4a}-positive tumor cells in the non-oropharynx significantly correlated with HPV-transformed carcinomas in those locations. Excellent and good inter-

observer reproducibility were observed for those parameters (Cohen´s κ=1.0 (95%-confidence interval: 1.0-1.0) and 0.851 (0.564-1.0), respectively). These observations may support the agreement on a uniform definition of $p16^{INK4a}$-positivity to reliably identify HPV-driven oropharyngeal and non-oropharyngeal head and neck carcinomas on histology. Co-expression of $p16^{INK4a}$ and the proliferation-associated antigen Ki-67 within the same cell specifically identifies HPV-transformed cells at the uterine cervix. Performance of this staining was analyzed for the first time in the head and neck. It was demonstrated that a clonal $p16^{INK4a}$/Ki-67 expression pattern exclusively occurred in transformed cells of this location. While this pattern was observed in 100% of the HPV-transformed HNSCC, it was also found in a minority of HNSCC (4.8%) and 23.5% of dysplastic lesions in the absence of HPV. This observation points to a further, HPV-independent mechanism of clonal $p16^{INK4a}$/Ki-67 expression in a subset of malignant head and neck lesions. At the same time, $p16^{INK4a}$/Ki-67 IHC may have substantial diagnostic impact on histo- and cytopathology in the head and neck as it was shown to specifically identifiy transformed cells in this location.

In the third part, the accuracy of four widely applied tests to identify HPV-driven oropharyngeal cancers was objectified by a comprehensive meta-analysis. A broad search string identified 1310 studies, of which 13 qualified for data extraction. HPV DNA detection by polymerase chain reaction (PCR) showed high pooled sensitivity and specificity (99% (91-100%); 91% (79-98%)) among the four eligible studies. HPV DNA in-situ hybridization was moderately sensitive but highly specific among the five eligible studies (84% (71-94%); 91% (78-99%)). $p16^{INK4a}$ IHC was highly sensitive and moderately specific in the 11 eligible studies (93% (86-98%); 84% (77-90%)). Considerable differences in test conduction were noted among studies, which may considerably impact diagnostic accuracy. Combined testing of HPV DNA detection by PCR and $p16^{INK4a}$ IHC demonstrated high pooled sensitivity and specificity (95% (84-100%); 100% (96-100%)) and could thus represent a promising testing strategy to diagnose HPV-induced oropharyngeal carcinomas with high accuracy. As shown in part two of this thesis this evaluation might be supported by a uniform definition of $p16^{INK4a}$-positivity.

In conclusion, the obtained data from this thesis provide insight into the significance of HPV detection in head and neck squamous epithelium of different dignity. It is demonstrated that the early detection of HPV-induced lesions in this location may be hampered by the lack of a currently identifiable precursor state. At the same time, the data suggest promising testing strategies to reliably identify HPV-transformed oropharyngeal and non-oropharyngeal head and neck carcinomas by surrogate markers. Considering future differential treatment of HPV-driven head and neck cancers a reliable diagnosis of HPV attribution in those cancers is a major clinical goal.