Towards uncovering polyomavirus-carrying human cancers

Summary

Although the incidence of cancer is rapidly growing worldwide, the etiology of many malignant neoplasms remains largely unknown and thus also their cure. The link between the development of cancer and viral infections has been established since long. Globally, viruses have been demonstrated to be the causative agents of up to 15% of all human malignancies. During the recent two decades, the invention of new molecular techniques such as Next Generation Sequencing technology has marked a turning point by helping to discover new viruses, including novel members of human polyomaviruses (HPyVs). However, only very few HPyVs have been involved in neoplastic human diseases, which is mainly MCPyV, which was identified to be the major etiological agent of 80% of Merkel cell carcinomas (MCC). Much remains to be investigated concerning the putative role of HPyVs in human carcinogenesis. The main aim of the research described in this thesis was to screen and test for the presence of novel HPyVs in diverse human cancer tissues to get more insight into the role and possible involvement of these viruses in human tumorigenesis. For this purpose, we used FFPE tissues and applied a diverse sensitive, and specific set of molecular techniques to detect HPyV's at a single-cell level within the histomorphological context.

Chapter one is a general introduction summarizing the direct or indirect role of DNA and RNA tumor viruses in carcinogenesis. Furthermore, this chapter focused more on describing the polyomaviruses in general, then a brief background of each known HPyV. In addition, the outline and the aim of the research works in this thesis are explained. In **chapter two**, the current knowledge on the emerging role of the skin-associated HPyV6 and 7 in human cancers was comprehensively reviewed. Notably, previous studies revealed that the seropositivity of HPyV6 was found to be higher than HPyV7 and increased with age. Although an interesting finding, HPyV6 and 7 prevalence were higher in malignancy tissues than in non-neoplastic tissues. Furthermore, HPyV6 was detected more frequently in skin malignancies while HPyV7 was found to be more frequent in other cancers such as thymoma and cholangiocarcinoma. Based on the reviewed studies, we concluded that HPyV6 and 7 remain important putative candidates possibly contributing to the etiopathogenesis of human disease especially skin tumors.

In **chapter three**, the presence of novel HPyVs in cholangiocarcinomas (CCA) was assessed to evaluate their possible contribution to the etiology of this human malignancy. Only recently, the presence of HPyV6 DNA (27%) has been reported by a Chinese research group in the bile fluid of CCA patients by PCR. Here, we aimed to investigate the prevalence of HPyVs in CCA tissues to elucidate possible clinicopathological correlations between HPyVs and CCA. Interestingly, HPyV7 (69%) was highly prevalent in the CCA cohort, the next most frequent was MCPyV (24%) followed by HPyV6 (14%). An important finding of this study was that HPyV7, HPyV6, and MCPyV are hepatotropic viruses and able to infect non-neoplastic human hepatocytes, bile duct

epithelium, and CCA tumor cells. Yet, it remains unclear if and how HPyVs might contribute to the tumorigenesis of CCA. An interpretation of these results could be that the frequent finding of HPyV's in the adjacent peritumoral hepatocytes might suggest a robust indirect role of these viruses by leading to chronic inflammation which ends up inducing CCA transformation.

BKPyV is well known to reactivate under immunosuppression, especially in the context of solid organ transplantations, e.g. kidney transplantations. BKPyV has been suspected to be a putative oncogenic virus in the development of urothelial cell carcinomas (UCC) in immunocompromised patients. In **chapter four** we assessed whether reactivation of BKPyV in Decoy-positive urine cytology specimens (UCS) predicts or plays a role in the development of UCC of the urinary bladder. We showed that BKPyV detection was not restricted to the urine samples of patients who were diagnosed with UCC of the urinary bladder but also detected in the urine of non-UCC patients. In contrast to the UCC tissue specimens, all tested FFPE tissues were BKPyV-negative by IHC and PCR. Therefore, BKPyV reactivation is not restricted to immunosuppression but also can be found in the UCC of the immunocompetent patients. Therefore, the role of BKPyV in the oncogenesis of the majority of urothelial cell carcinoma of the bladder is rather unlikely.

In **chapter five** we investigated the prevalence of MCPyV in human thymoma, which is a rare malignancy of unknown etiology. In animal models, Murine polyomavirus (MuPyV) had been reported to induce thymomas after injecting MuPyV capsid protein (VP1) in neonatal mice. In addition, a few years ago our research group demonstrated the high prevalence of HPyV7 in thymic epithelial tumors. However, we aimed to uncover if the recent tumor virus identified MCPyV is playing a role in the etiopathogenesis in human epithelial thymic tumors. The presence of MCPyV-DNA was detected in 19.4% of the cohort and the DNA, RNA, and protein of MCPyV were showed in the single-cell level of epithelial thymoma. The most likely conclusion for these findings is that MCPyV expression does not act the same mechanism as seen in MCC. Thus, the contribution of MCPyV to the etiopathogenesis of thymoma is unlikely at least based on our dataset which revealed less expression of viral DNA, RNA, and proteins in tumor cells.

In **chapter six**, tissues obtained from 119 patients with head and neck squamous cell carcinoma (HNSCC) who had no history of smoking tobacco or drinking alcohol were investigated for the presence of human papillomavirus (HPV), Epstein-Barr virus (EBV), and MCPyV. HPV and EBV have previously been shown to be important etiological factors in the development of HNSCC. Our data confirmed the presence of HPV and EBV in patients with HNSCC. In contrast, MCPyV-LTAg expression was not detected by both RNA-ISH and IHC. This is in contrast with previous studies which reported that MCPyV-DNA was detected in tongue SCC. The most likely explanation for this

overt contradiction is simply explained by the fact that we assessed the presence of MCPyV-LTAg expression on the single-cell level using RNA-ISH and IHC while the other studies tested their cohort by DNA PCR only. Our findings strongly support that there is no etiological role for MCPyV in HNSCC tumorigenesis. In **chapter seven**, a general discussion of the major findings of this thesis is presented.