# Inside cancer pathology

## Impact paragraph

### Social impact

Cancer is one of the world's top causes of death, with 10 million deaths in 2020 (1). With population aging and low socioeconomic communities, the global burden of cancer is rapidly increasing (1, 2). The underlying cause behind many cancers remains a mystery. Therefore, it is essential to discover what triggers them in order to reduce this burden through preventive measures or new therapies.

Studies suggest that 15-20% of cancers worldwide may be due to high-risk infectious agents (3, 4, 5, 6, 7). The 2018 GLOBOCAN report indicates that nearly 2.2 million cancer cases are associated with certain infections per year (3). As reported by the International Agency for Research on Cancer (IARC), several infectious agents are classified as carcinogenic to humans. These include eight viruses, three parasites, and one bacterium (2, 4, 8). In addition to promoting novel prevention, identifying novel human tumor viruses may serve as a potential tool to prevent early-stage pre-malignant lesions and the progression to cancer, thus enhancing cancer research. The vaccination of humans can prevent some of the cancers that are known to be caused by viruses. Members of the polyoma- and papilloma virus families are associated with tumor development, due to their proteins interacting with tumor suppressor proteins such as p53 and pRb, (9, 10) allowing for uncontrolled cell growth. This is of benefit for the virus though it then can spread more readily; however, worse for the host since it can lead to tumor development. The best-known instance of this phenomenon is in cervix cancer, where human papillomavirus (HPV) is believed to be responsible for 93 percent of cases (11, 12). Recently, bovine meat and milk factors (BMMFs) have been identified which were detected in colon, breast, lung, and pancreatic cancers (13, 14, 15). Identifying the viral genome status in the future will provide further insight into the potential tumorigenic role of these human polyomaviruses. In addition to significantly advancing our understanding of known human polyomaviruses in human cancers, this thesis also has the potential to identify novel potential tumorigenic human polyomaviruses and detect human polyomaviruses and BMMFs in clinical specimens.

### Scientific impact

In this thesis, the promising results were published as open-access manuscripts in peer-reviewed journals, making them freely available online and presented at international and national congresses to contribute to scientific knowledge (16, 17, 18) . The results of this thesis may also be used as a basis for future studies on HPyVs, BMMFs and human tumorigenesis. Except for

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MCPyV, which is responsible for 80% of MCC, the oncogenic potential of the novel HPyVs for human tumorigenesis has not yet been fully uncovered (19, 20, 21). Compared to MCPyV-negative MCC, MCPyV-positive MCC appears to have better clinical outcomes, better drug responses, and a higher survival rate (22, 23, 24). There is still much more to be done to understand how HPyVs and BMMFs are involved in human carcinogenesis. Our objective was to provide insight into the presence of these viruses in human malignancies and assess their association with tumors by utilizing a broad spectrum of molecular techniques in order to meet the immediate needs of elucidating the role of HPyVs and BMMFs.

Currently, it is unclear how HPyV6 and 7 contribute to human cancer. Studies have shown that HPyV6 and 7 have high seroprevalences in humans and can infect newborns (18). Hence, HPyV6 and 7 remain putative oncogenic viruses in humans, such as skin tumors as reviewed **in chapter 2**. In addition, much remains to be learned about HPyV6 and 7, including oncogenic potential, replication, latency, and tropism. This information will eventually help to understand the possible role of HPyV6 and 7 in carcinogenesis. Thus, these findings will be of significant interest to researchers so that they can conduct further studies in order to make advances in this area.

Reports from early research highlighted BK polyomavirus (BKPyV) as highly oncogenic in animal models (25), prompting the question of whether it is a potential cause of urothelial cell carcinomas (UCC) in urinary bladders in humans (26). This led to the International Agency for Research on Cancer (IARC) placing BKPyV into group 2B, possibly carcinogenic, according to findings in 2012 (27). Subsequent data from **chapter 3** concluded that reactivation of BKPyV detected in urine cytology had no association with UCC, and also that reactivation was not limited only to those who are immunosuppressed but applied also to immunocompetent patients (17). This new evidence has urged further study to elucidate any possible connection between intravesical treatment for UCC and BKPyV reactivation.

In **Chapter 4**, the presence of four polyomaviruses, JCPyV, HPyV6, HPyV7 and MCPyV, was studied in UCC samples and voided urine from patients with Decoy cells in their cytology. JCPyV-DNA was found in both urine and urothelial cells, while MCPyV was only observed in the urothelial cell carcinoma (submitted in Frontiers). However, both HPyV6 and 7 were not detected in all UCCs and urine specimens. Since there is inadequate evidence of a role for

JCPyV in carcinogenicity in UCC, these findings support the hypothesis that JCPyV infection could play a role in urothelial carcinoma tumorigenesis.

The etiology of Cholangiocarcinoma (CCA) remains largely unknown. **Chapter 5** showed that HPyV7, HPyV6 and MCPyV are hepatotropic viruses which can infect bile duct epithelium and hepatocytes, for the first time (16). The effect of HPyV's on CCA carcinogenesis has not been resolved yet, but it is speculated that HPyV7 might provoke an indirect inflammation similar to Hepatitis B and C viruses which can result in HCC. The results from **chapter 5** will provide a basis to explore the potential involvement of HPyV's in other hepatobiliary diseases.

Renal cell Carcinoma (RCC) is the most common type of kidney cancer (28). The etiology of RCC is still obscure (29). **Chapter 6** strongly suggest that MCPyV, HPyV7, HPyV6, BKV, JCV and WUPyV could potentially infect both RCC and surrounding tumor tissues. While all six HPyVs have shown a tendency to target the kidneys, we observed that MCPyV and HPyV7 were more commonly present in neoplastic and non-neoplastic cells within our subset of RCC samples compared to HPyV6, BKV, JCV and WUPyV. This study is the first to not only map these HPyVs in various distances of RCC tissues but also report the presence of MCPyV and HpyV6 on a single cell and protein level to confirm the bioactivity of these viruses in human kidney (Manuscript to be submitted in IJMS).

Epidemiological studies on diet and kidney cancer, of which renal cell carcinoma (RCC) is the most common type, have shown conflicting results. Conflicting associations have been found with diet in general and meat and dairy consumption in particular (30, 31). The recently discovered BMMFs represent  a specific novel class of infectious agents which that are intermediate in origin between bacterial plasmid and single-stranded circular DNA viruses (13, 32). **Chapter 7**, we aimed to test the most common subtypes of RCC, i.e., CCRCC, and PRCC for the possible presence of BMMFs in formalin-fixed and paraffin-embedded (FFPE) RCC tissues. In this first study of screening FFPE tissues with consensus BMMF1- and BMMF2-PCR, BMMF-sequences were detected more frequently in non-tumoral kidney tissues than in RCC tissues. It is highly interesting that BMMF-DNA is more frequently found in non-tumoral tissues compared to RCC in both collection groups. These findings are potentially in line with the proposed model for BMMF-induced indirect colon carcinogenesis, which includes the presence of BMMFs in adjacent non-tumoral tissues (33). We have shown that a broad-range PCR

approach for the detection of BMMF1- and BMMF2-DNA is a suitable tool for screening large FFPE tissue collections in general, and here RCC tissues in particular.

This dissertation aims to provide further insight into the possible role of HPyVs and BMMFs in human tumorigenesis. It has been very rare for these viruses to be detected at the single-cell level in a disease-specific histomorphological context. The presence of HPyVs on a single cell level was accurately determined using advanced sensitive and specific molecular techniques such as FISH, RISH, and IHC in this thesis. The molecular techniques we used (FISH, RISH, and IHC) for detecting the DNA, RNA, and protein of HPyVs are valuable assessment methods, but are insufficient for confirming their role in tumorigenesis. The HPyVs genome structure has many similarities with that of HPV, prompting speculation that these viruses may act similarly in terms of tumor formation (34, 35). Our datasets could be valuable for researchers specializing in viral-induced cancer development. We hope that the results of this work will serve as a useful resource for professionals and researchers delving into the field of cancers related to viruses (16, 17, 18). Nevertheless, our findings are not yet able to inform preclinical or clinical practice regarding HPyVs and tumorigenesis until more research is carried out through advanced molecular techniques such as NGS to further understand their potential role in oncogenesis and assess a possible role of viral integration or mutation of the LTAg in cancer development. Therefore, we recommend that HPyV's and BMMFs remain prospective agents involved in human tumorigenesis, but considerable effort is needed to confirm this hypothesis.

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