Inside cancer pathology

Summary

Even though cancer incidence is increasing rapidly around the world, the cause and cure of many malignant neoplasms remain largely unknown. Studies suggest that 20% of cancers worldwide may be due to high-risk infectious agents. There has been a long-standing link between the development of cancer and viral infections. Up to 15% of all human malignancies have been linked to viruses. In the last two decades, new molecular technologies such as Next Generation Sequencing have enabled us to come across new members of human polyomaviruses (HPyVs), however few have been found to be connected to cancerous diseases in humans; mainly Merkel cell carcinoma (MCC) which is caused by MCPyV in 80% of cases. Researching further into HPyVs and their involvement in tumorigenesis needs more exploration. Other infectious agent such as BMMFs have been recently found in colon, pancreases, lung and in our study of RCC cohort. The primary objective of this thesis was to screen and test for novel HPyVs and BMMFs in various cancer tissues to gain a better understanding of the role and possible involvement of these infectious agents in human tumorigenesis. Our goal was to detect HPyVs at single-cell levels within the histomorphological context using FFPE tissues and a variety of sensitive and specific molecular techniques for BMMFs DNA detection.

Chapter 1 provides a general introduction of how DNA and RNA tumor viruses and BMMFs contribute to carcinogenesis, either directly or indirectly. Moreover, this chapter focused more on describing the polyomaviruses in general, then giving a brief background of each known HPyV as well as BMMFs. Furthermore, the outline and the purpose of this thesis are discussed.

Chapter 2 reviews the current evidence to evaluate a possible role of HPyV6 and 7 in the etiopathogenesis of neoplastic human diseases. The frequent prevalence of HPyV6 and 7 DNA in non-neoplastic and neoplastic tissues along with their high seropositivity in the normal population indicate that both viruses have long-term latency in humans. Interestingly, HPyV6 prevalence was higher in skin malignancies than that of HPyV7. In contrast, HPyV7 was more frequently detected in non-cutaneous malignancies such as cholangiocarcinoma and renal cell carcinoma . Notably, previous studies revealed that the seropositivity of HPyV6 was found to be higher than HpyV7 and increased with age. In conclusion, HpyV6 and 7 remain important putative candidates that may contribute to the etiopathogenesis of human diseases, especially skin cancer.

Chapter 3, we assess the relation of BKPyV-positive urine cytology specimens (UCS) to the detection of UCC in a large UCS database and the following evaluation of BKPyV in the UCC of

the urinary bladder. In addition, we aimed to evaluate the relation of BKPyV to intravesicular BCG or mitomycin treatment of UCC patients. Our results shown that PCR detected BKPyV-DNA in urine samples of patients with either in situ or invasive UCC of the urinary bladder, while BKPyV-IHC and PCR were negative at the FFPE level of primary UCCs and metastases. However, the BKPyV detected in urine was not linked to previously resected urothelial cell carcinomas, excluding this virus as a possible cause of conventional type UCC in our cohort. BKPyV-reactivation has been observed not only in immunocompromised individuals, but also among those with urothelial cell carcinoma and no prior history of transplantation, malignancy or chronic diseases. The intravesical treatment could be associated with the reactivation of the latent BKPyV. Moreover, Cystitis may be behind the reactivation of the latent BKPyV in immunocompetent patients. Therefore, evaluating for BKPyV presence in post-UCC patients might be pertinent for assessing the risk of BKPyV-nephropathy and further studies are necessary to understand this complex relationship.

Recently, we studied the association between the BKPyV infection and urothelial cell carcinoma in patients with urine cytology positive for Decoy cells. However, in our patient cohort, both primary and recurrent UCC tissues tested negative for BKPyV by PCR and immunohistochemistry (IHC) as outlines in **chapter 3**.

Chapter 4 evaluates the presence of JCPyV, HPyV6, HPyV7, and MCPyV in the UCC samples and in the voided urine in the patients, diagnosed with UCC and with Decoy cells in urine cytology. JCPyV-DNA was detected in the urine and urothelial cell and MCPyV was detected in urothelial cell carcinoma. 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. Going forward, it is important to define whether or not both JCPyV and MCPyV are involved in UCC tumorigenesis.

The Chinese research group recently detected HPyV6 DNA in 27% of the bile fluid from cholangiocarcinoma (CCA) patients, prompting us to look further into this possible link. In **Chapter 5,** 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. However, it's still not known if they contribute to or cause CCA. The frequent finding of HPyVs in adjacent peritumoral hepatocytes could suggest a robust indirect role for these viruses in inducing CCA transformation through chronic inflammation as outlines in **chapter 1**.

Chapter 6, to investigate possible clinicopathological correlations between HPyVs and RCC, we screened for the prevalence of HPyVs in in various formalin-fixed and paraffin-embedded (FFPE) RCC tissues by human polyomavirus consensus and virus specific PCR in RCC tissues including adjacent non-neoplastic kidney tissues. PCR positive cases were further tested by FISH, RISH and IHC. Of note, 80% (44/55) of RCC and its non-tumoral tissues tested positive for one or more of the HPyVs (i.e., MCPyV, HPyV6, HPyV7, BKV, JCV and WUyV) in the same specimen. 27 (61%) specimens were positive for only one of HPyV, 13 (29.5%) specimens were positive for 2 HPyVs, 3 (6.8%) specimens were positive for 3 HPyVs, and only one (2.27%) RCC specimen was positive for 4 HPyVs on the same specimen. However, 11 (20%) RCC specimens were negative for all PCR approaches. Interestingly, MCPyV was seen in 22/55 (40%) of RCC tissues, HPyV7 was observed in 13/55 (23.6%) of RCC, and HPyV6 in 7 (12.7%) of RCC tissues. However, 9/55 RCC specimens share positivity for both MCPyV and HPyV7, while 2/55 RCC specimens have positivity for both HPyV6 and HPyV7. 0/55 was seen among MCPy and HPyV6 or for these 3 viruses on the same time. Our findings 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 level. We utilized various molecular techniques to examine the presence and bioactivity of these viruses from DNA to protein levels. The frequent identification of HPyVs, particularly MCPyV and HPyV7 in kidney tissues may also indicate a possible involvement in other kidney diseases.

our results may suggest an indirect link between these HPyVs and carcinogenesis through inflammation, further investigation is needed to fully understand their role in the development of RCC.

There have been contradictory results from epidemiological studies on diet and kidney cancer, including renal cell carcinoma (RCC). Interestingly, the geographic distribution of RCC incidence also reveals a significant degree of epidemiological agreement with the geographic distribution of colon and breast cancer incidences, suggesting a link with diet. In the recent discovery of bovine meat and milk factors (BMMFs), a novel class of infectious agents is distinguished between bacterial plasmids and single-stranded circular DNA viruses in terms of their ancestral origins. Recently, it has been shown that exogenous BMMF DNA derived from milk or meat is able to replicate in human embryonic kidney (HEK) cells and found in other cancer such as colon, pancrease and lung. **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. Indeed, we were able to reliably detect BMMFs-DNA in the RCC FFPE 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. Due to the frequent finding of BMMF2-DNA in the non-tumoral FFPE kidney tissues of the retrospective RCC collection, we also tried to amplify other parts of this specific BMMF2 genotype. By using seven different BMMF2-specific primer pairs covering the rest of the BMMF2 genome, we were able to amplify all parts of the respected BMMF genome in the tested cases. This approach confirmed the results of the BMMF2 broad-range PCR and indirectly possibly suggests that the whole BMMF2 genome is present in these tissues.