Towards uncovering polyomavirus-carrying human cancers

Impact

Social relevance

Cancer is a leading global cause of death, accounting for nearly 9.6 million deaths worldwide in 2018 [1]. The global burden of cancer is rapidly rising because of population aging and low socioeconomic communities [1, 2]. To date, the causative agent of many cancers remains unknown. Therefore, unraveling the underlying causes of diverse human cancers is crucial to mitigate the cancer burden by protecting the population from exposure to the respective carcinogens or at least limiting it by promoting novel prevention and therapeutic approaches.

Among all human cancers, approximately 10-15% of cancers are attributed to viral infection agents [1, 3-5]. Shedding light on the detection of novel human tumor viruses is an important opportunity for cancer research by promoting novel prevention, and as a potential tool, early detection of these viruses might contribute to preventing early-stage pre-malignant lesions and thus progression to cancer. Importantly, some of the known human cancers induced by viruses can be prevented by vaccination [1, 6, 7]. Human papillomavirus (HPV) which is directly linked to the etiopathogenesis of cervical carcinoma is a successful example from the past. The incidence of cervical cancer has already markedly declined in countries implementing the HPV vaccinations [1, 6, 8]. Additionally, the finding of HPV as an etiological factor of cervical carcinoma opened the gate to uncover the association of HPV with several other neoplasms including vagina, vulva, head, neck, anal, and penile carcinomas [1, 2]. Therefore, there is a global call to all researchers to elucidate if currently known viruses or still to be discovered ones are implicated in human tumorigenesis. There is a continuous ongoing scientific debate regarding the role of human polyomaviruses (HPyVs) in human tumorigenesis. Here we focused to explore and providing more information on the role of HPyVs in human malignancies.

Target group

This dissertation aims to broaden and deepen our understanding of the presence and the possible role of HPyVs in human tumorigenesis. The HPyVs genome organization shares many similarities with the HPVs genome. Based on this it has been speculated that HPyVs might be involved in human tumorigenesis analogous to HPV [9-11]. Our datasets could be of interest to many researchers, especially in the field of viral-related tumorigenesis. Additionally, we hope the outcome of this work will serve as a beneficial reference for researchers and professionals in viruses related to cancers in the future [12-16]. Yet, the presence of HPyVs defined in this thesis is insufficient to be translated into preclinical or clinical practice until further confirmation of their role in the tumorigenesis. The recommendation of this thesis is that HPyV's remain candidates for human tumorigenesis, but more effort is needed to develop more advanced molecular techniques to elucidate the role of HPyV's in cancer.

Scientific impact

The promising results obtained from the studies in this thesis have added to scientific knowledge since all the outcomes were published in a peer-reviewed journal as an open-access manuscript to make them freely accessible online and presented at international and national congresses [12-16]. Additionally, the results of this thesis possibly provide the basis for relevant follow-up studies on HPyV's and human tumorigenesis.

The oncogenic potential role of the novel HPyVs in human tumorigenesis has not yet been completely uncovered except for MCPyV, which is the causative agent of 80% of MCC [17-20]. It is important to note that MCPyV-positive MCC appears to have better clinical outcomes and drug responses and a favorable survival rate compared to MCPyV-negative MCC [21-25]. Given the potential implications of HPyV's in tumorigenesis, much more needs to be done to broaden the understanding of the involvement of these viruses in human carcinogenesis. To meet the immediate needs of elaborating the role of HPyV's, we aimed to provide insight into the presence of these viruses in various human malignancies and assess their association with tumors using a broad spectrum of molecular techniques.

HPyV6 and 7 contributions to human tumorigenesis are not yet elucidated. Studies about HPyV6 and 7 which shown a high seroprevalence in the human population and the ability to infect newborns [13]. Therefore, HPyV6 and 7 remain putative oncogenic viruses in humans for e.g. skin tumors as we discussed in detail in **chapter 2**. Moreover, a lot remains to be elucidated about HPyV6 and 7 such as their oncogenic potential, replication, latency, and tropism. This information will eventually help to understand the possible role of HPyV6 and 7 in carcinogenesis. As a consequence, these findings will be of major interest to stimulate further studies by researchers to bring advances in this field.

The etiology of Cholangiocarcinoma (CCA) is still largely unknown HPyV7, HPyV6, and MCPyV are reported for the first time as hepatotropic viruses and infecting bile duct epithelium and hepatocytes as shown in **chapter 3** [14]. The contribution of HPyV's to CCA carcinogenesis has not been definitely elucidated, but it is speculated that HPyV7 might act through an indirect mechanism to induce (chronic) inflammation as has been convincingly shown for Hepatitis B and C viruses in leading to hepatocellular carcinoma. In addition, the results and knowledge obtained from the study in **chapter 3** will pave the way to study the possible role of HPyV's in the etiopathogenesis of other hepatobiliary diseases.

Early reports showed that that BK polyomavirus (BKPyV) is highly oncogenic in animal models [30]. This raised the question of whether BK polyomavirus (BKPyV) is a potential oncogenic virus in the development of urothelial cell carcinomas (UCC) in the urinary bladder [31]. In 2012, the International Agency for Research on Cancer

(IARC) classified BKPyV as a group 2B possibly carcinogenic candidate to humans [32]. that the data in **chapter 4** described that the reactivation of BKPyV detected in urine cytology is not associated with UCC [15]. In addition, BKPyV-reactivation is not restricted to immunosuppression but is also found in the urine of immunocompetent patients. Therefore, those findings open a new direction for the research which is that the intravesical treatment of UCC is possibly causing BKPyV reactivation, there is a need to perform further research to elucidate this relationship.

Thymoma is a rare tumor of unknown etiology. Previously, Murine polyomavirus was shown to induce thymomas in an animal model [33]. In addition, a high prevalence of HPyV7 in thymic epithelial tumors was reported [34]. MCPyV is closely linked to the etiopathogenesis of MCC and yet the only human carcinogenic polyomavirus [20, 35, 36]. Therefore, screening if MCPyV is involved in other human tumors is highly needed such as if MCPyV is playing a role in the etiopathogenesis in human thymoma [16]. The contribution of MCPyV to the etiopathogenesis of thymoma is unlikely as described in **chapter 5**. Altogether, HPyVs remain suspected as a putative oncogenic virus in the development of thymomas. Therefore, further research is needed to investigate other HPyV's in thymomas.

In 2018, about 453,000 deaths of head and neck squamous cell carcinoma (HNSCC) and 888,000 new cases were diagnosed [1]. Importantly, HPV is linked to the etiopathogenesis of HNSCC, particularly the oropharynx tumor. Given the potential role of involvement of HPV in HNSCC, other important factors correlated to the tumorigenesis such as aging, smoking, alcohol consumption, and gender [1, 2]. A unique large cohort of non-smokers and non-drinkers with HNSCC is investigated for the presence of three known human tumor viruses (HPV, EBV, and MCPyV) in **chapter 6** [12]. the results confirmed the presence of HPV and EBV in patients with HNSCC and no MCPyV was detected in this subset. This indicating that no etiological role of MCPyV in HNSCC tumorigenesis. Therefore, screening for other HPyVs in a cohort diagnosed with HNSCC without a history of smoking and alcohol drinking is needed,

Finally, the presence of HPyV's DNA and their association with human malignancies have commonly been tested by PCR alone which is insufficient to prove causality. Very few studies have shown the presence of these viruses on the single-cell level in the histomorphological context of the specific disease. In this thesis, advanced sensitive and specific molecular techniques were implemented such as FISH, RISH, and IHC to accurately determine the presence of HPyVs on a single cell level as we have shown in **chapters 3 to 6**. Even though, the molecular techniques we used (FISH, RISH, and IHC) for detecting the DNA, RNA, and protein of HPyVs are valuable assessment methods but remain insufficient to confirm the role of the respective virus in tumorigenesis.

Implementing new powerful molecular technologies such as NGS to study the role of these viruses as potential oncogenic will seem to be a promising interesting avenue for further research. Therefore, there is an urgent need to apply additional molecular techniques to unravel the molecular basis of virus-host interactions and assess a possible role of viral integration or mutation of the LTAg in cancer development.

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