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First detection and complete genome sequence of a phylogenetically distinct human polyomavirus 6 highly prevalent in human bile samples

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Summary Oncovirus-associated malignancies are potentially preventable diseases with major public health significance. Human polyomaviruses (HPyVs) may be associated with oncogenesis or symptomatic illnesses in immunocompromised patients, but the site of viral shedding of most recently discovered HPyVs remains obscure. Using real-time PCR assay using specific primers targeting the HPyV6 large tumor antigen gene, we detected a phylogenetically distinct HPyV6 which was highly prevalent in the bile samples of patients with malignant biliary

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obstruction (18.8%) and acute gallstone cholangitis (5.5%). The prevalence rate and mean viral load of this HPyV6 were highest in the cholangiocarcinoma subgroup (27.6% and 2.41 $\times$ 10^4copies/ml). These findings were confirmed with another real-time PCR assay using specific primers targeting the HPyV6 viral capsid protein 2 gene. These bile HPyV6 strains may represent a novel clade of HPyV6 as they formed a distinct cluster from the other HPyV6s and exhibited >2% differences in amino acid sequences in their major proteins. While HPyV6 was unlikely the cause of the patients' acute symptoms and liver dysfunction, the virus may be related to immunosuppression in patients with malignancy and/or important in the oncogenesis of cholangiocarcinoma in patients without coinfection with other oncogenic microbes. Further studies to ascertain a causative role of HPyV6 in cholangiocarcinoma should be conducted.

Introduction

Malignancies caused by oncoviruses are potentially preventable conditions with major public health significance. Examples of oncoviruses causally linked to human malignancies include human papillomaviruses (cervical carcinoma), hepatitis B (HBV) and hepatitis C (HCV) viruses (hepatocellular carcinoma), Epstein—Barr virus (Burkitt's lymphoma, Hodgkin's lymphoma, post-transplant lymphoproliferative disease, and nasopharyngeal carcinoma), human herpesvirus 8 (Kaposi's sarcoma), and human T-lymphotropic virus (adult T-cell leukemia).[1] Moreover, Merkel cell polyomavirus (MCPyV), a novel human polyomavirus discovered in 2008, is also known to be associated with carcinoma of the skin (Merkel cell carcinoma).[2] Together, these seven oncoviruses account for about 12% of all human malignancies.[1]

Polyomaviridae is a family of small, non-enveloped, circular, double-stranded DNA viruses predominantly found in mammals and birds.[3] Polyomaviruses may cause asymptomatic or mild primary infections and remain latent in human, and some may be reactivated to cause severe disease during immunosuppression or are potentially oncogenic.[4] In addition to the better known BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV) that were discovered in 1971, more than 10 novel polyomaviruses infecting humans have been discovered since 2007.[5] Seroprevalence studies suggest that many of these polyomaviruses are acquired in early childhood and the seroprevalence rates increase with age.[6] Besides MCPyV-associated Merkel cell carcinoma, BKPyV has also been linked to urothelial malignancies in transplant recipients.[7] Moreover, other polyomaviruses may cause nephropathy in hematopoetic stem cell transplant recipients (BKPyV), progressive multifocal leukoencephalopathy in HIV-infected patients (JCPyV), and proliferative skin disorder in immunocompromised patients (trichodysplasia spinulosa-associated polyomavirus (TSPyV)).[8—11] In contrast to these few human polyomaviruses (HPyVs), the pathogenic roles of the other novel HPyVs, such as KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), HPyV6, HPyV7, HPyV9, HPyV10, Saint Louis polyomavirus (STLPyV), HPyV12, and New Jersey polyomavirus (NJPyV) are less clear.

These novel HPyVs have been detected in various types of bodily fluids and tissues of healthy volunteers and/or patients, most commonly respiratory tract secretions and swabs (KIPyV, WUPyV, HPyV6, HPyV7 and HPyV10), skin swabs (MCPyV, HPyV6, HPyV7 and HPyV9), and urine (HPyV7, HPyV9 and STLPyV).[12—17] Moreover, a number of these HPyVs have been detected in feces (KIPyV, WUPyV, MCPyV, HPyV6, HPyV9, HPyV10, STLPyV and HPyV12), but their causative roles as diarrheal agents have not been confirmed because these viruses may also be found in asymptomatic hosts.[17—24] We suspect that biliary excretion of these HPyVs may be an alternative explanation of their presence in feces. More importantly, we also investigated the association between these polyomaviruses and hepatobiliary tract and pancreatic malignancies or acute cholangitis.

Materials and methods

Ethical consideration

The study was approved by the institutional review board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Patients and specimens

Between 1 July 2011 and 30 June 2015, a total of 171 bile samples were collected by endoscopic retrograde cholangiopancreatography and/or percutaneous transhepatic biliary drainage from 171 adult Chinese patients admitted to Queen Mary Hospital, Hong Kong, China, for acute gallstone cholangitis or malignant biliary obstruction due to histologically-proven hepatobiliary tract or pancreatic malignancies. Their relevant demographic, clinical, and laboratory characteristics were recorded into a predefined database for analysis.

Consensus PCR screening for human polyomaviruses

Screening of 11 species of known HPyVs by PCR using consensus primers was performed as we previously described with slight modifications.[25] Briefly, total nucleic acid extraction was performed on 200 μl of each bile sample using EZ1 virus Mini Kit v2.0 (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The total nucleic