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BK virus in cancer development

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ABSTRACT

Polyomavirus (PyV) was discovered by accident in 1950 in the course of describing an infectious factor causing multiple tumours in rodents. The term is derived from two Greek words: poly (many) and oma (tumour). At present the family of human polyomaviruses (HPyV) consists of 10 members. One of the first members was BK virus, isolated in 1971 from the urine of a renal transplant patient. Serological examinations have shown that due to its ability to cause latent infection, about 90% of the general population can have specific antibodies attesting infection. In the case of infected persons with normal immunity, this virus is not dangerous. In the impaired immunity, however, loss of immunity results in virus reactivation and development of many life-threatening illnesses. Serological examinations have also reveal that BK polyomavirus considerably affects the development of cancers in humans. Hence, in 2012 a group of 26 researchers from 11 countries associated with the International Agency for Research on Cancer (a part of the World Health Organisation) classified BK polyomavirus within group 2B – "potentially carcinogenic to humans".

INTRODUCTION

In the course of describing an infectious factor causing multiple tumours in rodents, polyomavirus (PyV) was discovered by accident in 1950. The term is derived from two Greek words: poly (many) and oma (tumour) [1]. At present, the family of human polyomaviruses (HPyV) consists of ten members: BK virus (BKPyV) and JC virus (JCPyV), isolated for the first time about 45 years ago, and eight viruses identified more recently: KI (KIPyV) and WU (WUPyV), Merkel cell polyomavirus (MCPyV), HPyV6, HPyV7, trichodysplasia spinulosa polyomavirus (TSPyV), HPyV9 and MWPyV. The infectious factor PyV belongs to the *Polyomaviridae* family of viruses which are classified as group 1 in the Baltimore classification, wherein the genetic material consists of a single, circular double-stranded DNA particle. There is only one type of genome within the polyomaviruses (consisting of about 5000 base pairs), which is closed in an unenveloped icosahedral capsid. Viruses belonging to the Polyomaviridae family are usually typical of a given "host" species and are specific as to the selection of cells where the virus becomes latent or where lytic activation occurs [1,2]. In the case of most common species attacking people, JC and BK viruses choose urinary tract cells, KI and WU viruses attack respiratory tract cells and MC virus enters Merkel cells [3]. This individual nature is related to transcription and replication factors specific for a given species. Many of these, though, can also cause transformations in cells where lytic cycle activation does not normally occur [2].

The polyomavirus genome organisation consists of three functional regions: the non-coding control region (NCCR) and two coding regions: early and late. The non-coding region is the site of replication origin (ORI). Transcription from one side of ORI results in mRNAs encoding early proteins, and transcription from the other side of ORI generates the late structural proteins. The early non-structural proteins are called tumour antigens (T-Ag) because they affect cell cycle regulation, and, in some cases, induce cell transformation or tumour formation. T-Ag binds cancer suppressive proteins such as RB and p53, and initiates bi-directional viral genome replication from ORI. It also initiates the transcription of late genes which are transcribed from the opposite side of ORI from a strand complementary to that used for early gene transcription. The late region usually encodes three structural proteins (VP1, VP2 and VP3). Out of three structural proteins, VP1 constitutes above 70% of the total protein content in virus particles, therefore, it is also called the major virus protein. As far as BKPyV is concerned, its main task is binding to host cell surface receptors, thus making it possible for the virus to enter the cell. In addition, the VP1 protein sequence demonstrates significant genetic variability, giving rise to four basic BKPyV

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genotypes. The late region of many polyomaviruses also encodes a non-structural protein, known as the agnoprotein. This protein can interact within a few replication cycle positions and can play a role in facilitating the capsid assembly, but is not a part of the mature virion [4].

BKPYV CHARACTERISTICS

Polyomavirus infections, including BK virus, are widespread both in developed and developing countries [4,5]. It is estimated that about 90% of the entire population has antibodies against BKPyV particles. This high percentage may be related to the period when the primary infection occurs, usually in early childhood. However, immediately after birth, maternal antibodies remain for the first few months, therefore, between the age of 4 and 11 months, antibodies are detected only in 5% of infants [5]. With regard to BKPyV, virus transmission routes are not fully recognised. It is assumed that infection is transmitted via the respiratory tract, the faecal-oral tract, the blood, or through organ transplant. In the case of the normal immune system, infection is asymptomatic, sometimes manifested by a mild inflammation of the upper respiratory tract, a slight increase in body temperature and urinary tract disorders. As a result of productive infection, the virus passes to the latent stage, where urinary tract cells are the main site of persistence. Except for the urinary tract, BKPyV also localises in peripheral blood mononuclear cells (PBMC), tonsils and hematopoietic tissues [3]. A slightly impaired immune system, usually observed in the elderly, in pregnant women and diabetics, can initiate active virus replication. Still, in the case of impaired immunity accompanied by intensive immunosuppression therapy such as in organ transplantation or through acquired immunodeficiency syndrome (AIDS), evident clinical infection occurs [6,7]. Most often, attention is paid to three types of pathologies related to BK virus infection: interstitial nephritis and ureteral stenosis in patients after renal transplantation, as well as hemorrhagic cystitis in patients after transplantation of hemorrhagic stem cells [7].

CANCERS

The very name "polyomavirus" suggests that they can take part in cancer pathogenesis, and cell culture research, as well as some reports confirm the role of BKPyV in the development of carcinogenesis. Hence, in 2012, a group of 26 scientists from 11 countries associated with the International Agency for Research on Cancer, a part of the World Health Organisation, classified BK polyomavirus, as a group 2B - "potentially carcinogenic to humans" [8].

CELL TRANSFORMATION MECHANISM

Three mechanisms of HPyV infection affecting cancer development can be distinguished: hit-and-run, passenger and by-stander. In the first, the hit-and-run mechanism, a cell is infected with polyomavirus at the early stage of neoplasia. This results in the increase of chromosomal instability (CIN) and accelerates the process of carcinogenesis. However, in the advanced form, in the course of diagnosis, the virus genetic material is not detected. In the passenger mechanism, the virus attacks the tumour cell where it finds favourable conditions for its genome replication, but it does not have direct influence on cancer development. It can, however, engender side effects, like cell weakening and carcinogenesis facilitation. The last, the by-stander mechanism, does not affect the process of oncogenesis. Instead, the virus infects cells adjacent to tumour cells and is detected in anatomically attached compartments of these cells [1].

A further role in the process of carcinogenesis is played by one of the functional parts of the virus genetic material, the early region which encodes two non-structural proteins: the large-T antigen (T-Ag) and the small-t antigen (t-Ag). T-Ag is crucial in initiating cell transformations and their unlimited proliferation due to its influence on proteins produced by the host. Polyomavirus genomes do not code replication proteins, and, therefore, they use proteins produced by the infected cell in the S phase of the cell cycle. T antigen is engaged in the mechanism of taking control over the cell by disrupting the activity of suppressor proteins: pRb and p53 [9].

The disruption of TP53 gene coding p53 protein takes place in about 50% of all cancers. T-Ag of BK virus binds to p53 protein causing its inactivation, thus disabling the cell division cycle inhibition, which, consequently, results in an unlimited number of divisions. The exclusion of apoptosis mechanisms is crucial for providing the optimum environment for the virus genetic material multiplication and the submission of new virions. This action facilitates transformation in non-permissive cells and supports lytic infection in permissive cells [9].

The pRb protein (Retinoblastoma protein), belonging to the family of the so-called pocket proteins, is responsible for inhibiting the E2F factor (from the family of transcription factors) which is crucial for cell transition through the G1/S check point. In the case of DNA damage, the unphosphorylated active pRb protein binds to E2F, blocking the further cell division. Usually, in the course of a normal cell cycle, at the end of the G1 phase, CDK4 and CDK6 cytokines phosphorylate pRB protein. Its deactivation results in releasing transcription factors, which allows for the S phase of cell division. In the infected cell, despite the genetic material transformation, the cell cycle is not inhibited. This comes about from the ability of T-Ag viral binding to pRb, which causes the release of E2F factor and the cell cycle progression in spite of the occurring changes. It is the main mechanism used by the virus due to which T-Ag favours the abnormal proliferation of transformed cells of oncogenic character [9].

The next mechanism depends on taking control over the gene of DNA-methylotransferase (DNMT1), being the target gene for the E2F transcription factor. Its overexpression is related to the cancer suppressive gene, hypermethylation, which can result in oncogenesis development. It has been determined that T antigens of human BK polyomavirus and E1a adenovirus are capable of strong activation of transcription from the DNMT1 promoter. This activation requires Rb protein inactivation by oncogene and then the active E2F factor release. Results of this research, combined with earlier

research on DNMT1 overexpression effects, suggest that the abnormal regulation of DNA methylotransferase activity by pRb/E2F can be important, not only in viral lytic infections, but also in virus induced transformation and cancer development. In the case of virus mutations incapable of binding pocket proteins (RB), the lack of effectiveness in DNMT1 activation is observed, compared with their wild type equivalents. Moreover, E2F mutations inside DNMT1 promoters considerably neutralize transcription activation. These data suggest that DNMT1 viral induction via pRB/E2F route modulation can result in cancer transformation [10].

ROLE OF BKPYV IN CANCER DEVELOPMENT IN HUMANS

BKPyV in kidney and urinary tract cancer

A lot of research made on cell cultures and animals has confirmed the direct role of BKPyV in the process of carcinogenesis and cell transformation. Due to its ability of passing to latent state and locating in the urinary tract, it is assumed that the viral infection can mainly contribute to the increase of urinary tract cancer development. The latent infection supporting cell transformation is crucial for neoplasm. It has been observed that the growth of immunosuppression related to organ transplantation, very often a kidney, can lead to virus reactivation and initiate its replication. This can explain why the increased risk of malignant transformation related to BKPyV most often results in kidney cancer [11]. Indeed, research conducted by Narayanan *et al*. [12] confirmed the role of BK virus in the pathogenesis of cancer development. A case of a patient after kidney transplantation was described, where a poorly differentiated cancer of this organ was evident, after development of BKPyV-related nephropathy. The presence of BK virus was confirmed both in the transplanted organ and cancer, as well as the patient's blood and urine. It is worth noting that the donor's healthy kidney showed no sign of infection. Likewise, Geetha *et al*. [13] reported a case of bladder cancer in a patient with BKPyV nephropathy, where the virus genetic material was found in the bladder, in the metastasis (except for surrounding stromal cells) and in the nondysplastic urothelium. The absence of BKPyV in the intact tissues revealed that the virus was a casual transforming agent. The next report which confirms the role of BK virus in cancer development is the research made by Emerson *et al*. [14], who reported a case of a paediatric recipient of a kidney from an adult donor, where kidney cancer was probably developed due to BKPyV infection-related nephropathy. Reports concerning the influence of BK virus infection on cancer development happen to contradict each other. In research conducted by Kausman *et al*. [15] in a 10-year-old boy with a kidney tumour after BKPyV infection-related nephropathy, the removal of the primary tumour and discontinuation of immunosuppression resulted in the tumour regression and complete recovery. Immunohistochemical staining, nonetheless, indicated the absence of virus genetic material in the tumour. Knoll *et al*. [16] researched the BKPyV DNA and T-Ag expression in 55 patients with kidney cancer. In the group of examined persons, the presence of viral DNA was detected in only 9 patients, out of which in four cases, it was detected in only the tumour, and in three cases, in intact tissues. Moreover, only one sample indicated the presence of BKPyV T-Ag in the tumour. The presence of viral T antigen, nevertheless, was also detected in the healthy tissue, which let the authors assume that the virus had no influence on the tumour development.

Adequately sensitive and specific diagnostics is vital in the assessment of cancer development in infected persons. This became evident to Rogers *et al*. [17] when they examined 646 patients to estimate the relative risk of urinary tract cancer development related to BKPyV infection in patients with kidney transplantation between the year 2000 and 2009. The research results actually confirmed the 8-fold growth of UCC cancer development related to infection, but they were not statistically significant. Still, it is worth noting that in 33% of the examined patients, no PCR tests for the presence of virus in blood or urine were conducted, and the frequency of tests rose from 18% in 2000 to 84% in 2009, thus the noted percentage of detected BKPyV infections rose from 7 to 24%.

BKPyV in other cancers

Apart from urinary tracts, the BKPyV sequence is also frequently found in other cancers: brain, pancreas, lung and liver tumours, rhabdomysarcoma or Kaposi's sarcoma. The test results, however, are not always repetitive and the influence of virus infection on neoplastic transformation of cells raises a lot of doubts [18]. According to Tagahavi *et al*. [19], the BK virus can be a predisposing factor for prostate cancer development. In their study, viral DNA was identified in 15% of all patients with benign prostatic hyperplasia and in 28% of all patients suffering from prostate cancer. Furthermore, in patients with cancer, the viral DNA was observed more often in persons with lower Gleason grading. This situation could confirm the "hit and run" theory of viral infection.

Burger-Calderon *et al*. [20] suggest a connection between BKPyV and the oral cavity. BKPyV binds to cellular receptors such as N-linked glycoprotein with a 2,3-linked sialic acids and gangliosides GD1b and Gt1b. The aforementioned is true for both kidney (Vero) and oral (HSG) cells *in vitro*. Moreover, the virus genetic material was successfully detected with the use of Southern blot hybridisation in 19 out of 74 cases of patients with brain cancer and in 4 out of 9 patients with pancreatic islets cancer [21].

Research conducted by Flaegstad *et al*. [22] confirms the presence of the virus in brain cancers. BKPyV DNA was detected with the PCR method in 17 out of 18 patients with neuroblastoma, and in 16 of them, T-Ag expression was detected with the use of immunohistochemistry. Such results are contradicted by the results obtained by Arthur *et al*. [23], which indicated a lack of relation of BKPyV DNA to brain cancers (mainly glioblastoma multiforme).

A similar situation is observed in the case of research concerning the relation of BK virus infection to colorectal cancer. From among seven examinations assessing the role of BKPyV in human colorectal cancer, only two research groups detected BKPyV DNA and proteins in

the adenocarcinoma tissue of the large intestine. What is more, none of the remaining five teams managed to gather evidence for the existence of BKPyV DNA in a large range of colorectal cancers, adenocarcinomas and intact mucous membranes [24].

CONCLUSION

A lot of viral oncogenes are capable of transforming and inhibiting the host cell DNA replication to multiply their own genetic material. Contradictory reports concerning BKPyV influence on the process of neoplasia in humans can be explained by false positive results being the effects of contamination in the course of conducting the experimental part, the diversity of the test population, sample size changes and geographic differences in the occurrence of BKPyV. Another possible explanation is the loss of BKPyV DNA and protein which can result in obtaining false negative results. The absence of virus can result from differences in the selection of antibodies aimed at its antigens. Moreover, it is worth paying attention to the "hit and run" infection mechanism which can explain the lack of genetic material in non-differentiated cancer.

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