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Review

Mechanisms of BK virus infection of renal cells and therapeutic implications

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ABSTRACT

BK virus (BKV) causes BKV nephritis in renal transplant patients and contributes significantly to the increase of probability of graft loss. BKV, being latent in the urogenital tract, is likely to be transported with the donor kidney to recipients and following reactivation replicates in the nucleus of renal epithelial tubular cells. BKV daughter viruses are released and enter other renal epithelial cells to spread infection. There are still a lot of unknown factors about the mechanism and kinetics of BKV infection. The treatment of BKV infection, with exception of reduction in immunosuppression which increases the risk of allograft rejection, is almost exclusively limited to application of anti-viral drugs with rather inconsistent results. The shortcomings of anti-viral therapies demand the understanding of early steps of infection of permissive cells by BK virus in hope that adequate interventional therapies preventing infection of cells with BK virus could be developed. This review describes the BKV entry in target human cells, intracellular trafficking pathways of BKV particles and potential therapeutic implications based on understanding of mechanisms of BKV infection of renal cells.

Contents

Abbreviations: BKV, BK virus; JCV, JC virus; BKVN, BKV nephropathy; VP1, viral protein 1; HRPTEC, human renal proximal tubular epithelial cells; Cav-1, Caveolin-1; ER, endoplasmic reticulum; GA, Golgi system; mPyV, mouse polyomavirus.

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1. General information about BK virus

BKV is a small double-stranded DNA virus assigned to the Polyomaviridae family, which other well-known members include the JC virus (JCV) and simian virus SV40 [1]. Polyomaviruses are ubiquitous viruses with high seroprevalence rates in general population. Following primary infection, the virus remains latent in the host in different sites, particularly the kidneys and uroepithelial cells. Reactivation from latency may occur in normal subjects with asymptomatic viruria, while it can be associated with nephropathy in kidney transplant recipients [2]. It has been shown that during immunosuppression, virus reactivation causes cytopathic changes in the uroepithelium, and consequently, there is increased shedding of BKV in the urinary tract [3]. This review discusses the clinical aspect of BKV nephropathy as well as mechanism of renal cells infection.

2. BK virus associated nephropathy– clinical aspect

2.1. Clinical presentation

Declining renal allograft function is the most common presentation. The patient is asymptomatic with progressive rise in serum creatinine [4]. Interstitial nephritis associated with BKV infection is difficult to distinguish from acute cellular rejection. Subsequent fibrotic changes can contribute to chronic allograft nephropathy. Ascending infection along uroepithelium from urinary bladder has been reported to cause ureteral stenosis and subsequently allograft obstruction. Higher level of BKV has also been associated with hemorrhagic cystitis, and may be the initial presentation of BKV nephropathy in renal transplant recipient [4].

2.2. Diagnosis

Decoy cells have been observed in urine cytology of patients with BKV Nephropathy (BKVN). These cells originated from infected renal tubular cells with nuclei altered by viral inclusions. The presence of decoy cells is a sensitive (100%) measure but has a low positive predictive value of 29% for the diagnosis of BKVN [5]. Quantification of viral load in the plasma and urine with either viral DNA or viral protein-1 (VP-1) mRNA has been used to diagnose BKVN [5]. There is a general agreement that BK viruria of greater than 10^7 BKV copies/ml of urine and BKV viremia of 10^4 copies BKV/ml of plasma are typical in patients with biopsy-proven BKVN [3]. A transplant kidney biopsy remains the gold standard for diagnosing BKVN. Pathologic findings of infection include viral cytopathic changes in the tubular epithelium, glomeruli, and collecting ducts with interstitial inflammation and varying degrees of tubular atrophy or fibrosis [5]. These histological changes can be focal or isolated to the medulla and missed on one third of biopsies if only a single core is evaluated [6]. Therefore, at least two cores including the medulla should be examined. If there are no cytopathic changes on histology and there is a high clinical suspicion, then adjunctive tests such as immunohistochemistry staining with cross-reacting SV40 large T antigen should be performed since the histopathology of BKV infections may be misinterpreted as acute rejection [6].

2.3. Treatment

The goal in treating BKV infection is to eliminate the virus while preserving renal function and preventing acute or chronic rejection [5]. The principal treatment of BKV nephropathy is reduction in immunosuppression, which carries a risk of acute allograft rejection [4,6]. Various strategies for altering immunosuppressive therapy include discontinuation of an agent, decreasing an agent, switching immunosuppressant within the same class or to another class, steroid avoidance, or adjunctive therapies [5,6]. Antiviral therapy with leflunomide or cidofovir has been used in conjunction with decreasing immunosuppression in some cases [5]. Therapy with rituximab the anti-CD20 mAb has also been used with promising results. The administration of IVIG with concomitant reduction in immunosuppressive therapy has been successful; however, efficacy of IVIG is unclear [5]. Close monitoring of BKV DNA and renal function with any therapy is critical to improving outcome for patients with BKV infection [5]. Failure to clear BKV leads to worse graft function and outcomes. Monitoring should be performed with BKV PCR, until the viral level is undetectable or at least falls below the threshold value that is associated with BKV nephropathy [6]. On the basis of kinetic models and prospective monitoring, viremia clears in 7–20 weeks, but the initial decrease may be delayed by 4–10 weeks after immunosuppression reduction [6].

3. Mechanism of infection of renal cells: entry into cells and intracellular trafficking

The treatment of BKV infection, with exception of reduction in immunosuppression which increases the risk of allograft rejection, is almost exclusively limited to application of anti-viral drugs with rather inconsistent results. The shortcomings of anti-viral therapies demand the understanding of early steps of infection of permissive cells by BK virus in hope that adequate interventional therapies preventing infection of cells with BK virus could be developed. The efforts to uncover the mechanisms of BK virus infection have employed the preparations of human BK virus and cultured permissive cells. It is beneficial to use human renal proximal tubular epithelial cells (HRPTEC) since tubular epithelial cells are the main natural target of BKV infection [7,8].

The first indication that BK virus might enter target cells via caveolae-mediated endocytosis came from studies of BKV infection of permissive Vero cells derived from the kidney of an African green monkey [9]. Studies employing Vero cells allowed elucidating the early steps of BKV infection [10], but, since polyomaviruses are notoriously species specific and since Vero cells is not a primary, but an established aneuploid cell line, the mechanisms of entrance of BK into human renal epithelial cells could be different. The nature of host cells has a profound effect upon efficiency and consequences of viral infection. Experiments carried out with primary HRPTEC demonstrated that the depletion of cholesterol and decrease of Caveolin-1 (Cav-1) levels by Cav-1 siRNA inhibited cellular infection by BK virus [11]. Electron microscopic study of patients with BK allograft nephropathy revealed that BK virions appear to enter the renal tubular cells in non-coated vesicles that are morphologically consistent with caveolae [12] (caveolin-containing lipid rafts) which are small (diameter of 50–80 nm) flask-shaped plasma membrane invaginations, enriched in sphingolipids and cholesterol. Endocytosis of caveolin-containing lipid rafts occurs in the absence of functional clathrin-coated pits and is a highly regulated process [13]. BKV infection was not inhibited in HRPTEC transfected with clathrin siRNA. The colocalization of labeled BKV particles with Cav-1 and absence of significant colocalization with clathrin, as revealed by fluorescent microscopy and cross-correlation spectroscopy [14], further supports the conclusion that caveolar endocytosis is critical for BK virus infection of human renal epithelial cells [11].

The BKV passage through the endoplasmic reticulum (ER) on the way to nucleus [15]. Co-incubation of HRPTEC with BKV and microtubule disrupting agents prevented BKV infection [16]. It appears that after endocytosis via caveolae BKV particles are transported along the microtubules. This process requires the dynamicity of microtubules to be intact, but seems to be independent of motor protein dynein activity. In HRPTEC BKV particles are found in the ER

at 6–8 h after infection. Even though in morphological study with human patients the endosomes carrying virions appeared to fuse with a system of smooth vesicles and tubules that communicated with rough ER and was continuous with the Golgi system (GA) [12], a detailed analysis of intracellular trafficking pathways in HRPTEC revealed that BKV particles completely bypass the GA [16]. BKV infection of Vero cells was also sensitive to nocodazole-induced disassembly of the microtubule network for the initial 8 h following virus binding [17]. After that BKV particles enter the nucleus and following viral replication daughter viruses are delivered to other cells using the same cell entering and intracellular trafficking pathways. As a result, BKV infections expands and BKVN progresses. BKV course of infection in HRPTEC seemed to be relatively slow with process taking at least 24–48 h.

4. Cellular determinants of BK virus infection

The first step of infection is the interaction between the viral particle and target cells. All polyomaviruses utilize some kind of receptor molecules which present cellular determinants for interaction with viral particle. In pioneering work by the Atwood laboratory an N-linked glycoprotein containing alpha(2,3)-linked sialic acid was reported to constitute a critical component of the cellular receptor for BKV [18]. Site-specific mutagenesis, coupled with infectivity assays, allowed to generate a subset of BKV mutants deficient in binding to Vero cells and propose the existence of oligosaccharide receptor binding pocket on VP-1 [19]. Viral entry of not only BKV, but also JC virus (another human polyomavirus, which causes progressive multifocal leucoencephalopathy) is dependent on the ability to interact with sialic acid [20]. The nature of the sialyted glycoprotein, which serves as a receptor for BKV, remains to be uncovered. Since JC virus uses serotonergic receptor $5HT_{2A}R$ to infect human glial cells [21], it is possible that the BKV might also utilize a G-protein coupled receptor to enter renal epithelial cells. Polyomaviridae family of viruses displays a substantial variability with regard to their cellular receptors and have been shown to utilize molecules previously unknown to possess virus-related activities. The protein component of receptor of mouse polyomavirus (mPyV) appears to be $\alpha 4\beta 1$ integrin [20]. In innovative work by the Imperiale laboratory, sialyted glycosphyngolipid structures gangliosides GD1b and GT1b were also identified as receptors for BK virus [15].

5. Therapeutic implications and future directions of treatment

Although potent immunosuppressive therapies have proved to be useful for acute and chronic rejection after renal transplantation, they are a risk factor for the progression of latent BKV to BKVN. Retransplantation after renal allograft loss to BKVN remains a treatment option for some patients [22]. There are more than 100,000 patients with end stage renal disease waiting for kidney transplantation due to scarcity of organs which means loss of an allograft can put patients on dialysis for years. Moreover, the cost of retransplantation could exceed $500,000 per patient in some centers which includes laboratory charges for viral load testing, dialysis or other costs associated with managing BKV-related renal failure. Even after retransplantation there is a rate of recurrence of BKV in the new transplant [23]. Thus, BKVN remains a major threat to the success rate of most transplant centers, overall allograft longevity, and contributes significantly to escalation of health care costs in USA and worldwide. The treatment of BKVN pins on reducing immunosuppression and the treatment protocol is center specific. In general stopping or reducing antimetabolites like mycophenolate or azathioprine is common, which is usually combined with reducing calcinurin inhibitors. When immunosuppression has been reduced to the limit and still there is continuous decline of renal function antiviral therapy is initiated. Even with successful management of BKVN, the treatment is associated with an overall 1.69 hazard ratio of allograft loss. In view of limited success of routine anti-viral therapy and risk of drug renal toxicity the future directions of treatment might take advantage of the knowledge of early steps of BKV entry into the target cells. As described above, it has been established that BKV is taken up into cells via caveolae-associated endocytosis and that this is critical for BKV infection of HRPTEC [11,16]. Also, it seems very likely, that BKV uses unidentified sialyted glycoprotein as a cellular receptor to infect target cells. We believe that uncovering the identity of this glycoprotein will open up therapeutic approaches to interfere with binding of BKV to its cellular receptor. Human polyomavirus receptors may represent targets for therapeutic intervention to prevent and treat polyomavirus related diseases [24]. Thus, studies focusing on uncovering of the nature of BKV receptor in human renal epithelial cells represent a significant and clinically relevant area of investigation with immediate translational potential.

The other possible novel direction of therapeutic intervention of BKVN is interfering with BKV caveolar-mediated endocytosis. It is probable that agents, which are used in a clinical situation as cholesterol lowering agents, might decrease caveolae, interfering with BKV internalization and decreasing BKV infection of human renal epithelial cells. The ability of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors (statins), which is routinely used to treat hypercholesterolemia, to repress BK virus entry pathways in HRPTEC and, correspondingly, prevent BKV infection was demonstrated [25]. In this study HRPTEC were co-incubated with BKV and pravastatin, one of the hydrophilic statins. Both the percentage of BKV infected cells and the large T antigen expression, which suggested BKV infection, were significantly decreased in HRPTEC pretreated and co-incubated with pravastatin [25]. The pravastatin's inhibitory effect could be explained by a dramatic depletion of Cav-1, a critical element of caveolae, which happens due to inhibition of cholesterol synthesis. These data suggest that statins, acting via depletion of Cav-1, could prevent caveolar-dependent BKV entry and repress BKV infection of HRPTEC. Even though decrease of Cav-1 was demonstrated in pravastatin-treated HRPTEC [25], it must be taken into consideration that non-cholesterol mechanisms of statin action have been proposed [26]. Inhibition of the mevalonate pathway, which is inhibited by statins, also affects synthesis of isoprenoid precursors for prenylation of a number of signaling molecules, including Ras family of proteins [27]. The concentrations of statins needed to inhibit protein prenylation are much higher than those which prevent cholesterol synthesis suggesting that cells are likely to maintain the prenylation pathway at the expense of cholesterol synthesis [27]. Regardless their mechanism of action, statins unlikely to be efficient against progressed BKVN. Accordingly, the treatment with statins should be considered as soon as possible following detection of BKV viruria or viremia and it may be already too late when BKVN is diagnosed by renal biopsy, because at the time of diagnosis, BKV infection would be established by now and BKV will spread to other cells.

Recently it was demonstrated that viral attachment proteins coupled to beads can be used to screen large families of compounds that possessed similar chemical space as sialic acid for their ability to bind the virus. The high throughput search revealed several gallic acid-based small compounds which reduced binding and infection

of Vero cells by BKV [28]. These studies could set the base for the development of virion specific antagonists to treat BKV infection.

6. Conclusion

Nephritis induced by BKV, a non-enveloped double-stranded deoxyribonucleic acid (DNA) polyomavirus, remains a severe problem after renal transplantation. In view of limited success of routine anti-viral therapy the future directions of treatment should take advantage of the knowledge of early steps of BKV entry into the target cells. Caveolar endocytosis is critical for BKV infection of human renal tubular epithelial cells. An N-linked glycoprotein or ganglioside containing alpha (2,3)-linked sialic acid is a component of the cellular receptor for BKV. Novel potential directions of therapeutic intervention of BKV nephritis are strategies based either on prevention of binding of BKV particles to cellular receptors on renal tubular epithelial cells or interference of caveolin-mediated endocytosis and intracellular trafficking of BKV. The uncovering of the nature of BKV receptor in human renal epithelial cells, development of statin-based strategies to prevent caveolar-dependent BKV entry and depiction of small molecules - virion specific antagonists, would have immediate translational potential.

Conflict of interest

Authors do not have any financial or personal relationship with other people or organizations that could inappropriately influence their work.

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