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Macaque homologs of Kaposi's sarcoma-associated herpesvirus (KSHV) infect germinal center lymphoid cells, epithelial cells in skin and gastrointestinal tract and gonadal germ cells in naturally infected macaques

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ABSTRACT

We developed a set of rabbit antisera to characterize infections by the macaque RV2 rhadinovirus homologs of KSHV. We analyzed tissues from rhesus and pig-tailed macaques naturally infected with rhesus rhadinovirus (RRV) or Macaca nemestrina rhadinovirus 2 (MneRV2). Our study demonstrates that RV2 rhadinoviruses have a tropism for epithelial cells, lymphocytes and gonadal germ cells in vivo. We observed latent infections in both undifferentiated and differentiated epithelial cells with expression of the latency marker, LANA. Expression of the early (ORF59) and late (glycoprotein B) lytic markers were detected in highly differentiated cells in epithelial ducts in oral, renal, dermal and gastric mucosal tissue as well as differentiated germ cells in male and female gonads. Our data provides evidence that epithelial and germ cell differentiation in vivo induces rhadinovirus reactivation and suggests that infected epithelial and germ cells play a role in transmission and dissemination of RV2 rhadinovirus infections in vivo.

1. Introduction

The Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 is the most recently discovered member of the *Herpesviridae* that infects humans (Chang et al., 1994). KSHV is the causative agent of all forms of Kaposi's sarcoma (KS), including classical KS in elderly Mediterranean men, endemic KS in all populations in Sub-Saharan Africa and epidemic AIDS-associated KS in HIV-infected populations, and is also associated with several lymphoproliferative diseases, including primary effusion lymphoma (PEL) and a subset of multicentric Castleman Disease (MCD) (Schulz and Cesarman, 2015). KSHV is phylogenetically related to Epstein-Barr virus (EBV)/human herpesvirus 4, grouping within the tumor-inducing gammaherpesvirus subfamily. KSHV was originally identified in AIDS-KS lesions and analysis of the KSHV genome revealed a strong sequence similarity with herpesvirus saimiri (HVS), the prototype of the *Rhadinovirus* genus of gammaherpesviruses found in the New World squirrel monkey (Russo et al., 1996). We and others have shown that Old World primates are infected with two different Rhadinovirus lineages (Greensill et al., 2000; Schultz et al., 2000). KSHV belongs to the RV1 lineage, along with closely related homologs in chimpanzees, gorillas, macaques and other Old World primates. We identified macaque homologs of KSHV in KS-like retroperitoneal fibromatosis lesions associated with simian AIDS in different macaque species (Rose et al., 1997; Schultz et al., 2000). We obtained a partial sequence of the retroperitoneal fibromatosis herpesvirus (RFHVMm) infecting *Macaca mulatta*, the rhesus macaque (Bruce et al., 2009; Burnside et al., 2006; Rose et al., 2003, 1997; Schultz et al., 2000) and have sequenced the complete genome of the retroperitoneal fibromatosis herpesvirus (RFHVMn) infecting *Macaca nemestrina*, the pig-tailed macaque (Bruce et al., 2013). Both RFHVMn and RFHVMm have a very close similarity in sequence and gene structure to KSHV. The human and macaque RV1 genomes encode more than 90 genes, including a core of genes conserved across the herpesvirus family, which function in virus replication and virion

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structure. A group of latency associated genes, including the ORF73 latency-associated nuclear antigen (LANA), are conserved between the RV1 rhadinoviruses (Bruce et al., 2013; Burnside et al., 2006) In addition, the RV1 rhadinoviruses encode a number of novel genes exhibiting sequence homology to cellular genes that function in mitosis, cell cycle regulation and immunity (Bruce et al., 2013; Nicholas et al., 1998).

Macaques and other Old World primates, including chimpanzees and gorillas, are also infected with a more distantly related rhadinovirus, belonging to the RV2 rhadinovirus lineage (Greensill et al., 2000; Lacoste et al., 2000a, 2001, 2000b; Schultz et al., 2000). However, an RV2 rhadinovirus infecting humans has not been identified to date. The complete genome sequences have been determined for two distinct RV2 rhadinoviruses infecting the rhesus macaque, RRV-17577 (Searles et al., 1999) and RRV-2695 (Alexander et al., 2000), and we have determined the complete genome sequence for a closely related RV2 rhadinovirus infecting the pig-tailed macaque (MneRV2) (Bruce et al., 2015). Overall, the genome structure of the RV2 rhadinoviruses resembles that of KSHV, RFHV and the other RV1 rhadinoviruses, however, the RV2 rhadinoviruses lack a number of conserved RV1 genes implicated in immune evasion and pathogenesis and have an eight-fold amplification of an interferon regulatory gene (Bruce et al., 2015).

Significant sequence differences are observed between genes conserved between the RV1 and RV2 rhadinoviruses (Bruce et al., 2013, 2015). We compared the LANA homologs of the macaque and human RV1 rhadinoviruses, RFHVMn and KSHV, respectively, with the LANA homologs of the macaque RV2 rhadinoviruses, RRV and MneRV2. RFHVMn and KSHV LANA contain long acidic repeat sequences in middle of the proteins, which react with specific monoclonal antibodies (Bruce et al., 2006; Kellam et al., 1999). This repetitive region is missing in the LANA homologs of RRV and MneRV2 (Burnside et al., 2006). While the expression and localization of the KSHV and RFHVMn LANA homologs has been analyzed by immunohistochemistry and detected in tumor tissue in vivo (Bielefeldt-Ohmann et al., 2005; Bruce et al., 2006), no reagents have been developed to detect the LANA homologs of RRV or other RV2 rhadinoviruses. Sequence comparisons of the RV1 and RV2 ORF59 DNA polymerase processivity factors, which are critical markers of viral replication, revealed a high level of sequence similarity in the N-terminal domain (Bruce et al., 2009). While the C-terminal region was highly conserved between the macaque RV2 rhadinoviruses, there was minimal sequence similarity to the C-terminal domain of the RV1 ORF59 homologs. We have utilized this sequence difference to develop a panRV2 ORF59 polyclonal rabbit antiserum, which detects the ORF59 lytic marker of the macaque RV2 rhadinoviruses (Bruce et al., 2009). Using this antibody, a strong correlation was detected between the expression of RRV ORF59 in the nucleus of experimentally infected tissue culture cells and replication and production of infectious virions (Bruce et al., 2009). Strong nuclear expression of ORF59 was also observed in RRV- and MneRV2-infected tumor cells in simian AIDS-associated T-cell and B-cell lymphomas (Bruce et al., 2012). This wide-spread ORF59 expression was highly correlated with elevated levels of RRV and MneRV2 viral DNA, attaining nearly 1000 viral genomes per cell, further establishing ORF59 as a marker of lytic replication. In contrast, the major rhadinovirus virion glycoprotein, glycoprotein B (gB), is highly conserved between KSHV, RFHVMn, RRV and MneRV2 (Bruce et al., 2016). Sequence alignment revealed a peptide sequence that was completely conserved in the gB homologs of the different RV1 and RV2 rhadinoviruses. We have utilized this gB peptide sequence to develop a polyclonal panRV1/RV2 anti-gB rabbit (1508) antiserum, which can detect the gB homologs of KSHV and the macaque and chimpanzee RV1 rhadinoviruses as well as the gB homologs of the macaque RV2 rhadinoviruses. A strong characteristic staining of cytoplasmic gB is detected with this antiserum in RRV and MneRV2 infected lymphomas, which correlates with RV2 ORF59 expression and replication of viral DNA (Bruce et al., 2012).

KSHV and the chimpanzee and macaque RV1 rhadinovirus homologs contain a conserved “RGD” motif in the major virion glycoprotein B, which functions to bind the critical αVβ3 integrin entry receptor (Bruce et al., 2016; Garrigues et al., 2014a, 2008, 2014b). KSHV also utilizes the ephrin receptor tyrosine kinase A2 (Eph)A2 as an entry receptor, which binds to the virion-associated glycoprotein H/glycoprotein L (gH/gL) complex (Hahn and Desrosiers, 2014). The gH/gL complex of the macaque RRV also binds members of the Eph family receptors for entry into B-cells and endothelial cells, but uses an uncharacterized Eph-independent receptor for entry into fibroblasts and epithelial cells (Hahn and Desrosiers, 2013). A previous study suggested that KSHV utilizes the α3β1 integrin as an RGD-dependent entry receptor (Akula et al., 2002), however, we have shown that KSHV entry is independent of α3β1 and KSHV gB does not bind soluble α3β1 (Garrigues et al., 2008). The virion gBs of RRV and other RV2 rhadinoviruses lack an “RGD” motif (Bruce et al., 2016), and do not interact with either αVβ3 or α3β1 integrins (Garrigues et al., 2008). Other studies have identified the C-type lectin DC-SIGN and the cysteine transporter xCT as KSHV receptors (Kaleeba and Berger, 2006; Rappocciolo et al., 2006), however, it is not known whether these receptors are utilized by the RV2 rhadinoviruses.

Both the RV1 and RV2 rhadinoviruses show a broad cellular tropism in vitro infecting cells of lymphoid, endothelial, epithelial and mesenchymal origin. In general, KSHV infection of a wide variety of tissue culture cell types is latent, with high level expression of LANA and other latency-associated genes and only minimal expression of ORF59, gB and other genes associated with the pathway of lytic replication (Bechtel et al., 2003; Blackbourn et al., 2000; Lagunoff et al., 2002; Renne et al., 1998). In contrast, RRV and MneRV2 infection of fibroblasts in culture is permissive with high level expression of ORF59 and production of infectious virions (Bruce et al., 2009; Desrosiers et al., 1997). While RRV infection of EBV-transformed B-cell lines resulted in low levels of virus replication (Bilello et al., 2006), our studies showed that RRV infection of mucosal epithelial cells, including the HeLa cervical and AGS gastric adenocarcinoma cell lines, displayed a strict latency with no expression of the critical ORF59 marker of lytic replication (DeMaster and Rose, 2014). In pathological tissue in vivo, KSHV is detected in the spindled tumor cells of endothelial origin in HIV+ and HIV- KS (Chang et al., 1994; Huang et al., 1995; Schalling et al., 1995) and blastoid lymphocytes of B-cell origin in AIDS-related pleural effusion lymphoma and multicentric Castleman disease (Cesarman et al., 1995; Dupin et al., 1999; Foreman et al., 1997; Orenstein et al., 1997). The macaque RV1 rhadinovirus, RFHV, is detected in spindled tumor cells in AIDS-related retroperitoneal fibromatosis lesions in macaques infected with simian retrovirus 2 (SRV-2) or simian immunodeficiency virus (SIV) (Bielefeldt-Ohmann et al., 2005; Bruce et al., 2006; Burnside et al., 2006). KSHV is also detected in endothelial cells and monocytes in KS lesions and keratinocytes of the epidermis overlying lesions, as well as adjacent eccrine ductular epithelial cells (Blasig et al., 1997; Boshoff et al., 1995; Foreman et al., 1997; Reed et al., 1998). The macaque RV2 rhadinoviruses RRV and MneRV2 are detected in blastoid lymphocytes of B-cell and T-cell origin in simian AIDS-related lymphomas and in circulating B-cells (Bergquam et al., 1999; Bruce et al., 2012; Orzechowska et al., 2008). In experimental infections, the macaque RV2 rhadinoviruses show a tropism for B lymphocytes (Mansfield et al., 1999; Orzechowska et al., 2009) and are detected in retroperitoneal fibromatosis lesions (Bruce et al., 2006; Orzechowska et al., 2008). Little is known regarding the sites and status of rhadinovirus infections outside of pathological lesions, although there is some evidence of KSHV infection in epithelial cells in oral tissues, including tonsils, adenoids and buccal epithelium (Chagas et al., 2006; Pauk et al., 2000; Webster-Cyriaque et al., 2006).

In the current study, we have utilized the panRV2 anti-ORF59 and the panRV1/RV2 anti-gB antiserum to examine the sites of infection and replicative status of the macaque RV2 rhadinoviruses in non-tumor tissue in naturally infected rhesus macaques (RRV) and pig-tailed macaques (MneRV2) undergoing long-term retroviral infections with