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Molecular epidemiology of Kaposi's sarcoma herpesvirus (KSHV) in Cuban and German patients with Kaposi's sarcoma (KS) and asymptomatic sexual contacts

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Abstract

Kaposi's sarcoma (KS) shows a distinct geographical and ethnic distribution. The variable K1 gene serves to differentiate the KSHV subtypes A–E, M, N, and Q. Phylogenetic characterization of 19 classical and epidemic German KS specimens revealed the Eurasian KSHV subtypes C (n = 13, including 6 classical KS) and A (n = 6), while 27 Cuban specimens showed a variety of different subtypes (A: n = 16, 4 being A5; C: n = 8; B: n = 2; and the new subtype E: n = 1). Three pairs of isolates from KS patients and peripheral blood mononuclear cells (PBMC) of their sexual partners without KS were studied for the first time and found identical, strongly arguing for sexual transmission of KSHV in this unique cohort. The unique ethnic background of the Cuban population may explain the variety of different KSHV strains.

Keywords: HHV-8; KSHV subtypes; Sexual transmission

Introduction

The human herpesvirus-8 (HHV-8) or Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent involved in all forms of Kaposi's sarcoma (KS) (Boshoff et al., 1995). KS is a tumor of mixed cellularity, occurring frequently in human immunodeficiency virus type 1 (HIV-1) infection (epidemic KS) and in transplant recipients (iatrogenic KS) (Hengge et al., 2002). More rarely, this tumor occurs among elderly men of Mediterranean and Middle eastern origin (classic KS) or in individuals from East and Central Africa (endemic KS) (Hengge et al., 2002). Furthermore, KSHV has been involved in the etiology of pleural effusion lymphoma (PEL) and multicentric Castleman's disease (MCD) (Cesarman et al., 1995).

While the complete genome of KSHV is conserved, both ends of this γ-herpesvirus have an incredible rate of variation. The ORF K1 gene encodes a highly variable membrane glycoprotein of 289 amino acids (aa), consisting of an extracellular domain (aa 1–228) with an amino terminal signal peptide, an immunoglobulin receptor-like domain with two variable regions (VR), called VR1 and VR2, a transmembrane domain (aa 229–261), and a C-terminal cytoplasmic domain (aa 262–289) which contains an immunoglobulin receptor tyrosine-based activation motif (ITAM) (Cook et al., 1999; Lee et al., 2003; Zong et al., 1999).

Due to the distinct geographical and ethnic pattern of distribution (Hengge et al., 2002), the phylogenetic analysis using ORF K1 sequences yielded eight major subtypes A–E, M, N, and Q with 15–30% of amino acid variability (Meng et al., 1999; Zong et al., 1999). Interestingly, subtypes A5 and B are almost exclusively associated with patients from sub-Saharan Africa and subtype D is primarily

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found in patients of Pacific Island heritage (Lacoste et al., 2000; Zong et al., 1999). Recently, the new E subtype has been described in Amerindians from Brazil (Biggar et al., 2000) and Ecuador (Whitby et al., 2004). The Eurasian subtypes A and C are distributed throughout the USA, Europe, Asia, and the Middle East (Meng et al., 1999; Zong et al., 1999).

Until August 2004, 82 AIDS-associated KS patients have been diagnosed in Cuba. This represents about 1.6% of the Cuban HIV population (Kouri et al., 2004). While only 21 cases of AIDS-associated KS were reported between 1986 and 1997, 61 patients were newly diagnosed with KS within the past 6 years, reflecting its current dramatic increase. From European countries molecular epidemiology data from Italy, Greece, Scandinavian, and Russia are available (Cesarman et al., 1995; Kadyrova et al., 2003; Zong et al., 1999). However, there are no reports regarding KSHV subtypes from Germany, although both, classical and AIDS-KS, are relatively frequent.

Therefore, we have analyzed the molecular epidemiology of the KSHV K1 gene from Cuban AIDS-KS patients and compared it with German samples from both classical and epidemic KS. Moreover–for the first time–we studied the presence of KSHV DNA in peripheral blood mononuclear cells (PBMC) from asymptomatic sexual contact persons of KS patients.

Results and discussion

The clinical and epidemiological features, including age, sex, ethnic origin, HIV status, and KS type of the 44 KSHV-positive patients, are summarized in Table 1. Consistent with other studies demonstrating a male predominance of KS, 9 out of 10 Cuban asymptomatic KSHV-infected patients (90%), 14 out of 16 (87.5%) from Cuban KS samples, and 17 out of 18 (94.44%) German KS patients were males. The mean age was similar for Cuban KS patients and their sexual contacts (mean: 38.2; range 28–63; and mean: 33.1; range 18–58, respectively), while German patients with classical KS were significantly older (mean: 72.2; range 65–84) than individuals with AIDS-KS (mean: 41.1; range 28–57) (Table 1). Regarding sexual behavior, most Cuban patients were homosexual (n = 19, 73.1%), three (11.5%) were bisexual, and four (15.3%) were heterosexual, including three women and one male patient. All German patients were homosexuals (n = 13, 72.2%), except for the classical KS patients.

Thirteen of the 24 (54.2%) Cuban asymptomatic sexual contacts (mean age: 38.1 for 20 men and 35.5 for 4 women, respectively) of KS patients were antibody-positive by the LANA IFA method; data not shown), similar to previous results obtained by our group (Kouri et al., 2004). Our previous study has shown that the prevalence rate of KSHV antibodies in HIV-infected Cuban patients was 32 of 154 (20.8%). This is significantly less than the frequency of 54.2% reported in this paper for individuals having a sexual relation with a KS patient (Kouri et al., 2004). These results support the notion that KSHV may be transmitted by homosexual intercourse besides other transmission modes such as kissing, breastfeeding, and shared utensils (Kedes et al., 1996).

To analyze KSHV subtypes in Cuban and German samples, we amplified and sequenced 621 bp of the extracellular domain of the ORF K1 gene (nt 117–738). We obtained 27 Cuban ORF K1 sequences using the two-step nested PCR technique: 10 from asymptomatic sexual contacts and 17 from 16 KS patients (one patient contributed two samples). In addition, we obtained 19 sequences from 18 German KS patients (one patient contributed two samples).

ORF K1 sequences were blast searched and appropriate reference sequences were selected from the GenBank database for phylogenetic tree analysis (Fig. 1a). As shown in Fig. 1a, three already known main subtypes A, B, and C and the newly described E subtype were identified based on topological associations and high bootstraps values of the nucleotide sequences. The phylogenetic characterization showed that the majority of the German samples fell in subtype C (n = 13, 68.4%), while 6 were grouped as A (32.6%). A and C subtypes are considered of Eurasian origin and may have evolved from a common ancestor about 35,000 years ago (Zong et al., 2002). In agreement with a previous report by Boshoff et al. all classical KS of our study belonged to the C subtype, while AIDS-KS belonged to the subtypes A or C (Boshoff et al., 1995). Cuban samples, in contrast to German samples, showed a wide range of subtypes, including A (16 samples; 4 of them A5), C (n = 8), and B subtype (n = 2), and the newly described E subtype (n = 1) (Fig. 1a), which has been recently found exclusively in Amerindians from Brazil and Ecuador (Biggar et al., 2000; Whitby et al., 2004). The A5 and B subtypes have previously been reported as African genotypes (Treurnicht et al., 2002; Zong et al., 1999). Interestingly, 5 of 6 of these “out-of-place” strains were detected in patients of Caribbean White ethnicity, whereas the three patients classified as Caribbean Black and all but one of those classified as Mulatto had classical Eurasian K1 subtypes. These results strongly support the Cuban melting pot idea of the parent strains probably originating in the classical sources from America, Africa or Europe, but later being transmitted across these ethic boundaries within the AIDS epidemic (Cook et al., 1999; Meng et al., 1999; Zong et al., 1999). All of our 46 sequences (Cuban and German) were different from each other with the exception of the samples obtained from the same individuals (C38-C40 and G22-G24). German samples tended to be more conserved compared to Cuban samples which were more distant from each other (Fig. 1a).

Upon pairwise comparison, the amino acid divergence ranged from 0% to 33% (Fig. 1b) while the nucleotide divergence accounted for up to 23%. The variation of