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DETECTION OF HERPESVIRUS-LIKE DNA SEQUENCES IN KAPOSI'S SARCOMA IN PATIENTS WITH AND THOSE WITHOUT HIV INFECTION

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Abstract *Background.* Herpesvirus-like DNA sequences have recently been found in lesions from patients with Kaposi's sarcoma and the acquired immunodeficiency syndrome (AIDS). It is not known whether these sequences are also present in classic Kaposi's sarcoma or in the Kaposi's sarcoma that occurs in homosexual men who are seronegative for the human immunodeficiency virus (HIV).

Methods. We analyzed DNA in tissue samples from patients with AIDS-associated Kaposi's sarcoma, patients with classic Kaposi's sarcoma, and HIV-seronegative homosexual men with Kaposi's sarcoma. We also analyzed DNA in samples of uninvolved tissue from these patients and in control tissue from healthy subjects. All samples were tested blindly by polymerase chain reaction (PCR) with specific primers to amplify $KS330_{233}$, a herpesvirus-like DNA sequence.

Results. The $KS330_{233}$ PCR product was found in 20 of 21 tissue samples (95 percent) from the patients with Kaposi's sarcoma, including 10 of the 11 samples from the patients with AIDS-associated Kaposi's sarcoma, all 6 samples from the patients with classic Kaposi's sarcoma, and all 4 samples from the HIV-negative homosexual men with Kaposi's sarcoma. Only 1 of the 21 control samples (5 percent) was positive (odds ratio, 400; 95 percent confidence interval, 19 to 17,300). Of the 14 samples of uninvolved skin from the patients with Kaposi's sarcoma, 3 were positive for $KS330_{233}$. Representative PCR-product sequences were more than 98 percent identical for the three types of Kaposi's sarcoma, suggesting that all three are caused by the same agent.

Conclusions. The same herpesvirus-like DNA sequences are present in AIDS-associated Kaposi's sarcoma, classic Kaposi's sarcoma, and the Kaposi's sarcoma that occurs in HIV-negative homosexual men. Therefore, this presumably new human herpesvirus is not solely an opportunistic infection in patients with AIDS, and the three forms of Kaposi's sarcoma may be caused by the same infectious agent. (N Engl J Med 1995;332:1181-5.)

KAPOSI'S sarcoma is the most common neoplasm in patients with the acquired immunodeficiency syndrome (AIDS),[1] and in some cohorts of homosexual men with AIDS, the lifetime risk of Kaposi's sarcoma approaches 50 percent.[2] Epidemiologic evidence suggests an infectious cause of AIDS-associated Kaposi's sarcoma.[3] Surveillance data indicate that Kaposi's sarcoma is roughly 20 times more likely to develop in patients with AIDS who are homosexual or bisexual than in those who have hemophilia.[1,4] Among homosexual men with AIDS, the risk of Kaposi's sarcoma is associated with specific sexual practices[5] and geographic locations.[4,6]

Histopathologically similar forms of Kaposi's sarcoma also occur in people who are seronegative for the human immunodeficiency virus (HIV). The classic form is an indolent sarcoma that usually occurs on the lower extremities,[7] most often in elderly men of Mediterranean, Middle Eastern, or Eastern European ethnic origin.[8] Evidence that classic Kaposi's sarcoma may have an infectious cause comes from studies in Sweden indicating an upsurge in cases of classic Kaposi's sarcoma in the 1970s, before the AIDS epidemic.[9] Serologic studies suggest that this form of Kaposi's sarcoma may be associated with cytomegalovirus infection.[10,11]

The frequency of Kaposi's sarcoma in HIV-negative homosexual men is higher than expected, supporting the hypothesis that the etiologic agent can be sexually transmitted and is distinct from HIV type 1.[12] In HIV-seronegative homosexual men with Kaposi's sarcoma, there is no detectable immunodeficiency and the tumor resembles classic Kaposi's sarcoma in its presentation and clinical course (unpublished data). Endemic Kaposi's sarcoma in Africa[13,14] and post-transplantation Kaposi's sarcoma[15] are additional forms that occur in immunocompetent and immunocompromised persons, respectively.

Chang et al.[16] used representational difference analysis[17] to identify unique DNA sequences associated with Kaposi's sarcoma in patients with AIDS. These DNA sequences were shown to be of nonhuman origin and closely homologous to minor capsid and tegument protein genes of the gammaherpesviruses, Epstein–Barr virus, and herpesvirus saimiri. A polymerase-chain-reaction (PCR) primer set amplifying a sequence of 233

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Tissue DNA specimens were prepared and provided by Dr. Yao-Qi Huang, Dr. Jian J. Li, and Dr. Alvin Friedman-Kien of the Departments of Microbiology and Dermatology, New York University School of Medicine, New York.

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base pairs (bp), designated $KS330_{233}$, identified these herpesvirus-like sequences in all 25 tissue specimens from patients with AIDS-associated Kaposi's sarcoma that were both amplifiable and histologically confirmed. In addition, these sequences have been found in specimens of involved tissue from patients with AIDS and body-cavity–based lymphomas[16,18] but not in most samples of uninvolved tissue from these patients and not in tissue from patients without AIDS.

Although these findings suggest that a new human herpesvirus is the cause of AIDS-associated Kaposi's sarcoma, it is possible that this agent preferentially colonizes preexisting Kaposi's sarcoma in immunosuppressed patients and does not have an etiologic role.[16] To determine whether these herpesvirus-like DNA sequences are also present in lesions from immunocompetent persons with Kaposi's sarcoma, we performed a randomized, blind evaluation to determine the presence of the sequences in tissue samples from patients with AIDS-associated Kaposi's sarcoma, patients with classic Kaposi's sarcoma, and HIV-seronegative homosexual men with Kaposi's sarcoma.

Table 1. Demographic and Clinical Characteristics of 21 Patients with Kaposi's Sarcoma.*

Patient No.	Age (yr)	Sex	Homosexual or Bisexual	HIV Serologic Status	CD4+ Count/mm³†	$KS330_{233}$	
						Lesion	Uninvolved tissue
AIDS and Kaposi's sarcoma							
1	34	M	No	Pos	143	Pos	Neg
2	52	M	Yes	Pos	201	Pos	Neg
3	50	M	Yes	Pos	82	Neg	Neg
4	32	M	Yes	Pos	14	Pos	Neg
5	26	M	Yes	Pos	65	Pos	Neg
6	33	M	Yes	Pos	122	Pos	Neg
7	38	M	Yes	Pos	14	Pos	Pos
8	50	M	Yes	Pos	87	Pos	ND
9	34	M	Yes	Pos	21	Pos	ND
10	27	M	Yes	Pos	163	Pos	ND
11	39	M	Yes	Pos	74	Pos	ND
Classic Kaposi's sarcoma							
12	82	M	No	Neg	825	Pos	Neg
13	68	M	No	Neg	780	Pos	Neg
14	61	M	No	Neg	ND	Pos	Neg
15	58	M	No	Neg	ND	Pos	Neg
16	54	M	No	Neg	ND	Pos	Pos
17	78	F	No	Neg	ND	Pos	ND
Kaposi's sarcoma without HIV infection							
18	58	M	Yes	Neg	956	Pos	Neg
19	39	M	Yes	Neg	1122	Pos	Pos
20	42	M	Yes	Neg	1050	Pos	ND
21	49	M	Yes	Neg	884	Pos	ND

*Pos denotes positive, Neg negative, and ND not determined.

†Count obtained closest to the date of the biopsy (generally within six months before the biopsy).

Methods

Patient Enrollment

Patients with AIDS-associated Kaposi's sarcoma, patients with classic Kaposi's sarcoma, and HIV-seronegative homosexual men with Kaposi's sarcoma seen in a private practice in New York City were enrolled in the study. All patients provided informed consent to participate in the study and were tested to determine their HIV serologic status. Tissue specimens were collected during routine clinical examinations. Data on demographic characteristics and risk factors were collected at the time of enrollment. The diagnosis of Kaposi's sarcoma was confirmed by histopathological examination. The serologic status of the HIV-seronegative homosexual patients was confirmed by repeated enzyme-linked immunoassays and Western blot tests at the time of the diagnosis of Kaposi's sarcoma.

Tissue Collection, Storage, and Preparation

Tissue specimens were obtained from lesions and from uninvolved skin at the time of the biopsy. Control skin samples were obtained from patients with neither Kaposi's sarcoma nor AIDS who were undergoing elective plastic surgery. Control samples of peripheral-blood mononuclear cells were obtained from healthy, HIV-seronegative donors.

The tissue specimens were fresh-frozen immediately after removal and stored at −70°C. Tissue was generally obtained by punch biopsy, which limited the amount of DNA available for analysis.

DNA was extracted with chloroform phenol, resuspended in deionized distilled water at a concentration of 0.1 μg per microliter, and stored at 4°C.[19] Control DNA specimens were extracted from peripheral-blood mononuclear cells and excess skin tissue obtained from patients without Kaposi's sarcoma who had undergone cosmetic surgery. All identifying information on control samples was removed to maintain confidentiality. To ensure blinding, DNA specimens from Kaposi's sarcoma lesions and from control tissues were extracted and coded in a separate laboratory by persons uninvolved in PCR testing. Control specimens were randomly distributed among the batches of samples to be tested. Each batch of samples tested included negative controls (those without DNA) and positive controls (those containing DNA with the $KS330_{233}$ sequence).

PCR Amplification for $KS330_{233}$

All DNA samples were confirmed to be amplifiable with PCR primers specific for a conserved region of a human interferon gene.[19] PCR primers were synthesized (Operon, Alameda, Calif.) to amplify the 233-bp $KS330_{233}$ region of the sequence associated with Kaposi's sarcoma and AIDS. This sequence is homologous to portions of the minor capsid genes *ORF26* and *BDLF1* of herpesvirus saimiri and the Epstein–Barr virus, respectively.[16]

Each PCR reaction used approximately 0.2 μg of genomic DNA, 100 pmol of each primer (5′TCCGTGTTGTCTACGTCCAG3′ and 5′AGCCGAAAGGATTCCACCAT3′), 2 units of *Taq* polymerase, 100 μM of each deoxynucleotide triphosphate, 1.5 mM magnesium chloride, 50 mM potassium chloride, 10 mM TRIS–hydrochloride (pH 9.0), and 0.1 percent Triton X-100 in a final volume of 50 μl. PCR amplification was carried out at 94°C for 2 minutes (1 cycle); 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1.5 minutes (35 to 40 cycles); and 72°C for 5 minutes (1 cycle). Amplifications were performed in a Perkin–Elmer 480 Thermocycler (Perkin–Elmer Cetus, Norwalk, Conn.) with ramp times of 1 second between steps. Amplification products were visualized on 2 percent agarose gel containing ethidium bromide and were scored for the presence or absence of the 233-bp fragment. PCR products with a band in the expected 233-bp region were transferred to nitrocellulose paper and subjected to Southern blot hybridization with a 25-bp internal oligomer end-labeled with [γ-^{33}P]deoxycytidine triphosphate.[16] PCR reactions were considered positive only if the PCR products specifically hybridized in the expected 233-bp region. Significance testing, with exact confidence intervals, was performed with Epi-Info (USD, Stone Mountain, Ga.).

Representative amplification products from each form of Kaposi's sarcoma were purified on gel and cloned into pCRII vector with the TA cloning system (Invitrogen, San Diego, Calif.). Both sense and

antisense strands of the cloned $KS330_{233}$ product were sequenced by standard methods.[16]

RESULTS

Demographic Data

Sarcoma tissue was available from 11 patients with AIDS-associated Kaposi's sarcoma, 6 patients with classic Kaposi's sarcoma, and 4 HIV-seronegative homosexual men with Kaposi's sarcoma (Table 1).

Among the patients with AIDS-associated Kaposi's sarcoma, the median age at the time of the diagnosis was 34 years (range, 26 to 52). All the patients with AIDS-associated Kaposi's sarcoma were homosexual or bisexual men, except for Patient 1, who reported no history of sexual relations with men, intravenous drug use, or receipt of blood products and was assumed to have been infected with HIV through heterosexual activities. Of the six patients with classic Kaposi's sarcoma, five were men and one was a woman; their median age was 65 years (range, 54 to 82). None of the five men reported a history of sexual relations with men or intravenous drug use, and all were HIV-seronegative at the time of the biopsy. The HIV-seronegative homosexual men had a median age of 41 years (range, 39 to 58) and were also HIV-seronegative at the time of the biopsy. The patients with AIDS-associated Kaposi's sarcoma had depressed CD4+ T-cell counts (median count, 87 cells per cubic millimeter; range, 14 to 201), whereas the HIV-seronegative homosexual men with Kaposi's sarcoma and the patients with classic Kaposi's sarcoma had normal counts.

One of the patients with classic Kaposi's sarcoma was of Arabic ancestry and had been born in Lebanon (Patient 17), two were of Russian Jewish ancestry (Patients 12 and 13), and two were of Italian ancestry (Patients 14 and 15). Among the homosexual men with Kaposi's sarcoma, only one (Patient 19) was of Italian ancestry. All the other patients were of Northern European or Hispanic ancestry.

PCR Analysis of $KS330_{233}$

Tissue samples from the three groups of patients with Kaposi's sarcoma and control samples were randomly distributed and blindly tested in three batches (Fig. 1). Of the 11 samples from the patients with AIDS-associated Kaposi's sarcoma, 9 were initially positive for $KS330_{233}$ and 1 (the sample from Patient 6) was positive on repeated blind testing (Table 1); only 1 sample (from Patient 3) was negative for $KS330_{233}$ with PCR performed blindly on two separate DNA samples. Tissue samples from all six patients with classic Kaposi's sarcoma and all four HIV-seronegative homosexual men with Kaposi's sarcoma were positive for $KS330_{233}$. $KS330_{233}$ was found in samples of uninvolved skin from 3 of 14 patients with Kaposi's sarcoma (Table 1). In comparison, 13 of these 14 patients had lesions that were positive for $KS330_{233}$ (unmatched odds ratio, 48; 95 percent confidence interval, 3.6 to 2201).

Control specimens included 10 samples of peripheral-blood mononuclear cells from healthy HIV-seronegative donors and 11 skin samples from patients without

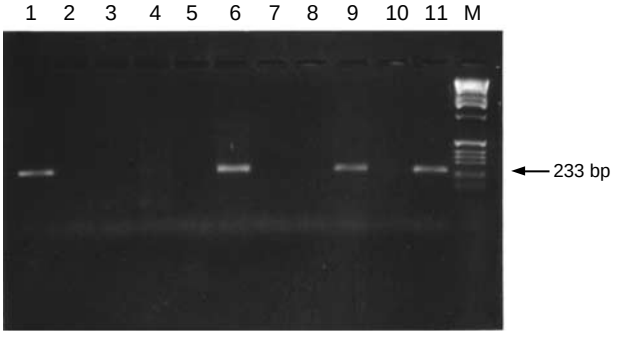

Figure 1. Example of $KS330_{233}$ PCR Amplifications from the First Batch of Tissue Samples from Patients with Kaposi's Sarcoma and Controls.

Amplification products are detectable in DNA samples from an HIV-seronegative homosexual man with Kaposi's sarcoma (lane 1, Patient 18), a patient with classic Kaposi's sarcoma (lane 6, Patient 12), and a patient with AIDS-associated Kaposi's sarcoma (lane 9, Patient 4). One sample from a patient with AIDS-associated Kaposi's sarcoma (lane 4, Patient 3) failed to generate a PCR product detectable by Southern hybridization in repeated blind evaluations. The PCR product was amplifiable in all the remaining 17 samples from the patients with Kaposi's sarcoma (not shown). Samples in lanes 2, 5, and 8 (DNA from uninvolved tissue from patients with Kaposi's sarcoma) and lanes 3 and 7 (control DNA samples from patients without Kaposi's sarcoma) are negative. Lane 10 contains a negative control (no DNA), lane 11 contains a positive control (DNA from a patient with AIDS-associated Kaposi's sarcoma) previously shown to contain Kaposi's sarcoma–associated herpesvirus sequences,[16] and lane M is a molecular-weight marker.

Kaposi's sarcoma (Table 2). Only 1 of the 21 control specimens, a skin sample, was positive for $KS330_{233}$, as compared with 20 of the 21 sarcoma specimens (odds ratio, 400; 95 percent confidence interval, 19 to 17,300). After unblinding, repeated PCR examination of the single positive control sample was negative, suggesting the possibility of a false positive result initially.

DNA Sequencing Studies

PCR products from representative samples of the three forms of Kaposi's sarcoma (tissue specimens ob-

Table 2. PCR Analysis of $KS330_{233}$ in DNA Samples from Patients with Kaposi's Sarcoma and Controls.*

SAMPLE TYPE	NO. TESTED	POSITIVE FOR $KS330_{233}$
		no. (%)
Kaposi's sarcoma tissue		
AIDS and Kaposi's sarcoma	11	10 (91)
Classic Kaposi's sarcoma	6	6 (100)
Kaposi's sarcoma without HIV infection in homosexual men	4	4 (100)
Total	21	20 (95)
Control		
PBMC from seronegative healthy subjects	10	0
Skin from healthy subjects	11	1 (9)
Total	21	1 (5)

*PBMC denotes peripheral-blood mononuclear cells. The odds ratio for the comparison of the total number of Kaposi's sarcoma lesions with the total number of control samples is 400 (95 percent confidence interval, 19 to 17,300).

tained from Patients 1, 2, 4, 12, 13, 16, and 19) were cloned and sequenced (Fig. 2). Five $KS330_{233}$ products (in samples from Patients 1, 4, 13, 16, and 19), representing all three forms of Kaposi's sarcoma, differed from the prototypic sequence originally derived from a genomic library made from a Kaposi's sarcoma lesion[16] at a single base-pair position (47) coding for a proline-to-leucine substitution. PCR products from lesions in one patient with AIDS-associated Kaposi's sarcoma (Patient 2) and one with classic Kaposi's sarcoma (Patient 12) had a second genotype with five base-pair substitutions (at positions 46, 47, 69, 146, and 153). Base-pair substitutions at positions 46 and 47 code for a proline-to-isoleucine substitution and a base-pair substitution at position 146 codes for an aspartate-to-glycine substitution, as compared with the prototypic sequence. The remaining base-pair substitutions do not involve amino acid substitutions. The high degree of sequence conservation among PCR products suggests that the same herpesvirus-like agent is present in all three forms of Kaposi's sarcoma.

Discussion

Epidemiologic evidence strongly suggests that both the AIDS-associated and non–AIDS-associated forms of Kaposi's sarcoma have infectious courses.[3] Chang et al. derived unique DNA sequences from a lesion in a patient with AIDS-associated Kaposi's sarcoma; these sequences are homologous to but distinct from portions of three gammaherpesvirus genes found in two separate locations.[16] Subsequent sequencing studies of a 21-kilobase insert derived from a genomic library of a lesion in a patient with Kaposi's sarcoma have confirmed its colinearity with members of the gammaherpesvirus subfamily (unpublished data). These sequences thus appear to mark a new human herpesvirus, although isolation and identification of the virus are pending. This agent has been given the descriptive name Kaposi's sarcoma–associated herpesvirus (KSHV); a formal designation has not yet been made.[16]

In our blinded evaluation, the $KS330_{233}$ sequence was specifically associated with Kaposi's sarcoma lesions from both patients with HIV infection and those without infection. This PCR product is a highly sensitive and highly specific marker for the presence of KSHV sequences in DNA from patients with AIDS-associated Kaposi's sarcoma. DNA from patients with other common human herpesviruses, such as Epstein–Barr virus and cytomegalovirus, does not amplify $KS330_{233}$.[16] Detection of these sequences in patients with AIDS-associated Kaposi's sarcoma, patients with classic Kaposi's sarcoma, and HIV-seronegative homosexual men with Kaposi's sarcoma suggests that these three forms of Kaposi's sarcoma are not distinct and that KSHV is an etiologic agent in Kaposi's sarcoma. Although an interaction between endogenous growth factors and

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                10        20        30        40        50        60        70        80
KSHV            AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCCCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG
Patient 1       AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCTCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG
Patient 2       AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACATCGT GTTCCCCATG GTCGTGCCTC AGCAACTGGG
Patient 4       AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCTCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG
Patient 12      AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACATCGT GTTCCCCATG GTCGTGCCTC AGCAACTGGG
Patient 13      AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCTCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG
Patient 16      AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCTCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG
Patient 19      AGCCGAAAGG ATTCCACCAT TGTGCTCGAA TCCAACGGAT TTGACCTCGT GTTCCCCATG GTCGTGCCGC AGCAACTGGG

                90       100       110       120       130       140       150       160
KSHV            GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG
Patient 1       GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG
Patient 2       GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGGTGAT GTCAATATGG
Patient 4       GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG
Patient 12      GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGGTGAT GTCAATATGG
Patient 13      GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG
Patient 16      GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG
Patient 19      GCACGCTATT CTGCAGCAGC TGTTGGTGTA CCACATCTAC TCCAAAATAT CGGCCGGGGC CCCGGATGAT GTAAATATGG

                170       180       190       200       210       220       230
KSHV            CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 1       CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 2       CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 4       CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 12      CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 13      CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 16      CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
Patient 19      CGGAACTTGA TCTATATACC ACCAATGTGT CATTTATGGG GCGCACATAT CGTCTGGACG TAGACAACAC GGA
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Figure 2. DNA Sequences for $KS330_{233}$ PCR Products Amplified from Tissue Samples Obtained from Seven Patients with Kaposi's Sarcoma.

The sequences are almost identical, suggesting that all three forms of Kaposi's sarcoma are caused by the same agent. Two genotypes were found that differed from the prototypic sequence for Kaposi's sarcoma–associated herpesvirus (KSHV), derived from a genomic library.[16] Products from lesions in Patients 1, 4, 13, 16, and 19 had a single base-pair substitution, and products from lesions in Patients 2 and 12 had five base-pair substitutions (substitutions are bold and underlined). No genetic divergence according to the type of Kaposi's sarcoma was detected at this locus for these North American forms of the disease. Patients 1, 2, and 4 had AIDS-associated Kaposi's sarcoma; Patients 12, 13, and 16 had classic Kaposi's sarcoma; and Patient 19, a homosexual man, had Kaposi's sarcoma without HIV infection.

HIV Tat protein has been hypothesized to play a part in the pathogenesis of Kaposi's sarcoma,[20,21] our findings indicate that coincident HIV infection is not necessary for the development of the Kaposi's sarcoma phenotype.

Sequence analysis of $KS330_{233}$ PCR products demonstrates that the genome in all three forms of Kaposi's sarcoma is the same, within expected limits of strain variation. Random errors of *Taq* polymerase incorporation are unlikely to result in these conserved genotype patterns. No relation was seen between the different types of Kaposi's sarcoma and these divergent genotypes at this locus. The $KS330_{233}$ PCR product was not detected initially in two samples of lesions from patients with AIDS-associated Kaposi's sarcoma but was detected in one of these samples on repeated blind examination. The inability to detect the amplifiable product in the other sample may have been due to either sequence polymorphism at the PCR priming site or the absence of infection with KSHV.

The variable detection of $KS330_{233}$ in samples of normal skin from patients with Kaposi's sarcoma confirms previous studies[16] indicating that $KS330_{233}$ is not a portion of the human genome and is also consistent with an exogenous infectious process. These findings indicate that the agent may disseminate to tissue that is phenotypically normal, in both patients with AIDS and those without AIDS. It is possible that the agent is latent in this tissue, given the high sensitivity of PCR in detecting herpesvirus DNA.[22]

Despite the detection of $KS330_{233}$ in association with all three forms of Kaposi's sarcoma, our study has several important methodologic limitations. The tissue specimens were obtained from punch biopsies, which limited the amount of DNA available for testing. We were therefore unable to perform confirmatory Southern hybridization directly on the DNA samples. Although Southern hybridization is a less sensitive test for specific DNA sequences, it is also less likely to have false positive results. Of the 21 control samples tested, 1 was initially positive for $KS330_{233}$ by PCR but negative on repeated examination. As a conservative assumption, this sample was considered to be positive, but it was probably falsely positive. Alternatively, the positive result may reflect the prevalence of skin infection with KSHV in persons at low risk for Kaposi's sarcoma. The variability of sequenced PCR products from Kaposi's sarcoma DNA, however, indicates that our results are not due to carryover PCR contamination or contaminating DNA sequences. Recent reports have confirmed our findings in HIV-seronegative persons with classic Kaposi's sarcoma.[23-25]

The presence of KSHV sequences in both patients with AIDS-associated Kaposi's sarcoma and HIV-seronegative homosexual men with Kaposi's sarcoma is consistent with epidemiologic data suggesting that the etiologic agent is sexually transmitted.[1] These sequences are also found in lesions from women and heterosexual men with Kaposi's sarcoma, however, so male homosexual activity is not the exclusive mode of transmission. Although additional studies are needed to determine the modes of transmission, our study provides evidence that this herpesvirus-like agent has an etiologic role in the development of Kaposi's sarcoma in both people with HIV infection and those without infection.

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