J Korean Med Sci 1998; 13: 54-9
ISSN 1011-8934

Human Herpesvirus 8 in Kaposi's Sarcoma and Kaposi's Sarcoma-mimicking Vascular Tumors

Kaposi's sarcoma (KS) had been a rare and unusual vascular tumor until a recent epidemic of a disseminated and fulminant form of KS in AIDS patients. Infectious agents have been suspected of causing KS, and recently partial genomic DNA sequences of human herpesvirus 8 (HHV8) have been identified in AIDS-associated KS lesions. Since then, genomic DNA sequences of HHV8 have been isolated in other forms of KS. Although the partial genomic DNA sequence of HHV8 was reported to be, if rare, identified in vascular tumors other than Kaposi's sarcoma (KS), the presence of HHV8 in a very large fraction of KS indicates that detection of HHV8 by PCR is a useful auxiliary tool in differentiating KS from other KS-mimicking vascular tumors. We examined whether the 233-bp segment of the viral DNA was detected in Korean patients with KS and other KS-mimicking vascular tumors. HHV8 sequences were identified in all of nine classic type of KS but not in three epithelioid hemangioendotheliomas and seven angiosarcomas. Our results confirm the relatively restricted distribution of HHV8 and also argue against the likelihood of secondary colonization of KS cells by HHV8.

Key Words : *Angiosarcoma, Human herpesvirus 8, Kaposi's sarcoma*

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Received : July 23, 1997
Accepted : September 2, 1997

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INTRODUCTION

Kaposi's sarcoma (KS) has been recognized as one of the malignant vascular tumors and presents as one of four forms; 1) acquired immunodeficiency syndrome (AIDS)-related KS; 2) classic KS; 3) organ transplant-associated KS; 4) African KS. Although the clinical manifestations and course of the disease differ among the four forms, the histopathology of the different forms is essentially identical. The light microscopic features of KS include a highly vascularized lesion with a proliferation of spindle shaped cells infiltrating between collagen bundles, often accompanied by extravasated erythrocytes and a variable number of inflammatory cells, including lymphocytes and plasma cells. For a long time there has been a dispute about the etiology of KS and the histogenetic identity of the spindle cells of KS. Recently Chang et al. (1) identified unique DNA sequences associated with KS in patients with AIDS. These unique DNA sequences were shown to be of nonhuman origin and closely homologous to two herpesviruses, Epstein-Barr virus (EBV) and herpes saimiri, both of which belong to the subfamily of Gammaherpesvirinae. These data suggested the presence of a previously unidentified human herpesvirus, referred to as Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) (2). Further progress in understanding this virus has been provided by development of a cell culture system for this virus and demonstration of its ultrastructural features (3). HHV8 has also been identified in human immunodeficiency virus (HIV)-negative classic KS (4-7).

Histopathologically it is sometimes difficult to differentiate KS from angiosarcoma and hemangioendothelioma because some of these show spindle cell areas. The differentiation of KS from angiosarcoma is clinically very important because of the far better prognosis of the former (8). When the partial genomic DNA sequence of HHV8 was detected in KS, HHV8 was initially thought to be a specific cause for KS. However, the partial genomic DNA sequence was reported to be, although rare, identified in other vascular tumors, including angiosarcoma and capillary hemangioma (9). We performed PCR-based analysis to determine whether HHV8 is present in vascular tumors, including epithelioid hemangioendothelioma, classic KS, and angiosarcoma.

MATERIAL AND METHODS

Tissues and DNA preparation

We retrospectively analyzed 22 formalin-fixed paraffin-embedded tissue samples obtained from 19 patients who had undergone surgical resection or biopsy for angiosarcoma, epithelioid hemangioendothelioma, or KS at Seoul National University Hospital. All KS patients were confirmed to be serologically negative for HIV by enzyme linked immunoassays.

DNA was extracted from the formalin-fixed, paraffin-embedded tissues as follows. After examination of hematoxylin and eosin (H&E)-stained sections, blocks were chosen that contained areas of hemangioendothelioma, KS, or angiosarcoma. Ten 5-μm sections were cut and mounted on glass slides and the area of interest was outlined with a diamond pen. With a clean scalpel blade, the outlined portion was excised and scraped into a microfuge tube. The remaining procedure was performed with the QIAamp tissue kit (QIAGEN, Chatsworth, CA) following the manufacturer's instructions. All specimens were deparaffinized in 1 ml of xylene and lysed by overnight incubation in 180 μl of ATL lysis buffer (QIAamp tissue kit) with 20 μl of proteinase K (20 mg/ml). The lysis mixture was centrifuged for 1 minute to remove undigested tissue; the eluent (DNA) was combined with 200 μl of AL buffer (QIAamp tissue kit), vortexed, and incubated at 70℃ for 10 minutes. After the addition of 210 μl of 100% ethyl alcohol, the specimen was vortexed, added to a QIAamp spin column, and centrifuged at 9,000 rpm for 1 minute. The filtrate was discarded, and the preceding step was repeated. The DNA was eluted from the column with 50 μl of deionized H_2O preheated to 70℃. After the deionized H_2O addition, the column was centrifuged at 8,000 rpm for 1 minute; the filtrate (DNA) was retained and the column was discarded. The DNA was then stored at 4℃ until further use.

PCR amplification

To evaluate whether all the DNA samples were amplifiable, PCR was done first with a primer pair specific for a region of β-globin gene (10). Only the specimens which showed an amplified β-globin were included for further study. A 2-μl volume of the DNA samples was used as a template for the PCR reaction. Primers for amplification of the $KS330_{233}$ were described by Chang et al. (1): 5'-TCCGTGTTGTCTACGTCCAG-3' and 5'-AGCCGAAAGGATTCCACCAT-3'). 2 μl of each DNA sample was added to a 28 μl reaction mixture containing 3 μl of standard PCR buffer, 2.4 μl of dNTP (200 μmol/L), 1 μl of each primer (15 pmol each), 0.12 μl of *Taq* polymerase and deionized H_2O to make a total volume of 28 μl. Thirty cycles of amplification were carried out using cycling parameters of 95℃ for 1 minute, 55℃ for 1 minute 30 seconds, 72℃ for 1 minute, and (last cycle) 72℃ for 10 minutes. Then 10 μl of each PCR product was added to 2 μl of loading dye. Each sample was loaded onto a 2% agarose gel and electrophoresed at 100V.

RESULTS

Clinicopathological features of the patients are given in Table 1. Three cases of hemangioendothelioma were epithelioid hemangioendothelioma of the liver and eleven cases of KS from seven patients were all classic KS. Five patients with KS have a single tumor but patients 8 and 9 had three synchronous KS in the scalp, neck, and flank (Fig. 1) and three metachronous KS in the scalp (Fig. 2), respectively. Two samples of KS were not amplified with

Table 1. Demographic and clinicopathological characteristics of 13 patients

Patient No.	Sex	Age(yr)	Site	β-globin	$KS330_{233}$
Epithelioid hemangioendothelioma					
1	F	2	Liver	Pos	Neg
2	F	2	Liver	Pos	Neg
3	M	12	Liver	Pos	Neg
Classic Kaposi's sarcoma					
4	M	59	Hand	Neg	Neg
5	M	69	Chin	Pos	Pos
6	M	56	Hand	Neg	Neg
7	M	72	Leg	Pos	Pos
8	M	64	Scalp (tumor1)*	Pos	Pos
			Neck (tumor2)*	Pos	Pos
			Flank (tumor3)*	Pos	Pos
9	M	52	Scalp (tumor1)**	Pos	Pos
			Scalp (tumor2)**	Pos	Pos
			Scalp (tumor3)**	Pos	Pos
10	F	72	Sole	Pos	Pos
Angiosarcoma					
11	M	66	Scalp	Pos	Neg
12	M	34	Orbit	Pos	Neg
13	M	62	Scalp	Pos	Neg
14	F	45	Spleen	Pos	Neg
15	M	61	Spleen	Pos	Neg
16	M	37	Face	Pos	Neg
17	M	76	Scalp	Pos	Neg
18	M	53	Scalp	Pos	Neg

* Triple synchronous tumors

** Tumor 3 was a recurred tumor after resection of tumor 2 which was also a recurred tumor after resection of tumor 1.

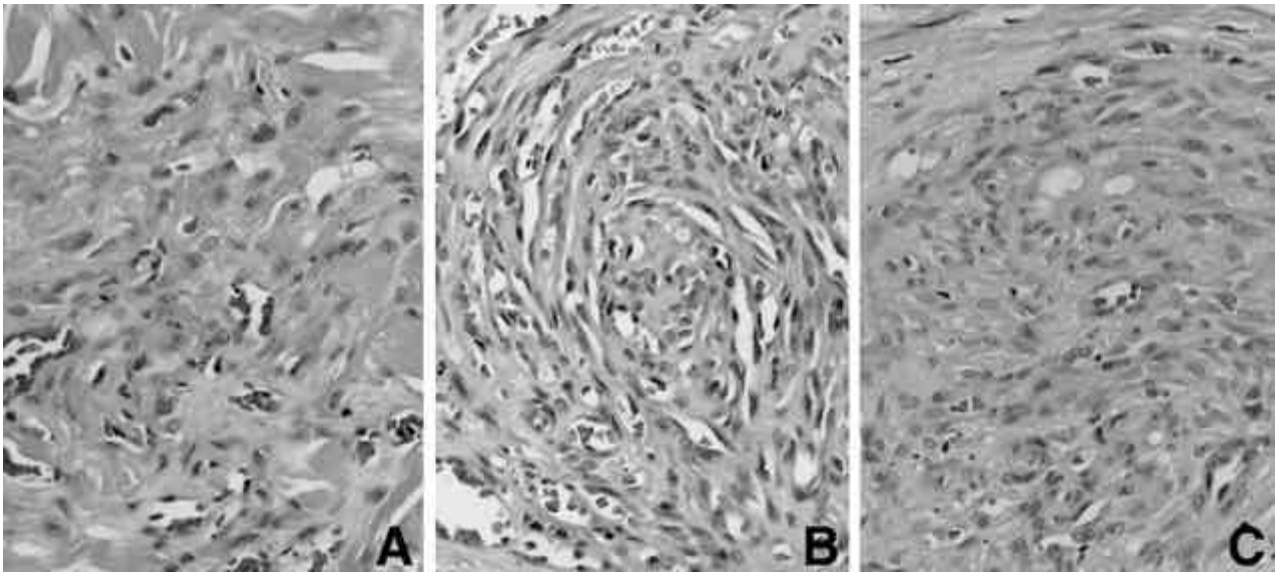

Fig. 1. Photomicrographs of synchronous triple Kaposi's sarcoma in the scalp (A), neck (B), and flank (C). Histopathologically, the scalp lesion is in the earliest stage, the neck lesion is in more advanced stage and the flank lesion in between. The neck lesion demonstrates typical histopathologic features of Kaposi's sarcoma (B).

both β-globin primers and $KS330_{233}$ primers. All of nine tissue samples from five patients with KS, including the three synchronous KS of patient 8 and three metachronous KS of patient 9, were positive for KS 330_{233} product (Fig. 3). Eight samples from patients with angiosarcoma and three samples from patients with epithelioid hemangioendothelioma were negative for $KS330_{233}$ product.

DISCUSSION

KS first described by Moritz Kaposi (11), had remained a rare and unusual tumor until recently. The recent epidemic of a disseminated, fulminant form of KS was first observed among young homosexual men in large urban centers in the United States (12). KS is the most common neoplasm in patients with the acquired

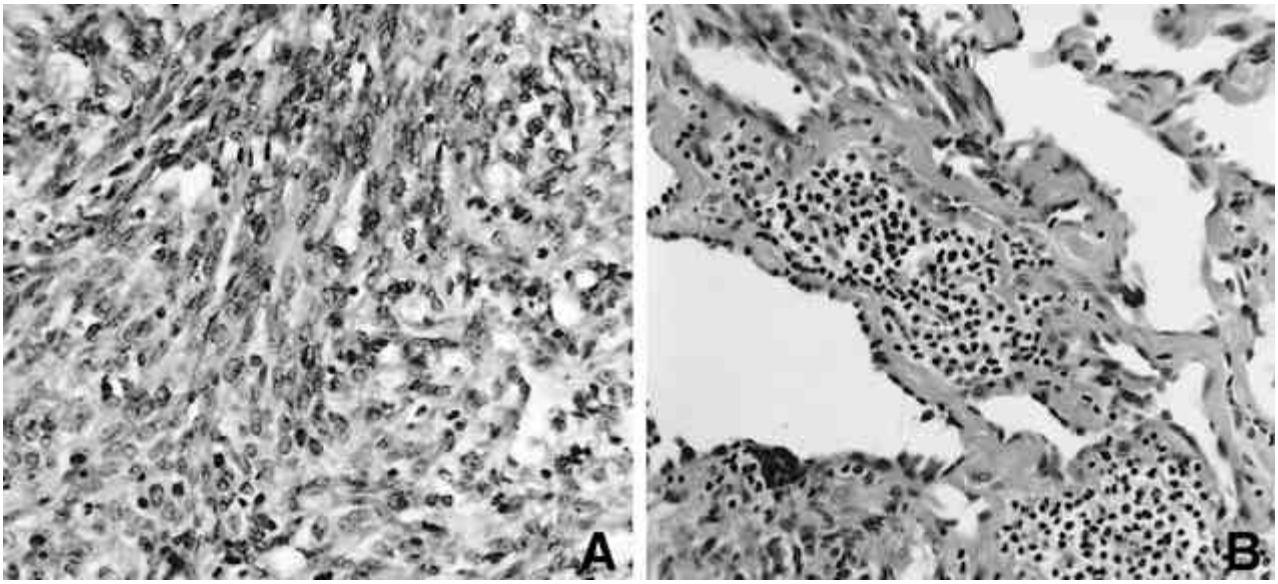

Fig. 2. Photomicrographs of primary KS lesion in patient 9. The lesion showed short, cellular fascicles of spindled cells with small vascular spaces containing erythrocytes and dispersed chronic inflammatory cells (A) and peripheral angiomatous areas (B). Recurrent lesions showed essentially the same histopathological features.

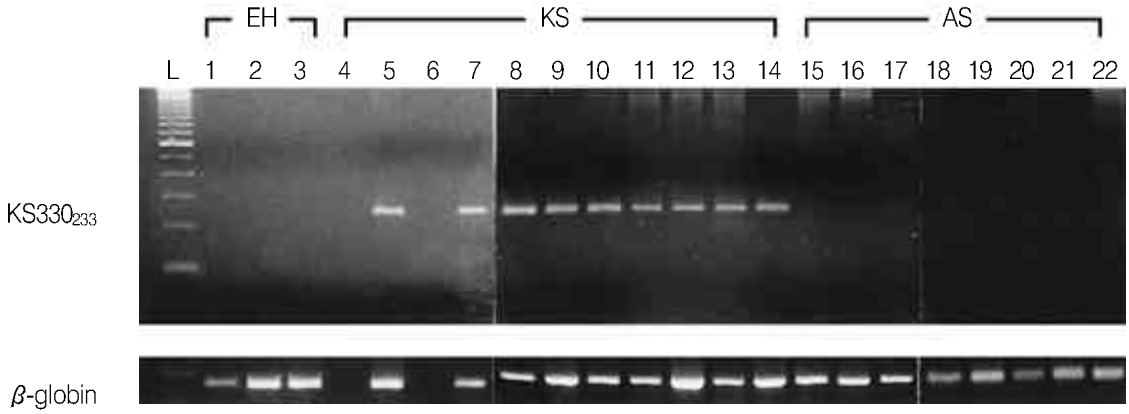

Fig. 3. Electrophoresis of polymerase chain reaction products from patients with epithelioid hemangioendothelioma (lane 1-3), Kaposi's sarcoma (lane 4-14), and angiosarcoma (lane 15-22) with $KS330_{233}$ primers and β-globin primers. Of DNA samples with efficient amplification of globin DNA, all DNA samples from KS patients revealed $KS330_{233}$ products. Lane L is 100-bp molecular weight ladder; lane 8-10, DNA from triple synchronous KS tumors of patient 8; lane 11-13, DNA from primary and recurrent lesions of patient 9. Abbreviations: EH, epithelioid hemangioendothelioma; KS, Kaposi's sarcoma; AS, angiosarcoma.

immunodeficiency syndrome (AIDS). Although HIV is an important risk factor in AIDS-associated KS, epidemiologic evidence suggests an infectious cause, but other than HIV; KS also occurs in selected HIV-negative group, including classic KS, transplant-associated KS, and some African KS (13, 14); KS is roughly 20 times more likely to develop in patients with AIDS who are homosexual or bisexual than in those who are hemophiliacs (15, 16); Among homosexual men with AIDS, the risk of KS is associated with specific sexual practices (17) and geographic locations (18); The frequency of KS in HIV-negative homosexual men is higher than expected (19).

Recently, genomic sequences of a novel herpesvirus, termed KS-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8), have been identified in AIDS-associated KS tissues by polymerase chain reaction (PCR)-based methods (1). These sequences have been demonstrated in more than 90% of tumor specimens of KS obtained from AIDS patients and even in non-neoplastic tissue such as skin from AIDS patients, although the frequency was low (7). This viral DNA sequence was proved to be present in KS tissues from non-AIDS patients by several recent reports including the present study (5, 7, 10, 20, 21). Using PCR-based methods, Huang et al. (5) detected HHV8 in 89% of classic KS. Most materials in these reports were KS tissue samples from American or African patients with or without AIDS. There have been few reports on identification of HHV8 in KS from Asian regions (4) and the present study is the first report that HHV8 DNA sequences have been identified in KS specimens from non-AIDS patients in Korea where the prevalence of AIDS is very low (22).

Our study material included a patient (patient 9) who was erroneously diagnosed as having angiosarcoma. He presented in the scalp a malignant vascular tumor which was diagnosed as "consistent with angiosarcoma" (Fig. 2). After resection of the tumor, two recurrences occurred and the recurred tumors demonstrated essentially the same histologic features as those of the primary tumor. These three specimens demonstrated the amplified $KS330_{233}$ sequence and a histological review of the three specimens revealed that these tumors were composed of central spindle cell areas with high cellularity and a few hyaline globules and peripheral angiomatous areas, which are the findings frequently seen in KS.

Histological differential diagnosis between angiosarcoma and KS is important because the prognosis of the former is dismal (8) whereas classic KS usually runs a relatively benign, indolent course for 10 to 15 years or more (23). Histologically, typical cases of angiosarcoma demonstrate a tubular, vasoformative growth pattern lined by atypical tumor cells. However, in poorly differentiated spindle cell angiosarcoma demonstration of endothelial differentiation is difficult and it requires differentiation from the histologically aggressive form of KS. As demonstrated in our results, PCR amplifying $KS330_{233}$ could be a useful auxiliary tool for diagnosing KS and even differentiating between KS and other KS-mimicking tumors. Although the partial genomic DNA sequences of HHV8 were reported to be present in angiosarcoma (9), the positive rate (from 0% to 29%) is very low compared with that of KS (over 90%) (24-27). Considering there are discordant results about the presence of HHV8 in angiosarcoma, three possibilities may be raised: 1) contamination during PCR, 2) overdiag-

nosis of angiosarcoma (KS erroneously diagnosed as angiosarcoma), and 3) the presence of a subset of angiosarcoma which is HHV8-associated.

$KS330_{233}$ PCR product is a highly sensitive and specific marker for the presence of HHV8 sequences in DNA from patients with AIDS-associated or non-AIDS-associated KS. DNA from patients with other common human herpesviruses, such as Epstein-Barr virus and cytomegalovirus, does not amplify $KS330_{233}$. Because PCR methods carry the possibility of a false positive result, Southern hybridization using KS330Bam or KS631Bam may be an alternative. However, it is less sensitive and requires fresh tissue. Most of the specimens from KS patients in this study were small punch biopsies having a limited amount of DNA and were formalin-fixed. We were therefore unable to perform confirmatory Southern hybridization directly on the DNA samples.

The question has been raised whether KS represents a neoplastic or reactive lesion. The exact cell of origin is not known. It is possible that at least two distinct transforming mechanisms may be involved in the pathogenesis of KS. Hypothetically the first step may be initiated by infection of HHV8 that causes the transformation of certain susceptible cells, i.e. endothelial cells or dermal dendrocytes, which was demonstrated by Boshoff et al. (28) using PCR in situ hybridization. With progression of the disease, increased KS tumor proliferation may result from the selection of a more malignant population resulting from genetic alterations such as amplification or mutation of K-*ras* gene (29).

Acknowledgement

The authors are indebted to Bo Young Lee for her technical support.

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