Detection of herpesvirus-like DNA sequences in Mediterranean Kaposi's sarcoma

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Abstract. The aetiology of Kaposi's sarcoma remains obscure, however, epidemiological studies indicate that the disease possesses an infectious aetiology. Recent data revealed the presence of specific herpesvirus-like DNA sequences (KHSV) in all forms of Kaposi's sarcoma indicating that a novel virus may be the infectious agent which causes the disease. The aim of the present investigation was to assess the incidence of this herpesvirus-like DNA sequence in 28 Mediterranean Kaposi's sarcomas. DNA was extracted from formalin-fixed paraffin-embedded tissues and analysed by a sensitive PCR based assay. The KSHV specific DNA sequences were found in 22 of 28 (79%) cases suggesting a potential important role in the development of the disease.

Introduction

Kaposi's sarcoma (KS) is a multifocal hyperproliferative tumour of the skin and mucous membranes. Four main types of KS have been described, KS affecting elderly men, usually of Mediterranean origin (classical), endemic African KS, KS found in HIV positive patients (epidemic) and an iatrogenic form in association with immunocompromised patients (1). Apart from the involvement of endogenous factors such as cytokines (2) and oncogenes (3), epidemiological studies pointed in addition to the observation that immunosuppression predisposes to the development of KS, to the involvement of an infectious agent important for the development of the disease (4). A recent report of Chang *et al* (5) provided evidence for a novel herpes virus (KSHV) which is present in nearly 100% of the HIV-associated KS. Further studies (6-10) indicated that these KSHV sequences are also present in other forms of KS indicating an important role for this virus in the development of this type of tumour. We investigated the presence of KSHV sequences by the polymerase chain reaction (PCR) in 28 Greek patients with KS suffering from the Mediterranean type of the disease. In Greece this type of KS is endemic in the Peloponnese region with an incidence of 8 cases/10^6 people/year and thus it would be of particular interest to assess the presence of the novel virus in these specimens.

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Materials and methods

DNA from paraffin-embedded tissues was extracted by proteinase K, phenol-chloroform extraction and ethanol precipitation as previously described (11). The primers and the conditions for the specific amplification of KSHV sequence have been described by Chang *et al* (5). 157 bp of the K-*ras* proto-oncogene were amplified using the primers 5-ACTGAATATAAACTTGTGGTAGTTGGACCT-3 and 5-TCAAAGAATGGTCCTGGACC-3. PCR programs were as follows: 95°C for 5 min and 35 cycles consisting of 95°C, 56°C and 72°C for 20 sec, 40 sec and 1 min respectively. PCR products were electrophoresed in a 2% agarose gel, stained with ethidium bromide and scored for the presence of the specific 233 bp (KSHV) and 157 bp (K-*ras*) bands.

Results and Discussion

In the present study we investigated the incidence of KHSV DNA in 28 Mediterranean KS and in 2 HIV related KS in patients from Greece. The presence of KSHV DNA was revealed in both the HIV-positive specimens and in 22 among 28 (79%) Mediterranean KS (Table I, Fig. 1). In order to evaluate the quality of the DNA for the PCR amplification, we amplified the K-*ras* proto-oncogene in all specimens. Although K-*ras* was amplifiable in all specimens by the first PCR, in KSHV 15 of the 22 (68%) positive specimens were scored as positive by the first PCR, while for the remaining 7 specimens a second round of PCR amplification was required. Chang *et al* (5,6) reported that KSHV was present in approx. 1-2 copies per cell in KS. We may postulate that the inability of PCR to reveal the presence of the virus genome on the first round of amplification is due to the low copy number of the virus. Thus, the presence of the KSHV genome in the remaining 6 KSHV negative specimens should

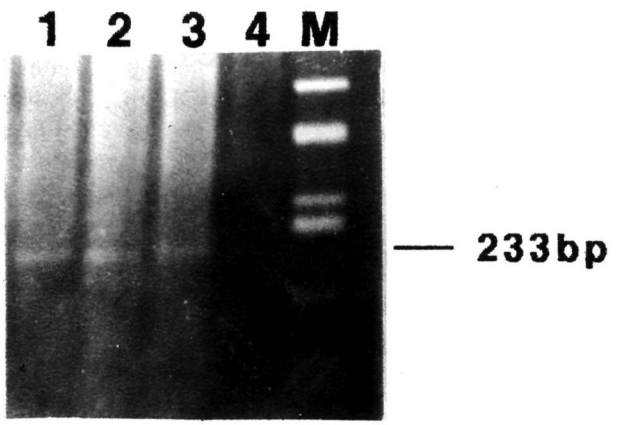

Figure 1. Detection of KSHV by PCR. Arrow indicates the 233 bp KSHV product. Lanes 1-3, positive specimens; lane 4, blank sample; M, pUC18/HaeIII.

Table I. The presence of KSHV in association with clinicopathological parameters in Mediterranean Kaposi's sarcoma.

Patient no.	KSHV	Sex	Age
1	+	M	62
2	+	M	
3	+	F	
4	+	F	52
5	-	M	76
6	-	M	66
7	-	M	60
8	+*	F	85
9	+	F	52
10	-	M	79
11	+	F	52
12	+*	M	69
13	+	M	
14	+	M	
15	+	F	80
16	+	M	73
17	+*		
18	+		
19	+*		
20	+		
21	+*	M	72
22	+	M	68
23	-		
24	+	F	80
25	+*		
26	+*	F	83
27	+	M	66
28	-		

*indicates that a second round of amplification was required.

be considered as a possibility but the low copy number of the virus in these specimens, in addition to the bad quality DNA (archive material from paraffin-embedded tissues) did not permit the amplification of these KSHV specific sequences. We also used two cases of HIV-positive KS as a positive control since KSHV was initially detected in this particular form of the disease. Negative specimens were reamplified by a second round of PCR in order to increase the sensitivity of the assay. In order to avoid false positive signals due to PCR contamination, samples without DNA template were included in the second round of PCR amplification.

Although the presence of KSHV DNA is apparent in a significant proportion of KS specimens, several lines of evidence have cast doubt on the aetiological association of the virus with the disease. If indeed this novel virus causes KS, it should be expected to be present in a high copy number in the positive specimens. Furthermore, several KS cell lines did not harbour the KSHV specific sequences (12).

In the present study we detected the novel herpesvirus-like DNA sequences in a significant proportion of Mediterranean KS indicating an association between KSHV and the development of the disease. However, the real significance of KSHV infection should be considered with caution since particular data (low copy number of the virus in the infected cells and the controversy between *in vivo* and *in vitro* results) suggest that KSHV may simply be an opportunistic infection. Further experiments are required in order to reveal the particular role of KSHV in the development of KS.

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