Human herpes viruses in non-melanoma skin cancers

A. Zafiropoulos, E. Tsentelierou, K. Billiri, D.A. Spandidos *

Laboratory of Virology, Medical School, University of Crete, Heraklion, Crete 71100, Greece

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Abstract

We examined the possible involvement of human herpes viruses in sporadic non-melanoma skin cancer of Greek patients. Polymerase chain reaction (PCR) based detection assays were utilized for the detection of viral cytomegalovirus (CMV), herpes simplex virus (HSV) and Epstein–Barr virus (EBV) genomes in 24 squamous cell carcinomas (SCC), five Bowen’s disease, 72 basal cell carcinomas (BCC) specimens and eight premalignant lesions. Forty-two of 109 (38.5%) skin lesions were found positive for CMV DNA. The highest incidence was 6/8 (75%) observed in specimens with premalignant lesions. The incidence was 37.5% (27/72) in BCC, 33% (8/24) in SCC and 20% (1/5) in extragenital Bowen’s disease. All samples were negative for HSV-1/2 and EBV DNA as assessed by our PCR based assay. The CMV infection showed no statistically significant correlation with the histological type, age, site of lesion or sex. Our results give a strong indication of the possible involvement of CMV in non-melanoma skin cancer development.

Keywords: Cytomegalovirus; Epstein–Barr virus; Herpes simplex virus; Skin cancer

1. Introduction

Skin cancer is a common neoplasia of the Caucasian population [1,2]. Squamous cell carcinomas (SCC) and basal cell carcinomas (BCC), account for 25 and 75% of the non-melanoma skin cancers, respectively [3,4]. Precursor lesions, such as Bowen’s disease (i.e. SCC in situ) when left untreated could develop into invasive SCC [5]. Although SCC and BCC share the same origin in respect of cell type, their clinical and biological behaviors show distinct differences [2,6]. BCC is a locally aggressive, slow growing tumor that rarely metastasizes, and is mostly seen in the elderly population. SCC is more aggressive, locally invasive and has metastatic potential. SCC appears sporadically. The rare autosomal dominant form of the disease is called Ferguson–Smith syndrome and displays multiple self-healing SCC lesions [7,8]. Even though information is growing on molecular defects leading to cell transformation in many types of human cancers, the contribution of environmental factors or pathogens seems to be extremely important. Facts that underline the above statement are the difference in cancer incidence observed between developed and developing countries as well as the difference between immunosuppressed and immunocompetent subjects.

The family of human herpes viruses has been incriminated as etiological factors for several human cancers. Epstein–Barr virus (EBV) has been implicated in nasopharyngeal carcinoma and African
Burkitt’s lymphoma [9]; herpes simplex virus type 2 (HSV-2) was linked by serologic and molecular studies to cervical carcinoma [10–12]; human cytomegalovirus (HCMV) was associated with cervical carcinoma [13,14], adenocarcinomas of the prostate [15,16] and colon [17,18], and Kaposi’s sarcoma (KS) [19-21]; human herpesvirus 6 (HHV-6) was associated with lymphoproliferative disorders [22,23]; and Kaposi’s sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8) was associated with KS [24–26].

The aim of the present study was to examine the involvement of human herpesvirus in an array of skin lesions. We examined the presence of genetic material from HSV-1/2, EBV and cytomegalovirus (CMV) in 109 specimens of benign and malignant cutaneous lesions obtained from immunocompetent hosts. We demonstrate a very high incidence of CMV infection while HSV-1/2 and EBV were absent.

2. Materials and methods

2.1. Tumor specimens and DNA extraction

Twenty-three SCC, five Bowen’s disease, 72 BCC and eight other premalignant lesions were obtained from the ‘A. Sygros’ Hospital (Athens, Greece) with the approval of the donors and the institute’s ethics committee. The diagnosis of the samples was histologically confirmed. The specimens were stored at −70°C immediately after dissection until DNA extraction. DNA was extracted as previously described [27] and stored at −20°C until polymerase chain reaction (PCR) amplification.

The age of the patients with SCC and Bowen’s disease lesions ranged between 40 and 88 (average 68) years and those with BCC lesions between 39 and 90 (average 68) years (27 females and 40 males). All the lesions examined were located on sun-exposed parts of the body. As the sun-exposed parts of the body were characterized the head and neck for both sexes, the chest and shoulders for males and the legs for females [28]. Twenty-three of the 24 SCC tumors were located on the head and neck, and one on the leg of a female patient. Three of the five Bowen’s disease specimens were located on the head and neck, and the other two on the chest of two male patients. Fifty of the 72 BCC tumors were located on the head and eight on the neck, two on the chest and three on the shoulders and four on the legs.

2.2. Oligonucleotide primers and PCR amplification

All specimens were examined for the presence of amplifiable DNA using a set of primers for alpha-globin gene. In each PCR reaction, two negative controls were employed to make sure that no contaminants were introduced in the initial PCR. To establish specificity of primer-directed amplification, each set of primers was tested with a relevant control template: (a) EBV (supernatant from B19 marmoset cell line ATCC), (b) HSV-1 (supernatant of HSV-1 infected BHK cells) and CMV (supernatant of CMV infected cells). The primers used in the present study are as follows. CMV_Forward: gtgaccaaggccacgacgtt, CMV_Reverse: tctgccaggacatctttctc, HSV-1/2_Forward: cagtacggccccgagttcgtga, HSV-1/2_Reverse: gtagatggtgcgggtgatgtt, EBV_Forward: gtgtgcgtcgtgccggggcagccac, EBV_Reverse: acctgggagggccatcgcaagctcc.

PCR analysis was performed in a 50 μl reaction volume containing 500 ng of genomic DNA, 1 μM of each primer, 250 μM dNTPs, 5 μM of 10 X buffer (670 mM Tris–HCl, pH 8.5, 166 mM ammonium sulphate, 67 mM magnesium chloride, 1.7 mg bovine serum albumin per ml, 100 mM β-mercaptoethanol, and 1% (wt/vol) Triton X-100) and 1 U of Taq DNA polymerase. The DNA was denatured for 3 min at 94°C and subsequently amplified for 35 cycles at 94, 55 and 72°C in each step. PCR products were analyzed on a 3% agarose gel and photographed on a UV light transilluminator.

2.3. Automated DNA sequencing

The PCR products were resolved through 2% agarose gels, excised and processed with the Wizard PCR Preps DNA purification Clean kit (Promega, Southampton, UK) to remove unincorporated primers and dNTP. The sequencing reaction contained: 4 μl Big Dye Terminator ready-reaction mix (PE ABI, Warrington, UK); 2 μl of cleaned PCR product; and 1.6 pmol of sequencing primer in a total reaction volume of 10 μl. Reaction conditions were 96°C for 10 s, 50°C for 10 s, 60°C for 4 min, 25 cycles.
Sequencing products were precipitated with isopropanol to remove unincorporated dye terminators and resuspended in 4 μl of loading buffer (formamide: dextran sulfate/ethylenediamine tetraacetic acid, 5:1). Products were run on a 377 ABI PRISM automatic sequencer and analyzed with the Sequencing Analysis software (PE ABI, Warrington, UK). Both strands were sequenced for each PCR product from at least two independent PCR experiments.

2.4. Statistical methods

Statistical analysis of the results was performed with the software package SPSS 10.0 (for Windows) using the chi-square test. Statistical significance threshold was set at the 0.05 level. The samples to facilitate statistical analysis were categorized according to the donor age in three groups: 40–60, 61–75 and 76–90. The Bowen and the other sample group contained very few samples and were excluded from the statistical analysis of the CMV infection status with the histology although there was a definitive difference in the trend.

3. Results

The presence of amplifiable DNA, using primers for a fragment of β-globin gene, was confirmed in all of the 109 skin lesion specimens examined (data not shown). The histological classification of the samples was 72 BCC, 24 SCC, five specimens with extragenital Bowen’s disease and eight with other premalignant lesions.

Forty-two of 109 (38.5%) skin lesions were found positive for CMV DNA (Fig. 1A). The highest incidence was 6/8 (75%) observed in specimens with premalignant lesions. The incidence was 37.5% (27/72) in BCC, 33% (8/24) in SCC and 20% (1/5) in extragenital Bowen’s disease. The results of PCR analysis of the 109 samples are summarized in Table 1. All positive samples were repeated for verification. Two representative samples were additionally verified by sequencing of the PCR products where homology of the product with the CMV gene was established in both cases. All samples were negative for HSV-1/2 (Fig. 1B) and EBV (Fig. 1C) DNA as assessed by our PCR based assay. The CMV infection showed no statistically significant correlation with the histological type, age, site of lesion or sex.

4. Discussion

Many genetic and environmental factors are known to contribute to the development of skin cancer, the most important being repeated exposure to...
UV radiation in sunlight [29–32]. UV radiation can cause mutations to cellular DNA which if not repaired could lead to tumor formation [33]. UV radiation is responsible for a state of relative immunosuppression of the skin due to its effect on the cutaneous immune system which leads to inadequate prevention of tumor development [34].

Because of the ubiquitous distribution of HCMV and the high seroconversion rates, an etiological association between HCMV infection and human cancer has been difficult to establish. However, evidence based on virological, epidemiologic, and molecular studies, which have demonstrated the presence of viral DNA or antigens in tumor tissues suggests its involvement in specific cancers. Both infectious and UV-inactivated HCMV were shown to transform a variety of mammalian cells in vitro, and these transformed cells were tumorigenic in nude mice. In addition, HCMV infection was shown to modulate the expression of various proteins involved in cell cycle regulation and apoptosis, providing a rationale for studying specific viral genes and their role in cellular transformation.

HCMV is a beta herpesvirus that infects 50–90% of adults and establishes latency. In immunocompromised individuals, it is reactivated and can even cause focal colonic epithelial lesions [35]. CMV proteins have been shown to promote mutagenesis, modulate cell cycle progression, angiogenesis and cell invasion [36, 37]. Several groups reported detection of CMV DNA in colorectal cancer [18, 38]. Furthermore, a recent report demonstrated both the presence of viral genome and expression of viral proteins IE1-72 in a tumor cell specific pattern in colorectal polyps and adenocarcinomas but not in adjacent non-neoplastic tissue [39]. The data strongly suggested an important role for CMV in the multistep model for the transformation of a normal cell to a malignant one. We have demonstrated a considerable frequency of CMV infection in the group of non-melanoma skin cancers. It is a fact that CMV can infect epithelial cells but its ability to transform them is not established. Since in the non-melanoma skin cancer the major etiological factor is considered to be the UV-induced mutation, it could be argued that the CMV infection with its proven mutagenic capacity [40] might contribute to the genomic instability, increasing in this way the possibility of deregulating the cell cycle genes. Further experiments in biological models are necessary to test the above hypothesis for the non melanoma skin cancers based on clinical observations.

References


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