Analysis of Prognostic Significance of Merkel Cell Polyomavirus in Chronic Lymphocytic Leukemia

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Abstract

Merkel cell polyomavirus (MCPyV) has been found to be associated with chronic lymphocytic leukemia (CLL). We evaluated its prognostic significance in CLL. MCPyV occurrence seems to be a relatively rare event during the course of CLL. MCPyV is also unlikely to influence the outcome of CLL patients.

Background: Merkel cell polyomavirus (MCPyV), a ubiquitous DNA tumor virus, has been found to be associated with Merkel cell carcinoma and chronic lymphocytic leukemia (CLL). Previous studies have reported conflicting results on the frequency and potential pathogenetic role of MCPyV in CLL. The aim of this study was to evaluate MCPyV's association with CLL and its prognostic significance. **Patients and Methods:** Between 2006 and 2013, DNA samples obtained from CLL patients (n = 119) before treatment were tested for MCPyV using quantitative real-time polymerase chain reaction analysis and verified by gel electrophoresis. Only samples testing positive by both methods were considered valid. **Results:** We found that 13 (11%) of 119 CLL cases were positive for MCPyV. Between the groups of MCPyV-positive and -negative patients, there was no significant difference in the sex, age, cytogenetics, presence of p53 defect, or immunoglobulin heavy chain (*IGHV*) mutational status. In the subset of MCPyV-negative patients, advanced Rai stage (III to IV) was found more frequently, and therapy was initiated more often. There was no difference in overall response rate, median progression-free survival, and overall survival between both groups. We did not observe any new positivity after treatment in initially MCPyV-negative patients. **Conclusion:** This study provides the first analysis of the prognostic role of MCPyV in CLL. MCPyV occurrence seems to be a relatively rare event during the course of CLL. MCPyV is also unlikely to influence the outcome of CLL patients.

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Introduction

Merkel cell polyomavirus (MCPyV), a ubiquitous DNA tumor virus, has been found to be associated with Merkel cell carcinoma (MCC) and chronic lymphocytic leukemia (CLL).[1] MCPyV sequences have also been detected in the normal tissues of tumor patients, such as those with Kaposi sarcoma, primary squamous cell carcinoma, or keratoacanthoma, and in the blood of healthy donors.[2]

Polyomaviruses are nonenveloped icosahedral viruses with a circular double-stranded DNA genome that encodes certain oncoproteins, and they are putative oncogenic viruses. Currently, 9 human polyomaviruses are known, but their precise roles in carcinogenesis remain poorly understood. The fifth polyomavirus, Merkel cell polyomavirus, was reported in 2008 by Feng et al.[3] It was detected in approximately 80% of MCC, which is an aggressive and rare neuroendocrine skin tumor.[4]

MCCs affect predominantly elderly white men on sun-exposed areas of the skin. MCC appears more frequently in elderly patients. It has a higher incidence in immunocompromised patients. In these patients, a highly aggressive course with fatal MCC outcome is often observed. MCC's incidence has been rising more dramatically in recent years than the increased incidence of cutaneous melanoma. More than one-third of MCC patients will

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die as a result of the carcinoma or its complications, making it twice as lethal as melanoma.[5]

CLL is the most common B cell leukemia, affecting more than 6 per 100,000 patients per year.[6] There has been a proposed limited antigenic etiology, at least in some cases, of CLL based on immunoglobulin heavy chain (*IGHV*) stereotypy found across unrelated cases, suggesting that a viral source may provide such antigenic stimulation. With an established epidemiologic link between CLL and MCC, there has been some interest in investigating the possible leukemogenic role of MCPyV, which is found in 80% of MCC cases.[7]

However, previous studies have reported conflicting results on the frequency and potential pathogenetic role of MCPyV in CLL. The association with MCPyV seems to represent a rare and late event during the natural history of CLL.[8] Furthermore, MCPyV sequences have also been detected in various normal tissues in tumor-affected patients. Immunologic studies have detected MCPyV antibodies in as many as 80% of healthy blood donors. This high seroprevalence suggests that MCPyV infection is widespread in humans.[9] In addition, MCPyV's prognostic significance is unknown in CLL and has not been previously studied.

The aim of this study was therefore to evaluate MCPyV's association with CLL and to investigate the occurrence of MCPyV positivity in relation to the course of CLL.

Patients and Methods

Patients

DNA CLL samples consecutively obtained from CLL patients (n = 119) before treatment were tested for MCPyV using quantitative real-time polymerase chain reaction (RT-PCR). Furthermore, 21 patients were tested repeatedly after CLL therapy. Therefore, 140 samples were tested in total. The incidence of MCPyV infection was compared with clinical (age, sex, CLL stage, therapy) and biologic prognostic CLL factors (cytogenetics, the presence of p53 defects, *IGHV* mutational status and C38 positivity). All blood samples were processed after obtaining written informed consent in accordance with the Declaration of Helsinki under protocols approved by the local ethical committee.

Methods

The MCPyV was detected in DNA samples by RT-PCR and verified by gel electrophoresis. Only samples positive by both methods were considered to be truly positive for MCPyV.

Primers and Probe Design

Primers and probes were designed to amplify MCPyV's DNA sequence using the Primer-3 software and NCBI Reference sequence (accession number NC_010277; http://www.ncbi.nlm.nih.gov/tools/primerblast). Primers and probes were tested to avoid selfdimer, heterodimer, and hairpin formation using the IDT DNA database (http://www.idtdna.com) and were also tested for specific hybridization only for MCPyV DNA sequence by the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast). The following set of primers and probe sequences were used: CPyVfw 5′-gcttcagactcccagtccag-3′, MCPyVrev 5′-ttggtggtctcctctctgct-3′, and MCPyV probe 5′-cccgaacaccatgaggaaccca-3′ BHQ1HEX (product length 98 bp) (GeneriBiotech, Hradec Kralove, Czech Republic).

RT-PCR and Gel Electrophoresis

RT-PCR for MCPyV was performed in 25 μL reaction volume containing 1 U of HotStart Taq polymerase, 75 nmol $MgCl_2$, 1 × PCR buffer (AB Gene, Epsom, UK), 5 nmol dNTPs (Promega, Madison, WI), 10 pmol MCPyVfw, 10 pmol MCPyVrev, 5 pmol MCPyVprobe and 100 ng of DNA using Rotor Gene 3000 (Corbett Research, Sydney, Australia). The optimized thermal profile for MCPyV amplification was initiated with 15-minute polymerase activation at 96°C, followed by 50 cycles of 95°C for 15 seconds and 60°C for 15 seconds. The RT-PCR data were verified by gel electrophoresis using the Agilent DNA 1000 kit on an Agilent Bioanalyzer (Agilent, Santa Clara, CA).

Fluorescence In-Situ Hybridization (FISH)

FISH analyses were performed on the interphase nuclei of cultured peripheral blood cells using a panel of DNA probes to detect prognostically significant aberrations: deletion 13q and trisomy 12 (LSI D13S319/LSI 13q34/CEP 12, Vysis, Downers Grove, IL; or XL DLEU/LAMP/12cen and XL DLEU/LAMP/12cen, MetaSystems, Altlussheim, Germany), and deletion 11q and deletion 17p (LSI p53/LSI ATM, Vysis; or XL ATM/p53 probe, MetaSystems). Two hundred interphase nuclei were evaluated. The cutoff level for each individual probe was determined on the basis of a negative sample analysis and calculated as the mean ± 3 SD. Chromosomal aberrations were categorized according to the hierarchical model of Döhner et al.[10]

IGHV *Mutation Status*

The *IGHV* gene's mutation status was examined using PCR followed by PCR amplicon sequencing. The sequences obtained were analyzed using an IMGT/VQUEST tool and database. The unmutated *IGHV* gene was defined using sequence identity to germ-line sequence ≥ 98%; the mutated *IGHV* gene was defined by identity < 98%. *IGHV* subsets were also analyzed to be compared with MCPyV positivity or negativity.

Results

We found that 13 (11%) of 119 CLL cases were positive for MCPyV. Between the groups of MCPyV-positive and -negative patients, there was no significant difference in the sex (5 of 49 positive women, 8 of 70 positive men), age (median age 60.5 years for positive and 60 years for negative patients), cytogenetics (unfavorable cytogenetics [del 17p or 11q] in 38% of positive and 24% of negative patients), presence of p53 defect (23% of positive patients vs. 20% of negative patients), or *IGHV* mutational status (unmutated in 69% of positive patients and 76% of negative patients). Only 2 patients from the MCPyV-positive group were from any CLL subset (both from subset 5; no MCPyV-negative patient had this subset 5 *IGHV*).

At baseline, advanced Rai stage (III and IV) was found more frequently in MCPyV-negative patients (15% vs. 41%; $P = .04$). Therapy (fludarabine, cyclophosphamide, and rituximab regimen or alemtuzumab) was also initiated more frequently in the negative group (46% vs. 68%; P = NS). There was no difference in median