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ASSOCIATION OF POLYOMAVIRUS JC WITH COLON CANCER

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JC virus (JCV) is a human polyomavirus that has recently been demonstrated to be associated with pediatric malignancies, ie, medulloblastomas. Recent controversial data suggests that JCV may play a role in the pathogenesis of colon cancer. Chromosomal instability, a hallmark of colon cancer, may also be induced by JCV.

We further tested this hypothesis by employing polymerase chain reaction (PCR) technology to detect JCV in normal and colon cancer tissues. DNA was extracted using commercial kits from paraffin-embedded tissue specimens from eight colon cancer patients; one normal and one tumor tissue specimen from each patient. Extracted DNA was PCR amplified using primers specific for T antigen (TAg) and 610-bp JCV V-T region, cloned into the pCR2.1 vector, and the kanamycin resistant bacterial transformants were sequenced using the ABI Sequencer. The sequences were analyzed using the Lasergene Software.

Our results showed 100% JCV infection of both the normal and tumor colon tissues, and 61% of the JCV transcriptional control region sequences were Mad1 type. Furthermore, genotyping based on the V-T intergenic region demonstrated that all eight patients were infected with JCV Type 1A. Presence of JCV in normal and colon cancer tissues suggests that JCV may act as a co-factor in colon cancer carcinogenesis.

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INTRODUCTION

Epidemiology of Colon Cancer

Colon cancer is the third leading cancer in the United States, accounting for 10% of all cancer deaths. In 2003, there were 105,500 new colon cancer cases with 57,100 deaths in the United States. The average age at diagnosis is 60 to 65 years old and men are more likely to develop colon cancer than women. Also, African Americans are more likely than Caucasians to be diagnosed with colon cancer. Non-genetic risk factors that can lead to colon cancer include old age, recurrent intestinal polyps, inflammatory bowel disease, low-fiber high-fat diet, lack of exercise, smoking and alcohol consumption. About 10%–30% of colon cancers are hereditary and 60%–85% have no genetic cause and are referred to as sporadic. Of all colon cancers, 85% are due to chromosomal instability (CIN) and 15% are due to microsatellite instability (MSI).

Background of Polyomavirus JCV

JCV has been isolated from the brain, lung, liver, kidney, spleen, bone marrow, bladder, prostate, tonsils, lymph nodes, and leukocytes. However, it remains controversial whether this virus could be a factor leading to the development of colon cancer. The mode of transmission is unclear, but the respiratory- and digestive-tracts have been suggested as possible routes. JCV is asymptomatic in an immunocompetent person, but can be reactivated in immunosuppressed patients such as those infected with HIV. The JCV genome is divided into three regions: early proteins, late proteins and the regulatory region also known as the transcriptional control region (TCR). Early proteins consist of large T-antigens and small t-antigens. Late proteins consist of VP1, VP2, VP3, and the auxilliary agnoprotein. Based on the TCR, JCV can be classified into rearranged type, which is predominantly found in the brain of patients with PML, and archetype, which is predominantly found in urine of healthy individuals. Theoretically, rearranged type JCV (Mad-1) results from deletion and/or duplication of archetype JCV (CY1). Archetype TCR contains all 6 regions, A–F. The Mad-1 TCR contains a 98-bp duplication of the A, C, E and deletion of region B and D.

The objectives of our study were to determine if JCV is present in the colon of colon cancer patients and to determine which JCV strain is most prevalent so as to determine if there is any association between JCV and colon cancer.

METHODS

Paraffin-embedded tissue was shaved from the tissue block and suspended in xylene to dissolve the paraffin. Total tissue DNA was extracted using QIAamp DNA Mini kit (Qiagen). PCR and nested PCR were performed using Takara *Taq polymerase* and primers encompassing the TCR and the 610-bp V-T intergenic region. Amplified PCR products were run on a 2% agarose gel in 0.5× TBE with 0.5 μg/μL ethidium bromide for 45 min at 100 volts. PCR products were cloned into pCR2.1 using the TOPO® Cloning Reaction pro-

tocol. DNA was isolated from Kanamycin resistant bacterial transformants using the QIA Plasmid Mini Kit and digested with EcoR1 to identify clones with JCV insert. The Biotechnology/ Molecular Biology Instrumentation and Training Facility at the University of Hawaii at Manoa sequenced positive transformants. Resulting sequences were aligned using DNASTAR Lasergene software Seqman and compared to archetype and Mad-1 JCV using the MegAlign program.

RESULTS

From the ethidium bromide stained agarose gel, it was evident that all patients were infected with JCV. From the sequenced clones, there were no JCV archetypes detected, but there were a total of four archetype-like. Mad-1 was the most prevalent strain, accounting for 61% of 36 sequenced samples. There were 22 Mad-1 type and 10 Mad-1-like clones. Based on the JCV genotyping chart on the 610-bp V-T intergenic,the point mutations demonstrated that all eight patients were infected with JCV Type 1A.

CONCLUSION

Based on our results, our hypothesis that JCV would be present in the colon and that Mad-1 would be the predominant type, were both correct. Mad-1 was the most prevalent JCV strain found in the colon irrespective of the site within the colon and the site of the tissue section (normal vs tumor). Archetype JCV was not detected in the specimens. All eight patients were infected with JCV Type 1A, the major type found in the United States. Based on our data and supportive evidence by other studies, we conclude that JCV is more likely associated with colon cancer.

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