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Research Paper

Microsatellite Chromosomal Instability in Bladder Cancer Patients Infected with Hepatitis C Virus

Medical Science

KEYWORDS :

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ABSTRACT

The objective of this study was to investigate the impact of HCV infection on MSI in cancer bladder. Tissue samples from 100 (50 HCV infected and 50 non-HCV infected) patients (aged 17- 89 years). Surgical specimens were obtained by radical cystectomy of bladder tumors. The frequency of MSI +LOH in D9S162 locus (40%) was higher than that of D9S171 locus (34%). Moreover, there was positive correlation between HCV infection and MSI in D9S171 and D9S162 loci 9. LOH and MSI of D9S171 and D9S162 loci were more frequent in cancer bladder tissues than normal tissues as well as the frequency was higher in HCV infected patients.

Introduction

Bladder cancer in Egypt is the most prevalent cancer in men (16%) and the second most common cancer in women, producing more than7900 deaths annually, which is strikingly higher than most other parts of the world (Khaled, H. 2005). The neoplastic changes in the urothelium of bladder are a multistep phenomenon involving the activation of oncogenes, and inactivation or loss of tumor suppressor genes (Sandberg, A. A. and Berger, C. S., 1994).Microsatellite instabilities (MSIs) are independent molecular markers for prognosis; it can help in detecting a germline mutation and therefore allows for the detection of possible hereditary cancers (Eltz,S et al., 2008). LOH of chromosome 9 is the most frequent event described in bladder carcinoma as well as aberrations on chromosome 4. LOH has been frequently reported in bladder carcinoma at specific regions of chromomosomes 4, 5, 8,9,11, and 17 (Utting et al., 2002).

Hepatitis C virus (HCV) infection may have some etiopathogenic role in the development of tumors. Also, HCV can infect Blymphocytes in vitro and in vivo (Sung, V. M et al., 2003)and its envelope protein E2 can bind to CD81 in the CD21/CD19/CD81 costimulatory complex, suggesting the ability of HCV to alter the intracellular signaling B cells (Pileri, P et al., 1998).Moreover, in HCV-infected individuals, oligoclonal lymphoproliferative disorders and chromosomal translocations have frequently been observed in B lymphocytes, suggesting that HCV may cause chromosomal instability (Kitay-Cohen, Y et al., 2000).So, the aim of this study was to investigate the MSI in cancer bladder in HCV infected patients.

Material and Methods

Patients and Sample Collection

Tissue samples from 100 patients with bladder tumors were obtained from the Urology& Nephrology Center, Mansoura. 50 HCV infected patients and 50 non-HCV infected patients (aged 17- 89 years). The pathologic diagnosis was confirmed on all tissue samples. Surgical specimens were obtained by radical cystectomy of bladder tumors. The experimental procedures were reviewed and accepted by the Ethics Committees of the National Cancer Institute.

Sample preparation, DNA extraction and PCR amplification

In each case, fresh tumor samples after dissection were frozen immediately in liquid nitrogen for 5 minutes and all samples are stored at −80 °C until DNA extraction.DNA was extracted from tissue samples using EZNA™ tissue DNA kit, following the manufacturer's recommendations (GE Healthcare, UK andOMEGA bio-tek, U.S.A). The concentration and the purity of DNA were quantified spectrophotometrically at absorbance of 260/280 nm and 260/280nm. The sequences of primers as follow; D9S171 (F)5' AGCTAAGTGAACCTCATCTCTGTCT 3', (R)5' ACCCTAGCACTGATGGTATAGTCT 3'and D9S162(F)5'GCAATGACCAGTTAAGGTTC 3'D9S162(R)5' AATTCCCACAACAAATCTCC 3'.PCR amplification was performed in a final volume of 10µL containing approximately 40ng of DNA template, 10x buffer contains 200 mMtrimethamine (tris) buffer pH 8.3, 500 mM KCI and 15 mM $Mgcl_2$, (5U/µl) of Taq polymerase (Perfect Taq Plus DNA polymerase (5 PRIME, Inc, USA), 10mM deoxynucleoside triphosphates mix consisted of 10mM each of dATP, dCTP, dGTP and 5mM dTTP, 1mM Cy5 dUTP-PCR and 10 pmoles of the primers. The thermal cycling profile on a biometra instrument consisted of a 30 sec denaturation step at 94° C, a 30s annealing step at the optimized temperature of 55° C and a 45 sec extension step at a temperature of 72° C, for a total of 40 cycles. Each PCR was initiated with a 3 min denaturation step at 94° C and terminated with a 5 min extension step at

Analysis of microsatellite instability

After amplification, five microliters of the PCR product were mixed with five microliters of stopping dye then this mixture was denatured at 95° C for 5 min, placed on ice and then loaded onto the sequencing gel.The gel from SuperFill® 6% sequencing gel cartilage is casted between two disposable glass plates (MicroCel™ Cassette) and toasted it in the Gel Toaster™ polymerizing unit for 5 minutes.Once the gel is ready, it is positioned into the sequencer and the buffer chambers filled with 1x TBE (Tris-Borate- EDTA) buffer, pH 8.3. The gel temperature and voltage are set at 54° C and 1200 V, respectively. A 5 min pre-run is initiated to bring the gel temperature to the set values.Each of the 4 lanes is loaded with 5 µL of denatured mixture of DNA which ex-

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tracted from tissue sample of the same patient so in total 20 patient samples can be loaded on each MicroCel plate. The laser is switched on and the electrophoresis started. Laser intensity can be varied accordingly in order to bring the signals within the dynamic range of the detectors.At the end of the run (60 min), the data can be analyzed by using the Fragment Tool™ of the Gene Objects™ software (Visible Genetics).

Statistical analysis

The Chi-square test was used to determine the correlation between tumor stage and grade, HCV infection, and loss of chromosome 9 as detected with polymorphic markers D9S171 and D9S162. A p-value of <0.05 was considered significant.

Results

Frequency of MSI and LOH in normal and bladder cancer tissues

Table 1 showed the frequency of MSI and LOH in D9S171 and D9S162 loci in normal and cancer bladder tissues in HCV infected and non-HCV infected patients. The normal tissues from non-HCV infected patients did not show any case of MSI. However, normal bladder tissues with HCV infection showed significant increase in MSI and LOH. The MSI and LOH were more frequent in cancer tissues of patients with HCV infection. Moreover, the frequency of MSI +LOH in D9S162 locus (40%) was higher than that of D9S171 locus (34%). Figure 1 showed the MSI in cancer bladder tissues with and without HCV infection.

Correlations between HCV and MSI in D9S171 and D9S162 loci

Table 2 showed positive correlation between HCV infection and MSI in D9S171 and D9S162 loci in chromosome 9. At the same time, there were no correlations between MSI in these loci and tumor stage, grade, age and sex of patients.

Discussion

The present study is the first study, up to the best of our knowledge, investigated the impact of HCV infection on microsatellite instability of chromosomes. In this study examined the MSI in two loci at chromosome 9 namely, D9S171 and D9S162 in HCV and non-HCV infected patients. We found that LOH was more frequent in bladder cancer tissues from patients with HCV infection.Microsatellites, also known as short tandem repeats are repeating sequences of 2-6 base pairs of DNA. Microsatellite analysis is a promising marker for the detection and prognosis of bladder cancer (Wadhwa N et al., 2012).

Aberrations concerning chromosome 9 have been observed by several investigators using techniques such as LOH analysis and FISH. However, there are some important differences between the techniques and this affects the significance of the obtained results with respect to the interpretation of tumor pathogenesis. When LOH is scored, in general this will imply that the intensity of the lost alle is visually much lower than that of the control. Thus, the loss is relatively clear-cut and it can safely be concluded that the region in which the marker(s) is located has been deleted in most of the tumor cells.

LOH is frequently found on the chromosomal arms 4p, 8p, 9p, 11p and 17p and plays an important role in the development of bladder cancer. Burger M et al., (2006) found that allelic loss of chromosome 9 is the most frequent event in 50-60% of bladder tumors. In this study, the frequency of LOH+MSI in D9S171 locus of chromosome 9 increased from 22% in non-HCV infected patients to 34% in HCV infected patients and the frequency of LOH+MSI in D9S162 locus of chromosome 9 increased from 18% in non-HCV infected patients to 40% in HCV infected patients. Moreover, the findings of this study showed that MSI and LOH was more frequent in cancer bladder tissues than normal bladder tissues. Moreover, there was a positver correlation between HCV infection and MSI in these loci.

MSI plays a significant role in evolution, initiation and progression in bladder tumors (Vaish M et al., (2005). Mutations in these loci in chromosome 9 caused deformity some tumor suppressor genes causing replication errors and genetic imbalance. One tumor suppressor gene identified on 9p is the CDKN2A (p16, MTS1) gene. Cairns (1995), showed that in 71% of primary bladder tumors homozygous deletions targeting this gene were detected. Besides the CDKN2A gene on 9p there is evidence for at least two other loci on chromosome 9q (van Tilborg et al., 1999).

Conclusion

LOH and MSI of D9S171 and D9S162 loci of chromosome 9 were more frequent in cancer bladder tissues than normal tissues and HCV increased the frequency of these instability of these loci. These findings might cause mutations of tumor suppressor genes regulated by these loci.

Table (1): Frequency of MSI and LOH in D9S171 and D9S162 loci of chromosome 9 in normal and cancer bladder tissues of non-HCV and HCV infected patients

	-ve MSI	MSI	LOH	MSI+LOH
D9S171 locus				
Normal tissue without HCV infection	(50) 100%	(0) 0%	(0) 0%	(0) 0%
Normal tissue with HCV infection	(40) 80%	(2) 4%	(3) 6%	(5) 10%
Cancer tissue without HCV infection	(36) 68%	(3) 6%	(2) 4%	(9) 18%
Cancer tissue with HCV infection	(19) 38%	(7) 14%	(7) 14%	(17) 34%
D9S162 locus				
Normal tissue without HCV infection	(50) 100%	(0) 0%	(0) 0%	(0) 0%
Normal tissue with HCV infection	(42) 84%	(2) 4%	(1) 2%	(5) 10%
Cancer tissue without HCV infection	(34) 68%	(3) 6%	(5) 10%	(8) 16%
Cancer tissue with HCV infection	(13) 26%	(8) 16%	(9) 18%	(20) 40%

HCV= hepatitis C virus infection, MSI= microsatellite instability, LOH= loss of homozygous. Chi square test

Table (2): Correlations between in D9S171 and D9S162 loci of chromosome 9 and HCV infection and tumor grade, stage, patient age and sex Pearson correlations.

	D9S1717	D9S162
Patient age	r= 0.002 P= 0.962	r= 0.062 P= 0.216
Patient sex	r= - 0.064 P= 0.198	r= - 0.067 P= 0.178
Tumor grade	r= 0.007 P= 0.883	r= 0.025 P= 0.618
Tumor grade	r= - 0.021 P= 0.675	r= 0.029 P= 0.562
HCV infection	R= 0.228 P= 0.000	0.290 0.000

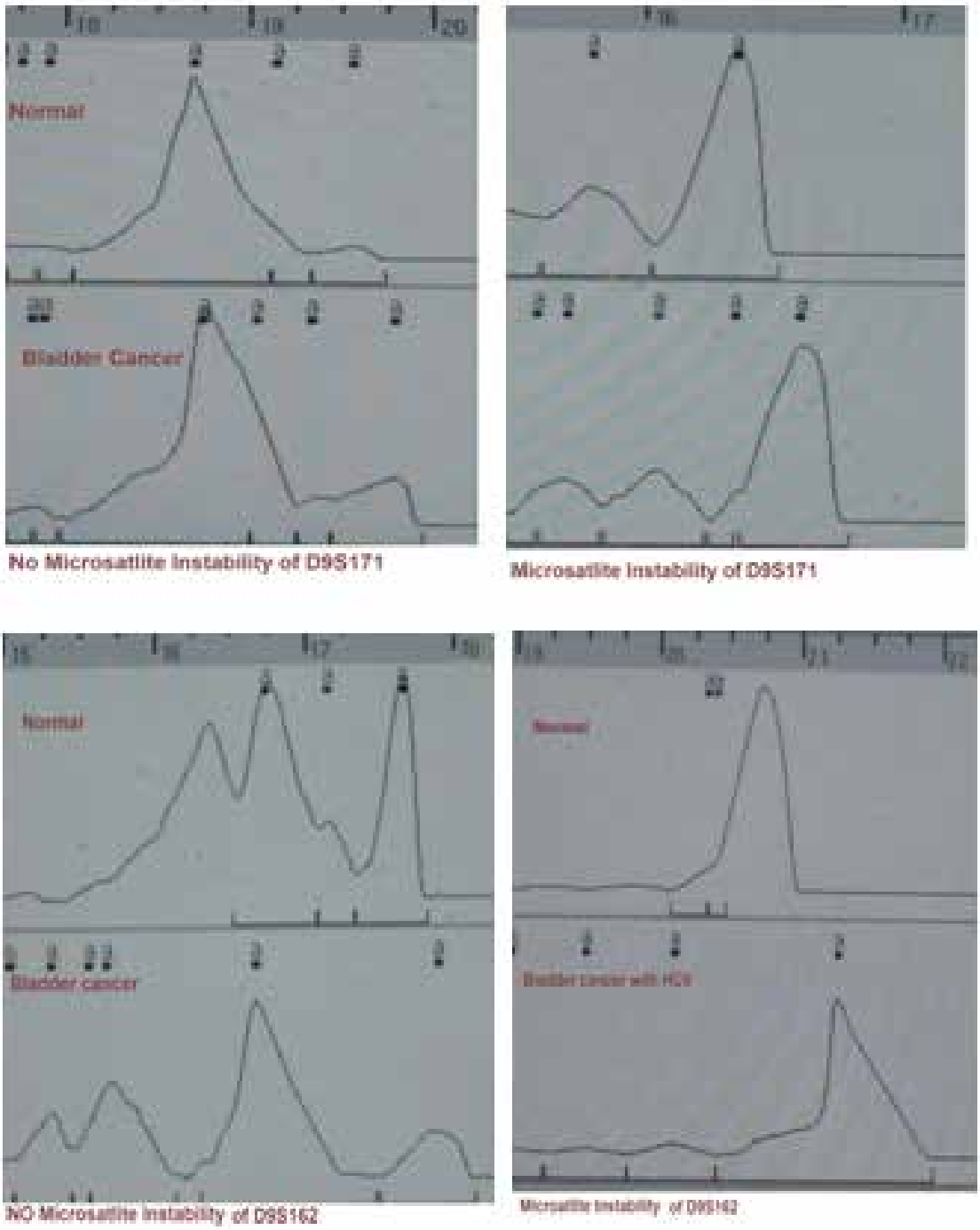

Figure (1): Microsatellite instability at D9S162 and D9S171 loci of chromosome 9 in cancer bladder tissues in HCV infected patients.

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