PO Box 2345, Beijing 100023, China
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RAPID COMMUNICATION

Frequent loss of heterozygosity at 8p22 chromosomal region in diffuse type of gastric cancer

Hedayat Allah Hosseini, Ali Ahani, Hamid Galehdari, Ali Mohammad Froughmand, Masoud Hosseini, Abdolrahim Masjedizadeh, Mohammad Reza Zali

Hedayat Allah Hosseini, Abdolrahim Masjedizadeh, Biochemistry Department, Medical school, Ahwaz Jondishapoor University of Medical Science, Ahwaz, Iran
Ali Ahani, Masoud Hosseini, Biology Department, Shahid Beheshti University, Tehran, Iran.
Hamid Galehdari, Ali Muhammad Froughmand, Genetics Department, Shahid Chamran University, Ahwaz, Iran
Mohammad Reza Zali, Research Center of Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran
Supported by Research Center of Gastric and Liver Disease in Tehran Taleghani hospital
Correspondence to: Hedayat Allah Hosseini, Biochemistry Department, Ahwaz Jondishapoor University of Medical Science, Ahwaz, Iran. hosseini.h@aujums.ac.ir
Telephone: +98-61-13332036 Fax: +98-61-13332036
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Abstract

AIM: To study the loss of heterozygosity (LOH) at 8p21-23 locus in diffuse gastric cancer.

METHODS: To evaluate the involvement of this region in gastric cancer, we used eight microsatellite markers covering two Mb of mentioned region, to perform a high-resolution analysis of allele loss in 42 cases of late diffuse gastric adenocarcinoma.

RESULTS: Six of these STS makers: D8S1149, D8S1645, D8S1643, D8S1508, D8S1591, and D8S1145 showed 36%, 28%, 37%, 41%, 44% and 53% LOH, respectively.

CONCLUSION: A critical region of loss, close to the *NAT2* locus and relatively far from *FEZ1* gene currently postulated as tumor suppressor gene in this region.

Key words: Loss of heterozygosity; Tumor suppressor genes; diffuse type of gastric cancer; STS marker; *N*-Acetyltransferase 2; *Fez1*

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INTRODUCTION

Adenocarcinoma of the stomach (ACS) is the second most common cancer worldwide and there are two distinct biological and etiological subtypes of ACS: (*a*) the intestinal and (*b*) the diffuse-infiltrative type. The individual's risk of the intestinal disease is dominant in countries with a high incident of gastric cancer. Although its incidence is decreasing, ACS is the second leading cause of cancer mortality in many countries[1,2].

A recent cancer survey by the Iranian Ministry of Health and Medical Education (IMHME) revealed that gastric adenocarcinoma is the most common fatal cancer in Iran, with a wide variation of death rate among different provinces. According to the recent cancer statistics, deaths due to gastric cancer constitute about 39% of all deaths due to cancer each year in some parts of Iran[3].

Diet and environment are important factors in the intestinal form of ACS, which is associated with chronic atrophic gastritis and intestinal metaplasia of the gastric mucosa. In addition, environmental factors have influence on incidence of the diffuse (i.e. infiltrative) form of ACS[4]. One of the strong tools to genetic analyses is loss of heterozygosity (LOH) that consequently leads to loss of function of tumor suppressor genes. Inactivation of first normal allele mainly occurs by point mutation, followed by deletion or loss of second allele[5]. Hence, to LOH analyzing in a region, usually the microsatellite STS markers are used. These markers enable to trail contemporary two alleles of a gene[6].

Frequent LOH at specific chromosomal regions in certain tumors implies the presence of suppressor genes. Recent allelotyping studies have shown that allelic losses on the short arm of chromosome 8, particularly at bands 21-23.1, are frequently associated with various tumors, including prostate cancer[7,8], breast cancer[9,10], head and neck squamous cell carcinoma[11,12], urinary bladder carcinoma[13,14], hepatocellular carcinoma[15], lung cancer[16] and colorectal cancer[17]. Additionally, frequent deletion at 8p22 has been shown strongly associated with gastric cancer progression[18]. These observations suggest that chromosomal region 8p21-23.1 plays a critical role in the development of various tumors.

Experimentally functional evidence by chromosome transfer into tumor cells at 8p region showed the presence of one or more putative tumor suppressor gene(s) in this region[19]. In addition, micro-cell fusion experiments suggested the possible location of metastasis suppressor

gene(s) at 8p[20, 21]. It is possible that at the locus 8p, two or more genes will be involved in suppressing of cancer development. Efforts toward positional cloning of the suppressor gene(s) allowed the isolation of an important candidate tumor suppressor *FEZ1* gene at 8p22. However, the significance of *FEZ1* in the tumor-development or progression remains confused[22-24].

Accordingly, in this first study from Iran, eight microsatellite STS markers were selected to analyze frequency of allelic loss in 42 cases of late diffuse type of gastric cancer.

MATERIALS AND METHODS

Subject

Paraffin embedded tissues of 42 patients were analyzed with advanced locally diffuse type of gastric cancer registered in RCGLD registry system from 2003 to 2005.

DNA Extraction: Formalin-fixed, paraffin-embedded tissue blocks were sectioned with 5 μm thickness. They stained (Hematoxylin and Eosin), and viewed to confirm histological grading. Using the stained dissected slides as templates, two 20-μm section fragment of paraffin-embedded tissue were placed in two sterile tubes as source of tumoral and normal samples, separately. Deparaffinization was performed with Xylene, followed by DNA extraction as described previously[25].

Microsatellite STS marker selection

LOH analysis by paired normal-tumor microsatellite PCR was performed using eight well-mapped microsatellite markers (Table 1). These markers cover approximately two Mb of 8p22 region. Figure 1 shows the chromosomal positions of the selected markers, based on the sequence tagged site database (http://www.ncbi.nlm.nih.gov), with supplementary mapping information, provided through the Cooperative Human Linkage Center database (http://www.chlc.org), the Genome Database (http://www.gdb.org), the Genetic Location Database (http://www.cedar.genetics.soton.ac.uk/public_html).

PCR of STS microsatellite markers

In a total volume of 25 μL the PCR contained 2 μL DNA sample solution, 200 μm of all four deoxynucleotide triphosphates (dNTP), 50 pmol of each forward and reverse primers, 0.2 μL super Taq polymerase (Roche), 2 μL of 10X PCR buffer (Roche), and 3 mmol/L $MgCl_2$ (Roche). The following thermal cycling conditions were employed for all reactions: an initial denaturation step of 5 min, followed by 35 cycles of denaturing, annealing, and extension (30 s each) and a final 20-min extension step. A denaturing temperature of 95℃ and an extension temperature of 72℃ were used and annealing temperatures for the different primer sets were optimized as necessary (Table 1)

Gel electrophoresis, staining and LOH analyzing

PCR-products were size-separated on a Biorad 165-3860 Sequi-gene using 5 μL of the sample was then loaded onto a 60 g/L polyacrylamide gel containing 7 mol/L urea, 450 mmol/L Tris-borate (pH 7.5) and 1 mmol/L EDTA (pH 7.0) running buffer. Loaded gels were electrophoresed for 2-4 h (depend on PCR product size bands) and stained with $AGNO_3$ method[26].

Because of low quality of specimens, 17 of 42 samples were excluded and the remains normal and tumor paired-samples from 25 late diffuse type of gastric cancer were screened for LOH at 8p22. LOH analysis was performed as described previously[27,28]. Each locus scored for LOH according to the absence (allelic loss) or the disequilibrium (allelic imbalance) of signal from one allele in the tumor-DNA-amplification product as compared with the normal one. Reduction of > 50% of band intensity was considered to loss. (Figure 2)

RESULTS

Allelic loss for at least one locus detected in 76% (19/25) cases examined. Eight loci were tested in all of the matched normal and tumor samples (Table 1). Two of these STS markers (D8S1948, D8S280) have been excluded because of non-informatively. In other markers allelic loss ranged were 36% (4/11) for D8S1949, 28% (5/18) for D8S1645, 37% (7/19) for D8S1643, 41% (5/12) for D8S1508, 44% (4/9) for D8S1591 and 53% (7/13) for D8S1145. Allelic imbalance for at least one locus found in 76% of tumor samples. Figure 2 shows the pattern of allelic loss for each case. In eight cases, loss of one allele tends to telomeric and in four cases to centromeric region. Other cases showed loss in the middle point of 8p22 region.

DISCUSSION

In this study we examined a region of chromosome 8p favored as potentially harboring tumor suppressor genes[7-18]. The allelotyping performed in a region less than two Mb at 8p22 to identify a common deletion in the late diffuse type of gastric carcinoma. Allelic imbalance for at least one locus found in 76% of tumor samples. However, the diffuse-type of gastric cancer has a higher normal cell content and the DNA impurities maybe superimposed to infrequent chromosomal losses[28]. Therefore, the frequency of 8p22 deletion in this study is considerable. The unique connection of this locus in the carcinogenesis introduces two possibilities: (1) 8p22 locus is one of phenotype-determined events tends to develop diffuse gastric cancer; (2) or alternatively, 8p22 locus participate in late stage of diffuse gastric cancer and is more likely to harbor chromosome instabilities. Furthermore, our data allowed us to define a minimal region of allelic loss at 8p22 to a segment around D8S1145 marker with a LOH rate of 53% (Figure 2); make it a good candidate to harbor putative TSG.

Several candidate cancer-susceptibility genes at 8p22, such leucine zipper tumor suppressor 1 (*LZTS1*) or *FEZ1*[22-24], deleted in liver cancer *DLC1*[29] and mitochondrial tumor suppressor gene1 *MTUS1*[30] are other candidate TSGs in this region. Nevertheless, the minimal region of loss in our tumor samples was telomeric

Table 1 Markers and their characteristics

Locus	Forward primer (5'-3')	Reverse primer (5'-3')	Size range (bp)	Annealing temperature (℃)	(%) Informative	(%) LOH
D8S1948	TTACAAAACATACCCAGTGTTTGG	CTTTTTAGTGCTTGAGACTGTCTCC	110-111	58	< 10	Exclude
D8S1949	TGTCTTACAGCTCTCCCTCTCC	CAGTAAGGATCACCAAGACAAGG	106-107	65	40	36
D8S1645	GTTCAACTGTTGATTTTTTGACAA	CTTTTTAGTTAATTCCCATCAGCA	176-177	62	72	28
D8S1643	AGGCCTTGTAAGTGATAAAGGC	TTCTCCATCAACCTTTTGGC	100-101	64	80	37
D8S280	CAATTTCATTGCTAGGTGTATATCC	CTGTTTTATGGCTGAATAGTGTCC	224-232	59.5	< 10	Exclude
D8S1508	AAAATTCCTACCTTGCTATGAACA	CTGCACGTAACTCTCCACCA	181-182	61.5	50	41
D8S1591	CAAAGATTTCTTTTTATTCACCTGC	TTTCTTTAGATGGAGTCCATTGC	208-209	59.5	36	44
D8S1145	TGCTAACTGGCACGGTCAC	CAATCCCAGTAATCTATAACTTCA	261-289	63	56	53

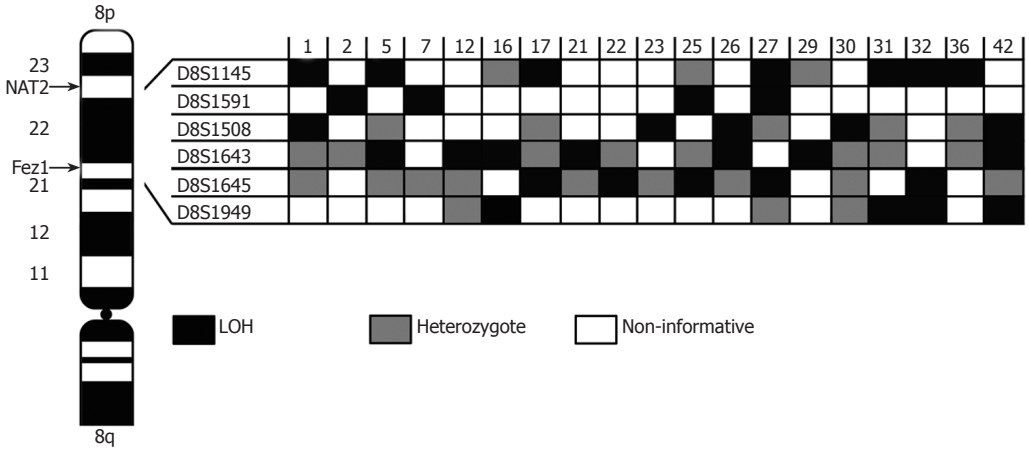

Figure 1 LOH pattern of markers and chromosomal positions. Case number is illustrating above each column. Microsatellite STS markers listed in order from telomere to centromere together with their genetic location. *Fez1* gene locus is close to D8S1949 marker with 36% LOH and *NAT2* gene locus is close to D8S1145 marker with 53% LOH, respectively.

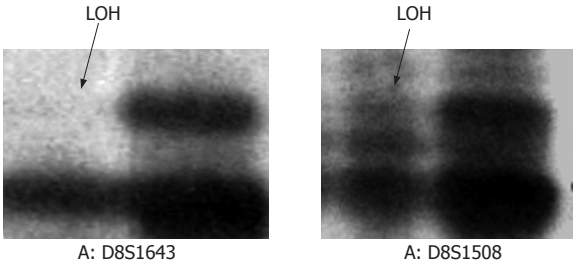

Figure 2 Showed LOH in two markers.

to these genes and the D8S1145 marker with the highest LOH rate is close to the *NAT2* locus.

The *N*-acetyltransferase isoezymes, *N*-acetyltransferase 1 (*NAT1*) and *N*-acetyltransferase 2 (*NAT2*) catalyze either *N*-acetylation of aromatic amine and hydrazine drugs or *O*-acetylation of *N*-hydroxy-aromatic and heterocyclic amines, and have a primary role in the activation and/or deactivation of a large and diverse number of environmental pollutants[31]. Because *NATs* activate and/or deactivate environmental pollutants, some of which have been implicated in the etiology of cancers, it has been suggested some polymorphisms that alter the function of *NAT* genes may be risk factors for the disease[32]. It is often suggested that human *NAT2* activity is highest in the liver and gastrointestinal tract[33]. Certain polymorphisms are associated with a decrease in *N*-acetyltransferase2 activity leading to a possible increased risk factor to develop bladder, gastric, lung and prostate cancers[32-35]. In other hand, occurring of diffuse gastric cancer in proximal gastric tissue is abundance than other section of gastric tissue[36,37] and *NAT2* only expressed in this region of stomach[38,39].

Furthermore, accumulating evidences indicate that both genetic and epigenetic changes associate with diffuse gastric cancers[23,40]. Ethnic background suggested being associated with differences in disease aggression and outcome in Asian populations[41,42]. Based on mentioned reports and our LOH rate around *NAT2* locus, we hypothesize that the loss of *NAT2* gene might influences the progression of this form of cancer or this region harbored another tumor suppressor gene far from of *FEZ1* locus.

Further studies will define the key gene targets of alteration on 8p22-23.1 in gastric cancers.

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