A partial allelotyping of urothelial carcinoma of bladder in the Chinese

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The cancer spectrum and genetic pathways underlying tumorigenesis vary among different ethnic populations due to genetic background and/or environment discrepancies. We have implied in our previous study that the genetic alterations found in bladder cancers of Chinese patients differ from those of Caucasians. We performed the present study to explore the genetic pathways of the urothelial carcinoma (UC) in Chinese patients. We carried out a partial allelotyping of Chinese UC on chromosome arms commonly deleted in Caucasian UC, and compared the allelotyping between Chinese and Caucasian UC. Forty-five Chinese UC specimens were allelotyped using 30 microsatellite markers on 18 chromosome arms. The most frequent regions of loss of heterozygosity included 9q (54.1%), 17p (51.2%), 9p (48.8%), 18q (42.2%), 3p (41.9%), 16q (33.5%) and 11p (30.0%). Compared with UC from the UK and US, the LOH frequencies on most chromosome arms in this study are higher, with statistically significant differences on 3p, 16q and 18q. Our results suggest that both consensus and different alterations exist between Chinese and Caucasian UC, indicating that genetic alterations of cancer can vary between different ethnic populations due to genetic and/or etiological discrepancies.

Introduction

Bladder cancer is the sixth most common malignancy world wide (1), the sixth most common cancer in men and the tenth most common cancer in women in China (2). Urothelial carcinoma (UC) is the predominant bladder cancer throughout Western countries and China. Somatic genetic alterations in UC specimens collected in the UK and the US have been well characterized (3). In these studies, loss of heterozygosity (LOH) in several specific regions of genome identified the probable location of tumor suppressors that may play a role in the development of UC (3).

Cancer types and genetic pathways initiating tumorigenesis in the bladder vary among different ethnic populations due to genetic background and/or environment exposure discrepancies. As an example, in Egypt and other regions of the Middle East where the trematode Schistosoma haematobium is endemic, bladder carcinoma is predominantly squamous cell carcinoma (SCC) and the genetic abnormalities are different from that of UC (4). Additionally, schistosomiasis associated UC showed different genetic alterations from that collected in the UK and the US (4). We have implied in our previous study that genetic alterations of bladder cancer in Chinese may be different from that of Caucasian patients (5). However, the molecular events underlying tumor development in the bladder cancer of Chinese are poorly understood.

In order to better understand the genetic pathways of UC in Chinese patients, we screened 45 Chinese patients with UC for LOH on 18 chromosome arms commonly deleted in UC of Caucasian patients, and compared the results from the two populations.

Materials and methods

Tissues

Samples from 45 patients with sporadic UC were obtained from the Department of Urology, Cancer Hospital, Chinese Academy of Medical Sciences. Age, sex, tumor stage, grade and smoking history of each patient are listed in Table I. Tumor stage and grade were classified according to the tumor-node-metastasis system and WHO criteria (6). Surgical specimens were obtained by transurethral resection of bladder tumors, radical cystectomy or nephroureterectomy. No medications were taken before surgery in these patients. In each case, freshly excised tumor samples were frozen immediately in liquid nitrogen for 5 min and stored at −80°C until DNA extraction. Histopathological examination was performed on representative sections of each tumor by a pathologist (S.Z.) before DNA extraction, and only samples that were comprised of >75% tumor cells were included in this study. Peripheral blood samples were collected before the patients underwent operations. Lymphocytes were isolated by addition of citrate (final concentration 0.2%) to 5 ml peripheral blood followed by dilution with lymphocyte isolating solution (Institute of Haematology, CAMS, Tianjin, China), and centrifugation at room temperature (800–1000 g for 10 min). The layer containing leukocytes was collected and washed twice with phosphate-buffered saline. Cell pellets were stored at −80°C until DNA extraction.

DNA preparation

Tumor tissues were pulverized in the presence of liquid nitrogen. Pulverized tumors and peripheral leukocytes were digested with 50 μg/ml proteinase K in 1% (w/v) sodium dodecyl sulfate for 8–14 h at 48°C, and twice extracted with phenol–chloroform. DNA was precipitated by addition of 2 vol of 95% ethanol and 1/3 vol of 3 M sodium acetate.

Microsatellite analysis

DNA from each sample was analyzed using 30 microsatellite markers (Research Genetics, Huntsville, AL) listed in Table II, as described previously (5). Briefly, the forward primer of each marker pair was end-labeled with [γ-32P]dATP (New England Nuclear, Boston, MA). In each polymerase chain reaction (PCR), 20–100 ng of DNA was subjected to 35 cycles, in which each
cycle consisted of: 95°C for 30 s (desaturating temperature), 52–62°C for 1 min (varying annealing temperature), and 72°C for 1 min (extension temperature). There was a single final extension at 72°C for 10 min. PCR products were separated by electrophoresis on a denaturing 7% urea–polyacrylamide–formamide gel, and the bands were detected by autoradiography.

Informative cases (i.e. cases occurring when the length of two alleles vary and can be separated by polyacrylamide–gel electrophoresis) were scored as a 'partial loss' were evaluated further by using the public domain of NIH Imagine (100 ng/PCR) in cases with LOH or PCR failure. All results were confirmed by at least two independent observers. Results were verified by independent PCR with increased DNA templates (100 ng/PCR) in cases with LOH or PCR failure.

Allelotyping data from the UK and the US UC for comparison

We used frequently cited data from the UK (7–9) and the US (10,11) consisting of 333 UC in these studies. Among the cases for which tumor stage or grade data were available, the stage distribution consisted of G1 9±44.4%, as shown in Table II. No correlation between LOH of any microsatellite marker/chromosome arm and sex, smoking history, tumor stage or grade. In addition, χ² tests were employed to compare the frequencies of LOH on each chromosome arm between Caucasian patients and Chinese patients, and to compare the stage distribution between Caucasian patients and Chinese patients. χ² tests were performed using the software package Epi Info version 5.0 (Center for Disease Control, Epidemiology Program Office, Atlanta, GA).

### Results

Thirty microsatellite markers on 18 chromosome arms were used in this study. The markers were selected on the basis of their close proximity either to tumor suppressor genes implicated in the development of UC (i.e. P16 at 9p21, RB1 at 13q13) or to minimum regions of deletion mapped in bladder or other tumors. We intended to use the same microsatellite markers as used for the UK and US UC patients (7–10). However, as we have identified previously (5), some of the markers gave very low rate of heterozygosity or unclear distinct patterns among this group of patients and were excluded. Two or three markers were used on 3p, 4q, 9p, 9q, 11q, 17p and 18q to increase the numbers of informative chromosome arms. Informative results were obtained for 89.4% (724/810) of chromosome arms examined. LOH results of each tumor arm were shown in Figure 2. The highest frequency of LOH detected is 9q (54.1%), followed by 17p (51.2%), 9p (48.8%), 18q (42.2%), 4q (41.9%), 16q (33.5%) and 11p (30.0%), as shown in Table II. No correlation between LOH of any microsatellite marker/chromosome arm with sex, smoking history, tumor stage or grade.
Fig. 1. Microsatellite analysis in tumor tissue (T) compared with peripheral blood leukocytes DNA (N). Representative autoradiograms are shown. Heterozygosity or lost alleles are suggested with arrows.

Fig. 2. Patterns of LOH of each microsatellite marker (A) and chromosome arm (B) in 45 bladder tumors. Status of each microsatellite marker or chromosome arm is indicated by shading as LOH (black), retention of heterozygosity (white) and non-informative (gray).
smoking history, tumor stage or grade were found, perhaps due to the relatively small case number. A partial allelotyping for this series of tumors is shown in Figure 3. There is considerable variation in the frequency of LOH in individual tumors. In one case, a pTa–G2 tumor (Case 25, Figure 2) showed retention of heterozygosity at all informative loci examined, whereas a pT4–G3 tumor (Case 19, Figure 2) showed LOH on 10 chromosome arms. In general, high-grade late-stage tumors are prone to show LOH on multiple arms. On chromosome arms where two or more markers were used, the same retention/LOH of the markers was observed in most cases. However, some cases showed different retention/LOH of the markers on the same chromosome arm, suggesting there may be more than one tumor suppressor on the same arm involved in the development of bladder cancer.

As shown in Table III and Figure 3, we compared LOH frequencies of chromosome arms between Chinese bladder tumors and Caucasian bladder tumors from the UK (7–9) and the US (10,11). The P value for difference of grade or stage distribution between Caucasian and Chinese UC is 0.9906 and 0.4165, respectively. Generally, the LOH frequencies on most chromosome arms in our group are higher than that of Caucasian patients (7–11). However, only the differences on 3p, 16q and 18q are statistically significant.

**Discussion**

Tumor incidences, tumor types and genetic abnormalities may be different among different geographic and/or ethnic populations. Different risk factor exposure is believed to contribute to the differences (12,13). However, since environment–genetic interactions play very important roles in the development of tumors, genetic predisposition is now taken as another explanation for the differences. A good example is that the mutation pattern of p53 differs considerably in breast cancers among different ethnic populations (14–17). We implied in our previous studies that the pattern of LOH in bladder cancer in Chinese might be different from that of Caucasian patients (5). Here, we assessed LOH in chromosomal regions known to be involved in the development of UC in the UK (7–9) and the US (10,11). In comparing the LOH frequencies in our study and previous studies of UC in Western countries, our results suggest that there are both consensus and different alterations between Chinese and Caucasian UC. Indeed, the Chinese
tumors showed significant LOH in all chromosomal regions identified previously in Caucasian UC.

The frequency of LOH on 17p almost certainly reflects the known high frequency of involvement of TP53 in these tumors. The involvement of TP53 is associated with high tumor grade and stage, which has been well documented (3). However, in our study, the frequency of LOH of TP53-linked microsatellite marker TP53 is lower than that of D17S695 on 17p13.3 (Table II), suggesting that there are other tumor suppressor genes on 17p besides TP53 involved in the development of bladder cancer.

LOH frequencies at 1p, 2p, 4q, 5p, 5q, 11q, 13q, 14q, 17q, 20p are found relatively low (<20%), similar to those of UC in Caucasian patients (see Table III and Figure 3). The most striking differences between the Chinese bladder tumors and all Caucasian tumors studied previously are in the relative frequencies of involvement of 3p, 16q and 18q (Table III; P<0.01). Frequencies of LOH on these chromosome arms in Chinese bladder tumors are >20% higher than those in Caucasian UC and statistically significant. These results suggest that tumor suppressors on chromosome arms 3p, 16q and 18q could play more important roles in the development of UC in Chinese. The target tumor suppressor genes of these deletions have not yet been identified.

In summary, we demonstrate in this study that Chinese bladder UC share genetic alterations with Caucasian bladder tumors and also possess different genetic abnormalities, which may be contributed by different genetic background and/or environment exposure. None of the tumors in this study are occupation or infection related. Thus, the different genetic abnormalities demonstrated in this study may be attributed to other etiological factors because of differences in dietary habits, social behavior or the genetic background discrepancy between Caucasian and Chinese patients. It is probable that the genetic and etiological discrepancies may exist in other malignancies and diseases and must be considered when exploring genetic alterations of diseases.

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