COMPARISON OF CHROMOSOMAL IMBALANCES DETECTED BY COMPARATIVE GENOMIC HYBRIDIZATION IN ARSENIC-INDUCED AND NON-ARSENIC-INDUCED TRANSITIONAL CELL CARCINOMA

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ABSTRACT

In order to compare the chromosomal imbalances between arsenic-induced and non-arsenic-induced TCC, we analyzed 24 arsenic-induced and 25 non-arsenic-induced TCC by comparative genomic hybridization. More arsenic-induced TCCs had at least one chromosomal imbalance (100%) than non-arsenic- induced TCCs (70%). The number of chromosomal imbalances (mean $\pm$ standard error) was also significantly higher in the arsenic- induced TCCs (5.7 ± 0.6) than non-arsenic-induced TCCs (2.9 ± 0.5). Arsenic exposure is significantly associated with an increased number of chromosomal imbalances after adjustment for cigarette smoking and grade and stage of TCCs in the multiple linear regression analysis. We also found a significantly higher frequency of chromosome gains at 1p12-13, 3q24-25, 4q12-13, 7q112 and 8q22 in arsenic-induced TCCs than in non-arsenic-induced ones. In addition, the frequency of chromosome losses in 16p132-p133 and 17p13 was also significantly higher in arsenic-induced TCCs than non-arsenic-induced ones.

INTRODCUTION

We have reported the dose-response relationship between transitional cell carcinoma (TCC) and ingested arsenic among residents in southwestern and northeastern arseniasis-endemic areas in Taiwan. The relative TCC risks were 1.9, 8.2 and 15.3 for those who drank well water with an arsenic concentration of 10.1-50.0, 50.1-100.0 and >100.0 μg/L, respectively, compared with those who drank well water with an arsenic concentration of $\leq$10 μg/L in the northeastern endemic area.

Inorganic arsenic has been reported to induce sister chromatid exchanges, chromosome abberations, micronuclei and aneuploidy in cultured cells. Genomic instatbility induced by inorganic arsenic may cause the development of various cancers.

The specific aim of this study was to compare chromosomal

imbalances between arsenic-induced and non-arsenic-induced TCCs by comparative genomic hybridization.

MATERIALS AND METHODS

Patients, Tumor Tissues and Risk Factors Exposure

Patients were recruited from Chi-Mei Hospital in Tainan County from 1998-1999. Specimens of transitional cell carcinomas were obtained from 49 patients affected with cancers of the urinary bladder, ureter, and renal pelvis. Grades and stages of the tumors were assessed according to World Health Organization and TNM classifications. Blood samples were also collected from TCC patients to derive reference DNA for CGH analysis.

The information on the exposures to ingested high-arsenic well water, cigarette smoking, and other risk factors was obtained from the standardized interview based on a structured questionnaire. The TCCs were considered related to arsenic exposure from whom having lived in the arseniasis-endemic area and consumed high-arsenic artesian well water for more than 10 years. Other TCCs were classified as the non-arsenic-related.

DNA Preparation

Control genomic DNA samples were prepared from the blood of each patient using TALENT genomic DNA Extraction kit (TALENT, Triste, Italy). DNA was extracted from each TCC tissue using a QIAmp Blood Kits (QIAGEN).

CGH Analysis

Control and tumor DNA were labeled with Spectrum Red dUTP or Spectrum Green dUTP by the CGH nick translation kit (Vysis, Illinois, USA). The DNase and DNA polymerase I concentration in the labeling solution was adjusted to reveal an average fragment size of 300-3000bp. A total of 200 ng Spectrum Green-labeled probe, 100 ng of Spectrum Red-labeled probe, and 10 μg of the unlabeled Cot-1 DNA were precipitated with ethanol. The DNAs were dissolved in 10 μL hybridization buffer, denatured at 70°C for 5 min and hybridized to denatured normal metaphase spreads.

The slides were washed after a 3 day hybridization. Chromosomes were counterstained with 4’,6-diamidino-2- phenylinodole (DAPI) in an antifading solution. The results were evaluated using a digital image analysis system based on a fluorescence microscope and charge-coupled camera (Photometrics, Tucson, Arizona, USA) interfaced to a Quips XL workstation. The mean green/red ratio of 1.5 and 0,75 was used as cut-off levels for gains and loses, respectively.

Statistical Analysis

All results were analyzed by χ^2 tests or multiple linear regression

analysis. P values <0.05 were considered statistically significant.

RESULTS

The mean age was similar in patients affected with arsenic-related and non-arsenic-related TCC. There were more males and cigarette smokers among arsenic-related TCC patients than non-arsenic- related TC patients. Most arsenic-related and non-arsenic-related TCCs were at grade 2 and stage 2 (Table 1).

There were less DNA losses and gains in non-arsenic- related TCCs (Table 2) than in arsenic-related TCCs (Table 3). More arsenic- induced TCCs had at least one chromosomal imbalance (100%) than non-arsenic- induced TCCs (70%). The number of chromosomal imbalances (mean $\pm$ standard error) was also significantly higher in the arsenic- induced TCCs (5.7 ± 0.6) than non-arsenic-induced TCCs (2.9 ± 0.5).

A significantly higher frequency of chromosome gains at 1p12-13, 3q24-25, 4q12-13, 7q112 and 8q22 in arsenic-induced TCCs than in non-arsenic-induced ones. In addition, the frequency of chromosome losses in 16p132-p133 and 17p13 was also significantly higher in arsenic-induced TCCs than non-arsenic-induced ones (Table 4).

Arsenic exposure is significantly associated with an increased number of chromosomal imbalances after adjustment for cigarette smoking and grade and stage of TCCs in the multiple linear regression analysis (Table 5).

Table 1. Demographical and histopathological characteristics of patients affected with arsenic-related and non-arsenic-related transitional cell carcinomas

Variables	Arsenic-related	Non-arsenic-related
Age		
<50	2 (9.1%)	3 (12.5%)
50-74	17 (77.3%)	19 (79.2%)
75+	3 (13.6%)	2 (8.3%)
Mean±SD	65.0±9.8	63.2±10.8
Gender		
Male	17 (70.8%)	14 (56.0%)
Female	7 (29.2%)	11 (44.0%)
Cigarette smoking		
No	11 (45.8%)	15 (60.0%)
Yes	13 (54.2%)	10 (40.0%)
Tumor grade		
1	1 (4.2%)	0 (0%)
2	12 (50.0%)	17 (68.0%)
3	7 (29.2%)	7 (28.0%)
4	3 (12.5%)	1 (4.0%)
Unknown	1 (4.2%)	0 (0%)
Tumor stage		
a	1 (4.2%)	3 (12.0%)
1	8 (33.3%)	4 (16.0%)
2	10 (41.7%)	12 (48.0%)
3	2 (8.3%)	5 (20.0%)
4	0 (0%)	1 (4.0%)
Unknown	3(12.5%)	0 (0%)

Table 2. Age, gender, tumor stage and grade, DNA losses and gains of 25 patients affected with non-arsenic-related transitional cell carcinomas

Record No.	Age	Gender	Grade	Stage	DNA losses	DNA gains
CM1011	76	F	3	1	2q, 4q32-qter, 5q21-q23, 9q31-q33, 17p	—
CM1013	?	M	2	2a	1q42-q44, 8q, 9p21-p23, 9q34, 17q24-q25, 20p112	6q25-q27, 8p
CM1014	67	M	3	2a	—	9q13-q21
CM1026	56	F	2	2a	8p21-p23, 15q112-q14	1q21-q23, 3, 7q112, 20q
CM1031	31	M	2	a	8q, 10q	9, 11p112-p13, 18
CM1033	72	F	2	3a	2q36-q37, 9q34	4q28
CM1043	77	M	2	a	—	—
CM1058	58	F	3	3a	—	8q12-22, 11q12-q14
CM1059	57	F	2	1	—	—
CM1064	49	M	2	2a	9q, 10q	—
CM1067	64	M	3	a/1	—	1p12-p21, 9p23-p24
CM1076	56	M	2	3,a,x	8q21-q24, 9q34, 11q24-q25, 13q12-q14	—
CM1080	73	F	2	2b	9p	1q21-q31, 2q23-q24, 7q112, 12q12-q13
CM1083	72	M	2	1	—	—
CM1085	67	M	2	2a	—	7q35-q36, 16q23-q24
CM1096	63	F	3	2	2q36-qter, 10q24-q25, 13q32-qter	1p,3p12, 17p112-p12
CM1106	66	M	2	2a	9p13-p21, 9q31-q34	21q
CM1107	57	F	2	2a	—	—
CM1113	70	M	2	3a	3q27-q29	15q15-q21
CM1115	45	F	2	2	17p13	5pter-q13
CM1119	69	F	4	4	1q42-q44,	1p12-p13,

					9q32-qter, 10q22-q23, 21q22	5q112-q12
CM1134	71	M	3	2a	—	—
CM1142	60	M	2	1	—	—
CM1143	70	F	3	3	—	—
CM1144	71	M	3	2b	1p21-p32, 3q	5q, 12p12-p13

Table 3. Age, gender, tumor stage and grade, DNA losses and gains of 24 patients affected with arsenic-related transitional cell carcinomas

Record No	Age	Gender	Grade	Stage	DNA losses	DNA gains
10043	77	F	—	—	17q25	1p12-p21
10049	66	M	2	—	20q112-q12	1p12-p13, 4q12-q13, 5q112-q21
CM1007	61	F	2	2a	3q, 5q15-q33, 6q15-qter, 8p21-pter, 10q, 13q	5p, 16q121-q122
CM1016	48	F	2	3a	2q, 8q112-q12, 9q31, 10, 13q12-q14, 15q15-q22, 17p	3p22-pter
CM1020	70	M	2	a	2p-q143, 9, 13q21-q32, 18q21-qter	3p, 4,8q
CM1021	46	F	3	3a	8p22-pter, 9q34	1q, 3q24-q263, 10q26, 12p
CM1024	69	M	3	2a	1q41-q44, 16p132-p133	1p, 5q112-q13, 6q12-q14
CM1030	68	M	2	1	9q34, 16p132-p133	—
CM1035	76	M	3	2	9q13-q21	7p112-p13
CM1071	52	M	2	1	—	4q12-q21
CM1093	65	M	2	2	5q34-q35, 9q13-q22, 16p131-pter, 17p12-p13	1q31-q32, 4p, 4q12-q22, 10p, 11q
CM1101	51	M	2	2a	2q37, 5p15, 10q25-q26, 14q31-q32	1p12-p13, 7q112, 11q12-q13, 16p, 20q112-q12
CM1120	68	M	4	1	4p16, 21q22	2p23-pter
CM1123	69	M	3	—	5p15, 8p23, 9q34, 10q25-q26, 17p13	1p13-p22, 3, 4q27-32, 9p
CM1131	63	F	3	1	9p23, 17p13	3q, 5p12-p14, 8q
CM1135	67	M	2	1	1p12-p13, 9p13-p21	4p152-p16, 5p, 8q13-qter, 12q21-q22, 16q121-q122

CM1139	72	F	1	1	10p15, 11p15	—
CM1153	72	M	2	2a	9p23-p24, 17p12-pter	1q21-q22, 7q112, 8q
CM1154	79	M	3	2,2	9p, 10q26, 11p15, 16, 17p13	1p12-p31
CM1159	84	M	poorly	—	16q24, 17p12-pter	1p12-p13, 4q12-q21
CM1160	59	M	poorly	1	5q33-q35, 10q25-q26, 11p15, 16p131-pter, 17p13	3q133-q25, 7q112
CM1163	64	M	2	2	15q	1p12-p13, 5q112, 7q112
CM1169	69	F	3	2	9, 10q25-q26, 17p13	3q, 8q22-qter, 10p13-pter, 14q21-q23, 18pter-18q12
CM1191	57	M	2	2	16p13, 17p12-pter, 20q132-q133	4q26-q28, 7p, 7q112

Table 4. Frequent DNA losses and gains detected by CGH in arsenic-related and non-arsenic-related transitional cell carcinomas

Chromosome	Minimal overlapping regions	Arsenic-related (n=24)	Non-arsenic-related (n=25)	P-value
DNA gains				
1p	1p12-13	33.3%	12.0%	0.07
3q	3q24-25	20.8%	4.0%	0.10
4p	4p152-pter	12.5%	0%	0.11
4q	4q12-13	20.8%	0%	0.02
	4q27-28	12.5%	4.0%	0.35
7q	7q112	20.8%	4.0%	0.10
8q	8q22	20.8%	4.0%	0.10
DNA losses				
9p	9p23-24	25.0%	16.0%	0.50
9q	9q13-21	16.7%	4.0%	0.19
	9q34	25.0%	24.0%	1.00
10q	10q26	29.2%	12.0%	0.17
11p	11p15	12.5%	0%	0.11
16p	16p132-p133	25.0%	0%	0.01
17p	17p13	41.7%	8.0%	<0.01

Table 5. Linear regression analysis of the association with the number of DNA losses and gains for arsenic exposure, tumor stage, tumor grade and cigarette smoking

Variable (comparison)	β	Standard error	P value
Arsenic exposure（Yes vs. No）	3.39	0.84	<0.001
Tumor stage（2+ vs.1/a）	1.34	0.86	0.13
Tumor grade（3+ vs.1/2）	-0.24	0.89	0.79
Cigarette smoking（Yes vs. No）	0.02	0.87	0.98

COMPARISON OF LOSS OF HETEROZYGOSITY ON CHROMOSOMES 15 AND 16 IN ARSENIC-INDUCED AND NON-ARSENIC-INDUCED TRANSITIONAL CELL CARCINOMAS

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ABSTRACT

Transitional cell carcinoma (TCC) has been documented to be associated with ingested arsenic among residents in southwestern and northeastern arseniasis-endemic areas in Taiwan. In order to compare the chromosomal deletions between arsenic-induced and non-arsenic-induced TCCs, we analyzed 26 arsenic-induced and 28 non-arsenic-induced TCC by analysis of loss heterozygosity (LOH) on chromosome 15 and 16. More arsenic-induced TCCs had at least one LOH than non-arsenic-induced TCCs. The number of LOH was also significantly higher in the arsenic-induced TCCs than non-arsenic-induced TCCs. A higher frequency of LOH was observed at D15S205, D15S994, D16S3046, D16S3075, S16S515, D16S516 and D16S3091 in arsenic-induced TCCs than in non-arsenic-induced ones. In addition, a higher frequency of LOH was observed at D15S1002, D16S3103 and D16S415 in non-arsenic-induced TCCs than in arsenic-induced ones.

MATERIALS AND METHODS

Tumor and Normal DNA samples

TCCs were obtained by transurethral resection and stored at -80℃. Blood samples were obtained from patients to derive normal DNA samples. Peripheral blood was collected from each subject into an EDTA tube for the isolation of buffy coat and plasma, which were stored at -80 ℃ until analysis. DNA was extracted from buffy coat using a QIAmp Blood Kits (QIAGEN).

Polymerase Chain Reaction (PCR)-based Amplification of Microsatellite Markers

PCR reactions were performed in a total volume of 30 uL containing 50 ng of the extracted DNA, 50 mM potassium chloride, 10 mM Tris-HCl (pH=8.0), 5 mM magnesium chloride,.2.5 mM each dNTPs, 0.3 pmol primer of Linkage Mapping Set-MD10 kits (ABI, Prism), and 0.5 U Gold Taq. The DNA amplification was performed as follows; initial denaturatin at 94℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 40 s, annealing at 55℃ 30 s, with an extension at 72℃ 40s and a final extension at 72℃ for 10 min. The PCR products were analyzed by electropheresis on a 2% agar gel stained with ethidium bromide.

Analysis of LOH

The LOH repeat region resides in the first exon of the gene. A system was established to rapidly analyze the LOH repeat sequence length in a large number of samples. We constructed a set of kits (ABI Prims, Linkage Mapping Set-MD10). For rapid and accurate assessment of fragment length, the DNA fragments were run on a 6% denaturing

polyacrylamide gel by automated fluorescence detection (Genescan; Applied Biosystems 377).

RESULTS

The frequency of LOH at chromosomes 15 and 16 in all 54 TCCs ranged 10% at D16S404 to 56% at D16S515 (Table 1). More arsenic-induced TCCs had at least one LOH than non-arsenic-induced TCCs. The number of LOH was also significantly higher in the arsenic-induced TCCs than non-arsenic-induced TCCs.

As shown in Tables 2 to 3 and Figures, a higher frequency of LOH was observed at D15S205, D15S994, D16S3046, D16S3075, S16S515, D16S516 and D16S3091 in arsenic- induced TCCs than in non-arsenic-induced ones. In addition, a higher frequency of LOH was observed at D15S1002, D16S3103 and D16S415 in non-arsenic-induced TCCs than in arsenic-induced ones.

Discussion

The findings of a high frequency (30% or higher) of LOH at chromosome 15 and 16 in TCCs are consistent with those reported in previous studies. More over, we also found a higher frequency of LOH in arsenic-induced TCCs than in non-arsenic- induced ones, especially at chromosome 16. The striking difference in LOH frequency was found to cluster at D15S205(35%>17%) and D15S127(39%>25%) on chromosome 15, and D16S515 (75%>47%), D16S516 (60%>25%), D16S3091 (71%>36%) on chromosome 16. The markers D16S515、D16S516 and D16S3091 are in a region about 20cM, which indicate that there may be some gene associated with the mechanism of arsenic-induced TCCs deserving further investigation.

Table 1. Frequency of LOH of genetic markers on chromosomes 15 and 16 in all transitional cell carcinomas

Marker	Total	NI	MSI	H	LOH	LOH %
Chromosome 15						
D15S128	**38**	**9**	**3**	**26**	**5**	**19%**
D15S1002	**54**	**14**	**0**	**40**	**9**	**23%**
D15S165	**44**	**37**	**0**	**7**	**2**	**29%**
D15S1007	**48**	**12**	**8**	**28**	**7**	**25%**
D15S1012	**39**	**9**	**1**	**29**	**8**	**28%**
D15S994	**49**	**16**	**1**	**32**	**9**	**28%**
D15S978	**35**	**10**	**0**	**25**	**7**	**28%**
D15S117	**45**	**17**	**0**	**28**	**13**	**46%**
D15S153	**38**	**8**	**2**	**28**	**6**	**21%**
D15S131	**51**	**20**	**0**	**31**	**9**	**29%**
D15S205	**52**	**7**	**0**	**45**	**12**	**27%**
D15S127	**47**	**6**	**3**	**38**	**12**	**32%**
D15S130	**53**	**9**	**1**	**32**	**11**	**26%**
D15S120	**35**	**13**	**3**	**19**	**6**	**32%**
Chromosome 16						
D16S423	**29**	**1**	**2**	**26**	**8**	**31%**
D16S404	**42**	**13**	**0**	**29**	**3**	**10%**
D16S3075	**50**	**5**	**1**	**44**	**13**	**30%**
D16S3103	**50**	**29**	**4**	**17**	**3**	**18%**
D16S3046	**50**	**17**	**4**	**29**	**6**	**21%**
D16S3068	**51**	**11**	**1**	**39**	**9**	**23%**
D16S3136	**49**	**18**	**0**	**31**	**6**	**19%**
D16S415	**48**	**20**	**1**	**27**	**12**	**44%**
D16S503	**45**	**24**	**1**	**20**	**8**	**40%**
D16S515	**52**	**16**	**2**	**34**	**19**	**56%**
D16S516	**30**	**8**	**0**	**22**	**9**	**41%**
D16S3091	**41**	**14**	**1**	**26**	**11**	**42%**
D16S520	**49**	**2**	**0**	**47**	**15**	**32%**

NI: non-informative; MSI: Microsatellite instatbility; H: heterozygosity

Table 2. Frequency of LOH of genetic markers on chromosomes 15 in arsenic-related and non-arsenic-related transitional cell carcinomas

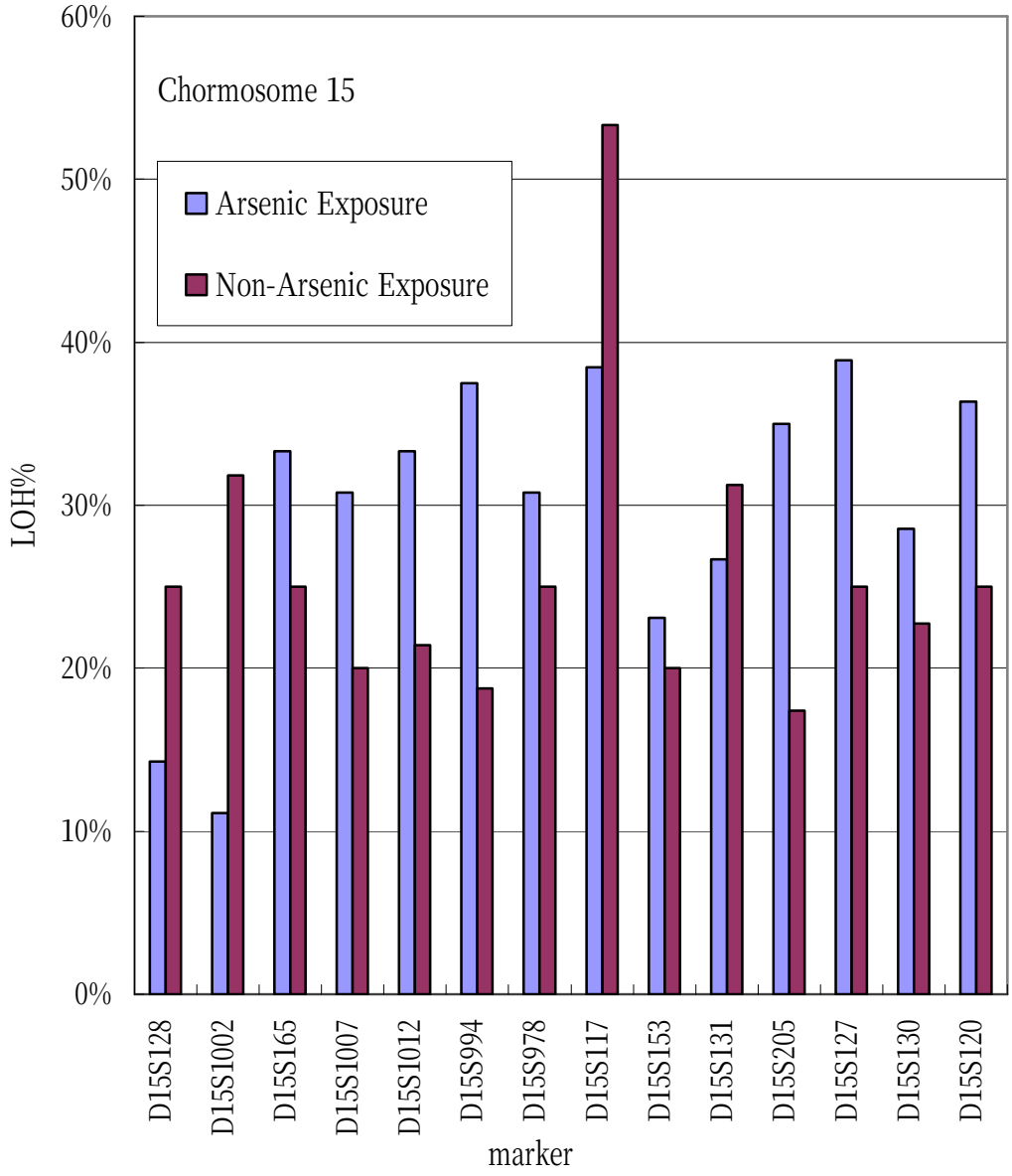
Markers	**Arsenic-related TCCs**					
	Total	**NI**	**MSI**	**H**	**LOH**	**LOH %**
D15S128	**21**	**5**	**2**	**14**	**2**	**14%**
D15S1002	**26**	**8**	**0**	**18**	**2**	**11%**
D15S165	**21**	**18**	**0**	**3**	**1**	**33%**
D15S1007	**24**	**6**	**5**	**13**	**4**	**31%**
D15S1012	**20**	**5**	**0**	**15**	**5**	**33%**
D15S994	**23**	**7**	**0**	**16**	**6**	**38%**
D15S978	**17**	**4**	**0**	**13**	**4**	**31%**
D15S117	**23**	**10**	**0**	**13**	**5**	**38%**
D15S153	**20**	**6**	**1**	**13**	**3**	**23%**
D15S131	**25**	**10**	**0**	**15**	**4**	**27%**
D15S205	**24**	**4**	**0**	**20**	**7**	**35%**
D15S127	**23**	**4**	**1**	**18**	**7**	**39%**
D15S130	**25**	**3**	**1**	**21**	**6**	**29%**
D15S120	**20**	**7**	**2**	**11**	**4**	**36%**
Markers	**Non-arsenic-related TCCs**					
	Total	**NI**	**MSI**	**H**	**LOH**	**LOH %**
D15S128	**17**	**4**	**1**	**12**	**3**	**25%**
D15S1002	**28**	**6**	**0**	**22**	**7**	**32%**
D15S165	**23**	**19**	**0**	**4**	**1**	**25%**
D15S1007	**24**	**6**	**5**	**15**	**3**	**20%**
D15S1012	**19**	**4**	**1**	**14**	**3**	**21%**
D15S994	**26**	**9**	**1**	**16**	**3**	**19%**
D15S978	**18**	**6**	**0**	**12**	**3**	**25%**
D15S117	**22**	**7**	**0**	**15**	**8**	**53%**
D15S153	**18**	**2**	**1**	**15**	**3**	**20%**
D15S131	**26**	**10**	**0**	**16**	**5**	**31%**
D15S205	**26**	**3**	**0**	**23**	**4**	**17%**
D15S127	**24**	**2**	**2**	**20**	**5**	**25%**
D15S130	**28**	**6**	**0**	**22**	**5**	**23%**
D15S120	**15**	**6**	**1**	**8**	**2**	**25%**

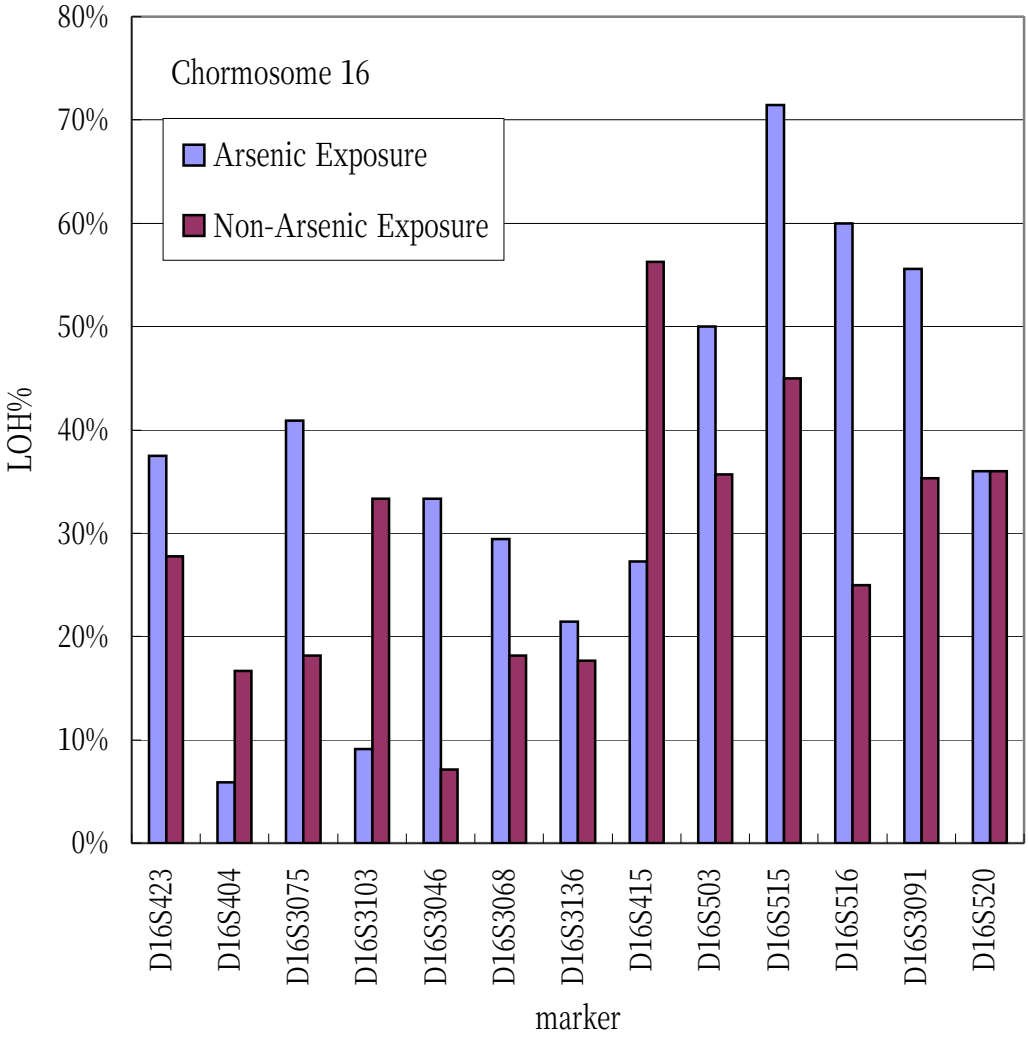
NI: non-informative; MSI: Microsatellite instatbility; H: heterozygosity

Table 3. Frequency of LOH of genetic markers on chromosomes **16** in arsenic-related and non-arsenic-related transitional cell carcinomas

Markers	Arsenic-related TCCs					
	Total	NI	MSI	H	LOH	LOH %
D16S423	10	1	1	8	3	38%
D16S404	23	6	0	17	1	6%
D16S3075	25	3	0	22	9	41%
D16S3103	23	12	0	11	1	9%
D16S3046	25	8	2	15	5	33%
D16S3068	24	6	1	17	5	29%
D16S3136	24	10	0	14	3	21%
D16S415	23	11	1	11	3	27%
D16S503	22	16	0	6	3	50%
D16S515	26	10	2	14	10	71%
D16S516	13	3	0	10	6	60%
D16S3091	19	9	1	10	5	56%
D16S520	23	1	0	22	9	41%
Markers	Non-arsenic-related TCCs					
	Total	NI	MSI	H	LOH	LOH %
D16S423	19	0	1	18	5	28%
D16S404	19	7	0	12	2	17%
D16S3075	25	2	1	22	4	18%
D16S3103	27	17	4	6	2	33%
D16S3046	25	9	2	14	1	7%
D16S3068	27	5	0	22	4	18%
D16S3136	25	8	0	17	3	18%
D16S415	25	9	0	16	9	56%
D16S503	23	8	1	14	5	36%
D16S515	26	6	0	20	9	45%
D16S516	17	5	0	12	3	25%
D16S3091	22	5	0	17	6	35%
D16S520	26	1	0	25	9	36%

NI: non-informative; MSI: Microsatellite instatbility; H: heterozygosity


Chormosome 15
Arsenic Exposure
Non-Arsenic Exposure
LOH%
60%
50%
40%
30%
20%
10%
0%
D15S128
D15S1002
D15S165
D15S1007
D15S1012
D15S994
D15S978
D15S117
D15S153
D15S131
D15S205
D15S127
D15S130
D15S120
marker


Chormosome 16
Arsenic Exposure
Non-Arsenic Exposure
LOH%
80%
70%
60%
50%
40%
30%
20%
10%
0%
D16S423
D16S404
D16S3075
D16S3103
D16S3046
D16S3068
D16S3136
D16S415
D16S503
D16S515
D16S516
D16S3091
D16S520
marker

COMPARISON OF MUTATION SPECTRA OF *p53* GENE IN ARSENIC-RELATED AND NON-ARSENIC-RELATED TRANSITIONAL CELL CARCINOMAS

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Abstract

The dose-response relationship between transitional cell carcinoma (TCC) and ingested arsenic has been observed in arseniasis-endemic areas of southwestern and northeastern Taiwan. We carried out this study to compare the mutation spectra of *p53* gene between arsenic-related and non-arsenic-related transitional cell carcinomas.

A total of 114 TCCs were collected from 114 affected patients in 1998-2000. The sequences of exons 5 to 8 of *p53* gene were determined by polymerase chain reaction (PCR)-based direct DNA sequencing. The history of arsenic exposure through drinking well water, cigarette smoking, and exposures to other risk factors were obtained through standardized interview based on a structured questionnaire.

The *p53* gene mutation was observed in 41 (36%) of 114 tumors. The higher the grade or stage of the tumor, the more frequent the *p53* mutations. The mutation frequency was 44% for arsenic-related TCC and 30% for non-arsenic-related TCCs showing an odds ratio of 1.9 (95% confidence interval 0.8-4.9) after adjustment for age, gender, cigarette smoking, stage and grade. The longer the duration of consuming high-arsenic artesian well water, the higher the mutation frequency (trend test P=0.07).

Most frequent mutation type was G:C→A:T transition for both arsenic-related and non-arsenic-realted TCCs. The major mutation location were exons 8 (36%) and 6 (36%) for arsenic-related TCCs,

and exon 5 (34.8%) for non-arsenic-related TCCs. While only 9% of point mutations in arsenic-related TCCs were at CpG sites, 21% point mutations in non-arsenic-related TCCs were at CpG sites. The mutation hot spots were at codons 208, 280 and 285 for arsenic-related TCCs; and at codons 179 and 273 for non-arsenic-related TCCs.

Materials and Methods

Patients, Tumor Tissues and Risk Factors Exposure

Specimens of transitional cell carcinomas were obtained from 114 patients affected with cancers of the urinary bladder, ureter, and renal pelvis from 1998 to 2000. Grades and stages of the tumors were assessed according to World Health Organization（1）and TNM （2） classifications. The information on the exposures to ingested high-arsenic well water, cigarette smoking, and other risk factors was obtained from the standardized interview based on a structured questionnaire.

Detection of p53 mutation: Direct Sequencing

Mutation of *p53* gene from exons 5 to 8 was screened using PCR-based direct DNA sequencing. Exons 5-8 of the *p53* gene were individually amplified by PCR. All the PCR products were purified using Microcon-PCR Filter Unit (Millipore, U.S.A.), and then directly sequenced using BigDye™ Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems). The sequencing products were electrophoresed on 5％Long Ranger gel. The original data were analyzed by ABI prism DNA sequence analysis software (version 3.4.1). All PCR fragments were sequenced on both forward and reverse strands to exclude the possibility of cross-contamination.

Statistical Analysis

All results were analyzed by χ^2 tests or multiple logistic regression analysis.

P values <0.05 were considered statistically significant.

Results

Arsenic exposure was associated with an increased *p53* mutations in TCCs showing an OR of 1.9; and the longer the duration of consuming high-arsenic artesian well water, the higher the *p53* mutation frequency in TCCs (Table 1).
As shown in Table 2, the mean age of patients affected with arsenic-related TCCs was older than those with non-arsenic-related TCCs. The frequency distributions of gender, cigarette smoking and cancer site were similar in both groups. The exposure to TCC carcinogens was higher in arsenic-related TCC patients than non-arsenic-related ones. The stage and grade of the arsenic- related TCCs were more advanced than non-arsenic-related TCCs.

The more advanced the grade and stage, the higher the *p53* mutation frequency (Table 3). The OR of having *p53* mutation was 3.7 for the grade 3 TCCs than the grade 1 and grade 2 TCCs. There was a significant trend of *p53* mutation by stage (P=0.02) after adjustment for age and gender.

There was some difference in mutation site between arsenic-related and non-arsenic-related TCCs (Table 4). The frequent mutation sites were exons 6 and 8 for arsenic-related TCC, and exon 5 for non-arsenic-related TCCs. The majority of the mutation type was G：C→A：T transition for both types of TCC. There were less deletion mutations and CpG site mutations in the arsenic-related TCCs than non-arsenic-related TCCs.

Figure 1 shows the distribution of the mutation codons in the *p53* of arsenic-related and non-arsenic-related TCCs. The most frequent mutation hot spots were codons 208, 280 and 285 in arsenic-related TCCs, and codons 179 and 273 in non-arsenic-related TCCs.

Table 1. *p53* mutations in transitional cell carcinoma by exposure to arsenic through ingestion of artesian well water

Variable	Mutation		Crude odds ratio (95%CI)	P-value	Adjusted odds ratio (95%CI) *	P-value
	Yes	No				
Arsenic Exposure						
No	**19**	**45**	**1**		**1**	
Yes	**22**	**28**	**1.9（0.9-4.1）**	**0.12**	**1.9（0.8-4.9）**	**0.16**
Duration of consuming high-arsenic artesian well water (Year)						
0	**19**	**45**	**1**		**1**	
1-29	**6**	**11**	**1.3（0.4-3.9）**	**0.66**	**1.1（0.3-3.7）**	**0.94**
≥30	**16**	**17**	**2.2（0.9-5.4）**	**0.07**	**2.8（0.9-8.5）**	**0.05**
			P for trend 0.07		**P for trend 0.07**	

***Adjusted for stage, grade, cigarette smoking, gender and age.**

Table 2. Comparison of epidemiological and pathological characteristics between arsenic-related and non-arsenic-related transitional cell carcinoma

Variable		Arsenic-related TCCs (n=50)	Non-Arsenic-related TCCs (n=64)
		NO.（%）	NO.（%）
Gender	Male	29(58.0)	39(60.9)
	Female	21(42.0)	25(39.1)
Age*	30-59	10(20.0)	20(31.3)
	60-69	20(40.0)	21(32.8)
	≧70	20(40.0)	23(35.8)
Cigarette smoking[a]	No	30(60.0)	35(54.7)
	Yes	20(40.0)	29(45.3)
Carcinogen exposure[b]	No	19(38.0)	32(50.0)
	Yes	31(62.0)	32(50.0)
Cancer site	Renal pelvis	11(23.4)	12(18.8)
	Bladder	32(68.1)	43(67.2)
	Ureter	4(8.5)	9(14.1)
Grade	1	2(4.2)	1(1.6)
	2	25(52.1)	38(62.3)
	3	21(43.8)	22(36.1)
Stage	0a	4(9.3)	13(22.0)
	I	18(41.9)	16(27.1)
	II	13(30.2)	21(35.6)
	III	8(18.7)	9(15.3)

*Mean age (±standard deviation) was 67.5±9.2 years for arsenic-related TCCs and 63.9±11.4 years for non-arsenic-related TCCs

[a]Mean duration of cigarette smoking (±standard deviation) was 38.2±12.6 years for arsenic-related TCCs and 37.7±14.6 years for non-arsenic-related TCCs

[b]History of exposures to hair dyes, paints, pesticides, analgesic, and/or anti-inflammatory drugs.

Table 3. Association between *p53* mutation and stage and grade of transitional cell carcinoma

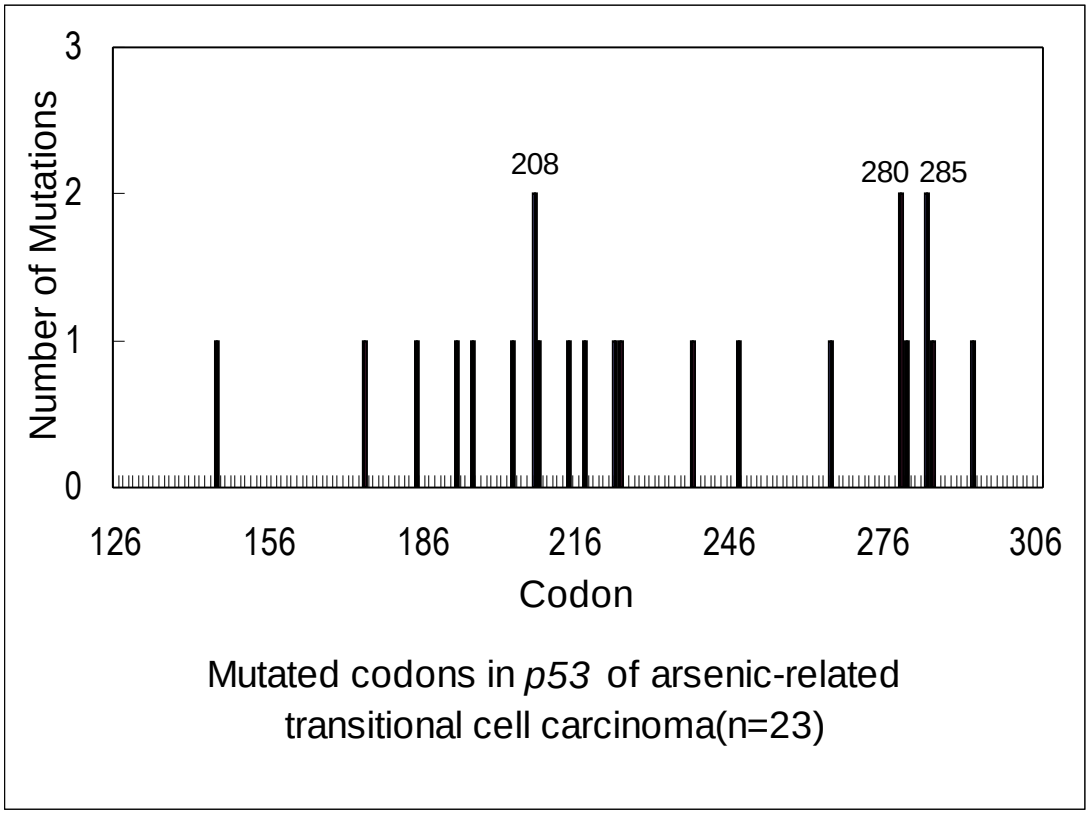
Variable	Mutation		Crude	P-value	Adjust	P-value
	Yes	No	OR （95%CI）		OR （95%CI） *	
Grade						
1+2	18	51	1		1	
3	21	19	3.1（1.4-7.2）	0.006	3.7（1.6-9.1）	0.003
Missing	2	3				
Stage						
0a+Ⅰ	13	38	1		1	
Ⅱ	14	20	2.1（0.8-5.3）	0.13	2.0（0.8-5.2）	0.16
Ⅲ+Ⅳ	10	7	4.2（1.3-13.8）	0.02	4.1（1.3-14.1）	0.02
Missing	4	8				
			P for trend＝0.01		P for trend＝0.02	

*Adjusted for gender and age.

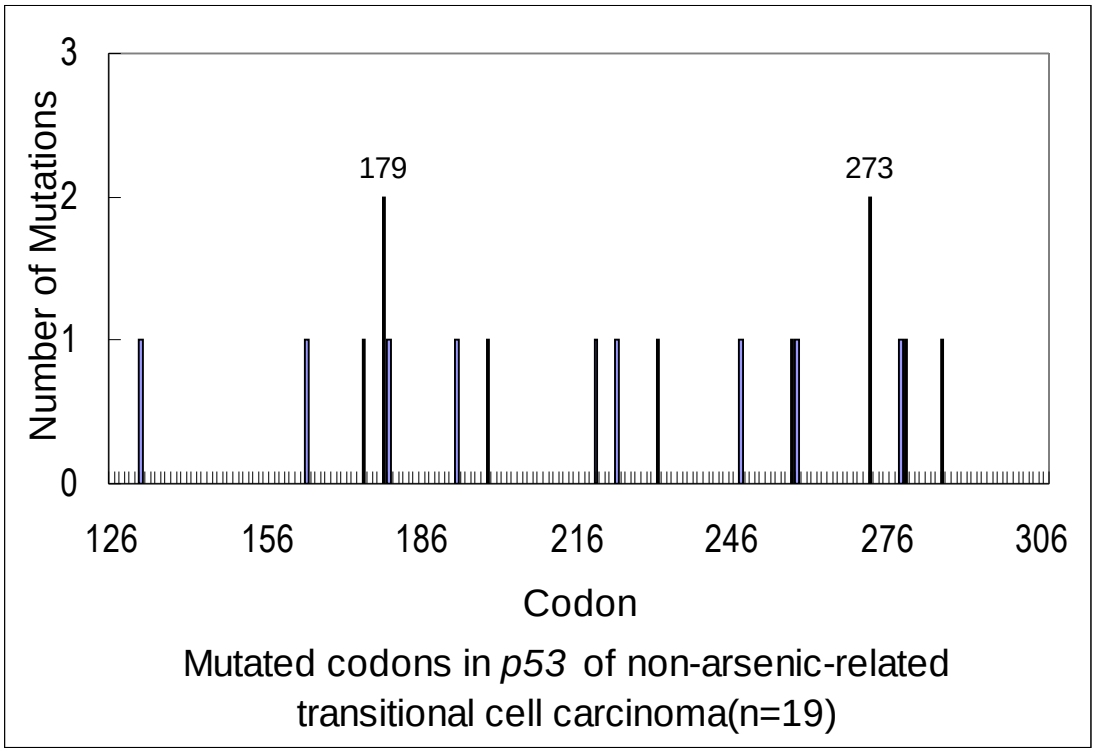
Table 4. Comparison of *p53* mutation spectra between arsenic- related and non-arsenic-related transitional cell carcinoma

Variable	Arsenic-related TCC（n=25）	Non-Arsenic related TCC（n=23）
	NO.(%)	NO.(%)
***p53* mutation site**		
exon 5	3(12.0)	8(34.8)
exon 6	9(36.0)	4(17.4)
exon 7	4(16.0)	5(21.7)
exon 8	9(36.0)	6(26.1)
***p53* mutation type**		
Transition	14(56.0)	9(39.1)
A：T→G：C	3(12.0)	2(8.7)
G：C→A：T	11(44.0)	7(30.4)
Transversion	9(36.0)	10(43.5)
A：T→T：A	5(20.0)	6(26.1)
G：C→C：G	0(0.0)	3(13.1)
G：C→T：A	4(16.0)	1(4.3)
Deletion	2(8.0)	4(17.4)
CpG site mutation*	2(8.7)	4(21.0)

*point mutations only



Mutated codons in *p53* of arsenic-related transitional cell carcinoma(n=23)



Mutated codons in *p53* of non-arsenic-related transitional cell carcinoma(n=19)

Figure 1. Distribution of mutation codons in *p53* gene of arsenic-related and non-arsenic-related transitional cell carcinomas

Conclusion

(1) Increased *p53* mutation was associated with the tumor grade and stage.

(2) Arsenic-related and non-arsenic-related TCCs had different *p53* mutation location, frequency and hot spots.

(3) The lower mutation frequency of CpG sites suggested that the *p53* mutation in arsenic-related TCCs may not be associated with endogenous carcinogens.