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Comparison of Genetic Changes in Transitional and Squamous Bilharzial-Related Bladder Cancers Using Fluorescence *in situ* Hybridization

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Abstract: Two types of bladder cancer, Squamous Cell (SC) and Transitional Cell (TC) differ in their histopathology, clinical outcome and etiology. Therefore, the underlying genetic effects of these two types of tumor may also be different. We compared numerical aberrations of different chromosomes in bilharzial-associated squamous and transitional cell carcinoma of the bladder and correlated the findings to p53 gene amplification. Cystectomy for invasive bladder was performed in 35 men and 15 women with a mean age of 54.6 years (range 28 to 82). Of these patients 33% had histologically verified squamous cell carcinoma and 17% had transitional cell carcinoma. We used fluorescence *in situ* hybridization to evaluate the numerical aberrations of chromosomes 7, 9 and 17 and alterations in p53. Present results demonstrated that aberrations of chromosome 7 were observed in 75% of the squamous cell carcinoma and in 93% of transitional cell carcinoma. Aberrations of chromosome 9 were also observed in 90% of squamous cell carcinoma, however, they were seen only in 51% of transitional cell carcinoma. Aberrations of chromosome 17 were detected in only 25% of squamous cell carcinoma, compared to 82% in transitional cell carcinoma. The p53 gene amplification was similar in both types with 84% for squamous cell carcinoma and 73% for transitional cell carcinoma. Our data clearly show difference between chromosomal patterns of invasive bilharzial squamous cell carcinoma and transitional cell carcinoma. However, overexpression of p53 in both types was similar. Additionally, aberrations of chromosome 9 were observed in both types, which confirm the 2 pathways in the oncogenesis of squamous cell and transitional cell carcinoma.

Key words: Genetics, bladder cancer, squamous cell carcinoma, transitional cell carcinoma, *in situ* hybridization, schistosoma

INTRODUCTION

In Western countries, more than 90% of primary bladder carcinomas are Transitional Cell Carcinoma (TCC), whereas, Squamous Cell Carcinoma (SCC) comprises less than 10% (Gibas and Gibas, 1997). Carcinoma of the urinary bladder is the most common malignancy in many tropical and subtropical countries due to endemic infection by *Schistosoma haematobium*, causing a disease most commonly known as bilharzia. Bilharzial-related bladder carcinoma is a major oncologic problem in

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Egypt and many parts of the Middle East and Africa. Egypt represents a hyperendemic area of *S. haematobium* infection, with an overall prevalence of ~50%. In Egypt, the frequency of bilharzial-related bladder carcinoma accounts for 31% of all types of cancer (39 and 11% of cancers in males and females, respectively) (World Health Organization, 1985, 1993).

Compared with nonbilharzial-related bladder cancer, *S. haematobium*-related bladder cancer has different clinical and pathological features. In contrast to Western countries, more than two-third of bladder cancer in Egypt is SCC with a peak incidence at around 50 years of age (El-Bolkainy *et al.*, 1972, 1981; Dean *et al.*, 1954; Mostofi *et al.*, 1988).

Most genetic studies focus on transitional cell cancer and chromosomal aberrations and mutations of tumor suppressor genes, known to have an important role in the pathogenesis of TCC (Sandberg and Berger, 1994). Partial deletions of chromosome 9 and numerous aberrations of chromosome 17 were defined as the most important alterations in transitional cell cancer and these alterations are associated with an increased expression of p53. Genetic data on squamous cell carcinoma are scarce. We have previously reported chromosomal aberrations in bilharzial-related bladder cancers from Egyptian patients by fluorescence *in situ* hybridization (FISH) (Aly and Khaled, 2002, 1999, 2004). Few studies have compared bilharzial-related bladder tumors of TCC and SCC subgroups (Kamel *et al.*, 1994; Warren *et al.*, 1995; Osman *et al.*, 1997; Chaudhary *et al.*, 1997; Wu *et al.*, 1998).

Several studies on mutations in the tumor suppressor gene p53 demonstrated differences between SCC and TCC in type and distribution of mutations. Correlation with chromosomal alterations and different pathways of oncogenesis of the 2 tumor types were also discussed (Kamel *et al.*, 1994; Warren *et al.*, 1995; Chaudhary *et al.*, 1997; Gonzalez-Zulueta *et al.*, 1995).

In the current study, FISH was used to compare chromosomal alterations in both transitional and squamous subgroups of bilharzial-related bladder cancers. We investigated whether, in addition to histological and etiological differences, SCC and TCC have differences in genetic alterations involved in the development of both types of bilharzial-bladder cancer. We compared numerical aberrations of chromosomes 7, 9 and 16 in both types and correlated the results to p53 amplification in both types.

MATERIALS AND METHODS

Tumor Samples

Tumor specimens were obtained from the National Cancer institute, Cairo, Egypt. A cohort of 50 patients with bilharzial-related bladder carcinomas was evaluated. All samples were from primary tumors following radical cystectomy. Thirty-three SCC tumors and 17 TCC tumors were studied. All tumors were stage pT2-4. Tumor stage and grade were defined according to UICC (UICC, 1978) and World Health Organization classifications (Mostofi *et al.*, 1973). No preoperative radiation therapy or chemotherapy was administered to any of the patients before removal of specimen. All samples were from paraffin-embedded archival specimens. Mixed tumors were excluded from the study. Schistosomiasis infection was confirmed in all cases by the presence of ova in urine of patients.

The biotin-conjugated DNA probes specific for chromosomes 7, 9, 17 and 18 were commercially obtained (Oncor, Gaithersburg, MD). In order to allow a proper evaluation of FISH signals, the previously published criteria (van Dekken *et al.*, 1990) were adapted. Briefly, 350-450 intact and non-overlapping interphase nuclei, which had signals with more or less the same homogenous fluorescence intensity, were counted and the number of bright fluorescent spots per nucleus was scored for each probe. Minor hybridization spots were not counted. Spots in a paired arrangement (split spots) were counted as one signal. The percentage of nuclei, which had one, two, three, four or more signals per one nucleus, were calculated for each specimen.

Gene alterations were determined by FISH using a probe spanning approximately 140 kb of the chromosomal region containing the p53. This probe was utilized to detect the presence or absence of p53 alterations.

Details of FISH protocol used in this study have been extensively described elsewhere (Khaled *et al.*, 2004). Briefly, formalin fixed, paraffin embedded tissue sections (4 µm thick) were deparaffinized in xylene, dehydrated in 100% ethanol and air dried. After a brief wash in phosphate buffer saline, the slides were dehydrated serially and air-dried in the dark. Hybridization with the centromeric probes or p53 DNA probe was carried out according to the manufacture's instructions. Nuclei were counterstained with PI (propidium iodide) 1 g mL⁻¹ in Vectashield mounting media (Vector Laboratories, Burlingame, CA).

The procedure for the detection of oncogene alterations has been previously described (Ozakyol *et al.*, 2006).

RESULTS

Patients' characteristics in study were shown in Table 1. Data of the chromosomal pattern in both SCC and TCC bilharzial patients are detailed in Table 2.

Of the 33 patients with SCC, 10 (30%) had aberrations of all 3 analyzed chromosomes, 14 (42%) had aberrations of chromosomes 7 and 9, 5 (15%) had monosomy 9, 3 (9%) had a single trisomy 7 and 1 (3%) had trisomy 7 and 17. The p53 alterations were detected in 26 of the 33 patients (79%) (Fig. 1).

Table 1: Patient characteristics

	Squamous cell carcinoma	Transitional cell carcinoma
No. of patients	33	17
Patient mean age (range)	45.5 (28-64)	49.9 (32-66)
No. of grade		
1	18	1
2	11	9
3	4	7
No. of stage		
Ta	-	-
T1	2	2
T2	24	12
T3	4	1
T4	3	2

Table 2: Chromosomal pattern in both SCC and TCC patients

Numerical chromosomal aberrations	No. of patients (%)	
	Squamous cell carcinoma	Transitional cell carcinoma
Chromosome 7		
Diploid	7 (21%)	-
Trisomy	25 (75.7%)	12 (70.5%)
Polysomy	1 (3%)	5 (29.4%)
Chromosome 9		
Diploid	3 (9%)	8 (47%)
Monosomy	-	1 (5.9%)
Trisomy	28 (84.8%)	4 (23.5%)
Polysomy	2 (6%)	4 (23.5%)
Chromosome 17		
Diploid	23 (69.7%)	3 (17.6%)
Trisomy	9 (27%)	10 (58.8%)
Polysomy	1 (3%)	4 (23.5%)

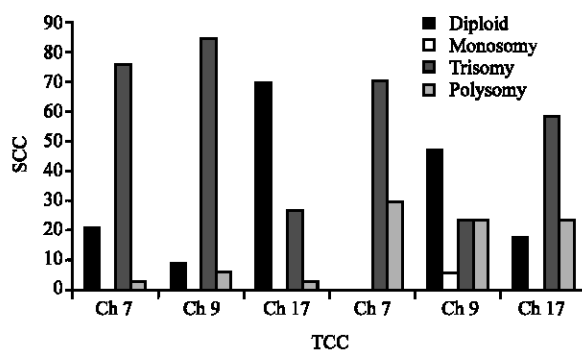


Fig. 1: Correlation between the percentages of aberrations in chromosomes 7, 9 and 17 in SCC and TCC patients

Of the 17 patients with TCC, 6 (35%) had aberrations of all the analyzed chromosomes, 4 (23%) had aberrations of chromosomes 7 and 17, 3 (17%) had a single trisomy 7, 2 (11%) had monosomy 9 and 2 (11%) had monosomy 9 and trisomy 7. The p53 alterations were detected in 12 of the 17 patients (70%).

Statistical analysis revealed highly significant differences for numerical aberrations among the two groups with $p \leq 0.05$ for chromosome 7, $p \leq 0.01$ for chromosome 9 and $p \leq 0.001$ for chromosome 17. For p53 no significant differences were observed among the two groups. Highly significant difference was seen between p53 and aberrations of chromosome 17.

DISCUSSION

Carcinoma of the bladder in Western countries is thought to be caused by chronic exposure of the urothelium to exogenous carcinogens, which are concentrated in the urine, leading to genetic damage. In contrast, bilharzial-related bladder cancer is suggested to be related to the chronic inflammatory response which occurs following *S. haematobium* infection. Western bladder cancer predominantly shows transitional cell histology, while bilharzial-related cancer is largely of squamous type. Little is known about the genetic distinction between these two tumor types. In this study, we show that histology of squamous and transitional cancer in bilharzial-infected patients show distinct patterns of chromosomal alterations.

Most studies on the molecular genetics of bladder cancer have focused on transitional cell carcinoma and much less are known about genetic alterations in squamous cell cancer.

Comparison of the chromosomal pattern of both types revealed a distinct difference between squamous and transitional cell cancer. Chromosome 7 showed similar aberrations in both groups. Trisomy or polysomy 7 was observed in at least 73% of the patients in association with advanced tumor stage which confirms previous reports in which trisomy 7 has been shown to identify more aggressive tumor types (Waldman *et al.*, 1991; El-Rifai *et al.*, 2000).

While in squamous cell carcinoma chromosome 9 was altered in 87% of the cases, in transitional cell carcinoma specimens were observed in only 57% (1.5 times more in squamous cell carcinoma than in transitional cell carcinoma).

Statistical differences were highly significant between both groups. Monosomy 9 has been reported as an early event in the carcinogenesis of bilharzial-associated SCC (Aly and Khaled, 1999, 2002; Ghaleb *et al.*, 1996). Gonzalez-Zulueta *et al.* (1995) observed allelic loss of 9p twice frequent in squamous cell carcinoma. A different genetic pathway for the evolution of bilharzial related bladder cancer was suggested by Khaled *et al.* (2004). Our present study revealed the contribution of chromosome 9 in the oncogenesis of both tumor types.

Chromosome 17 was altered in only 33% of the SCC in contrast to a nearly 58% of TCC.

The majority of human tumors seem to have mutations in the p53 locus on chromosome 17. The p53 gene encodes a nuclear phosphoprotein important for the control of transcription and replication of DNA in eukaryotic cells. There have been several studies on increasing p53 overexpression that might be directly related to higher grade or stage in TCC (Spruck *et al.*, 1994; Olumi *et al.*, 1990; Pycha *et al.*, 1997; Sidransky *et al.*, 1991). An increased p53 overexpression was related to higher degree and stage tumors in both tumor types but type and position of mutations different between them (Aly and Khaled, 2004; Warren *et al.*, 1995; Gonzalez-Zulueta *et al.*, 1995). In this study, similar frequency of p53 amplification was observed in both types, which were (84%) for SCC and (73%) for TCC. This is in agreement with previously presented data (Kamel *et al.*, 1994; Warren *et al.*, 1995; Gonzalez-Zulueta *et al.*, 1995).

CONCLUSIONS

Present findings clearly showed differences in chromosomal patterns between bilharzial squamous cell and transitional cell carcinoma but similar frequencies of p53 alterations in both types. Aberrations of chromosome 9 were observed in both types which confirm the 2 pathways in the oncogenesis in squamous and transitional cell carcinoma at the cytogenetic level as suggested by molecular studies previously described (Gonzalez-Zulueta *et al.*, 1995; Waldman *et al.*, 1991; Spruck *et al.*, 1994; Pycha *et al.*, 1997). In these studies, an alteration of chromosome 9 was described as an early event in oncogenesis of bladder cancer and additional alterations of p53, located on chromosome 17, may be responsible for progression of the disease. The prognostic value of these findings remains to be determined.

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