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FU-MK-1 ANTIBODY AS TUMOR MARKER TOOL IN URINARY BLADDER CANCER DIAGNOSIS

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ABSTRACT

The present study was conducted to investigate the power of MK-1 as a tumor marker using the FU-MK-1 for the diagnosis of the urinary bladder cancer tumor depending on the altitude of expression in different grades and types of bladder cancer tissues. Eighty urinary bladder cancer tissues were sectioned for immunohistochemistry using FU-MK-1 monoclonal antibody for screening of the MK-1 activity. Activity of the MK-1 was found in 46 cases out of 80 patients. The distribution of MK-1 expression in different types of the bladder cancer tumors was found as follows: ACC (50.0%), SCC (62.5%) and TCC (57.1%). On the other hand, MK-1 expression was significantly increased in grade III of all types of urinary bladder cancer when compared to that of other grades. Also, MK-1 expression was correlated with the change in abnormal DNA ploidy. In conclusion, the expression of MK-1 is significantly associated with urinary bladder cancer.

INTRODUCTION

Urinary bladder cancer is the commonest oncogenic problem in Egypt; it represents about 20.6% of all malignancies. This could be attributed to the infection with *Schistosoma haematobium* (El-Bolkainy, 1991; Ibrahim and Khaled, 2006).

Tumor markers considered as useful tool for diagnosis or prognosis (Diamandis, 2002). The MK-1 antigen is widely expressed in carcinomas of various tissues as stomach, colon, pancreas, gall bladder, bile duct, breast and lung (Watanabe et al., 1993). Recent molecular characterization demonstrated that MK-1 is a transmembrane glycoprotein with a molecular weight of 40 kDa and is encoded by the GA733-2 gene. Furthermore, immunoblotting analysis indicated that FU-MK-1 binds to a small fragment (6 kDa) generated from a tumor cell line under hypotonic conditions, suggesting that the FU-MK-1 epitope exists on the distal 6-kDa peptide of the extracellular domain of the GA733-2 molecule. It was concluded that the MK-1 antigen is the GA-733-2 antigen, which is currently

being used as a target in clinical trials with monoclonal antibodies (Tomita et al., 2000). Only few investigators, however, have described the expression of MK-1 in urological cancers (Anagnostaki et al., 1990; Zorzos et al., 1995).

This study was done to investigate the relation between the expression of MK-1 as a tumor marker in different stages and grades of urinary bladder cancer by using the FU-MK-1 monoclonal antibody.

MATERIALS AND METHODS

Subjects:

Eighty urinary bladder cancer patients, mean age 54.7 ± 8 years, from Urology and Nephrology Center, Mansoura University were divided according to the type and grade into 20 patients with adenocarcinoma tumor *(ACC)* (8 grade I, 8 grade II and 4 grade III), 32 patients with squamal cell carcinoma *(SCC)* (6 grade I, 10 grade II and 16 grade III) and 28 with transitional cell carcinoma *(TCC)* (8 grade I, 6 grade II and 14 grade III). Another 30 patients with non-malignant urinary bladder tissue were selected as controls.

Immunohistochemistry Methodology:

Tissue sectioned at 4μm was mounted on silanized capillary gap slides (Ventana), baked at 60°C for a minimum of 60 min, deparaffinized with xylene, and rehydrated through graded alcohols to distilled water. Sections were then placed in 0.01-M citrate buffer at pH 6.0 and heated in a steamer (Black & Decker, Model H390) for 20 min at 90°C. Paraffin sections were blocked by treatment with 0.03% H_2O_2 in methanol for 20 minutes and 3% H_2O_2 for 5 minutes. In most paraffin sections, proteolytic digestion was carried out with 0.4% pepsin for 20 minutes at 37°C. FU-MK-1 monoclonal antibody, kindly a gift from Prof. Dr. M. Kuroki, Department of Biochemistry, School of Medicine, Fukuoka University, Fukuoka – Japan, was used at a dilution of 1:2000. The slides were preincubated with 10% normal horse serum for 20 minutes to minimize non-specific binding. The slides were sequentially incubated at room temperature with the primary MoAb for 1 hour followed by biotinylated secondary antibody antibody (Vector Laboratories, Burlingame, CA, USA) for 40 minutes and avidin-peroxidase conjugates (Dakopatts, Glostrup, Denmark) for 40 minutes. A secondary biotinylated antibody was used and reactivity detected by an avidin-biotin immunoperoxidase system employing diaminobenzidine tetrahydrochloride as the chromogen. The IHC test was initially calibrated against negative control samples slides to yield negative and strong membrane staining on the appropriate cells. It was then parallel tested with the FU-MK-1/MK-1 testing for negative controls by replacing the primary antibody with normal saline.

DNA ploidy:

Flowcytometry DNA pattern study was done by using the propidium iodide (50μg/ml). Fresh tissue specimens in isotonic saline were cut into pieces and gently rubbed through fine nylon gauze. Tris-EDTA buffer was used for washing and centrifuged for 10 minutes at 1800 rpm. Pellet was fixed with 1ml ice cold absolute ethanol. After at least 12 hours of fixation, the sample was again centrifuged, removal of the ethanol and the tumor cells were stained according to the method of Vindelov et al. (1983). Briefly, 1800μl of trypsin was added to 200 μl of the cell suspension in citrate buffer and the tube was inverted to mix the contents gently. Sample suspensions were kept for 10 minutes at room temperature, during which the tube was inverted five to six times. 1500 μl Trysinhibitor was added and mixed again by gently inversion. After 10 minutes at room temperature 1500 μl ice cold propidium iodide solution was added. The solution was mixed and the samples were filtered through 30 μm nylon membrane into tubes and kept in an ice bath until analysis. Samples were run in the flowcytometer between 2.5 – 3 hours after the addition of propidium iodide solution.

Statistical Analysis: Chi-square test was used to evaluate the significance level of the results.

RESULTS

This study showed a highly significant difference in the expression of MK-1 between malignant and non malignant cells of urinary bladder tissues by using the immunohistochemical technique as shown in table (1).

Table (1): MK-1 expression in urinary cancer bladder patients and non-malignant tissue

	MK-1 activity		*P* value
	-ve	+ve	
Non-malignant subjects (n =30)	30	-	< 0.001 (Highly significant)
Urinary bladder cancer (n = 80)	34	46	

MK-1 Immunohistochemistry activity with different types of cancer bladder:

Table (2) shows the frequency of MK-1 expression among the different types of urinary bladder cancer. The results illustrated that no statistically significance of MK-1 distribution in different types of urinary bladder cancer was found.

Table (2): MK-1 expression in different types of malignant.

	Types of urinary bladder cancer		
	ACC (n = 20)	SCC (n = 32)	TCC (n = 28)
MK-1 activity	10	20	16
%	50.0	62.5	57.1
P value	Non-significant		

P value shows no significance between different types of bladder cancer.

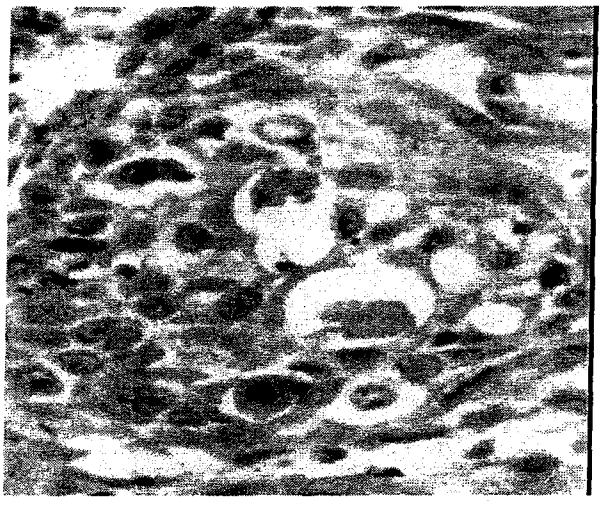

Figure (1): Immunohistochemistry done for urinary bladder cancer tissues (Squamous cell carcinoma) showing brown cytoplasmic staining with FU-MK-1 monoclonal antibody.

Correlation between MK-1 immunohistochemistry by FU-MK-1 and different grades of cancer bladder cell types

Our results showed a direct correlation between the grades of all types of cancer bladder and MK-1 expression by immunohistochemistry using FU-MK-1 monoclonal antibody, in comparison with non malignant bladder tissues as shown at table (3).

Table (3): MK-1 expression percents with different bladder cell types and grades.

Type of Bladder Tissues	Grades of Cancer Bladder Tissues		
	No. of + ve cases (%)		
	GI	GII	GIII
ACC Group (n = 20)	2 (25.0)	5 (62.5)	3 (75.0)
SCC Group (n = 32)	2 (33.3)	6 (60.0)	12 (80.0)
TCC Group (n = 28)	2 (25.0)	4 (66.6)	10 (71.4)
No. of + ve cases (%)	6 (27.8)	15 (63.0)	25 (75.5)

MK-1 expression and DNA ploidy in urinary bladder cancer

DNA ploidy exhibits a negative ploidy in non-malignant bladder tissue, where DNA index was less than 1.05. Patients with urinary bladder cancer showed a positive ploidy (diploidy, tetraploidy and aneuploidy).

Table (4): Relation between MK-1 expression and DNA ploidy in urinary bladder cancer.

DNA ploidy	MK-1 activity using the IHC		
	- ve	+ ve	*P* value
Diploidy	14	23	
Tetraploidy	18	11	0.586
Aneuploidy	12	12	

DISCUSSION

Current methods of investigation of urinary bladder cancer involve cystoscopy, ultrasound scanning and contrast urography with additional information provided by cytology. These methods, although having a high detection rate, are expensive, time-consuming, invasive and uncomfortable. There is, therefore, a need for an inexpensive, noninvasive, quick and simple investigation with a high sensitivity and specificity for the detection of bladder cancer. There are an increasing number of molecular assays available for the detection of bladder cancer. From bladder tumour antigens to nuclear matrix proteins to adhesion molecules, cytoskeletal proteins and growth factors, urology

has looked at them all to support the early detection and diagnosis of bladder cancer (Saad et al., 2001).

This study included 30 non-malignant bladder tissues as a control group, and 80 patients with urinary bladder cancer as a study group.

Our results showed that there is a highly significant difference in MK-1 expression between malignant and non malignant cells of bladder tissues, using the immunohistochemical technique as shown in table (1). This finding agrees with the study of Watanabe et al., (1993) who found that MK-1 had the expression in malignant epithelial neoplasms of various tissues such as stomach, colon, pancreases, gall bladder bile duct, breast and lung. Also, it was mentioned that GA733-2 antigen is a cell surface glycoprotein highly expressed on most human gastrointestinal carcinoma and at a lower level on most normal epithelia (Chong and Speicher, 2001). This over expression of MK-1 in malignancy can be explained on the basis that MK-1 (GA733-2) is functioning as a Ca^{++}-independent homotypic cell adhesion molecule (CAM) (Litvinov et al., 1994). CAMs are belonging to a novel class distinct, by its structural features, belongs to the four known super families of CAMs (immunoglobulin-type, selectins, cadherins, and integrins) (Chothia and Jones, 1997). While, cell-cell adhesion is a dynamic process essential for the normal development and function of multicellular organisms and is known to play an important role in tumor progression and metastasis (Litvinov et al., 1996).

As shown in table (2), results showed no significant change in MK-1 expression in different types of urinary bladder cancer. On the other hand, our results revealed that there is a significant increase of MK-1 expression in grade III of all types of urinary bladder cancer (table 3). This direct correlation between the grades of different types of urinary bladder cancer with MK-1 expression was reported by Litvinov et al. (1996). They found an increase of GA733 protein during progression of cervical lesions from cervical intraepithelial neoplasia grade I to grade III. They have suggested that the increase of cell proliferation in cervical squamous epithelia (which GA733-2 is normally negative) correlates with the expression of GA733-2.

Furthermore, the results of this study showed that the entire control group exhibited a normal ploidy (DNA index was < 0.95 and > 1.05); but for urinary bladder cancer patients, there was a positive ploidy (diploidy, tetraploidy and aneuploidy) as shown in table (4).

In conclusion, this work, to our knowledge, is the first study to investigate the expression of MK-1 receptor in Egyptian urinary bladder cancer patients using non-commercially FU-MK-1 monoclonal antibody. The study revealed that MK-1 has a power expression in differentiating between the grades of the bladder cancer. Also, MK-1 expression is correlated with the change in DNA ploidy.

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