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Mitochondrial Translocase Expression Profile in Renal Cancer

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ABSTRACT

Background: mitochondrial appropriate function depends on ideal mitochondrial translocation machinery, of which translocase of outer mitochondrial membrane (TOMM) have a vital role in normal functioning cell. Any disturbance in the translocation machinery leads to either tumorigenesis or apoptosis.

Aim of the work: This study aimed to investigate the expression of TOMM34 in renal cell carcinoma (RCC). TOMM34 expression was assessed in 42 samples of RCC patients using immunohistochemistry technique.

Results: immunohistochemical staining revealed significant accumulation of TOMM34 protein in RCC cases compared to their corresponding non-cancerous renal tissues. TOMM34 protein expression was significantly associated with age and gender categories in contrast to the other clinicopathological features.

Conclusion: according to protein expression level this study demonstrated that TOMM34 is a marker of poor outcome in RCC.

Keywords: RCC, TOMM34, IHC.

INTRODUCTION

Renal cell carcinoma accounts for 3% of all malignant tumor types and is considered the most lethal of urologic cancers. Renal cell carcinoma arises from the renal epithelium and accounts for roughly 90% of renal malignant tumors. These tumors are more common in male cases than females by 3:2 ratio. This type of malignant tumors is hereditary in only 3-5% of cases and is more common to be sporadic. Among many of signs, pain and hematuria are potential representing signs, also vascular tumor thrombus may present. Renal cell carcinoma showed metastatic feature in many sites including, lung, lymph nodes, liver, bone and brain [(1)].

For this lethal tumor, many markers have been studied in order to predict the behavior of renal cell carcinoma, this prediction of invasiveness and metastatic potential of renal cell carcinoma at an early stage is one of the most important challenges of cancer research. None of the available markers considered an ideal marker for prognosis, diagnosis, monitoring the treatment response of renal cell carcinoma [(2)].

Mitochondria are a vital organelle in cell providing the needed energy and regulating metabolism for survival. Dysfunction of mitochondrial bioprocesses lead to either cell death or development in to tumor cell; also they have been reported as a common and consistent phenotype of cancer cells and suspected feature of cancer development and progression [(3,4)].

Mitochondria possesses translocation machineries for proper function, including translocase of outer mitochondrial membrane (TOMM) machinery. A part of TOMM components, TOMM34 were identified from expressed sequence tag (EST) and cDNA databases as an important component of protein importing [(3)]. TOMM34 plays a vital role in the delivery of preproteins from cytosol to the TOM complex; the protein interacts with the mature portion of the targeted protein. This protein is found in the cytoplasm and associated with the outer mitochondrial membrane. TOMM34 was described to be found on the surface of the outer membrane, later studies reported that TOMM34 is localized in the cellular cytosol and works as a component of a large chaperone, which transports protein to mitochondria [(3,5)].

A recent study identified TOMM34 as a co-chaperone with HSP70 and HSP90 by their simultaneously scaffolding for importing and maturation of preproteins [(5,6)]. Inhibition of TOMM34 could be a good choice as an anticancer drug and promising target for immunotherapy, as suppression of TOMM34 resulted in inhibition of cancerous cell growth [(4)].

MATERIAL AND METHODS

Patients and tumors

The study was conducted on a well-characterized series of renal cell carcinoma retrieved from archives of Pathology Department,

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Faculty of Medicine, Suez Canal University. The targeted samples were in the form of formalin fixed paraffin embedded (FFPE) archival blocks, 42 patients with primary RCC were compared to 42 disease-free healthy renal tissues from the same patients. All specimens were obtained by cytoscopic resection of whole tumors during the period from 2009 to 2013, the archived blocks showed good quality and sufficient tissues. Patients with secondary RCC and patients with history of any other malignancies were excluded from this study. This study was approved by Research Ethics Committee, Faculty of Medicine, Suez Canal University.

Immunohistochemistry (IHC)

Expression of TOMM34 in the series of RCC (n= 84) was assessed using IHC technique, all sections were cut at 5 µm and they were mounted on super frost plus slides. Heat-induced retrieval of antigen epitopes was performed in citrate buffer (pH 6). The primary antibody of TOMM34 mouse monoclonal antibody (**Novus Biologicals, Catalog no. (2A9)[NBP2-00892])**, was used followed by detection kit Power Stain™ Poly HRP DAB Kit **(Genemed, Catalog no. 52-0017)** following the manufacturer's guidelines. The counter stain, Hematoxylin solution, Meyer's **(Sigma-Aldrich, Catalog no. MHS1)** were used through the technique. A positive control (a known TOMM34 positive tissue section) and negative control were included in each staining run.

Scoring of IHC expression

The scoring of TOMM34 IHC expression was performed using H-score. Staining intensity was scored as 0, 1, 2 or 3 as negative, mild, moderate or marked, respectively. The percentage of positive cells at each intensity was subjectively estimated to produce a final score ranging from 0 to 300. All cases were scored without prior knowledge of the patients' pathological or outcome data.

Statistical analysis of IHC data

Statistical analysis was performed by using SPSS version 22.0 software. Cut-off point values for TOMM34 were chosen before statistical analysis. Correlation analysis using Spearman rank correlation (r_s) and Pearson's correlation coefficient were performed where appropriate and paired t-tests were used to assess related samples. All tests were two tailed and *P* values were considered statistically significant below 0.05.

The study was done after approval of ethical board of Suez Canal university.

RESULTS

Forty two cancer tissue specimens and nine normal non-cancer specimens were stained according to immunohistochemistry technique for TOMM34 and assessed using H scoring system. TOMM34 immunoreactivity provided diffuse cytoplasmic stain. There was minimal stromal staining reported in the examined specimens. There was significant difference in protein expression between cancer and non-cancer tissues (P=0.009). All normal tissues and 45% of cancer specimens did not show expression of TOMM34. Nevertheless, there was cytoplasmic staining of TOMM34 antibody in 55% of renal cancer tissues with variable degrees **(Figs. 1,2).** RCC specimens showed mild expression (H scoring from 10 to 50) and marked expression (H scoring 100 to 200) with equal percentage of 13%, While the majority of tissues showed moderate expression (H scoring from 50 to 100) by 74%. The expression of TOMM34 in the specimens using H scoring system ranged from 10 to 200 with mean±SD of 94.78±43.88 (**Table 1).**

Table 1: showing association between TOMM34 protein expression and clinicopathological characteristics. (without non-expressors) (n=23)

Variable		No	Mild	Moderate	Marked	P value #
No		23	3 (13)	17 (74)	3 (13)	
Age	**Age between 20 and 40**	2 (8.7)	0 (0)	0 (0)	2 (66.7)	0.005**
	Age between 40 and 60	14 (60.9)	2 (66.7)	11 (64.7)	1 (33.3)	
	Age over 60	7 (30.7)	1 (33.3)	6 (35.3)	0 (0)	
Gender	**Female**	7 (30.4)	1 (33.3)	3 (17.6)	3 (100)	0.017*
	Male	16 (69.6)	2 (66.7)	14 (82.4)	0 (0)	
Side	**Right**	15 (65.2)	3 (100)	10 (58.8)	2 (66.7)	0.385
	Left	8 (34.8)	0 (0)	7 (41.2)	1 (33.3)	
Nuclear grade	**Grade I**	4 (17.4)	1 (33.3)	2 (11.8)	1 (33.3)	0.611
	Grade II	15 (65.2)	2 (66.7)	12 (70.6)	1 (33.3)	
	Grade III	4 (17.4)	0 (0)	3 (17.6)	1 (33.3)	
Tumor size	**T1**	6 (26.1)	1 (33.3)	5 (29.4)	0 (0)	0.407
	T2	11 (47.8)	2 (66.7)	8 (47.1)	1 (33.3)	
	T3	6 (26.1)	0 (0)	4 (23.5)	2 (66.7)	
Capsular Infiltration	**Negative**	17 (73.9)	2 (66.7)	12 (70.6)	3 (100)	0.538
	Positive	6 (26.1)	1 (33.3)	5 (29.4)	0 (0)	
HPD	**Clear cell**	15 (65.2)	1 (33.3)	12 (70.6)	2 (66.7)	0.410
	Chromophobe	3 (13)	1 (33.3)	1 (5.9)	1 (33.3)	
	Papillary	5 (21.7)	1 (33.3)	4 (23.5)	0 (0)	

Values are represented as number (percentage). No: number of tested samples, HPD: histopathological diagnosis. # Chi square test was used. Statistical significance at p values <0.05.

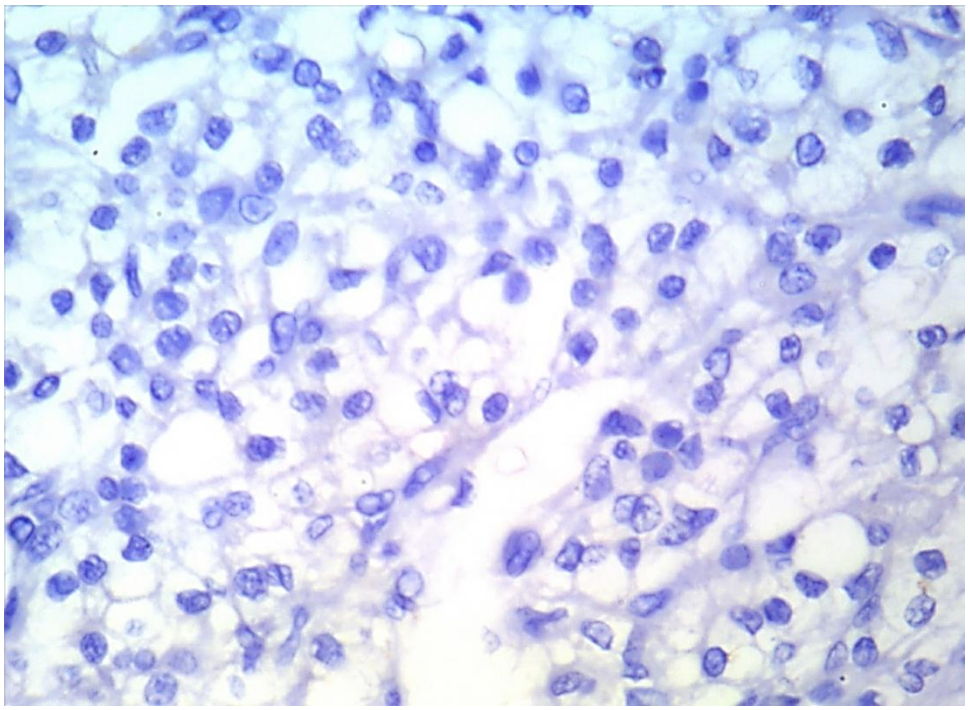

Fig. 1: clear cell RCC, GIII, showing negative TOMM34 IHC expression (TOMM34 X400)

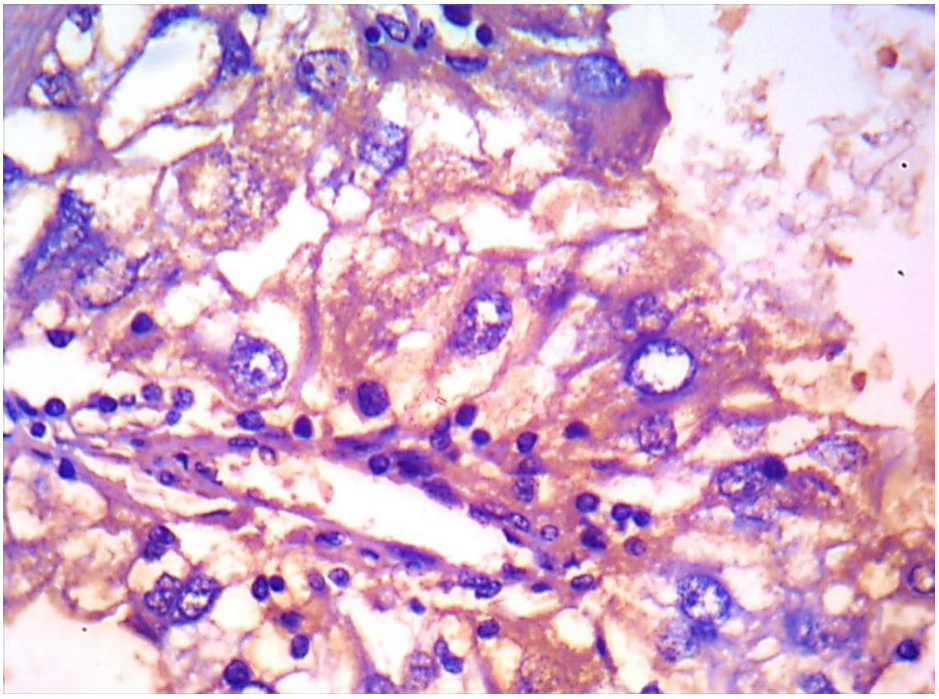

Fig. 2: **clear cell RCC, grade II, showing marked TOMM34 IHC expression (TOMM34 X400)**

DISCUSSION

Renal cell carcinoma (RCC) is the most lethal urological malignancy, accounting for 100,000 deaths worldwide annually [7,8]. Approximately 20–30% of patients were diagnosed at the metastatic stage of disease and half of the remaining patients will experience recurrence after an initially curative treatment [8]. Predicting the clinical outcome of individual RCC patients is challenging and not always possible with classic prognostic factors, staging and grading, which are primarily used when assessing cancer prognosis.

During the last decade, treatment of metastatic RCC had dramatically changed, giving new hope to patients who were suffering from this malignancy, where survival has traditionally been regarded as poor in advanced stages. In the era of new molecular targeted therapies there was a definite need for better tools to predict the clinical course of RCC. Accurate prognostication would help in patient counselling and to plan and individualize patient treatment and follow-up. High-risk patients could be selected for more effective treatments, more careful surveillance and clinical trials with adjuvant therapies. On the other hand, patients with indolent disease could be spared from over-treatment, psychological stress and adverse effects of follow-up, such as radiation exposure, which would also save health care costs and resources [9].

Biomarkers are objectively measured and evaluated indicators of biological or pathological processes or treatment responses. Cancer biomarkers are typically produced by the tumor or by the body in response to the tumor. Prognostic biomarkers were used to categorize patients into different risk groups and to predict the course of a disease. The ideal prognostic biomarker provided prognostic information that was not provided by available clinicopathological indices. Other criteria for prognostic biomarkers were statistical significance, reproducibility, standardization, external validation and feasibility, such as suitability for daily clinical practices, for example evaluations based on urine and blood samples and reasonable costs [10].

Immunohistochemical techniques are used to determine the expression and cellular and subcellular locations of proteins of interest in tissue samples [11]. Renal cell carcinoma is recognized as a family of cancers that originate from the renal tubular epithelium and have distinct genetic and molecular backgrounds, unique morphological features and a characteristic clinical course [8]. The advances in gene technology, molecular biology and proteomics have enabled the identification of underlying molecular pathways of RCC as well as therapeutic targets for advanced disease[12].

Mitochondria have a vital role in the proper cellular function, maintaining production of

energy, consequently controlling cell fate either by cell replication or apoptosis. Interruption and dysfunction of mitochondria suspected to be contributor of cancer progression and development. Mitochondria possesses translocation machineries for proper function, including translocase of outer mitochondrial membrane (TOMM) machinery. A part of TOMM components, TOMM34 was identified from expressed sequence tag (EST) and cDNA databases as an important component of protein importing[3], forming a co-chaperone with HSP70 and HSP90 and interact with the mature portion of preprotein and importing of preproteins form cytoplasm into mitochondria[6].

In vitro study showed that TOMM34 interacts with VCP playing an important role in normal cellular function[13]. TOMM34 in normal tissues was overexpressed in testis and ovary and mild expression in colon, spleen and prostate. Kidney, lung, heart and liver. TOMM34 was overexpressed in CRC cells and siRNA targeted to TOMM34 has a remarkable effect on reducing colon cancer cells. The study recommended using TOMM34 as a useful diagnostic marker and inhibitors of TOMM34 as an anticancer drug[4].

To the best of our knowledge, this is the first study describing the association between TOMM34 protein expression and cases with RCC. The current study aimed to reveal the association between TOMM34 protein expression and clinicopathological features including, age, gender, tumor side, tumor size, capsular infiltration, tumor grade and histopathological diagnosis in specimens taken from patients with RCC compared to normal (non-cancer) normal renal tissues. Forty-two RCC specimens were enrolled in this study, the patient's age was ranged from 20 to 79 years old with mean age was 53 years old (53 ± 12.2), nearly two thirds of cases were males and represented 62.5% and the female cases represented 37.5%. The majority of cases showed negative capsular infiltration (80%), they were categorized according to tumor size into three groups **T1, T2** and **T3** represented 35.3%, 44% and 20.6% respectively. Over half of cases were intermediate differentiated tumors (57.6%) and 24.2% were poorly differentiated tumors and the rest were well differentiated. The histological examination showed four subtypes, clear cell RCC, chromophobe RCC, papillary RCC and unclassified RCC resembled 59.5%, 19%, 15.4% and 7.1% respectively. The association between the clinicopathological features showed significant difference between: age and gender, gender and capsular infiltration, nuclear grade and tumor size, nuclear grade and capsular infiltration with P values 0.003, 0.007, 0.009 and 0.04, respectively.

The immunohistochemical expression of TOMM34 protein was categorized into three groups, mild ,moderate and marked expression that agree with a study of TOMM34 expression in BC cases[3]. There was a significant difference between TOMM34 protein expression in RCC tissues and normal non-cancerous tissues (P=0.009), the study on CRC cases revealed the same result as the study reported that the expression of TOMM34 protein was upregulared in invasive CRC.

Expression of TOMM34 protein in RCC tissues by using H scoring system with mean 94.78 ± 43.88. TOMM34 protein expression showed high significant difference between the age categories (P=0.005) and between gender categories (P=0.0017) as all marked expression were of female cases, these findings are contrast to the study which reported that TOMM34 protein expression and clinicopathological features of BC cases were not statistically significant [3]. This difference may be attributed to the difference in protein epitope detected by the different antibodies used to detect TOMM34 immunohistochemically, usage of different commercially available antibodies, variety in analytic techniques (scoring system or immunostaining techniques), difference in the sample size, variant organs and different population on each study was performed could also cause those contradictory results.

The other clinicopathological features, tumor side, tumor size, capsular infiltration and histopathological diagnosis were not statistically significant with TOMM34 protein expression, which agree with findings in breast cancer study [3].

In our study, TOMM34 protein expression was localized in cytoplasmic regions. This localization is proper for interaction with HSP90 for importing new synthesized preproteins for maturation [4].

Another study on BC, TOMM34 was differentially expressed in cases, upregulated in cases with distant recurrence compared to metastases cases. TOMM34 expression was associated with large tumor size, high tumor grade, advanced nodal stage, definite lymphovascular invasion and histological types of known adverse clinical outcomes. The association of TOMM34 protein expression and clinicopathological parameters was not statistically significant [3].

In conclusion, the present report correlated the immunohistochemical expression of TOMM34 protein with clinicopathological

parameters including, age, gender, tumor side, tumor size, nuclear grade, capsular infiltration and histopathological diagnosis. TOMM34 protein expression was up-regulated in high grade tumors in left kidney.

Investigations of the functions of TOMM34 may be useful in understanding the complex process of tumor progression and this marker could be useful as a prognostic factor in cases of renal cell carcinoma.

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