Open Access

Baghdad Science Journal **Vol.15(3)2018**

DOI: http://dx.doi.org/10.21123/bsj.2018.15.3.0244

The Prognostic Value of some Epithelial-Mesenchymal Transition Markers and Metastasis-Related Markers in Human Transitional Cell Carcinoma of the Bladder

May Khaleel Ismael

Received 1/4/2018, Accepted 15/7/2018, Published 13/9/2018

Abstract:

Recent reports provided evidence that epithelial to mesenchymal transition (EMT) and some matrix metalloproteinases (MMPs) contribute to the invasion and metastasis of cancer cells. This study investigated the expression pattern of some EMT markers (E-cadherin and Vimentin) and some MMPs (MMP-2 and MMP-9) in transitional cell carcinoma (TCC). Fifty five paraffin embedded biopsies were included in this study. Expression pattern of E-cadherin and Vimentin was evaluated by immunohistochemistry while cytoplasmic mRNA expression of both MMP-2 and MMP-9 were determined by *in situ* hybridization. The expression of all markers were significantly increased with the increase of patient's age (≥ 50 years), and furthermore an increase in men expression when compared to women. Interestingly, all healthy tissues showed positive E-cadherin expression while they did not show any expression of Vimentin, MMP-2 and MMP-9. E-cadherin expression decreased, whereas expression of Vimentin increased according to the grade and stage of the tumor. Similarly, both MMP-2 and MMP-9 expression were increased with the progression of TCC. The current study conclude that a decrease in E-cadherin together with increased Vimentin, MMP-2 and MMP-9 are significant markers that correlate with poor prognosis of TCC.

Keywords: E-cadherin, MMP-2, MMP-9, TCC, Vimentin.

Introduction:

In terms of morbidity and mortality, TCC of the urinary bladder is considered one of the most prevalent solid malignancies worldwide, with a high incidence in industrialized nations (1-3). It accounts for 90% of bladder carcinoma and is classified into superficial (75%) and muscle invasive tumors (25%) (4). In Iraq, bladder cancer is fifth most prevalent of all cancer types, with 1163 registered cases in 2011, and the ratio of men to women at 3:1. For men bladder cancer is second most common cancer at 9.3 % and in women ranked tenth at 2.7 %. According to the latest WHO data published in 2014, bladder cancer deaths in Iraq reached 984 or 0.67 % of total deaths (5). The extracellular matrix (ECM) is a vital structure that is essential for a normal organ development and tissue homeostasis, and deregulated ECM remodeling leads to many diseases, including cancer (6). Since micro metastasis, is the initial and extremely important point in tumor metastasis (4), it is pivotal to recognize the molecular mechanisms leading to invasion and metastasis of bladder cancer cells.

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: maykhaleelismael@scbaghdad.edu.iq

Exons 7 and 9 mutations showed an association with bladder cancer initiation and metastasis (7, 8).The invasiveness of epithelial cells depends on the activation of the EMT (9). The molecular features of the EMT involve down regulation of the epithelial adhesion protein E-cadherin and expression of the mesenchymal intermediate filament protein Vimentin (10). The E-cadherin belongs to the diverse family of cadherin, membrane-associated glycoproteins, and is expressed in epithelial cells intermediating cell-cell cohesion and playing a crucial role in epithelial cell brow and tissue remodeling (11). In ordinary cells, E-cadherin's cytoplasmic domain binds with subtypes β or γ of the catenin proteins, which ensure the connection to the actin microfilament, thus insuring cytoskeleton integration and establishment of cellular adhesion (12). In tumor progression, loss of cellular adhesion is a crucial point, resulting in invasive and poorly differentiated tumors (13), therefore, the loss of epithelial cell polarity is the essential feature of EMT and mesenchymal cell properties occupation (2, 14, 15). Oppositely, the main characteristic features of mesenchymal cells are loosely associated cells and disorganized cellular layers that lack polarity and tight cell-to-

cell adhesion proteins, making these cells favorable to cell migration (16). Vimentin is a member of type III intermediate filament protein family, and known to be one of the most widely expressed and highly conserved proteins, responsible for changing the cellular shape and strengthening the cytoskeleton exists in cells of mesenchymal origin such as leukocytes, endothelial cells, and smooth muscle cells (17). Recently, it has been observed on apoptotic cells and thrombin-activated platelets (18). It was also detected with a diverse expression patterns in bladder cancer and normal urothelia (19, 20). Increased Vimentin expression has been reported in various epithelial cancers, and correlates with tumor growth, invasion, metastasis and poor prognosis (10). Thus, the main characteristic features of EMT are correlated with partition of intracellular tight junctions and loss of cell-cell cohesion and these results in the loss of epithelial features and tend to acquire the mesenchymal morphology (21). Finally, they boost cell motility, resulting in the freeing of cells from the parental epithelial tissue site and gain the ability to reconstitute metastatic colonization at distant sites (16). Meanwhile, invasion through basement membranes and interstitial ECM necessitate the action of proteolytic enzymes. Among these enzymes, MMPs are able to degrade almost all of the ECM components (6, 22). MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are secreted, zinc-dependent, have a fibronectin-like domain, cancer-associated endopeptidases. They cleave various targets (ECM, cytokines, chemokines and growth factor receptors) that conversely regulate signaling pathways in cell growth, inflammation, migration, angiogenesis and invasion (4). Accordingly, the aim of this study was to evaluate the relationship of E-cadherin, Vimentin, MMP-2 and MMP-9 expressions as probable prognostic factors with relevance to tumor grade and stage in TCC of the urinary bladder.

Material and Methods:

Tissue specimens

Samples were collected from fifty- five paraffin embedded biopsies belonging to patients with TCC, with an age ranged from 36 to 70 years, were included in this retrospective study. The tissue specimens were collected from the archives of histopathology laboratories in Baghdad, Iraq from August 2015 to May 2016. These tissue blocks were diagnosed primarily according to the histopathological records of bladder biopsy specimens. Based on Tumor /Node/ Metastasis (TNM) system (23). Bladder cancer was classified into grades (GI, GII, GIII) and stages (Ta, T1, T2). Twenty five patients had GI, 18 had GII, and 12 had GIII. While 12 had Ta, 24 had T1, and 19 patients had T2. Each obtained tissue sample was histopathologically re-evaluated by specialist pathologist in order to confirm the records and choose the best sections of tissues. Furthermore, ten normal bladder tissues were obtained from the archives of Forensic Medicine Institute in Baghdad, Iraq. They were from eight males and two females and the mean age was the same as patients group. Formalin-soaked, paraffin embedded tissue blocks were divided with microtome into sections 4μm thick. From each tissue case, the first section was stained with standard haematoxylin and eosin (H&E), and the other (4) sections were placed on positively charged slides for Immunohistochemical staining for the detection of E-cadherin and Vimentin and *In situ* hybridization for the MMP-2 and MMP-9 investigations.

Immunohistochemical staining

The wax embedded sections were deparaffinized with xylene then rehydrated with decreasing concentrations of alcohol. The slides were rinsed in 0.01mol/L citrate buffer (pH 6.0), and heated in the oven (100º C) to regain the antigen. Slides were placed in 3% hydrogen peroxide to cover the endogenous peroxidase activity and rinsed with phosphate buffered saline (PBS). The primary antibody was placed on the tissue sections and the mouse monoclonal anti human E- cadherin NCH- 38 clone and the mouse monoclonal Vimentin antibody V9 clone (DAKO, USA) were applied at a dilution of 1:100 for each and secondary antibody was added to the sections then washed with PBS. The slides were stained with diaminobenzidine (DAB) chromogen, then counterstained with haematoxylin, dehydrated، and finally cover slips were placed. The positive control was provided with the kit (DAKO, USA), while the negative control was made by using the procedure described above, but PBS was used instead of the primary antibody.

In situ hybridization technique

After dewaxing and dehydration, the slides were treated with proteinase K solution. Probe for human MMP-2 (Maxim Biotech Cat. No. IH-60025) and MMP-9 (Maxim Biotech Cat. No.: IH-60028) and the hybridization/ detection kit (provided from Maxim Biotech/USA Cat. Number IH-6001(IHD-0050)) was placed on the slide and incubated overnight incubation at 37°C, which allow hybridization of the probe with the target nucleic acid. The slides were rinsed in detergent wash then treated with RNase A solution and streptavidin conjugate. 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate-

chromogen solution (BCIP/NBT) conjugate was dropped on tissue section and finally, slides were examined under a light microscope. In positive cells; a blue colored precipitate appeared at the site of the probe. Slides were then counterstained with nuclear fast red (NFR) and DPX was used as a permanent-mounting medium to mount sections.

Scoring system:

All tissue specimens were blindly investigated by two independent pathologists. The stained sections were examined and scored using Olympus light microscope (Japan) at 400X magnification. In case of E-cadherin and Vimentin which had been examined by immunohistochemistry, the tissue sections were scored quantitatively according to the scoring system of Sumi *et al* (24).Examination and scoring of MMP-2 and MMP-9 expression were done according to the scoring system of Blancato *et al* (25).

Statistical analysis

In this study we used the Statistical Analysis System-SAS 2012 program for difference factors in study parameters. For significant compare between percentages; Chi-square test was used (26).

Results

Fifty –five tissue specimens belonging to patients with TCC were included in this study, with a mean age of 57 years and the number of patients less than 50 years was 13, with a male to female ratio of 3:1(41 male and 14 female). E-cadherin, Vimentin, MMP-2 and MMP-9 markers were evaluated according to age, sex, and histological grades and stages.

Expression of markers and their interpretation according to age and sex

TCC tissue specimens were investigated for E-cadherin, Vimentin, and MMP-2 and MMP-9 markers. The TCC cases were divided into two groups according to age; <50 years and ≥ 50 years. Notably, the expression of all markers was significantly increased with the increase of patient's age (≥ 50 years). Interestingly, all healthy tissues showed positive E-cadherin expression while, there was no expression of Vimentin, MMP-2 and MMP-9 according to age and sex for grade and stage of the tumor. From Table 1, it can be noticed that there was significant differences between male and female in E- cadherin expression in association with grade of tumor. While, these significant differences were showed an expression of Vimentin and MMP-2 in association with stages of the tumors (Table 2).

Table 1. Expression of markers and its association with grade of the tumor according to sex

Parameters	Male (N=41)		Female (N=14)		Chi-square value
	Positive	Negative	Positive	Negative	
E-cadherin	18 (73.26%)	23 (26.74%)	4 (28.57%)	10 (71.43%)	11.36 **
Vimentin	15 (36.59%)	26 (63.41%)	6 (42.86%)	8 (57.14%)	1.88 NS
MMP-2	22 (53.66%)	19 (46.34%)	8 (57.14%)	6 (42.86%)	1.06 NS
MMP-9	33 (80.49%)	8 (19.51%)	11 (78.57%)	3 (21.43%)	0.661 NS

** (P<0.01), NS: Non-Significant.

Table 2. Expression of markers and its association with stage of the tumor according to sex

Parameters	Male		Female		Chi- square value
	Positive	Negative	Positive	Negative	
E-cadherin	15 (36.59%)	26 (63.41%)	5 (35.71%)	9 (64.29%)	1.06 NS
Vimentin	10 (24.39%)	31 (75.61%)	7 (50.00%)	7 (50.00%)	8.72 **
MMP-2	30 (73.17%)	11 (26.83%)	12 (85.71%)	2 (14.29%)	6.03 **
MMP-9	34 (82.93%)	7 (17.07%)	12 (85.71%)	2 (14.29%)	1.16 NS

** (P<0.01), NS: Non-Significant.

Immunohistochemical analysis of E-cadherin and Vimentin

According to grade of the tumor; E-cadherin expression was noticed in the cytoplasm of 22 (40%) of TCC cases, this include 15 (60%) in grade I, 5 (27.8%) in grade II, and 2 (16.7%) in grade III; whereas 33 (60%) of TCC did not show any expression of E-cadherin as shown in Table 3 and Fig.1. Furthermore, Table 4 revealed the association between E- cadherin expression and stage of tumor. It can be noticed that, E-cadherin expression was positive in the cytoplasm of 20 (36.4 %) of TCC cases, consisting of 8 (14.5%) in stage Ta, 9 (16.3%) in stage T1, and 3 (5.4%) in stage T2; however 35 (63.6%) of TCC did not appear any expression of E-cadherin protein.

Table 3. Expression of E- cadherin with grade of the tumor

Grade	Positive		Negative	
	No.	**(%)**	**No.**	**(%)**
GI (N=25)	15	60.00	10	40.00
GII (N=18)	5	27.78	13	82.22
GIII (N=12)	2	16.67	10	83.33
Control (N=10)	10	100	0	0.00
Chi-square value	---	14.72 **	---	14.72 **
	** (P<0.01).			

Table 4. Expression of E- cadherin with stages of the tumor

Stage	Positive		Negative	
	No.	**(%)**	**No.**	**(%)**
Ta (N=12)	8	66.67	4	33.33
T1 (N=24)	9	37.50	15	62.50
T2 (N=19)	3	15.79	16	84.11
Control (N=10)	10	100	0	0.00
Chi-square value	---	11.53 **	---	11.53 **
	** (P<0.01).			

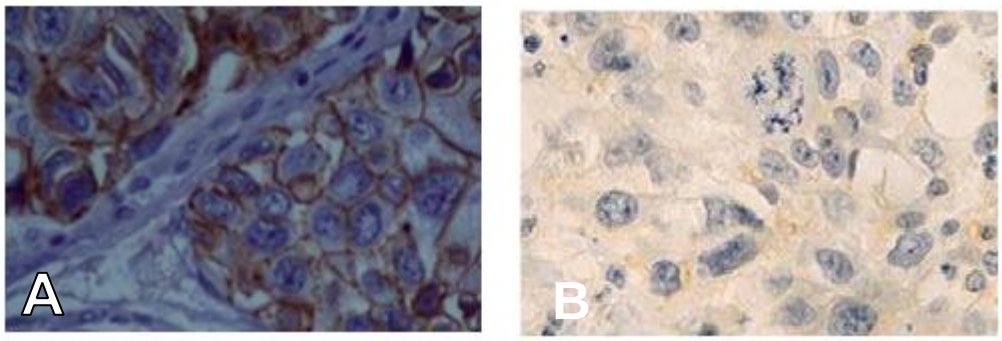

Figure 1. (A) E- cadherin expression in TCC (B) no expression of E- cadherin in TCC (mag.400X)

Vimentin expressions were evaluated in TCC cases and it was observed that Vimentin was expressed in 22(40%) of TCC cases and 33(60%) did not show any expression of Vimentin according to grades of the tumor (Table 5 and Fig. 2). Table 6 shows that the expression of Vimentin with stages of the tumor; in which 17(30.9%) of TCC cases were positive and 38(69.1%) were negative.

Table 5. Expression of Vimentin with grades of the tumor

Grade	Positive		Negative	
	No.	**(%)**	**No.**	**(%)**
GI (N=25)	6	24.00	19	76.00
GII (N=18)	7	38.89	11	61.11
GIII (N=12)	9	75.00	3	25.00
Control (N=10)	0	0.00	10	100
Chi-square value	---	12.65 **	---	12.65 **
	** (P<0.01).			

Table 6. Expression of Vimentin with stages of the tumor

Stage	Positive		Negative	
	No.	**(%)**	**No.**	**(%)**
Ta (N=12)	3	25.00	9	75.00
T1 (N=24)	7	29.17	17	70.83
T2 (N=19)	7	36.84	12	63.16
Control (N=10)	0	0.00	10	100
Chi-square value	---	9.42 **	---	9.42 **
	** (P<0.01).			

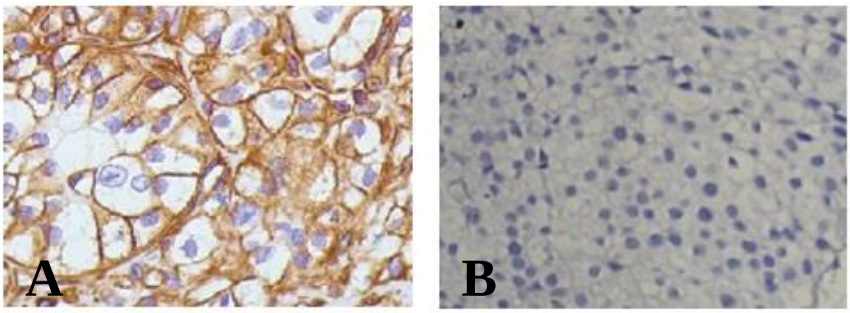

Figure 2. (A) Vimentin expression in TCC (B) no expression of Vimentin in TCC (mag.400X)

In situ mRNA hybridization for MMP-2 and MMP-9

The results demonstrated that there was an increase of MMP-2 expression with the increase of grade and stage of the tumor compared to healthy control group. (Tables 7, 8 and Fig. 3A).

Table 7. Expression of MMP-2 with grades of the tumor

Grade	Positive		Negative	
	No.	(%)	No.	(%)
GI (N=25)	10	40.00	15	60.00
GII (N=18)	13	72.22	5	27.78
GIII (N=12)	10	83.33	2	16.67
Control (N=10)	0	0.00	10	100
Chi-square value	---	13.68 **	---	13.68 **
** (P<0.01).				

Table 8. Expression of MMP-2 with stages of the tumor

Stage	Positive		Negative	
	No.	(%)	No.	(%)
Ta (N=12)	5	41.67	7	58.33
T1 (N=24)	19	79.17	5	20.83
T2 (N=19)	18	94.74	1	5.26
Control (N=10)	0	0.00	10	100
Chi-square value	---	14.27 **	---	14.27 **
** (P<0.01).				

Similarly, MMP-9 expression was increased with the increase of grade and stage of the tumor and there was statistically significant differences compared with control group. (Tables 9, 10 and fig. 3B).

Table 9. Expression of MMP-9 with grades of the tumor

Grade	Positive		Negative	
	No.	(%)	No.	(%)
GI (N=25)	19	76.00	6	24.00
GII (N=18)	14	77.78	4	22.22
GIII (N=12)	11	91.67	1	8.33
Control (N=10)	0	0.00	10	100
Chi-square value	---	14.07 **	---	14.07 **
** (P<0.01).				

Table 10. Expression of MMP-9 with stages of the tumor

Grade	Positive		Negative	
	No.	(%)	No.	(%)
G1 (N=12)	7	58.33	5	41.67
G2 (N=24)	21	87.50	3	12.50
G3 (N=19)	18	94.74	1	5.26
Control (N=10)	0	0.00	10	100
Chi-square value	---	14.62 **	---	14.62 **
** (P<0.01).				

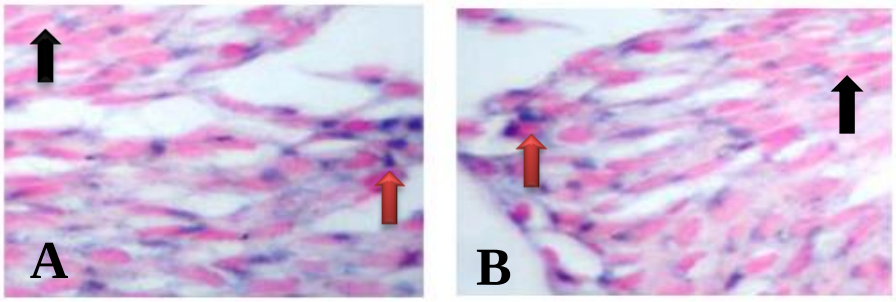

Figure 3. (A) *In situ* MMP-2 expression in TCC (mag.400X), (B) MMP-9 expression in TCC (mag.400X). Red arrow= positive expression, black arrow= negative expression.

Discussion:

Bladder cancer is ranked as the ninth most diagnosed cancer worldwide (27) and it ranks 13th, in mortality rates, which decrease in the most advanced countries (28). Although the modern therapeutic strategies of bladder cancer have made great progress, the undesirable molecular events, especially susceptibility to invasion and metastasis, are still puzzling in clinical treatment, often resulting in an insufficient therapy and dissatisfying prognosis (4). Since the molecular markers hold considerable promise for predicting patient prognosis, various markers are in practice to diagnose the TCC by immunohistochemistry and *in situ* hybridization techniques (1, 4, 29, 30, and 31). Our study tried to evaluate four biomarkers; two of which linked to EMT (E-cadherin and Vimentin) and the rest linked to tumor invasion and metastasis (MMP-2 and MMP-9) to determine the occurrence of expression of these molecules *in vivo* and the capability of using such markers as prognostic tools of TCC. The incidence of the disease in women is increasing, but remains 3-4 times lower compared with the incidence in men, but gender differences varied greatly between countries (28). Our findings showed the same pattern and most of TCC patients were male (41 cases) and only 14 cases were female. Also, The current results show that expression of all studied markers were among age groups, while it was noticed that older age group (≥50) and men showed high expression of all markers. The spotted patterns and tendency of bladder cancer incidence worldwide reflect the expansion of tobacco smoking. An Iraqi study suggest that carcinogens exist in cigarettes could be induce mutations in *K-ras* gene which showed to play an important role in bladder cancer developing. The homozygous genotype of some mutations of the *H-ras* proto-oncogene is detected at an increased risk of bladder cancer (32). On the other hand, the current study revealed that the expression pattern of E-cadherin decreased and Vimentin increased according to the grade and stage of the TCC (from Grades I to III and stages Ta to T2) and these differences were statistically significant. Several findings showed different types of results in this issue and some results were in accordance with our study (1, 29, and 30). One of the study results demonstrated that an increase of E-cadherin was associated with increasing tumor grade, and it was observed that 59.5% of cases were positive for E-cadherin via immunohistochemical analysis and their positivity was higher in low grade papillary carcinoma than in high grade. In the same study, E-cadherin expression was found to have a statistically significant decrease with stage of the tumor while Vimentin positivity showed an expression pattern opposite to that of E-cadherin (29). Favaretto *et al* found a significant association between E-cadherin expression and adverse clinicopathological features such as advanced pathological tumor stage, high pathological tumor grade, and lymph node metastases (13). Another study also confirms that Vimentin was found to have statistically significant correlations with grade, recurrence, and progression; it demonstrated that Vimentin was overexpressed in 69.56% of TCC cases and its positivity increased according to grade of the TCC, it was 42.85%, 72.22% and 92.85% in grade I, grade II, and grade III respectively(1). Many arguments support the theory that EMT plays a vital role in tumor invasion and metastasis, because the cells will acquire a more motile and invasive phenotype (2). Several studies have revealed that decreased E-cadherin and increased Vimentin correlates with poor survival in patients with bladder cancers (29, 30). Other studies of bladder carcinoma cell lines reported that the loss of E-cadherin expression is accompanied by novel expression of N-cadherin, representing cadherin switching from an epithelial-specific classic cadherin to a mesenchymal- specific cadherin, such EMT-related events accompany increased motility and invasion in bladder carcinoma cells (20). However, the present study results confirmed that Vimentin expression is associated with the grade and stage of the tumor with an expression pattern opposite to that of E-cadherin. For a tumor cell to

invade the extracellular membrane, it must first overcome homotypic forces that maintain cell-to-cell adhesion within the primary tumor and then traverse through an altered extracellular membrane to enter the systemic circulation (4). The MMPs are degradative enzymes that alter the surrounding extracellular membrane and allow tumor cells to migrate into the surrounding matrix (6). In addition to the ability of MMPs to remodel ECM, some of these molecules, such as MMP-3, MMP-9, MMP-14, and MMP-28, are known to induce EMT directly (4, 33). Elevated expression levels of MMP-2 and MMP-9 are found in the tissue, serum, and urine of patients with bladder tumors (1, 31), a study result reported that virulent TCC is characterized by a high MMP-9:E- cadherin ratio (34). Our results were consistent with those findings, specifically that a high expression level of both MMP-2 and MMP-9 coupled with low level of E-cadherin and high level of Vimentin expression and this was associated significantly with aggressiveness of TCC. Several studies have shown a direct regulation of MMPs by EMT-associated transcription factors (33, 35). It was recently demonstrated that transforming growth factor TGF-β1 which is one of the pivotal factors regulating EMT, can lead to an increase in the expression of MMPs, such as MMP-2 and MMP-9, in breast and oral cancers (33). In conclusion, E-cadherin, Vimentin, MMP-2 and MMP-9 expressions in TCC showed significant relation with tumor grade and stage and using of these markers together will be helpful in the prognosis of TCC.

Conflicts of Interest: None.

References:

1. Arshad HR, Ali YB, Wanian MA, Shawgi AE, Hassan EF, Salah MA. Association of Cytokeratin and Vimentin Protein in the Genesis of Transitional Cell Carcinoma of Urinary Bladder Patients. Disease Markers. 2015; Article ID 204759, 5 pages.
2. Yang L, Yong-Tong Z, Li-Li M, Shi-Yu P, Li-Jie W, Cheng Y, *et al.* Characteristics of bladder transitional cell carcinoma with E-cadherin and N-cadherin double-negative expression. Oncology Letters, 2016; 12: 530-536.
3. Zahra MH, Abdolaziz Kh, Zahra A. Circulating Levels of M30 and M65 Molecules in Transitional Cell Carcinoma of the Bladder and Their Relation to Tumor Progression.Iran J Cancer Prev, 2016; 9(2):1-8.
4. Yi Y, Shengjie Y, Chuan L, Xunhua L, Guangyong X, Weili Z. Metadherin knockdown suppresses bladder cancer cell invasion and metastasis by inhibiting the epithelial to mesenchymal transition. Int.J.Clin.Exp.Pathol., 2016; 9(2):802-814.
5. AL-Faisal AHM, Nafeh MA. Detection of five novel mutations in *K-ras* gene for Iraqi patients with bladder cancer. IJSBAR, 2015; 24(5):76-86.
6. Caroline B, Jonathan Ch, Zena W. Remodeling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol., 2014; 15(12): 786–801.
7. AL-Faisal AHM, Bresam S. Detection of Three Novel Mutations in Exon 7 of *FGFR3* Gene in Iraqi Patients with Bladder Cancer. Journal of Biology, Agriculture and Healthcare, 2015; 5:218-225.
8. AL-Faisal AHM, Bresam S. Association of Exon 9 *FGFR3* Mutations and Cancer Grads in Patients with Bladder Cancer. IJB. 2016; 15 (2): 109-118.
9. Sarah H, Genevieve H, Meghan L, Mckenna L, Shannon B, Karolina L, *et al*. EMT and tumor metastasis. Clinical and Translational Medicine, 2015; 4(6):1-13.
10. Yuan L, Yanan W, Chunqin Ch, Jiawen Z, Wenyan Q, Yu D, *et al*. LSD1 binds to HPV16 E7 and promotes the epithelial-mesenchymal transition in cervical cancer by demethylating histones at the Vimentin promoter. Oncotarget, 2017; 8(7): 11329-11342.
11. Sadiqa K. Quadri Cross talk between focal adhesion kinase and cadherins: Role in regulating endothelial barrier function. Microvasc. Res., 2012 ; 83(1): 3–11.
12. Clairotte A, Lascombe I, Fauconnet S, Mauny F, Félix S, Algros M.P, *et al.* Expression of E-cadherin and α-, β-, γ-catenins in patients with bladder cancer identification of γ-catenin as a new prognostic marker of neoplastic progression in T1 superficial urothelial tumors.AmJ.Clin Pathol., 2006; 125(1):119–126.
13. Ricardo LF, Atessa B, Romain M, Andrea H, Bernhard G, Vitaly M, *et al*. Prognostic role of decreased E‐cadherin expression in patients with upper tract urothelial carcinoma: a multi‐institutional study. World J Urol., 2017; 35:113–120.
14. Ombretta R, Paolo DP, Valli DR, Vincenzo C, Renato C. Levels of Soluble E-Cadherin in Breast, Gastric, and Colorectal Cancers. BioMed Res.Inter. 2014; Article ID 408047: 7 pages.
15. Pratima UP, Julia D, Landon JI, Robert WM, Ayyappan KR. Carcinoma cells induce lumen filling and EMT in epithelial cells through soluble E-cadherin-mediated activation of EGFR. J. Cell Sci., 2015; 128:4366-4379.
16. Yanyuan W, Marianna S, Jaydutt VV. Epithelial-Mesenchymal Transition and Breast Cancer. J. Clin. Med., 2016; 5(13): 1-18.
17. Arun S, and Shulin L, Vimentin as a potential molecular target in cancer therapy Or Vimentin, an overview and its potential as a molecular target for cancer therapy. Cell Mol Life Sci., 2011; 68(18): 3033–3046.
18. Leong H S, Mahesh B M, Day J R, Smith J D, McCormack A D, Ghimire G, Podor T J, Rose M L. Vimentin autoantibodies induce platelet activation and formation of platelet-leukocyte conjugates via platelet-activating factor. JLB, 2008; 83:263-271.
19. Ding X, Wang Y, Ma X, Guo H, Yan X, Chi Q, *et al*. Expression of HMGA2 in bladder cancer and its association with epithelial-to-mesenchymal transition. Cell Proliferation, 2014; 47(2): 146–151.

20. Richard T B. Cell adhesion and urothelial bladder cancer: the role of cadherin switching and related phenomena, 2016; Downloaded from http://rstb.royalsocietypublishing.org/ on September 22.
21. Samy L, Jian X, Rik D. Olecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol., 2014; 15(3): 178–196.
22. Chou-Kit C, Chang-Yi W, Jeff Yi-Fu C, Ming-Chong N, Hui-Min D W, Jen-Hao C, *et al*. BubR1 Acts as a Promoter in Cellular Motility of Human Oral Squamous Cancer Cells through Regulating MMP-2 and MMP-9. Int. J. Mol. Sci., 2015; 16, 15104-15117.
23. Rosai J. Ackerman's surgical pathology, 1996; 8th(ed). Mosby-Year Book, Inc.1: 1185-1211.
24. Sumi T, Yoshida H, Hyun Y, Yasui T, Matsumoto Y, Hattori K, *et al*. Expression of matrix metalloproteinases in human transitional cell carcinoma of the urinary bladder. Oncol. Rep., 2003; 10:345-349.
25. Blancato J, Singh B, Liao D J, Dickson R B. Correlation of amplification and over expression of the c-myc oncogen in high-grade breast cancer, FISH, *in situ* hybridization and Immunohistochemical analysis. Br J Cancer, 2004; 90: 1612-1619.
26. SAS. Statistical Analysis System, User's Guide. Statistical, 2012; Version 9.1th (ed). SAS. Inst. Inc. Cary. N.C. USA.
27. Taha N Y, Ayhan D, Ebru Ş A, Handan Ö, Hakan Ö. Clinical Use of Tumor Markers for the Detection and Prognosis of Bladder Carcinoma: A Comparison of CD44, Cytokeratin 20 and Survivin. Urol. Oncol. 2016; 13(3):2677-2683.
28. Sebastien A, Jacques F, Isabelle S, Ariana Z, Ahmedin J, Freddie B. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. Europ. Urol. 2017; 71: 96-108.
29. Jun Z, Dahai D, Lingling S, Guiming Z, Lijiang S. Prognostic significance of the epithelial-tomesenchymal transition markers e-cadherin, vimentin and twist in bladder cancer. IBJU, 2014; 40 (2): 179-189.
30. Egbert B, Michael S C, Brasil S N, Micah A J, Chad W, Kimberly M, *et al*. Identification and Prognostic Significance of an Epithelial-Mesenchymal Transition Expression Profile in Human Bladder Tumors. Clin Cancer Res., 2007; 13(6): 1685-1694.
31. Areej AH, Jasim M K, Alaa Gh H. The Role of Matrix Metalloproteinase-2 and -9 *in situ* Hybridization in Bladder Cancer Progression. Iraqi J Med Sci., 2011; 9 (3):247-254.
32. AL-Faisal A H M, Amer M K, Ahmed A S. Detection of Codon 12/13 g.6262G>A Mutation of H-ras Gene in Iraqi Bladder Carcinoma Patients. IJB, 2015; 14(1): 44-52.
33. Bai X, Li Y, Zhang H, Wang F, He H, Yao J, *et al*. Role of matrix metalloproteinase-9 in transforming growth factor-β1-induced epithelial–mesenchymal transition in esophageal squamous cell carcinoma. Onco Targets Ther., 2017; 2(10): 2837—2847.
34. Joel W S, Takashi K, Paul P, Keiji I, Sun J K, Jonathan I, *et al*. Treatment with Low-Dose Interferon- Restores the Balance between Matrix Metalloproteinase-9 and E-Cadherin Expression in Human Transitional Cell Carcinoma of the Bladder. Clin Cancer Res, 2001; 7: 2840–2853.
35. Yokoyama K, Kamata N, Fujimoto R, Tsutsumi S, Tomonari M, Taki M, *et al*. Increased invasion and matrix metalloproteinase-2 expression by Snail-induced mesenchymal transition in squamous cell carcinomas. Int. J Oncol., 2003; 22(4):891–898.

التعبير عن بعض المعلمات الطلائية- الميزنكيمية وانتشار الاورام السرطانية للتنبؤ بمستقبل مرضى سرطان الخلايا الانتقالية للمثانة البولية

مي خليل اسماعيل

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة:

من بين السمات الأكثر تميزًا للأورام الخبيثة هي غزو الورم الخبيث وانتشار الخلايا السرطانية. أثبتت الدراسات الحديثة أن الانتقال الطلائي- الميزنكيمي يساهم في هذا الحدث المهم ويرتبط مع فقدان الخواص الطلائية واكتساب ميزات الخلايا الميزنكيمية. علاوة على ذلك ، فقد تم مؤخرًا إثبات أن بعض انواع الانزيمات المحللة للبروتين المعروفه باسم Matrix metalloproteinases (MMPs) والتي تلعب دورا مهما في غزو الأورام الخبيثة عن طريق إعادة تشكيل المادة الخارج خلوية (ECM) يمكن أن تحفز الانتقال الطلائي - الميزنكيمي بشكل مباشر. في هذه الدراسة ، تم التحري عن نمط تعبير بعض علامات الانتقال الطلائي- الميزنكيمي (E-cadherin و Vimentin) وبعض الانزيمات المحللة للبروتين(MMP-2 و MMP-9) في الجسم الحي لتحديد ما إذا كانت علامات الانتقال الطلائي - الميزنكيمي ذات الصلة و MMPs يمكن أن تتكهن بمستقبل مرضى سرطان الخلايا الانتقالية للمثانة البولية. شملت الدراسه 55 خزعة سرطانية مطمورة بشمع البارافين مأخوذه من المرضى الذين يعانون من سرطان الخلايا الانتقالية و10 خزع نسيجية طبيعية ماخوذه من المثانة خلال التشريح.

تم تقييم نمط تعبير E-cadherin و Vimentin بواسطة تقنية التصبيغ المناعي النسيجي immunohistochemistry بينما تم تحديد تعبير mRNA السيتوبلازمي لكل من MMP-2 و MMP-9 بواسطة التهجين الموضعي *in situ* hybridization . لوحظ زيادة التعبير عن جميع العلامات بشكل ملحوظ مع زيادة عمر المريض (≤ 50 سنة) ولدى الذكور مقارنة بالإناث. ومن المثير للاهتمام أن جميع الأنسجة السليمة أظهرت تعبيرًا إيجابيًا عن E-cadherin في حين أنها لم تظهر أي تعبير عن Vimentin و MMP-2 و MMP-9. بينما انخفض تعبير E-cadherin مع تقدم درجة ومرحلة الورم وكان هذا الفرق ذو دلالة إحصائية معنوية ؛ في حين زاد التعبير عن Vimentin وفقا لدرجة ومرحلة الورم. وبالمثل ، سجلت زيادة في التعبيرعن كل من MMP-2 و MMP-9 مع تطور السرطان. ويمكن أن نستنتج أن انخفاض E-cadherin جنبا إلى جنب مع زيادة Vimentin، MMP-2 و MMP-9 كانت علامات هامة تنذر بسوء مستقبل سرطان الخلايا الانتقاليه.

الكلمات المفتاحية: E-cadherin ، MMP-2، MMP-9 ، TCC ، Vimentin.