OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Evaluation of Cytoskeleton-Associated Proteins; Ezrin, Podocalyxin and Paxillinin Urothelial Carcinoma of the Urinary Bladder

Manal A. khalaf[1-2*], Nagwa M. Zenhom[3-4], Muhammed Gamal Mekhemar[4], Rash Fouad[3], Maram El-Hussieny[1]

[1]Department of Pathology, Faculty of Medicine, Minia University, 61511, El-Minia, Egypt
[2]Department of Pathology, Faculty of Medicine, Albaha University, 65527, Albaha, Saudia Arabia
[3]Department of Biochemistry, Faculty of Medicine, Minia University, 61511, El-Minia
Egypt
[4]Department of Urology, Minia University Hospital, Minia University, 61511, El-Minia, Egypt

DOI: 10.36348/sjpm.2021.v06i12.001 | **Received:** 03.01.2021 | **Accepted:** 13.01.2021 | **Published:** 08.12.2021

***Corresponding author:** Manal A. khalaf

Abstract

Ezrin, podocalyxin and paxillin are cytoskeleton-associated proteins, implicated in several malignancies, but their role in Urothelial carcinoma of the bladder (UCB) is still controversy. We investigated the expression of those markersin UCB and the association of their expression pattern with clinicopathological parameters.Quantitative rt-PCR analyses used to examine mRNA level of ezrin and paxillinin23 fresh specimens of UCB and adjacent normal mucosal tissues from patients undergoing cystoscopy.Also, immunohistochemical evaluation of ezrin, podocalyxin and paxillin was performed on 123 paraffin blocks of UCB. Ezrin mRNA levelwas significantly high in normal mucosa than in UCB (p=0.012) while paxillinmRNA level wasn'tsignificant (p=0.34). Significant negative correlation was found between membranous ezrin immunoexpression and prognostic parameters as grade,T-stage and recurrence(p=0.001, p<0.0001and p= 0.002 respectively). Regarding paxillin immunoexpression,significant negative correlation was detected with grade andT-stage (p<0.0001). Membranous expression of podocalyxin was significantly correlated with grade, T-stage and recurrence (p=0.016, p= 0.003 and p<0.0001respectively). A significant correlation was found between three markers.These results suggest that ezrin, podocalyxin and paxillin could be valuable prognostic molecules in evaluation of UCB and this might lead to establishment ofnew molecular therapeutic strategy and prognostic biomarkers in UCB.

Key words: Ezrin; Podocalyxin; Paxillin; Urothelial Carcinoma.

1. INTRODUCTION

Urinary bladder cancer (UBC) is a serious problem; it ranks the 7th in men and the 17thin women. In developed countries, 90% of UBC is urothelial carcinoma [1]. In Egypt, UBC represents about 12.7% of total malignant tumors.It ranks the2ndbetween malignant tumors in males after liver cancer, with 4:1 male to female ratio, andthe median age at diagnosis is 60.5 years. About 73% of all UBCare due tourothelial carcinoma of the bladder (UCB) [2, 3].

Ezrinis a significant member of the ezrin/radixin/moesin (ERM) family of proteins.ERM proteins act as a group of adaptor molecules linking actin cytoskeleton to plasma membrane.They controlthe functions of cytoskeleton as cell adhesion, survival and motility [4,5]. The expression of ezrin and its prognostic significance are variable in different cancer types.Ezrinexpression is associated with aggressive phenotype in several tumors, such asmelanoma and endometrial cancer [6, 7]. Furthermore,it induced metastasis by several downstream effectors, depending on which residue of ezrin is phosphorylated. Generally, phosphorylation of tyrosine residues activates a signaling cascade that subsequently activates the Phosphatidylinositol-3kinase (PI3K) pathway to promote tumorprogression and metastasis [8].

Podocalyxin (podocalyxin-like protein, PODXL) is a CD34-relatedanti-adhesive transmembranesialomucin. It is expressed bypodocytes and a variety of normal cells during embryonic development, where it plays an important role inmorphogenesis. In the kidney, itfunctions as an anti-adhesion molecule which keepsfiltration pores between neighboring podocyte foot processes [5, 9, 10]. It plays a critical role inepithelial–mesenchymal transition as it could regulate and interact with E-cadherin[11].Many

studies haveconfirmed PODXL as a prognostic indicator of poor outcome in a variety of malignancies including; colorectal [10] and breast [12].

Paxillin is an essential component of focal adhesions (FAs) and has a significant role in the transduction of extracellular signals into intracellular responses. It contributes to the recruitment of specific kinases, oncoproteins and structural proteins which are involved in intracellular signaling cascades. The activation of these pathways leads to reorganization of cytoskeleton needed for cell attachment and migration [13, 14]. Its involvement in cell migration was firstly suggested by the high levels of expression of its phosphorylated protein form found in multiple cancers and metastatic malignant cells [15]. Paxillin was significantly relevant to poor prognosis of patients with gastric cancer [16] and colorectal cancer [17].

The aim of this work is to evaluate the expression of ezrin, PODXL and paxillin in UCB and the correlations between these proteins and clinicopathological data to evaluate the utility of them as therapeutic modalities and prognostic markers. In addition, we evaluated the relationship between these markers. Wealso analyzed mRNA levels of both ezrin and paxillin in order to demonstrate their role in carcinogenesis of UCB.

2. MATERIAL AND METHODS

2.1. Clinical Samples and Cases selection

This study was in accordance with the ethical standards and carried after approval of the Ethics Committee at Faculty of medicine, Minia University. The present study included 123 cases of UCB, which were chosen from the Hospital archive of the Department of Pathology, Minia University Hospital. Tumor tissues were obtained from 86 patients who had undergone radical cystectomy and 37 patients had done transurethral resection of the bladder.

Concerning real-time polymerase chain reaction (rt-PCR), multiple biopsies were taken from any suspected malignant lesions and adjacent normal mucosa. Samples were obtained from patients undergoing cystoscopy at the Department of Urology, Minia University Hospital, after obtaining written consent of the patients. The fresh specimens were divided into 2 parts: part one was frozen by liquid nitrogen for rt-PCR, and part two was fixed in10%formalin, processed and embedded in paraffin wax. Clinicopathological data were retrieved from the medical records and pathology reports.

2.2. Immunohistochemical procedures forezrin, PODXL and paxillin

Five µm sections were prepared for immunohistochemistry for primary antibodies, utilising the avidin biotin-peroxidase complex with diaminobenzidine (DAB) chromagen detection system. Slides were stained withezrin antibody (Monoclonal mouse antibody, clone MS – 661 – R7, 7 ml ready to use, Lab Vision Laboratories, USA),podocalyxin antibody (Polyclonal rabbit antibody, 7ml Ready to use, Lab Vision Laboratories, USA) andpaxillinantibody (Monoclonal mouse antibody clone 5H11, 7ml Ready to use, Lab Vision Laboratories, USA).Slides were incubated overnight using standard techniques. Normal kidneywas used as positive control for PODXL expression while, normal colorectal epithelium was the positive control for ezrin and finally, paxillin positive control was colon adenocarcinoma.

Assessment of the slides was performed by two independent pathologists, who were blinded to both clinicaland outcome data, under light microscopy using an Olympus microscope, Japan.

Ezrin staining of the membrane, and cytoplasm was evaluated for each case. The percentage of cells with membranous staining was evaluated and the median value of each case was used in statistical analysis [18]. PODLX expression was recorded as: (0) = negative expression, (1) = weak cytoplasmic positivity in any proportion of cells, (2) = moderate cytoplasmic positivity in any proportion, (3) = distinct membranous positivity in <50% of cells, (4) = distinct membranous positivityin >50% of cells.

Positive expression was preserved for both scores 3 and 4 [19]. Paxillin cytoplasmic staining was considered positive. The frequency of staining was scored as: (%0)=0, (≤10%)=1, (11-50%)=2, (51-80%)=3, (≥81)=4. The intensity of staining was classified as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The final staining score (0-7) was obtained by combining staining intensity and frequency. Further cases were classified into 4 grades: “0”, <10% of cells stained positive regardless of intensity; “1+”, 3 points; “2+”, 4–5 points; and “3+”, 6–7 points. For statistical analyses, specimens were classified by a three-tier semiquantitative scheme: “0”, negative expression; “1+”, low expression; and “2+” and “3+”, high expression [20].

2.3. Quantitative rt-PCR for ezrin and paxillin gene expression

Total RNA was extracted from bladder tissue homogenate using RiboZol reagent (Amresco, Solon, USA) following the manufacturer’s instructions. Quantitative rt- PCR was performed with 50 ng RNA template per reaction using Thermo Scientific one step kits in 25 µL reaction volumes containing 70 nM of specific primers inrt-PCR Detection System (Kappa Biosystems, USA). The SYBR green data were analyzed with a relative quantification to 18s RNA as reference gene. The sets of primers used were in table (1).

Then the samples were placed in a thermal cycler (Applied Biosyst 7500 fast, Techne (Cambridge) LTD., UK).The relative expression level of ezrinand paxillingenes was calculated using the formula 2 (-ΔΔCt) according to Van Guilder *et al*. [21]. They were scaled relative to controls where control samples were set at a value of 1. Thus, results for all experimental samples were graphed as relative expression compared with the control.

2.4. STATISTICAL ANALYSIS

Descriptive data are presented as range (minimal and maximal) and median for continuous non-parametric variables (ezrin expression) and percentages for categorical variables. Mann Whiteny U test and Kruskal-Wallis H test were used for ezrin expression. Chi-square (X2) test and Fishers exact test were done to test for the differences between categorical data. Statistical analysis was done using SPSS® Release 17 (SPSS, Inc.). Statistical significance was determined at p value of ≤0.05.

3. RESULTS

3.1. Ezrinimmunohistochemicalexpression

Membranous ezrin expressionwas significantly reduced in high grade tumors, advanced T-stage and in recurrent tumors (p=0.001,p< 0.0001 and p= 0.002 respectively). In addition, there was a significant association between reduced membranous ezrin expression and female gender (p= 0.015) (Table 2).

3.2. PODXL immunohistochemical expression

PODXL displayed membranous expression in 25.2% of cases.Our results revealed strong significant positive associations between membranous PODXL expression and high grade, advanced T-stage and recurrent tumors (p= 0.016,p=0.003and p<0.0001 respectively). Moreover, significant association was observed between membranous PODXL expression and female gender (p= 0.007) (Table 3).

3.3. Paxillinimmunohistochemical pattern

Paxillin showed cytoplasmic expression**.** Positive expression was found in 73% of cases. Analyses of data revealed significant associations between reduced paxillin expression and high grade tumors, advanced T-stage and recurrent tumors. There was a significant association between reduced expression and female gender (Table 4).

3.4. Relation between ezrin, PODXL and paxillin expression

Table (5) demonstrates statistically significant inverse association between reduced ezrin expression and membranous PODXL expression (p< 0.0001). Also significant association between ezrin and paxillin expression (p<0.0001) and a significant inverse association between membranous PODXL expression and paxillin expression (p<0.001) was found.

3.5. Quantitative rt-PCR for ezrin and paxillin gene expression

Analyses of mRNA level ofezrin and paxillin revealedthatezrin was highly elevated in urothelial carcinoma group when compared to control group (adjacent normal mucosa) (p=0.001). Furthermore, relative mRNA expression of paxillin was significantly elevated in urothelial carcinoma group incomparison with control group (p=0.001).

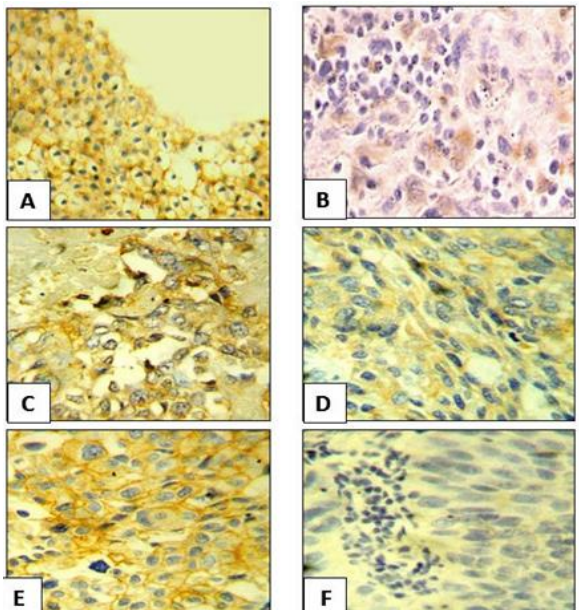

Fig-1: Immunohistochemical expression of ezrin,paxillinand podocalyxin in urothelial carcinoma: (A)Ezrin positive membranous staining (B) negative ezrin staining. (C)Paxillin positive cytoplasmic staining (D) negativepaxillin staining (E)Podocalyxin positive membranous staining and (F) Podocalyxin negative staining. (Streptavidin-biotin-immunoperoxidase 400X)

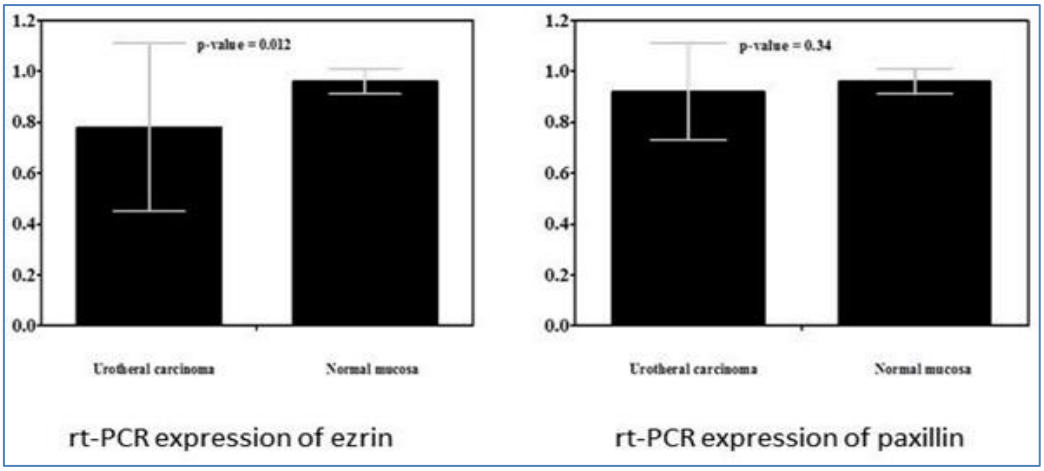

Fig-2: RT-PCR expression of ezrinand paxillin

Relative mRNA expression of ezrin was significantly decreased in urothelial carcinoma group as compared to control group (adjacent normal mucosa) (p=0.012). While there was a non-significantrelation between mRNA expression in urothelial carcinoma group and control group (adjacent normal mucosa) (p=0.34).

Table-1: The sets of primers

Gene	Primer sequence
ezrin	*sense: 5′-AGCTGTGAAGAGACTCTGTTTG-3′* *antisense: 5′-CTTAGCTGTGAAGGAGAAAGC-3′*
paxillin	*Sense*: *5′- CATATCGCCTGAGTTGCTT-3′* *Antisense*: *5′-CACCTGCTTGTGCAAGAAA-3′*
18s RNA	*sense: 5′-CCTGGATACCGCAGCTAGGA-3′* *anti sense: 5′-GCGGCGCAATACGAATGCCCC-3′*

Table-2: Associations of ezrin expression with clinicopathological characteristics

Clinicopathological characteristics	n = 123	Ezrin expression		*p*-value
	No (%)	Range	Median	
Mean Age ± SD				
< average	59 (48%)	0-100	30	0.3
≥ average	64 (52%)	0-100	45	
Gender				
Male	85 (69.1%)	0-100	17.5	0.015*
Female	38 (30.9%)	0-100	50	
Grade				
Grade 1	27 (22%)	0-100	80	
Grade 2	50 (40.6%)	0-90	45	0.001*
Grade 3	46 (37.4%)	0-70	10	
Stage				
Ta	19 (15.5%)	0-100	80	
T1	30 (24.4%)	0-100	57.5	<0.0001*
T2-4	74 (60.1%)	0-80	15	
Recurrence				
Occurred	24 (19.5%)	0-55	17.5	0.002*
Not occurred	99 (80.5%)	0-100	45	

Mann Whitney U-test: for comparison between 2 groups; Kruskal-Wallis H-test, for comparison between more than 2 groups. * Significant if p value<0.05.

Table-3: Associations of PODXL expression and clinicopathological characteristics

Clinicopathological Characteristics	n = 123	PODXL expression		p-value
		Negative No = 92 (74.8%)	Positive No = 31 (25.2%)	
	No (%)	No (%)	No (%)	
Mean Age ± SD				
< average	59 (48%)	45 (48.9%)	14 (45.2%)	0.84
≥ average	64 (52%)	47 (51.1%)	17 (54.8%)	
Gender				
Male	85 (69.1%)	70 (76.1%)	15 (48.4%)	0.007*
Female	38 (30.9%)	22 (23.9%)	16 (51.6%)	
Grade				
Grade 1	27 (22%)	24 (26.1%)	3 (9.7%)	
Grade 2	50 (40.6%)	40 (43.5%)	10 (32.2%)	0.016*
Grade 3	46 (37.4%)	28 (31.4%)	18 (58.1%)	
Stage				
Ta	19 (15.5%)	19 (20.7%)	0 (0%)	
T1	30 (24.4%)	25 (27.2%)	5 (16.1%)	0.003*
T2-4	74 (60.1%)	48 (52.1%)	26 (83.9%)	
Recurrence				
Occurred	24 (19.5%)	9 (9.8%)	15 (48.4%)	<0.0001*
Not occurred	99 (80.5%)	83 (91.2%)	16 (51.6%)	

Chi-square test, *Significant difference (p value <0.05)

Table-4: Associations of paxillin expression with clinicopathological Characteristics

Clinicopathological Characteristics	n = 123	Paxillin expression n (%)			*p*-value
		Negative 33(26.82%)	Low 68(55.28%)	High 22(17.88%)	
Mean Age ± SD					
< average	59 (48%)	12 (36.4%)	31 (45.6%)	9 (40.9%)	0.67
≥ average	64 (52%)	21 (63.6%)	37 (54.4%)	13 (59.6%)	
Gender					
Male	8569.1%)	17 (51.5%)	58 (85.3%)	10 (45.5%)	<0.0001*
Female	38 (30.9%)	16 (48.5%)	10 (14.7%)	12 (54.5%)	
Grade					
Grade 1	27 (22%)	3 (9.1%)	8 (11.8%)	16 (72.7%)	
Grade 2	50 (40.6%)	12 (36.4%)	33 (48.5%)	5 (22.8%)	<0.0001*
Grade 3	46 (37.4%)	18 (54.5%)	27 (39.7%)	1 (4.5%)	
Stage					
Ta	19 (15.5%)	1 (3%)	5 (7.4%)	13 (59%)	
T1	30 (24.4%)	8 (24.3%)	18 (26.5%)	4 (18.2%)	<0.0001*
T2-4	74 (60.1%)	24 (72.7%)	45 (66.1%)	5 (22.8%)	
Recurrence					
Occurred	24 (19.5%)	15 (45.5%)	7 (10.3%)	2 (9.1%)	<0.0001*
Not occurred	99 (80.5%)	18 (54.5%)	61 (89.7%)	20 (90.9%)	

Chi-square test, **Fishers exact test,*Significant difference (p value <0.05)

Table-5: Associations of ezrin expression with PODXL and paxillin expression

Marker expression	n = 123	Ezrin expression		p-value
	No (%)	Range	Median	
PODXL expression				
Negative	92 (74.8%)	0-100	50	<0.0001*
Positive	31 (25.2%)	0-70	10	
Paxillin expression				
Negative	33 (26.8%)	0-100	80	
Low	68 (55.3%)	0-80	30	<0.0001*
High	22 (17.9%)	0-70	10	

Mann Whitney U-test: for comparison between 2 groups; Kruskal-Wallis H-test, for comparison between more than 2 groups. * Significant if p value<0.05.

4. DISCUSSION

The results from this study demonstrate that membranous ezrin expression was significantly reduced in high grade tumors, advanced T-stage and in recurrent tumors. These findings were in accordance with Andersson *et al*. [22] as they observed that low membranous expression was significantly associated with advanced T-stage and high grade tumors.Another study detected that low ezrinmembranous expression was significantly correlated with high grade tumors and with muscle invasion [23]. These findings support the hypothesis that reducedezrin membranous expression in UCB is associated with aggressive tumor phenotype.

A significant association between reduced membranous ezrin expression and female gender was observed in our study. The same significant association was detected in a previous study [22].While a former study foundno correlation between ezrin expression and gender [18]. Thus ezrin may be a relevant biomarker in researches studying the molecular epidemiology of UCB and in determining the influence of sex hormones and reproductive factors on cancer risk factors.

In further agreement with our results, down-regulationof ezrin has been previously reported in othertumors such as gastric carcinoma, where weak ezrinimmuoreactivity correlated with clinicopathologicalparameters and adverse prognosis [24]. Theseresults suggest that down-regulation of ezrin in UCB may be involved in tumor progression.

In contrast to our findings, several results demonstrated that overexpression of ezrinis found to be associated with aggressive phenotype in several tumors, such as melanoma and endometrial cancer [6, 7]. Hence, both down-regulation and up-regulation of ezrin in relation to increased metastatic ability and poor prognostic parameters have been reported in tumors. This apparent discrepancy might be due to the fact that ezrin shows multiple functions in adhesion, migration, survival and proliferation that may differ among various cell types [25, 26].

To our Knowledge, this is the first time to analyzeezrinmRNA levelby rt-PCR in UCB and adjacent normal mucosa. It was decreased in malignant tissue significantly than the adjacent normal mucosa. These results are in accordance with a previous study which evaluated ezrin mRNA level in normal esophageal mucosa and esophageal squamous cell carcinoma. Although the mechanism of its down-regulation in neoplastic cells is not obvios, it could be explained by corresponding changes in mRNA level according to what was detected by rt-PCR [27].

In this study, PODXL displayed membranous expression in 31/123 (25.2%) of cases of UBC.A higher proportion (25.2%) of cases with membranous PODXL expression compared with other studies carried onUBC, as they showed percentages ranged from (10.2%-21.0%) [19, 22, 28].

In line with studies on UBC, our results revealed strong significant associations between membranous, not cytoplasmic, PODXL expression andunfavorable tumor parameters including; high grade, advanced T-stage and recurrent tumors [22, 28]. Moreover, we found significant association was observed between membranous PODXL expression and female gender.

A study developed a monoclonal antibody that targets PODXL in a preclinical mouse study, and it inhibited tumor growthand metastatic progression in breast tumor cells [12]. Therefore, PODXL mayrepresent a novel therapeutic target in metastatic carcinoma.

Concerning paxillin, positive cytoplasmic expression was found in 90/123 (73%) of cases.Additionally, High expression was significantly low in grade 2 (22.8%) than grade 3 (4.5%) tumors. Also it showed significant low expression in advanced tumor stage. This agreed with Athanasopoulou *et al*. [23], who found positive expression in (93.7%) of UCB. Moreover, they showed low paxillin protein expression in high grade UCB compared with grade 1 and a significantly lower expression in invasive tumors. Consistently, decreased paxillin expression had been detected in breast carcinoma, where it correlated with positive lymph nodes indicating its effect in the metastasis [29].

On contrast to our findings, high paxillinexpression was accompanied with a great possibility of malignant behavior through the effect of paxillin on cell proliferation regulated by tyrosine/serine phosphorylation in colorectal cancer 30.

To our knowledge, no previous study did examine paxillin mRNA level in UBC and adjacent normal mucosa. We found that paxillinm RNA level was decreased in urothelial carcinoma when correlated with adjacent normal mucosa but this wasn't significant ($p<0.34$). Paxillin mRNA was evaluated in esophageal carcinoma and was significantly higher ($P<0.05$) than normal esophageal mucosa [31].

A positive correlation was demonstrated between ezrin and paxillin immunoexpression in urothelial carcinoma. This could suggest a significant synergistic effect of these two genes incarcinogenesis**.** So, impaired ezrin expression has beenshown to alter focal adhesion mediated cell–matrix adhesion, suggesting a cross talk between these two actincytoskeleton-associated proteins [32].

A statistically significant inverse association was found between reduced ezrin and membranous PODXL expression.PODXL expression was found to be correlated with change in the subcellular localization of ezrin and ezrin phosphorylation. Thus, PODXL could affect ezrin regulated signaling pathways [5,22].

On the other hand, inverse association was detectedbetween of paxillin and membranous PODXL expression. No previous studies havedemonstrated the association between paxillin and PODXL in UBC. Lin *et al*. [33] reported that PODXL could regulate the phosphorylation of FAK and paxillin in oral squamous cell carcinoma.

From ourobservations in this study, we could suggest that ezrin, PODXL and paxillin can play essential roles in the pathogenesis, aggressiveness, invasion, and progression of urothelial carcinoma. So further molecular studies of those key molecules could help in detecting prognosis and treatment of UCB, and may assist in the identification of cases that are at greater risk and may have an unfavorable clinical outcome.

Prospective studies on the efficacy of PODXL-targeted therapies in urothelial carcinomas are warranted. Also studying the efficacy of PODXL/ezrin complex and ezrin/paxillin complex could be valuable biological target molecules in the treatment of patients with UCB.Further studies are recommended to demonstrate the relation between ezrin, PODXL and paxillinon molecular basis. These should include in vitro molecular studies and transfer data to a large scale of cases with survival information to confirm their prognostic significance. This in turn will help in identification of patients with aggressive tumors in order to select cases that can benefit from therapy.

Funding

- This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

- The authors reported no conflict of interests regarding the publication of this article.

REFERENCES

1. Torre, L. A., Bray, F., Siegel, R. L., & Ferlay, J. (2012). Lortet− Tieulent J., Jemal A.(2015). *Global cancer statistics*, 87-108.
2. 2-Mokhtar, N., Gouda, I., & Adel, I. (2007). Malignant urinary system tumors. Cancer Pathology Registry, 2003-2004 And Time Trend Analysis. Cairo, Egypt: *Nat Canc Inst. Cairo University. P: 32*
3. Ibrahim, A. S., Khaled, H. M., Mikhail, N. N., Baraka, H., & Kamel, H. (2014). Cancer incidence in Egypt: results of the national population-based cancer registry program. *Journal of cancer epidemiology*, *2014*.
4. Gautreau, A., Poullet, P., Louvard, D., & Arpin, M. (1999). Ezrin, a plasma membrane–microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proceedings of the National Academy of Sciences*, *96*(13), 7300-7305.
5. Sizemore, S., Cicek, M., Sizemore, N., Ng, K. P., & Casey, G. (2007). Podocalyxin increases the aggressive phenotype of breast and prostate cancer cells in vitro through its interaction with ezrin. *Cancer research*, *67*(13), 6183-6191.
6. Mäkitie, T., Carpén, O., Vaheri, A., & Kivelä, T. (2001). Ezrin as a prognostic indicator and its relationship to tumor characteristics in uveal malignant melanoma. *Investigative ophthalmology & visual science*, *42*(11), 2442-2449.
7. Ohtani, K., Sakamoto, H., Rutherford, T., Chen, Z., Kikuchi, A., Yamamoto, T., ... & Naftolin, F. (2002). Ezrin, a membrane-cytoskeletal linking protein, is highly expressed in atypical endometrial hyperplasia and uterine endometrioid adenocarcinoma. *Cancer letters*, *179*(1), 79-86.
8. Elliott, B. E., Meens, J. A., SenGupta, S. K., Louvard, D., & Arpin, M. (2005). The membrane cytoskeletal crosslinker ezrin is required for metastasis of breast carcinoma cells. *Breast cancer research*, *7*(3), 1-9.
9. Nielsen, J. S., & McNagny, K. M. (2009). The role of podocalyxin in health and disease. *Journal of the American Society of Nephrology*, *20*(8), 1669-1676.
10. Kaprio, T., Fermér, C., Hagström, J., Mustonen, H., Böckelman, C., Nilsson, O., & Haglund, C. (2014). Podocalyxin is a marker of poor prognosis in colorectal cancer. *BMC cancer*, *14*(1), 1-7.
11. Taniuchi, K., Furihata, M., Naganuma, S., Dabanaka, K., Hanazaki, K., & Saibara, T. (2016). Podocalyxin-like protein, linked to poor prognosis of pancreatic cancers, promotes cell invasion by binding to gelsolin. *Cancer science*, *107*(10), 1430-1442.
12. Snyder, K. A., Hughes, M. R., Hedberg, B., Brandon, J., Hernaez, D. C., Bergqvist, P., ... & McNagny, K. M. (2015). Podocalyxin enhances breast tumor growth and metastasis and is a target for monoclonal antibody therapy. *Breast cancer research*, *17*(1), 1-14.
13. Deakin, N.O., Turner, C.E. (2008). Paxillin comes of age. J Cell Sci. 121:2435–44.
14. López-Colomé, A. M., Lee-Rivera, I., Benavides-Hidalgo, R., & López, E. (2017). Paxillin: a crossroad in pathological cell migration. *Journal of hematology & oncology*, *10*(1), 1-15.
15. Deakin, N. O., Pignatelli, J., & Turner, C. E. (2012). Diverse roles for the paxillin family of

proteins in cancer. *Genes & cancer*, *3*(5-6), 362-370.
16. Liu, C. J., Kuo, F. C., Hu, H. M., Chen, C. Y., Huang, Y. B., Cheng, K. H., ... & Kuo, C. H. (2014). 17β-Estradiol inhibition of IL-6–Src and Cas and paxillin pathway suppresses human mesenchymal stem cells–mediated gastric cancer cell motility. *Translational Research*, *164*(3), 232-243.
17. Zhao, C. J., Du, S. K., Dang, X. B., & Gong, M. (2015). Expression of paxillin is correlated with clinical prognosis in colorectal cancer patients. *Medical science monitor: international medical journal of experimental and clinical research*, *21*, 1989.
18. Palou, J., Algaba, F., Vera, I., Rodriguez, O., Villavicencio, H., & Sanchez-Carbayo, M. (2009). Protein expression patterns of ezrin are predictors of progression in T1G3 bladder tumours treated with nonmaintenance bacillus Calmette-Guerin. *European urology*, *56*(5), 829-836.
19. Larsson, A., Fridberg, M., Gaber, A., Nodin, B., Levéen, P., Jönsson, G., ... & Jirström, K. (2012). Validation of podocalyxin-like protein as a biomarker of poor prognosis in colorectal cancer. *BMC cancer*, *12*(1), 1-9.
20. Gao, W., Zhang, C., Feng, Y., Chen, G., Wen, S., Huangfu, H., & Wang, B. (2012). Fascin-1, ezrin and paxillin contribute to the malignant progression and are predictors of clinical prognosis in laryngeal squamous cell carcinoma. *PloS one*, *7*(11), e50710.
21. VanGuilder, H. D., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*, *44*(5), 619-626.
22. Andersson, G., Wennersten, C., Gaber, A., Boman, K., Nodin, B., Uhlén, M., ... & Jirström, K. (2014). Reduced expression of ezrin in urothelial bladder cancer signifies more advanced tumours and an impaired survival: validatory study of two independent patient cohorts. *BMC urology*, *14*(1), 1-8.
23. Athanasopoulou, A., Aroukatos, P., Nakas, D., Repanti, M., Papadaki, H., & Bravou, V. (2013, August). Decreased ezrin and paxillin expression in human urothelial bladder tumors correlate with tumor progression. In *Urologic Oncology: Seminars and Original Investigations* (Vol. 31, No. 6, pp. 836-842). Elsevier.
24. Bal, N., Yildirim, S., Nursal, T. Z., Bolat, F., & Kayaselçuk, F. (2007). Association of ezrin expression in intestinal and diffuse gastric carcinoma with clinicopathological parameters and tumor type. *World Journal of Gastroenterology: WJG*, *13*(27), 3726.
25. Bretscher, A., Edwards, K., & Fehon, R. G. (2002). ERM proteins and merlin: integrators at the cell cortex. *Nature reviews Molecular cell biology*, *3*(8), 586-599.
26. Wang, H. J., Zhu, J. S., Zhang, Q., Sun, Q., & Guo, H. (2009). High level of ezrin expression in colorectal cancer tissues is closely related to tumor malignancy. *World journal of gastroenterology: WJG*, *15*(16), 2016.
27. Zeng, H., Xu, L., Xiao, D., Zhang, H., Wu, X., Zheng, R., ... & Li, E. (2006). Altered expression of ezrin in esophageal squamous cell carcinoma. *Journal of Histochemistry & Cytochemistry*, *54*(8), 889-896.
28. Boman, K., Andersson, G., Wennersten, C., Nodin, B., Ahlgren, G., & Jirström, K. (2017). Podocalyxin-like and RNA-binding motif protein 3 are prognostic biomarkers in urothelial bladder cancer: a validatory study. *Biomarker research*, *5*(1), 1-10.
29. Madan, R., Smolkin, M. B., Cocker, R., Fayyad, R., & Oktay, M. H. (2006). Focal adhesion proteins as markers of malignant transformation and prognostic indicators in breast carcinoma. *Human pathology*, *37*(1), 9-15.
30. Chen, H. Y., Shen, C. H., Tsai, Y. T., Lin, F. C., Huang, Y. P., & Chen, R. H. (2004). Brk activates rac1 and promotes cell migration and invasion by phosphorylating paxillin. *Molecular and cellular biology*, *24*(24), 10558-10572.
31. Cai, H. X., Yang, L. C., Song, X. H., Liu, Z. R., Chen, Y. B., & Dong, G. K. (2012). Expression of paxillin and FAK mRNA and the related clinical significance in esophageal carcinoma. *Molecular medicine reports*, *5*(2), 469-472.
32. Hiscox, S., & Jiang, W. G. (1999). Ezrin regulates cell-cell and cell-matrix adhesion, a possible role with E-cadherin/beta-catenin. *Journal of cell science*, *112*(18), 3081-3090.
33. Lin, C. W., Sun, M. S., & Wu, H. C. (2014). Podocalyxin-like 1 is associated with tumor aggressiveness and metastatic gene expression in human oral squamous cell carcinoma. *International journal of oncology*, *45*(2), 710-718.