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Diagnostic Significance of Immunohistochemical Expression of E-26 Transformation-Specific (ETS)-Related Gene (ERG) and Golgi Membrane Protein 1 (GOLM1) in Prostatic Carcinoma

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What's known on the subject? and What does the study add?

Many practical problems have been encountered in the accurate diagnosis of prostatic carcinoma and its differentiation from benign mimicker lesions, even when using the traditional panel of immunohistochemistry that fails to solve the problems. Thus, our research was motivated by the aim to reduce the undiagnosed cases of carcinoma, and our conclusion was encouraging. The combination of *ERG* and Golgi membrane protein 1 (*GOLM1*) as a diagnostic panel has not been discussed before. According to studies on both elements independently, their combination could be promising. We have found that the combination of *ERG* and *GOLM1* is a promising diagnostic panel for PCa, solving many practical diagnostic problems as traditionally encountered with the older panel, thereby leading to proper treatment and better survival outcomes.

Abstract

Objective: Diagnosis of prostatic adenocarcinoma (PAC) and differentiation from benign mimickers' lesions represent one of the most challenging problems. *ERG* and Golgi membrane protein 1 (*GOLM1*) have a role in PAC and may aid in solving this diagnostic dilemma for appropriate treatment, better prognosis, and survival. The aim of our study is to evaluate the diagnostic accuracy of *ERG* and *GOLM1* co-expression as a panel in PAC and the association between their expression and clinicopathological parameters.

Materials and Methods: This cross-sectional study was conducted on forty cases of PAC and twenty-four cases of benign prostatic lesions. Paraffin blocks of all studied cases were cut, and hematoxylin and eosin slides were examined. Immunohistochemical expressions of *ERG* and *GOLM1* were evaluated.

Results: Nuclear *ERG* and paranuclear *GOLM1* expression were observed in 55% and 92.5% of PAC cases, respectively. *ERG* showed 55% sensitivity, 100% specificity and 71.9% diagnostic accuracy, while *GOLM1* showed 92.5% sensitivity, 70.8% specificity and 87.5% diagnostic accuracy. The combined use of markers synchronously revealed 97.5% sensitivity, 70.8% specificity, and 87.5% accuracy. There was a statistically significant inverse association between *ERG* and prostate-specific antigen, Gleason grade groups, ki-67, and a direct association with metastasis. There was a statistically significant association between *GOLM1* and metastasis.

Conclusion: Our study recommends using both *ERG* and *GOLM1* as a panel for improving diagnostic validity of PAC. *ERG* expression could be a favorable prognostic marker, while *GOLM1* may also be a prognostic marker, albeit with limited value.

Keywords: Diagnosis, prostatic adenocarcinoma, *ERG*, *GOLM1*, immunohistochemistry

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Introduction

Prostate cancer (PC) is the second most common cancer (14.2%) and the fifth leading cause of death (7.3%) among men globally. About 1.5 million new cases have been diagnosed since 2022 (1).

One of the challenges in the diagnosis of PC using biopsy is the benign lesions that closely mimic prostatic adenocarcinoma (PAC) and differ in treatment from it. Although the light microscopic findings remain the gold standard for the diagnosis of PAC, difficult cases may benefit from immunohistochemical (IHC) studies (2). The most common diagnostic panel includes amethylacyl CoA racemase (AMACR) and high molecular weight cytokeratin (HMWCK) or p63, but multiple drawbacks have appeared in this panel (3). *ERG* and Golgi membrane protein 1 (*GOLM1*) are two potential IHC markers that are still under validation for use in PAC diagnosis.

ERG, *ETS*-related gene, is a member of the E-26 transformation-specific family of transcription factors. In 1987, ERG was first discovered in human colorectal carcinoma cells by Reddy et al. (4). In 2005, Tomlins et al. (5) identified gene fusions between the androgen receptor (AR)-regulated gene *TMPRSS2* and *ERG*. This fusion is caused by chromosomal translocation or interstitial deletion on chromosome 21. Nuclear expression of *ERG* by IHC correlates with the fusion. *ERG* plays a role in PC by disrupting the differentiation of the prostate epithelium, triggering tumor growth, progression, and angiogenesis through the activation of *MYC*, *AR*, and the nuclear factor-kappa B pathways (6,7).

GOLM1 is type II glycosylated protein residing on cis-Golgi cisternae. It was first isolated from viral hepatitis patients by Kladney et al. (8). It was reported that *GOLM1* acts as a key oncogene in PC by promoting tumor growth, invasion, migration, and metastasis through the Transforming growth factor-beta (TGF-β)/Smad, *AR* and *PIK3-AKT* signaling pathways (9-11).

To our knowledge, this is the first study to investigate the combination of *ERG* and *GOLM1* as a diagnostic panel in PAC. According to the studies on each of them, their combination could be useful in the accurate diagnosis of PAC and solving difficult cases to receive appropriate treatment and a better outcome (8-13).

Our study aimed to evaluate the diagnostic accuracy of *ERG* and *GOLM1* co-expression as a diagnostic panel for PAC and to accurately discriminate PAC from benign mimickers for proper management. Additionally, we sought to assess the association between the expression of *ERG* and *GOLM1* and clinicopathological parameters in PAC.

Materials and Methods

This cross-sectional study included 64 selected cases of prostatic lesions, comprising 40 cases of primary PAC and 24 cases of benign prostatic mimickers, collected between May 2023 and May 2024. Specimens were obtained through transrectal ultrasound-guided biopsy (TRUS), transurethral resection of the prostate (TURP), or radical prostatectomy. Clinicopathological data, including patient age, tumor size, lymph node involvement, metastasis, and preoperative prostate-specific antigen (PSA) serum levels, were retrieved from clinical reports accompanying the specimens. PAC slides were reviewed by two experienced pathologists to assess tumor characteristics. PAC cases were histologically classified according to the current World Health Organization Classification of Urinary and Male Genital Tumors (5th Edition) (14), graded using the Gleason grading system (15), and staged according to the 8th Edition of the The American Joint Committee on Cancer TNM staging system (16).

- The inclusion criteria were cases that were diagnosed as primary PAC or benign prostatic lesions and had clinical data.

- The exclusion criteria include cases with insufficient tissue for staining or a history of chemotherapy or radiotherapy.

Ethical Statement

All procedures were conducted in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki Declaration, along with its later amendments or equivalent ethical guidelines. The study was approved by the Institutional Review Board of the Zagazig University Faculty of Medicine (IRB approval no: 10498, date: 26.02.2023). Written informed consent from participants was obtained.

Immunohistochemistry

IHC staining was performed using the EnVision system technique (DAKO, North America Inc., CA, USA). Tissue sections (3-5 µm) from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, rehydrated, and incubated for 10 minutes in an antigen retrieval solution (pH 6.0). Finally, the slides were incubated with *ERG* (DAKO, Rabbit Monoclonal, Code IR659, ready to use), *GOLM1* (Santa Cruz Biotechnology, sc-365817, 200 µg/mL, mouse monoclonal, dilution 1:500), and Ki-67 (DAKO, Monoclonal, Mouse, Anti-Human, ready to use).

Interpretation and Evaluation of Immunostaining

ERG immunoreactivity was recorded as nuclear staining in neoplastic cells. Staining intensity was classified as follows: 0 (no staining), +1 (mild), +2 (moderate), and +3 (strong). The H-score was calculated by multiplying the intensity score by the percentage of stained cells, resulting in a score ranging from 0 to 300. Cases were categorized as follows: ≤10 (no expression),

11-100 (low expression), 101-200 (intermediate expression), and >200 (high expression). *ERG* positivity was defined as an H-score ≥11. Endothelial cell reactivity served as an internal positive control (12).

GOLM1 immunoreactivity was observed as juxtanuclear staining located on the luminal side of neoplastic cells. A semiquantitative scoring system was used to evaluate both staining intensity (0=no staining, 1=mild, 2=moderate, 3=strong) and the percentage of stained cells (≤5%=0, 6%-25%=1, 26%-50%=2, 51%-75%=3, ≥75%=4). The total score, generating an immunoreactivity score (IRS) for each case, was calculated by multiplying the percentage score and the intensity score. *GOLM1* positivity was defined as IRS ≥4. Human gallbladder tissue was used as a positive control for *GOLM1* (10).

For ki-67 staining, only nuclear staining in tumor cell nuclei was considered positive. A 10% positivity threshold was used as the cut-off point to differentiate between low and high proliferation indices (12).

Statistical Analysis

All data were collected, tabulated, and analyzed using SPSS (Statistical Package for the Social Sciences, version 26, Chicago, Illinois, USA). Mean, standard deviation, median, and range were calculated for quantitative variables, while frequency and percentage were used for qualitative variables. The chi-square test and Fisher's exact test were used to assess the association between two categorical variables. The t-test was used to compare the means of two normally distributed groups to determine whether there was a significant difference between them. The Mann-Whitney U test was used to compare two independent groups that were not normally distributed to assess whether there was a significant difference. The Spearman rank correlation test is used to measure the strength and direction of the relationship between two ordinal variables. Specificity [true negative/(true negative + false positive) × 100%), sensitivity (true positive/(true positive + false negative) × 100%), negative predictive value (NPV) (true negative/(true negative + false negative) × 100%), positive predictive value (PPV) (true positive/(true positive + false positive) × 100%), accuracy (true positive+ true negative)/(true positive + true negative + false positive + false negative) × 100%)], receiver operating characteristic curve, and area under the curve, along with their respective 95% confidence intervals, were calculated. A p-value <0.05 was considered statistically significant, while a p-value <0.001 was regarded as highly statistically significant.

Results

Patients' characteristics: The age of studied cases ranged from 49 to 86 years. All cases of prostatic carcinoma were of the adenocarcinoma type (PAC). Most of the PAC cases (29/40) (72.5%), were above 65 years with a high mean age (68.4), standard deviation (SD) (±8), and interquartile range (11), while 14/24 (58.3%) of benign prostatic lesion cases were below 65 years with a lower mean age (60.6), SD (±8), and interquartile range (12). Regarding PSA, 38/40 (95%) of PAC cases were above 10 ng/mL, whereas all cases of benign prostatic lesions were below 10 ng/mL. Most PAC cases presented with had intermediate grades (group 2, 3) 17/40 (42.5%), low ki-67 expression 21/40 (52.5%), T2 19/40 (47.5%), absence of perineural invasion 21/40 (52.5%), and absent lympho-vascular invasion 35/40 (87.5%) Table 1.

Immunohistochemical Results

ERG: Nuclear *ERG* expression was observed in 22 out of 40 PAC cases (55%) with homogeneous staining in 20 PAC cases, and only two cases were heterogeneous. Strong and diffuse immunostaining of *ERG* (H-score above 200) was detected in 12 cases (30%). The remaining positive cases, eight cases with H-score 100-200, showed moderate staining, while two cases with H-score <100 showed mild staining. Eighteen out of 40 cases (45%) showed negative expression of *ERG* with an H-score of 0. All cases of benign prostatic lesions showed negative *ERG* expression (H-score=0). Highly statistically significant differences have been detected between malignant and benign prostatic tissue regarding *ERG* expression (p<0.001) Table 2/ Figure 1 (a-d), 3 (a, b).

GOLM1: Cytoplasmic granular *GOLM1* expression was observed in 37 out of 40 PAC cases (92.5%). A diffuse and strong *GOLM1* expression [immunoreactivity score (IRS)=12) was observed in 21 out of 40 cases (52.5%). Moderate intensity of *GOLM1* was detected in 16 out of 40 cases, in which 11 cases showed a diffuse pattern with IRS=7-9. While 5 cases showed less diffuse pattern expression with IRS=4-6. Mild intensity and diffuse *GOLM1* expression (IRS=3) were detected in 3 out of 40 PAC cases. Regarding benign prostatic lesions, positive *GOLM1* expression was detected in 7 out of 24 cases (29.2%). *GOLM1* expression was statistically significantly upregulated high in PAC compared with benign prostatic tissue (p<0.001): Table 2, Figure 2 (a-d), Figure 3 (c, d).

Statistical analysis of *ERG* and its diagnostic power in PAC at H-score ≥ 11 revealed 55% sensitivity, 100% specificity, 100% PPV, 57.1% NPV, and 71.9% accuracy. Statistical analysis of the diagnostic power of *GOLM1* at IRS ≥4 revealed 92.5% sensitivity, 70.8% specificity, 84.1% PPV, 85% NPV and 71.9% accuracy. Co-expression of *ERG*+ or *GOLM1*+ (positive expression was considered if either *ERG* or *GOLM1* was positive, whereas both needed to be negative to consider them negative) showed 97.5% sensitivity, 70.8% specificity, 84.8% PPV, 94.4% NPV, and 87.5% accuracy. The combined expressions of *ERG*+ and

Table 1. Clinico-pathological features of the studied cases		
Variables	**Prostatic adenocarcinoma n=40**	**Benign lesions n=24**
Age		
Mean ± SD	6 8.4±8	60.6±8
Median	6 8	60
Range	56-86	49-82
IQR	11	13
Age:	1	
<65	1 (27.5%)	14 (58.3%)
≥65	29 (72.5%)	10 (41.7%)
PSA		
Mean ± SD	74±39	4±2.5
Median	80	3
Range	9-180	1-9
PSA (ng/mL)		
<4	0	13 (54.2%)
4-10	2 (5%)	11 (45.8%)
>10	38 (95%)	0
Procedure used		
-TRUS	21 (52.5%)	4 (16.7%)
-TURP	5 (12.5%)	10 (41.7%)
-RP	14 (35%)	10 (41.7%)
Gleason grading group		
Group 1	8 (20%)	
Group 2,3	17 (42.5%)	
Group 4,5	15 (37.5%)	
Ki-67 expression		
Low	21 (52.5%)	
High	19 (47.5%)	
cT2	19 (47.5%)	
cT3	5 (12.5%)	
cT4	16 (40%)	
Nodal metastasis		
Nx	26 (65%)	
N0	10 (25%)	
N1	4 (10%)	
Metastasis		
Mx	21 (52.5%)	
M0	10 (25%)	
M1	9 (22.5%)	
PNI		
Absent	21 (52.5%)	
Present	19 (47.5%)	
LVI		
Absent	35 (87.5%)	
Present	5 (12.5%)	

GOLM1+ (positivity of both *ERG* and *GOLM1* was required to consider positive, while negative expression was considered if either *ERG* or *GOLM1* was negative) showed 50% sensitivity, 100% specificity, 100% PPV, 54.5% NPV, and 68.8% accuracy. Table 3 and Figure 4.

Regarding the association between *ERG* expressions and clinico-pathological parameters, an inverse significant difference has been detected between positive and negative *ERG* expression cases, regarding PSA level, Gleason grade group, and ki-67 (p-value=0.019, 0.024, 0.005) respectively. Furthermore, a significant association was observed between *ERG* expression and metastasis (p=0.029) Table 4.

Statistical analysis of the association between *GOLM1* expressions and clinico-pathological parameters of PAC cases revealed a statistically significant association between *GOLM1* expression and metastasis (p=0.021). No association could be detected between other parameters and *GOLM1* expression. There was a highly significant association between *ERG* and *GOLM1* expression (p<0.001) Table 4.

Discussion

PC is a major health care challenge and one of the leading causes of mortality among men, often attributed to late diagnosis. Many practical problems have been encountered in the accurate diagnosis of PAC even when using the traditional panel of immunohistochemistry (AMACR and HMWCK or p63) (2,3). Multiple research projects have been documented for other immuno-histochemical markers that can overcome the drawbacks of the old panel and diminish the missing cases of PAC (11,12). *ERG* and *GOLM1* have shown potential roles in PAC diagnosis and prognosis (5-13). Both *ERG* and *GOLM1* as a diagnostic panel haven't been discussed before. According to studies conducted individually on both, their combination could be promising (9-13). In the present study, we evaluated *ERG* and *GOLM1* expression in all studied cases, their diagnostic validity in PAC as well as their potential prognostic role.

TMPRSS2-ERG gene fusion is responsible for *ERG* overexpression in PACs, leading to the activation of other subsequent oncogenes and the *PTEN/AKT/PIK3/mTOR* pathway. Moreover, *ERG* decreases the number of cells arrested at G0 and increases cells at G_1 (5).

In the current study, nuclear *ERG* expression was found in 55% of PAC cases, predominantly with a homogenous pattern; with negative expression in all benign lesion cases, showing a significant difference (p<0.001). Our results were in agreement with the previous studies using the same methods (12,13,17). However, other studies have demonstrated higher

Table 2. Immunohistochemical expression of *ERG*, *GOLM1* in benign prostatic lesions and prostatic adenocarcinoma

ERG expression					GOLM expression				
	Benign prostatic lesions=24	Prostatic adenocarcinoma n=40	X^2	p-value	Benign prostatic lesions n=24		Prostatic adenocarcinoma n=40	X^2	p-value
Negative	24 (100%)	18 (45%)			17 (70.8%)		3 (7.5%)		
Positive	0	22 (55%)			7 (29.2%)		37 (92.5%)		
H-score:					**IRS**				
0-10	24 (100%)	18 (45%)			**<4**	17 (70.8%)	3 (7.5%)		
					≥4:				
11-100	0	2 (5%)			4-6	7 (29.2%)	5 (12.5%)		
100-200	0	8 (20%)			7-9	0	11 (27.5%)		
>200	0	12 (30%)			**10-12**	0	21 (52.5%)		
Staining intensity			20.114	<0.001**				28.005	<0.001**
Negative	24 (100%)	18 (45%)			17 (70.8%)		0		
Mild	**0**	2 (5%)			5 (20.8%)		3 (7.5%)		
Moderate	0	8 (20%)			2 (8.3%)		16 (40%)		
Strong	0	12 (30%)			0		21 (52.5%)		
% of positivity									
Mean ± SD	0	77.9±15			34±40		80.7±12		
Median	**0**	80			0		80		
Range	0	50-100			0-100		60-100		

x^2: Chi-square test, **: Highly significant, TRUS: Transrectal ultrasound, TURP: Transurethral resection of the prostate, RP: Radical prostatectomy, PNI: Perineural invasion, LVI: Lympho-vascular invasion, PSA: Prostatic specific antigen. IQR: Interquartile range, GOLM: Golgi membrane protein, SD: Standard deviation

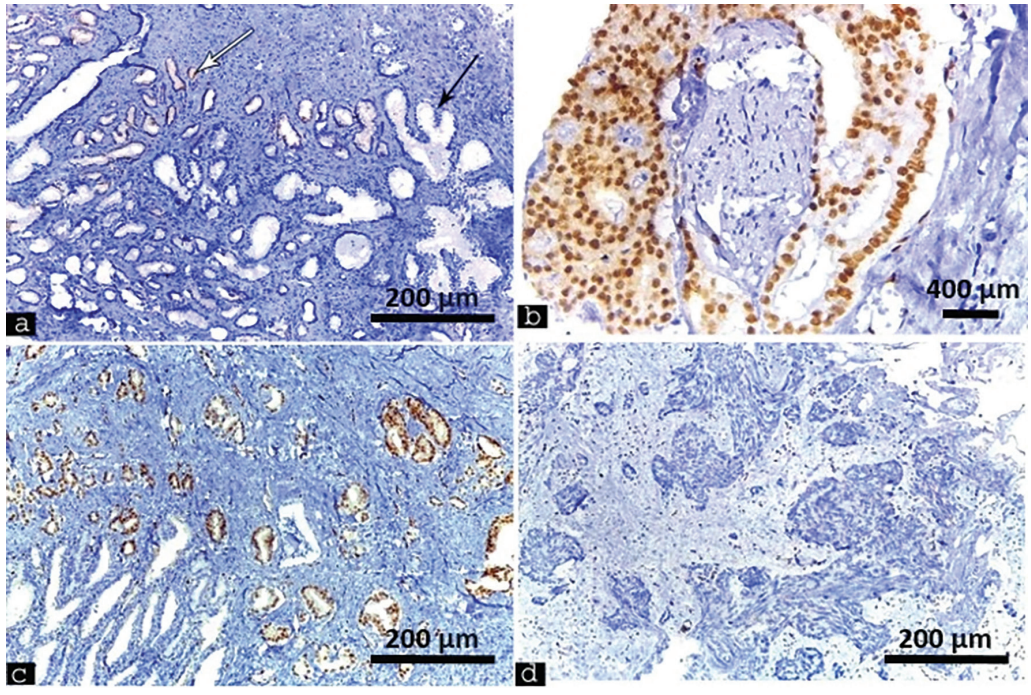

Figure 1. Immunohistochemical expression of *ERG*: **(a):** Mild nuclear expression of PAC (White arrow) and negative staining in adjacent benign glands (Black arrow) (x100) **(b):** Moderate nuclear expression of malignancy (pattern 4) with perineural invasion (x400) **(c):** Strong nuclear expression of malignancy (pattern 3) invading seminal vesicles, T3, with internal control positive) (x100) **(d):** Negative nuclear expression of malignancy (pattern 5) invading bladder wall T4, with positive internal control (X100)

PAC: Prostatic adenocarcinoma

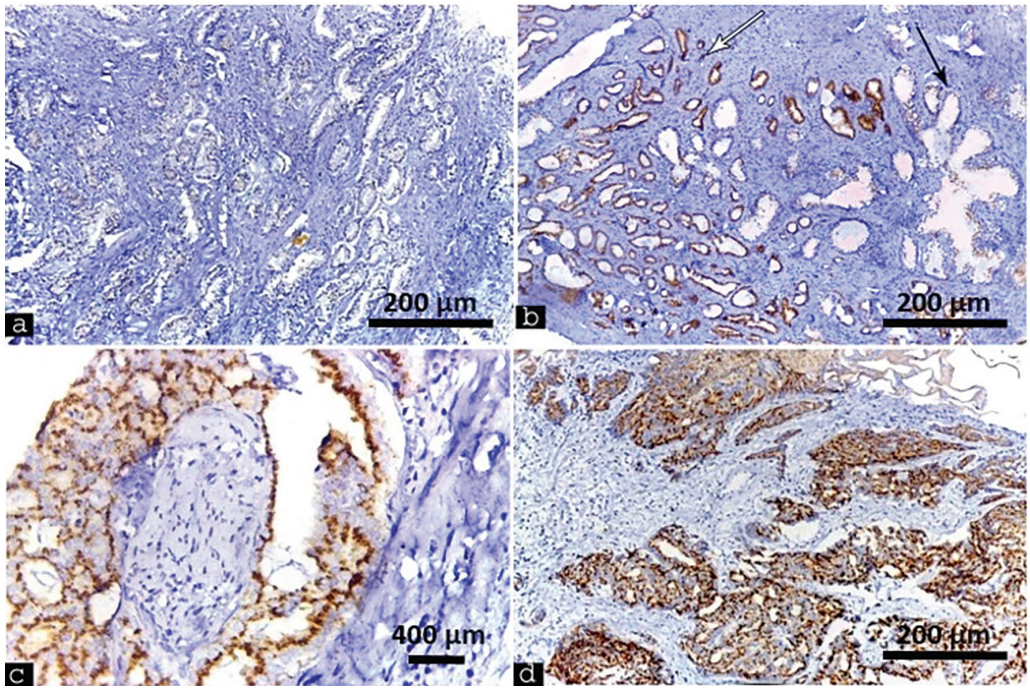

Figure 2. Immunohistochemical expression of *GOLM1*: **(a):** Mild paranuclear expression of malignancy (pattern 3, foamy), (x100) **(b):** Moderate paranuclear expression of malignancy (Pattern 3) (White arrow), and mild expression in adjacent benign glands (Black arrow), (x100) **(c)** Moderate expression of malignancy (pattern 4) with perineural invasion (x400) **(d):** Strong expression of malignancy (pattern 5) with bladder invasion T4 (x100)

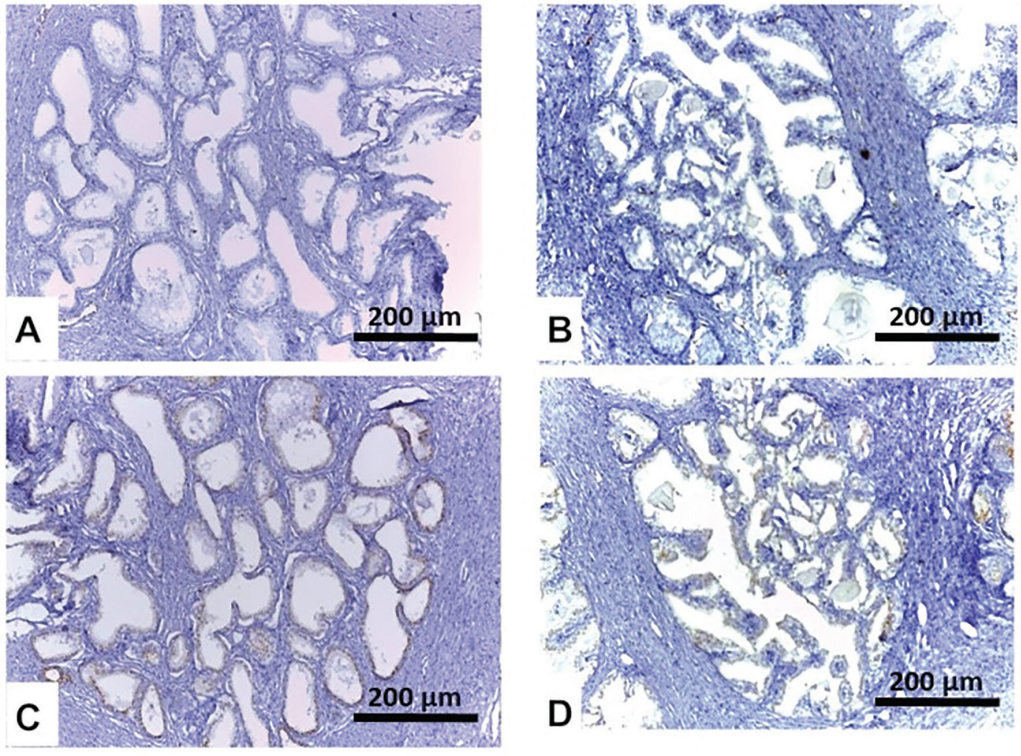

Figure 3. Immunohistochemical expression of benign prostatic lesion: **(a):** Negative nuclear *ERG* expression in adenosis with positive internal control (x100). **(b):** Negative nuclear *ERG* expression in clear cell cribriform hyperplasia (x100). **(c):** moderate granular *GOLM1* expression in adenosis (x100). **(d):** Mild fine granular *GOLM1* expression in clear cell cribriform hyperplasia (x100)

Table 3. The diagnostic validity of *ERG* and *GOLM1* expression in the diagnosis of prostatic adenocarcinoma				
	ERG	**GOLM1**	**Combination (*ERG*⁺ or *GOLM1*⁺)**	**Combination (*ERG*⁺ and *GOLM1*⁺)**
Sensitivity	55%	92.5%	97.5%	50%
Specificity	100%	70.8%	70.8%	100%
PPV	100%	84.1%	84.8%	100%
NPV	57.1%	85%	94.4%	54.5%
Accuracy	71.9%	84.4%	87.5%	68.8%
AUC	0.775	0.817	0.842	0.750
P-value	<0.001**	<0.001**	<0.001**	0.001*
Confidence interval	0.663-0.887	0.696-0.937	0.725-0.958	0.633-0.867

IRS: Immunoreactivity score, **: Highly significant, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the ROC curve, *GOLM1*: Golgi membrane protein 1, ROC: Receiver operating characteristic

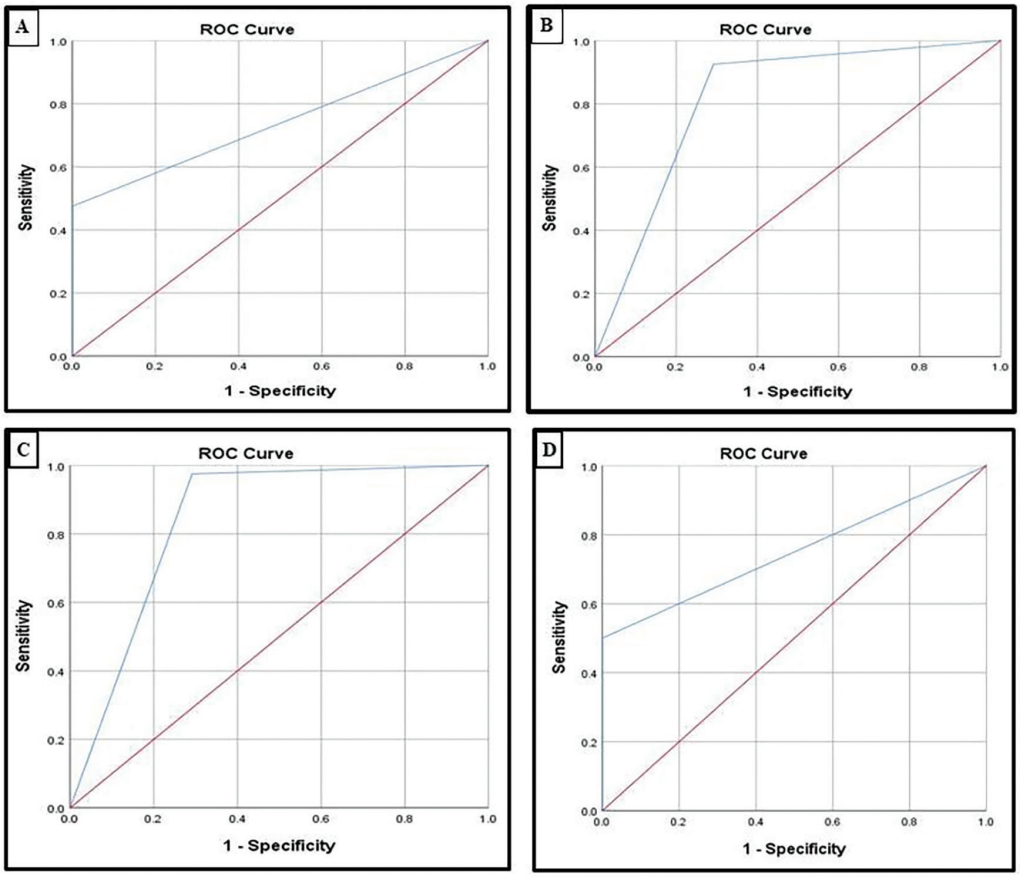

Figure 4. A) ROC curve of *ERG*, **B)** ROC curve of *GOLM1*, **C)** ROC curve of combination (*ERG*⁺ or *GOLM1*⁺). **D)** ROC curve of combination (*ERG*⁺ and *GOLM1*⁺)
ROC: Receiver operating characteristic

percentages of *ERG* expressions (18-20), while others have observed a lower percentage in PAC cases (21-23). Based on types of biopsies, tumor site, methods of assessment, race, and genetic variation, *ERG* expression differs between studies (6,7). The highest ERG expression was observed in cases analyzed using polymerase chain reaction (PCR) and IHC compared to those analyzed using fluorescence *in situ* hybridization, and was higher among Caucasians than among Americans and Asians. It was also higher in prostatectomy cases than in TURP cases (6,7,17). In our study, we used IHC as one of the two most effective methods, alongside PCR, for detecting *ERG* expression. Additionally, Caucasians were more susceptible to ERG expression than other racial groups. Moreover, utilizing different types of biopsy procedures expanded the scope of the results.

Table 4. Association between *ERG*, *GOLM1* and clinicopathological parameters of prostatic adenocarcinoma cases

Variables	Total	*ERG* n=40		p	*GOLM1* n=40		p
		Negative 18 (45%)	Positive 22 (55%)		Negative 3 (7.5%)	Positive 37 (92.5%)	
Age Mean ± SD		70.2±8	66.3±7	0.11f	60.7±5	69±8	0.06 f
Less than 65	11 (27.5%)	4 (36.4%)	7 (63.6%)	0.583 x	2 (18.2%)	9 (81.8%)	0.178 F
More than 65	29 (72.5%)	14 (48.3%)	15 (51.7%)		1 (3.4%)	28 (96.6%)	
PSA Mean ± SD		87.4±44	58.1±24	0.019* f	76.7±25	74.5±40	0.661 ¶
Procedure used							
TRUS	21 (52.5%)	11 (52.4%)	10 (47.6%)	0.053 F	2 (9.5%)	19 (90.5%)	0.702 F
TURP	5 (12.5%)	4 (80%)	1 (20%)		0	5 (100%)	
RP	14 (35%)	3 (21.4%)	11 (78.6%)		1 (7.1%)	13 (92.9%)	
Gleason group							
Group 1	8 (20%)	2 (25%)	6 (75%)	0.024* F	0	8 (100%)	0.587 F
Group 2, 3	17 (42.5%)	5 (29.4%)	12 (70.5%)		1 (5.9%)	16 (94.1%)	
Group 4, 5	15 (37.5%)	11 (73.3%)	4 (26.7%)		2 (13.3%)	13 (86.7%)	
Ki-67 expression							
Low	21 (52.5%)	5 (23.8%)	16 (76.2%)	0.005* x	3 (14.3%)	18 (85.7%)	0.233 F
High	19 (47.5%)	13 (68.4%)	6 (31.6%)		0	19 (100%)	
Tumor size							
cT2	19 (47.5%)	6 (31.6%)	13 (68.4%)	.304 F	3 (15.8%)	16 (84.2%)	0.338 F
cT3	5 (12.5%)	3 (60%)	2 (40%)		0	5 (100%)	
cT4	16 (40%)	9 (56.3%)	7 (43.7%)		0	16 (100%)	
Nodal metastasis							
Nx	26 (65%)	14 (53.8%)	12 (46.2%)	0.340 F	3 (11.5%)	23 (88.5%)	0.671 F
N0	10 (25%)	3 (30%)	7 (70%)		0	10 (100%)	
N1	4 (10%)	1 (25%)	3 (75%)		0	4 (100%)	
Metastasis							
Mx	21 (52.5%)	8 (38.1%)	13 (61.9%)	0.029* F	0	21 (100%)	0.021* F
M0	10 (25%)	8 (80%)	2 (20%)		3 (30%)	7 (70%)	
M1	9 (22.5%)	2 (22.2%)	7 (77.8%)		0	9 (100%)	
PNI							
Absent	21 (52.5%)	11 (52.4%)	10 (47.6%)	0.360 x	2 (9.5%)	19 (90.5)	1 F
Present	19 (47.5%)	**7 (36.8%)**	12 (63.2%)		1 (5.3%)	18 (94.7%)	
LVI							
Absent	35 (87.5%)	16 (45.7%)	19 (54.3%)	1 F	3 (8.6%)	32 (91.4%)	1 F
Present	5 (12.5%)	2 (40%)	3 (60%)		0	5 (100%)	
ERG expression							
Negative	18 (45%)				1 (5.6%)	17 (94.4%)	<0.001** r
Positive	22 (55%)				2 (9.1%)	20 (90.9%)	

f: t-test, ¶: Mann-Whitney U test, x^2: Chi-square test, *f*: Fisher's exact test, r: Correlation coefficient. *: Significant. **: Highly significant, TRUS: Transrectal ultrasound, TURP: Transurethral resection of the prostate, RP: Radical prostatectomy, PNI: Perineural invasion, LVI: Lympho-vascular invasion, PSA: Prostatic specific antigen, *GOLM1*: Golgi membrane protein 1, SD: Standard deviation

GOLM1 is a Golgi-specific transmembrane protein that functions as an oncogene, activating the PI3K-AKT-mTOR pathway and is characterized by para-nuclear granular expression. The granules are much coarser and stain deeply brown in malignancy compared to benign glands (8).

In the current study, *GOLM1* expression was homogeneously positive in 92.5% of PAC cases and 29.2% of benign lesion cases, showing a significant difference (p<0.001). These results were similar to the observations from several previous studies (9,10,24,25). Kristiansen et al. (25), reported the upregulation of *GOLM1* in PAC (92.3%) compared to benign lesions (20%) using both PCR and IHC staining on tissue. On the other hand, Varambally et al. (26) detected lower *GOLM1* expression, observing it in 75% of PAC and 28% of benign lesions using the cytological urine sample. Li et al. (27) found a higher percentage of *GOLM1* expression in benign lesions (50%), using different methods of assessment. In IHC, the assessment depends only on the color score to compare between benign and malignant lesions. The difference in results may be due to differences in genetic backgrounds or techniques of assessment (PCR, Western blot, Immunofluorescence). *GOLM1* expression can be more effectively demonstrated using IHC or PCR on tissue samples rather than through cytology. Although cytology is a non-invasive diagnostic method, its results, as reported by Varambally et al. (26), were not encouraging due to low sensitivity. Additionally, *GOLM1* expression has been observed to be higher in Asian and Caucasian populations compared to American populations (10,11,24-27). However, it has been reported that African Americans exhibit higher *GOLM1* upregulation than European Americans (24).

In our study, positive *ERG* can predict PAC with a sensitivity of 55%, specificity 100%, PPV 100%, NPV 57.1%, and accuracy 79.1%. *ERG*-IHC is a reliable diagnostic test for PAC. These findings align with several studies conducted by the same assessment methods (12,17,28,29). In contrast to our findings, Sayed et al. (21) and Navaei et al. (30) reported lower sensitivity (22%, 27.8%, respectively), but observed the same specificity (100%). Similarly, Positive *GOLM1* can predict PAC with sensitivity of 92.5%, specificity of 70.8%, PPV of 84.1%, NPV of 85% and accuracy of 84.4%. *GOLM1*-IHC is a good diagnostic test for PCa. We found that the optimal cutoff value for *GOLM1* expression in our study was IRS=5, where the sensitivity reached 92.5%, specificity 100%, PPV 100%, NPV 88.9%, and accuracy 95.3%. Based on this, we recommend using IRS=5 to achieve better diagnostic accuracy. Our results are consistent with the study done by Kristiansen et al. (25) and Li et al. (27). However, Varambally et al. (26), and Wei et al. (31) reported lower sensitivity and specificity.

Our study concluded that positive *ERG* expression is defined as at least mild staining in more than 10% of tumor cells; this can confirm PAC. However, negative ERG expression does not exclude malignancy, as some PAC cases lack ERG expression. Additionally, since PC is the only tumor with *ERG* rearrangement, *ERG* expression in a metastatic lesion of unknown origin strongly suggests PC. Similarly, positive *GOLM1* expression is defined as at least moderate intensity in more than 50% of tumor cells and can confirm PAC. However, negative or mild *GOLM1* expression in malignant cases does not exclude the diagnosis of PAC.

Owing to the low sensitivity but high specificity of *ERG*, and high sensitivity but lower specificity of *GOLM1*, along with their cost-effectiveness as IHC tools, it was motivating to test the combination of both markers for PAC diagnosis. Here, 17 out of 18 *ERG*-negative cases (94.4%) were identified by *GOLM1*, demonstrating its complementary role in detection. Two out of 3 cases (66.7%) without *GOLM1* upregulation were *ERG*-positive, highlighting the benefit of dual-marker assessment. One case was negative for both markers, a finding that was associated with a high Gleason grade. Twenty cases showed positivity for both markers, reinforcing their combined diagnostic potential. The combined expression of *ERG*+ or *GOLM1*+ (positivity for either marker) showed 97.5% sensitivity, 70.8% specificity, 84.8% PPV, 94.4% NPV, and 87.5% accuracy.

Thus, the combined use of *ERG* and *GOLM1* significantly improves diagnostic accuracy, making it a valuable approach for PAC diagnosis.

Statistical analysis of *ERG* in the present study revealed a significant association between *ERG* positivity and low PSA, low ki-67, and low and intermediate Gleason grade groups (p=0.019, 0.005, 0.024, respectively). These findings agree with several studies (12,13,17,32,33). Notably, a study done by Dawoud et al. (12), reported a significant association between *ERG* expression and low Gleason grading group and ki-67, where most cases of low and intermediate Gleason grading groups were *ERG*-positive expression (93%). However, Hashmi et al. (34) found that 64.5% positive cases were more related to high grades with significant association with aggressive disease. Our findings support the role of *ERG* in early prostatic carcinogenesis, as *ERG* expression is commonly detected in early-stage or lower-grade tumors. The variation in *ERG* expression across different tumor grades might be explained by the number of gene fusions. Lower copy fusion is linked to low-grade tumors. Higher-grade tumors may exhibit an increased number of fusion copies, leading to more aggressive disease (7).

Regarding surgical biopsy procedures, our study found that most radical prostatectomy cases were *ERG*-positive, although the association was not statistically significant (p=0.053). This finding is consistent with previous studies by Kong et al. (35)

and Xu et al. (36), but inconsistent with Mosquera et al. (29). A key factor influencing *ERG* positivity in TRUS and TURP samples may be tumor heterogeneity and multifocality, leading to lower *ERG* detection rates. Additionally, it is well established that *TMPRSS2-ERG* fusion is less frequent in transition zone tumors, which may explain the lower *ERG* positivity observed in TURP and TRUS biopsy specimens (37).

In our study of tumor staging and metastasis, we found a statistically significant association between distant metastasis and *ERG* expression, where 77.8% of metastatic cases were *ERG*-positive (p=0.029). These findings agree with previous studies (37-39), supporting the role of *ERG* in tumor progression and metastasis. However, our results are inconsistent with the study by Tabriz et al. (40), in which all cases were obtained via radical prostatectomy, potentially influencing the findings. It has been suggested that PAC harboring *ERG* gene fusions caused by deletion has a worse prognosis than those resulting from translocation (38). Additionally, aberrant *ERG* expression plays a key role in epithelial-mesenchymal transition by reducing E-cadherin expression, leading to increased tumor invasiveness. Furthermore, *ERG* upregulates CXCR4 expression in about 80% of primary PAC cases, which enhances bone metastasis (7).

For the prognostic significance of *GOLM1*, our study found no significant association between *GOLM1* expression and clinico-pathological parameters except metastasis. These results are consistent with the previous studies done by Kristiansen et al. (25), and Yan et al. (10), suggesting that *GOLM1* plays a role in the initiation and persistence of PAC tumor proliferation and migration (27). In the current study, *GOLM1* expression is significantly associated with metastasis (p=0.021). A similar observation was reported by Qin et al. (9), who detected the relation between *GOLM1* and E-cadherin, metastasis, and poor survival via the TGF-β1/Smad2 signaling pathway. There is a significant association between *ERG* and *GOLM1* in PAC cases. This suggests that *ERG* and *GOLM1* intersect in key oncogenic pathways, contributing to PAC progression (7,10).

Study Limitations

- Other types of PC (transitional, squamous, basal cell carcinomas) were not available.
- The sample size was relatively small and should be further evaluated on a larger scale. The sample size was determined based on the number of cases received at the institution during the study period. A small sample size may introduce a risk of error due to false negatives, making it difficult to detect significant differences.

Conclusion

Our study is the first to discuss the combination of *ERG* and *GOLM1* as a diagnostic panel in PAC. We concluded that the co-expression of *ERG* and *GOLM1* is a useful diagnostic panel and represents an important aid in solving the diagnostic difficulties associated with PAC and prostatic benign mimickers' lesions for proper management and better prognosis. *ERG* is a potential prognostic marker in PAC and is associated with favorable clinicopathological features. *GOLM1* may be a prognostic marker with limitations.

Ethics

Ethics Committee Approval: All procedures were conducted in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki Declaration, along with its later amendments or equivalent ethical guidelines. The study was approved by the Institutional Review Board of the Zagazig University Faculty of Medicine (IRB approval no: 10498, date: 26.02.2023).

Informed Consent: Written informed consent from participants was obtained.

Footnotes

Authorship Contributions

Concept: S.A.A., M.M.O., H.A.A., M.A., A.E.A., Design S.A.A., M.M.O., H.A.A., A.E.A., Data Collection or Processing: S.A.A., M.A., Analysis or Interpretation: M.M.O., H.A.A., A.E.A., Literature Search: S.A.A., Writing: S.A.A., M.M.O., H.A.A., M.A., A.E.A.

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