

Introduction: Forkhead box M1 (FOXM1) is considered as a novel anti-cancer target, because it has many essential functions such as mitosis regulation, cell cycle transition, and other carcinogenesis signaling pathways. Dachshund homolog 1 (DACH1) is a member of the Sno/Ski co-repressor family.

Material and methods: Expression of DACH1 has been detected in many cancers. Patients and pathologic specimens: 50 patients with endometrial cancer (EC) were included in the study: ten specimens of normal endometrium and twenty specimens of endometrial hyperplasia. All samples underwent processing to investigate FOXM1 and DACH1 expression using immunohistochemistry.

Results: FOXM1 expression was detected in EC tissues more than normal endometrium and endometrial hyperplasia tissues ($p = 0.001$) and 0.01 . Increased FOXM1 expression was positively associated with larger tumor size ($p = 0.002$), high grade ($p = 0.004$), myometrial invasion, presence of lymph node metastases, higher Federation of Gynecology and Obstetrics (FIGO) stage ($p < 0.001$), and worse progression-free survival (PFS) and overall survival (OS) rates. The expression of DACH1 was lower in EC cells than normal endometrium and endometrial hyperplasia tissues ($p = 0.071$) and 0.252 . Low DACH1 expression was associated with high grade ($p = 0.001$), presence of lymph node metastases ($p = 0.49$), higher FIGO stage ($p = 0.022$), and unfavorable PFS and OS rates ($p = 0.037$). We found an inverse association between expression of FOXM1 and DACH1 in EC tissues and in non-neoplastic endometrial tissues ($p = 0.007$).

Conclusions: FOXM1 over-expression and DACH1 down-regulation in EC were related to poor clinical and pathological parameters and unfavorable prognosis.

Key words: endometrial cancer, FOXM1 and DACH1, prognosis, immunohistochemistry.

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Forkhead box M1 over-expression and dachshund homolog 1 down-regulation as novel biomarkers for progression of endometrial carcinoma in Egyptian patients

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Introduction

Endometrial cancer (EC) is one of the most commonly diagnosed malignant gynecological tumors among females worldwide, and it is the most common malignant gynecological tumors in developed countries [1, 2]. There are two identified types of EC: type I tumors which are often preceded by endometrial hyperplasia, and type II tumors, which are derived from intraepithelial carcinoma, a precancerous lesion, and are predominantly serous carcinomas arising in atrophic endometrium [3]. Although there are many newly discovered treatments for EC, the prognosis of EC patients with advanced or recurrent EC is still unfavorable [4]. There is an urgent need to understand the genetic and molecular pathogenesis of EC oncogenesis. The forkhead box (FOX) proteins (which originate from a huge family of different transcriptional factors) play essential roles in various biological processes, such as cell proliferation, differentiation, and cell cycle regulation [5]. Forkhead box M1 (FOXM1) has a 100-amino-acid winged helix DNA binding domain and it is considered a novel anti-cancer target, because it has many important functions in mitosis regulation, cell cycle transition, as well as other carcinogenesis signaling pathways [6]. Nevertheless, the detailed data about its expression and cellular functions in EC remain unclear [7]. Dachshund homolog 1 (DACH1), a member of the Sno/Ski co-repressor family, forms a part of the retinal determination (RD) signaling pathway, it has a vital role in drosophila eye and limb development [8]. Interestingly, the expression of DACH1 was detected in many cancers and it was found to be associated with patients' prognosis [9, 10]. Although DACH1 is hypothesized to be a tumor suppressor in EC tissue, its expression in EC pathogenesis remains vague. We decided to analyze FOXM1 and DACH1 expression, because both of them have been linked to many functions in mitosis regulation, cell cycle transition, and carcinogenesis signaling pathways, however, no previous study has assessed them together in EC. The aim of the study is to clarify the expression of FOXM1 and DACH1 in EC tissues and the relationship between their expression level, clinico-pathological features, and the prognosis of EC patients.

Patients and pathologic specimens

Our retrospective study was conducted on eighty formalin-fixed and paraffin-embedded biopsies, which were randomly selected from the archive

of the Pathology Department, Zagazig University. The patients were operated on, diagnosed, treated, and followed up from February 2015 to February 2020. The hysterectomy specimens were retrieved: 50 patients with EC, 10 specimens of normal endometrium, and 20 specimens of endometrial hyperplasia. The endometrial hyperplasia specimens were divided into two sections: ten simple hyperplasia without atypia and ten with atypical hyperplasia. The normal endometria were obtained in two phases: five specimens in the proliferative endometrial phase and five specimens in the secretory endometrial phase. The clinicopathological characteristics of our patients including demographic information, age, tumor size, tumor stage, pathological subtype, degree of myometrial invasion, cervical stromal invasion, adnexal and serosal invasion, parametrial extension, regional lymph node metastasis, peritoneal cytology, type of treatment received, follow-up data, and the patients' outcomes were obtained from the patients' medical records at the Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University.

According to patients' files, all patients were operated on in the Department of Gynecology and Obstetrics, Faculty of Medicine, Zagazig University, whereas tissue specimens were diagnosed in the Pathology Department, Faculty of Medicine, Zagazig University. EC was graded and staged according to the International Federation of Gynecology and Obstetrics (FIGO) classification and the patients received radiotherapy and chemotherapy according to their staging at Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University.

All samples underwent processing and preparation to investigate FOXM1 and DACH1 expression changes in EC patients and non-neoplastic endometrial samples.

Immunohistochemistry method of staining and assessment of forkhead box M1 and dachshund homolog 1 expression

All tissue samples were immune-stained and incubated with primary mouse monoclonal anti-FOXM1 (ab207298) and primary rabbit polyclonal anti-DACH1 antibody (ab205718) dilution 1:1000 (Abcam, Cambridge, MA). The semi-quantitative scoring system was used to assess nuclear FOXM1, cytoplasmic DACH1 staining intensity, and extent of stain. Each specimen was assigned a score according to the intensity of nuclear FOXM1 staining and cytoplasmic DACH1 staining (no detected stain = 0), weak stain means slight yellow stain = 1, moderate stain means yellowish brown stain = 2, and strong stain means brown stain = 3. The extent of stained cells was scored as follows: 0% = 0, 1–24% = 1, 25–49% = 2, 50–74% = 3, and 75–100% = 4. The final stain score was obtained by multiplication of the two scores, it ranged from 0 to 12 taking 4 as a cut-off point to divide marker expression into high and low stain [7].

Statistical analysis

Categorical variables' percentages were compared using the χ^2 test or Fisher's exact test. The correlations between

FOXM1 and DACH1 expression were determined using the phi coefficient (ϕ). Overall survival (OS) was calculated as the time from the start of treatment to the date of patients' death or last follow-up visit (censored). Progression free survival (PFS) is the duration from date of the start of treatment to the date of disease progression. Assessment of OS and PFS was done according to markers, they were estimated using the Kaplan-Meier plot method, and they were compared using the two-sided exact log-rank test. OS was done for all EC patients (50 patients). PFS was done for 25 EC patients (7 stage II patients with maximal stromal invasion, all stage III, and all stage IV). A p -value < 0.05 was considered significant. The statistics were performed using SPSS 22.0 for Windows and MedCalc for Windows.

Results

FOXM1 expression was detected in EC tissues more than normal endometrial tissues and more than endometrial hyperplasia tissues, these results were statistically significant ($p = 0.001$) and 0.01 respectively (Table 1, Fig. 1).

Forkhead box M1 expression and association with histopathological, clinical, and pathological findings in endometrial cancer samples

Levels of tissue protein expression of FOXM1 were positively associated with larger tumor size ($p = 0.002$), higher grade ($p = 0.004$), presence of lymph vascular invasion (LVI), myometrial invasion, cervical stromal invasion, adnexal invasion, positive peritoneal cytology, presence of lymph node metastases, higher FIGO stage ($p < 0.001$), presence of distant metastases ($p = 0.029$), and parametrial and serosal invasion ($p = 0.003$).

No significant association was found between FOXM1 and older age of the EC patients or histopathological subtype (Tables 2, 3).

Patients with increased FOXM1 expression have higher incidence of tumor progression ($p = 0.399$) and poorer response to therapy ($p < 0.001$) in contrast to patients with low FOXM1 expression levels.

Kaplan-Meier survival analysis revealed that patients with higher expression of FOXM1 have worse 5-year PFS and 5-year OS rates than patients having lower FOXM1 expression levels ($p = 0.26$) and < 0.001 respectively (Tables 4, 5, Fig. 2).

DACH1 expression was lower in EC cells than normal endometrial tissue and endometrial hyperplasia, these results were statistically nonsignificant ($p = 0.071$) and 0.252 respectively (Table 1).

Dachshund homolog 1 expression and association with histopathological, clinical, and pathological findings in endometrial cancer samples

DACH1 expression was inversely associated with larger tumor size, presence of myometrial invasion ($p = 0.002$), older age of the EC patients, higher grade ($p = 0.001$), presence of LVI ($p = 0.045$), presence of distant metastases and adnexal invasion ($p = 0.043$), presence of lymph nodes metastases ($p = 0.49$), and higher FIGO stage ($p = 0.022$).

Table 1. Comparison between endometrial carcinoma and non-cancerous endometrial tissue regarding forkhead box M1 and dachshund homolog 1 expression

	Endometrioid carcinoma (N = 50)		Endometrial hyperplasia (N = 20)		Normal endometrium (N = 10)		p-value ¹
	No.	%	No.	%	No.	%	
Age							
≤ 55 years	38	76	12	60	0	0	< 0.001
> 55 years	12	24	8	40	10	100	
FOXM1							
Low	23	46	16	90	–	–	0.01
High	27	54	4	10	–	–	
FOXM1							
Low	23	46	–	–	10	100	0.001
High	27	54	–	–	0	0	
DACH1							
Low	13	26	8	20	–	–	0.252
High	37	74	12	80	–	–	
DACH1							
Low	13	26	–	–	0	0	
High	37	74	–	–	10	100	0.071

Categorical variables are expressed as number (percentage), 1 – χ^2 , $p < 0.05$ is significant. FOXM1 – forkhead box M1, DACH1 – dachshund homolog 1

No significant associations were found between DACH1, serosal invasion, parametrial invasion, cervical stromal invasion, positive peritoneal cytology, or histopathological subtype (Table 2, Figs. 4, 5).

Patients with increased DACH1 expression have lower incidence of tumor progression ($p = 0.001$), lower mortality ($p = 0.026$), and better response to therapy ($p = 0.003$) in contrast to patients with low DACH1 expression levels.

5-year PFS and 5-year OS rates were higher in patients having elevated DACH1 protein than those having suppressed DACH1 expression ($p = 0.013$) and 0.017 respectively) (Tables 4, 5).

We found an inverse association between expression of FOXM1 and DACH1 in EC tissues and non-neoplastic endometrial tissues ($\phi -0.250$), $p = 0.007$.

Discussion

The histological factors could affect EC prognosis such as clinical stage, depth of myometrial invasion, lymph vascular space invasion (LVSI), and histological subtype [7]. However, it was found that EC patients with the same clinical and pathological prognostic features had variable outcomes. So, specific prognostic biomarkers associated with clinical features and prognosis of EC were needed. We found that FOXM1 is over-expressed in tissues of EC and its high expression level was related to poorer prognosis of EC patients, unfavorable patients' outcomes, and undesirable prognostic parameters. Our findings are consistent with results of Hu *et al.* [6] and results of Feng *et al.* [7], who reported a similar association between FOXM1 expression and dismal patients' outcome in he-

patocellular carcinoma and EC. Previous studies indicated that FOXM1 is an essential regulator of a plethora of biological processes, and its dysregulation could contribute to oncogenesis and cancer progression [11]. FOXM1 expression is considered a significant predictor of dismal clinical outcome in patients with many solid tumors [12, 13], and it regulates many proliferative signals in the cell cycle [5]. If FOXM1 (a transcription factor) is over-expressed, it is considered a potential therapeutic target in a wide range of human cancers [14]. Moreover, FOXM1 is involved in oncogenesis, epithelial-mesenchymal transition (EMT), malignant cell invasion, and metastasis [15]. Similar results were reported by Wen *et al.* [16], who stated that high FOXM1 expression in ovarian cancer patients is related to an unfavorable outcome as it can promote ovarian cancer cell proliferation, invasion, and metastasis. In addition, FOXM1 plays a vital role in progression of esophageal cancer, as it can increase the expression of MMP-2 and MMP-9, which leads to promoting malignant cell invasion and spread [17]. Furthermore, FOXM1 can promote angiogenesis in several cancers through enhancing vascular endothelial growth factor expression, so it promotes cancer progression [18, 19]. Feng *et al.* [7] found a reduction in proliferative and invasive ability of cells of EC after knocking down FOXM1, which proved the impact of FOXM1 in progression of EC and its role as a new prognostic biomarker for EC.

Another important impact of FOXM1 in EC progression is its role in resistance to chemotherapy that was increasingly identified [6].

Based on the plethora of identified impacts of FOXM1 in many cancers, further studies are needed to clarify its

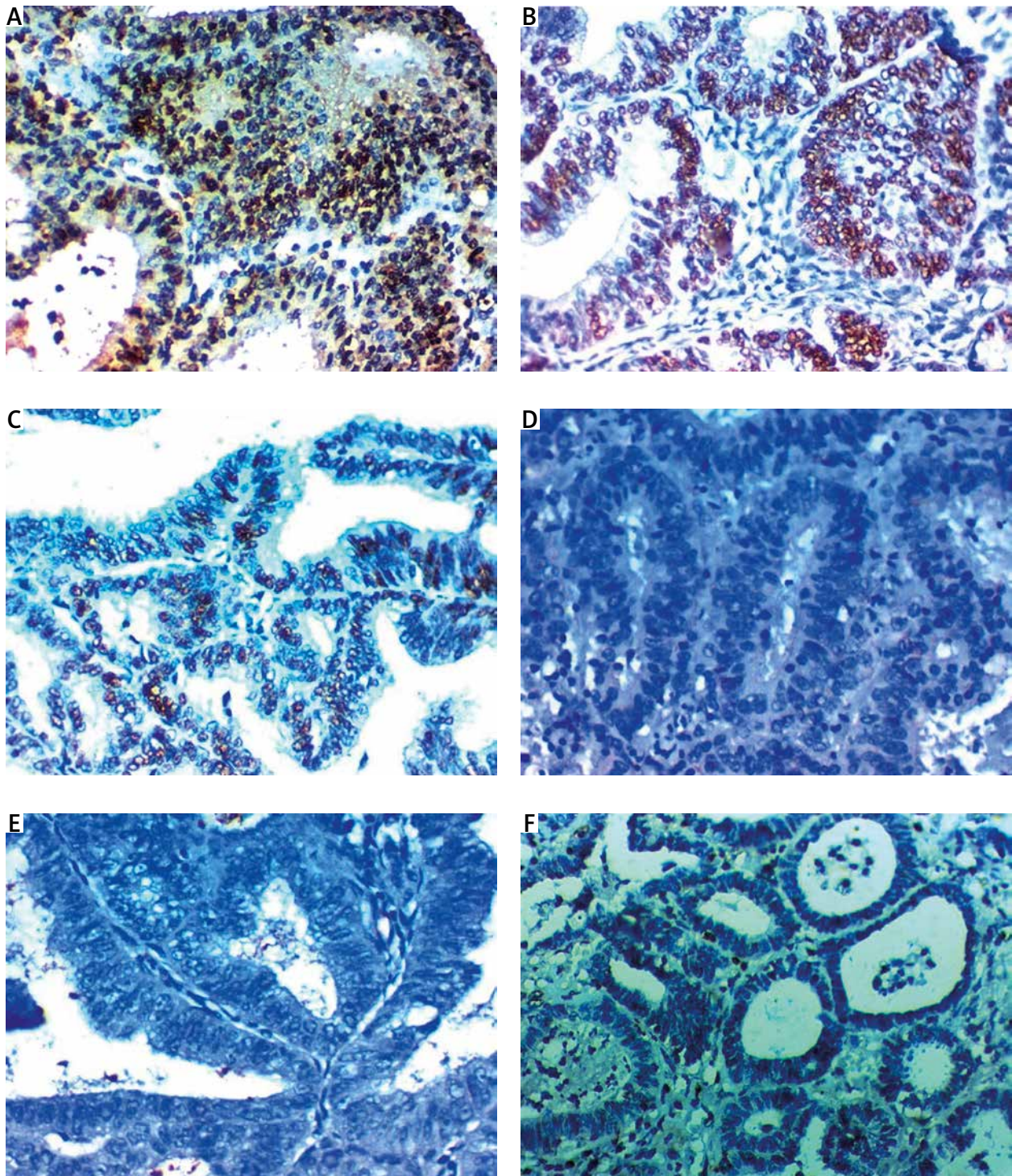


Fig. 1. Expression of forkhead box M1 in nuclei of endometrial carcinoma cells. **A, B** – high nuclear expression in high grade and stage endometroid endometrial carcinoma 400×, **C** – high nuclear expression in high grade and stage serous endometrial carcinoma 400×, **D, E** – low nuclear expression in low grade and stage endometroid endometrial carcinoma 400×, **F, G** – low nuclear expression in endometrial hyperplasia 400×

relevance to EC progression. Due to the lack of detailed studies about mechanisms of action of FOXM1 in EC, we assessed the expression of another marker, DACH1.

We found that DACH1 is down-regulated in EC tissues in comparison to normal endometrium, and its expression was associated with a favorable outcome and better pathological parameters. Previous studies showed very

similar results to ours: in EC, squamous cell carcinoma cells, and in renal carcinoma tissues [2, 20].

Zhou *et al.* [2] noted that DACH1 is down-regulated in EC tissues compared to non-malignant endometrium and is positively associated with better outcome.

Zhang *et al.* [20] reported that the expression of DACH1 was more down-regulated in squamous cell carcinoma

Table 2. Clinicopathological features, immunohistochemical markers and outcome of 50 patients with endometrial carcinoma

Characteristics	All endometrial carcinoma patients (N = 50)		Characteristics	All endometrial carcinoma patients (N = 50)	
	No.	%		No.	%
Age			FIGO stage		
≤ 55 years	38	76	Stage I	22	44
> 55 years	12	24	Stage II	10	20
Size			Stage III	8	16
< 4 cm	17	34	Stage IV	10	20
> 4 cm	33	66			
Grade			Distant metastasis		
Grade I	17	34	Negative	40	80
Grade II	18	36	Positive	10	20
Grade III	15	30			
LVSI			FOX M1		
Absent	30	60	Low	22	44
Present	20	40	High	28	56
Extent of myometrial invasion			DACH1		
<50 %	17	34	Low	13	26
> 50%	33	66	High	37	74
Cervical stromal invasion			Treatment		
Absent	22	44	Surgery	22	44
Present	28	56	Surgery + adjuvant radiotherapy	3	6
			Concurrent chemoradiation	15	30
			Chemotherapy	10	20
Parametrial extension			Response to CTH & CRT	(N = 25)	
Absent	32	64	OAR	14	56
Present	18	36	NR	11	44
Serosal invasion			Follow-up duration (months)		
Absent	32	64	Mean ± SD	36.50 ± 15.77	
Present	18	36	Median (range)	38.50 (12– 60)	
Adnexal invasion			Recurrence	(N = 40)	
Absent	32	64	Absent	34	85
Present	18	36	Present	6	15
Peritoneal cytology			Progression	(N = 25)	
Negative	32	64	Absent	11	44
Positive	18	36	Present	14	56
Lymph node			Metastasis	(N = 40)	
Negative	32	64	Absent	32	80
Positive	18	36	Present	8	20
Histopathology			Mortality	(N = 50)	
Endometrioid EC	40	80	Alive	32	64
Non-endometrioid EC	10	20	Died	18	36

FIGO – Federation of Gynecology and Obstetrics, LVSI – lymph vascular space invasion, FOX M1 – forkhead box M1, DACH1 – dachshund homolog 1, CTH & CRT – chemotherapy and radiotherapy, EC – endometrial cancer

Table 3. Relation between clinicopathological features and immunohistochemical staining for endometrial carcinoma patients $N = 50$

Characteristics	All		FOXM1				p-value	DACH1				p-value
	(N = 50)		Low (N = 22)		High (N = 28)			Low (N = 13)		High (N = 37)		
	No.	%	No.	%	No.	%		No.	%	No.	%	
Age												
≤ 55 years	38	76	19	50	19	50	0.1281	5	13.2	33	86.8	0.0011
> 55 years	12	24	3	25	9	75		8	66.7	4	33.3	
Size												
< 4 cm	17	34	15	88.2	2	11.8	0.0021	0	0	17	100	0.0021
> 4 cm	33	66	7	21.2	26	78.8		13	39.4	20	60.6	
Histopathology												
Endometrioid EC	40	80	21	52.2	19	47.5	0.0591	9	15	31	85	0.0531
Non-endometrioid EC	10	20	1	10	9	90		4	40	6	60	
Grade												
Grade I	17	34	15	88.2	2	11.8	0.0042	0	0	17	100	0.0012
Grade II	18	36	6	33.3	12	66.7		5	27.8	13	72.2	
Grade III	15	30	1	6.7	14	93.3		8	53.3	7	46.7	
LVSI												
Absent	30	60	19	63.3	11	36.7	0.0011	5	16.7	25	83.3	0.0451
Present	20	40	3	15	17	85		8	40	12	60	
Extent of myometrial invasion												
< 50%	17	34	15	88.2	2	11.8	< 0.0011	0	0	17	100	0.0021
> 50%	33	66	7	21.2	26	78.8		13	39.4	20	60.6	
Cervical stromal invasion												
Absent	22	44	17	77.3	5	22.7	< 0.0011	4	18.2	18	81.8	0.2641
Present	28	56	5	17.9	23	82.1		9	32.1	19	67.9	
Parametrial extension												
Absent	32	64	19	59.4	13	40.6	0.0031	6	18.8	26	81.2	0.1801
Present	18	36	3	16.7	15	83.3		7	38.9	11	61.1	
Serosal invasion												
Absent	32	64	19	59.4	13	40.6	0.0031	6	18.8	26	81.2	0.1801
Present	18	36	3	16.7	15	83.3		7	38.9	11	61.1	
Adnexal invasion												
Absent	32	64	21	65.6	11	34.4	< 0.0011	5	15.6	27	84.4	0.0431
Present	18	36	1	5.6	17	94.4		8	44.4	10	55.6	
Peritoneal cytology												
Negative	32	64	21	65.6	11	34.4	0.0011	5	15.6	27	84.3	0.0651
Positive	18	36	1	5.6	17	94.4		8	44.4	10	55.5	
Lymph node												
Negative	32	64	21	65.6	11	36.7	0.0011	5	15.6	27	84.3	0.0491
Positive	18	36	1	5.6	17	94.4		8	44.4	10	55.5	
Distant metastasis												
Negative	40	80	21	52.2	19	47.5	0.0291	8	20	32	80	0.0431
Positive	10	20	1	10	9	90		5	50	5	50	
FIGO stage												
Stage I	22	44	17	77.3	5	22.7	< 0.0012	3	13.6	19	86.4	0.0222
Stage II	10	20	2	20	8	80		2	20	8	80	
Stage III	8	16	2	25	6	75		3	37.5	5	62.5	
Stage IV	10	20	1	10	9	90		5	50	5	50	

Categorical variables are expressed as number percentage, $1 - \chi^2$, $2 - \chi^2$ for trend, $p < 0.05$ is significant. FOXM1 – forkhead box M1, DACH1 – dachshund homolog 1, EC – endometrial cancer, FIGO – Federation of Gynecology and Obstetrics

Table 4. Relation between immunohistochemical staining for forkhead box M1, dachshund homolog 1 and outcome in endometrial carcinoma patients (N = 50)

Outcome	Total		FOX M1				P	DACH2				p
			Low		High			Low		High		
	N	%	N	%	N	%		N	%	N	%	
Response												
CR	7	14.0	2	9.1	5	17.9	< 0.001	0	0.0	7	18.9	0.003
PR	7	14.0	1	4.5	6	21.4		3	23.1	4	10.8	
NR	11	22.0	0	0.0	11	39.3		7	53.8	4	10.8	
Total	25	50.0	19	86.4	6	21.4		3	23.1	22	59.5	
Survival status												
Alive	32	64.0	22	100.0	10	35.7	< 0.001	5	38.5	27	73.0	0.026
Dead	18	36.0	0	0.0	18	64.3		8	61.5	10	27.0	
Total	50	100.0	22	100.0	28	100.0		13	100.0	37	100.0	
Progression												
Absent	11	44.0	2	66.7	9	40.9	0.399	0	0.0	11	73.3	< 0.001
Present	14	56.0	1	33.3	13	59.1		10	100.0	4	26.7	
Total	25	100.0	3	100.0	22	100.0		10	100.0	15	100.0	

Categorical variables are expressed as number (percentage), $p < 0.05$ is significant. FOX M1 – forkhead box M1, DACH1 – dachshund homolog 1, CR – complete tumor response, PR – partial tumor response, NR – not reported

Table 5. Relation between immunohistochemical staining for forkhead box M1, dachshund homolog 1 and survival in endometrial carcinoma patients

	Total N	n of events	Censored		Survival rate %	p-value
			N	%		
Overall survival (n = 50)						
FOX M1						
Low	22	0	22	100.0	100.0	< 0.001
High	28	18	10	35.7	0.0	
DACH2						
Low	13	8	5	38.5	0.0	0.017
High	37	10	27	73.0	57.9	
Progression-free survival (n = 25)						
FOX M1						
Low	3	1	2	66.7	66.7	0.26
High	22	13	9	40.9	28.4	
DACH2						
Low	10	10	0	0.0	0.0	0.013
High	15	4	11	73.3	71.1	

Continuous variables are expressed as mean (95% CI), categorical variables are expressed as number (percentage), $p < 0.05$ is significant. FOX M1 – forkhead box M1, DACH – dachshund homolog

cells than adjacent non-neoplastic mucosa, and reduction of its expression was found in the cancers that spread to lymph nodes and distant organs. Moreover, up-regulation of DACH1 expression led to suppression of malignant cell proliferation, invasion and spread, and evoked induction of apoptosis in malignant cells. Therefore, DACH1 can be considered one of the novel therapeutic targets which is expected to improve EC patients' prognosis.

Chu *et al.* [21] observed reduced expression of DACH1 in renal carcinoma tissues, it was inversely associated with tumor cell proliferation, grade, and TNM stage. Additionally, they demonstrated that DACH1 function restoration in renal clear cell cancer cells could lead to inhibition of tumor growth.

DACH1 expression leads to down-regulation of transcription of cyclin D1, which is the key in controlling malig-

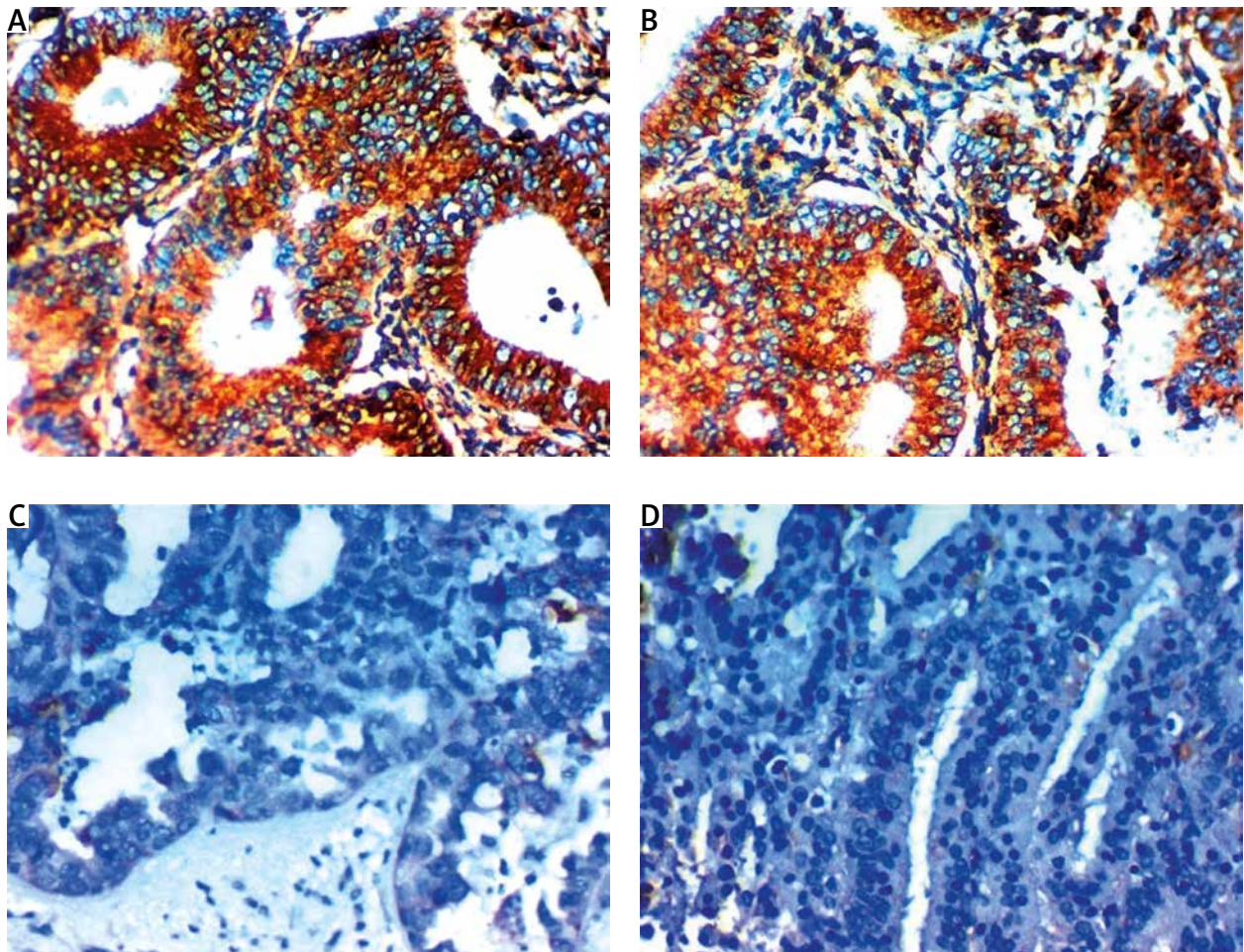


Fig. 2. Expression of dachshund homolog 1 in the cytoplasm in cells of endometrial carcinoma. **A** – high cytoplasmic expression in low grade and stage endometrial carcinoma 400×, **B** – high cytoplasmic expression in endometrial hyperplasia 400×, **C** – low cytoplasmic expression in high grade and stage serous endometrial carcinoma 400×, **D** – low cytoplasmic expression in high grade and stage endometrial carcinoma 400×

nant cell proliferation, so, DACH1 re-activation represents a potential therapeutic target of cancer.

Recently, it was found that DACH1 inhibition leads to stimulation of oncogene dependent cancer cell proliferation, invasion, and migration, which was established in breast cancer cells [9, 10]. Our results demonstrated that over-expression of DACH1 leads to suppression of proliferation and metastatic ability of EC cells. Similar results were observed in cells of gastric cancer, where there is marked reduction in DACH1 expression in chemo-resistant cancers compared with chemo-sensitive cancer, consequently, it could be a predictor for chemo-resistance in malignant cells [22]. Overall, all these previous results and our results suggest that DACH1 leads to suppression of tumor progression. Studies have confirmed that DACH1 expression leads to inhibition of SNAIL and TGF- β , thus, it inhibits EMT, which includes mechanisms that are involved in breast cancer cell invasion and metastases. Consequently, this adds to our results indicating that DACH1 leads to regulation of EMT in EC [23, 24]. Zhou *et al.* [2] observed that up-regulation of invasion and metastasis markers (N-cadherin and vimentin) and down-regulation of epithelial markers (E-cadherin and β -catenin) in DACH1

negative cancer cells indicate a rise of cancer cell invasion and spread. On the other hand, up-regulation of DACH1 resulted in increased expression of E-cadherin and β -catenin, these observations suggested that increased DACH1 expression might be able to inhibit EMT by inhibition of the Notch1 pathway through c-Jun. Nevertheless, over-expression of DACH1 decreased cyclin D, c-Jun, Notch1, and Hes1 expression. All these agents vastly promote cancer progression, so knocking down DACH1 leads to down-regulation of these proteins.

Our data showed that FOXM1 leads to EMT induction in EC cells in contrast to DACH1, we found a negative correlation between them in both EC and non-malignant endometrial tissues. FOXM1 and DACH1 influence cancers of various organs, and this is the first study to assess their expression together in EC and show the association with patients' prognosis. Both FOXM1 and DACH1 can affect EC patients' prognosis, but their underlying mechanism remains unclear. Further studies are needed to understand the molecular mechanism between them and EC prognosis, which will be used to design better intervention strategies to predict and improve prognosis of EC patients.

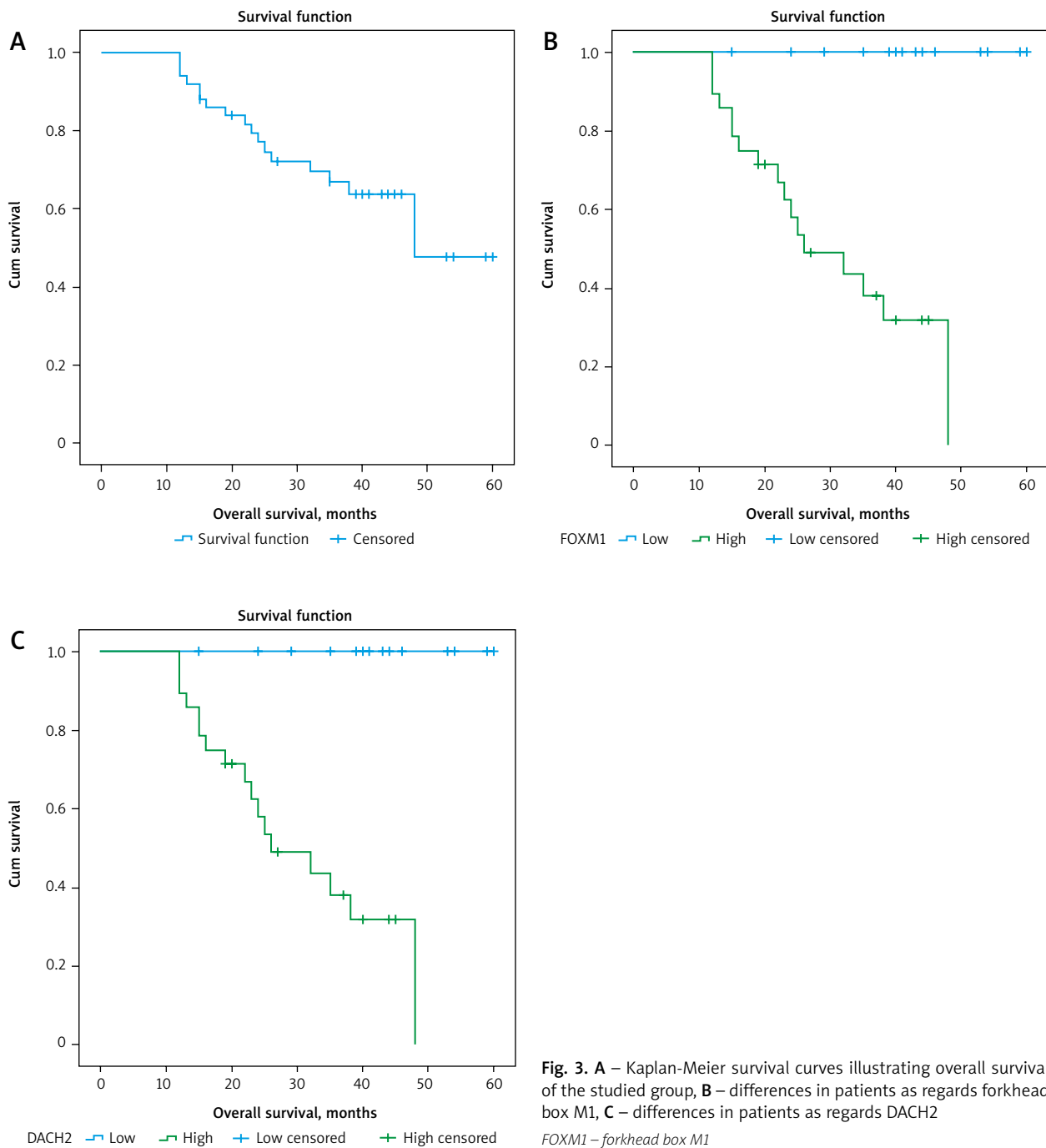


Fig. 3. A – Kaplan-Meier survival curves illustrating overall survival of the studied group, B – differences in patients as regards forkhead box M1, C – differences in patients as regards DACH2

FOXM1 – forkhead box M1

Conclusions

Our study identified FOXM1 over-expression in addition to down-regulation of DACH1 in EC compared to normal tissues. Furthermore, we explained the positive relationship between their expression and clinical, pathological data, and patients’ prognosis. We found an inverse association between FOXM1 and DACH1, therefore, all of our data suggest that FOXM1 and DACH1 can be considered as

attractive therapeutic targets and prognostic biomarkers for EC and possibly for other cancers which are characterized by FOXM1 over-expression and/or down-regulation of DACH1 expression.

The authors declare no conflict of interest.

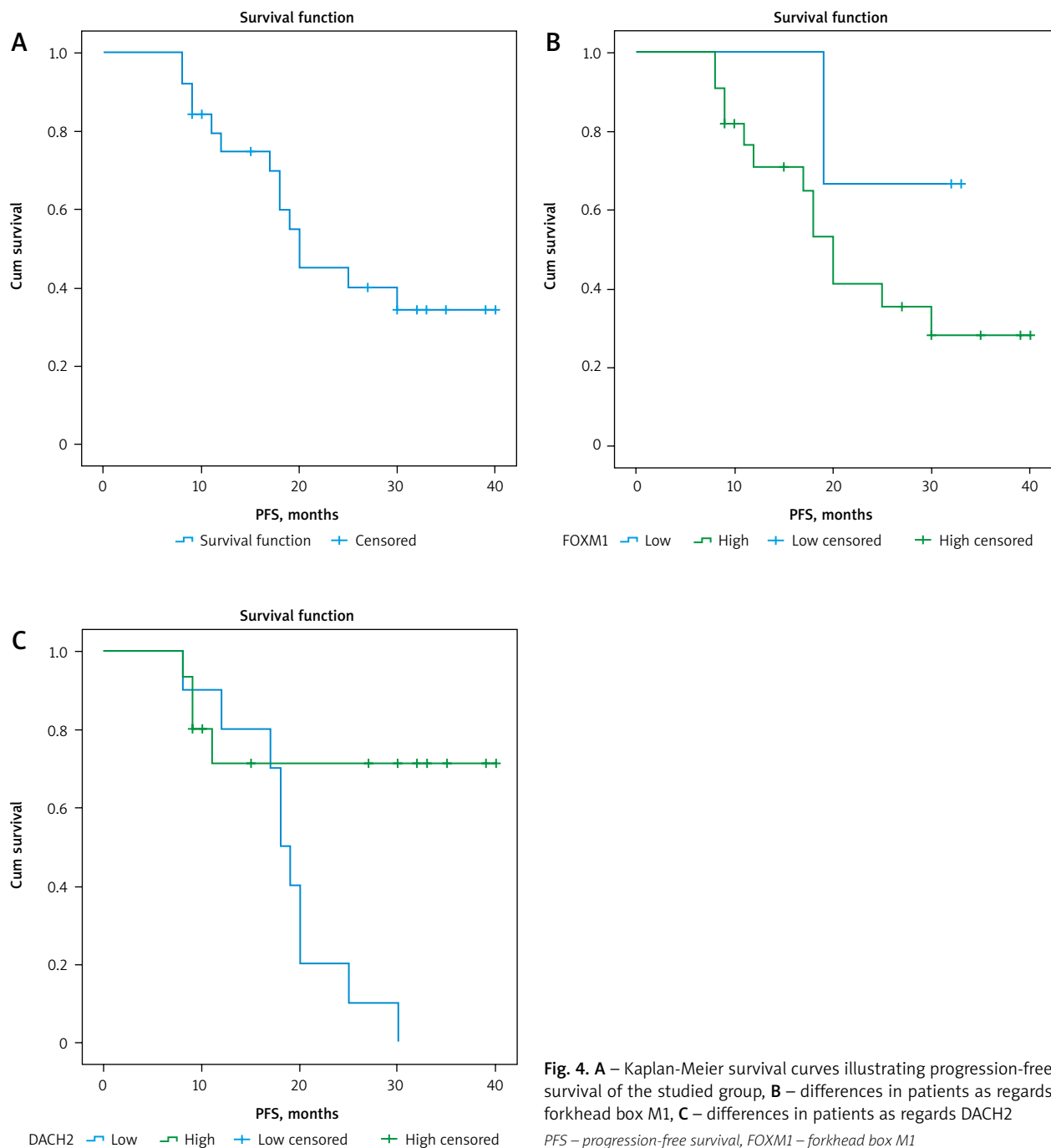


Fig. 4. A – Kaplan-Meier survival curves illustrating progression-free survival of the studied group, **B** – differences in patients as regards forkhead box M1, **C** – differences in patients as regards DACH2

PFS – progression-free survival, FOXM1 – forkhead box M1

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