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ORIGINAL RESEARCH

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Immunohistochemical expression and predictive value of PAX3 and FOXO1 proteins in high-grade serous ovarian carcinoma

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Abstract

Background: The transcription factors Forkhead box protein O1 (FOXO1) and paired box 3 (PAX3) are involved in various cellular functions and oncogenesis. In this study, we aimed to determine their involvement in high-grade serous ovarian cancer (HGSOC) through immunohistochemical expression. **Methods**: Immunohistochemical analysis was performed on 128 paraffin-embedded specimens of HGSOC to evaluate the expression of FOXO1 and PAX3. The data were correlated with various clinicopathological variables. **Results**: FOXO1 and PAX3 were expressed in a significant proportion of cancer tissues, 90% and 59%, respectively. The Kaplan-Meier plots indicated that patients who exhibited positive FOXO1 expression had better overall survival (OS) and disease-free survival (DFS) rates. Patients with positive PAX3 expression had a slightly shorter overall survival period. Chemoresistance and advanced International Federation of Gynecology and Obstetrics (FIGO) stage were found to have the strongest association with poorer survival. **Conclusions**: We found an increased immunohistochemical expression of PAX3 and FOXO1 in HGSOC. This finding indicates a need for deeper exploration of their links to signalling pathways, which could lead to the development of new therapeutic strategies to combat chemoresistance.

Keywords

Ovarian cancer; Immunohistochemistry; PAX3 protein; FOXO1 protein

1. Introduction

High-grade serous ovarian cancer (HGSOC) is typically diagnosed in its later stages. It affects an estimated 230,000 women annually and results in the death of 150,000 women worldwide [1, 2]. The preferred therapeutic regimen for HGSOC consists of cytoreductive surgery and subsequent adjuvant chemotherapy. Although there have been significant improvements in the diagnosis and treatment, more than 70% of women are diagnosed at an advanced stage and the majority relapse and die. The clinical presentation of HGSOC is characterized by nonspecific symptoms, which frequently result in a delay in diagnosis. The most commonly reported symptoms include abdominal bloating, pelvic pain, early satiety and urinary frequency. As the disease progresses, patients may experience more severe symptoms such as ascites, bowel obstruction and pleural effusions. At present, there is a lack of effective screening strategies for the early detection of ovarian cancer [3]. The diagnostic criteria for HGSOC are based on a combination of histopathological evaluation and molecular testing. Histologically, HGSOC is characterised by high-grade nuclear atypia, significant mitotic activity, and the presence of destructive stromal invasion. Immunohistochemistry is the gold standard for confirming the diagnosis, with markers such as p53, Wilms tumor 1 (WT1) and p16 being commonly expressed in HGSOC tumors. Molecular diagnostics, including the identification of Breast cancer gene 1/2 (BRCA1/2) mutations and other genomic alterations, are essential for supporting the diagnosis and guiding treatment decisions [4]. The standard treatment for HGSOC is a combination of cytoreductive surgery and platinum-based chemotherapy. Chemotherapy, often consisting of carboplatin and paclitaxel, follows surgery to target any remaining cancer cells. While initial responses to chemotherapy are promising, many patients experience recurrence within a few years. Recently, the addition of targeted therapies, such as PARP inhibitors for BRCA-mutated tumors and anti-angiogenic agents, has been shown to improve progression-free survival [5]. However, resistance to these treatments remains a significant challenge, necessitating ongoing research into new therapeutic strategies to comprehend the molecular pathogenesis of ovarian cancer and promote the generation of precise and efficacious prognostic markers to enhance patient outcomes [6]. Therefore, we aim to investigate the immunohistochemical expression of FOXO1 and PAX3 in HGSOC in order to establish their involvement in HGSOC prognosis and future treatment options.

The transcription factor FOXO1 belongs to the FOX fam-

ily of forkhead transcription factors. FOXO1, a member of the FOX family of forkhead transcription factors, regulates a wide range of targets, including genes involved in apoptosis, autophagy, antioxidant enzymes, cell cycle inhibitors and immune and metabolic processes [7]. Due to its multifaceted roles, FOXO1 is often considered a "super" transcription factor. It plays a key role in maintaining tissue homeostasis and responding to various stimulations. Tissue culture studies have shown that FOXO1 is downregulated in several cancers, including breast, kidney, prostate, and cervical [8–12]. Therefore, FOXO1 might be a critical target for both therapy and prevention of HGSOC.

The Paired box (PAX) gene family of transcription factors is now acknowledged to potentially play vital roles in cellular proliferation, differentiation, migration and tissue development [13]. As part of the *PAX* gene family, PAX3 exhibits significant expression in glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, and gastric cancer [14–17]. However, the role of PAX3 in HGSOC has not been explored so far. Therefore, investigating its involvement in HGSOC may provide insights into potential therapeutic targets driving the disease.

The dysregulation of FOXO1, frequently driven by the hyperactivation of the Phosphatidylinositol 3-kinase/Protein kinase B (PI3K/AKT) pathway, is a common feature in ovarian cancer, resulting in FOXO1 inactivation. This functional inactivation impairs FOXO1's role in promoting apoptosis, thereby contributing to tumour progression and resistance to chemotherapy. Furthermore, recent findings highlight the molecular mechanisms through which FOXO1 regulates Structural Maintenance of Chromosomes 4 (SMC4), and a clinical association between the expression of FOXO1/Methyltransferase-like 14 (METTL14)/SMC4 has been identified in ovarian cancer [18, 19].

Mutations in *PAX* genes have been implicated in a wide range of human diseases, from hypothyroidism and diabetes to various cancers. Of particular interest is the oncogenic potential attributed to PAX fusions, exemplified by PAX3-FOXO1 fusion protein (PAX3::FOXO1). This fusion protein exhibits a striking ability, up to 100-fold greater than PAX3 alone, to enhance transcription at critical regulatory sites involved in target gene expression. Such enhanced transcriptional activity underscores its profound influence in oncogenesis. In the context of ovarian cancer, PAX3::FOXO1 has been implicated in promoting epithelial-mesenchymal transition (EMT), a key process that facilitates metastasis. By regulating genes critical for cellular migration and invasion, PAX3 plays an important role in determining the aggressive behaviour of ovarian tumours. Furthermore, its involvement in stem cell biology suggests an additional dimension: the potential to promote cancer stem cell populations within ovarian tumours. These populations are notorious for their role in tumour recurrence and metastasis, thus adding to the clinical relevance of PAX3 in ovarian cancer progression [13, 20].

2. Materials and methods

2.1 Patient data

A total of 128 paraffin-embedded HGSOC samples were included in the study. Tissue samples were collected from patients who underwent primary or interval cytoreductive surgery and platinum-based chemotherapy with paclitaxel between 2016 and 2020 and had pathological confirmation at the Clinic for Women's Diseases and Child-birth, University Clinical Centre Zagreb. The study excluded all patients with a history of other invasive carcinomas and all patients who received a chemotherapy regimen other than a platinum-based chemotherapy regimen with paclitaxel as their first treatment. Tumor staging was evaluated using the classification system established by the International Federation of Gynecology and Obstetrics 2014 FIGO staging for ovarian, fallopian tube and peritoneal cancer [21]. Clinical information, such as age at diagnosis, Body Mass Index (BMI), reproductive history, surgical details, disease stage upon diagnosis, information on oncological interventions and response to therapy, BRCA status, platinum-free interval, disease-free survival (DFS), and overall survival, were extracted from available medical records. The duration from the date of diagnosis to death or last follow-up was defined as overall survival (OS). All tumor tissues underwent histological examination by a single gynaecological pathologist. This study received approval from the Ethics Committee at the Clinic for Women's Health and Childbirth, University Clinical Centre Zagreb.

2.2 Immunohistochemistry and data analysis

Tissue samples were fixed in 10% buffered formalin for 24 hours. After fixation, the tissue was dehydrated in a series of ascending alcohols (70%, 96%, 100%). The dehydrated tissue was impregnated with xylene, an intermediate between alcohol and paraffin. Tissue dehydration was performed using a Tissue-Tek VIP Sekura system in a fully enclosed and automated system under controlled conditions. After dehydration, the tissues were embedded in paraffin blocks. The cooled paraffin blocks were sectioned using a microtome (Leica). The thickness of the resulting tissue sections was 3–4 microns. Deparaffinisation and antigen retrieval were performed using the PT Link Dako system with EnVision FLEX Target Retrieval Solution High pH 9.0 (DakoOmnis) and EnVision FLEX Target Retrieval Solution Low pH 6.0 (DakoOmnis). This process took 20 minutes at a maximum temperature of 97 °C. After washing with EnVision FLEX Wash Buffer (DakoOmnis), the primary rabbit monoclonal antibodies PAX3 (ab216683, Abcam) and FOXO1A (ab52857, Abcam) were incubated. A high pH buffer was used for deparaffinisation and antigen retrieval for the PAX3 primary antibody and a low pH buffer for the FOXO1A primary antibody. The primary antibody PAX3 was diluted 1:50 and FOXO1A 1:200 with EnVision FLEX Antibody Diluent and incubated for 30 minutes. To block tissue peroxidase and prevent non-specific staining, EnVision FLEX Peroxidase Blocking Reagent (DM841, RTU DakoOmnis, Agilent Technologies, Glostrup, Denmark) was added for 10 minutes after washing with buffer. The preparations were washed again with buffer and EnVision FLEX/HRP secondary antibody (RTU DakoOmnis) was added for 30 minutes. The

resulting antibody-antigen complex was visualised with 3,3′-diaminobenzidine (DAB) for 10 minutes, which oxidises in the presence of peroxidase to form brown coloured precipitates. EnVision FLEX Substrate Working Solution was prepared by adding 1 mL EnVision FLEX Substrate Buffer (DakoOmnis) + 1 drop of EnVision FLEX DAB+ Chromogen (DakoOmnis). Stained slides were rinsed with distilled water, counterstained with hematoxylin using Mayer's Lillie's Modification Histological Staining Reagent® Dako for 1 minute. The slides were then immersed in tepid water for 10 minutes and coverslipped. The entire process was automated and performed under fully enclosed and controlled conditions in the DakoOmnissystemin University Clinical Centre Zagreb, Department of Pathology and Cytology.

One pathologist assessed the level of immune staining of each formalin fixed, paraffin-embedded section. Theanalysis was conducted using the Immunoreactive Scale (IRS) as proposed by Remmele and Stegner [22]. This scale employs the percentage of positively stained cells and the staining intensity of the reaction as parameters. The final result is the product of these two parameters. Protein expression levels were calculated using the following equation: Immunohistochemical (IHC) score (IS) = staining intensity (0: no staining; 1: weak, light brown; 2: moderate, brown; 3: strong, brown) × percentage of positive cells (1: <10%; 2: 10%–35%; 3: 35%–70%; 4: >70%).

For the purpose of further statistical analysis and to clarify the results, all patients with less than 10% positively stained cells and weak staining intensity were considered negative.

2.3 Statistical analysis

The Chi-squared and Fisher's exact tests were used to analyse the relationship between FOXO1 and PAX3 protein expression levels and clinicopathological characteristics. Kaplan-Meier method was used to assess the overall survival (OS) and disease-free survival (DFS), and survival was analysed by log-rank test. The Cox proportional hazards model was used to estimate the hazard ratios and confidence intervals (CIs) in both univariate and multivariate models. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 13.0, SPSS Inc.; Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 FOXO1 and PAX3 protein expression in association with clinicopathological parameters in HGSOC

To determine whether FOXO1 and PAX3 protein expression is associated with clinicopathological parameters in HGSOC, we analysed 128 Formalin-fixed, paraffin-embedded cancer tissue samples by immunohistochemical staining. Out of the total number of cancer tissues stained, 13 (10%) showed negative staining for FOXO1, while 115 (90%) exhibited positive staining. For PAX3, 53 (41%) had negative staining, and 75 (59%) showed positive staining (Fig.1).

The results of the expressions of FOXO1 and PAX3 in relation to clinicopathological characteristics are presented in Tables 1 and 2. The outcomes of the χ^2 or Fisher's Exact test did not reveal a significant relationship between FOXO1 and PAX3 protein expression and clinicopathological parameters but there was a positive correlation between PAX3 immunoreactivity and FIGO stage ($p = 0.044$).

3.2 The relationship between FOXO1 and PAX3 expression and outcomes in HGSOC patients

Kaplan-Meier plots were used to analyse OS and DFS and identify the relation between FOXO1 and PAX3 expression and survival (Fig. 2). The analysis of survival comprised 128 patients with HGSOC. Although the difference was not statistically significant, Kaplan-Meier plots showed that patients with positive FOXO1 expression had better OS and DFS (log-rank $p = 0.454$ and $p = 0.256$, respectively, Fig. 2A,B). Patients who exhibit positive PAX3 expression demonstrate a slightly shorter overall survival period. However, this difference was not statistically significant (log-rank $p = 0.735$, Fig. 2C).

3.3 Correlation between FOXO1 and PAX3 expression and OS or DFS.

Univariate and multivariate Cox proportional hazards analyses demonstrate that there is no correlation between FOXO1 and PAX3 expression and OS or DFS (Table 3). However, the study did find that chemoresistance and advanced FIGO stage, among other prognostic factors, had the strongest association with poorer survival. In univariate analysis, interval cytoreduction was found to be a statistically significant prognostic variable for adverse outcomes, although not in multivariate analysis (Table 3).

4. Discussion

Although significant progresshas been made in the treatment of high-grade serous ovarian cancer (HGSOC), the mortality rate remains high. Therefore, understanding the molecular mechanisms related to the progression, chemoresistance, and metastasis of HGSOC is essential.

We found that FOXO1 was expressed at higher levels in the majority of samples (90%), while PAX3 was expressed at higher levels in 59% of samples, suggesting a possible oncogenic role in HGSOC. The results of increased FOXO1 expression align with the findings of the study conducted on a mixed group of all types of epithelial ovarian cancer by Liu *et al*. [23]. Recent studies have shown that the expression of FOXO1 plays a significant role in paclitaxel-induced drug resistance in ovarian cancer [24]. This opens up the possibility for further molecular research in the direction of downregulating FOXO1 in HGSOC. In contrast, other studies have shown that the expression of FOXO1 is reduced in cancers such as breast, cervical and prostate [9, 10, 12].

The PAX3-FOXO1 fusion oncoprotein is unequivocally linked to alveolar rhabdo-myosarcoma. It exerts control over multiple signaling pathways that are crucial for cell proliferation, migration and death [25]. The role of FOXO1 in tumorigenesis is contradictory and requires

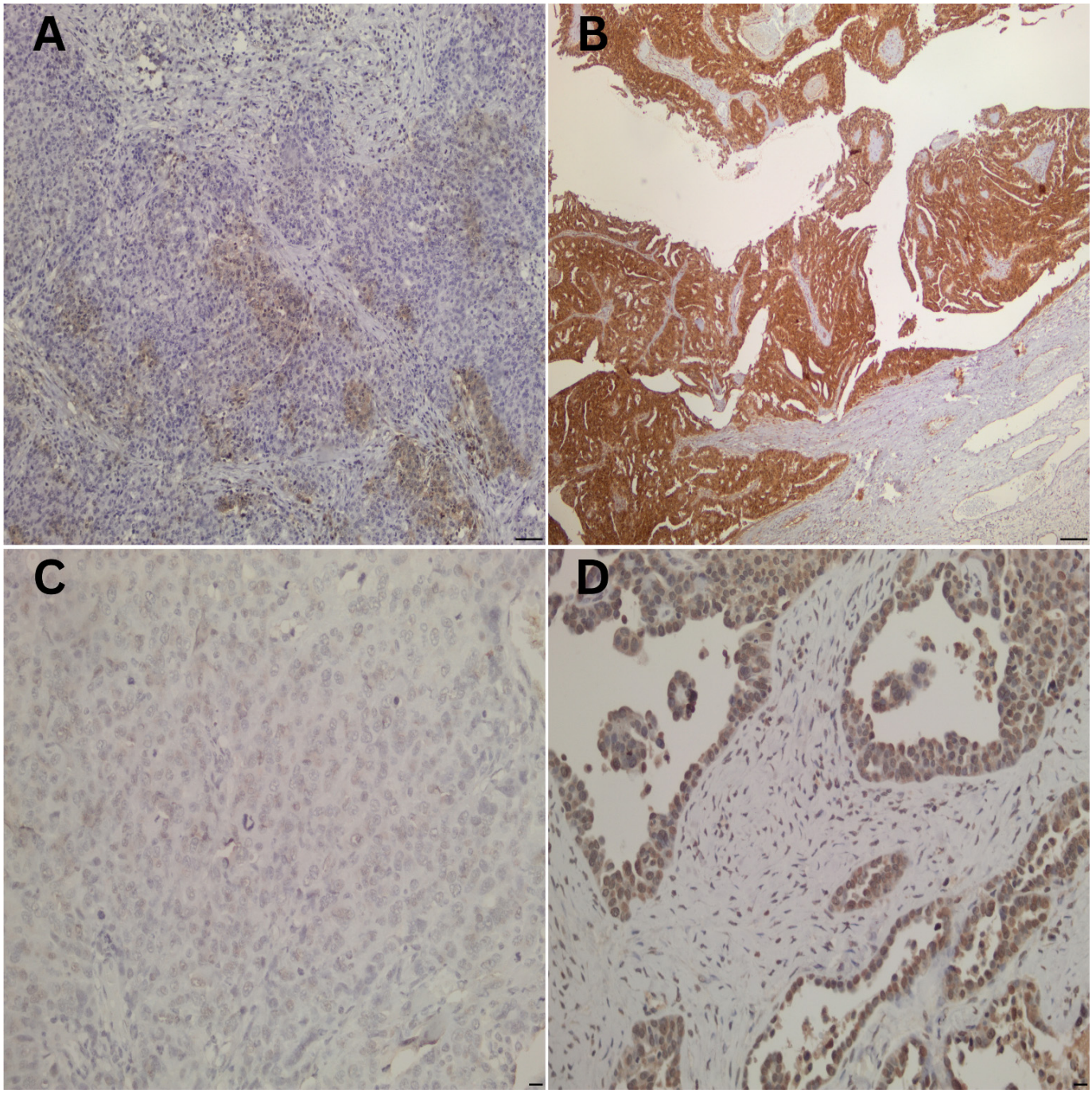


FIGURE 1. Immunohistochemical staining of FOXO1 and PAX3 in HGSOC samples (scale bar: 100 µm). (A) FOXO1 <10% positively stained cells and weak staining intensity (negative). (B) FOXO1 >10% positively stained cells and strong staining intensity (positive). (C) PAX3 <10% positively stained cells and weak staining intensity (negative). PAX3 >10% positively stained cells and moderate staining intensity (positive).

further investigation. However, it has been shown to have antiocogeniceffects through anti-progression, anti-proliferation, pro-apoptosis and pro-autophagy mechanisms. These mechanisms ultimately contribute to cell death and tumor suppression in esophageal, gastric, bladder, breast, colorectal and lung carcinomas [26–29]. However, other studies have demonstrated pro-oncogenic effects, such as inducing acute myeloid leukemia and promoting the growth of renal carcinoma [30, 31]. Muratovska *et al*. [32] reported that PAX3 is frequently expressed in cancer and required for the survival of melanoma cell lines.

Furthermore, we investigated the relationship between the expression of FOXO1 and PAX3 and overall survival and disease-free survival using Kaplan-Meier curves. Positive expression of FOXO1 protein was associated with slightly better overall survival and disease-free survival. However, positive expression of PAX3 protein was associated with slightly shorter overall survival. These results contradict the findings of Han *et al*.'s [6] study, which reported opposite results. The disparate results can be explained by the fact that Han *et al*. [6] conducted their research on a heterogeneous group of epitel ovarian carcinomas with different grades and histopathologies,

TABLE 1. FOXO1 expression in relation to standard clinicopathological variables using the χ^2 or Fisher's exact test.

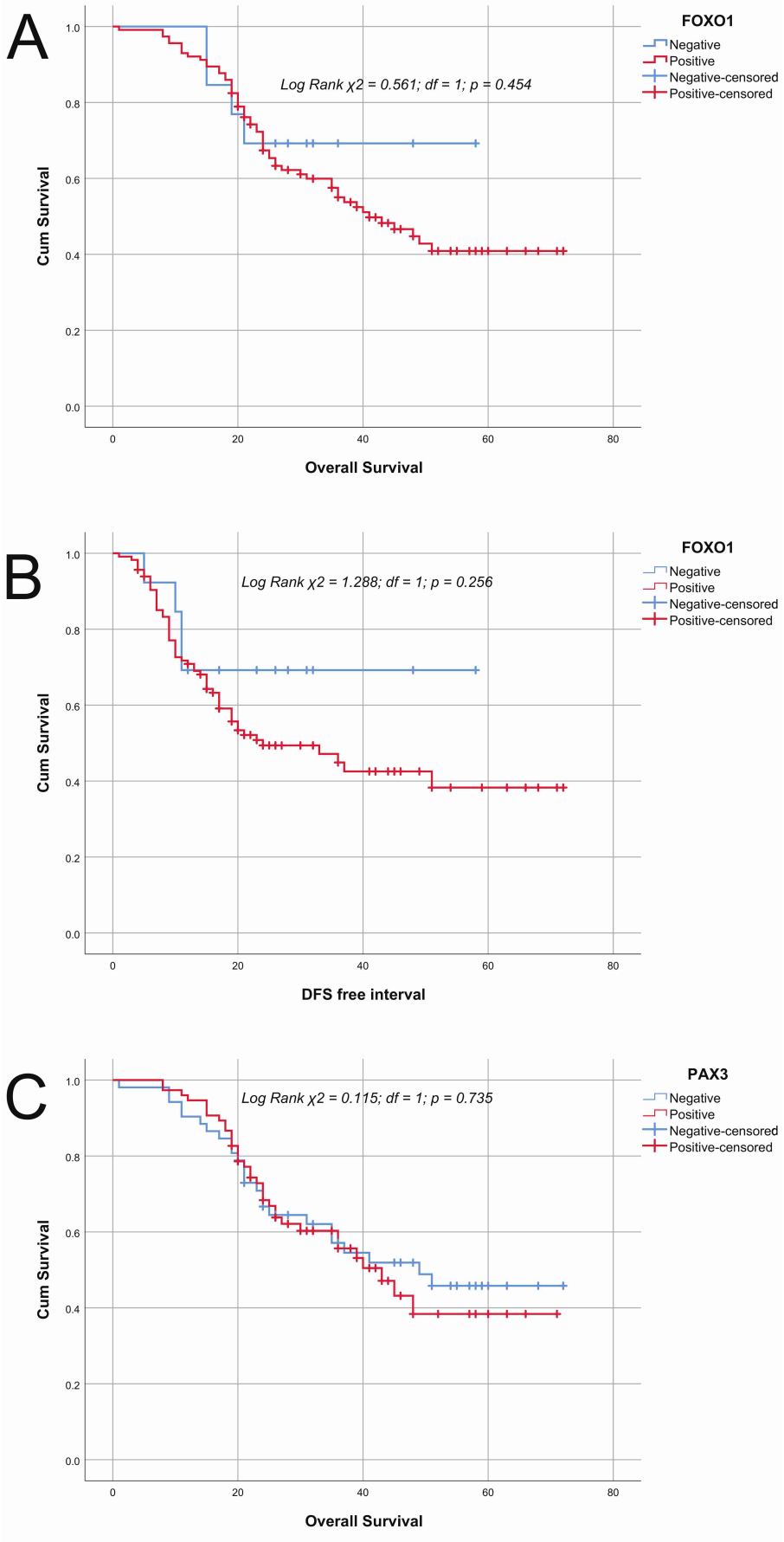
	FOXO1				χ^2	*p*
	Negative		Positive			
	n	%	n	%		
Total	13	(10)	115	(90)		
Age (yr)						
≤50	1	(8)	22	(19)	0.406	0.461*
>50	12	(92)	93	(81)		
Chemosensitivity						
Sensitive	8	(62)	76	(66)	0.001	0.985
Resistant	5	(38)	39	(34)		
Thrombocytes						
≤400	11	(85)	90	(78)	0.030	0.735*
>400	2	(15)	25	(22)		
Menopausal status						
Premenopausal	1	(8)	26	(23)	0.794	0.284*
Postmenopausal	12	(92)	89	(77)		
Vascular invasion						
Yes	4	(31)	33	(29)	0.000	1.000*
No	9	(69)	82	(71)		
BRCA1/2 status						
Unknown	5	(39)	54	(47)	2.520	0.474*
Mutation of BRCA1	2	(15)	12	(10)		
Without mutation	4	(31)	42	(37)		
Mutation of BRCA2	2	(15)	7	(6)		
FIGO stage						
IA	0	(0)	5	(4)	11.308	0.141*
IB	0	(0)	2	(2)		
IC	2	(15)	6	(5)		
IIA	1	(8)	7	(6)		
IIB	1	(8)	1	(1)		
IIIA	0	(0)	3	(3)		
IIIB	0	(0)	7	(6)		
IIIC	4	(31)	63	(55)		
IVA	3	(23)	8	(7)		
IVB	2	(15)	13	(11)		
CA125						
≤35	2	(15)	11	(10)	0.030	0.621*
>35	11	(85)	104	(90)		
Surgical procedure						
PDS	9	(69)	95	(83)	0.634	0.264*
IDS	4	(31)	20	(17)		

**Fisher's exact test. FIGO: Federation International of Gynecology and Obstetrics; CA: Carbohydrate antigen; PDS: Primary debulking surgery; IDS: Interval debulking surgery; FOXO1: Forkhead box protein O1; BRCA1/2: Breast cancer gene 1/2.*

TABLE 2. PAX3 expression in relation to standard clinicopathological variables using χ^2 or Fisher's exact test.

	PAX3				χ^2	*p*
	Negative		Positive			
	n	%	n	%		
Total	53	(41)	75	(59)		
Age (yr)						
≤50	13	(24)	10	(13)	1.936	0.164
>50	40	(76)	65	(87)		
Chemosensitivity						
Sensitive	37	(74)	47	(63)	0.422	0.516
Resistant	16	(30)	28	(37)		
Thrombocytes						
≤400	38	(72)	63	(84)	2.133	0.144
>400	15	(28)	12	(16)		
Menopausal status						
Premenopausal	16	(30)	11	(15)	3.611	0.057
Postmenopausal	37	(70)	64	(85)		
Vascular invasion						
Yes	16	(30)	21	(28)	0.005	0.943
No	37	(70)	54	(72)		
BRCA1/2 status						
Unknown	28	(53)	31	(41)	4.406	0.221
Mutation of BRCA1	6	(11)	8	(11)		
Without mutation	18	(34)	28	(37)		
Mutation of BRCA2	1	(2)	8	(11)		
FIGO stage						
IA	4	(7)	1	(1)	15.651	0.044*
IB	0	(0)	2	(3)		
IC	1	(2)	7	(9)		
IIA	6	(11)	2	(3)		
IIB	0	(0)	2	(3)		
IIIA	0	(0)	3	(4)		
IIIB	1	(2)	6	(8)		
IIIC	29	(55)	38	(51)		
IVA	6	(11)	5	(7)		
IVB	6	(11)	9	(12)		
CA125						
≤35	6	(11)	7	(9)	0.005	0.944
>35	47	(89)	68	(91)		
Surgical procedure						
PDS	47	(89)	57	(76)	2.498	0.114
IDS	6	(11)	18	(24)		

**Fisher's exact test. FIGO: Federation International of Gynecology and Obstetrics; CA: Carbohydrate antigen; PDS: Primary debulking surgery; IDS: Interval debulking surgery; PAX3: paired box 3; BRCA1/2: Breast cancer gene 1/2.*



F I G U R E 2. Kaplan-Meier survival curves for patients with high-grade serous ovarian cancer (HGSOC). HGSOC patients with FOXO1+ (>10% stained tumor cells) showed better (A,B) overall and disease-free survival (log-rank $p = 0.454$ and $p = 0.256$, respectively). HGSOC patients with PAX3+ (>10% stained tumor cells) have a slightly shorter (C) overall survival (log-rank $p = 0.735$). FOXO1: Forkhead box protein O1; PAX3: paired box 3; DFS: disease-free survival; *df*: degrees of freedom.

TABLE 3. Univariate and multivariate analyses of the associations between prognostic variables and overall and disease-free survival rates in HGSOC.

	Overall survival hazard ratio [95% CI], *p*-value		Disease-free survival hazard ratio [95% CI], *p*-value	
	Univariate	Multivariate	Univariate	Multivariate
FIGO stage (III–IV)	5.56 [2.01–15.41], <0.001	2.42 [0.73–8.00], 0.15	3.72 [2.05–6.75], <0.001	2.13 [1.06–4.27], 0.03
CA125+ (>35 U/mL)	2.83 [0.89–9.06], 0.08	0.89 [0.23–3.49], 0.87	2.36 [1.14–4.89], 0.02	1.31 [0.57–3.00], 0.53
Age (>50 yr)	0.99 [0.53–1.87], 0.98	0.46 [0.22–0.97], 0.04	1.14 [0.68–1.90], 0.62	1.47 [0.85–2.56], 0.17
FOXO1+	1.46 [0.53–4.03], 0.46	0.64 [0.14–2.96], 0.57	1.45 [0.70–2.99], 0.31	1.14 [0.34–3.82], 0.83
PAX3+	1.07 [0.64–1.79], 0.80	0.28 [0.04–2.24], 0.23	0.99 [0.67–1.48], 0.97	0.55 [0.12–2.40], 0.42
FOXO1+/PAX3+	1.22 [0.73–2.02], 0.45	3.38 [0.39–29.61], 0.27	1.10 [0.74–1.64], 0.62	1.68 [0.36–7.98], 0.51
Chemoresistance	7.03 [4.10–12.04], <0.001	6.67 [3.52–12.64], <0.001	18.03 [10.54–30.84], <0.001	17.20 [9.50–31.14], <0.001
Thrombocytes (>400,000/mL)	1.53 [0.87–2.67], 0.14	1.19 [0.64–2.20], 0.58	1.49 [0.95–2.35],0.08	1.94 [1.18–3.20], 0.01
Interval cytoreductive surgery	2.12 [1.16–3.87], 0.01	1.62 [0.85–3.10], 0.15	2.24 [1.36–3.70], <0.001	1.17 [0.68–2.01], 0.57

CI: confidence intervals; FIGO: Federation International of Gynecology and Obstetrics; CA: Carbohydrate antigen; FOXO1: Forkhead box protein O1; PAX3: paired box 3.

while our study included only HGSOC.

In addition, univariate and multivariate Cox proportional hazards analyses indicate no correlation between FOXO1 and PAX3 expression and overall survival (OS) or dis-ease-free survival (DFS). This finding contradicts the results of immunohistochemical studies performed on heterogeneous groups of ovarian epithelial cancers with different grades and histopathologies [6, 23], raising concerns about comparability. Parameters significantly associated with poorer survival in univariate and multivariate analyses included FIGO stages III–IV, chemoresistance, and interval cytoreduction only in univariate analyses.

Treating chemoresistant ovarian cancer continues to be a significant challenge. It has been suggested that components involved in DNA damage repair and apoptosis could be targeted to treat platinum-resistant ovarian cancer. A recent study has shown that FOXO1 may play a crucial role in increasing the sensitivity of ovarian cancer cells to a combination of cisplatin and XPO1 inhibitors [33], Linlin Ma *et al.* [34] demonstrated that silencing FOXO1 in a paclitaxel-resistant cell line reduced its resistance. FOXO1 is a key mechanism in the PI3K/AKT signalling pathway for the chemosensitisation of endometrial cancer [35]. Liu *et al.* [36] reported that PAX3 exhibits tumor suppressor activity, which is regulated by the same signalling pathway in thyroid cancer.

We acknowledge that our study has several limitations. Firstly, the focus on immunohistochemical analysis has examined protein expression levels. Whilst this provides valuable insights, a more comprehensive understanding could be achieved by additional genomic and functional analyses to unravel the underlying mechanisms at the molecular level. Despite the trends observed in the Kaplan-Meier plots, the lack of statistical significance in the survival outcomes may be due to the limited statistical power associated with the sample size. Addressing these limitations in future studies would contribute to a more comprehensive understanding of the role of PAX3 and FOXO1 in HGSOC and their potential implications for prognosis and therapeutic strategies.

5. Conclusions

Our data indicate an increase in the immunohistochemical expression of PAX3 and FOXO1 in HGSOC. Although there were no statistically significant differences in survival, further research is required to explore the implications of this finding and the role of FOXO1 and PAX3 in the progression of HGSOC. In addition, the increased expression of these proteins may be relevant for further molecular investigation and identification as targets for therapeutic intervention.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

DT and DS—designed the research study. DT, DS and MMP—performed the research. VT, NP and KK—provided help and advice with formal analysis and visualization; analyzed the data. DT and MMP—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee at the Clinic for Women's Health and Childbirth, University Clinical Centre Zagreb (Protocol number: 02/013AG) approval date 12 March 2022. Informed consent was obtained from all subjects involved in the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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