Am J Transl Res 2025;17(1):144-161
www.ajtr.org /ISSN:1943-8141/AJTR0161455

Original Article

Clinicopathologic significance of FUT8, STX4, and calpain2 expression in ovarian cancer

Yumei Yang[1], Mei Wang[1], Linlin Chen[2], Xiangnan Chen[3], Yuhua Wang[1], Wenfeng Ye[1,4]

[1]Department of Obstetrics and Gynecology, Pudong New Area People's Hospital, Shanghai 201299, China; [2]Department of Obstetrics and Gynecology, Taizhou Second People's Hospital of Jiangsu Province, Taizhou 225511, Jiangsu, China; [3]Department of Obstetrics and Gynecology, The First People's Hospital of Nantong, Nantong 226000, Jiangsu, China; [4]Anhui Medical University, Hefei 230022, Anhui, China

Received October 25, 2024; Accepted December 12, 2024; Epub January 15, 2025; Published January 30, 2025

Abstract: Objectives: To investigate the expression of FUT8, STX4, and calpain2 in ovarian tumor tissues and their association with clinicopathological characteristics. Methods: This retrospective study analyzed the expression of FUT8, STX4, and calpain2 in ovarian tumor samples from patients treated at Shanghai Pudong New Area People's Hospital between January 1, 2008, and December 31, 2017. The association between marker expression and histopathological features was evaluated. Binary logistic regression was used to assess the diagnostic value of these markers in malignant ovarian tumors. Results: The expression of FUT8, STX4, and calpain2 was significantly higher in malignant ovarian tumors compared to benign tumors (all $P < 0.05$). A positive correlation was observed among the expressions of FUT8, STX4, and calpain2 (all $P < 0.001$). FUT8 expression was associated with the International Federation of Gynecology and Obstetrics (FIGO) stage ($P = 0.013$), STX4 expression was related to patient age ($P = 0.012$) and lymph node metastasis ($P = 0.019$). Patients with high co-expression of all three markers had a significantly higher likelihood of having malignant ovarian tumors compared to those with low expression of at least one marker ($P = 0.002$). Conclusions: Simultaneous detection of FUT8, STX4, and calpain2 expression in ovarian tumor tissues provides valuable diagnostic insights for malignant ovarian tumors.

Keywords: FUT8, STX4, calpain2, ovarian cancer, biomarkers, clinicopathological features

Introduction

Ovarian cancer, a significant malignancy of the female reproductive system, ranks among the three most common gynecological cancers and poses a substantial threat to women's health and survival [1]. It has the highest mortality rate among gynecological cancers, highlighting the urgency of addressing this critical health challenge [1]. Due to its complex histopathology, lack of specific early clinical manifestations, and insidious progression, ovarian cancer is often referred to as the "silent killer" [2]. In 2022, China reported 57,090 new cases of ovarian cancer and 39,306 related deaths [3]. The absence of effective early screening methods and diagnostic markers frequently results in late-stage diagnoses, with over 70% of patients presenting at intermediate to advanced stages, requiring comprehensive treatments such as surgery and chemotherapy [4]. Advanced ovarian cancer often metastasizes to organs like the liver, lungs, bones, and brain, leading to poor prognoses and reducing the 5-year survival rate to less than 40% [4]. Despite its significant impact, the mechanisms underlying distant metastasis remain poorly understood, and effective early diagnostic strategies are lacking [5].

Biomarkers play a crucial role in predicting cancer prognosis, monitoring treatment response, detecting tumor recurrence, and guiding clinical decision-making [6]. Therefore, identifying reliable early biomarkers and elucidating the molecular mechanisms of metastasis are essential for improving early diagnosis, controlling metastasis and recurrence, and enhancing survival outcomes in ovarian cancer [6].

Fucosyltransferases (FUTs), part of the glycosyltransferase superfamily, catalyze the transfer

https://doi.org/10.62347/SQKL1957

of fucose from guanosine diphosphate (GDP)-fucose to N-, O-, or glycosylphosphatidylinositol (GPI)-anchored sugar chains, forming fucosylated oligosaccharides, glycoproteins, and glycolipids [7]. Among these, FUT8 uniquely adds fucose to the first N-acetylglucosamine (GlcNAc) residue of N-glycans via an α-(1,6)-linkage [8]. Aberrant expression of FUT8 has been implicated in various malignancies, playing a pivotal role in tumorigenesis and progression [8].

FUT8 modulates glycan chain structures of substrate proteins such as immunoglobulins, alpha-fetoprotein (AFP), and cell adhesion molecules, influencing tumor cell behavior [8]. Core fucosylation enhances the binding affinity of receptors like epidermal growth factor receptor (EGFR) and integrin α3β1, while its absence reduces receptor-ligand binding and downstream signaling, impacting gene expression, proliferation, migration, invasion, apoptosis, adhesion, and angiogenesis [9]. FUT8 has been shown to promote tumor cell proliferation and drug resistance through upregulation of EGFR expression and signaling [10]. Additionally, increased core fucosylation of E-cadherin enhances tumor cell adhesion, migration, and invasion [11].

Research demonstrates that FUT8 mRNA, protein levels, and enzymatic activity are significantly elevated in various cancers, including lung, breast, ovarian, and prostate cancers, correlating with tumor severity [11-14]. These findings underscore the importance of investigating FUT8 as an early and reliable biomarker and elucidating its role in metastasis to improve ovarian cancer prognosis.

Syntaxin (STX) proteins are key components of the SNARE complex, a protein family comprising synaptosome-associated proteins (SNAPs), STXs, and vesicle-associated membrane proteins (VAMPs) [15]. Emerging evidence highlights the critical role of SNARE proteins in the etiology, progression, invasion, and metastasis of cancer, positioning them as promising therapeutic targets [15, 16]. The STX family consists of 16 members, including STX1, STX2, STX3, and STX4, which are primarily localized to the plasma membrane [17]. Among them, STX4-mediated integrin transport has been identified as a pivotal factor in cancer cell migration and survival, making it a potential target for cancer therapeutics [18].

Our prior research demonstrated STX4 overexpression in ovarian cancer, which appears to promote tumor progression and suggests its viability as a target to suppress cancer cell invasiveness [19]. This observation aligns with studies showing a positive correlation between STX4 expression and clinical stage as well as lymphatic metastasis in ovarian cancer, further implicating its role in tumorigenesis and progression [4]. Targeting STX4 as a therapeutic strategy for ovarian cancer is, therefore, a promising area for future exploration [4].

Calcium-activated neutral protease (calpain) is a member of the calcium-dependent cysteine protease family and it acts as a downstream signaling molecule of the G-protein-coupled estrogen receptor (GPER) [20, 21]. Calpain, a class of calcium-dependent proteases, promotes tumor migration and invasion by cleaving substrates such as focal adhesion kinase (FAK) and E-cadherin [22, 23]. Studies have shown that calpain regulates various biological processes, including cytoskeletal remodeling, neurodevelopment, cell cycle regulation, apoptosis, cell proliferation, migration, and signal transduction, through cleavage modifications [24].

Among the calpain family, Calpain1 and Calpain2 are the most extensively studied members [21]. Notably, Calpain2 has been shown to mediate ductal cancer cell migration through the mitogen-activated protein kinase (MAPK) pathway [25]. Additionally, Guo et al. reported that Calpain2 regulates estrogen (E2)-stimulated tumor growth by upregulating the breast tumor cells 1 (GREB1) gene, enhancing cell proliferation, and promoting tumor progression [11, 26]. These findings suggest that the combined evaluation of FUT8, STX4, and Calpain2 expression in tumors could be instrumental in predicting malignant metastasis [11, 26].

To address existing research gaps and enhance the understanding of the clinicopathological features of ovarian tumors, this study aims to investigate the correlations among FUT8, STX4, and Calpain2 expression and their association with the clinicopathological characteristics of ovarian malignancies. Exploring these relation-

ships is crucial for identifying biomarkers that can predict malignant metastasis, aiding in the differential diagnosis of aggressive ovarian tumors. By analyzing the joint expression patterns of FUT8, STX4, and Calpain2 in ovarian cancer tissues, this study seeks to provide valuable insights into their prognostic significance and potential as therapeutic targets. This comprehensive analysis aims to advance our understanding of ovarian cancer's molecular mechanisms and contribute to the development of more effective diagnostic and treatment strategies.

Materials and methods

Patients and specimens

In the initial phase of this study, preliminary trials were conducted to determine FUT8 expression levels in both the control and ovarian cancer groups. The mean expression values were 93.49 ± 35.21 in the control group and 116.58 ± 37.72 in the ovarian cancer group. With the significance level (α) set at 5% and statistical power (1-β) at 80%, we used equal group allocation to ensure the robustness of our comparative analysis. Based on calculations using the Sample Size module in PASS software, a sample size of 19 cases per group was required to achieve the desired statistical power. To account for potential dropouts and improve reliability, we increased the sample size by approximately 20%. Consequently, a total of at least 92 cases, with an equal distribution of 46 per group, was required.

This retrospective study included 93 patients diagnosed with ovarian tumors and hospitalized at Shanghai Pudong New Area People's Hospital between January 1, 2008, and December 31, 2017. The inclusion criteria were as follows: (1) age ≥ 18 years; (2) histopathological confirmation of ovarian cancer; and (3) initial diagnosis with no prior exposure to chemotherapy, radiotherapy, immunotherapy, or targeted therapy. Exclusion criteria included incomplete clinical records, secondary ovarian cancer, concurrent malignancies, autoimmune diseases, or mental disorders.

Formalin-fixed, paraffin-embedded (FFPE) surgical tissue samples were collected from all eligible patients. Histological diagnoses were confirmed by two independent pathologists through blinded reviews of histological slides. Clinical and pathological data, including age, tissue type, tumor type, FIGO stage, degree of differentiation, tumor location, lymph node metastasis, and distant metastasis, were meticulously collected. The clinical and pathological staging of ovarian cancer patients followed the 2014 International Federation of Gynecology and Obstetrics (FIGO) criteria [27].

All samples were handled in strict compliance with ethical guidelines and standards. Ethical approval for the study was obtained from the Review Board of Shanghai Pudong New Area People's Hospital (Approval Number 2022-D-36). This rigorous methodology ensures the reliability and validity of the study findings, contributing to a deeper understanding of the clinical and pathological features of ovarian cancer.

Immunohistochemistry

Immunohistochemical analysis was performed to evaluate protein expression in ovarian tissue samples. Tissue sections were processed using a standardized protocol for antigen retrieval and rehydration. The slides were microwaved for 10 minutes in a 0.01 M citric acid buffer and then cooled for 30 minutes to facilitate antigen retrieval. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide in methanol for 20 minutes. To reduce non-specific binding, the slides were incubated with 5% bovine serum albumin in phosphate-buffered saline (PBS) at room temperature.

Following three washes with PBS, the samples were incubated overnight at 4°C with the following mouse monoclonal primary antibodies: anti-FUT8 (1:500, clone ab191571, Abcam), anti-STX4 (clone ab77037, Abcam, Cambridge, UK), and anti-calpain2 (Cell Signaling Technology, Danvers, USA). Immunoreactivity was visualized by applying rat anti-mouse IgG2b-peroxidase, followed by 3,3'-diaminobenzidine tetrahydrochloride substrate in a Tris-HCl buffer (pH 7.6) containing 0.02% hydrogen peroxide. The slides were counterstained with hematoxylin, dehydrated, and mounted for microscopic analysis. Negative controls were prepared by replacing the primary antibody with PBS to confirm staining specificity.

Interpretation and evaluation of immunohistochemical results

Immunostaining results were independently evaluated by two clinical pathologists, with a senior expert resolving any discrepancies. Immunoreactivity was detected in both the cytoplasm and nucleus of tumor cells, with uniform cytoplasmic staining and variable nuclear staining intensity. A scoring system was employed to quantify staining density and intensity. Five high-power fields (200× magnification) were randomly selected for each sample to assess staining intensity and the proportion of positively stained tumor cells.

The proportion of positive cells was quantified using Image J software [28]. Protein expression was evaluated semi-quantitatively using the H-score system, which integrates the percentage of positive cells and staining intensity. The H-score, ranging from 0 to 300, was used to categorize protein expression as weak positive (< 100), positive (100-200), and strong positive (> 200). For analysis, FUT8, STX4, and calpain2 expression levels were dichotomized into high (H-score ≥ median) and low (H-score < median) groups based on the median H-score for each protein.

The H-score system, which calculates the product of the percentage of positive cells and the staining intensity. The protein expression for each marker was subsequently grouped accordingly [29]. The H-score was determined using the formula: H-score = (percentage of strong positive cells × 3) + (percentage of positive cells × 2) + (percentage of weak positive cells × 1) [30].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics Version 27.0.1 (SPSS Inc., Chicago, IL, USA). The normality of continuous data was assessed using graphical methods (e.g., histograms) and the Shapiro-Wilk test. FUT8 and calpain2 expression scores followed a normal distribution and are reported as the mean ± standard deviation ($\bar{x}$±sd). Inter-group comparisons for these variables were conducted using the independent samples t-test. Conversely, STX4 protein expression scores that exhibited a non-normal distribution are presented as the median (interquartile range). Inter-group comparisons for STX4 were performed using the Mann-Whitney U test. Statistical significance was defined as a *P*-value < 0.05 for all comparisons.

Clinical-pathological characteristics, including age, tissue type, tumor location, distant metastasis, lymph node metastasis, differentiation, and FIGO stage, were treated as categorical variables. The nine possible combinations of high and low expression levels for FUT8/STX4/calpain2 and the eight combinations of their respective expression levels were analyzed as categorical data and reported as frequency (n) and percentage (%). Pearson's chi-square test was used for inter-group comparisons if the theoretical frequency exceeded 5 and the total sample size was at least 40. Yates' correction was applied when the theoretical frequency was between 1 and 5 with a total sample size of at least 40. Fisher's exact test was employed when the theoretical frequency was less than 1 or the total sample size was below 40.

Protein expression scores were dichotomized into high and low expression groups based on the median value. Scores equal to or above the median were classified as high expression, while scores below the median were classified as low expression. Spearman's rank correlation coefficient was used to evaluate the correlation between the expression scores of FUT8, STX4, and calpain2 proteins. Multivariate binary logistic regression analysis was conducted to identify predictive factors. The predictive performance of the model and individual risk factors was assessed by calculating the area under the receiver operating characteristic (ROC) curve (AUC), Youden index, optimal cutoff value, sensitivity, and specificity. Predictive efficacy was evaluated based on the AUC, with values between 0.5 and 0.7 indicating low predictive efficacy, and values between 0.7 and 0.9 indicating moderate predictive efficacy, as described by Chen Y et al. [31].

Results

Clinical and pathological characteristics of patients

This study included 93 patients with ovarian tumors, comprising 12 cases (12.90%) of be-

Table 1. Clinical and pathological characteristics of ovarian cancer patients

Clinicopathologic parameters	Case No.
Age (years)	
< 50	25 (31.25)
≥ 50	55 (68.75)
Tissue type	
Serous ovarian cancer	67 (83.75)
Other types of ovarian cancer	13 (16.25)
FIGO stage	
I/II	33 (35.48)
III/IV	47 (58.75)
Differentiation	
Poorly/Moderately differentiated	63 (78.75)
Well-differentiated	5 (6.25)
Undifferentiated	12 (15.00)
Tumor location	
Unilateral	53 (66.25)
Bilateral	27 (33.75)
Lymph node metastasis	
Negative	29 (36.25)
Positive	8 (10.00)
Unscanned	43 (53.75)
Distant metastasis	
Negative	19 (23.75)
Positive	61 (76.25)

Results expressed as the number of patients and percentage (%); FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

nign tumors, 1 case (1.08%) of a borderline tumor, and 80 cases (86.02%) of malignant tumors. Among patients with malignant ovarian tumors, 55 were aged ≥ 50 years, and 25 were aged < 50 years. There were 67 cases of serous ovarian cancer and 13 cases of other histological subtypes. Based on FIGO staging, 33 cases were classified as stage I/II, while 47 cases were classified as stage III/IV. In terms of differentiation, there were 63 cases of poorly/moderately differentiated tumors, 5 cases of well-differentiated tumors, and 12 cases of undifferentiated tumors. Regarding tumor laterality, 53 cases were unilateral, and 27 cases were bilateral. Lymph node metastasis was observed in 8 cases, while 29 cases showed no lymph node involvement. Additionally, 61 cases had distant metastases, while the other 19 cases did not (**Table 1**).

Comparison of FUT8, STX4 and calpain2 expression between benign and malignant ovarian tumors

The cohort included 80 cases of malignant tumors, 12 cases of benign tumors, and 1 case of borderline tumors. The expression levels of FUT8, STX4, and calpain2 were compared between the benign and malignant ovarian tumors. Results demonstrated that the expression of FUT8, STX4, and calpain2 was significantly higher in malignant ovarian tumors compared to benign tumors ($P < 0.05$; **Table 2**; **Figures 1**-**3**).

Correlation analysis of FUT8, STX4, and calpain2 expression in ovarian cancer

Spearman's rank correlation analysis was performed on 80 ovarian cancer samples. The median expression scores were 177.50 (interquartile range [IQR]: 135.00-198.75) for FUT8, 207.50 (IQR: 166.25-250.00) for STX4, and 205.00 (IQR: 152.50-240.00) for calpain2. Significant positive correlations were observed between the expression levels of these proteins: FUT8 and STX4 ($r = 0.405$, $P < 0.001$), FUT8 and calpain2 ($r = 0.632$, $P < 0.001$), STX4 and calpain2 ($r = 0.495$, $P < 0.001$) (**Table 3**; **Figures 4**-**6**).

Correlation of FUT8, STX4 and calpain2 expression with clinicopathologic features in ovarian cancer

In this study of 80 ovarian cancer patients, the median H-scores for FUT8, STX4, and calpain2 were 177.5, 207.5, and 205, respectively. Patients were grouped into high and low expression categories based on these median scores: 40 patients each for high and low FUT8 expression, 39 with high STX4 expression and 41 with low expression, and 40 patients each for high and low calpain2 expression. The correlation between the expression of these proteins and clinicopathological features was analyzed.

FUT8 expression was not significantly associated with age ($P = 0.228$), differentiation ($P = 0.053$), distant metastasis ($P = 0.189$), tissue type ($P = 0.762$), tumor location ($P = 0.813$), or lymph node metastasis ($P = 0.835$), but it was significantly associated with FIGO stage ($P = 0.013$). STX4 expression showed no significant

Table 2. Comparison of FUT8, STX4 and calpain2 expression between benign and malignant ovarian tumors

	Benigne ovarian tumors (n = 12)	Malignant ovarian tumors (n = 80)	U value	*P* value
FUT8	83.33±48.45	177.50 (135.00, 198.75)	-4.128	< 0.001
STX4	117.50±55.08	207.50 (166.25, 250.00)	-3.880	< 0.001
calpain2	45.00 (20.00, 131.25)	205.00 (152.50, 240.00)	-4.315	< 0.001

Results expressed as means ± SD or median (Q1, Q3); FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

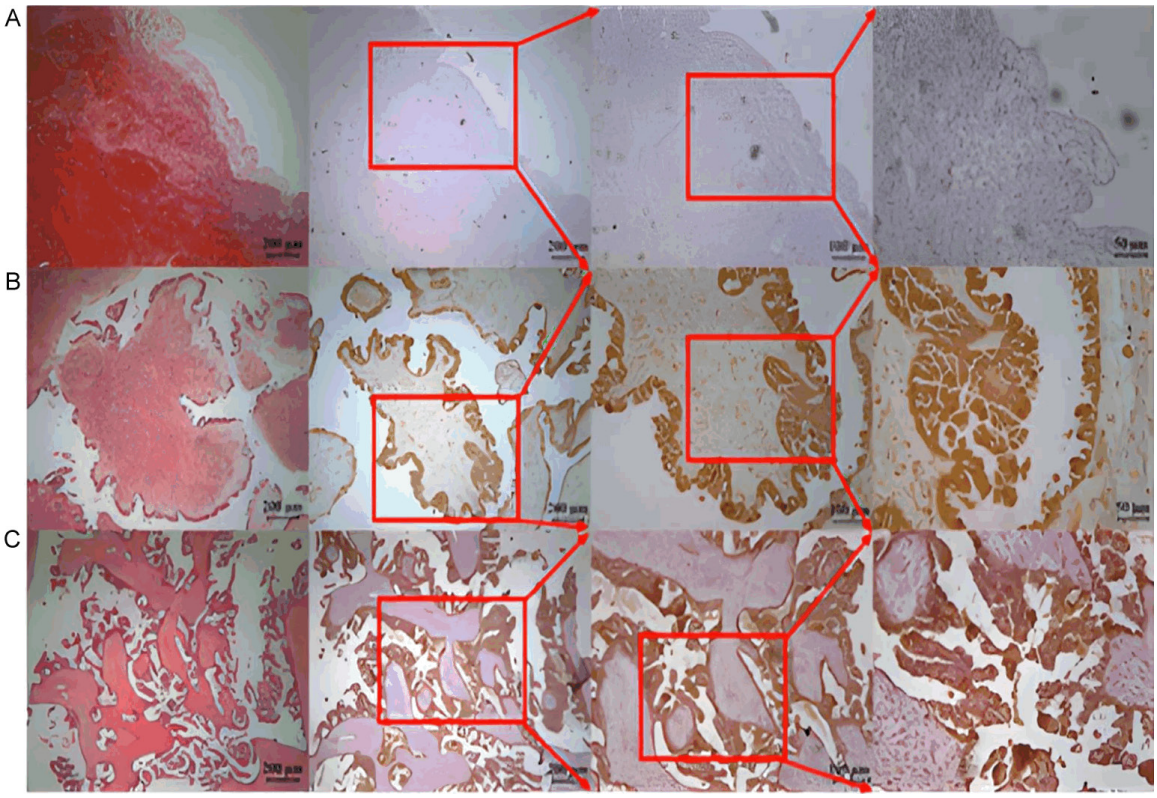

Figure 1. FUT8 immunohistochemical staining in three types of OC (HE ×200). A. Benign ovarian tumor; B. Borderline ovarian tumor; C. OC. FUT8, Glycosyltransferase 8; OC, ovarian cancer.

relationship with differentiation (P = 0.158), tissue type (P = 0.417), FIGO stage (P = 0.621), tumor location (P = 0.692), or distant metastasis (P = 0.086), but it was significantly associated with age (P = 0.012) and lymph node metastasis (P = 0.019). Calpain2 expression was not significantly associated with differentiation (P = 0.842), age (P = 0.469), tissue type (P = 0.762), FIGO stage (P = 0.496), tumor location (P = 0.813), lymph node metastasis (P = 0.246), or distant metastasis (P = 0.793) (**Tables 3**-**5**).

Predictive value of FUT8, STX4, and calpain2 in ovarian cancer

Multivariate binary logistic regression analysis was performed to compare the predictive effects of FUT8, STX4, calpain2, and their combined model. The statistical analysis revealed that FUT8, STX4, and calpain2 were not independent predictors of ovarian cancer (P > 0.05). However, the individual diagnostic performance of each protein was evaluated: FUT8: AUC = 0.879 (95% CI: 0.803-0.955, P < 0.001), sensitivity = 0.763, specificity = 0.917, Youden index = 0.680, cutoff value = 132.5. A score ≥ 132.5 indicated a high risk of ovarian cancer. STX4: AUC = 0.848 (95% CI: 0.762-0.935, P < 0.001), sensitivity = 0.638, specificity = 1.000, Youden index = 0.638, cutoff value = 182.5. A score ≥ 182.5 identified high-risk ovarian cancer cases. Calpain2: AUC = 0.888 (95% CI: 0.814-0.961, P < 0.001), sensitivity = 0.688, specificity = 1.000, Youden index = 0.688, cut-

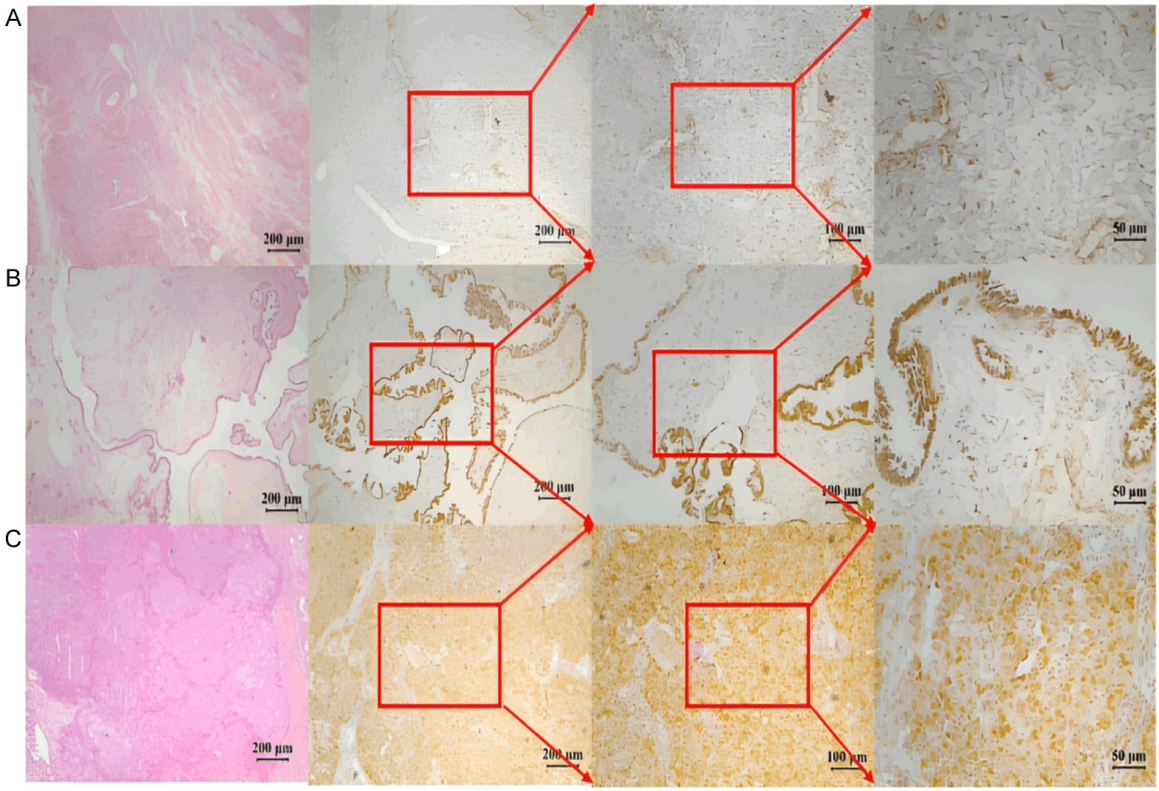

Figure 2. STX4 immunohistochemical staining in three types of OC (HE ×200). A. Benign ovarian tumor; B. Borderline ovarian tumor; C. OC. STX4, Synaptotagmin-4; OC, ovarian cancer.

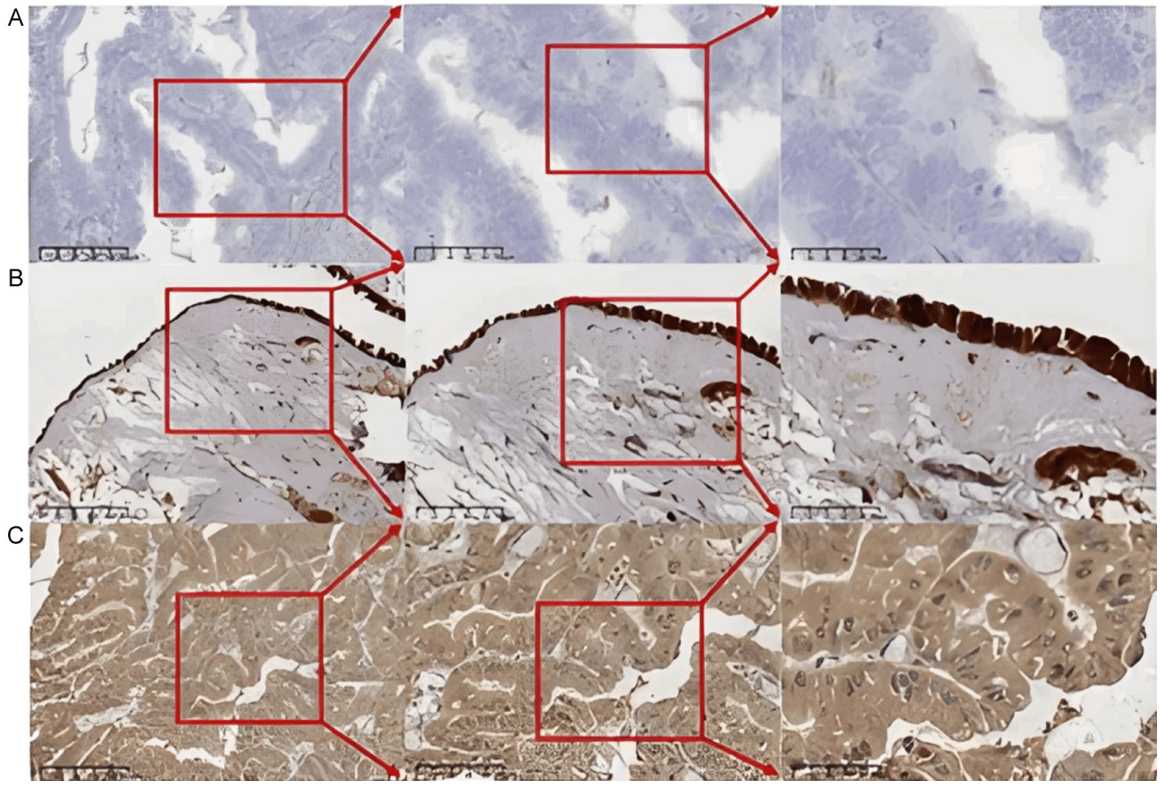

Figure 3. Calpain2 immunohistochemical staining in three types of OC (HE ×200). A. Benign ovarian tumor; B. Borderline ovarian tumor; C. OC. OC, ovarian cancer.

Table 3. Correlation analysis of FUT8, STX4 and calpain2 expression in ovarian cancer

FUT8	STX4	calpain2	r value	*P* value
177.50 (135.00, 198.75)	207.50 (166.25, 250.00)	-	0.405	< 0.001
177.50 (135.00, 198.75)	-	205.00 (152.50, 240.00)	0.632	< 0.001
-	207.50 (166.25, 250.00)	205.00 (152.50, 240.00)	0.495	< 0.001

Results expressed as median (Q1, Q3), Correlation coefficients (r) were calculated by Spearman rank correlation analysis; FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

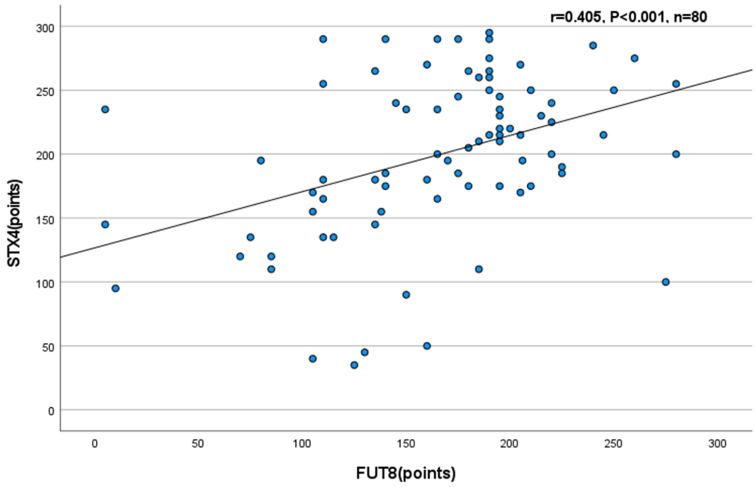

Figure 4. Analysis of the correlation between FUT8 expression and STX4 expression.

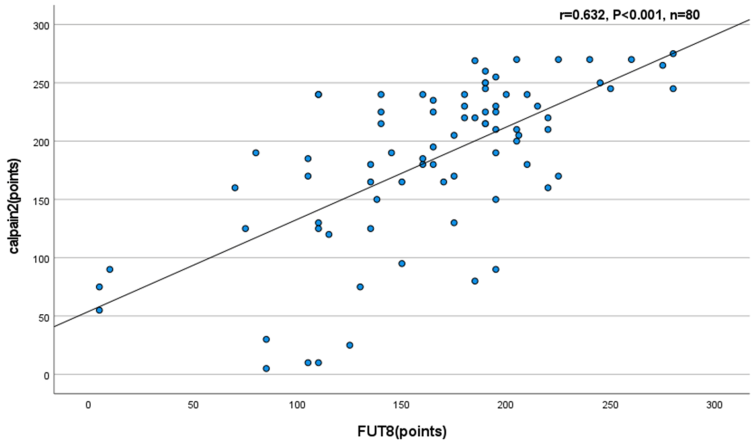

Figure 5. Analysis of the correlation between FUT8 expression and calpain2 expression.

off value = 167.5. A score ≥ 167.5 classified patients as high risk for ovarian cancer, demonstrating the highest diagnostic efficiency among the three indicators.

The combined FUT8/STX4/calpain2 model had an AUC of 0.894 (95% CI: 0.819-0.968, P < 0.001), sensitivity = 0.713, specificity = 1.000, Youden index = 0.713, and cutoff value = 0.9227803, indicating excellent diagnostic performance. The Hosmer-Lemeshow test yielded a *P*-value of 0.587 (> 0.05), suggesting a good model fit. This indicates that the model aligns well with the observed data and accurately reflects the characteristics and trends of ovarian cancer cases (**Tables 6**, **7**; **Figure 7**).

Correlation analysis of joint expression of two factors out of FUT8, STX4, and calpain2 with malignant ovarian tumors

In this study involving 92 patients with ovarian tumors, the median H-scores for FUT8, STX4, and calpain2 were 165, 187.5, and 195, respectively. Patients were categorized based on these median scores into high and low expression groups: high FUT8 (44 patients) and low FUT8 (48 patients); high STX4 (46 patients) and low STX4 (46 patients); high calpain2 (44 patients) and low calpain2 (48 patients). Statistical analyses revealed that high expression of FUT8, STX4, or calpain2 was significantly associated with malignant ovarian tumors, with higher malignancy rates observed in patients with high marker expression compared to those with low expression (P < 0.001; **Table 10**).

Further analysis examined the association between malignant ovarian tumors and the

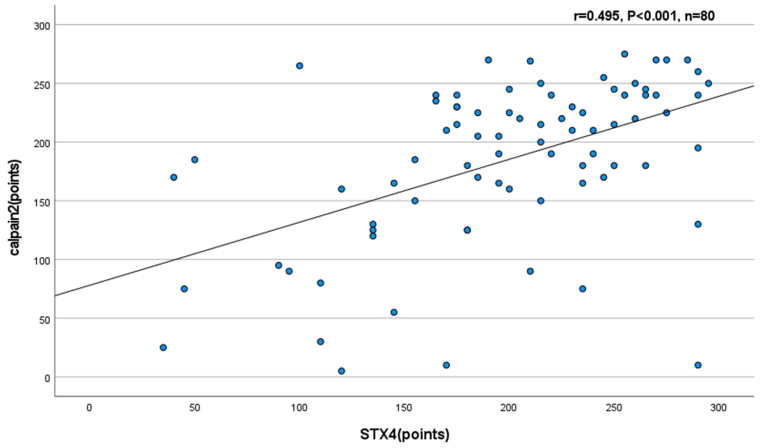

Figure 6. Analysis of the correlation between STX4 expression and calpain2 expression.

concurrent high expression of two markers (FUT8, STX4, and calpain2). The findings were as follows: FUT8 and STX4: The malignancy rate was 100.00% in patients with concurrent high expression of FUT8 and STX4, which was not significantly different from those with only one marker highly expressed (100.00%, P = 1.000). However, it was significantly higher than in patients with both markers showing low expression (67.57%, P < 0.001). FUT8 and Calpain2: The malignancy rate was 100.00% in patients with concurrent high expression of FUT8 and calpain2, showing no significant difference from those with one high marker (100.00%, P = 1.000) but significantly higher than in those with both markers showing low expression (67.57%, P < 0.001). STX4 and Calpain2: The malignancy rate was 100.00% in patients with concurrent high expression of STX4 and calpain2, which was not significantly different from those with one high marker (100.00%, P = 1.000) but significantly higher than in those with both markers showing low expression (65.71%, P < 0.001; **Table 8**).

Correlation analysis of joint expression of all three factors of FUT8, STX4, and calpain2 with malignant ovarian tumors

Among the 92 patients, 27 exhibited high expression of all three markers (FUT8, STX4, and calpain2). Compared to patients with at least one marker showing low expression, those with concurrent high expression of all three markers had a significantly higher malignancy rate (P = 0.002; **Table 9**).

Discussion

In this study, we identified that elevated expression levels of FUT8, STX4, and calpain2 are correlated with an increased risk of malignant ovarian tumors, highlighting their potential as biomarkers for malignancy prediction in patients with ovarian tumors. Furthermore, concurrent high expression of all three markers was significantly associated with a higher risk of malignant ovarian tumors in the studied cohort. These findings suggest that the simultaneous detection of FUT8, STX4, and calpain2 expression in ovarian tumor tissues could serve as a valuable diagnostic tool for identifying malignant ovarian tumors.

Ovarian cancer is the fifth most prevalent cancer among women worldwide, often developing during menopause, with a recent trend of earlier onset. The subtlety of early symptoms and the lack of effective diagnostic methods contribute to the increasing incidence of ovarian cancer, which has the highest mortality rate among gynecological malignancies [32-34]. Traditional screening methods, including transvaginal ultrasound and serum tumor markers such as carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4), have limitations in clinical practice [32-34].

Recently, novel molecular markers derived from liquid biopsy techniques, such as circulating tumor cells (CTCs), circulating tumor RNA (ctRNA), circulating tumor DNA (ctDNA), and tumor-educated platelets (TEPs), have expanded the targets for early ovarian cancer detection [32-34]. However, their diagnostic efficacy remains suboptimal when used independently. Emerging techniques, including gene chips, proteomics, and immunohistochemistry, are gaining prominence in research on the early diagnosis of ovarian cancer. Despite these advances, the heterogeneity of ovarian cancer's pathological types and the unclear mechanisms underlying its development mean that no single biomarker currently offers high sensitivity and specificity for early clinical diagnosis.

Table 4. Correlation of FUT8 expression with clinicopathologic features in ovarian cancer

Clinicopathologic parameters	Case No.	FUT8 expression		χ^2 value	*P* value
		Low	High		
Age (years)				1.455	0.228
< 50	25 (31.25)	15 (18.75)	10 (25.00)		
≥ 50	55 (68.75)	25 (81.25)	30 (75.00)		
Tissue type				0.092	0.762
Serous ovarian cancer	67 (83.75)	33 (82.50)	34 (85.00)		
Other types of ovarian cancer	13 (16.25)	7 (17.50)	6 (15.00)		
FIGO stage				6.241	0.013
I/II	33 (35.48)	22 (55.00)	11 (27.50)		
III/IV	47 (58.75)	18 (45.00)	29 (72.50)		
Differentiation				5.989	0.053
Poorly/Moderately differentiated	63 (78.75)	28 (70.00)	35 (87.50)		
Well-differentiated	5 (6.25)	5 (12.50)	0 (0.00)		
Unknown	12 (15.00)	7 (17.50)	5 (12.50)		
Tumor location				0.056	0.813
Unilateral	53 (66.25)	27 (67.50)	26 (65.00)		
Bilateral	27 (33.75)	13 (32.50)	14 (35.00)		
Lymph node metastasis				0.044	0.835
Negative	29 (78.38)	18 (62.07)	11 (37.93)		
Positive	8 (21.62)	4 (10.00)	4 (10.00)		
Distant metastasis				1.726	0.189
Negative	19 (23.75)	12 (30.00)	7 (17.50)		
Positive	61 (76.25)	28 (70.00)	33 (82.50)		

Results expressed as the number of patients and percentage (%); FUT8, Glycosyltransferase 8.

Table 5. Correlation of STX4 expression with clinicopathologic features in ovarian cancer

Clinicopathologic parameters	Case No.	STX4 expression		χ^2 value	*P* value
		Low	High		
Age (years)				6.267	0.012
< 50	25 (31.25)	18 (43.90)	7 (17.95)		
≥ 50	55 (68.75)	23 (56.10)	32 (82.05)		
Tissue type				0.658	0.417
Serous ovarian cancer	67 (83.75)	33 (80.49)	34 (87.18)		
Other types of ovarian cancer	13 (16.25)	8 (19.51)	5 (12.82)		
FIGO stage				0.244	0.621
I/II	33 (35.48)	18 (43.90)	15 (38.46)		
III/IV	47 (58.75)	23 (56.10)	24 (61.54)		
Differentiation				3.522	0.158
Poorly/Moderately differentiated	63 (78.75)	29 (70.73)	34 (87.18)		
Well-differentiated	5 (6.25)	3 (7.32)	2 (5.13)		
Unknown	12 (15.00)	9 (21.95)	3 (7.69)		
Tumor location				0.157	0.692
Unilateral	53 (66.25)	28 (38.29)	25 (64.10)		
Bilateral	27 (33.75)	13 (31.71)	14 (35.90)		
Lymph node metastasis				-	0.019
Negative	29 (78.38)	18 (62.07)	11 (37.93)		
Positive	8 (21.62)	1 (2.44)	7 (17.95)		
Distant metastasis				2.941	0.086
Negative	19 (23.75)	13 (31.71)	6 (15.38)		
Positive	61 (76.25)	28 (68.29)	33 (84.62)		

Results expressed as the number of patients and percentage (%); STX4, Synaptotagmin-4.

Table 6. Correlation of calpain2 expression with clinicopathologic features in ovarian cancer

Clinicopathologic parameters	Case No.	calpain2 expression		χ^2 value	*P* value
		Low	High		
Age(years)				0.524	0.469
< 50	25 (31.25)	14 (35.00)	11 (27.50)		
≥ 50	55 (68.75)	26 (65.00)	29 (72.50)		
Tissue type				0.092	0.762
Serous ovarian cancer	67 (83.75)	34 (85.00)	33 (82.50)		
Other types of ovarian cancer	13 (16.25)	6 (15.00)	7 (17.50)		
FIGO stage				0.464	0.496
I/II	33 (35.48)	15 (37.50)	18 (45.00)		
III/IV	47 (58.75)	25 (62.50)	22 (55.00)		
Differentiation				0.633	0.842
Poorly/Moderately differentiated	63 (78.75)	31 (77.50)	32 (80.00)		
Well-differentiated	5 (6.25)	2 (5.00)	3 (7.50)		
Unknown	12 (15.00)	7 (17.50)	5 (12.50)		
Tumor location				0.056	0.813
Unilateral	53 (66.25)	27 (67.50)	26 (65.00)		
Bilateral	27 (33.75)	13 (32.50)	14 (35.00)		
Lymph node metastasis				-	0.246
Negative	29 (78.38)	15 (51.72)	14 (48.28)		
Positive	8 (21.62)	2 (5.00)	6 (15.00)		
Distant metastasis				0.069	0.793
Negative	19 (23.75)	10 (25.00)	9 (22.50)		
Positive	61 (76.25)	30 (75.00)	31 (77.50)		

Results expressed as the number of patients and percentage (%).

Table 7. Multivariate binary logistic regression analysis for predicting ovarian cancer patients with ovarian tumors

Relevant factors	B	Standard error	Wald χ^2 value	*P* value	*OR* value	95% *CI* value	
						Lower limit	Upper limit
FUT8	0.009	0.008	1.388	0.239	1.009	0.994	1.025
STX4	0.007	0.006	1.138	0.286	1.007	0.994	1.020
calpain2	0.012	0.007	3.357	0.067	1.012	0.999	1.025

FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

Therefore, the search for reliable biomarkers remains critical for improving early diagnosis and treatment, potentially enhancing the quality of life and prognosis of affected women [35].

Ovarian cancer is characterized by advanced-stage presentation in most patients, with 70%-80% diagnosed only after disease progression beyond the early stages. This delay significantly worsens outcomes, with a five-year survival rate of 20%-30% for advanced cases [34]. The surgical management of ovarian lesions requires distinct approaches based on tumor type. For benign ovarian tumors, simple tumorectomy or adnexectomy is typically sufficient, while malignant ovarian tumors necessitate a multimodal approach involving surgery, chemotherapy, radiotherapy, integrative traditional Chinese and Western medicine, and gene therapy [36].

Failure to promptly address benign tumors may lead to unnecessary destruction of healthy ovarian tissue, whereas delayed treatment of malignant tumors results in missed therapeutic windows, poorer prognoses, and increased mortality risk. These factors underscore the critical importance of precise preoperative diagnostics and early detection of ovarian cancer, currently regarded as the most effective strategy for reducing mortality. Despite this urgency, there remains a lack of reliable biomarkers and effective diagnostic methods for

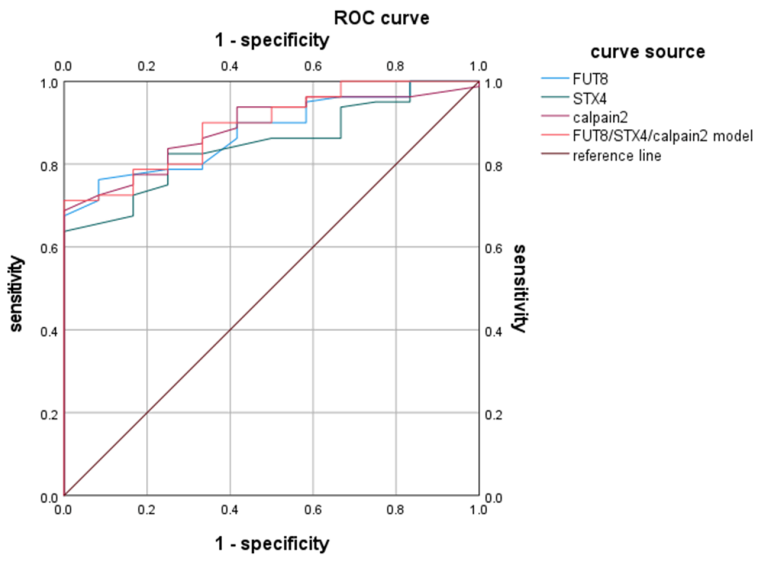

Figure 7. ROC curves for predicting malignant ovarian tumors patients based on related factors and the FUT8/STX4/calpain2 model.

early detection and screening [32-34]. Identifying biomarkers with improved sensitivity and specificity, as well as predictive value for accurate ovarian cancer diagnosis, is therefore paramount.

The dynamic process of tumor invasion and metastasis involves multiple factors and sequential steps, including the detachment of cancer cells from the primary tumor, invasion into adjacent tissues and organs, entry into blood or lymphatic vessels, and eventual colonization at distant sites, where they proliferate to form metastatic tumors [37]. This process is often influenced by aberrant protein glycosylation, which affects tumor growth, differentiation, transformation, adhesion, metastasis, and immune surveillance, thereby playing a critical role in tumorigenesis and migration [38, 39].

Our study revealed that high expression of FUT8 is significantly correlated with the FIGO stage of ovarian cancer and the benign/malignant classification of ovarian tumors. This association may be attributed to FUT8's role in upregulating core fucosylation modifications of substrate molecules, contributing to the malignant transformation, progression, and metastasis of tumors [40].

Additionally, high STX4 expression was significantly associated with patient age, lymph node metastasis, and the benign/malignant classification of ovarian tumors. This correlation may result from age-related variations in tumor biological characteristics, which influence STX4 expression [41]. For example, advancing age is associated with changes in the tumor microenvironment, cellular signaling pathways, and hormonal levels, particularly declining estrogen and progesterone levels in women, all of which could affect the onset and progression of ovarian cancer and STX4 expression [42]. Older patients may also experience cumulative genetic and epigenetic alterations, leading to changes in gene expression patterns, including STX4 [42]. Furthermore, age-related declines in immune system function may impact the tumor immune microenvironment and, consequently, STX4 expression and function [42, 43].

Tumor metastasis is a complex process involving interactions between cancer cells and their microenvironment. During metastasis, cancer cells infiltrate the extracellular matrix (ECM), a process heavily influenced by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, which are critical for intracellular vesicular transport [43]. Evidence indicates that specific SNARE proteins, particularly those involved in the transport of invasive sodium-associated proteins, play a pivotal role in enabling the invasive and migratory properties of malignant cancer cells [15, 16, 42, 44].

STX4, a member of the syntaxin family, is involved in guiding MT1-MMP to the plasma membrane [45]. Studies have demonstrated that STX4 mediates increased invasiveness in breast and ovarian tumors by promoting cell adhesion, proliferation, invasion, and chemotactic migration, while reducing apoptosis [19, 46, 47]. The integrin transport mediated by STX4 is essential for cancer cell migration and survival, thereby contributing to ovarian cancer progression [19].

Despite these findings, significant knowledge gaps remain regarding the direct impact of

Table 8. Predictive value of individual related factors and FUT8/STX4/calpain2 model for ovarian cancer patients with ovarian tumors

Prognostic indicators	AUC	95% CI	*P* value	Youden index	Cut-off value	Sensitivity	Specificity
FUT8	0.879	0.803-0.955	0.000	0.680	132.500	0.763	0.917
STX4	0.848	0.762-0.935	0.000	0.638	182.500	0.638	1.000
calpain2	0.888	0.814-0.961	0.000	0.688	167.500	0.688	1.000
FUT8/STX4/calpain2 model	0.894	0.819-0.968	0.000	0.713	0.9227803	0.713	1.000

FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

Table 9. Correlation analysis of joint expression of two of FUT8, STX4, and calpain2 with tumor type

	Case No.	Tumor type		χ^2 value	*P* value
		Benign (%)	Malignant (%)		
FUT8/STX4					
(1) Both of FUT8/STX4 low expression	37	12 (32.43)	25 (67.57)	19.408	< 0.001
(2) One of FUT8/STX4 high expression	20	0 (0.00)	20 (100.00)	(2) versus (3)	
(3) Both of FUT8/STX4 high expression	35	0 (0.00)	35 (100.00)	-	1.000
FUT8/calpain2					
(1) Both of FUT8/calpain2 low expression	37	12 (32.43)	25 (67.57)	19.371	< 0.001
(2) One of FUT8/calpain2 high expression	22	0 (0.00)	22 (100.00)	(2) versus (3)	
(3) Both of FUT8/calpain2 high expression	33	0 (0.00)	33 (100.00)	-	1.000
STX4/calpain2					
(1) Both of STX4/calpain2 low expression	35	12 (34.29)	23 (65.71)	20.935	< 0.001
(2) One of STX4/calpain2 high expression	24	0 (0.00)	24 (100.00)	(2) versus (3)	
(3) Both of STX4/calpain2 high expression	33	0 (0.00)	33 (100.00)	-	1.000

Results expressed as the number of patients and percentage (%); FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

Table 10. Correlation analysis of joint expression of FUT8, STX4, and calpain2 factors with tumor type

	Case No.	Tumor type		χ^2 value	*P* value
		Benign (%)	Malignant (%)		
FUT8/STX4/calpain2				18.731	0.002
(1) Fewer than FUT8, STX4, and calpain2 high expression	65	12 (18.46)	53 (81.54)		
$FUT8^{low}/STX4^{low}/calpain2^{low}$	32	12 (37.50)	20 (62.50)		
$FUT8^{low}/STX4^{high}/calpain2^{high}$	6	0 (0.00)	6 (100.00)		
$FUT8^{high}/STX4^{low}/calpain2^{high}$	6	0 (0.00)	6 (100.00)		
$FUT8^{high}/STX4^{high}/calpain2^{low}$	8	0 (0.00)	8 (100.00)		
$FUT8^{low}/STX4^{low}/calpain2^{high}$	5	0 (0.00)	5 (100.00)		
$FUT8^{low}/STX4^{high}/calpain2^{low}$	5	0 (0.00)	5 (100.00)		
$FUT8^{high}/STX4^{low}/calpain2^{low}$	3	0 (0.00)	3 (100.00)		
(2) All of FUT8, STX4, and calpain2 high expression	27	0 (0.00)	27 (100.00)		

Results expressed as the number of patients and percentage (%); FUT8, Glycosyltransferase 8; STX4, Synaptotagmin-4.

STX4 on ovarian cancer cell proliferation and invasion, as well as its potential influence on the tumor microenvironment. Further research is required to elucidate the precise mechanisms by which STX4 regulates these processes and to explore its potential as a therapeutic target in ovarian cancer [48]. Our study revealed a significant association between high calpain2 expression and the classification of ovarian tumors as benign or malignant. This correlation may stem from calpain2's role in cytoskeletal remodeling during cell motility and fusion, regu-

lation of enzymatic degradation within the cell cycle, hydrolytic modification in signaling pathways, modulation of gene expression, degradation of apoptotic pathway substrates, and its effects as a long-range enhancer [37].

Calpain2, a highly conserved calcium-dependent protease, requires sufficient calcium ions for activation. Under physiological conditions, intracellular calcium levels are typically insufficient to activate calpain2. However, in malignant tumor cells, intracellular calcium concentrations are significantly elevated, disrupting calcium homeostasis and leading to increased calpain2 activity or expression. This elevation promotes tumor cell migration and invasion, highlighting calpain2's potential as a therapeutic target and its involvement in the malignant transformation and metastatic cascade of ovarian cancer [49]. Further research is necessary to elucidate the mechanisms by which calpain2 contributes to these processes, which could inform the development of novel therapeutic strategies targeting ovarian cancer progression.

Given the significant associations of FUT8, STX4, and calpain2 with ovarian tumor classification, we utilized these markers in a combined diagnostic model to differentiate between benign and malignant ovarian tumors. The combined model demonstrated an AUC of 0.894, with a sensitivity of 0.713 and a specificity of 1.000, reflecting high diagnostic accuracy. ROC curve analysis identified optimal cutoff values of 132.5, 182.5, and 167.5 for FUT8, STX4, and calpain2, respectively. These findings suggest that tumors with H-scores of FUT8 $\geq$ 132.5, STX4 $\geq$ 182.5, and calpain2 $\geq$ 167.5 can be classified as malignant. Achieving these cutoff values for all three markers simultaneously enhances diagnostic precision.

We recommend that medical professionals assess the expression of FUT8, STX4, and calpain2 comprehensively to distinguish between benign and malignant ovarian tumors. Timely and accurate diagnosis based on these markers can improve patient prognosis and quality of life.

Glycosylation, a pivotal epigenetic modification, reflects cellular environmental changes without altering the genetic sequence and is one of the most prevalent post-translational modifications in all organisms. Aberrant glycosylation is a hallmark of tumorigenesis, profoundly influencing various stages of tumor development and directly promoting tumor progression and metastasis [50].

FUT8, the sole fucosyltransferase responsible for core fucosylation, adds fucose to asparagine residues in glycoproteins. Abnormal FUT8 activity has been identified in multiple cancers, including breast, prostate, and gastric cancers, and has been linked to prognosis. FUT8 influences tumorigenesis and progression by regulating critical processes such as cell proliferation, apoptosis, migration, and metastasis [10, 51, 52].

In this study, we observed elevated FUT8 expression in malignant ovarian tumor tissues. These findings suggest that core fucosylation of the N-glycan structures of key receptors in ovarian cancer may be influenced by additional factors beyond FUT8 alone. Further exploration of these mechanisms may provide insights into the molecular pathways driving ovarian cancer progression and identify potential therapeutic targets.

The tumor microenvironment consists of a diverse array of cellular components, including inflammatory cells, fibroblasts, neurons, and vascular endothelial cells. These cells either directly activate proliferative signals in cancer cells or indirectly modulate the tumor milieu by secreting various molecules [53]. Our study identified a significant correlation between FUT8 and STX4 or calpain2, suggesting a potential interplay among these factors that may synergistically enhance the proliferation and metastatic potential of ovarian tumor tissues through distinct mechanisms. However, the biological interrelationships between FUT8 and these markers in ovarian cancer remain unexplored, necessitating further investigation to elucidate the underlying mechanisms.

In our analysis, the co-expression of FUT8 and STX4 or calpain2 did not yield significantly greater diagnostic accuracy in differentiating benign from malignant ovarian tumors compared to the use of individual markers. This observation implies that the independent expression of FUT8, STX4, or calpain2 in ovarian tumors is already sufficient to influence tumor differentiation. Therefore, it is recommended

that medical practitioners assess the expression levels of these three markers collectively to accurately classify ovarian tumors as benign or malignant. Early and precise diagnosis, coupled with timely intervention, could improve patient prognoses and enhance quality of life.

Calpain2, a calcium-activated neutral protease in the cysteine proteinase superfamily, consists of an 80 kD catalytic subunit and a 30 kD regulatory subunit, forming a heterodimer [54]. This enzyme is involved in numerous cellular processes, including cytoskeletal reorganization, proteolytic modification of signaling proteins, substrate degradation in apoptotic pathways, and enzyme turnover within the cell cycle. In oncology, calpain2 is critical for maintaining the malignant phenotype of tumor cells [24].

Despite its involvement in calcium-mediated cellular functions, the precise physiological role of calpain2 remains incompletely understood. As a highly conserved protease, calpain2 activation depends on sufficient calcium ions [55]. Under normal conditions, intracellular calcium levels are insufficient to activate calpain2. However, in malignant tumor cells, elevated intracellular calcium disrupts cellular equilibrium, suggesting that calpain2 operates under high calcium concentrations in cancer cells [56].

Interestingly, studies on vertebrate heart development show that arrhythmias caused by STX4 dysfunction can be ameliorated by calcium supplementation [47]. Using immunohistochemical staining, we analyzed the relationship between STX4 and calpain2 expression in human ovarian tumor tissues and observed a significant correlation between the two. This finding suggests that aberrant STX4 expression and activation may contribute to calpain2 overexpression in ovarian cancer.

We hypothesize that reducing calcium ion levels in tumors could potentially downregulate both STX4 and calpain2 expression. Clinical evidence from our study suggests that regulating STX4 and calpain2 expression may partially influence the progression and metastasis of ovarian tumors. However, further research is required to elucidate the mechanisms of calpain2 activation through STX4-mediated signaling pathways and their synergistic roles in tumor progression.

This study has several limitations that warrant consideration. Firstly, the precise mechanisms by which FUT8, STX4, and calpain2 contribute to the malignant biological behaviors of ovarian cancer remain incompletely understood. These mechanisms are likely influenced by complex regulatory factors, and further in-depth research is needed to clarify their roles. Secondly, the relatively small sample size and the single-center design of this study may introduce selection bias. To address these limitations, future studies should aim to increase the sample size, incorporate high-quality cases, and perform multicenter investigations to improve the precision and generalizability of the findings. While clinical evidence suggests that the regulation of STX4 and calpain2 expression may partially contribute to ovarian tumor progression and metastasis, additional studies are required to elucidate the activation mechanism of calpain2 through STX4-mediated signaling pathways and their synergistic effects in tumor development.

In conclusion, early diagnosis of ovarian cancer remains a challenging yet critical goal, as it is essential for initiating timely treatment and improving patient outcomes. The identification of reliable biomarkers is vital for guiding the selection of effective diagnostic methods and enhancing the accuracy of ovarian cancer detection. Biomarkers also provide a valuable reference for the combined detection of ovarian cancer markers, contributing significantly to improving diagnostic and treatment outcomes.

This study demonstrates that FUT8, STX4, and calpain2 are highly expressed in ovarian cancer and that their expression is interrelated. Individually, these biomarkers may aid in differentiating tumor types, while their combined use in a diagnostic model offers superior efficacy in distinguishing between benign and malignant ovarian tumors. These findings suggest that the combined expression of FUT8, STX4, and calpain2 could serve as a valuable predictor of malignancy in ovarian tumors.

Additionally, our results highlight the potential role of these three proteins in promoting tumor angiogenesis and metastasis. Targeting FUT8, STX4, or calpain2 may offer a promising adjuvant therapeutic strategy for ovarian cancer. To further validate these findings, future research should focus on expanding sample sizes, con-

ducting multicenter cohort studies, and investigating the impact of FUT8, STX4, and calpain2 on ovarian cancer prognosis. Moreover, in-depth exploration of the complex mechanisms underlying the involvement of these biomarkers in the malignant progression of ovarian cancer is essential to advance our understanding and develop novel therapeutic strategies.

Acknowledgements

This study was supported by Pudong New Area Health and Family Planning Commission Health Science and Technology Project (PW2022A-18) and Pudong New Area Health and Family Planning Commission Discipline Construction Project (PWZxk2022-28).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wenfeng Ye, Department of Obstetrics and Gynecology, Pudong New Area People's Hospital, Shanghai 201299, China. Tel: +86-13801509020; E-mail: yewenfengcz@163.com

References

[1] Siminiak N, Czepczyński R, Zaborowski MP and Iżycki D. Immunotherapy in ovarian cancer. Arch Immunol Ther Exp (Warsz) 2022; 70: 19.

[2] Sasamoto N and Elias KM. Early detection of ovarian cancer. Cold Spring Harb Perspect Med 2023; 13: a041337.

[3] Xia C, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N and Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl) 2022; 135: 584-590.

[4] Peng JX, Li Y, Ma XH and Liu L. Analysis of death and life loss of four common gynecological malignant tumors in Yinchuan city from 2011 to 2015. J Ningxia Med Univ 2019; 41: 254-258, 267.

[5] Ayyagari V, Li M, Pasman Z, Wang X, Louis S, Diaz-Sylvester P, Groesch K, Wilson T and Brard L. Assessment of the diagnostic and prognostic relevance of ACAT1 and CE levels in plasma, peritoneal fluid and tumor tissue of epithelial ovarian cancer patients - a pilot study. BMC Cancer 2022; 22: 387.

[6] Weimer J, Bozorghadad H, Schoonover K, Carll C and Repco K. Familiarity and perceptions of ovarian cancer biomarker testing and targeted therapy: a survey of oncology nurses in the United States. Oncol Nurs Forum 2023; 50: 437-448.

[7] Schneider M, Al-Shareffi E and Haltiwanger RS. Biological functions of fucose in mammals. Glycobiology 2017; 27: 601-618.

[8] Matsushita T, Nozaki M, Sunaga M, Koyama T, Hatano K and Matsuoka K. Preparation of N-linked-type GlcNAc Monomers for glycopolymers and binding specificity for lectin. ACS Omega 2023; 8: 37329-37340.

[9] Ouchida T, Maeda H, Akamatsu Y, Maeda M, Takamatsu S, Kondo J, Misaki R, Kamada Y, Ueda M, Ueda K and Miyoshi E. The specific core fucose-binding lectin Pholiota squarrosa lectin (PhoSL) inhibits hepatitis B virus infection in vitro. Sci Rep 2023; 13: 6175.

[10] Höti N, Lih TS, Pan J, Zhou Y, Yang G, Deng A, Chen L, Dong M, Yang RB, Tu CF, Haffner MC, Kay Li Q and Zhang H. A comprehensive analysis of FUT8 overexpressing prostate cancer cells reveals the role of EGFR in castration resistance. Cancers (Basel) 2020; 12: 468.

[11] Guo H, Sun Q, Huang X, Wang X, Zhang F, Qu W, Liu J, Cheng X, Zhu Q, Yi W, Shu Q and Li X. Fucosyltransferase 8 regulates adult neurogenesis and cognition of mice by modulating the Itga6-PI3K/Akt signaling pathway. Sci China Life Sci 2024; 67: 1427-1440.

[12] van Pul L, Maurer I, Boeser-Nunnink BDM, Harskamp AM, van Dort KA and Kootstra NA. A genetic variation in fucosyltransferase 8 accelerates HIV-1 disease progression indicating a role for N-glycan fucosylation. AIDS 2023; 37: 1959-1969.

[13] Tudor L, Nedic Erjavec G, Nikolac Perkovic M, Konjevod M, Uzun S, Kozumplik O, Mimica N, Lauc G, Svob Strac D and Pivac N. The association of the polymorphisms in the FUT8-related locus with the plasma glycosylation in post-traumatic stress disorder. Int J Mol Sci 2023; 24: 5706.

[14] Kyunai YM, Sakamoto M, Koreishi M, Tsujino Y and Satoh A. Fucosyltransferase 8 (FUT8) and core fucose expression in oxidative stress response. PLoS One 2023; 18: e0281516.

[15] Wang S, Xie K, Ren ZJ and Ren BH. Research progress on the role of SNARE protein in tumorigenesis. Journal of Medical Postgraduates 2022; 35: 872-876.

[16] Liu H, Dang R, Zhang W, Hong J and Li X. SNARE proteins: Core engines of membrane fusion in cancer. Biochim Biophys Acta Rev Cancer 2024; 189148.

[17] Huang Y, Su Y, Shen L, Huo Z, Chen C, Sun T, Tian X, Li N and Yang C. A novel IFNbeta-induced long non-coding RNA ZAP-IT1 interrupts Zika virus replication in A549 cells. Virol Sin 2022; 37: 904-912.

[18] Tebbe L, Kakakhel M, Al-Ubaidi MR and Naash MI. The role of syntaxins in retinal function and health. Front Cell Neurosci 2024; 18: 1380064.
[19] Ye W, Zhu D, Xu B, Xu M, Liu Y, Xie J, Wang Q and Jiang J. STX4 expression is associated with classification, clinical stage and lymphatic metastasis in ovarian cancer. Transl Cancer Res 2019; 8: 466-472.
[20] Tian X, Gao Z, Yin D, Hu Y, Fang B, Li C, Lou S, Rao Z and Shi R. 17beta-estradiol alleviates contusion-induced skeletal muscle injury by decreasing oxidative stress via SIRT1/PGC-1α/Nrf2 pathway. Steroids 2023; 191: 109160.
[21] Williams JK, Ngo JM, Murugupandiyan A, Croall DE, Hartzell HC and Schekman R. Calpains orchestrate secretion of annexin-containing microvesicles during membrane repair. bioRxiv [Preprint] 2024; 2024.09.05.611512.
[22] Samuels TL, Blaine-Sauer S, Yan K, Plehhova K, Coyle C and Johnston N. Topical alginate protection against pepsin-mediated esophageal damage: E-cadherin proteolysis and matrix metalloproteinase induction. Int J Mol Sci 2023; 24: 7932.
[23] Jiang Q, Zhang D, Liu J, Liang C, Yang R, Zhang C, Wu J, Lin J, Ye T, Ding L, Li J, Gao S, Li B and Ye Q. HPIP is an essential scaffolding protein running through the EGFR-RAS-ERK pathway and drives tumorigenesis. Sci Adv 2023; 9: eade1155.
[24] Vella MA, García DC, De Boeck M, Valdecantos PA and Roldán-Olarte M. Role of steroid hormones in the maintenance of focal adhesions in bovine oviductal epithelial cells. Domest Anim Endocrinol 2024; 88: 106839.
[25] Mostoufi-Moab S, Kelly A, Mitchell JA, Baker J, Zemel BS, Brodsky J, Long J and Leonard MB. Changes in pediatric DXA measures of musculoskeletal outcomes and correlation with quantitative CT following treatment of acute lymphoblastic leukemia. Bone 2018; 112: 128-135.
[26] Qi Z, Bai X, Wu L, Zhang P, Guo Z and Yu Y. CAPN2 promotes apalutamide resistance in metastatic hormone-sensitive prostate cancer by activating protective autophagy. J Transl Med 2024; 22: 538.
[27] Phippen NT, Wang G, Bateman NW, Lowery WJ, Stany MP, Hamilton CA, Maxwell GL, Conrads TP and Darcy KM. FIGO staging criteria is a meaningful clinical threshold for evaluating molecular signatures in serous ovarian cancer patients. Gynecologic Oncology 2015; 137: 188-188.
[28] Varghese F, Bukhari AB, Malhotra R and De A. IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. PLoS One 2014; 9: e96801.
[29] Bitirim CV. The role of zinc transporter proteins as predictive and prognostic biomarkers of hepatocellular cancer. PeerJ 2021; 9: e12314.
[30] Dabbs DJ. Diagnostic Immunohistochemistry 3rd ed. Elsevier Health Sciences 2013.
[31] Chen Y, Du H, Wei BH, Chang XN and Dong CM. Development and validation of risk-stratification delirium prediction model for critically ill patients: a prospective, observational, single-center study. Medicine (Baltimore) 2017; 96: e7543.
[32] Dai J, Fanf XX and Fan BH. Advances in the diagnostic methods of ovarian tumor. Journal of Inner Mongolia Medical University 2020; 42: 662-665.
[33] Sun L, Tan J and Xin H. Research progress and application of tumor markers in the diagnosis of ovarian cancer. Chemical Industry Times 2020; 34: 22-25.
[34] Wang XG, Xu ZY, Liu SJ and Xing JL. Research progress of tumor markers in the diagnosis of ovarian cancer. Chinese Journal of Oncology Prevention and Treatment 2022; 14: 111-116.
[35] Xin YQ, Ju YY and Wang XH. Advances in biomarkers and early diagnosis of ovarian cancer. Journal of International Obstetrics and Gynecology 2020; 47: 544-549.
[36] Jiao XF and Gao QL. Research progress of biomarkers for ovarian cancer. Practical Oncology 2024; 39: 401-406.
[37] Nagae M, Yamaguchi Y, Taniguchi N and Kizuka Y. 3D structure and function of glycosyltransferases involved in N-glycan maturation. Int J Mol Sci 2020; 21: 437.
[38] Tingting Y, Xiaoyan G, Mingli X, Chaoyi Y and Chengcheng L. Research progress of fucosyltransferase 8 in tumors. Cancer progression 2023; 21: 465-467+534.
[39] Khorami-Sarvestani S, Hanash SM, Fahrmann JF, León-Letelier RA and Katayama H. Glycosylation in cancer as a source of biomarkers. Expert Rev Proteomics 2024; 21: 345-365.
[40] Wei X, Liu X, Shi L, Yun F and Jia YF. The effect of fucosyltransferase 8 expression on prognosis and immune cell infiltration in breast cancer. Practical Journal of Clinical Medicine 2021; 25: 76-81, 87.
[41] Kang HJ, Bai SH, Li R, Gao Y, Yan YL, Li GZ, Feng ZD, Ma W, Zhang JZ and Ren J. To construct a prognostic model for ovarian cancer patients based on immune-related genes. Journal of Practical Oncology 2023; 38: 347-354.
[42] Pan LL and Li L. Bioinformatics analysis and clinical verification of prognostic markers in patients with advanced epithelial ovarian cancer undergoing neoadjuvant chemotherapy.

Chinese Journal of Obstetricsand Gynecology 2023; 58: 121-128.
[43] Yang ZX, Qin SY, Zou D and Yang LH. Bioinformatics-based screening of chemokines related to the prognosis of ovarian cancer patients and construction of a prognostic model. Cancer Progression 2023; 21: 718-723.
[44] Matsuguchi S and Hirai Y. Syntaxin4, P-cadherin, and CCAAT enhancer binding protein β as signaling elements in the novel differentiation pathway for cultured embryonic stem cells. Biochem Biophys Res Commun 2023; 672: 27-35.
[45] Livingstone R, Bryant NJ, Boyle JG, Petrie JR and Gould GW. Diabetes is accompanied by changes in the levels of proteins involved in endosomal GLUT4 trafficking in obese human skeletal muscle. Endocrinol Diabetes Metab 2022; 5: e361.
[46] Brasher MI, Chafe SC, McDonald PC, Nemirovsky O, Gorshtein G, Gerbec ZJ, Brown WS, Grafinger OR, Marchment M, Matus E, Dedhar S and Coppolino MG. Syntaxin4-Munc18c interaction promotes breast tumor invasion and metastasis by regulating MT1-MMP trafficking. Mol Cancer Res 2022; 20: 434-445.
[47] Perl E, Ravisankar P, Beerens ME, Mulahasanovic L, Smallwood K, Sasso MB, Wenzel C, Ryan TD, Komár M, Bove KE, MacRae CA, Weaver KN, Prada CE and Waxman JS. Stx4 is required to regulate cardiomyocyte Ca(2+) handling during vertebrate cardiac development. HGG Adv 2022; 3: 100115.
[48] Zeng K, Li Q, Wang X, Liu C, Chen B, Song G, Li B, Liu B, Gao X, Zhang L and Miao J. STX4 as a potential biomarker for predicting prognosis and guiding clinical treatment decisions in clear cell renal cell carcinoma. Heliyon 2024; 10: e23918.
[49] Tang J, Wu YM, Zhao P, Yang XM, Jiang JL and Chen ZN. Overexpression of HAb18G/CD147 promotes invasion and metastasis via alpha-3beta1 integrin mediated FAK-paxillin and FAK-PI3K-Ca2+ pathways. Cell Mol Life Sci 2008; 65: 2933-2942.
[50] Liu S, Tu C, Zhang H, Huang H, Liu Y, Wang Y, Cheng L, Liu BF, Ning K and Liu X. Noninvasive serum N-glycans associated with ovarian cancer diagnosis and precancerous lesion prediction. J Ovarian Res 2024; 17: 26.
[51] Ma M, Han G, Wang Y, Zhao Z, Guan F and Li X. Role of FUT8 expression in clinicopathology and patient survival for various malignant tumor types: a systematic review and meta-analysis. Aging (Albany NY) 2020; 13: 2212-2230.
[52] Duran A, Guerrero PE, Ortiz MR, Pérez Del Campo D, Castro E, Garcia-Velasco A, Fort E, de Llorens R, Saldova R, Llop E and Peracaula R. Characterization of mesothelin glycosylation in pancreatic cancer: decreased core fucosylated glycoforms in pancreatic cancer patients' sera. Biomedicines 2022; 10: 1942.
[53] Oya Y, Hayakawa Y and Koike K. Tumor microenvironment in gastric cancers. Cancer Sci 2020; 111: 2696-2707.
[54] Yang Q, Wang JY, Wang L, Zhou PF, He KQ, Yan M, Mao DH, Chen TX and Zhang JJ. Estradiol promotes migration and invasion of SK-Br3 cells through calcium-activated neutral protease 2 promoting the limited hydrolysis of integrin β4. Journal of Guizhou Medical University 2023; 48: 147-152.
[55] Zeng ZR, Lei S, Zhang JJ, Yang YS, Xue Y, Lan JZ, Sun YM, Hu XX and Chen TX. Hypoxia promotes migration and invasion of pancreatic cancer cells via inducing calpain 2 expression. Tumor 2019; 39: 595-605.
[56] Zhang X, Ren Z, Liu B and Wei S. RUNX2 mediates renal cell carcinoma invasion through calpain2. Biol Pharm Bull 2022; 45: 1653-1659.