ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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CLINICOPATHOLOGIC VALUE OF IMMUNOHISTOCHEMICAL PINCH/LIMS-1 ANTIBODY EXPRESSION IN OVARIAN SEROUS CARCINOMA AND BORDERLINE SEROUS TUMOR

OVER SERÖZ KARSİNOMUNDA VE BORDERLİNE SERÖZ TÜMÖRDE İMMÜNHİSTOKİMYASAL PINCH/LIMS-1 ANTİKOR EKSPRESYONUNUN KLİNİKOPATOLOJİK DEĞERİ

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Öz

Amaç

Özellikle ilginç cys-his zengin proteinin (PINCH/LIMS-1), tümörlerdeki ve tümörle ilişkili stromadaki kanser hücrelerinin gelişimini ve yayılımını denetlediği varsayılmaktadır. Bu çalışmanın amacı, seröz borderline tümör (SBT) ve seröz karsinomda (SC) tümör ve peritümöral stromadaki PINCH-1 ekspresyonunu değerlendirmek ve ekspresyonu ile çeşitli klinik ve patolojik parametreler arasındaki ilişkileri incelemektir.

Gereç ve Yöntem

Yirmi bir SBT ve 89 SK vakasında PINCH-1 antikorunun ekspresyonu streptavidin/HRP-biotin ile indirekt immünoperoksidaz tekniği kullanılarak analiz edilmiştir. PINCH-1'in tümör ve peritümöral stromadaki boyanma paterni semikantitatif skorlama yöntemi kullanılarak değerlendirilmiştir. Çalışmada kullanılan boyama yöntemi PINCH-1 ekspresyonunun tanımlanmasına olanak sağlamış ve semikantitatif skorlama yöntemiyle PINCH-1 boyanmasının yaygınlığı ve yoğunluğu değerlendirilmiştir. Böylece, PINCH-1 ekspresyonu ile hasta yaşı, tümör boyutu, FIGO evresi, intraabdominal yıkama sitolojisi, kapsül invazyonu, tümör yerleşimi, tümör derecesi ve tanı anındaki kanser antijen 125 (CA125) seviyeleri gibi çeşitli klinik ve patolojik faktörler arasındaki korelasyon incelenmiştir.

Bulgular

Çalışmada PINCH-1'in SK vakalarında SBT vakalarına göre daha yaygın olduğu bulunmuştur. SK'ler SBT vakalarındakilere göre daha güçlü boyanma göstermiştir (p<0,001). Çalışmada ayrıca, tümörü çevreleyen dokudaki PINCH-1 boyanmasının yaygınlığı ve yoğunluğu ile tümörün tek ya da bilateral overde yer alması arasında pozitif bir korelasyon bulunmuştur (dağılım için p = 0,038, yoğunluk için p = 0,024). Bununla birlikte, PINCH-1 boyanması ile tümör boyutu arasında negatif bir korelasyon vardı (yaygınlık için p=0.019, yoğunluk için p=0.007). Ayrıca, PINCH-1 tümör boyanmasının yoğunluğu, FIGO evresi ve tümör dereceleri arttıkça istatistiksel anlamlılık sergilemiştir (sırasıyla p = 0,032 ve p = 0,001).

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Sonuç
Bu çalışmanın sonuçları, SK'lerin SBT'lere göre daha yüksek düzeyde PINCH-1 boyanma yoğunluğu sergilediğini göstermektedir. Ayrıca, FIGO evresindeki ve tümör derecesindeki artış, tümör dokusunda artan PINCH-1 boyanma yoğunluğu ile ilişkilidir. Peritümöral stromadaki PINCH-1 boyanmasının yayılımı ve yoğunluğu bilateral tümör vakalarında daha dikkat çekicidir, ancak tümör boyutuyla ters orantılıdır. PINCH-1 ekspresyonu ile önemli klinikopatolojik faktörler arasında gözlenen ilişki, bu molekülün seröz over kanseri gelişiminde rol oynayabileceğini düşündürmektedir. Çalışmamız PINCH-1'in tümörogenezdeki rolünün daha iyi anlaşılmasına katkıda bulunabilir ve bu yolağı hedefleyen yeni terapötik stratejilerin geliştirilmesi için yol gösterici olabilir.

Anahtar Kelimeler: FIGO evresi, PINCH/LIMS-1, Seröz borderline tümör, Seröz karsinom, Seröz over karsinomunda PINCH ekspresyonu

Abstract

Objective
Particularly interesting cys-his rich protein (PINCH/LIMS-1), a protein implicated in cell adhesion, is assumed to oversee the development and invasion of cancer cells in tumors and tumor-associated stroma. This study aimed to assess PINCH-1 expression in serous borderline tumor (SBT) and serous carcinoma (SC) in the tumor and peritumoral stroma and scrutinize any associations between its expression and various clinical and pathological parameters.

Material and Method
In this study, the expression of the PINCH-1 antibody was analyzed in 21 cases of SBT and 89 cases of SC using the indirect immunoperoxidase technique with streptavidin/HRP-biotin. The staining pattern of PINCH-1 in the tumor and peritumoral stroma was evaluated using a semiquantitative scoring method. The staining procedure used in the study allowed for the accurate identification of PINCH-1 expression, and the data obtained through the semiquantitative scoring method provided a reliable of assessing the degree and intensity of PINCH-1 staining. Thus, the correlation between PINCH-1 expression and various pathologic factors such as patient age, tumor size, FIGO stage, intra-abdominal washing cytology, capsule invasion, tumor location in the ovary, tumor grade, and cancer antigen 125 (CA125) levels at the time of diagnosis was examined.

Results
The study found that PINCH-1 was more prevalent in cases of SC than in SBT cases. The tumors in SC cases had stronger staining than those in SBT cases ($p<0.001$). The study also found a positive correlation between the diffusiveness and intensity of PINCH-1 staining in the tissue surrounding the tumor and whether the tumor was located on one or both sides of the ovaries ($p = 0.038$ for diffusiveness, $p = 0.024$ for intensity). However, there was a negative correlation between PINCH-1 staining and tumor size ($p=0.019$ for diffusiveness, $p=0.007$ for intensity). Furthermore, the intensity of PINCH-1 tumor staining exhibited statistical significance in Figo stage and tumor grades as these increased ($p = 0.032$ and $p = 0.001$, respectively).

Conclusion
The results of this study indicate that SCs exhibit a higher level of PINCH-1 staining intensity than SBTs. Furthermore, an increase in the FIGO stage and tumor grade is associated with increased intensity of PINCH-1 staining in the tumor tissue. Additionally, the diffusiveness and intensity of PINCH-1 staining in the peritumoral stroma is more remarkable in cases of bilateral tumors but is inversely correlated with tumor size. The observed association between PINCH-1 expression and important clinicopathologic factors suggests that this molecule may be involved in developing serous ovarian cancer. Overall, these findings may contribute to a better understanding of the role of PINCH-1 in ovarian tumorigenesis and may have implications for developing novel therapeutic strategies targeting this pathway.

Keywords: FIGO stage, PINCH/LIMS-1, PINCH expression in serous ovarian carcinoma, Serous borderline tumor, Serous carcinoma

Introduction

Ovarian cancer constitutes approximately 30% of malignancies of the female genital system, ranking second among gynecological cancers in developed societies following endometrial cancers (1). It ranks as the seventh most frequently detected cancer in women worldwide. The etiology of the majority of tumors, accounting for approximately 60-70 percent, can be traced back to an epithelial origin. Approximately 30-50 percent of these tumors are bilateral. Epithelial malignancies account for 90-95 percent of ovarian

malignancies, with SC comprising 60-75 percent of these cases. Among these, high-grade serous carcinoma (HGSC) is the most frequently encountered, with 50-70 percent of cases being bilateral (2–4). Presently, the challenges pertaining to early detection and intervention remain considerable, often leading to diagnoses occurring during advanced stages of the disease. This delayed diagnosis contributes significantly to the elevated mortality rates associated with ovarian cancer, which stands as the leading cause of gynecologic cancer-related deaths on a global scale. Regrettably, owing to its asymptomatic character, about 75% of patients receive a diagnosis during an advanced stage. Consequently, the prognosis for ovarian cancer cases frequently remains unfavorable, as evidenced by a five-year survival rate hovering around 45-50 percent (5, 6).

Ovarian cancer can be categorized into Type I and Type II tumors. Type I tumors are low-grade and typically have a favorable prognosis, except for clear cell carcinoma. Type II tumors are high-grade and aggressive, with advanced stage and chromosomal instability when contrasted with type I tumors. P53 mutations accompany most instances. Type I tumors arise from atypical proliferative (borderline) tumors, whereas Type II tumors originate from the background of serous tubal intraepithelial carcinoma (STIC). On the other hand, ovarian serous carcinoma is divided into low-grade serous carcinomas (LGSC) and HGSC. HGSCs are the most frequent type and account for about 90% of cases, while LGSCs represent approximately 10%. Type I ovarian cancer is typically associated with LGSCs, while HGSCs fall under Type II ovarian cancer. LGSC has minimal nuclear atypia, infrequent mitosis, and fewer molecular abnormalities. HGSC has significant nuclear atypia and mitosis (more than 12 per 10 high-power fields) along with more molecular abnormalities. In high-grade tumors, the possibility of mixed ovarian tumors (less than 1%) exists, yet it is currently understood that these actually represent distinct morphological variants of HGSCs (6, 7).

In these aspects, if we can identify new IHC markers to predict the prognosis of ovarian SC, treatment options could be improved. For this purpose, guided by the insights provided by the literature pertaining to the possible effects of the PINCH on tumor carcinogenesis (8), we aimed to investigate its implications within clinicopathologic features of serous ovarian tumors.

PINCH is a member of the LIM family and relates the molecular steps that accompany many intracellular mechanisms. The PINCH gene is located on chromosome 2q12.2. It encodes a protein that functions and participates in cell adhesion by interacting with integrin-linked kinase (ILK) through its LIM domain, serving as an adapter (9). The expression of PINCH-1 is observed in tumor-associated stroma, fibroblasts, myoblasts, and endothelial cells. The PINCH family consists of two members, namely PINCH-1 (LIMS-1) and PINCH-2 (10, 11).

The relationship between PINCH and other intracellular mechanisms is still being investigated today. PINCH-1 serves as a focal adhesion protein associated with integrin-linked kinase to constitute an intracellular component of growth factor receptor signaling. It is essential for the survival of primitive endodermal cells. It supports the development of many anti-apoptotic, angiogenic, and tumorigenic pathways (12–16). Also, Its expression was previously presented in the literature, such as prostate carcinoma, breast carcinoma, lung carcinoma, colorectal adenocarcinoma, pancreatic adenocarcinoma, and skin cancer (9, 17, 18). However, the literature did not broadly cover the relation between PINCH and ovarian SC. Therefore, we considered studying PINCH-1 for the purpose of clinicopathologic value.

Material and Method

Patient Characteristics vnd Tissue Samples

For this study, we randomly selected 110 patients who had undergone surgery for salpingo-oophorectomy, hysterectomy, and debulking surgery. These cases were categorized according to the FIGO Surgical Staging System for Ovary Carcinoma, 2017, and were typed and graded based on the WHO Lyon consensus, 2014. We obtained patient information from the data system of Gazi University Hospital between 2008 and 2014, including surgical and pathological records. Patient records and tissue slides were examined and scored by two pathologists without data beforehand. The cases were divided into two groups: SBT and SC, which consisted of 21 serous borderline tumors and 89 serous carcinomas. The patients in both groups had a median age of 54.5 years (with a range of 10-82 years). We carefully selected blocks that accurately depicted the histopathological characteristics of each case, including the tumor and peritumoral stroma. We then conducted immunohistochemical procedures on those selected blocks to determine the expression of PINCH-1.

Immunohistochemistry

PINCH immunohistochemical staining was performed on formalin-fixed, paraffin-embedded selected cases` blocks. At that point, for the comprehending

expression of PINCH, was used PINCH/LIMS-1 (rabbit polyclonal antibody, used 1/150 dilution, clone GTX114984, Genetex, USA) with Streptavidin- biotin immunoperoxidase indirect method. We evaluated renal medulla tissue for positive control of PINCH antibody.

To prepare a tissue sample, a five-micrometer section from paraffin-embedded tissue was first placed and deparaffinized into an incubator for one hour. Then, sections were cleared, hydrated, and dehydrated by xylene and graded alcohols, respectively. After this procedure, to reveal masked epitopes, the sections were microwaved with Tris -EDTA buffer (PH 9.0) at 85 0C for 10 minutes, then at 70 0C for 10 minutes, and left to cool down at room temperature for thirty minutes. They were then washed three times with distilled water. The sections were incubated with 3% H2O2 solution for ten minutes to eliminate endogenous peroxidase. The following step was the incubation of the PINCH-1 antibody; the section was incubated primary antibody at +4 0C overnight and then was washed three times with PBS. After PBS washing, streptavidin-biotin elution (multi-species ultra streptavidin detection system-HRP; Signet, Massachusetts, USA) was performed for twenty minutes at room temperature. Then, the slides were rewashed with PBS, and amino-ethyl-carbazole (AEC) was used as a chromogen and incubated for ten minutes. Finally, the sections were counterstained with hematoxylin, and slides were read over a light microscope (Olympus CX 51).

We assessed the staining in the tumor and surrounding tissue, focusing on the expression of PINCH in stromal fibroblasts, myofibroblasts, epithelial cells, and tumor cells. The intensity and diffusiveness of the PINCH stain were examined. Three levels were used to categorize the staining diffuseness (0-3): "grade 0" indicated less than or equal to 5%, "grade 1" between 6% and 34%, "grade 2" between 35% and 49%, and "grade 3" more than 50%. Additionally, the intensity was assessed using a scale ranging from negative (0), mild (+1), moderate (+2), and strong (+3) (Figure 1) (9, 19–21).

Statistical Analysis and Ethical Considerations

The research data were imported into a computer environment and analyzed using "SPSS 15.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL) for Windows." Descriptive statistics were presented, including the mean (±) standard deviation, median (minimum-maximum), and percentages. The suitability of variables for normal distribution was assessed through visual methods (histograms and probability plots) and analytical techniques (Kolmogorov-Smirnov/Shapiro-Wilk tests). Variables found not to follow a normal distribution were compared using the Mann-Whitney U Test, while independent samples T-tests were employed for normally distributed variables. The Spearman Correlation Test was used to evaluate relationships between non-normally distributed variables. For the assessment of categorical variables, the Chi-Square test was applied. A statistical significance level of p-value < 0.05 was considered as the threshold for significance. Ethical approval was approved by the ethical committee of Gazi University. On December 08, 2014, permission was obtained from the local ethics committee with decision number 544 at the Faculty of Medicine.

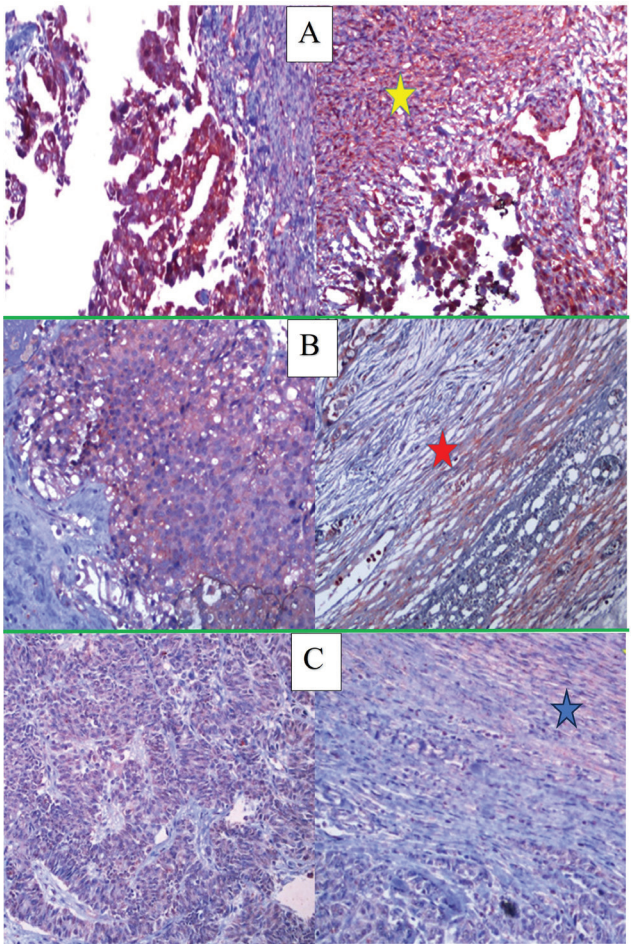

Figure 1

Photographs taken at 200 X magnification showing the intensity of PINCH-1 antibody tumor and peritumoral staining (A, B, C); (A) +3 strong tumoral and peritumoral (yellow asterisks) staining, (B) +2 moderate tumoral and peritumoral (red asterisks) staining, (C) +1 mild tumoral and peritumoral (blue asterisks) staining.

Results

Patient Characteristics

Among the entire cohort, 21 cases (19.09%) were characterized as SBT, whereas 89 cases (80.91%) were classified as the SC. The comprehensive assessment of all cases, encompassing parameters such as age, tumor size, FIGO stage, intra-abdominal wash cytology, capsule invasion, tumor localization within the ovary, grade, and cancer antigen 125 (CA125) levels at the time of diagnosis, is presented in Table 1. Statistically significant differences emerged, revealing that SBT cases were notably younger, predominantly at FIGO stage I, and exhibited lower CA125 levels compared to SC cases ($p<0.001$). Furthermore, SC cases showed a higher prevalence of bilateral localization ($p=0.023$), capsule invasion ($p<0.01$), and a higher tumor grade ($p<0.001$). Notably, no significant difference was detected between the two subtypes with regard to tumor diameter ($p=0.864$).

PINCH-1 Expression Patterns

Upon the comprehensive evaluation of all 110 patients, the diffusiveness of PINCH-1 tumor staining was most prominent in grade 3 (40%), while peritumoral stromal staining diffusiveness was predominantly observed in grade 0 (80%). Concerning the intensity of PINCH-1 peritumoral staining, a negative pattern was prevalent in the majority of cases (79.1%), whereas the intensities of tumor staining exhibited a nearly uniform distribution (negative: 28.2%, weak: 23.6%, moderate: 27.3%, and strong: 20.9%) (Table 2).

The diffusiveness and intensity of PINCH-1 tumor staining and peritumoral stromal staining in SBT and SC cases were compared using the Chi-Square test.

Table 1 Comparison of the characteristics of SBT and SC cases.

	SBT (n=21)	SC (n=89)	p-value	Total (n=110)
Age	39 (±16.41)	56.9 (±10.03)	**<0.001**	53.95± 13.41
Tumor size (cm)	7 (1-34)	7 (0.1-21)	**0.864**	7 (0.1-34)
FIGO stage I II III IV	 19 (90.5%) 2 (9.5%) 0 0	 6 (6.7%) 4 (4.5%) 65 (73%) 14 (15.8%)	**<0.001**	 25 (22.7%) 6 (5.5%) 65 (59.1%) 14 (12.7%)
Peritoneal Lavage Cytology Positive Negative Suspicious	 1 (4.8%) 13 (61.9%) 7 (33.3%)	 57 (64%) 20 (22.5%) 12 (13.5%)	**<0.001**	 58 (52.7%) 33 (30%) 19 (17.3%)
Capsular invasion Yes No	 2 (9.5%) 19 (90.5%)	 73 (82%) 16 (18)	**<0.001**	 35 (3.8%) 75 (68.2%)
Tumor Over Localization Unilateral Bilateral	 13 (61.9%) 8 (38.1%)	 29 (32.6%) 60 (67.4%)	**0.023**	 42 (38.2%) 68 (61.8%)
Grade Low High	 21 (100%) 0	 7 (7.9%) 82 (92.1%)	**<0.001**	 28 (25.5%) 82 (74.5%)
CA 125 level (U/ml)	33 (10-3031)	607 (16-6363)	**<0.001**	**375.5(10-6363)**

SBT: serous borderline tumor, SC: serous carcinoma; nonparametric data is presented as median (min-max), and parametric data is presented as mean ± standard deviation. Statistically significant p values are in bold.

Table 2 Comparison of tumoral and peritumoral stromal PINCH-1 staining diffusiveness and intensity in SBT and SC

	SBT (n=21)	SC (n=89)	p-value	Total (n=110)
Diffusiveness of PINCH-1 tumor staining				
Grade 0	2 (9.5%)	31 (34.8%)	0.134	33 (30%)
Grade 1	6 (28.6%)	16 (18%)		22 (20%)
Grade 2	2 (9.5%)	9 (10.1%)		11 (10%)
Grade 3	11 (52.4%)	33 (37.1%)		44 (40%)
Diffusiveness of PINCH-1 peritumoral staining				
Grade 0	16 (76.2%)	72 (80.9%)	0.711	88 (80%)
Grade 1	3 (14.2%)	13 (14.6%)		16 (14.5%)
Grade 2	1 (4.8%)	3 (3.4%)		4 (3.6%)
Grade 3	1 (4.8%)	1 (1.1%)		2 (1.8%)
Intensity of PINCH-1 tumor staining				
Negative	1 (4.8%)	30 (33.7%)	**<0.001**	31 (28.2%)
Mild	7 (33.3%)	19 (21.3%)		26 (23.6%)
Mid	12 (57.1%)	18 (18%)		30 (27.3%)
Strong	1 (4.8%)	22 (24.7%)		23 (20.9%)
Intensity of PINCH-1 peritumoral staining				
Negative	16 (76.2%)	71 (79.8%)	0.256	87 (79.1%)
Mild	0	6 (6.7%)		6 (5.5%)
Mid	3 (14.3%)	10 (11.2%)		13 (11.8%)
Strong	2 (9.5%)	2 (2.2%)		4 (3.6%)

SBT: serous borderline tumor, SC: serous carcinoma

No significant differences were observed between SBT and SC cases in terms of the diffusiveness and intensity of PINCH-1 peritumoral stromal staining, as well as the diffusiveness of PINCH-1 tumor staining (p=0.711, p=0.256, p=0.134, respectively). However, a statistically significant difference was found only in the intensity of PINCH-1 tumor staining between SBT and SC cases (p<0.001) (Table 2).

Relationship Between PINCH-1 Expressions and Clinicopathological Features

The Spearman correlation test was conducted to investigate whether there was any relationship between the diffusiveness and intensity of PINCH-1 tumor and peritumoral stromal staining and various clinical parameters, including patient age, FIGO stage, peritoneal lavage cytology, tumor ovary localization, tumor size, histopathological subtype (SBT/SC), capsule invasion, tumor grade, and CA 125 levels. No statistically significant correlations were found between the intensity and diffusiveness of PINCH-1 tumor staining and these aforementioned characteristics. However, when assessing the correlations between the diffusiveness and intensity of PINCH-1 peritumoral stromal staining and these clinical parameters, a positive correlation was observed with the tumor's unilateral/bilateral ovary localization (p=0.038 for diffusiveness and p=0.024 for intensity). In contrast, a negative correlation was identified with tumor size (p=0.019 for diffusiveness and p=0.007 for intensity) (Table 3).

Additionally, the clinical and pathological characteristics were examined with respect to the diffusiveness and intensity scores of PINCH-1 staining using the Chi-Square test. Concerning the diffusiveness of PINCH-1 tumor staining, no statistically significant differences were found among the clinical and pathological

Table 3 Correlation results of PINCH-1 peritumoral staining patterns with clinicopathological features

	Diffusiveness, Extent		Intensity	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value
Age	0.080	0.407	0.088	0.362
FIGO stage	-0.014	0.882	-0.023	0.809
Peritoneal lavage cytology	0.079	0.410	0.065	0.500
Tumor Over Localization	**0.198**	**0.038**	**0.215**	**0.024**
Tumor size	**-0.223**	**0.019**	**-0.257**	**0.007**
Histopathologic subtype (SBT/SC)	-0.054	0.572	-0.057	0.554
Capsul invasion	0.044	0.647	0.043	0.656
Tumor grade	-0.025	0.792	-0.020	0.836
Ca 125 level	-0.025	0.900	0.019	0.845

SBT: serous borderline tumor, SC: serous carcinoma

Table 4 Chi-square test results of PINCH-1 tumor staining patterns with clinicopathological features (p-values)

	Diffusiveness	Intensity
FIGO stage (I-II-III-IV)	0.396	**0.032**
Peritoneal lavage cytology (positive-negative)	0.894	0.170
Tumor Over Localization (unilateral-bilateral)	0.840	0.742
Histopathologic subtype (SBT vs. SC)	0.134	**<0.001**
Capsul invasion (yes or no)	0.781	0.287
Tumor grade (low or high)	0.436	**0.001**

SBT: serous borderline tumor, SC: serous carcinoma

characteristics. However, the intensity of tumor staining exhibited statistically significant differences in relation to the FIGO stage, histopathologic subtype, and tumor grade groups (p=0.032, p<0.001, and p=0.001, respectively) (Table 4). Regarding the diffusiveness and intensity of PINCH-1 peritumoral stromal staining, no statistically significant differences were detected across any of the clinical and pathological parameters.

Discussion

Clinical prognostic factors in ovarian cancer may be considered to include disease stage at the time of diagnosis, age, success of optimal debulking surgery, tumor volume, presence of residual tumor after primary debulking surgery, response to neoadjuvant-adjuvant CT, preoperative and postoperative CA125 levels, presence of a second malignancy, and recurrence after primary cytoreductive surgery.

Different types of ovarian cancers may have distinct genetic alterations driving their development, which underscores the heterogeneity of the disease. The histological type of the tumor, its grade, deletions in tumor suppressor genes (such as Rb, PTEN, chromosomes 1, 6, 7, 8, 9, 14, 17), amplifications and

mutations causing gene activation in oncogenes (such as KRAS, BRAF, MYC, EGFR, HER2, β-catenin), familial inheritance, somatic mutations (TP53, BRCA1/2, PIK3CA), and mismatch repair gene defects (MLH1, MSH2, PMS2, and MSH6) can be considered as pathological and genetic factors that contribute to tumor heterogeneity and influence prognosis (22–26). Certainly, among these prognostic factors, there are two that stand out due to their repeatability, strong correlation, and undeniable impact on prognosis: stage and post-primary debulking recurrence. They play an essential role in treatment planning and monitoring patients, contributing substantially to the overall clinical management of cancer cases (27).

Although stage is an essential determinant of cancer prognosis, it is also true that other factors, such as histologic type, can influence prognosis even in low-stage cancers. In particular, rare histologic types can have a worse prognosis even at low stages. For example, rare histological types, such as clear cell carcinoma, may exhibit more aggressive biological behavior. Therefore, not only the stage but also the histologic type should be considered. This is an important approach in cancer management, where each patient is unique, and personalization of response to treatment and prognosis is vital (28, 29).

PINCH-1 expression has been detected in breast, colon, prostate, lung, squamous cell carcinomas, and many other malignant tumors. In the literature, PINCH-1 expression is associated with poor prognosis in colon, pancreas, and oral cancers (17,30). In addition, in some tumors, PINCH-1 expression is especially prominent in the invasive tumor margin and stroma. PINCH-1's role as a molecular switch allows it to modulate cellular signaling pathways. Targeting molecules involved in signal transmission can offer novel therapeutic avenues for developing targeted treatments (30, 31).

In this study, PINCH-1 expression in tumor and peritumoral stroma was examined in 110 cases of SBT and SC. PINCH-1 expression was evaluated separately according to staining intensity and staining diffuseness, and statistical comparison was made with clinicopathologic parameters affecting prognosis. PINCH-1 tumor staining intensity increased with increasing FIGO stage. Even though the clinical follow-up of the cases is unknown, the statistically significant correlation of PINCH-1 expression with an important prognostic parameter, such as the FIGO stage, suggests that PINCH-1 expression may be a determinant that may affect survival. When PINCH-1 expression was evaluated according to tumor types, PINCH-1 tumor staining intensity was statistically significantly increased in SC cases.

There is also a weak negative correlation between PINCH-1 peritumoral staining intensity and staining diffusiveness with tumor size. However, PINCH-1 peritumoral stromal staining intensity and prevalence increased in bilateral tumors. The intensity of PINCH-1 tumor staining was found to be statistically significantly different between tumor histopathological grades. This difference manifested itself as increased PINCH-1 expression in high-grade cases. When analyzed in all findings, our study seems to be consistent with the literature in many aspects.

In certain studies, it has been emphasized that the expression of PINCH-1 is more intense in tumor invasive margins and tumor-associated stroma compared to the main tumor (21). In contrast to this finding in our study, when PINCH-1 expression in tumor and peritumoral stroma was considered, PINCH-1 expression was more intense in epithelial cells forming the tumor. In some cases, PINCH-1 expression in the stroma and vascular structures within the stroma, which have no apparent relationship with the tumor and are relatively distant to the tumor in the ovarian parenchyma, showed PINCH-1 expression at least as much as tumor epithelial cells and even in some PINCH-1 negative cases, PINCH-1 expression was observed in the components of the stroma distant to the tumor. The reason for this is thought to be tumor-associated stroma and microenvironmental extracellular matrix (ECM) content or some mediators. As mentioned in the article by Scaife et al., the increase in ECM may lead to tumor development and metastasis (32). Intracellular communication of PINCH-1 with ECM is mediated by integrins and is known to be regulated by growth factor (PDGF, EGF, IGF, etc.) dependent ligand-receptor relationships. ECM elements and growth factor concentration are increased in tumor-associated stroma. Therefore, PINCH-1 signaling complexes are also increased accordingly. Although the degradation of stimulatory factors that rise from the stroma by many mechanisms is faster, the degradation of intracellular signaling complexes is much slower. Once the ligand binds, the signaling complexes formed activate or inhibit many dynamic pathways. As a result, no matter how low the stimulus is, once stimulation occurs, PINCH-1 protein and its associated signaling pathway (RTK and MAPK signaling pathway) interactions increase (33). Consequently, the PINCH-1 protein is a cell adhesion molecule linked to integrins and ECM, and there are studies in the literature that refer to PINCH-1-related carcinogenesis and emphasize the need for drug

studies that can target the PINCH-1 pathway (16, 31). However, we obtained the survival information of only nine patients in our hospital data. For this reason, we could not compare PINCH expression with survival. We could not perform Kaplan-Meier survival curve analysis, log-rank test, Cox proportional hazards model, and other related statistics. Hence, this aspect will be a potential critique point for our study. In conclusion, the intensity of PINCH-1 staining in SC increases concomitantly with higher tumor grade and advanced FIGO stages. Moreover, in SC cases, the intensity of tumor staining is notably more pronounced compared to SBT. The correlation of PINCH-1 staining with substantial clinicopathologic prognostic parameters suggests it may have a potential association with the molecular pathogenesis and aggressive course of high-grade ovarian tumors.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
Ethical approval was approved by the ethical committee of Gazi University. On December 08, 2014, permission was obtained from the local ethics committee with decision number 544 at the Faculty of Medicine. The study was conducted in line with the principles of the Helsinki Declaration.

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

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Availability of Data and Materials
Authors can confirm that all relevant data are included in the article and/or its supplementary information files.

Authors Contributions
OE: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

ÖE: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing.

ZAK: Data curation; Visualization; Validation; Writing-original draft.

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