Advances in Obstetrics and Gynecology Research, 2025, Volume 3, Issue 2
https://ojs.bbwpublisher.com/index.php/AOGR
Online ISSN: 2981-8060

Expression of CLDN18.2, CDX2, SATB2, and PAX8 in Primary and Gastrointestinal-Derived Mucinous Ovarian Carcinoma

Fujun Wang[1]*, Yanli Li[1], Yanhua Cui[1], Lixin Zhao[2]

[1]Gynecology Department, Junnan County People's Hospital, Linyi 276600, Shandong, China
[2]Pathology Department, Junnan County People's Hospital, Linyi 276600, Shandong, China

***Corresponding author:** *Fujun Wang, 531747651@qq.com*

Abstract: *Objective*: Primary and metastatic mucinous ovarian carcinomas share similar morphologies but have significant differences in prognosis. This study aims to explore the immunophenotypic characteristics of primary and gastrointestinal-derived mucinous ovarian carcinomas. *Methods*: A total of 230 cases of primary and gastrointestinal-derived mucinous ovarian tumors surgically removed at Junnan County People's Hospital and the Affiliated Hospital of Qingdao University from 2014 to 2024 were randomly selected. These included 67 cases of primary mucinous ovarian carcinoma, 56 cases of primary borderline mucinous ovarian tumor, 61 cases of colorectal-derived mucinous ovarian carcinoma (including 26 cases from the appendix and 35 cases from the colorectum), 26 cases of gastric-derived mucinous ovarian carcinoma, and 20 cases of mucinous cystadenoma of the ovary. All specimens were reviewed and confirmed by two experienced pathologists according to the 2020 WHO classification criteria. Immunohistochemistry was used to detect the expression differences of CLDN18.2 in primary mucinous ovarian tumors. Furthermore, the expressions of CLDN18.2, CDX2, SATB2, and PAX8 were jointly detected in primary and gastrointestinal metastatic mucinous ovarian tumors to explore the immunoexpression characteristics of multiple immune markers in primary ovarian and upper and lower gastrointestinal-derived ovarian metastatic mucinous carcinomas. *Results*: 1. CLDN18.2 showed varying degrees of expression in mucinous cystadenoma of the ovary, borderline mucinous ovarian tumor, and primary mucinous ovarian carcinoma, but was not expressed in normal ovarian and fallopian tube tissues. 2. In primary mucinous ovarian carcinoma, CLDN18.2 and PAX8 showed high expression, while CDX2 and SATB2 showed lower expression. In gastric-derived mucinous ovarian carcinoma, CLDN18.2 and CDX2 showed high expression, while SATB2 and PAX8 were almost not expressed. In colorectal-derived mucinous ovarian carcinoma, CDX2 and SATB2 showed high expression, while CLDN18.2 and PAX8 showed low expression. *Conclusion*: CLDN18.2 shows high expression in both primary and gastric-derived mucinous ovarian carcinomas and can be used as an auxiliary method for differentiating primary and gastrointestinal-derived mucinous ovarian carcinomas along with CDX2, SATB2, and PAX8.

Keywords: Mucinous ovarian carcinoma; Gastrointestinal-derived mucinous ovarian carcinoma; CLDN18.2; Immunohistochemistry; Differential diagnosis

Online publication: July 11, 2025

1. Introduction

Ovarian cancer poses a severe threat to women's health. Although it ranks third in the incidence of female reproductive system tumors, it has the highest fatality rate [1]. Most women in developed countries die from ovarian cancer [2]. Epithelial ovarian carcinoma (EOC) is the main pathological type of ovarian cancer [3], accounting for approximately 90% of cases [4]. Mucinous ovarian carcinoma (MOC) is one of the subtypes of EOC, with unique clinical, histological, and molecular characteristics [5]. However, it is difficult to distinguish the primary source of the tumor [6]. Compared to primary mucinous ovarian carcinoma (PMOC), metastatic mucinous ovarian carcinoma (MMOC) is more common, mainly originating from the gastrointestinal tract and pancreatobiliary system [7]. There are differences in the prognosis of PMOC and MMOC [8], so correctly identifying the source of ovarian tumors is crucial for clinical management.

CLDNs are a family of tight junction proteins composed of at least 27 protein members [9]. Evidence suggests that CLDNs proteins are key structural and functional components of tight junction proteins, playing a critical role in tumorigenesis and inflammation [10]. CLDN18, a member of the CLDNs family, has two subtypes: CLDN18.1 and CLDN18.2. In normal tissues, CLDN18.1 is expressed in the lungs [11], while CLDN18.2 is only expressed in differentiated gastric mucosal epithelial cells [12]. CLDN18 is highly expressed in gastric cancer, esophageal cancer, pancreatic cancer, ovarian cancer, cholangiocarcinoma, and lung adenocarcinoma [13]. Studies by Halimi *et al.* [14,15] have shown positive expression of CLDN18 in gastric-type mucinous cystadenoma and gastric-type mucinous borderline tumors of mucinous ovarian tumors.

CDX2 encodes a transcription factor that plays an important role in regulating the development and differentiation of intestinal epithelial cells [16]. Research indicates that CDX2 is expressed in both normal and neoplastic intestinal epithelial tissues, serving as a marker for intestinal-derived tumors [16,17].

SATB2 is a human DNA-binding protein involved in transcriptional regulation and chromatin remodeling, with high expression specifically localized in the epithelial cells of the lower digestive tract [18]. Recently, the role of STAB2 in the differential diagnosis of colorectal cancer has gradually been recognized. Aldaoud's [19] study shows that SATB2 is a sensitive and highly specific marker in colorectal cancer. Besides CDX2, CK7, and CK20, PAX8 has also been mentioned for distinguishing the primary source of mucinous ovarian cancer [19]. The paired box gene (PAX) encodes a family of nine transcription factors involved in organogenesis during human development [20]. Bowen *et al.*'s [21] studies have confirmed that PAX8 is an important marker of genital tract origin.

Currently, no research has been found on the immunohistochemical expression characteristics of combined applications of CLDN18.2, CDX2, SATB2, and PAX8 in primary ovarian and gastrointestinal-derived mucinous carcinomas. Therefore, this study intends to investigate the immunophenotypic characteristics of different sources of ovarian mucinous carcinomas by jointly detecting the expression of several immune markers in primary ovarian and gastrointestinal-derived ovarian mucinous carcinomas.

2. Materials and methods

2.1. General information

A total of 230 cases of primary and gastrointestinal-derived mucinous ovarian tumors were randomly selected from the surgical resection cases at Junnan County People's Hospital and the Affiliated Hospital of Qingdao University from 2014 to 2024. These included 67 cases of primary mucinous ovarian cancer, 56 cases of primary borderline mucinous ovarian tumors, 61 cases of mucinous ovarian cancer derived from the large intestine (including 26 cases from the appendix and 35 cases from the colon and rectum), 26 cases of ovarian mucinous carcinoma derived

from the stomach, and 20 cases of ovarian mucinous cystadenoma. Additionally, normal ovarian and fallopian tube tissues from 10 patients who underwent total hysterectomy due to uterine fibroids were selected as controls. All specimens were reviewed and confirmed by two experienced pathologists based on the 2020 WHO classification criteria. None of the selected patients received radiotherapy or chemotherapy before surgery. All study subjects signed informed consent, and the study protocol was approved by the Medical Research Ethics Committee of Junnan County People's Hospital.

2.2. Reagents and methods

Surgical specimens were routinely fixed and embedded in paraffin. The paraffin-embedded tissues were continuously sectioned at a thickness of 4μm and subjected to immunohistochemical staining for CLDN18.2 (abcam, ab203563; 1:500), CDX2 (ABclonal Technology Co, a20222; 1:50), SATB2 (abcam, ab92446; 1:50), and PAX8 (abcam, ab53490; 1:100). Result interpretation: The slides were reviewed by experienced pathologists in a double-blind manner. Positive expression of CLDN18.2 was defined as staining of the outer basement membrane, while CDX2, SATB2, and PAX8 were all expressed in the cell nucleus. A semi-quantitative scoring system was used to evaluate staining intensity and the percentage of stained cells. Staining intensity was scored as follows: 0 for no color; 1 for light yellow, 2 for brown yellow, and 3 for tan. The percentage of stained cells was scored as follows: < 5% positive cells were scored as 0; 5–25% as 1; 26–50% as 2; 51–75% as 3; and 76–100% as 4. The sum of the two scores represented the staining score, with a total score > 2 considered positive and ≤ 2 considered negative.

2.3 Statistical methods

SPSS 26.0 software was used for statistical analysis. The chi-square test (χ^2 test) was selected as the statistical method, and a *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Expression of CLDN18.2 in mucinous ovarian tumors

The results showed that CLDN18.2 immunohistochemical staining was mainly localized to the outer edge of the cell basement membrane, and positive CLDN18.2 staining appeared as brown yellow or tan. CLDN18.2 was not expressed in normal ovaries but showed varying degrees of expression in mucinous cystadenomas, borderline mucinous ovarian tumors, and primary mucinous ovarian cancers (**Table 1**, **Figure 1**).

Table 1. Expression of CLDN18.2 in mucinous ovarian tumors

Category	Staining intensity				Staining percentage					CLDN18.2	
	0	1	2	3	0	1	2	3	4	Positive	Negative
Primary mucinous Ovarian cancer	3	4	25	35	3	4	15	15	30	56 (83.6%)	11 (16.4%)
Borderline mucinous Tumor	5	7	28	16	6	17	28	5	0	26 (46.4%)	30 (53.6%)
Mucinous Cystadenoma	3	12	5	0	3	10	4	3	0	3 (15%)	17 (85%)

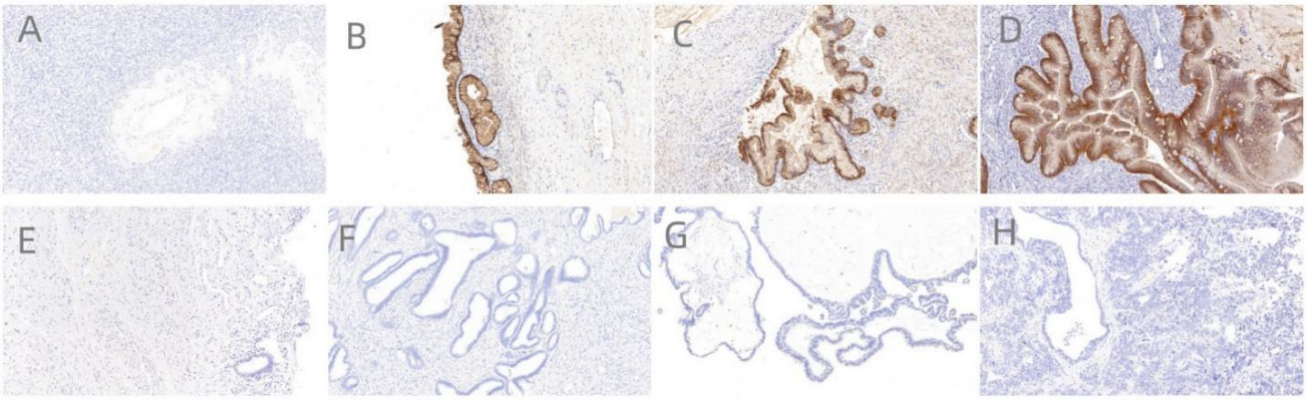

Figure 1. The expression of CLDN18.2 in normal ovary (A), mucinous cystadenoma (B), borderline mucinous tumor (C), primary mucinous ovarian cancer (D), normal fallopian tube (E), serous cystadenoma (F), borderline serous tumor (G), and high-grade serous ovarian cancer (H) at 200x magnification.

3.2. Immunohistochemical characteristics of CLDN18.2, CDX2, SATB2, and PAX8 in primary mucinous ovarian cancer and gastrointestinal-derived mucinous ovarian cancer

3.2.1. Comparison between primary mucinous ovarian cancer (PMOC) and gastrointestinal-derived mucinous ovarian cancer

The positive expression rate of CLDN18.2 in PMOC was 92.5% (62/67), which was significantly higher than that in colon-derived mucinous ovarian cancer (37.7%). The difference between the two was statistically significant ($P < 0.05$), but there was no significant difference compared to stomach-derived mucinous ovarian cancer (96.2%) ($P > 0.05$). CDX2 had a positive expression rate of 29.9% (20/47) in PMOC, which was significantly lower than that in stomach-derived mucinous ovarian cancer (61.5%) and colon-derived mucinous ovarian cancer (90.2%). The differences were statistically significant ($P < 0.05$). The positive expression of SATB2 in PMOC and stomach-derived mucinous ovarian cancer accounted for only 4.5% (3/67) and 4.8% (1/26), respectively, while the positive expression in colon-derived mucinous ovarian cancer was as high as 91.8% (56/61). The difference between PMOC and colon-derived mucinous ovarian cancer was statistically significant ($P < 0.05$), but there was no significant difference compared to stomach-derived mucinous ovarian cancer ($P > 0.05$). The expression rate of PAX8 in PMOC was 43.3% (29/67), while only 1 case (1.6%) of colon-derived mucinous ovarian cancer showed positive expression, and no positive expression was observed in stomach-derived mucinous ovarian cancer. Compared with gastrointestinal-derived mucinous ovarian cancer, the positive expression rate of PAX8 in PMOC was higher, and the difference was statistically significant ($P < 0.05$).

3.2.2. Comparison between colon-derived mucinous ovarian cancer and stomach-derived mucinous ovarian cancer

CLDN18.2 was positively expressed in 37.7% (23/61) of colon-derived mucinous ovarian cancers, while the positive expression rate of CLDN18.2 in stomach-derived mucinous ovarian cancer was 96.2% (25/26). The positive expression rate of CLDN18.2 in stomach-derived mucinous ovarian cancer was significantly higher than that in colon-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). CDX2 was positively expressed in 90.2% (55/61) of colon-derived mucinous ovarian cancers, while the positive expression rate in stomach-derived mucinous ovarian cancer was 61.5% (16/26). The positive expression rate

of CDX2 in colon-derived mucinous ovarian cancer was higher than that in stomach-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). SATB2 had a positive expression rate of 91.8% (56/61) in colon-derived mucinous ovarian cancer, but only 1 case (4.8%) showed positive expression in stomach-derived mucinous ovarian cancer. The positive expression rate of SATB2 in colon-derived mucinous ovarian cancer was significantly higher than that in stomach-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). PAX8 was positively expressed in only 1 case (1.6%) of colon-derived mucinous ovarian cancer and was not expressed in stomach-derived mucinous ovarian cancer. There was no significant difference between the two ($P > 0.05$).

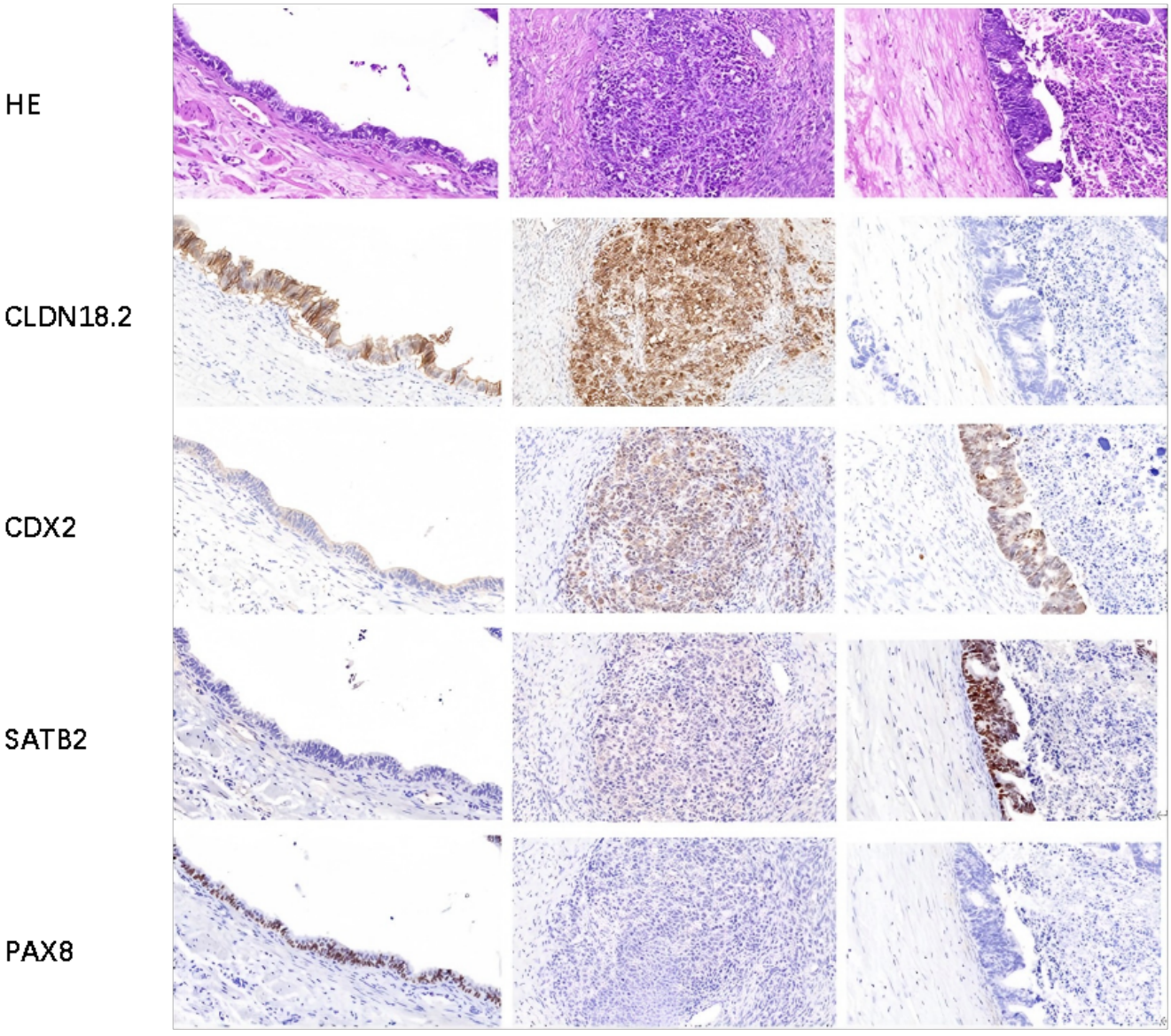

Figure 2. Expression of CLDN18, CDX2, SATB2, and PAX8 in PMOC, metastatic mucinous ovarian cancer from gastrointestinal origin.

Table 2. Expression of CLDN18.2, CDX2, SATB2, and PAX8 in PMOC and mucinous ovarian cancer of gastrointestinal origin

		PMOC (n = 67) (%)	Metastatic colorectal (n = 61) (%)	Gastric metastatic (n = 26) (%)	*P*-value 1	*P*-value 2	*P*-value 3
CLDN18.2	+	62 (92.5%)	23(37.7%)	25(96.29%)	< 0.001	0.867	< 0.001
	-	5 (7.5%)	38(62.3%)	1(4.8%)			
CDX2	+	20 (29.9%)	55 (90.2%)	16(61.5%)	< 0.001	0.005	0.004
	-	47 (70.1%)	6 (9.8%)	10 (28.5%)			
SATB2	+	3 (4.5%)	56 (91.8%)	1 (4.8%)	< 0.001	1.000	< 0.001
	-	64(95.5%)	5(8.2%)	25(96.2%)			
PAX8	+	29 (43.3%)	1 (1.6%)	0 (0.0%)	< 0.001	< 0.001	1.000
	-	38 (56.7%)	60(98.4%)	26(100%)			

P-value 1: Comparison between PMOC and mucinous ovarian cancer of colonic origin; *P*-value 2: Comparison between PMOC and mucinous ovarian cancer of gastric origin; *P*-value 3: Comparison between colonic and gastric origin groups.

4. Discussion

Mucinous ovarian tumors are classified as benign, borderline, and malignant, but these three forms can coexist within the same pathological tissue [8,22]. Many scholars believe that mucinous ovarian cancer (MOC) progresses from benign to borderline and then to malignant pathology [23]. Distinguishing between metastatic and primary ovarian malignancies is crucial. CLDN18 is one of 27 proteins that constitute the claudin family, essential for forming tight junctions and maintaining the polarity of epithelial and endothelial cells [24]. Experimental evidence suggests that abnormal expression of CLDN18 and CLDN18.2 in gastric cancer is significant for diagnosis, treatment, and prognosis [12,25–28]. This study demonstrates that CLDN18.2 is positively expressed in both mucinous cystadenoma and borderline mucinous ovarian tumors, often focal but sometimes diffuse. In PMOC, the positive expression rate of CLDN18.2 protein is significantly increased, with 51 out of 60 positive cases showing diffuse expression. Based on CLDN18.2 expression levels in mucinous ovarian tumors, it has certain diagnostic value for PMOC.

Eighty percent of MOC cases originate from metastases, primarily from the gastrointestinal tract [26]. Distinguishing the primary source of MOC can be challenging due to the histological similarity between some primary and metastatic mucinous carcinomas. Although numerous studies have confirmed that clinical features like tumor size and laterality can distinguish between PMOC and MMOC [6,29–31], no single feature can unequivocally differentiate them [6]. Immunohistochemistry is the most commonly used method to distinguish between primary and metastatic tumors. Experiments have confirmed the differential diagnostic value of CK7, CK20, CDX2, SATB2, PAX8, and others in ovarian cancer [17–21,32]. However, relying on a single antibody is insufficient for determining the tumor's origin, and several antibodies are typically required for differential diagnosis. In our study, we first used CLDN18.2, CDX2, SATB2, and PAX8 to differentiate the primary source of mucinous ovarian cancer. Our results show that CLDN18.2 expression is higher in PMOC and gastric-derived mucinous ovarian cancer than in colonic-derived mucinous ovarian cancer. CDX2 expression is higher in colonic and gastric-derived mucinous ovarian cancers than in PMOC. SATB2 shows high expression in colonic-derived mucinous ovarian cancer but minimal expression in PMOC and gastric-derived mucinous ovarian cancer. PAX8 is positively expressed in about half of PMOC cases but rarely in gastrointestinal-derived mucinous ovarian cancer. Based on these results, the

common immunohistochemical phenotype in PMOC is CLDN18.2(+), CDX2(-), SATB2(-), PAX8(+), in colonic-derived mucinous ovarian cancer is CLDN18.2(-), CDX2(+), SATB2(+), PAX8(-), and in gastric-derived mucinous ovarian cancer is CLDN18.2(+), CDX2(+), SATB2(-), PAX8(-).

5. Conclusion

In summary, our findings provide an experimental basis for the differential diagnosis of CLDN18.2 in mucinous ovarian cancer. However, further research is needed to understand the mechanism of CLDN18.2's role in the development of mucinous ovarian cancer and whether it can be a new therapeutic target.

Funding

Qingdao University Medical Group Special Key Research Project (Project No.: YLJT20231011)

Disclosure statement

The authors declare no conflict of interest.

References

[1] Menon U, Karpinskyj C, Gentry-Maharaj A, 2018, Ovarian Cancer Prevention and Screening. Obstetrics and Gynecology, 131(5): 909–927.

[2] Kossai M, Leary A, Scoazec J, et al., 2018, Ovarian Cancer: A Heterogeneous Disease. Pathobiology, 85(1–2): 41–49.

[3] Heinzelmann-Schwarz V, Gardiner-Garden M, Henshall S, et al., 2006, A Distinct Molecular Profile Associated with Mucinous Epithelial Ovarian Cancer. British Journal of Cancer, 94(6): 904–913.

[4] Lheureux S, Gourley C, Vergote I, et al., 2019, Epithelial Ovarian Cancer. The Lancet, 393(10177): 1240–1253.

[5] Cree I, White V, Indave B, et al., 2020, Revising the WHO Classification: Female Genital Tract Tumours. Histopathology, 76(1): 151–156.

[6] Dundr P, Singh N, Nozickova B, et al., 2021, Primary Mucinous Ovarian Tumors vs. Ovarian Metastases from Gastrointestinal Tract, Pancreas and Biliary Tree: A Review of Current Problematics. Diagnostic Pathology, 16(1): 20.

[7] Kubecek O, Laco J, Spacek J, et al., 2017, The Pathogenesis, Diagnosis, and Management of Metastatic Tumors to the Ovary: A Comprehensive Review. Clinical and Experimental Metastasis, 34(5): 295–307.

[8] Prat J, D'Angelo E, Espinosa I, 2018, Ovarian Carcinomas: At Least Five Different Diseases with Distinct Histological Features and Molecular Genetics. Human Pathology, 80: 11–27.

[9] English D, Santin A, 2013, Claudins Overexpression in Ovarian Cancer: Potential Targets for Clostridium Perfringens Enterotoxin (CPE) Based Diagnosis and Therapy. International Journal of Molecular Sciences, 14(5): 10412–10437.

[10] Tabaries S, Siegel P, 2017, The Role of Claudins in Cancer Metastasis. Oncogene, 36(9): 1176–1190.

[11] Li J, Zhang Y, Hu D, et al., 2020, Analysis of the Expression and Genetic Alteration of CLDN18 in Gastric Cancer. Aging, 12(14): 14271–14284.

[12] Liang J, Zhang H, Huang Y, et al., 2021, A CLDN18.2-Targeting Bispecific T Cell Co-Stimulatory Activator for Cancer Immunotherapy. Cancer Management and Research, 13: 6977–6987.

[13] Li J, Yu Z, Hu D, et al., 2020, Analysis of the Expression and Genetic Alteration of CLDN18 in Gastric Cancer. Aging,

12(14): 14271–14284.

[14] Halimi SA, Maeda D, Shinozaki-Ushiku A, et al., 2013, Claudin-18 Overexpression in Intestinal-Type Mucinous Borderline Tumour of the Ovary. Histopathology, 63(4): 534–544.

[15] Halimi S, Maeda D, Ushiku-Shinozaki A, et al., 2021, Comprehensive Immunohistochemical Analysis of the Gastrointestinal and Mullerian Phenotypes of 139 Ovarian Mucinous Cystadenomas. Human Pathology, 109: 21–30.

[16] Yu J, Liu D, Sun X, et al., 2019, CDX2 Inhibits the Proliferation and Tumor Formation of Colon Cancer Cells by Suppressing Wnt/Beta-Catenin Signaling. Cell Death and Disease, 10(1): 26.

[17] Bayrak R, Hacıhanefioglu A, Savaş H, 2012, The Value of CDX2 and Cytokeratins 7 and 20 Expression in Differentiating Colorectal Adenocarcinomas. Diagnostic Pathology, 7: 9.

[18] Li Z, Roth R, Rock J, et al., 2017, Dual Immunostain With SATB2 and CK20 Differentiates Appendiceal Mucinous Neoplasms From Ovarian Mucinous Neoplasms. American Journal of Clinical Pathology, 147(5): 484–491.

[19] Aldaoud N, Erashdi M, AlKhatib S, et al., 2019, The Utility of PAX8 and SATB2 Immunohistochemical Stains in Distinguishing Ovarian Mucinous Neoplasms. BMC Research Notes, 12(1): 770.

[20] Danialan R, Assaad M, Burghardt J, et al., 2013, The Utility of PAX8 and IMP3 Immunohistochemical Stains in the Differential Diagnosis of Benign, Premalignant, and Malignant Endocervical Glandular Lesions. Gynecologic Oncology, 130(2): 383–388.

[21] Bowen N, Logani S, Dickerson E, et al., 2007, Emerging Roles for PAX8 in Ovarian Cancer and Endosalpingeal Development. Gynecologic Oncology, 104(2): 331–337.

[22] Ricci F, Affatato R, Carrassa L, et al., 2018, Recent Insights Into Mucinous Ovarian Carcinoma. International Journal of Molecular Sciences, 19(6): 1569.

[23] Morice P, Gouy S, Leary A, 2019, Mucinous Ovarian Carcinoma. New England Journal of Medicine, 380(13): 1256–1266.

[24] Mineta K, Yamamoto Y, Yamazaki Y, et al., 2011, Predicted Expansion of the Claudin Multigene Family. FEBS Letters, 585(4): 606–612.

[25] Athauda A, Chau I, 2021, Claudin 18.2 – A FAST-Moving Target in Gastric Cancer? Annals of Oncology, 32(5): 584–586.

[26] Baek J, Park D, Kim G, et al., 2019, Clinical Implications of Claudin18.2 Expression in Patients With Gastric Cancer. Anticancer Research, 39(12): 6973–6979.

[27] Kim S, Shin K, Park J, et al., 2020, Clinical Significance of CLDN18.2 Expression in Metastatic Diffuse-Type Gastric Cancer. Journal of Gastric Cancer, 20(4): 408–420.

[28] Kiyokawa T, Pesci A, et al., 2021, Claudin-18 as a Promising Surrogate Marker for Endocervical Gastric-Type Carcinoma. American Journal of Surgical Pathology, 46(5): 628–636.

[29] Kelemen L, Köbel M, 2011, Mucinous Carcinomas of the Ovary and Colorectum: Different Organ, Same Dilemma. The Lancet Oncology, 12(11): 1071–1080.

[30] Yemelyanova A, Vang R, Judson K, et al., 2008, Distinction of Primary and Metastatic Mucinous Tumors Involving the Ovary. American Journal of Surgical Pathology, 32(1): 128–138.

[31] Simons M, Bolhuis T, Haan A, et al., 2019, A Novel Algorithm for Better Distinction of Primary Mucinous Ovarian Carcinomas and Mucinous Carcinomas Metastatic to the Ovary. Virchows Archiv, 474(3): 289–296.

[32] Gucer H, Caliskan S, Kefeli M, Mete O, 2020, Do You Know the Details of Your PAX8 Antibody? Endocrine Pathology, 31(1): 33–38.