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Advances toward improved understanding and treatment of uncommon ovarian cancer types and subtypes

Edited by
Robb Hollis, Mihaela Carmen Cristea and Mignon Van Gent

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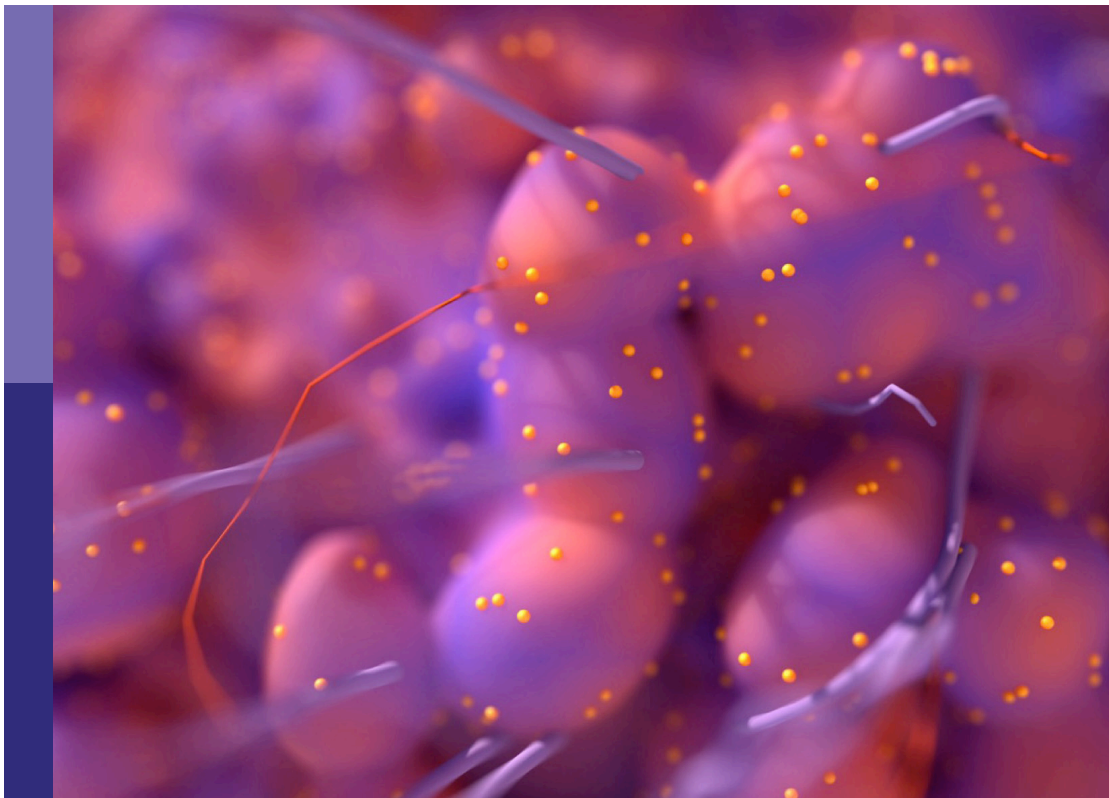

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Advances toward improved understanding and treatment of uncommon ovarian cancer types and subtypes

Topic editors

Robb Hollis — University of Edinburgh, United Kingdom

Mihaela Carmen Cristea — Regeneron Pharmaceuticals, Inc., United States

Mignon Van Gent — Amsterdam University Medical Center, Netherlands

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Sophia George,
University of Miami, United States

*CORRESPONDENCE
Robert L. Hollis
robb.hollis@ed.ac.uk

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Editorial: Advances toward improved understanding and treatment of uncommon ovarian cancer types and subtypes

Robert L. Hollis [1*] and Mignon D. J. M. van Gent [2]

[1]The Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom, [2]Department of Gynaecologic Oncology, Center for Gynaecologic Oncology Amsterdam, Cancer Center Amsterdam, Amsterdam University Medical Centres, Amsterdam, Netherlands

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Editorial on the Research Topic
Advances toward improved understanding and treatment of uncommon ovarian cancer types and subtypes

Introduction

Ovarian cancer is an umbrella term for a multitude of distinct disease entities identified in and around the ovary, fallopian tube and peritoneum. These include epithelial ovarian cancers (ovarian carcinomas), of which there are six major types: high grade serous (HGSOC), endometrioid (EnOC), clear cell (CCOC), mucinous (MOC), low grade serous (LGSOC) and ovarian carcinosarcoma (OCS) (1). Non-epithelial cancers include malignant germ cell tumors (teratoma, dysgerminoma, yolk sac tumor and others), sex chord stromal tumors (granulosa cell tumors, Sertoli-Leydig cell tumors and more), Brenner tumors and mesenchymal tumors, among others (2). These various types have been shown to arise from distinct developmental origins, have unique molecular profiles, varied response rates to conventional and targeted therapies, and distinct overall clinical behavior (2–8).

HGSOC is by far the commonest, and the vast majority of research has accordingly focused on this tumor type. These studies have advanced our knowledge of HGSOC at the genomic, transcriptomic and proteomic levels (4, 9–11), identifying therapeutically–exploitable disease biology that has led directly to the design and utilization of additional targeted treatment strategies, including poly(ADP–ribose) polymerase (PARP) inhibitors (12, 13). After approximately 30 years of limited progress in improving ovarian cancer survival, the integration of these agents into routine clinical practice is now shifting the survivorship landscape in HGSOC.

However, progress within the other, less common, ovarian cancer types has been lacking, and many remain critically understudied with a corresponding lack of targeted therapeutic options. Indeed, the fundamental molecular landscape in many of these tumor types have either only recently been established, or have yet to be described in large numbers of samples (3, 7, 8, 14, 15). In this Research Topic, we aimed to provide a platform for communication of research in uncommon and understudied forms of ovarian cancer, in the hope of advancing our understanding of these discrete disease entities.

Ovarian carcinosarcoma

OCS represents approximately 4% of ovarian cancer diagnoses, is characterized by the presence of both high grade carcinomatous and high grade sarcomatous components (2), and is exceptionally aggressive (median survival <2 years) with higher levels of intrinsic chemoresistance compared to HGSOC (16).

Three contributions on OCS are presented in this Research Topic that augment our current understanding of this uncommon and aggressive tumor type. Zheng et al. report a case of a 76 year–old female diagnosed with FIGO stage IIIC OCS. The report provides an excellent example of OCS histopathology, with contrasting cytokeratin immunohistochemical profiles between carcinomatous (CK+) and sarcomatous components (CK–), but shared aberrant p53 immunophenotype indicative of *TP53* mutation. They also demonstrate the presence of chondrosarcomatous differentiation, which has been reported as the most frequent heterologous element in OCS (16).

In a clinical cohort study, McFarlane et al. make use of two contrasting data sources to compare the clinical behavior of OCS patients versus those with other ovarian carcinomas: one from The Edinburgh Ovarian Cancer Database, the other from Surveillance Epidemiology and End Results (SEER) database. The findings identify OCS as the histotype with the least favorable overall survival profile, and this is especially the case in the context of early stage diagnosis, with FIGO stage I–II OCS patients demonstrating a median survival time of just two years in their primary cohort. The study also demonstrates that OCS patients represent an older patient population compared to other histotypes, with the median age at diagnosis being 67 years.

Finally, a molecular profiling study is presented by Dhillon et al., analyzing a cohort of OCS samples by targeted sequencing and immunohistochemical profiling. They show that the *TP53* mutation rate in this tumor type is high, but that a minority of cases (15–20%) are p53 wildtype. The p53 wildtype population demonstrated poorer survival, and this is one of the first reported molecular prognostic factors in OCS. Moreover, they demonstrate that a proportion of OCS harbor *BRCA1/2* mutation, highlighting the potential for some OCS patients to benefit from PARP inhibition. The *BRCA1/2*–mutant cases were suggested to experience more favorable survival, with 100% 3–year survival, though the number of *BRCA1/2*–mutant cases was limited.

Low grade serous ovarian carcinoma

LGSOC accounts for 3–5% of ovarian cancer diagnoses, demonstrates high levels of intrinsic chemoresistance, and affects younger women compared to HGSOC. Advancements have recently been made in treatment of LGSOC, with MEK inhibitors now recognized as a useful therapeutic option at recurrence (17), and endocrine maintenance therapy demonstrating substantial clinical activity (18).

A case report by Al–Aloosi et al. depicts an ex vivo drug testing study performed on organoids derived from a metastatic site of a patient with progressing LGSOC. Molecular tumor testing had previously revealed a somatic Y537S *ESR1* mutation likely associated with acquired resistance to letrozole, alongside absence of *KRAS*, *BRAF* or *NRAS* mutation. Characterization of organoid sensitivity to a panel of compounds and rational combinations resulted in the subsequent use of the endocrine therapy fulvestrant with the mTOR inhibitor everolimus. The authors report CA125 stabilization and a disease control period of 7 months on this treatment.

A sub–cohort analysis of a phase I study, presented by Nakamura et al., examines the safety of cisplatin–doxorubicin pressurized intraperitoneal aerosolized chemotherapy (PIPAC) in four heavily pre–treated LGSOC patients. The authors report the regimen to be well tolerated, and recommend further consideration of this strategy for recurrent LGSOC, where new treatment options are urgently needed to improve patient outcomes.

Endometriosis–associated ovarian cancers: endometrioid and clear cell carcinoma

EnOC and CCOC each represent up to 10% of ovarian cancer diagnoses, and are both recognized to be related to endometriosis. CCOC is highly chemoresistant, while EnOC reportedly demonstrates intermediate chemosensitivity that is lower than that of HGSOC. Both EnOC and CCOC are usually diagnosed at earlier stage compared to HGSOC (1), and both are among the epithelial types that appear to benefit most from complete surgical resection (19).

Two cohort studies using data from the SEER database are presented by (Liu et al. and Tian et al.). The former constructs a prognostic nomogram for CCOC showing the importance of log odds of positive lymph nodes (LODDS) in predicting ovarian cancer–specific survival, the latter uses a cohort of 4257 CCOC patients to demonstrate improvement in survival across time within the diagnosis period of 2000–2015.

Two review articles cover key topics in the field of endometriosis–associated ovarian cancers (Chen et al., Tang and Bian). Both cover key research progress made within CCOC and EnOC. In particular, they cover our contemporary understanding of the molecular drivers in these tumor types, key risk factors and summarize progress in the diagnosis and management of CCOC and EnOC.

Finally, a case report from Zhao et al. presents an individual with simultaneous EnOC and CCOC alongside endometriosis. Complementary molecular analysis demonstrated shared *ARID1A*, *KRAS*, *PIK3CA* and other mutational events in the two malignant populations, evidencing their clonal relationship.

Non–epithelial tumors

There are a large number of non–epithelial tumor types diagnosed at the ovary, the majority of which are poorly characterized at the molecular level. Many of these types are rare individually, but collectively non–epithelial tumors account for 10% of ovarian cancer cases. Accordingly, approximately 30,000 new diagnoses of these cancers are made worldwide each year (20). The vast majority of research beyond HGSOC has focused on other epithelial cancer types, leaving non-epithelial tumors critically understudied.

A review of ovarian steroid cell tumors, presented by Wei and Fadare, explores the clinical, radiological and histopathological features of these tumors alongside an overview of known molecular features. A retrospective study presented by Marino et al. examines patients with stage I immature teratoma that underwent either adjuvant chemotherapy or surveillance following fertility–sparing surgery, demonstrating excellent outcomes in both groups across the study period (100% overall survival in both groups, 87% and 90% disease–free survival in the surveillance and chemotherapy–treated groups, median follow–up time >15 years).

Two case reports of uncommon phenomena occurring in patients subsequent to teratoma diagnoses are presented: Tao et al. report a case of growing teratoma syndrome following treatment for immature teratoma with a review of the literature, highlighting this rare phenomenon, of which there is currently limited awareness. A second case report presents an individual with ovarian yolk sac tumor subsequent to mature cystic teratoma (Li et al.). Both of these clinical situations are uncommon, but worthy of highlighting to clinicians.

Variants of HGSOC

While HGSOC has received substantial research attention to date, subtypes within HGSOC are now widely recognized at the molecular level. In particular, around 50% of HGSOC are homologous recombination DNA repair deficient (HRD) and these tumors have been the focus of intense study (1). These investigations have culminated in the discovery and integration of PARP inhibitors into ovarian cancer management, which are most efficacious in HGSOC patients with identifiable HRD (13, 21). By contrast, the various homologous recombination repair proficient (HRP) molecular subtypes have been less extensively studied, such as those that demonstrate copy number gain of *CCNE1*.

Stiegeler et al. provide a comprehensive overview of HRP HGSOC in their review article, highlighting key potential therapeutic strategies particularly in the context of platinum–resistant relapse. The authors include targeted inhibitors of CDK1/2, WEE1, PI3K, AKT and ATR as options in their potential future HRP–HGSOC treatment algorithm, alongside the folate receptor alpha–targeted antibody–drug–conjugate Mirvetuximab.

A case report from Giancontieri et al. depicts an unusual case of high grade serous carcinoma of unknown primary at an inguinal node. Pathological examination demonstrated WT1, CK7 and PAX8 positivity, leading to a suspicion of tubo–ovarian origin. Subsequent surgery revealed only a serous tubal intraepithelial carcinoma (STIC), and a multidisciplinary team determined occult non–invasive STIC with node metastasis, and the authors propose this is likely from exfoliation and peritoneal spread rather than lymphatic spread.

An *in vitro* study presented by Iida et al. describes suppression of CRY1 as a potential mechanism by which anti–angiogenics may improve the efficacy of PARP inhibition in HRP HGSOC. They propose that CRY1 inhibition may be a potential strategy for improving PARP inhibitor efficacy, particularly for tumors that are considered HRP.

Concluding remarks

Uncommon forms of ovarian cancer are critically understudied, despite collectively representing around one third of ovarian cancer diagnoses, and some of these patient groups are markedly underserved by currently available treatment regimens. If patients diagnosed with these tumor types are to benefit from expanded treatment options in a similar manner to those with more common HGSOC, then it is clear that additional research attention will be critical for defining targetable disease drivers.

Author contributions

RH: Writing – original draft. MV: Writing – original draft.

Conflict of interest

RH: consultancy fees from GlaxoSmithKline and DeciBio, outside the scope of this work.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Stergios Boussios,
King's College London, United Kingdom
Wuliang Wang,
Second Affiliated Hospital of Zhengzhou University, China

*CORRESPONDENCE
Hua Hao
haohua410@tongji.edu.cn

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Carcinosarcoma of the ovary: a case report and literature review

Jian Zheng[1], Cui Tang[2], Ping Liu[3] and Hua Hao[1]*

[1]Department of Pathology, Yangpu Hospital, School of Medicine, Tongji University, Shanghai, China, [2]Department of Radiology, Yangpu Hospital, School of Medicine, Tongji University, Shanghai, China, [3]Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Objective: Carcinosarcoma of the ovary is a rare pathological type of ovarian cancer that is highly aggressive and occurs most frequently in the female reproductive tract at the site of the uterus. Herein, we explore the clinicopathological features, diagnosis, differential diagnosis, and treatment options for carcinosarcoma of the ovary.

Methods: We analyzed the clinical data of a case of carcinosarcoma, observed its histological morphology and immunohistochemical characteristics, detected the homologous recombination repair deficiency gene mutation, and reviewed the relevant literature.

Results: A 76-year-old menopausal woman visited our hospital because of abdominal distension, difficulty in urination, and constipation. Ultrasonography demonstrated abnormalities in the uterus and pelvic cavity, suggesting that the patient should undergo surgery. Immunohistochemical findings of carcinosarcoma of the right ovary were as follows: CK fraction (+), vimentin fraction (+), CK5/6 foci (+), p16 (+), p53 in approximately 70% (+), WT-1 foci (+), ER foci (+), PR part (+), Her-2 (1+), CK7 fraction (+), CK20 foci (+), CD99 fraction (+), CD10 fraction (+), CD56 foci (+), c-kit foci (+), SMA part (+), desmin foci (+), PD-L1 (-), SALL4 (-), OCT3/4 (-), p63 (-), p40 (-), D2-40 (-), inhibin (-), PLAP (-), CD30 (-), and Ki67 hotspot in approximately 80% (+). The patient underwent tumor cytoreduction and adjuvant chemotherapy. Currently, she is being followed up for 16 months and has a good general condition.

Conclusion: The diagnosis of carcinosarcoma relies on histopathological examination and differentiation of carcinosarcoma from immature teratoma. The current therapeutic regimen for carcinosarcoma is still based on tumor cytoreduction and platinum-containing chemotherapy; research on targeted therapy is still in progress.

KEYWORDS

ovary, carcinosarcoma, clinicopathology, differential diagnosis, treatment, case report

Introduction

As known, 90% of ovarian cancers are of an epithelial cell type and comprise multiple histologic types, with various specific molecular changes, clinical behaviors, and treatment outcomes. The remaining 10% are non-epithelial ovarian cancers, which include mainly germ cell tumors, sex cord-stromal tumors, and some extremely rare tumors such as small cell carcinomas (1). Ovarian carcinosarcomas follow a distinct natural history (1). Carcinosarcoma of the ovary, also known as ovarian carcinosarcoma and malignant mixed mesodermal tumor of the ovary, is a rare pathological type of ovarian cancer that is highly aggressive and occurs most frequently in the female reproductive tract at the site of the uterus (2). Carcinosarcomas occurring in the ovary account for only 1–4% of all pathological types of ovarian cancer (3). They have an atypical clinical presentation, advanced stage at the time of diagnosis, poor prognosis, and recur within 1 year after the end of the initial treatment in most patients. Currently, there is a lack of a uniform, standardized, diagnostic and therapeutic protocol for carcinosarcoma of the ovary. Herein, we report a case of carcinosarcoma of the ovary and summarize its clinicopathological features and treatment options, in light of the relevant domestic and international literature, to improve the understanding of this tumor.

Case description

Clinical data

The patient was a 76-year-old woman who experienced menopause for >20 years, without vaginal bleeding or other discomfort after menopause. Two weeks earlier, she experienced abdominal distension with difficulty in urination and defecation without obvious causes. On March 23, 2022, an abdominal ultrasonogram obtained outside the hospital showed an anterior uterus measuring 36 mm × 24 mm × 32 mm, endothelial thickness of 2 mm, cervical length of 25 mm, unequal echoes in the pelvic cavity, which measured 116 mm × 101 mm, unclear border, pelvic-free echogenic area of 110 mm × 96 mm, and the pelvic abdominal cavity in the echogenic area, with a depth of approximately 150 mm. The patient complained of abdominal distension, urinary difficulties, constipation, no abdominal pain, no irregular vaginal bleeding, and no increase in vaginal secretions. Therefore, she was admitted to our hospital as an emergency case for further investigation into the nature of the pelvic mass, as a malignant ovarian tumor was suspected. Physical examination revealed the following: bilateral adnexa not obvious to touch, pelvis could be touched a size of about 7 cm mass, activity check, no pressure pain. On admission, pelvic computed tomography (CT) showed a round, huge mass in the pelvic cavity, approximately 120 mm × 122 mm × 98 mm in size, with clear borders and uneven density. Low-density cystic necrosis was found inside. The CT value of the solid component of the lesion during plain scan was 24 HU. After enhancement, it appeared uneven. There was uniform, mild-to-moderate enhancement. The CT values in the arterial phase and venous phase were approximately 42 HU and 51 HU respectively. There was no obvious enhancement in the cystic necrosis area. It implied that the possible malignant pelvic tumor from the ovary may be an abdominopelvic cavity with a large amount of fluid or a small ascending colon diverticulum (Figures 1A, B). Enhanced magnetic resonance imaging (MRI) showed a huge mixed signal mass in the pelvic cavity. T1WI showed slightly low signal, T2WI showed obviously high and low mixed signal, T1WI-fs showed low mixed signal, T2WI-fs showed high and low mixed signal, and DWI (b=800) showed uneven signal. High signal, ADC value was about 1167×10^{-6} mm^2/s; the size of the lesion was about 120 mm×122 mm×98 mm, with clear boundary and smooth edge, and the lesion was unevenly enhanced after enhancement. The left ovary was unclearly displayed, and the uterus was compressed. There was no obvious thickening of the endometrium. The signal was uniform, the junction zone was clear, and the muscle layer signal was uneven. There was no obvious enhancement of the endometrium and myometrium after enhancement. The cervical parenchyma showed multiple, abnormal, and round signal shadows of varying sizes, with low signal on T1WI and high signal on T2WI, and with clear boundaries and uniform signals. No obvious enhancement was seen after enhancement. The bladder was poorly filled and a urinary catheter was visible in the cavity. A large amount of fluid accumulation was seen in the abdominal and pelvic cavity. It revealed a huge malignant pelvic space, possibly originating from the ovary, a large amount of fluid in the abdominopelvic cavity, and multiple nasal cysts in the cervix (Figures 1C–H). The preliminary diagnoses were ovarian malignancy and pelvic-abdominal effusion. Laboratory tests revealed the following: CA125 level: 16.73 U/ml, CA199 level: <2 U/ml, and CEA level: 2.82 ng/ml, all of which were normal. However, human epididymis protein 4 (HE4) level was 283.5 pmol/L, which was higher than the normal level. The patient underwent surgery on March 31st, and the patient intraoperative observation revealed a large amount of bloody ascites (5000 ml) in the pelvic and abdominal cavity. A huge mass of about 12×11×10 cm, was seen in the right appendage, originating from the right ovary. The surface of the tumor was smooth without rupture, and no obvious tumor was found on the surface. The uterus was slightly smaller, about 4×3×2 cm, with a regular outline. There was dense adhesion between the bladder and the lower segment of the uterus. No obvious abnormality was found in the left appendage. The greater omentum was thickened and had a hard texture. The cancer had metastasized. The greater omentum was thickened in a pie shape, measuring about 13×12×6 cm. The surface of the liver was detected. Miliary metastasis was detected. The stomach and pelvic intestines had smooth serosal surfaces, the posterior leaf of the left broad ligament showed flaky thickening and frizzy peritoneal surfaces, and the rest of the pelvic and abdominal peritoneum was smooth. Based on the above intraoperative observations, the International Federation of Gynecology and Obstetrics (FIGO) stage of the patient was IIIc T3cNxM0.

Pathological examination

There was a clear indication for surgery, and open laparotomy was later performed. Results of the intraoperative rapid pathology

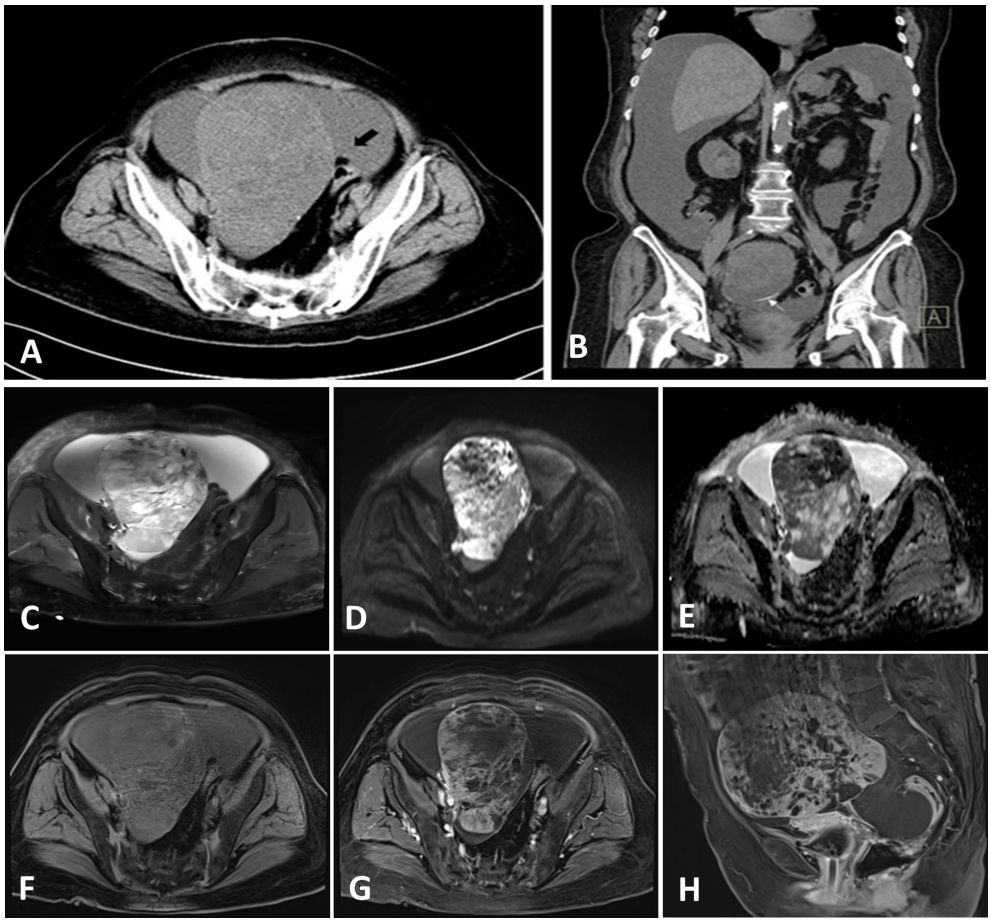

FIGURE 1
Computed tomography images **(A, B)** and enhanced magnetic resonance images **(C–H)** of the case.

report indicated a malignant tumor (right ovary), to be classified after paraffin sectioning and immunohistochemical examination. Collection and use of all specimens were approved by the Ethics Committee of Yangpu Hospital, School of Medicine, Tongji University. Abdominal tumor reduction, appendectomy, and partial salpingo-oophorectomy were performed. Postoperative gross pathological observation showed the following: right adnexa, soft, grayish dark red nodular mass measuring 14 cm × 12 cm × 8 cm with honeycombing in some areas; attached fallopian tube measuring 3 cm long, 5 cm in diameter; and nodular umbrella end measuring 2 cm × 2 cm × 1.5 cm with a medium texture (Figures 2A, B). Microscopically, the right ovarian mass showed plasma carcinoma with a poorly differentiated epithelial component; the mesenchymal component was chondrosarcoma (Figures 2C, D). The immunophenotyping of the right ovarian mass was as follows: ovarian tumor cells CK fraction (+) (Figure 2E), vimentin fraction (+), CK5/6 foci (+), p16 (+) (Figure 2F), p53 in approximately 70% (+) (Figure 2G), WT-1 foci (+) (Figure 2H), ER foci (+) (Figure 2I), PR part (+) (Figure 2J), Her-2 (1+) (Figure 2K), CK7 fraction (+) (Figure 2L), CK20 foci (+) (Figure 2M), CD99 fraction (+), CD10 fraction (+), CD56 foci (+), c-kit foci (+) (Figure 2N), SMA part (+) (Figure 2O), desmin foci (+) (Figure 2P), PD-L1 (-), SALL4 (-), OCT3/4 (-), p63 (-), p40 (-), D2-40 (-), inhibin (-), PLAP (-), CD30 (-), and Ki67 hotspot in approximately 80% (+). The pathological diagnosis was carcinosarcoma of the right ovary (the carcinoma component was high-grade plasmacytoid carcinoma, whereas the sarcomatoid component was chondrosarcoma). The peritoneal biopsy results indicated metastatic adenocarcinoma; carcinoma was observed in the greater omentum, left ovary, and left fallopian tube; there was no invasion of the tumor in the parietal uterus, vascular tissues of the right ovary or appendix, or vasculature and nerves. The results of the homologous recombination repair deficiency (HRD) gene test were as follows: HRD status was positive, the breast cancer susceptibility gene 1 (*BRCA1*) gene mutation was a variant of undetermined significance, and the tumor protein p53 (*TP53*) gene missense mutation with 86.15% mutation abundance, was a pathogenic variant.

Treatment and follow-up

To control the development of the disease and prolong the patient's survival period, the proposed postoperative treatment was

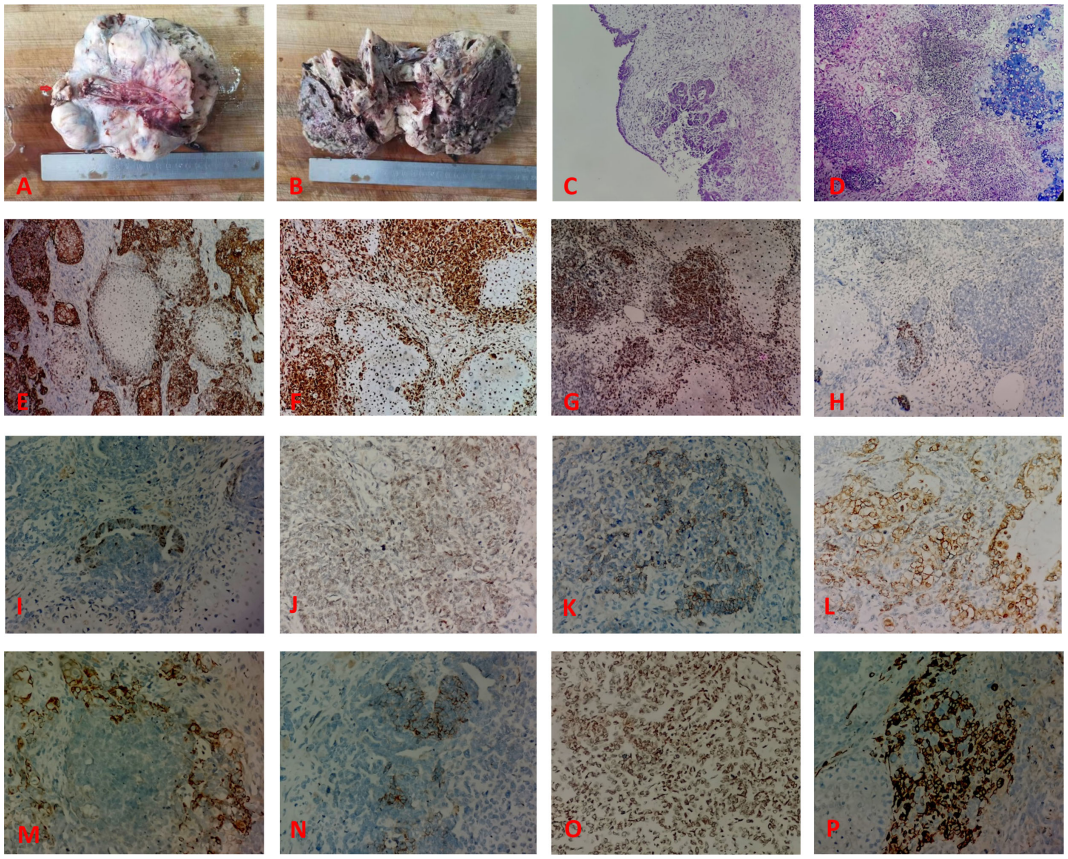

FIGURE 2
The pathological feature of the case. **(A)** The tumor is solid and nodular, with the umbilical end of the fallopian tube seen as a nodule on the surface (arrows). **(B)** The tumor is grayish dark red, solid, and has honeycombing in some areas. **(C)** Hematoxylin and eosin (HE) stain showing the epithelial component of the tumor (predominantly plasmacytoid carcinoma). **(D)** HE stain showing the poorly differentiated epithelial and mesenchymal components within the tumor (chondrosarcoma). Immunohistochemical findings: **(E)** epithelial component CK (+); **(F)** tumor cell p16 (+); **(G)** tumor cell p53 (+); **(H)** tumor cell WT-1 focal (+); **(I)** tumor cell ER foci (+); **(J)** PR part (+); **(K)** Her-2 (1+); **(L)** CK7 fraction (+); **(M)** CK20 foci (+); **(N)** c-kit foci (+); **(O)** SMA part (+); **(P)** desmin foci (+).

intravenous chemotherapy of albumin paclitaxel (400 mg) + carboplatin (500 mg) on day 1, every 21 days for six courses, and the first chemo treatment is April 14th (Figure 3). Currently, the patient is being followed up for 16 months and has a good general condition.

Discussion

Carcinosarcoma is a highly malignant tumor with both epithelial and mesenchymal components, most often occurring in the uterus and extremely rarely in the ovaries (3). Herein, we

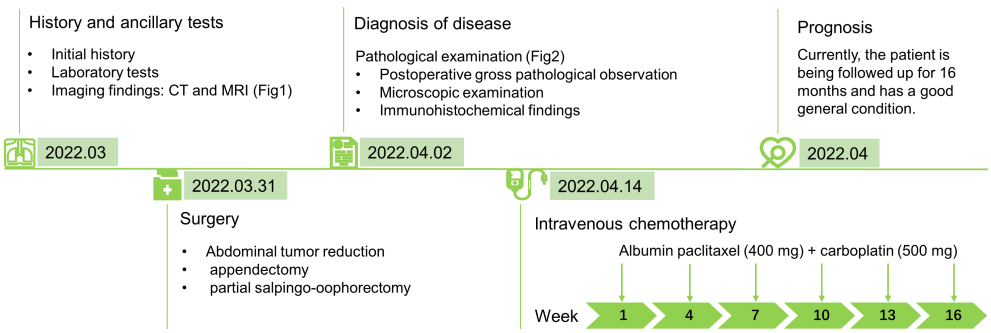

FIGURE 3
A timeline figure summarising the case diagnosis and treatment pathway.

identified a new case of carcinosarcoma and summarized the clinicopathological staging, treatment, and prognosis of the 58 previously reported cases (Table 1). Of the 59 cases reported to date, the age of onset was mostly 60–80 years in postmenopausal women (4). The risk factors associated with disease onset include obesity, childlessness, chronic estrogen use, and tamoxifen (5). Seventy-five percent of patients were reported to be at FIGO stages III–IV at the time of diagnosis, and 90% were at FIGO stages II–IV, with a significantly low survival rate (6).

Clinical manifestation

Carcinosarcoma is prevalent in postmenopausal elderly women. Our patient was 76 years old and was age compatible with other previously reported patients. Clinical manifestations of carcinosarcoma are similar to those of epithelial ovarian cancer, but aggressiveness is higher and the degree of malignancy is much higher. Carcinosarcoma is difficult to diagnose preoperatively, is not easily detected in the early stage, lacks specificity, has a rapid disease

TABLE 1 58 cases of clinic data and prognosis data.

Age	Stage	Treatment	Metastasis	Follow-up months	Outcome
55	Ic	Surgery, CT	Yes	NA	NA
47	Ia	Surgery, CT	NA	71	AWD
62	IIIc	Surgery, CT	NA	59	AWD
66	IIIc	Surgery, CT	NA	11	DOD
78	IIIc	Surgery, CT	NA	7	DOD
54	IIIc	Surgery, CT	NA	44	DOD
83	IIIb	Surgery, CT	NA	11	Alive
55	IIIc	Surgery, CT	NA	35	Alive with disease
58	IIIc	Surgery, CT	NA	10	DOD
65	IIIc	Surgery, CT	NA	18	Alive with disease
82	IV	Surgery, CT	NA	26	AWR
80	IIc	Surgery, CT	NA	6	AWD
62	IIIb	Surgery, CT	NA	41	DOD
65	IV	Surgery, CT	NA	14	Alive
60	IIc	Surgery, CT	NA	12	Alive
46	Ic	Surgery, CT	NA	30	Alive
64	IIIc	Surgery, CT	NA	46	Alive with disease
52	IIIc	Surgery, CT	Yes	18	AWD
65	IIIc	Surgery, CT	NA	24	Alive
51	IIIa	Surgery, CT	NA	14	DOD
37	NA	Surgery	Yes	5 days	DOD
48	IV	Surgery, CT	Yes	9	DOD
37	IIc	Surgery, CT	Yes	13	Alive
64	IIIc	Surgery, CT	Yes	3	Alive
69	IV	Surgery	Yes	10 days	DOD
60	NA	Surgery, CT	Yes	4	Alive
NA	IIIc	Surgery, CT	NA	33	AWD
NA	IIIc	Surgery, CT	NA	8	AWD
NA	IIIc	Surgery, CT	NA	19	DOD
NA	Ic	Surgery, CT	NA	15	DOD
NA	IIIc	Surgery, CT	NA	4	DOD

(Continued)

TABLE 1 Continued

Age	Stage	Treatment	Metastasis	Follow-up months	Outcome
NA	IIc	Surgery, CT	NA	24	Dead
NA	IIIc	Surgery, CT	NA	25	DOD
NA	IIIc	Surgery, CT	NA	110	AWD
NA	IIIc	Surgery, CT	NA	38	AWD
NA	IIIc	Surgery, CT	NA	18	DOD
NA	IIc	Surgery, CT	NA	13	DOD
NA	IIIc	Surgery, CT	NA	10	DOD
NA	IIIc	Surgery, CT	NA	23	DOD
50	NA	Surgery	Yes	2	DOD
55	IIc	Surgery, CT	NA	76	DOD
57	IIIc	Surgery, CT	NA	6	DOD
62	IIIc	Surgery, CT	NA	22	DOD
57	IIIc	Surgery, CT	NA	13	DOD
72	IIIc	Surgery, CT	NA	4	AWD
71	IIIc	Surgery, CT	NA	11	AWD
58	IIIc	Surgery, CT	NA	2	AWD
55	IV	Surgery, CT	NA	68	AWD
40	IIc	Surgery, CT	Yes	46	DOD
64	IV	Surgery, CT	NA	37	Dead
64	IIIc	Surgery, CT	NA	7	DOD
52	NA	Surgery, CT	NA	6	AWD
64	IIIa	Surgery, CT	Yes	26	AWD
75	IV	Surgery	Yes	NA	DOD
59	NA	Surgery	Yes	18	Dead
74	NA	Surgery	Yes	18	Alive
52	NA	Surgery, CT	Yes	12	AWD
78	IIIb	Surgery, CT	Yes	120	AWD

NA, data not available; CT, chemotherapy; AWD, alive without disease; DOD, dead of disease; AWR, alive with recurrence.

progression, is prone to metastasis, and most patients are in the late stage at the time of diagnosis. The main clinical manifestations include abdominal mass, abdominal distension, abdominal pain, ascites, occasional vaginal bleeding, and nonspecific gastrointestinal symptoms in some patients. A gynecological examination can reveal a pelvic mass with a large volume, irregular shape, unclear boundary, and poor activity. Gynecological ultrasonography can reveal a solid pelvic cystic mass. The present patient presented with abdominal distension and a pelvic mass.

However, the metastatic mechanism of carcinosarcoma is not fully understood. Direct spread, abdominal implantation, and lymphatic metastasis are considered important metastatic routes, similar to other malignant ovarian tumors. The rates of lymph node metastasis and vascular invasion in carcinosarcoma are high, and some researchers have reported that lymph node metastasis occurs in more than half of patients at the time of initial diagnosis (7–9). More than 90% of carcinosarcomas spread beyond the ovaries, and one-third of cases are associated with peritoneal effusion (7, 8). In our case, metastatic adenocarcinoma was observed from the peritoneal biopsy results, and carcinomatous involvement was observed in the greater omentum, left ovary, and left fallopian tube, which is consistent with findings in the literature.

Histopathological features

There are several theories regarding the organizational origins of carcinosarcoma (3, 9). These theories include the (1) transformation theory, which suggests that the sarcoma component is transformed from the cancer component during the

process of tumor derivation; (2) combinatorial theory, also known as the monoclonal origin theory, which suggests that the cancer and sarcoma components originate from a common pluripotent stem cell precursor that undergoes differentiation at the early stage of the tumor; and (3) collision theory, which suggests that the cancer and sarcoma components are independent of each other, originating from two different stem cells that ultimately collide to form the cancer/sarcoma. Currently, the theory of monoclonal origin is preferred. Additionally, some studies have reported the existence of gene mutations in carcinosarcoma, such as deletion of the breast cancer susceptibility gene 2 allele and *TP53* mutation (10, 11). In our patient, results of the HRD genetic test report showed that the patient was positive for HRD status, with a missense mutation in the *BRCA1* gene and a missense mutation in the *TP53* gene, which is located in exon 5 of the *TP53* gene; thereby, resulting in the substitution of amino acid 151 from proline to serine in the protein sequence encoded by the gene (10, 11). This mutation is considered pathogenic.

Microscopically, both epithelial and mesenchymal components were observed. The epithelial component can be an endometrioid or tubal epithelioid gland-like structure, squamous cell carcinoma, or clear cell carcinoma forming strips or nests; in this case, plasmacytoid carcinoma and poorly differentiated epithelial components were observed microscopically. The mesenchymal component can be endometrial mesenchymal sarcoma, smooth muscle sarcoma, chondrosarcoma, osteosarcoma, rhabdomyosarcoma, or liposarcoma, among which chondrosarcoma is the most common. The mesenchymal component in the present case was chondrosarcoma, which is in line with that in previous reports.

In addition, immunohistochemistry is valuable for the identification of different tissue components of carcinosarcoma (12, 13). In this case, immunohistochemistry findings were positive for CK and EMA in the epithelial component and diffusely positive for vimentin in the mesenchymal component; Ki-67 was also found to be positive in 60% of the cells (14). In our patient, the following findings were in accordance with those reported previously: CK fraction (+), vimentin fraction (+), CK5/6 foci (+), p16 (+), p53 in approximately 70% (+), WT-1 foci (+), ER foci (+), PR part (+), Her-2 (1+), CK7 fraction (+), CK20 foci (+), CD99 fraction (+), CD10 fraction (+), CD56 foci (+), c-kit foci (+), SMA part (+), desmin foci (+), PD-L1 (-), SALL4 (-), OCT3/4 (-), p63 (-), p40 (-), D2-40 (-), inhibin (-), PLAP (-), CD30 (-), and Ki67 hotspot in approximately 80% (+).

Imaging

The CT manifestation of carcinosarcoma is commonly a cystic-solid mixed density mass, mostly located unilaterally, more on the right side than on the left side, with multiple small cystic cavities of varying sizes in the capsule, varying thicknesses of the wall, and the solid part of the tumor is flocculent or nodular shape. The tumor parenchyma is mostly in the form of hypodensities on plain CT, and the area of necrotic cystic degeneration is in the form of lower densities. CT can accurately locate the carcinosarcoma and provide information on the size and shape of the lesion, internal structure, and growth characteristics. It is of great value to observe whether there is invasion of neighboring tissues and organs and whether there are lymph nodes and distant metastases. The CT examination of this patient showed a round mass in the pelvis, approximately 93 mm × 118 mm, with a clear boundary, uneven density, CT value of approximately 24 HU, and calcification at some edges; there was no obvious obstruction and dilatation of the lower abdomen and pelvic intestines; the bladder was well filled; the bladder wall was smooth and non-thick; there was no obvious abnormality in the bladder lumen; there were no obvious enlarged lymph nodes in the bladder; the abdominopelvic cavity was filled with a large amount of fluid; and a small cystic pouch protruding shadow was seen in the ascending colon. However, CT image staging and surgical pathology staging could not be matched. When a large amount of peritoneal fluid is present on CT images, it does not clearly show peritoneal implantation and regional lymph node metastasis. In particular, the sensitivity of discovering small nodes is poor, so pathology is still the gold standard for confirming the diagnosis of carcinosarcoma. Nevertheless, CT plays an important role in the observation of invasion of the tumor's neighboring organs and tissues, the presence or absence of pelvic effusion, and the metastasis of the peritoneum and lymph nodes, which provides an important basis for the clinical staging of the tumor.

Diagnosis and differential diagnosis

The clinical manifestations, imaging manifestations, and serological indices of carcinosarcoma are nonspecific and almost indistinguishable from other types of malignant ovarian tumors; therefore, preoperative diagnosis is difficult. Hence, diagnosis of carcinosarcoma requires a combination of clinical and pathological findings. In this case, the pathological diagnosis was an carcinosarcoma of the right ovary. The most important differential diagnosis of Carcinosarcoma is immature teratoma, which is distinguished by two factors. (1) Age of disease onset: Carcinosarcoma is almost always seen in postmenopausal women, whereas immature teratomas are more commonly seen in postmenopausal women, children, and young adults. (2) Pathomorphology: Carcinosarcoma is a simpler mixture of multiple malignant epithelia and mesenchyme, with immature embryonic organ-like structural changes without differentiation to the tertiary germ layer, and usually lacks the neural and germ cell components of teratomas, whereas immature teratomas tend to have embryonic neural ectodermal differentiation, such as neural tubes, which is important and can be distinguished from other teratomas. This information is important for differentiation.

Treatment

The current treatment for carcinosarcoma involves a combination of surgical procedures, often followed by adjuvant chemotherapy. Most retrospective studies have affirmed the role of tumor cytoreduction in the treatment of ovarian carcinosarcoma, and better survival has been achieved by optimal tumor reduction. Satisfactory tumor cytoreduction was defined as a maximum

residual focus of <1 cm in diameter after surgery. Doo et al. (2) reported that in 51 patients after tumor cytoreduction, the median durations of progression-free survival (PFS) of the three groups with no visible residual foci (n = 18), visible residual foci with a maximum diameter of ≤1 cm (n = 20), and >1 cm (n = 13) were 29, 21, and 2 months, respectively (P = 0.036); and the median durations of overall survival were 57, 32, and 11 months, respectively, with statistically significant differences (P < 0.05). Therefore, satisfactory tumor cytoreduction may improve patient prognosis, and residual lesions should be minimized during surgery to prolong patient survival.

Because of the lack of clinical studies with large datasets, the efficacy of first-line chemotherapy regimens is inconclusive, and platinum-based combination chemotherapy is currently used. Brackmann et al. (15) retrospectively analyzed 31 patients diagnosed with ovarian or primary peritoneal carcinosarcoma, and patients treated with carboplatin/paclitaxel had a significantly longer PFS than those receiving isocyclophosphamide/paclitaxel (17.8 versus 8.0 months). However, Yalcin et al. (16) evaluated the effect of satisfactory tumor cytoreduction followed by adjuvant paclitaxel in combination with platinum-based chemotherapy, on survival outcomes in 54 patients with ovarian carcinosarcoma and 108 patients with epithelial carcinoma of the ovary, both of whom underwent satisfactory tumor cytoreduction. They showed that treating patients with carcinosarcoma of the ovary and epithelial carcinoma of the ovary with the same regimen resulted in no significant difference in PFS durations of 29 and 27 months, respectively. Considering the present patient's condition, intravenous chemotherapy was administered on day 1 for 21 days.

Owing to the lack of therapeutic efficacy, several studies on biologically targeted therapies to improve efficacy are underway. Zhu et al. (17) detected the expression of programmed cell death ligand 1 (PD-L1) in 19 cases of carcinosarcoma and found that there was positive expression of PD-L1 in 52.6% of the cancer component and 47.4% of the sarcoma component; those with negative expression of PD-L1 in the sarcoma component had a significantly higher survival rate than those with positive expression (P = 0.036). The PD-1/PD-L1 signaling pathway may be a new target for tailoring immunotherapy. Vascular endothelial growth factor expression has also been reported in ovarian and uterine cancer sarcomas and is associated with tumor progression and poor prognosis (18). Tang et al. (19) found that a murine sarcoma virus oncogene (*KRAS*) mutation and p53 deletion in mouse ovarian epithelial cells can induce carcinosarcoma, the epithelial component of which is mainly endometrioid carcinoma, and that the tumor metastasizes quickly, with a significantly higher risk of death. We reviewed 58 cases, and the clinic data and prognosis data are shown in Table 1 (20–42). The maximum survival of 58 cases was 120 months; the disease-free survival of our case was 16 months, better than that of most of the cases. Results of the HRD genetic test report showed that our patient was positive for HRD status, with a missense mutation in the *BRCA1* gene and a missense mutation in the *TP53* gene, located in exon 5 of the *TP53* gene, resulting in the substitution of amino acid from proline to serine in its protein sequence. These germline mutations represent the most potent known genetic risk factors for epithelial ovarian cancers and are detected in 6–15% of women diagnosed with this condition. Knowledge of a patient's BRCA1/2 status can play a pivotal role in counseling, particularly in predicting their expected survival. Notably, BRCA1/2 carriers with epithelial ovarian cancers exhibit a more favorable response to platinum-based chemotherapies, resulting in enhanced survival rates. Determining the prevalence of BRCA1 and BRCA2 mutations in ovarian carcinosarcomas poses challenges. Nevertheless, compelling evidence suggests that BRCA-wild type tumors can also display a BRCA-like phenotype, often referred to as "BRCAness". Ovarian carcinosarcomas harboring loss-of-function mutations in homologous recombination genes may respond therapeutically to PARP inhibition (43). So, the medication recommendation suggests olaparib and niraparib as sensitive drugs and rucaparib, fluzoparib, pamiparib, and talazoparib as potentially beneficial drugs.

Prognosis

Carcinosarcoma is far more malignant than are tumors of the uterus and fallopian tubes; there exists a clear relationship between the prognosis of patients and the type of pathology, clinical stage, cancer antigen 125 level, size of the residual tumor after surgery, and chemotherapy regimen. Most patients have a short survival period, with a mean survival of 11–12 months. Patients with chondrosarcoma-containing components have a longer survival period than those without chondrosarcoma-containing components. In our case of carcinosarcoma containing a chondrosarcoma component, the patient has a good general condition and is still being followed.

In summary, the incidence of ovarian carcinosarcoma is low, the symptoms are atypical, there are no specific serological indexes and imaging manifestations, and the disease progresses rapidly with poor prognosis. Therefore, in practice, it is necessary to pay careful attention to the diagnosis based on the combination of clinical and pathological findings for cystic solid tumors of the ovary, and it is necessary to perform comprehensive and multi-location sampling to provide a sufficient basis for diagnosis and differentiation from immature teratoma. This may help improve the early diagnosis rate and reduce the morbidity and mortality rates. Additionally, the best therapeutic option is still uncertain, and targeted therapy is still being researched. If suitable therapeutic targets can be found, the prognosis of patients will be greatly improved. Lastly, as carcinosarcoma generally develops at an older age, new treatment options and associated toxic effects should be considered in future studies, as they may not be well tolerated in the older patient population. Treatment options with fewer toxic side effects and better efficacy should be actively explored, which will in turn improve the prognosis of carcinosarcoma.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

JZ: Conceptualization, Investigation, Writing – original draft. CT: Data curation, Formal Analysis, Resources, Writing – original draft. PL: Data curation, Resources, Writing – original draft. HH: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Yasushi Iida,
Jikei University Hospital, Japan
Kate Connolly,
Western General Hospital, United Kingdom

*CORRESPONDENCE
Aijun Liu
aliu301@126.com
Weng Yang
rubyyangwen@sina.com

†These authors have contributed equally to this work

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Simultaneous occurrence of two distinct histotypes of ovarian endometriosis-associated cancer in bilateral ovaries: implications for monoclonal histogenesis from a case report

Luyang Zhao[1†], Yunyue Liu[1†], Feng Liu[1], Xiangshu Jin[1], Jia Xu[1], Shuting Zhou[1], Yuanguang Meng[1], Aijun Liu[2*] and Weng Yang[1*]

[1]Department of Obstetrics and Gynecology, The Seventh Medical Centre of Chinese People's Liberation Army General Hospital, Beijing, China, [2]Department of Pathology, The Seventh Medical Centre of Chinese People's Liberation Army (PLA) General Hospital, Beijing, China

Background: Transformation of endometriosis to malignancy is a rare occurrence. Clear cell ovarian cancer and endometrioid ovarian cancer are the two histotypes most consistently linked to endometriosis. The exact pathways leading to malignant transformation of endometriosis remain elusive.

Case presentation: A 41-year-old woman presented to our hospital with a ten days history of abdominal pain which was not responsive to medication. Pathological examination revealed an unexpected finding of bilateral endometriosis associated with distinct malignancies: a clear cell carcinoma in the right ovary and a well-differentiated endometrioid carcinoma in the left ovary. Molecular analysis indicated a shared somatic driver mutation in ING1 in the eutopic endometrium and the bilateral ovaries while simultaneously exhibiting specific genetic alterations unique to each carcinoma. Notably, several common mutation sites were also identified, including previously reported common oncogenes (KRAS, PIK3CA, ARID1A). This finding prompts the hypothesis of a possible monoclonal origin of the two tumours.

Conclusion: This case represents an exceedingly rare occurrence of two different histotypes of ovarian endometriosis-associated cancer manifesting simultaneously in bilateral ovaries. Based on genetic analysis, we hypothesize that these malignancies may have a monoclonal origin, providing insights into understanding the different biological mechanisms underlying carcinogenesis.

KEYWORDS

endometriosis-associated ovarian cancer, clear cell ovarian cancer, endometrioid ovarian cancer, endometriosis, eutopic endometrium

1 Introduction

Endometriosis is a chronic and progressive inflammatory disease that affects 10% of women in their reproductive years (1). Previous research has indicated a significant link between endometriosis and increased risk of clear cell ovarian cancer (CCOC) and endometrioid ovarian cancer (EOC), with risks elevated by 3.4-fold and 2.3-fold, respectively (2). Although transformation of endometriosis to malignancy is uncommon, occurring in only approximately 0.7-1.6% of women (3), recent robust epidemiological studies have raised questions about the accuracy of these rates. Approximately one-third of all CCOC and EOC cases are now believed to originate from endometriosis (4). Nonetheless, the precise carcinogenic pathways underlying transformation of endometriosis to malignancy remain unclear. An increasing number of clinicopathological studies have suggested the existence of distinct pathways for malignant evolution of endometriosis-associated CCOC and EOC (4). Here, we describe a rare case involving a 41-year-old female patient with simultaneous bilateral tumours, with the right ovary showing primary CCOC and the left ovary EOC. This case provides evidence of a monoclonal origin for the different histotypes.

2 Case description

A 41-year-old woman, gravida 2 and para 1, presented with a ten days history of abdominal pain which was not responsive to medication. The patient had regular menstrual periods without significant dysmenorrhoea. Bilateral ovarian cysts were detected during a routine physical examination approximately one year prior. However, ten days before admission to a local hospital, she had persistent lower abdominal pain without any apparent reason. Notably, some serum tumour markers showed remarkable elevation: carbohydrate antigen 125 (CA125) of 1229 U/ml (normal range, 0–35), carbohydrate antigen 199 (CA199) of 7107 U/ml (normal range, 0–35) and carcino-embryonic antigen (CEA) 44.1 U/ml (normal range, 0–11). Conversely, carbohydrate antigen 153 and human epididymis protein 4 showed no significant increase. Abdominal computed tomography revealed a 15*10 cm cystic-solid tumour. Given the elevated white blood cell count of 19.35*10^9/l (with a neutrophil count of 93.2%), the local hospital initiated a one-week course of anti-infective treatment, which partially alleviated her symptoms. Her serum tumour marker levels decreased slightly, as follows: CA125 710 U/ml, CA199 2998 U/ml, and CEA 25.98 U/ml. Besides, she had no other medical conditions and she denied a family history of endometriosis or cancer. Subsequently, the patient sought further treatment at our hospital.

Physical examination showed that her body mass index was 21.3 kg/m2 (height: 165 cm, weight: 58 kg), with no significant recent changes. Abdominal assessment revealed slight pressure pain without obvious rebound pain in the lower abdomen. Gynaecological examination indicated a normally sized uterus with limited mobility. A tender, solid-cystic mass measuring 12 cm in diameter was noted posterior to the uterus. No palpable nodules were found on palpation of the anus. Her white blood cell count was within the normal range in our laboratory analysis. CA125 levels decreased to 417 U/ml, and CA199 levels decreased to 1772 U/ml. Gastroenteroscopy yielded normal results. Transvaginal ultrasonography revealed a 12*11*4.6 cm cystic-solid mass in the posterior uterus displaying a nonhomogeneous echo. The magnetic resonance imaging suggested potential malignancy due to multiple cystic-solid masses originating from the adnexal region accompanied by intracyst bleeding (Figure 1).

Robotic surgery was performed to explore the abdominal pelvic lesions. Chocolate-like discoloured deposits were distributed in the abdominal cavity, primarily within the greater omentum and peritoneal mesentery. The bilateral adnexa were adherent to the pelvic wall. The left ovary showed a mass of approximately 10*10 cm and the right ovary a mass of approximately 6*5 cm, both containing chocolate-like fluid and several papillary solid protrusions. Frozen pathology analysis of the left ovarian cyst suggested a well-differentiated carcinoma. Subsequently, a comprehensive surgical intervention, including total hysterectomy, bilateral salpingo-oophorectomy, omentectomy and lymphadenectomy, was performed. The final diagnosis was International Federation of Gynaecology and Obstetrics (FIGO) stage IC1, with a grade 2 endometrioid adenocarcinoma in the left ovary and a FIGO stage IC1 clear cell carcinoma in the right ovary (elaborated pathology

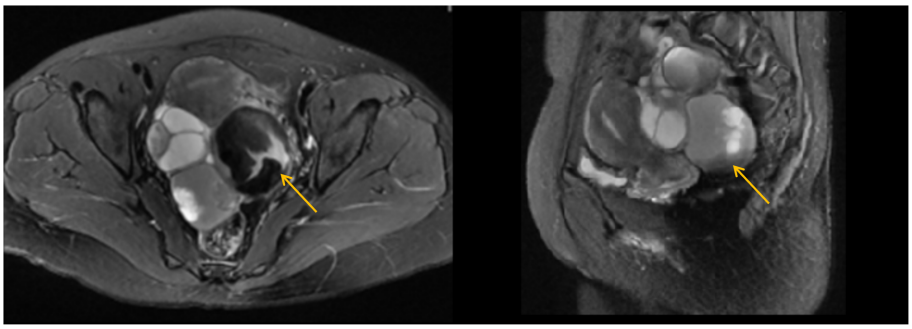

FIGURE 1
Magnetic resonance imaging (MRI) of the pelvic cavity (T2-weighted image). The MRI suggested potential malignancy due to multiple cystic-solid masses (the largest one 7.6*6.6cm, yellow arrow) originating from the adnexal region, accompanied by intra-cyst bleeding.

description provided below). The patient underwent a course of six chemotherapy cycles with paclitaxel-albumin and carboplatin, which resulted in complete relief. Postsurgery, her serum CA125 level decreased to 96.3 U/ml and the CA199 level to 239.7 U/ml. All serum biomarkers returned to normal levels after the second cycle of chemotherapy.

The final paraffin pathology report confirmed malignancy in the bilateral ovaries, without infiltration of the uterus, fallopian tube, omentum, or lymph nodes. Lymphatic vascular involvement was negative. The left ovarian mass was consistent with ovarian endometrioid carcinoma that was moderately differentiated with large areas of necrosis, and the surrounding glands showed atypical endometriotic lesions and endometriotic lesions (Figures 2A, B). Ectopic endometrial glands and mesenchymal components were observed within the localized wall of the left fallopian tube tissue, consistent with endometriosis with focal ectopic glands with atypia (Figure 2C). The right ovarian mass was consistent with ovarian clear cell carcinoma and was surrounded by endometriotic cysts and corpus luteum cysts with haemorrhage (Figures 2D, E). Immunohistochemistry (IHC) was conducted to provide confirmation of the diagnosis. The tumour cells in the left EOC showed positive monoclonal expression of estrogen receptor (ER) (+60%) and progesterone receptor (PR) (+60%), along with patchy expression of monoclonal p16 and p53. However, the expression of ER and PR were negative in the right CCOC. While, the expression of p16, and p53 was absent consistent with left EOC. The Ki-67 labelling index was approximately 50% in the left ovary and 40% in the right ovary. In particular, hepatocyte nuclear factor 1 beta (HNF-1β) showed strong positive expression in the CCOC, positive expression in the atypical endometriotic lesions of the left ovary, and patchy expression in the endometriotic lesions of both ovaries (Figure 3).

To explore potential aetiologies and therapeutic targets, whole-exome sequencing with next-generation sequencing was performed on eutopic endometrium (EU), bilateral tumours, and plasma samples. DNA sequencing results revealed somatic mutations in 29 genes for the EU, 66 genes for the CCOC, and 82 genes for the EOC (Supplementary Table S1). The shared mutated genes between the CCOC and EOC included ARIDIA, CCDC137, KHDRBS1, KRAS, PCDHB12, PIK3CA, SLC28A3 and ING1. ING1 was the sole gene mutated across all three samples (Supplementary Figure S1). Furthermore, NGS data analysis revealed microsatellite-stable status across all samples, with a low tumour mutational burden. Notably, only the EOC sample had a remarkably high homologous recombination deficiency score (50). Pathway enrichment analysis revealed different gene pathways for the three samples (Figures 4A-C). Twenty-nine gene pathways involving oestrogen metabolism, age, angiogenesis, apoptosis, and tumorigenesis, among others, were identified as enriched in both the EOC and CCOC samples (Figure 4D). The TGF-β signalling pathway and lysine degradation pathway were uniquely enriched in the EOC and CCOC, respectively (Figure 4E). Pearson correlation coefficients for signature features between the EU and CCOC were 0.9364 ($P<0.0001$), between the EU and EOC were 0.816 ($P<0.0001$), and between the EOC and CCOC were 0.8852 ($P<0.0001$) (Supplementary Figure S1).

3 Discussion

Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity, predominantly found within the pelvic cavity, ovary and fallopian tubes (1). Although it is a benign disease, it shares certain characteristics with cancer, such as local and distant invasion, resistance to apoptosis, and the ability to induce angiogenesis. Overall, the aetiology of this disease remains enigmatic (5). The pathogenesis of ovarian endometriomas is also a topic of debate, as no single theory can comprehensively explain the

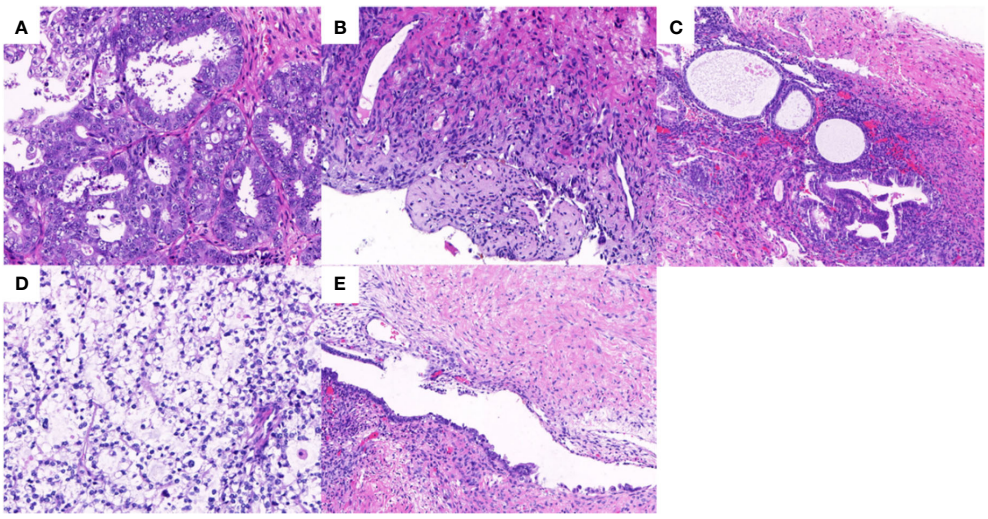

FIGURE 2
Hematoxylin-eosin staining in bilateral ovary tumors and endometrotic lesions. **(A)** The endometrioid adenocarcinoma in the left ovary with grade 2 (×20); **(B)** The borderline endometrioid adenocarcinoma area in the left ovary (×10); **(C)** The endometriotic area and atypical endometriotic area in the left fallopian tube (×10); **(D)** The clear cell carcinoma in the right ovary (×20); **(E)** The endometriotic lesion in the right ovary (×10).

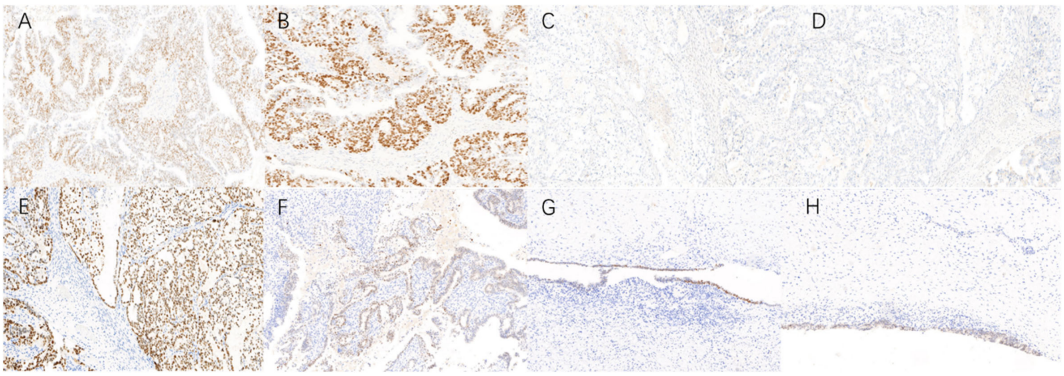

FIGURE 3
Immunohistochemistry (IHC) in bilateral ovary tumors and endometrotic lesions (×10). **(A, B)** Endometrioid ovarian cancer cells exhibited positive monoclonal expression of ER (+60%) and PR (+60%); **(C, D)** Clear cell ovarian cancer cells have no expression of ER, PR; **(E)** Clear cell ovarian carcinoma cells exhibited strong positive HNF-1β expression; **(F–H)** Atypical endometriotic lesion, endometriotic lesions of left ovary and right ovary also showed positive expression of HNF-1β.

histogenesis of endometriosis, and a contradiction between the implantation theory and the metaplasia theory persists.

The association between endometriosis and ovarian cancer was initially described in 1925 by Sampson (6). This was further substantiated by Scott in 1953, who observed the presence of benign endometriosis near ovarian cancer (7). Although transformation of endometriosis to malignancy is a rare occurrence, with an estimated incidence between 0.7% and 1.6% among women (3), recent evidence suggests that these data might be underestimated. CCOC and EOC are the two histotypes most consistently linked to endometriosis (8). Concurrent endometriosis has been observed in approximately 21%–51% of women with CCOC and in 23%–43% of women with EOC (9).

The exact pathways leading to malignant transformation of endometriosis remain elusive. Accumulated evidence shows that the process of endometriosis-associated ovarian carcinogenesis is intricate and involves multiple stages. The implanted ectopic endometrium accumulates key mutations over time, progressively undergoing genetic and epigenetic alterations. This transformation is further promoted by the inflammatory and hyperoestrogenic microenvironment, coupled with the oxidative stress present within the endometriotic lesion (10). Recurrent point mutations are restricted to few typical oncogenes and tumour suppressors. The most frequently observed oncogene mutations shared by both histotypes are ARID1A, PIK3CA and PTEN (11–13). However, an increasing body of evidence from clinicopathological studies suggests that distinct pathways might be involved in malignant degeneration of endometriosis leading to CCOC and EOC, which suggests that the relationship between these histotypes and endometriosis might be different (4).

The majority of researchers agree on the existence of a dichotomy in the aetiology of the two different ovarian tumours

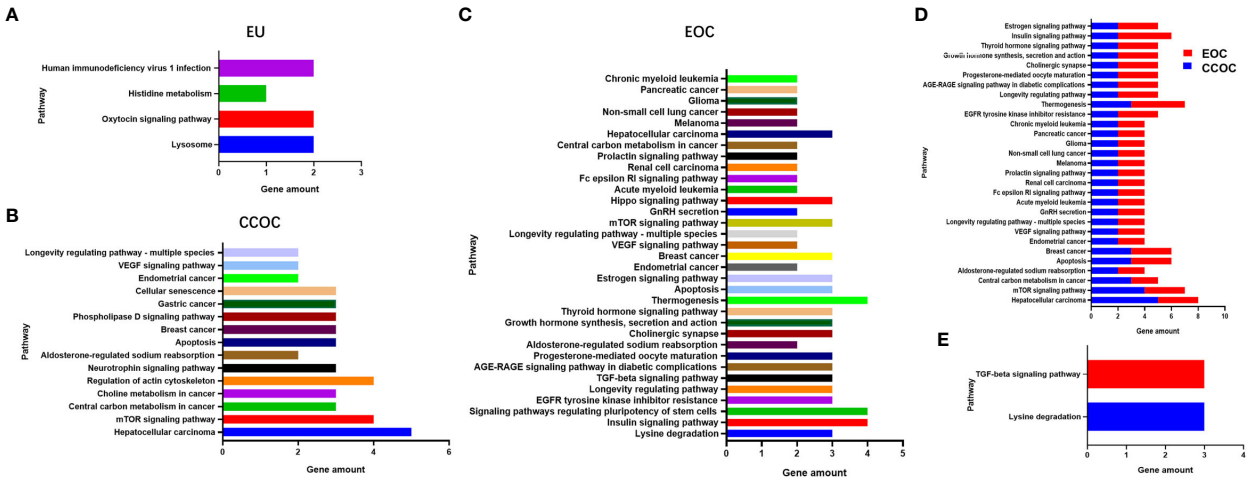

FIGURE 4
Pathway enrichment analysis revealed different gene pathways in eutopic endometrium **(A)**, clear cell ovarian cancer **(B)** and endometrioid ovarian cancer **(C)**. Twenty-nine gene pathways were identified enriched in both clear cell ovarian cancer and endometrioid ovarian cancer **(D)**. The TGF-β signaling pathway and lysine degradation pathway were uniquely enriched in endometrioid ovarian cancer and clear cell ovarian cancer, respectively **(E)**.

correlating with endometriosis. At present, two main mechanisms are proposed to explain the dichotomy aetiology. In an interesting paper, Kajihara et al. observed positive expression of HNF-1β during the late secretory or menstrual phase in EU, endometrioma and endometriosis-associated CCOC, which was absent in endometriosis-associated EOC and ovarian cortical inclusion cysts. Therefore, they proposed the theory that endometriosis-associated clear cell carcinoma originates from the HNF-1β-positive eutopic endometrium retrogradely transported via menstruation. However, endometrioid histology involves transformation from inclusion cysts to a Müllerian epithelium as a precursor for endometrioid tumour development (14). In a recent retrospective analysis by Bergamini and coworkers, comparison between endometriosis-associated CCOC and EOC patients revealed distinct clinical characteristics (15). For example, women with endometriosis-associated endometrioid ovarian cancer were significantly younger at diagnosis and exhibited lower disease stages, a lower prevalence of high-grade tumours, and a higher probability of simultaneous endometrial carcinoma in the uterus. Accordingly, they hypothesized that the original precursor of endometriosis-associated CCOC might be located in the endometrium, where an already mutated endometrial cell may lead to development of ovarian endometriosis via retrograde menstruation and that carcinogenesis for EOC might occur within uterine endometrial cancer.

In our study, distinct IHC staining biomarkers were indeed observed in the bilateral ovaries. ER and PR were strongly positively expressed in the EOC in the left ovary but absent in the CCOC in the right ovary. Conversely, HNF-1β was strongly positively expressed in the CCOC but absent in the EOC. Interestingly, HNF-1β also showed positive expression in the atypical endometriotic lesions and endometriotic lesions of both ovaries. To explore the key aetiological factors, we also sequenced the secretory endometrium tissue curettage from the uterus. Our gene sequencing results revealed different known cancer-associated mutations (CAMs) among the EU, CCOC, and EOC, with the number of CAMs progressively increasing. The overlapping genes between CCOC and EOC included ARIDIA, KRAS, PIK3CA, CCDC137, KHDRBS1, PCDHB12, SLC28A3 and ING1, most of which have been reported in previous research.

In addition, we noted that ING1 was the only gene shared across all three samples. The ING gene belongs to the tumour-suppressor gene family and has regulatory functions in cell proliferation, apoptosis and cell senescence (16). This family includes five members (ING1-5) that enhance p53 activity by inducing acetylation or increasing its stability (17). ING1 has been demonstrated to be a tumour suppressor in a variety of human cancers, including lung cancer (18), colorectal cancer (19), and prostate cancer (20). To date, no studies have established a direct correlation between ING1 and the incidence of ovarian cancer. Given that we found this gene for the first time in three related tissue species, it deserves subsequent deeper exploration.

In the endometrium, each menstrual cycle is analogous to classic tissue injury and repair, which includes inflammation and its resolution, angiogenesis, tissue formation and remodelling or re-epithelialization (21). Similar to EU, the ectopic endometrium (endometriotic lesion) sheds glandular epithelial cells during menstruation, but to a considerably lesser degree in endometriotic stromal cells. Based on their findings, Suda et al. proposed that endometrial cells already harbouring CAMs, which confer selective advantages, may retrograde and find ectopic sites conducive for their growth, thereby fostering endometriosis development (22). Based on the above evidence and our results, we hypothesize a monophyletic histogenesis in the aetiology of CCOC and EOC histotypes: endometrial glands possessing preexisting CAMs, potentially with selective advantages, can easily implant onto ectopic sites and undergo clonal expansion. The implanted ectopic endometrium faces a harsher microenvironment characterized by hyperoestrogenism, inflammation, and oxidative stress-individually and collectively mutagenic factors that generate a hotbed for DNA damage and subsequent CAMs. Hence, endometriotic lesions accumulate different and sufficient CAMs, ultimately driving the process of malignant transformation.

4 Conclusion

Endometriosis-associated ovarian carcinogenesis is a multistep process. Research is needed to advance understanding of the disease aetiology, identify risk factors, and develop early detection methods and effective targeted therapies. Here, we report the simultaneous presence of two different histotypes of ovarian endometriosis-associated cancer in bilateral ovaries. Based on our genetic analysis, we hypothesize that endometriosis-associated CCOC and EOC may have a monoclonal origin, providing insights into understanding the different biological mechanisms underlying carcinogenesis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Department of Obstetrics and Gynecology, the Seventh Medical Centre of Chinese PLA General Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LZ: Data curation, Investigation, Writing – original draft. YL: Investigation, Writing – original draft. FL: Investigation,

Writing – original draft. XJ: Formal Analysis, Writing – original draft. JX: Formal Analysis, Writing – original draft. SZ: Investigation, Writing – original draft. YM: Project administration, Writing – original draft. AL: Investigation, Methodology, Writing – review & editing. WY: Supervision, Writing – review & editing, Project administration.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1280529/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A) Pearson correlation coefficients for signature features between eutopic endometrium (EU) and clear cell ovarian cancer (CCOC) stood at 0.9364 (P<0.0001), between EU and endometrioid ovarian cancer (EOC) at 0.816 (P<0.0001), between EOC and CCOC at 0.8852 (P<0.0001) (Supplementary Figure S1). **(B)** Different loci in EU, CCOC and EOC, some of them (like ZNF469, CORO2A, and ZNF185) are not oncogenes. **(C)** The shared genes in EU, CCOC and EOC.

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EDITED BY
Massimo Broggini,
Mario Negri Institute for Pharmacological Research (IRCCS), Italy

REVIEWED BY
Robb Hollis,
University of Edinburgh, United Kingdom
Kathleen Pishas,
Peter MacCallum Cancer Centre, Australia

*CORRESPONDENCE
Eva J. Gordon
egordon@privatehealth.com
Carla Grandori
cgrandori@senginemedicine.com

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Case report: *ex vivo* tumor organoid drug testing identifies therapeutic options for stage IV ovarian carcinoma

Marwah Al-Aloosi[1], Amanda M. Prechtl[1], Payel Chatterjee[1], Brady Bernard[1,2], Christopher J. Kemp[3], Rachele Rosati[1], Robert L. Diaz[1], Lauren R. Appleyard[1], Shalini Pereira[1], Alex Rajewski[1], Amber McDonald[4], Eva J. Gordon[4]* and Carla Grandori[1]*

[1]SEngine Precision Medicine, Seattle, WA, United States, [2]Earle A. Chiles Research Institute, Providence Cancer Institute, Portland, OR, United States, [3]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, WA, United States, [4]Private Health Management, Los Angeles, CA, United States

Patients presenting with stage 4 ovarian carcinoma, including low-grade serous disease, have a poor prognosis. Although platinum-based therapies can offer some response, these therapies are associated with many side effects, and treatment resistance often develops. Toxic side effects along with disease progression render patients unable to receive additional lines of treatment and limit their options to hospice or palliative care. In this case report, we describe a patient with an unusual case of metastatic low-grade serous ovarian cancer with some features of high-grade disease who had received four previous lines of treatment and was suffering from atelectasis, pulmonary embolism, and hydronephrosis. A CLIA-certified drug sensitivity assay of an organoid culture derived from the patient's tumor (PARIS® test) identified several therapeutic options, including the combination of fulvestrant with everolimus. On this treatment regimen, the patient experienced 7 months of stable disease and survived nearly 11 months before succumbing to her disease. This case emphasizes the clinical utility of *ex vivo* drug testing as a new functional precision medicine approach to identify, in real-time, personalized treatment options for patients, especially those who are not benefiting from standard of care treatments.

KEYWORDS

low grade serous ovarian cancer, functional precision medicine, tumor organoids, medium-throughput drug screen, fulvestrant, everolimus

1 Introduction

Low-grade serous ovarian carcinoma (LGSOC) comprises less than 5% of ovarian cancers (1). LGSOC usually presents in young women and has unique morphological and molecular features that distinguish it from high-grade tumors (2). Patients who have LGSOC with cancer cells that are limited to the ovary have an excellent prognosis with surgery alone, but most LGSOCs have spread beyond the ovaries and have a poor prognosis (3). Standard of care management for ovarian cancers includes cytoreductive surgery, and for stage 1C and stages 2–4, the addition of platinum-based chemotherapy is indicated (2, 4). However, LGSOC patients generally have poor responses to platinum-based chemotherapies in the neoadjuvant, adjuvant, and relapsed settings, resulting in an unmet need for additional systemic treatment options (5, 6).

Treatments that target hormone receptors are an attractive option, as studies have shown that ~70% of LGSOCs are positive for estrogen receptor (ER) and ~30% are positive for progesterone receptor (PR), defined as weak (1% to 50% of tumor cell nuclei) or strong (≥50%) (7). Hormonal therapy is available for LGSOC as adjuvant, maintenance, and salvage therapy, and data suggest that patients treated with maintenance hormone therapy may have similar outcomes to those treated with maintenance chemotherapy (8). However, despite promising outcomes achieved with these therapies, rates of overall response and progression-free survival (PFS) indicate that they may not work for all patients and may fall short in terms of long-term disease management (9, 10). A variety of additional therapeutic combinations have been proposed to treat LGSOC, including the addition of CDK4/6 inhibitors to hormone therapy regimens like letrozole or fulvestrant, which have improved overall survival rates in patients with metastatic ER-positive breast cancer (11–13).

Patient-derived tumor organoids (PDTOs) have recently been developed to enable *ex vivo* functional testing, including drug screening, of a patient's tumor cells (14–16). PDTOs retain biologic features and genetic alterations from the originating tumor but also share the entire germline profile as well as any treatment history (17). Because these variables can affect drug sensitivity and response to therapy, controlling for them could enhance the predictive accuracy of patient-derived models relative to other cancer models that are genetically unrelated to any given patient. The PARIS® assay is a CLIA-certified, medium-throughput drug sensitivity assay that employs organoids cultured directly from solid tumors to test drugs or drug combinations in real-time for their potential efficacy (15–19). A report suggesting possible treatment options is then provided to the oncologist in a clinically relevant time frame.

In this case report, we describe a patient with LGSOC whose disease progressed despite surgical intervention and several lines of chemo- and hormonal therapies and who was unable to tolerate further chemotherapy. Tumor organoids were derived from a core biopsy of an abdominal metastatic lesion that was superficial on the right flank and easily accessible and subjected to both single-agent and combination drug sensitivity testing (17, 18). The PARIS® test results identified several additional treatment options including ceritinib, lapatinib, and neratinib, as well as drug combinations, including the ER antagonist, fulvestrant, plus the mTOR inhibitor, everolimus. This combination has shown efficacy in treating hormone therapy-resistant, hormone receptor-positive, EGF-receptor-positive, and HER2-negative breast cancer in postmenopausal patients (20), but to our knowledge, it is not widely used to treat ovarian cancer. Based on the PARIS® test results, the patient was treated with fulvestrant and everolimus and experienced reduced/stabilized CA-125 levels and stable disease for 7 months until she succumbed to her disease after 11 months.

2 Case description

2.1 Patient history

A 27-year-old woman, G1P1A0, presented with bloating and abdominal distension for several weeks, along with oligomenorrhea. Imaging studies showed evidence of clinical-stage IIIC ovarian carcinoma. The patient underwent a CT-guided omental biopsy, and pathology revealed metastatic grade 1 ovarian papillary serous carcinoma with high-grade foci. The patient received three cycles of neoadjuvant chemotherapy with taxol and carboplatin, followed by an exploratory laparotomy, radical resection for tumor debulking, total abdominal hysterectomy, bilateral salpingo-oophorectomy, rectosigmoid resection, partial resection of the transverse colon with re-anastomosis, partial ileal resection with re-anastomosis, and descending colostomy in October of 2016 (Figure 1). Her postoperative course was complicated by ileus and by pulmonary embolism, for which the patient received anticoagulation therapy. For adjuvant therapy, the patient switched to carboplatin and liposomal doxorubicin for three cycles and achieved stable disease. In January 2017, the patient started taking the aromatase inhibitor letrozole as maintenance therapy; in March 2018, palbociclib was added to letrozole due to disease progression and the emergence of a right flank mass. This treatment was selected based on the loss of *CDKN2A* noted in genomic profiling of the tumor, discussed below. However, palbociclib was held after two cycles due to grade 3 fatigue. The dose was reduced for the following cycle and terminated after 25 weeks, when the patient was admitted for small bowel obstruction. Six weeks later, the patient started liposomal doxorubicin; however, she received only two cycles due to disease progression that involved recurrent pleural effusion, requiring multiple thoracenteses. Thereafter, the patient suffered from increased flank pain, and imaging studies in February 2019 (about 5 weeks after discontinuing liposomal doxorubicin) showed disease progression and the development of left-sided hydronephrosis.

2.2 Tumor stage, pathology, and genomics

The specific diagnosis for this patient was metastatic papillary serous carcinoma, stage IIIC LGSOC. The tumor exhibited classic low-grade serous morphology with prominent micropapillary

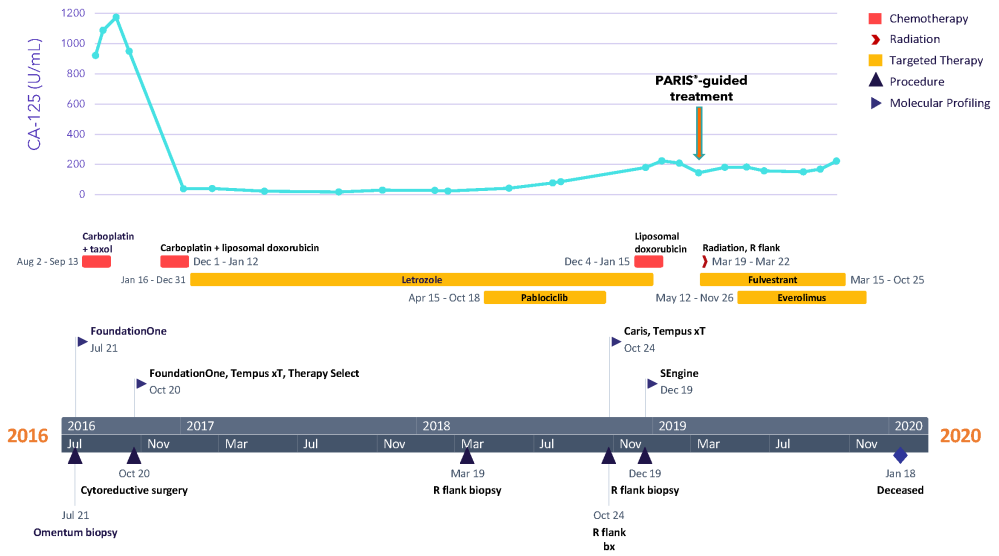

FIGURE 1
Clinical timeline.

features, and nuclear features were >95% low-grade. Foci of more pronounced atypia were noted with some increased mitotic activity, and p53 immunostaining was heterogeneous, consistent with wild-type p53. Additional molecular diagnostics (FoundationOne, December 2016) on a tumor sample from the omentum collected during surgery revealed a *CDKN2A* loss, wild-type TP53, KRAS, NRAS, and BRAF, and a microsatellite stable, mismatch repair proficient, PD-L1-negative tumor with a low mutational burden, indicating that this patient would likely not benefit from immune checkpoint inhibition. No significant germline variants were detected (OvaNext, July 2016), and no somatic mutations in *BRCA1* and *BRCA2* were identified (FoundationOne, December 2016). Further molecular testing (Caris MI Profile) on a right flank tissue sample from October 2018, after 9 months of letrozole, showed that the sample was ER positive, PR negative, and had acquired a somatic pathogenic alteration in the *ESR1* gene (Y537S), suggesting a possible resistance mechanism to letrozole (Figure 2A) (21). RNA expression analysis (Tempus xT) on the same tissue further identified overexpression of *TP53*, *MET*, *PAX8*, and *MUC16* (CA125) and underexpression of *PGR*. Full lists of genes included in molecular profiling tests are included in Supplementary Results.

2.3 Patient-derived tumor organoid-based drug testing

The patient was referred for the PARIS® test after exhausting all other standard of care treatment options. In December 2018, a core biopsy from an abdominal wall metastasis was obtained and shipped to SEngine Precision Medicine (Figures 2B, C). The sample was enriched for tumor cells and expanded as a 3D organoid culture for the drug screening assay; detailed methods for organoid culture have previously been described (17, 18). The *ESR1* mutation present in the biopsy tissue was confirmed in the organoids by targeted sequencing (Supplementary Materials). The screening assay consisted of a custom drug panel consisting of 12 single agents (cabozantinib, ceritinib, cobimetinib, crizotinib, enzalutamide, everolimus, fulvestrant, lapatinib, neratinib, palbociclib, ribociclib, and sorafenib) and five drug combinations informed by drugs that indicated a response in preliminary testing. Each drug was selected based on the genetic landscape of LGSOC, the genetic profile of this patient's tumor, and the physician's request. The drug combination study employed fulvestrant as a sensitizer agent, used at low concentrations, as a measure of the organoids for this patient (IC30). Organoids were then exposed to single drugs at six different concentrations, with or without the addition of fulvestrant. The assay was performed in 384-well plates, and the read-out was Cell Titer Glo measuring ATP concentration in the media as an indicator of cell viability, as previously reported. Drug combination methods were as described (17) and validated in animal PDX models.

The results of the drug screens were read after 6 days of incubation (Figure 2D; Table 1; Supplementary Table S2). The drugs were ranked from the most effective (SPM 15) to the least effective (SPM 1) with a proprietary metric, with scores of 15 to 9 considered active drugs. Exceptional and good single-agent drug responses were observed to ceritinib (SPM 14), lapatinib (SPM 13), fulvestrant (SPM 12), and neratinib (SPM 12), with low responses to everolimus (SPM 10), crizotinib (SPM 9), and enzalutamide (SPM 9). Cobimetinib (SPM 6) indicated a lack of response, while results for sorafenib and palbociclib were not evaluable. Given this patient's pathogenic mutation in the estrogen receptor gene *ESR1*, which may cause resistance to aromatase inhibitors (22), the selective estrogen receptor degrader (SERD) fulvestrant (Faslodex) was of particular interest and was used as the sensitizing agent for a subsequent five-drug combination screen consisting of fulvestrant plus either neratinib, lapatinib, palbociclib, ribociclib, or everolimus (Table 2).

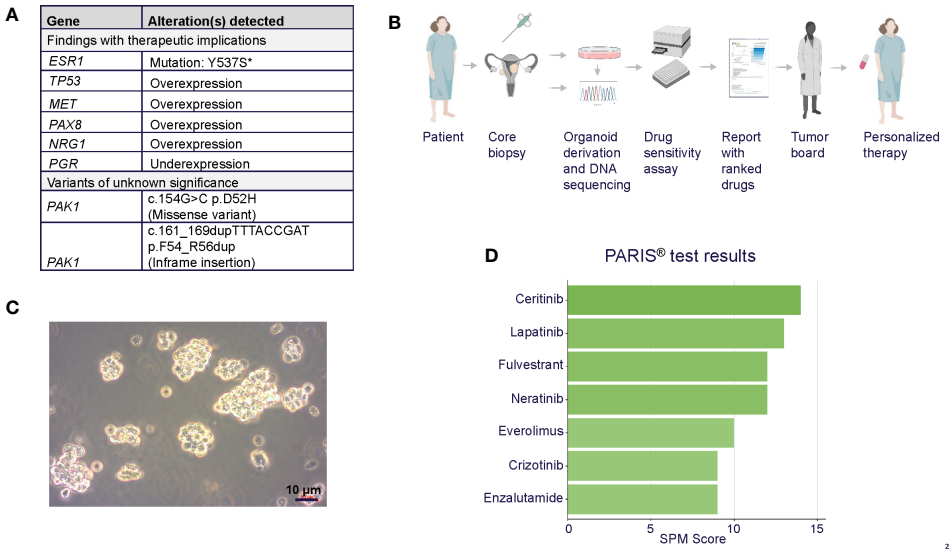

FIGURE 2
(A) Summary of tumor molecular profiling findings with therapeutic implications. **ESR1* mutation was confirmed in organoids. **(B)** PARIS® drug sensitivity assay workflow, including organoid generation from core biopsy, characterization, and report generation. Figure generated using Biorender. **(C)** Brightfield photomicrograph of the patient's cultured tumor organoids. Scale bar = 10 µm. **(D)** Table of top-scoring drugs in green from the PARIS® assay.

Despite the low response to everolimus in the single agent screen, this drug was included in the combination testing because it is approved for combination treatment with an ER antagonist for breast cancer and would thus be easier for the patient to obtain. In addition, our prior research found that the combination of fulvestrant plus everolimus was synergistic in a breast cancer patient. Combinations of fulvestrant with neratinib, lapatinib, ribociclib, and everolimus all demonstrated some degree of additive effect, with the best response seen with the HER2 inhibitors lapatinib and neratinib and the mTOR inhibitor everolimus. The combination of fulvestrant and palbociclib did not display an additive response. The evaluation of potential additive or enhanced effects of the drug combination was carried out in consideration of the sensitivity in relation to the overall sensitivity of the combination (single agent AUC) as well as the absolute difference in AUC (Δ AUC) with and without fulvestrant, as shown in Table 1. The results indicated that none of the drug combinations were enhanced, but instead, there were additive effects (less than ~10% increased sensitivity when the agents were combined, see ΔAUC column). A CLIA-certified test report describing these results was sent to the treating oncologist 43 days after the sample was received. Additional details about this test can be found in the Supplementary Materials and in previous preclinical research papers (15, 16, 23–25).

2.4 Post-PARIS® test

Based on genomic profiling and PARIS® test findings, along with consultation with the patient's oncologist and additional LGSOC experts, treatment with fulvestrant (500 mg on days 1, 15, 29, and subsequently every 28 days) was initiated in March of 2019, followed by palliative radiotherapy for the right flank mass (30 Gy in 10 sessions) the next week and placement of a nephroureteral stent in April 2019. Based on the patient's tumor organoid drug combination screen, everolimus (10mg, daily) was added to fulvestrant in May 2019. It is

TABLE 1 Single-agent PARIS® test drug screen results.

Drug	Target	C_{max}	IC_{50}	SPM
Ceritinib	ALK, IGF-1R, ROS1	1.43E−06	1.10E−06	14
Lapatinib	EGFR, HER2	4.04E−06	1.30E−06	13
Fulvestrant	Selective estrogen receptor degrader	2.08E−08	NA	12
Neratinib	EGFR, HER1, HER2, HER4	2.14E−07	8.50E−08	12
Everolimus	mTORC1	3.86E−08	7.60E−06	10
Crizotinib	ALK, ROS1, MET	9.48E−07	5.60E−06	9
Enzalutamide	Androgen receptor antagonist	3.57E−05	1.00E−05	9

A list of the drugs that indicated sensitivity according to the PARIS® test was ranked using the SPM score as single drugs. Drug name, gene product target, and maximal serum observed dose (C_{max}) as obtained from the literature; all drugs included are FDA-approved. SPM, SEngine Precision Medicine.

TABLE 2 Combination agent PARIS® test results.

Drug	Target	C_{max}	IC_{50}	Single agent AUC	Fulvestrant combination AUC	Absolute difference AUC
Everolimus	mTORC1	3.90E−08	7.60E−06	0.63	0.53	0.11
Lapatinib	EGFR, HER2	4.00E−06	1.30E−06	0.55	0.44	0.10
Ribociclib	CDK4, CDK6	7.10E−06	1.30E−06	0.6	0.51	0.09
Neratinib	EGFR, HER1, HER2, HER4	2.14E−07	8.50E−08	0.43	0.37	0.06

PARIS® testing using a combination of fulvestrant at 1 µM, the pretested IC_{30} concentration for this PDTO, along with either everolimus, lapatinib, ribociclib, or neratinib. The combinations are ranked by the largest differential area under the curve (AUC) obtained using six concentrations of each drug (10 µM, 3.16 µM, 1 µM, 316 nM, 100 nM, and 31.6 nM). Only the drugs that had enhanced activity with fulvestrant are shown.

noted that the patient received approval from her insurance company for this treatment. However, the malignant pleural effusion resulted in complete right lobe atelectasis, with scans in October showing disease progression. Fulvestrant was discontinued at the end of the month, and everolimus was discontinued a month later, when the patient's condition deteriorated further. The patient was given antibiotics and hospitalized 1 month later due to severe shortness of breath. Although a decision was made to start the combination of carboplatin, gemcitabine, and bevacizumab, the treatment was not initiated because the patient passed away 1 month later, at 30 years of age. Overall, since the start of fulvestrant and subsequent addition of everolimus 2 months later, the patient's CA-125 level stabilized (Figure 1), and she experienced disease control for 7 months and an overall survival of 11 months.

3 Discussion

Ovarian cancers are the second most common cancer of the female reproductive system and are associated with the highest risk of cancer-related death, with most women presenting with advanced-stage disease (26, 27). LGSOC tumors respond poorly to platinum-based chemotherapies (28), making them challenging to treat when there is residual disease following cytoreductive surgery (3, 8, 29). Thus, there is an unmet need to explore targeted treatment options for this subset of patients in the era of personalized medicine.

In this case, a young female patient with LGSOC who had disease progression after surgery and multiple lines of therapy, including neoadjuvant and adjuvant chemotherapies, adjuvant aromatase inhibitors, and CDK4/6 inhibitor treatment, sought further options to help treat her disease. Comprehensive molecular profiling of this patient's tumor provided information about several other important biomarkers. The patient was not a candidate for immune checkpoint inhibitors (ICI), based on the PD-L1-negative, microsatellite-stable, and mismatch repair-proficient status of the tumor, along with the loss of the cell-cycle regulatory gene *CDKN2A*. This tumor suppressor gene, which is commonly altered in many human cancers, has also been shown to be a marker for poor response to ICI (21). Notably, however, a somatic mutation in the *ESR1* gene was identified, which is significant because breast tumors with *ESR1* mutations have been shown to be resistant to letrozole both alone and in combination with other agents, including the PI3Kα inhibitor alpelisib (21, 30).

Tumor tissue was submitted for PARIS® testing to identify personalized treatment options with the potential to extend the life of this young patient. The results of the PARIS® test on tumor organoids derived from the patient's metastatic tissue identified multiple candidate single agent and combination treatment options, including fulvestrant plus everolimus. Studies in breast and gynecological cancers have shown promise for each of these agents in ER-positive cancers. For example, *ESR1* mutations do not result in resistance to fulvestrant in patients with metastatic breast cancer (22) as they do with letrozole. In fact, breast tumors harboring *ESR1* mutations have demonstrated greater sensitivity to selective estrogen receptor modulators such as tamoxifen and fulvestrant and to the combination of these endocrine therapies with CDK4/6, PI3K, or mTORC1 inhibitors (31).

It has been established that the PI3K-AKT-mTORC1 pathway plays an important role in endocrine resistance through ligand-independent activation of ER (31) and that one possible adaptive mechanism of resistance to PI3K inhibitors is stimulation of ER activity (32). Therefore, targeting PI3K and mTORC1 by combining their inhibitors with endocrine therapies can be of additive efficacy in endocrine-resistant and *ESR1*-mutated breast cancer (31). Clinical evidence has shown that the combination of fulvestrant and the mTOR inhibitor everolimus extended PFS in patients with breast cancer who became resistant to aromatase inhibitor therapy (20, 33). In the phase II PrE0102 trial, patients treated with everolimus plus fulvestrant had a PFS of 10.3 months, compared with 5.1 months in patients treated with placebo plus fulvestrant. In the phase II MANTA trial, PFS was extended for patients treated with fulvestrant plus everolimus (12.3 months) compared with fulvestrant alone (5.4 months) or fulvestrant plus the mTOR inhibitor vistusertib (7.6 months) (33). The addition of everolimus to letrozole in recurrent gynecologic cancers has also had promising results in heavily pretreated patients with ER-positive cancers (34, 35). It is noteworthy that novel agents are being explored in hormone-resistant breast cancers that harbor *ESR1* mutations, including giredestrant, proxalutamide, and enobosarm (36).

In addition to the combination of fulvestrant with everolimus, the PARIS® test identified several other targeted drugs, including enzalutamide, an oral androgen receptor inhibitor (37), as well as lapatinib and neratinib, which target members of the EGFR family.

Based on the results of the PARIS® test, the patient started fulvestrant in March 2019, and 2 months later, everolimus was added. Her disease remained stable until late October 2019; she ultimately succumbed to her cancer in January 2020. With the treatments

identified by the PARIS® test, the patient was able to experience 7 months of stable disease with manageable toxicities. This additional time of stable disease was notable given that the patient harbored many risk factors that are associated with poor prognosis, including being ≤ 35 years of age, having residual disease at the end of primary therapy, and lacking an alteration in the MAPK pathway (38–40).

A limitation of this approach is that challenges are often encountered in obtaining drugs that show effectiveness for individual patients but that are not approved for their specific cancer type. This issue has emerged alongside various precision oncology approaches to cancer treatment and must be urgently addressed by regulatory organizations and payers to enable patients to get the most effective treatments possible.

This case report highlights the successful application of the PARIS® test, a tumor organoid-based drug sensitivity assay, to identify effective targeted therapies for a patient with LGSOC who had progressed on multiple chemo- and targeted therapies. Together with other recent reports showing exceptional responses to organoid-guided therapies in patients who have failed standard of care (15, 19), this demonstrates that *ex vivo* functional testing is a novel precision medicine tool with clinical utility, especially for cancer types that have low responses to standard treatments, such as LGSOC. Given the rarity of this type of disease, this personalized *ex vivo* testing provides an avenue to identify treatments outside of conventional clinical trials. Using organoid-based drug testing to identify targeted therapies could dramatically influence a patient's outcome and, if employed earlier in the disease course, could preserve the overall patient wellness and quality of life while enhancing their chances for complementary treatment modalities such as immune-oncology interventions toward potential cures (15, 19, 24).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by and managed by Advarra IRB (Pro00036350) under SEngine IRB protocol (SE_IRB_001). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because SEngine provides a CLIA-certified test to cancer patients following physicians ordering request and also gives the option to patients to sign a consent to be included in an ongoing study enabling collection, aggregation and analysis of molecular and clinical data as they relate to the test. Written informed consent was not obtained from the individual for the publication of any potentially identifiable images or data included in this article because the patient passed away before manuscript writing was initiated. However, this publication does not contain identifiable information related to this patient. SEngine IRB protocol states in the Inclusion/Exclusion criteria the following: "Deceased patients that have passed away after the biological sample has been sent for a PARIS test and before they were able to sign consent, will automatically be included in the research study.

Author contributions

MA-A: Methodology, Writing – review & editing. AP: Methodology, Writing – review & editing. PC: Formal analysis, Methodology, Writing – review & editing. BB: Methodology, Writing – review & editing, Formal analysis. CK: Writing – original draft. RR: Methodology, Writing – review & editing. RD: Formal analysis, Methodology, Writing – review & editing. LA: Methodology, Writing – review & editing. SP: Formal analysis, Writing – review & editing. AR: Formal analysis, Writing – review and editing. AM: Writing – review & editing, Supervision. EG: Supervision, Writing – original draft. CG: Supervision, Writing – original draft, Conceptualization.

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Conflict of interest

Authors MA-A, AP, PC, RR, RD, LA, SP, BB, AR, and CG are or were employed by SEngine Precision Medicine and received stock options from the company. Authors AM and EG are employed by Private Health Management. Author CK is a founder and has ownership in SEngine Precision Medicine.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1267650/full#supplementary-material

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EDITED BY
Mignon Van Gent,
Amsterdam University Medical Center,
Netherlands

REVIEWED BY
Stergios Boussios,
Canterbury Christ Church University,
United Kingdom
Nektarios Koufopoulos,
University General Hospital Attikon, Greece

*CORRESPONDENCE
Lei Li
beileili@163.com
Manman Nai
13523046352@163.com

†These authors have contributed equally to this work and share last authorship

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Subsequent ovarian yolk sac tumor after operation of ovarian mature teratoma: a case report and review of the literature

Shuqing Li[1], Juan Peng[1,2], Yajun Zhang[1], Dongxia Liu[1], Lei Li[1*†] and Manman Nai[1*†]

[1]The Department of Obstetrics and Gynecology, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [2]Zhengzhou Key Laboratory of Endometrial Disease Prevention and Treatment, Zhengzhou Science and Technology Bureau, Zhengzhou, China

Ovarian mature teratoma represents a benign ovarian tumor, while ovarian yolk sac tumor (YST, endodermal sinus tumor) is a rare malignant tumor predominantly affecting young women, often associated with a grim prognosis post-metastasis. Both ovarian mature teratoma and ovarian YST are germ cell tumors. There are few studies on the correlation between ovarian YST and mature teratoma. Recurrence or malignant transformation may occur following the surgical intervention for ovarian mature teratoma. However, the occurrence of YST subsequent to such procedures is notably rare. In this investigation, we reported a case involving a 24-year-old unmarried woman with both mature ovarian teratoma and YST within a brief 1-year interval. Regular reexamination protocols facilitated the early-stage detection of YST. The patient underwent surgical treatment, chemotherapy, and measures to preserve ovarian function, resulting in a favorable prognosis. Our primary purpose is to distill clinical insights from the diagnostic and therapeutic journey of this patient. Our purpose is to enhance medical professionals' awareness that YST may be secondary to mature teratoma. Additionally, we underscore the critical importance of routine postoperative surveillance for ovarian mature teratoma, emphasizing its pivotal role in early malignant tumor detection—a factor paramount to the prognosis of patients.

KEYWORDS

ovary, teratoma, yolk sac tumor, case report, literature review

Introduction

Approximately 90% of ovarian cancers manifest as epithelial cell types, presenting a diverse array of histological variations. Conversely, the remaining 10% comprise non-epithelial ovarian cancers, which include a majority of germ cell tumors, sex cord-stromal tumors, and a subset of exceedingly rare entities such as small cell carcinomas. Ovary germ

cell tumors are the most common ovarian neoplasms in women until 30 years of age, originating from germ cells. With the exception of certain tissue types, such as mature teratoma, the majority of ovarian germ cell tumors are malignant and are often diagnosed at the early stage (60%–70%) (1). Ovarian mature teratomas are the most common ovarian germ cell tumor (2), accounting for 10%–20% of ovarian tumors (3). Typically, these teratomas measure 5–10 cm, with only approximately 9% exceeding 15 cm (2). Malignant ovarian germ cell tumors (MOGCTs) are thought to originate from primordial germ cells, featuring inherited or somatic acquired alterations (4). MOGCTs accounts for 5% of ovarian germ cell tumors (5), principally in the teenage years. The pathogenesis of malignant ovarian germ cell tumors remains elusive, potentially related to genetic or environmental factors. Surgery or surgery combined with chemoradiotherapy can significantly improve the prognosis (6). Among MOGCTs, YST accounts for 14%–20% of ovarian malignant germ cell tumors (7), and the incidence is approximately 0.048/100,000 (8). YST is highly malignant and easy to early metastasize and relapse. YSTs often present challenges in treatment due to chemotherapy resistance upon recurrence. We have conducted extensive literature reviews. There are many studies on the secondary occurrence of YST in immature teratoma (9–12).

However, the occurrence of YST subsequent to mature teratoma surgery is rare. In the literature that we searched, there were no documented instances of secondary YST after ovarian mature teratoma surgery. We report a case of giant ovarian YST only 1 year after surgery for mature ovarian teratoma, accompanied by a comprehensive review of pertinent literature. The diagnosis and treatment of this patient are meticulously summarized and analyzed. The patient underwent both surgery and chemotherapy, with a favorable prognosis attributed to the early detection of the YST.

Case presentation

The patient, a 24-year-old unmarried woman with no sexual history, sought medical attention at our hospital in August 2019 following the discovery of a pelvic cyst during a physical examination. 3D transrectal ultrasonography showed a mixed echo measuring 128 mm × 111 mm × 109 mm at the right anterior quadrant of the uterus. Before the operation, pelvic magnetic resonance imaging (MRI) indicated a large solid cystic mixed-signal mass in the pelvis, exhibiting well-defined boundaries and measuring a maximum cross-section of 158.56 mm × 83.74 mm × 112.54 mm. The mass exhibited closely proximity to the right ovary. The right ovarian mature teratoma was removed by conducting a single-hole laparoscopic operation at our hospital. During the operation, no significant abnormalities were found in the left ovary and bilateral fallopian tubes. The postoperative pathology was mature cystic teratoma. The patient recovered well from the operation.

In October 2020, a routine physical examination revealed a left adnexa cyst in the patient with a maximum diameter of 3 cm. Then, the color ultrasound at our hospital identified an echoless area measuring approximately 35 mm × 14 mm in the left accessory region. By January 2021, a follow-up color ultrasound of our hospital exhibited a cystic mass with a range of 104 mm × 105 mm × 68mm in the left ovary, with a high echo approximately 41 mm × 33 mm in the mass. The level of α-fetoprotein (AFP) was 72.20 IU/mL. A pelvic MRI showed a vast solid cystic mass in the pelvis, featuring clumpy solid components within the capsule. The mass measured approximately 112 mm × 78 mm × 128 mm and exerted compression on the adjacent uterus, bowel duct, and the right oviduct and ovary, encapsulated in its entirety.

Because the patient had not yet married and had not given birth, both the patient and her parents expressed a strong desire to preserve her fertility function as much as possible. Considering the potential malignancy of the ovarian tumor, we discussed fertility preservation for the patient and made a plan with the Department of Pathology and the Reproductive Center before the operation. Ultrasound showed that the dominant follicle was in the right ovary, with an average number of follicles observed in both ovaries. The patient's Anti-Müllerian Hormone (AMH) level measured 34.04 pmol/L, indicating a robust reserve function in both ovaries.

We performed an exploratory laparotomy, during which the left ovary exhibited a significant enlargement, measuring approximately 12 cm × 12 cm × 10 cm, with a smooth surface. The appearance of bilateral fallopian tubes and the right ovary was normal. There was approximately 50 ml of dark red bloody effusion in the pelvic cavity, with no abnormalities noted in the peritoneal of the pelvic abdominal cavity. A thorough examination of various surfaces, including the liver, spleen, ligaments, diaphragm, large omentum, and intestinal tubes, revealed no apparent abnormalities. A total of approximately 1,000 mL of dark red translucent liquid was aspirated from the left ovarian cyst, and the left ovarian cysts were completely excised. The yellow irregular meat-like tissue, approximately 4 cm × 4 cm, was identified in the ovarian cyst cavity. The intraoperative pathological analysis confirmed the presence of an ovarian YST. After consulting with the patient's parent, the left fallopian tube ovary and large omentum were removed, and a multi-point biopsy on the pelvic peritoneum was conducted. The right ovarian cortex, approximately 1 cm × 1 cm, was excised for cryopreservation of the ovarian tissue. Simultaneously, the reproductive physician cryopreserved the oocytes from the left ovary.

The postoperative pathological examination was a yolk cystic tumor. The postoperative diagnosis was a stage IC left ovarian yolk sac tumor. After surgery, she underwent chemotherapy with a combination of bleomycin, etoposide, and cisplatin for four cycles. Gonadotropin-releasing hormone agonist (GnRH-a) was administered to protect her ovarian function throughout chemotherapy with a total of four administrations, spaced at 28-day intervals. AFP was normal after the first cycle of chemotherapy. She resumed menstruating more than 3 months after the last

chemotherapy treatment. There has been no recurrence of the disease for 33 months follow-up to now.

Discussion

The occurrence of ovarian YST after the operation of ovarian mature teratoma is exceptionally rare. In this case, the patient sequentially developed ovarian mature teratoma and ovarian YST within only a 1-year interval. It has been documented that some non-ovarian YSTs are secondary to teratoma. We conducted extensive literature searches to gather clinical characteristics, summarizing them in Table 1. Yoshida's study revealed that the rate of YST developing after sacrococcygeal teratoma in children was 5.4%, while there was no statistically significant difference in the incidence of secondary to mature teratoma or immature teratoma (5.2% vs. 6.4%) (14). The possible mechanisms of recurrent YST after the surgery of mature teratoma are outlined below. First, yolk sac tumor is considered as malignant transformation of teratoma (18, 19), and mature teratoma is malignant prelesion (16). The possibility of subsequent malignant development exists even for mature teratoma (17). Second, YST lesions may be microscopic and often cannot be positive for AFP, so they are easily ignored (20). Some researchers believe the YST develops from undetected small yolk sac lesions in the original teratoma (18, 21). Both views are considered to be forms of teratoma recurrence, regardless of the presence or absence of YST lesions in the primary teratoma. YST has a variety of histological patterns, and it is difficult to identify specific subtypes. Therefore, when the pathological specimen is a mature teratoma, it is necessary to thoroughly sample the tumor and carefully examine the pathological section to identify the malignant tumor components (22, 23). Third, Yoshida et al. hold a different perspective, suggesting that teratoma and secondary YST are metachronous multifocal germ cell tumors, which are the presence of multiple *de novo* tumors arising in different sites after long intervals, rather than the transformation of residual teratoma (14). AFP level plays a crucial role in the early diagnosis of YST and monitoring treatment effect (24). Even in postmenopausal patients with ovarian tumors, AFP is recommended to be tested for early detection of ovarian malignant germ cell tumors (25). It is recommended that patients with sacrococcygeal teratoma should be tested for AFP every 3 months for 3 years after surgery to facilitate early detection of YST (14). Activation and malignant transformation of mature teratoma may occur after 7 years (15).

TABLE 1 Clinical features of reported cases of secondary YST after mature teratoma.

Author (year)	Age, Sex	Symptom	Initial tumor location, and diagnosis	Secondary tumor (YST) location	Adjuvant therapy	Prognosis	Time interval between initial and secondary tumor
Rahadiani N (13) (2019)	2-day-old infant, female	dyspnea	buccal mucosa, mature teratoma	around the site of previous surgery scar	no	/	16 months
Yoshida M (14) (2013)	mean age: 7.1 months. female and male	/	sacrococcygeal, mature teratoma(9 cases), immature teratoma (4 cases)	sacrococcygeal	platinum-based chemotherapy, or radiotherapy after chemotherapy	follow-up time: 1–24 years. 11 patients without recurrence; 2 patients died at 2 and 4 years after the diagnosis of YST.	5–30 months
Utsuki S (15) (2007)	9-year-old, male	anorexia, nausea, and vomiting without headache	intracranial, mature teratoma	in the third ventricle	chemotherapy: cisplatin + etoposide, local irradiation and spinal irradiation	died 15 months after the second hospitalization	7 years
Ohno Y (16) (1998)	18-month-old, female	an expanding abdominal girth	right retroperitoneal, a mature cystic teratoma with an area of endodermal sinus tumor differentiation	/	/	no recurrence at 50 months of age	/
Byard R W (17) (1991)	neonatus, female	a large polypoid mass	Nasopharynx, mature teratoma	nasopharynx	chemotherapy: doxorubicin hydrochloride (Adriamycin)+ cyclophosphamide + dactimomycin (Actinomycin D) + vincristine and local radiation	recurrence after 16 months, died 18 months after recurrence	3 years

/, not available.

Serum AFP concentration above 100 ng/dL almost always indicates the presence of a YST focus. However, the detection of AFP is not emphasized in the clinical periodic review of ovarian mature teratoma (20). In this case, if AFP was detected at the same time when the left ovarian tumor volume was 3 cm, the yolk sac tumor would possibly be detected earlier. While the existence of ovarian YST alongside the previous ovarian mature teratoma may be a random occurrence, further research is warranted on the correlation between mature ovarian teratoma and YST.

After surgery, mature ovarian teratoma may recur in the ipsilateral or contralateral ovaries. The intermediate and long-term recurrence rate in one study was 4.2%. The risk of recurrence increases if mature ovarian teratoma is bilateral, multiple, above 8 cm in diameter, with bone and central nervous system components (26, 27). Various tissue components of mature teratoma will have a secondary malignant transformation potentiality, a phenomenon more common in postmenopausal women. The malignant transformation rate is approximately 0.17%–2%, with 80% developing into squamous cell carcinoma, carrying a poor prognosis (28). Overexpression of p53, incomplete tumor resection, and tumor grade are risk factors for ovarian teratoma recurrence (29). In this case, the right ovarian mature teratoma was removed in 2019. The tumor was sizable, and the operation was performed using transumbilical single-incision laparoscopy, heightening the risk of ovarian cyst rupture (30). After the rupture of the tumor capsule wall, even a large amount of irrigation for the abdominal cavity could not avoid the residue of the contents, which increased the possibility of secondary malignant tumors (31). For giant teratoma, pathological examination is particularly challenging for the detection of malignant tumor components. Therefore, intraoperative rupture of tumor components should be minimized even for benign ovarian tumors to avoid tumor residue.

YST is highly malignant, prone to metastasize in the early stage, and carries a poor prognosis upon recurrence. Due to the absence of specific diagnostic markers, it is difficult to diagnose before surgery. In this case, the patient underwent regular color ultrasound after the operation of the ovarian mature teratoma. She promptly consulted a doctor when the ovarian cyst was detected so that the yolk sac tumor could be detected at an early stage without metastasis. In this case, ultrasound examination at an interval of 3 months showed that the ovarian tumor increased rapidly, threefold the ovarian tumor's initial volume. According to imaging examination and clinical characteristics, the possibility of malignancy could not be ruled out. For patients with ovarian tumors with rapid growth or large tumor volume in a short period, it may be caused by internal tumor bleeding or tissue necrosis (28), and physicians should be vigilant about whether it is a malignant tumor.

The preferred treatment option is surgery combined with chemotherapy for YST. As we know, high-grade serous ovarian cancer (HGSOC), constituting 75% of epithelial ovarian cancers, is highly chemosensitive, primarily characterized by uniform *TP53* mutants (32). While ovarian YSTs rarely exhibit *TP53* mutation (33), unlike HGSOC, they still demonstrate sensitivity to chemotherapy. Therefore, ovarian YST is sensitive to chemotherapy. Postoperative chemotherapy is recommended for all stages of ovarian YST to improve prognosis (34), and fertility preservation surgery is feasible regardless of stage. The thoroughness of initial surgical treatment and postoperative chemotherapy are independent risk factors for progression-free survival (35). Various factors, such as the malignant tumor itself, ovarian surgery, pelvic surgery, hyperthermic intraperitoneal chemotherapy, and chemoradiotherapy, may lead to a reduction in ovarian reserve function (36, 37). For MOGCTs, the fertility-sparing comprehensive surgical staging includes the excision of the affected unilateral salpingo-oophorectomy, preservation of the uterus and the contralateral ovary, or preservation of one or both normal ovarian tissues and uterus if both ovaries are involved, in addition to biopsy or excision of the omentum, and excision of lymph nodes depending on age and stage. Considering that YST is more common in children and young women, fertility-sparing surgery to preserve and protect fertility is particularly important for patients' quality of life in the future (38, 39). For unilateral malignant germ cell tumors, even in an advanced stage, fertility preservation surgery can be performed (40). The patient was 24 years old and unmarried in the case, and corresponding measures were taken to protect ovarian function before, during, and after surgery.

For women with fertility requirements, multidisciplinary consultation can be conducted before surgery to reduce the misdiagnosis rate and avoid over-treatment. Hormone levels and color ultrasound can determine initial ovarian reserve function. During the operation, immature oocytes can be extracted from the ovary for cryopreservation (41), and ovarian cortex cryopreservation can be performed (42). Frozen ovarian tissues can be used to isolate follicles or for transplantation. Resuscitation transplantation of cryopreserved ovarian tissue can increase autologous hormone levels and the probability of natural pregnancy. However, there may be a potential risk of malignant tumor cells implantation during transplantation, although this risk is low in other malignant tumors except in leukemia patients (43). For post-pubertal patients and patients with delayed chemoradiotherapy, it is feasible to obtain mature oocytes by promoting ovulation after surgery. Immature oocyte cryopreservation can be performed in preadolescent girls and patients with hormone-sensitive tumors (44).

Given the numerous previous studies on the protection of ovarian function by GnRH-a, the patient in this case received GnRH-a to protect ovarian function before chemotherapy. The GnRH-a inhibits ovarian function, prevents ovarian follicle recruitment, and prevents follicle growth and ovulation. The GnRH-a suppresses the secretion of endogenous gonadotropic hormone and follicle-stimulating hormone (FSH) levels and puts the follicle cells in a dormant state (45, 46). Therefore, reducing the sensitivity of follicles to chemotherapy drugs can minimize the destruction of follicles induced by chemotherapy and reduce the accumulation of chemotherapy drugs in ovarian tissue to reduce the damage to the ovary during chemotherapy. However, the role of GnRH-a in protecting ovarian function in patients with malignant tumors remains controversial (47–49). In this case, appropriate measures were taken to protect the patient's fertility before, during, and after the operation. The diagnostic and treatment process serves as a valuable learning experience for clinicians.

Conclusion

The genomic landscape and pathogenesis of ovarian YST remain elusive, posing challenges to study the diagnosis, treatment, and prognosis of the disease. The relationship between the occurrence of YST and ovarian mature teratoma cannot be determined. Because YST may be secondary to teratoma, patients with ovarian tumor after treatment of ovarian teratoma need to be vigilant about the possibility of YST. Attention should be paid to whether laparoscopic surgery increases the residual tumor lesions, especially single-incision laparoscopic surgery. Clinical surgeons are urged to minimize the exposure of tumor contents to the abdominal cavity during mature teratoma operations. Both physicians and patients should prioritize adherence to medical advice and engage in regular postoperative follow-up examinations, even for mature teratoma. These measures are paramount for the timely detection of ovarian malignant tumors, emphasizing the collaborative role of medical professionals and patients in ensuring comprehensive postoperative care.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

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EDITED BY
Mignon Van Gent,
Amsterdam University Medical Center,
Netherlands

REVIEWED BY
Pratibha Shukla,
New York University, United States
Stergios Boussios,
Canterbury Christ Church University,
United Kingdom
Wuliang Wang,
Second Affiliated Hospital of Zhengzhou
University, China

*CORRESPONDENCE
Oluwole Fadare
Oluwole.fadare@gmail.com

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Ovarian steroid cell tumors: what do we know so far?

Christina H. Wei[1] and Oluwole Fadare[2*]

[1]Department of Anatomic Pathology, City of Hope National Medical Center, Duarte, CA, United States, [2]Department of Pathology, University of California, San Diego, La Jolla, CA, United States

Steroid cell tumors (SCT) of the ovary are rare, which has limited advances in the understanding of this enigmatic neoplasm. In this review, we summarize currently known clinicopathologic information on SCT. SCT are frequently hormonally active, leading to elevated serum and/or urine levels of androgenic hormones or their metabolites, and associated symptomatology, including virilization. The reported age at diagnosis is broad and has ranged from as young as 1 year old to 93 years old, although most patients were between ages 20 and 40 years. Most tumors are stage I and unilateral. The tumors are usually well circumscribed with a solid or solid to cystic cut surface. The tumors in one series reportedly ranged in size from 1.2 to 45 cm (average 8.4 cm). MRI is a useful imaging modality, typically showing a well delineated mass with contrast enhancement and lipid content on T2 and T1 weighted images, respectively. Microscopically, SCT display polygonal to epithelioid cells with abundant eosinophilic to vacuolated/clear cytoplasm and display an immunoprofile that is consistent with sex cord-stromal differentiation. Most cases are benign, without any recurrences after primary resection, but a subset – probably less than 20% of cases –are clinically malignant. Pathologic criteria that can specifically predict patient outcomes remain elusive, although features that correlate with adverse outcomes have been proposed based on retrospective studies. The molecular characteristics of SCTs are similarly under characterized, although there is some evidence of an enrichment for hypoxia-signaling gene mutations in SCT. In malignant SCT, the tumors generally show greater global genomic instability, copy number gains in oncogenes, and occasional BAP1 mutation. Future studies involving multi-institutional cohort and unbiased molecular profiling using whole exome/transcriptome sequencing are needed to help advance our molecular understanding of SCTs.

KEYWORDS

ovarian steroid cell tumor, hyperandrogenemia, ovarian neoplasm/diagnosis, sex cord stromal tumor, virilization, ovary

Introduction and historical evolution

In the 5th edition of the World Health Organization (WHO) classification of female genital tumors, steroid cell tumor (SCT) is defined as "an ovarian parenchymal tumor comprised of steroid cells." (1) This simple definition is a reflection of the current understanding of this rare and enigmatic neoplasm. Historically, it has long been recognized that a subset of ovarian tumors that are associated with virilization are exclusively comprised of cells that closely resemble steroid hormone secreting cells, including the adrenocortical cortical cells, Leydig cells, and lutein cells (2). For several decades, different authors applied a variety of terms to these lesions, including androblastoma diffusum, arrhenoblastoma, Leydig cell tumor, adrenal or adrenocortical tumor, adrenal rest tumor, adrenal-like tumor, stromal luteoma, lipoid or lipid cell tumor, virilizing or masculinizing lipoid/lipid cell tumor, ovoblastoma, masculinovoblastoma, sympatheticotropic tumor, hilus cell tumor, and hypernephroma/hypernephroid tumor (2–7). The 1st edition of the WHO classification of ovarian tumors (1973) included Leydig cell tumors and lipoid cell (or lipid cell) tumors as separate entities, with the latter defined as a tumor comprised of one of the aforementioned steroid hormone secreting cells, but which "cannot be identified specifically as any one of the three types." (6) Given that many neoplasms of this class are comprised of tumor cells that contain no significant amounts of intracytoplasmic lipid, the term "lipoid or lipid cell tumor" was not ideal, and ultimately led to its replacement by "Steroid cell tumor", a term that was initially proposed by Dr. Robert E Scully in 1979 (8) as a better descriptor for the group of tumors that included stromal luteoma (9), Leydig cell tumor (10) and tumors in this class that could not be classified as either of these 2 entities - steroid cell tumor not otherwise specified (SCT NOS) (11). These 3 entities were thought to comprise 20%, 20% and 60% of steroid cell tumors respectively. A Leydig cell tumor is a benign, typically androgen producing tumor that is usually confined to the ovarian hilum and which commonly shows cytoplasmic Reinke crystals (1, 6). Stromal luteomas were initially conceptualized as benign, small, ovarian cortex-confined neoplasms that were mostly seen in postmenopausal patients (11, 12). Patients most frequently presented with abnormal vaginal bleeding that was probably attributable to hyperestrogenism (11, 12). Although stromal luteomas were thought to display distinctive clinicopathologic features (13, 14), starting with the 4th edition of the WHO classification of ovarian tumors (2014), stromal luteoma ceased to be recognized as a distinct entity (15). Tumors that were previously classified as stroma luteoma and SCT NOS were both subsumed under the SCT (15), and the latter has remained the preferred terminology for this tumor (1). SCTs are rare, with fewer than a thousand cases reported in the literature to date. This rarity has limited advances in the understanding of this enigmatic neoplasm. In this review, we summarize currently known clinicopathologic information on SCT.

Clinical and radiologic presentation

SCT are frequently hormonally active, leading to elevated serum and/or levels of androgenic hormones and their metabolites (11, 16, 17). In a subset of cases, ovarian SCT can induce ACTH secretion, leading to co-presentation of Cushing syndrome (18–21). Symptomatology is often related to androgenic excess, including virilization, hirsutism, balding, deepening of voice, acne, and clitoromegaly (11, 16). Overall, the most common initial manifestation in one series was virilization (41%), although 6.3% had estrogenic manifestations. In additional to symptoms related to androgen excess, there are age group-specific presentations. For example, in pediatric population, children may show isosexual precocious puberty (22). In child-bearing age group, women present with irregular menstrual cycles or infertility (23). In post-menopausal women, vaginal bleeding may occur (24, 25). In most cases, SCTs present as an unilateral ovarian tumor (11). However, it has been estimated that 6% of patients present with bilateral ovarian SCTs (11, 26). A subset of SCTs are malignant (11, 27)., and malignant SCTs has been reported in females as young as 4 years old (28). Malignant SCT presents with extra-ovarian disease, often involving the retroperitoneum, mesentery, omentum, and other intraabdominal organs such as colon (29). Distant metastasis includes the vertebral bone and brain (30). A rare case of malignant ascites from peritoneal dissemination has also been reported (31).

The age at diagnosis is broad, ranging from as young as 1 year old to 93 years old, but generally between 20s-40s. In one series (11), the average age was 43 years (range 2.5-80 years), and in one review of the literature, the median age was 33.5 years (range 3-93) (16). Accordingly, a significant number of ovarian SCT occurs in the pediatric population, wherein the tumors may initially be misdiagnosed with congenital adrenal hyperplasia, which may exhibit similar clinical symptomatology (32, 33). Along the same vein, women of reproductive age with ovarian SCT may be misdiagnosed with polycystic ovarian syndrome (PCOS) - a much more common hormonal disorder in this age group (34). Another critical point to underscore is that while the majority of the cases present with a unilateral ovarian mass (size ranging from 1.2-45 cm), smaller lesions may be missed by modern imaging techniques such as MRI, leading to underdiagnosis of ovarian SCT (35). Indeed, an integrative clinical, radiologic, and biochemical workup is necessary to achieve optimal screening. On rare occasion, for diagnostically occult cases, therapeutic oophorectomies has been performed to exclude the possibility of ovarian SCT (36). In general, MRI has the most specificity for a SCT, which typically demonstrates a well-defined solid mass. Key characteristics include contrast enhancement on T2-weighted image (37), and demonstration of lipid content on T1-weighted image with signal drop between pre-contrast T1-weighted opposed phase and T- weighted in phase images (38). On balance, clinical presentation of virilization, increased serum testosterone level, and

presence of a lipid-containing ovarian mass on MRI should raise the differential diagnosis of an ovarian SCT.

Ovarian SCT can occur in patients with germline mutations *in FH, VHL, and APC* genes. The most frequently reported cancer predisposition syndrome associated with ovarian SCT is VHL. There are 5 reported cases of SCT arising in VHL patients in the literature, four are unilateral on presentation and one is bilateral (39, 40). The onset age ranged from 16 to 46 years old (39). There is only one case report of a patient with germline *FH* mutation. This patient presented with asynchronous bilateral ovarian SCT, initially at age of 22 (left ovary, 2 cm), and later at 31 years old (right ovary, 6.3 cm) (41). There is also one case report of a benign, unilateral ovarian SCT in a 47-year-old woman with familial adenomatous polyposis syndrome (42).

Macroscopic, microscopic, and immunohistochemical features

In one series of 63 cases, 51, 4,7 and 1 case(s) were stage I, II, III, and IV respectively (11). 94% were unilateral and 6% bilateral (11). The tumors reportedly ranged in size from 1.2 to 45 cm (average 8.4 cm); 65% were described as well circumscribed and a smaller subset as encapsulated (11). Most were described as having a solid cut surface, with smaller subsets being solid to cystic or entirely cystic (11). The tumoral cut surfaces were mostly yellow, or in a minority of cases, brown, tan or gray white (11). Calcifications, hemorrhage or necrosis may be grossly observed. Microscopically, SCT comprises a proliferation of polygonal to epithelioid cells with abundant eosinophilic to vacuolated/clear cytoplasm (Figure 1). The nuclear and nucleolar size may vary from case to case or within a given case, as may the level of nuclear pleomorphism. The cells are arranged in sheet-like to nested patterns, separated by a delicate vascular network. Most cases have a low mitotic index, but this may vary as well. Necrosis, lymphovascular invasion, zones of hypercellularity, stromal hyalinization, lipid droplets, vague spindling and/or hemorrhage may be seen. Significantly, no Reinke crystals are present (a defining feature of Ledyig cell tumor). The immunoprofile of SCT is consistent with sex cord-stromal differentiation, with >80% expressing inhibin-A, SF1 and calretinin (41,42). A subset of SCT variably (30-70%) demonstrate positivity for CD99, androgen receptor, Melan A, estrogen receptor, progesterone receptor, SMA, CD10 and pancytokeratins (41,42,43). SCT do not express WT1 or epithelial membrane antigen (43).

Molecular pathogenesis

Three molecular studies have been reported on SCT (27, 43, 44). One of aforementioned studies included a "metastatic" ovarian Leydig cell tumor, which likely present a steroid cell tumor (44). Overall, there appear to be few, if any, pathognomonic recurrent mutations for SCT. This contrasts with other types of ovarian sex cord stromal tumors, such as Sertoli-Leydig cell tumor, Sertoli cell tumor of pure-type, sex cord-stromal tumors with annular tubules (SCTAT), adult granulosa cell tumor (AGCT), and juvenile granulosa cell tumor (JGCT). In one series, 60% of the Sertoli-Leydig cell tumors was found to have *DICER1* mutation, and some occurred in the setting of germline *DICER1* mutation (45). A subset of SCTAT and pure-type Sertoli cell tumor cases arise in association with germline *STK11* mutation that causes Peutz-Jegher syndrome (46). Interestingly, SCTAT occurring in context of syndromic germline STK11 mutation have improved outcomes compared to sporadic/non-syndromic patients (47). Over 95% of AGCT demonstrates recurrent somatic *FOXL2* mutation (48). JGCT may occur in the setting of Ollier disease and Maffucci syndrome, or

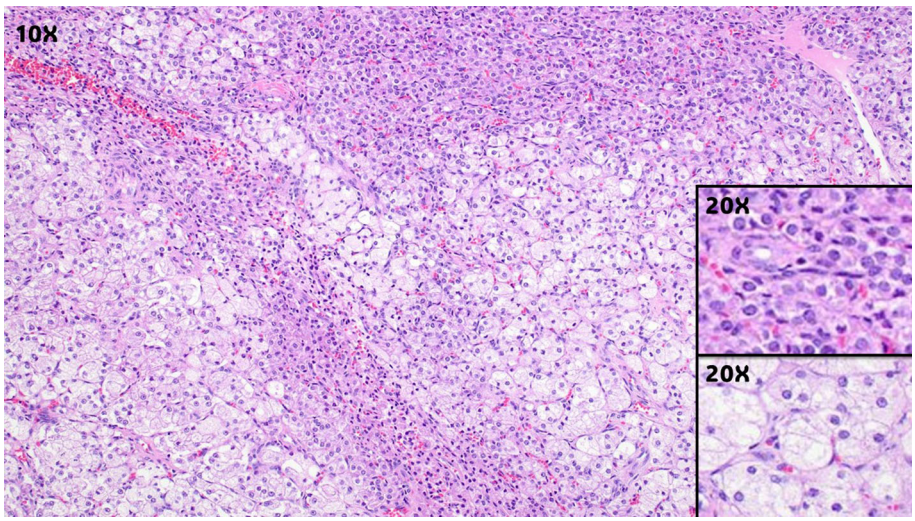

FIGURE 1
Ovarian steroid cell tumor, showing solid sheets of epithelioid to polygonal cell with eosinophilic to clear cytoplasm (Magnification, 10X). The insets show higher magnification of the tumor cells with eosinophilic cytoplasm (top inset, magnification 20X), or clear/vacuolated cytoplasm (lower inset, magnificent 20X).

somatic mosaic mutations in *IDH1* and *IDH2*, and somatic copy number changes in *AKT* (45).

In ovarian SCT, a more heterogeneous profile of genetic mutations has been reported, including *BAP1, FH, TP53, CTNNB1, CASP10, HIF1A, SRC, FOXO4, HOXA13, LHCGR, VHL, IDH2, SDHB*, and *BRCA2* (27, 43, 44). Most are missense mutations, except for *BAP1* and *FOXO4*, which are frameshift mutations (27, 43), and *SRC*, which is an in-frame deletion (43). In one of the studies, enrichment of hypoxia-associated gene mutation (*HIF1A*, *VHL*, *SDHB*, *SRC*, *IDH2*, and *FOXO4*) was observed in a retrospective case series of 5 benign and 2 malignant SCT patients (43). Interestingly, SCT has been reported in patients with germline mutation in *VHL* (39), suggesting a correlation between hypoxia signaling pathway in the tumorigenesis of SCT. Wnt signaling pathway is another implicated pathway dysregulated in SCT, since somatic *CTNNB1* mutation and biallelic *APC* loss molecular events have been reported in SCT (27, 42).

For malignant SCTs, we found a total of six malignant SCT with molecular information, reported by three independent studies (27, 43, 44). The molecular findings are not entirely consistent between series. However, two general observations were seen. First, malignant SCTs exhibited more global genomic instability by copy number analysis. This is supported by the identification of copy number gain in *MDM2* and CDK2 *genes*, *ATRX* rearrangement, and copy number amplification in *NPM1*, *DCM1*, and *SS18* genes (27, 44). However, it is important to note that these genes are sporadically reported and are not consistently found in all malignant SCT cases. More likely, these identified amplification and structural rearrangement events are passenger events secondary to global genomic instability. Second, *BAP1* mutation was found in two of the six malignant SCT cases sequenced to date, reported independently by two groups (27, 44). The mutation genotypes for *BAP1* were p.K453fs and p.S126Rfs*61 (personal communications with Dr. Vranic and Dr. Bennett). Interestingly, *BAP1* mutation has not been reported in benign SCTs to date. Other mutations found in malignant SCTs included *HIF1A* and *SDHB* (44).

Although the data is limited, other possibly negative molecular findings include: (1) The type of gene mutations does not appear to be correlated with the number of adverse histologic risk factors (27), and (2) Microsatellite instability was not identified in any tested sample, suggesting that SCTs are likely not hyper-mutated tumors (43, 44).

On balance, the malignant cases are genetically more unstable, characterized by global chromosomal number aberration, with occasional *BAP1* mutation. However, readers are cautioned to avoid overgeneralizing these findings due to the small sample size. The genomic profile of benign and malignant SCTs is still relatively under-characterized, secondary to limited samples of this rare tumor type, and the selective use of cancer gene panel assays to profile their genomic makeup in the published studies (27, 43). Indeed, some noncancer-related genes, such as metabolic or hormonal-related genes, may be important for the development or prognostication of SCTs. Future studies with larger sample size, and the use of more advanced, unbiased molecular techniques, such as whole exome and transcriptome molecular profiling, will ultimately provide a more comprehensive molecular profile of SCT.

We found one functional molecular study of SCT in the literature (49). Using telomerase repeat amplification protocol (TRAP) assay, this study showed intact telomerase activity in a malignant SCT. In a retrospective series of sex cord-stomal ovarian tumors, Dowdy et al. demonstrated that telomerase activity has a 94% specificity for malignancy. In the same study, none of the benign sex cord-cord-stomal ovarian tumors showed telomerase activity. The prognostic significance of telomerase activity in SCT, particularly in distinguishing benignity from malignancy warrants further investigation (46).

Patient outcomes and possible pathologic predictors

Most reported cases of SCT have been clinically benign without recurrences or death from disease following the primary resection of the tumor (11,16). In a recent review of the literature, Lin et al. found post-resection disease recurrence or progression occurred in 17.86% of cases, with a median tumor-free interval of 23 months (16). The authors noted that recurrences seemed to be associated with patient age, with a recurrence rate of 11.43% for patients aged 40 years or younger, and 28.57% for those older than 40 years, and no patients younger than 20 years of age reported with recurrence or progression. In the series of Mendoza et al, approximately 14% of cases were malignant (27). In the series of Hayes and Scully, most of which were consultation or referral cases, approximately one third of cases were clinically malignant (11). Overall, our impression is that the malignancy rate is probably less than 20%. The authors noted that the best pathological correlates of malignant behavior were: the presence of two or more mitotic figures per 10 high power fields (92% malignant); necrosis (86% malignant); a diameter of 7 cm or greater (78% malignant); hemorrhage (77% malignant); and grade 2 or 3 nuclear atypia (64% malignant) (11). In one case series, although all malignant SCTs demonstrated at least 4 atypical features, at least one atypical feature was present in benign cases as well (27). Thus, pathologic features that are specifically predictive of behavior have not been conclusively defined, although the data suggests that there may be features that correlative with adverse outcomes. A combination of pathogenomic classification may improve our ability to classify the prognosis of SCTs with atypical features.

In malignant cases, patients may either present with advanced extra-ovarian disease or recur after surgery. The disease recurrence timeline is variable and can recur within months or as long as 17 years after initial diagnosis and surgery, even in stage IA cases (27, 43). Metastatic SCT typically presents with intra-abdominal and retroperitoneal metastases, and on rare occasions, ascites. The clinical course for malignant SCTs are generally guarded, and most succumb to the disease 6-44 months following the diagnosis (11, 16, 27, 29, 30). However, as previously noted, recurrences may occur many years after primary resection. SCTs are generally insensitive to chemotherapy (29, 50). Rare case reports of disease

control with a GnRH agonist have been reported (51, 52). In benign cases, the serum testosterone level generally normalizes within days or weeks following surgical resection of SCT (16). Successful pregnancy is achievable following surgery, usually within 1 year of tumor removal (53, 54). Virilization and hirsutism are usually resolved within a year of surgical tumor removal. This underscores the importance of early detection and surgical management of SCT. However, to prevent overtreatment the readers are cautioned that increased use of prenatal ultrasound has led to increased detection of asymptomatic ovarian masses (55). Most adnexal masses detected during gestation are benign and functional (55). The most common sex cord stromal tumors detected during gestation are granulosa cell tumor (22%), thecoma (18.6%), and Sertoli-Leydig tumors (8.5%) (56). Fortunately, greater than 70% of sex cord stromal tumors found during pregnancy result in live births (56).

Summary and conclusions

Ovarian SCT are rare, with fewer than a thousand cases reported in the literature to date. SCT patients frequently display evidence of androgenic excess, with elevation in plasma testosterone level. A subset of SCT occurs in patients with germline mutations in *VHL, FH,* and *APC* genes. While most SCT are benign, a small subset are malignant and recurrences may occur many years after primary resection of an apparently localized tumor. Pathologic criteria that can specifically predict patient outcomes remain elusive, although features that correlate with adverse outcomes have been proposed based on retrospective studies. The molecular characteristics of SCTs are still under characterized, due to rarity of this entity. However, a few key observations have been made, including an enrichment of hypoxia-signaling gene mutations. In malignant SCT, the tumors generally show greater global genomic instability, copy number gains in oncogenes, and occasional *BAP1* mutation. Future studies involving multi-institutional cohort and unbiased molecular profiling using whole exome/transcriptome sequencing are needed to help advance our molecular understanding of SCTs.

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Robb Hollis,
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REVIEWED BY
Kate Connolly,
Western General Hospital, United Kingdom
Mihaela Carmen Cristea,
Regeneron Pharmaceuticals, Inc.,
United States

*CORRESPONDENCE
Robert Fruscio
robert.fruscio@unimib.it

†These authors share first authorship

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Outcome of patients with stage I immature teratoma after surveillance or adjuvant chemotherapy

Giuseppe Marino[1†], Tommaso Grassi[2†], Elena De Ponti[2], Serena Negri[1], Filippo Testa[1], Daniela Giuliani[2], Martina Delle Marchette[1], Cristina Dell'Oro[1], Diletta Fumagalli[1], Gianluca Donatiello[1], Giulia Besana[1], Liliana Marchetta[1], Cristina Maria Bonazzi[2], Andrea Alberto Lissoni[2], Fabio Landoni[1,2] and Robert Fruscio[1,2*]

[1]Department of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy, [2]Unit of Gynecology, Woman and Child Department, Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS) San Gerardo, Monza, Italy

Objective: Immature teratomas are rare malignant ovarian germ cell tumours, typically diagnosed in young women, where fertility-sparing surgery is the treatment of choice. The role of adjuvant chemotherapy in stage I disease remains controversial. We evaluated the impact of surveillance versus chemotherapy on the recurrence rate in stage I immature teratomas.

Methods: We collected a single centre retrospective series of patients with stage I immature teratomas treated with fertility-sparing surgery at San Gerardo Hospital, Monza, Italy, between 1980 and 2019. Potential risk factors for recurrence were investigated by multivariate logistic regression.

Results: Of the 74 patients included, 12% (9/74) received chemotherapy, while 88% (65/74) underwent surveillance. Median follow-up was 188 months. No difference in recurrence was found in stage IA/IB and IC immature teratomas [10% (6/60) vs. 28.6% (4/14) (P=0.087)], grade 1, grade 2, and grade 3 [7.1% (2/28) vs. 14.3% (4/28) vs. 22.2% (4/18) (p=0.39)], and surveillance versus chemotherapy groups [13.9% (9/65) vs. 11.1% (1/9)) (p = 1.00)]. In univariate analysis, the postoperative approach had no impact on recurrence. The 5-year disease-free survival was 87% and 90% in the surveillance and chemotherapy groups, respectively; the overall survival was 100% in both cohorts.

Conclusions: Our results support the feasibility of surveillance in stage I immature teratomas. Adjuvant chemotherapy may be reserved for relapses. However, the potential benefit of chemotherapy should be discussed, especially for high-risk tumours. Prospective series are warranted to confirm our findings.

What is already known on this topic: To date, no consensus has been reached regarding the role of adjuvant chemotherapy in stage I immature teratomas of the ovary. Some studies suggest that only surveillance is an acceptable choice. However, guidelines are not conclusive on this topic.

What this study adds: No difference in terms of recurrence was observed between the surveillance and the adjuvant chemotherapy group. All patients who relapsed were successfully cured with no disease-related deaths.

How this study might affect research, practice or policy: Adjuvant chemotherapy should be appropriately discussed with patients. However, it may be reserved for relapse according to our data.

KEYWORDS

immature teratoma of the ovary, germ cell tumor, chemotherapy, oncologic outcome, ovarian cancer

Introduction

Malignant ovarian germ cell tumours are rare malignancies accounting for approximately 5% of all ovarian cancers, with an estimated incidence of 3-4 cases/1,000,000 women in Europe (1). Immature teratomas represent approximately one-third of them and typically occur in young women, with a peak incidence between 15 and 30 years of age (1). Most patients are diagnosed with stage I disease and have an excellent prognosis (2). Disease grade and stage are two main prognostic factors (3). Given the young age at the diagnosis, the standard treatment is represented by fertility-sparing surgery with complete staging. In contrast, the need for adjuvant treatment is still controversial (4, 5). According to the National Comprehensive Cancer Network (NCCN) guidelines (4), patients diagnosed with stage IA grade 1 disease can avoid further treatments and undergo surveillance, while patients with stage I, grade 2 or 3 should receive adjuvant chemotherapy. However, due to the optimal prognosis with low rate of recurrence and the potential side effects of the therapy (6–11), the European Society for Medical Oncology (ESMO) guidelines suggest that close surveillance may also be considered in stage IA grade 2 or 3 and stage IB–IC, and chemotherapy reserved as salvage therapy for recurrence (5).

We report a large retrospective case series of post-pubertal patients with stage I, any grade, immature teratomas treated at our Institution. The primary aim of this study was to evaluate the impact of adjuvant chemotherapy or surveillance on the recurrence rate. Disease-free and overall survival were also assessed.

Methods

Patients characteristics

Patients with pathologically confirmed stage I pure immature teratoma treated at San Gerardo Hospital, Monza, between 1980 and 2019 were screened for inclusion. All cases were reviewed by a dedicated pathologist who categorised the tumours into three grades (3). The tumour stage was defined according to the 2014 Federal International Federation of Gynecology Oncology (FIGO) classification for ovarian cancer (12), adapting our cases previously diagnosed to this updated version.

Inclusion criteria were post-pubertal age (intended as post-menarche period) and treatment with primary fertility-sparing surgery, defined as preservation of the uterus and at least one adnexa. The type of ovarian surgery was defined as unilateral salpingo-oophorectomy (removal of the affected ovary and the ipsilateral fallopian tube with the preservation of the contralateral adnexa) or cystectomy (enucleation of the cystic lesion with preservation of both the adnexa). In bilateral cysts, fertility was preserved by performing a unilateral salpingo-oophorectomy + cystectomy. Complete surgical staging procedures, defined as omentectomy, peritoneal washing, and peritoneal biopsies, were also performed at the time of diagnosis or during surgical restaging. If primary surgery was not performed at our Institution, surgical restaging was performed within 90 days from the diagnosis and considered a complete staging. Despite these attempts, incomplete surgical staging was observed in most patients. All patients who did not undergo surgical staging were staged with imaging techniques, such as computed tomography (CT). Patient follow-up has changed over decades. From 1980 to 2000, the follow-up visit included a gynecologic examination with transvaginal ultrasound and alpha-fetoprotein measurement, CT, and laparoscopy ± biopsies. In the last two decades, with the improvement of imaging techniques, routine second-look laparoscopy was almost abandoned in the absence of suspected recurrence. Relapse was confirmed after a histological sampling obtained by biopsy or surgery. Follow-up was performed every 3 months for the first 2 years, then every 6 months until the fifth year, then yearly (5). Patients with less than 24 months of follow-up were excluded. Ethical approval from Comitato Etico Brianza was obtained (3930).

Statistical analysis

For descriptive statistics, frequencies and proportions were used for categorical variables, while for continuous variables, means or

medians were used with standard deviation or minimum-maximum range, respectively. Continuous variables were compared using the Wilcoxon rank sum test, while proportions were compared using the Chi-square test or Fisher's exact test. All p values are two-sided and were considered statistically significant if $p < 0.05$.

A multivariate logistic regression was performed to assess the event of disease recurrence and possible independent associations between patient, disease and treatment variables. Logistic regression was used for the analysis since the endpoint was binary. Disease-free survival curves were estimated with the Kaplan-Meier method. Stata Software 9.0 (Stata Corporation, College Station, TX, USA) was used for the analysis.

Results

Between 1980 and 2019, 110 post-pubertal patients with pure immature teratomas were referred to our Institution. Eighty of them had a stage I disease, as reported in Figure 1. Six patients were lost at follow-up, and 74 were included in the analysis. Patients' characteristics are shown in Supplementary Tables 1, 2A.

The median age at diagnosis was 27 years. Eighty percent of patients were stage IA (59/74), 1% were stage IB (1/74), and 19% stage IC (14/74). The rate of patients with grade 1, 2, and 3 was 38% (28/74), 38% (28/74), and 24% (18/74), respectively. Seventy-two percent of patients underwent unilateral salpingo-oophorectomy (53/74), whereas a cystectomy was performed in 28% (21/74). Seventy-four percent of patients (55/74) underwent a laparotomy, while 25% (19/74) underwent a laparoscopic procedure. Laparotomy was the preferred approach up to 2000, while 40% (10/25) of the procedures performed after 2000 were laparoscopic. Only 23% of patients (17/74) underwent complete surgical staging, while 77% (57/74) of cases did not, and further surgical staging was waived.

As shown in Table 1, 12% of patients (9/74) underwent adjuvant chemotherapy. Eight received 3 cycles of bleomycin/etoposide/cisplatin regimen, and only one received 3 cycles of bleomycin/vincristine/cisplatin schedule. Surveillance alone was recommended in 88% of patients (65/74). Among the 9 patients who received adjuvant chemotherapy 55.6% had stage IC disease while 44.4% had stage IA/B (p 0.010); also, 55.6% had grade 3while 33.3% had grade 2 and 11.1% grade 1 (p 0.058) (Table 1; Supplementary Table 5). A lower median age was observed in patients treated with chemotherapy [p value = 0.052]. Among patients who underwent adjuvant chemotherapy, 7 were treated before 2000, while 2 in the last two decades, favouring a "wait and see behaviour" (13). The type of ovarian surgery and the complete surgical staging did not influence the postoperative treatment (Table 1; Supplementary Tables 3, 4).

Oncologic outcomes are summarised in Table 2; Supplementary Table 2B. Among 10 relapsing patients, 1 (10%) received chemotherapy, while 9 (90%) underwent surveillance; the same percentages were observed among patient who did not have a relapse (12.5% and 87.5%, respectively, p: 1.00) (Figure 2). Six relapses were found in the IA+IB stage group (6/10 = 60%) and 4 in the IC stage group (4/10 = 40%) [p value = 0.087]. Among relapsed patients 20% had grade 1, 40% grade 2 and 40% grade 3 [p value = 0.390]. The recurrence rate was not different among patients who underwent a different surgical approach or type of ovarian surgery (Table 2; Supplementary Table 3). Moreover, no significant difference was observed in terms of recurrence rate among patients who underwent complete staging at the time of primary surgery (4/10 = 40.0%) and patients who did not (6/10 = 60.0%) [p value 0.224] (Table 2; Supplementary Table 4).

Among patients who received chemotherapy, the one who experienced a recurrence had stage IC grade 3 immature teratoma

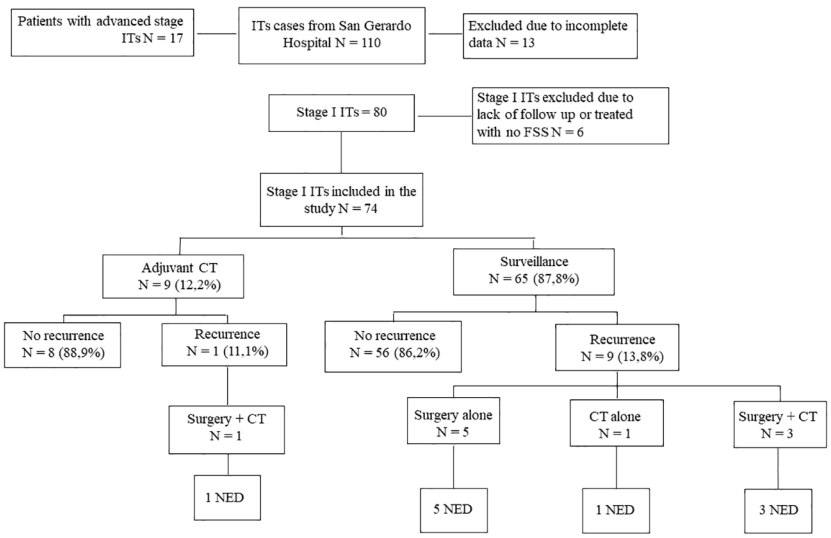

FIGURE 1
Study Flowchart. Flow chart relative to management and outcome of stage I ITs patients (from San Gerardo Hospital, Monza, between 1980 and 2019). ITs, Immature Teratomas; CT, Chemotherapy; NED, No Evidence of Disease.

TABLE 1 Patients' characteristics according to post-operative treatment.

Post-operative treatment	Surveillance (n=65)	Chemotherapy (n=9)	p value
Median age (min-max)	28	18	0.052
Decades of treatment			0.386
1980-1989 1990-1999 2000-2009 2010-2019	11 (16.9%) 30 (46.2%) 20 (30.8%) 4 (6.2%)	3 (33.3%) 4 (44.4%) 1 (11.1%) 1 (11.1%)	
Stage			0.010
IA + IB IC	56 (86.2%) 9 (13.8%)	4 (44.4%) 5 (55.6%)	
Grade			0.058
Grade 1 Grade 2 Grade 3	27 (41.5%) 25 (38.5%) 13 (20.0%)	1 (11.1%) 3 (33.3%) 5 (55.6%)	
Type of surgery			0.431
Cystectomy Unilateral Salpingo-Oophorectomy	20 (30.8%) 45 (69.2%)	1 (11.1%) 8 (88.9%)	
Complete staging			0.675
Yes No	16 (24.6%) 49 (75.4%)	1 (11.1%) 8 (88.9%)	
Relapse			1.00
	9 (13.9%)*	1 (11.1%)*	

*column percentage.

TABLE 2 Oncologic outcomes.

Relapse	Yes (N = 10)	No (N = 64)	p value
Median age (min-max)	22.5 (12-39)	27.5 (11-42)	0.304
Stage			0.087
IA+IB IC	6 (60.0%) 4 (40.0%)	54 (84.4%) 10 (15.6%)	
Grade			0.390
Grade 1 Grade 2 Grade 3	2 (20.0%) 4 (40.0%) 4 (40.0%)	26 (40.6%) 24 (37.5%) 14 (21.9%)	
Surgical approach			0.770
Laparotomy Laparoscopy Non available	7 (70.0%) 3 (30.0%) 0 (0%)	48 (75.0%) 14 (21.9%) 2 (3.1%)	
Type of surgery			0.715
Cystectomy Unilateral Salpingo-Oophorectomy	2 (20.0%) 8 (80.0%)	19 (29.7%) 45 (70.3%)	
Post-surgical approach			1.00
Surveillance Chemotherapy	9 (90.0%) 1 (10.0%)	56 (87.5%) 8 (12.5%)	
Complete staging			0.224
Yes No	4 (40.0%) 6 (60.0%)	13 (20.3%) 51 (79.7%)	

treated with a laparoscopic unilateral salpingo-oophorectomy and 3 subsequent cycles of bleomycin/etoposide/cisplatin. She developed an umbilical recurrence 7 months after the diagnosis, and she was successfully treated with surgery followed by two more cycles of bleomycin/etoposide/cisplatin. For the surveillance group, the time to relapse was between 3 and 168 months after surgery (median time 46 months; Figure 2). The characteristics of patients who experienced recurrence are summarised in Supplementary Table 6: 3 patients relapsed only in the contralateral ovary, 5 presented with peritoneal metastases (3 had only pelvic peritoneal involvement), and one patient experienced peritoneal and lymphatic recurrence. All patients were successfully treated with surgery ± chemotherapy. All patients were alive at the time of the last follow-up and with no evidence of disease. Only one patient was diagnosed with a second recurrence that was successfully treated. She had a stage IC2 (capsule ruptured before surgery) grade 3 disease at the time of the diagnosis; she underwent a complete surgical staging, and no adjuvant treatment was advised. Her first relapse was diagnosed nine months after surgery: she underwent 5 cycles of bleomycin/etoposide/cisplatin for diffuse intraperitoneal and visceral lesions, with complete remission. One year later, she developed a second localised relapse in the pouch of Douglas that was surgically removed, and a second-line adjuvant chemotherapy with Paclitaxel/Ifosfamide/Cisplatin was recommended (patient 9 in Supplementary Table 6).

Univariate analysis

Univariate analyses was performed to evaluate the prognostic role of the different clinicopathological variables on the recurrence rate (Table 3).

No factors showed a statistically significant impact on the relapse rate, and adjuvant chemotherapy did not show a protective effect. Stage of disease showed an Odd Ratio of 3.60 (CI: 95% 0.86 –15.1; stage IC vs. IA-IB), however, it did not reach statistical significance (p = 0.08).

The 5-year disease-free survival was 87.4% and 90.0% for patients who underwent surveillance and adjuvant chemotherapy, respectively (Figure 2). During follow-up, no patient died of the disease or by any means, with a disease-specific and overall survival of 100% for the whole cohort.

Discussion

Summary of main results

Patients in our study who underwent active surveillance did not show any worse oncological outcome when compared to those

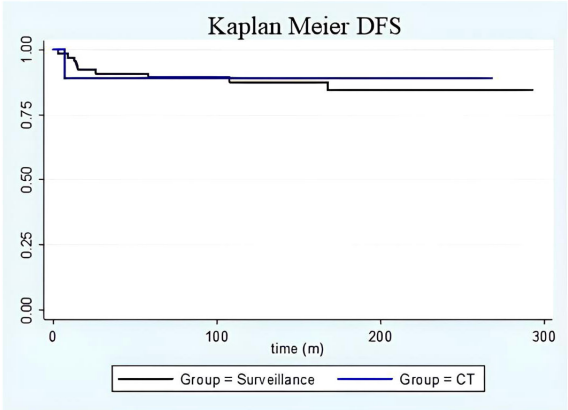

FIGURE 2
DFS Kaplan Meier. Kaplan-Meier curve on disease-free survival in the surveillance group (black line) versus adjuvant chemotherapy group (blue line). No differences were found between the groups in terms of disease-free survival. Moreover, time-to-relapse between the groups did not show any differences.

treated with chemotherapy. Disease-free survival was similar at five years between the two groups, suggesting that adjuvant chemotherapy neither improved oncologic outcome nor moved time-to-relapse further forward in the study population. Additionally, all patients who relapsed were successfully cured, with no disease-related deaths occurring during follow-up. There were some differences in the relapse rates between different grades (7.1% in grade 1 ITs, 14.3% in grade 2, and 22.2% in grade 3) and stages (28.6% for IC, while it was 10% in stage IA or IB). However, due to the small sample size, the present study was probably unable to reach statistical significance. Additionally, in our cohort, the absence of surgical staging was not a critical risk factor for a worse oncologic outcome, differently from other evidence (14).

Of note, these results corroborate the opinion of those who argue that chemotherapy can be omitted in the standard therapeutic approach for stage I disease.

TABLE 3 Association between clinical characteristics and relapse (univariate analysis).

	Univariate		
	OR	C.I. 95%	P value
Age at diagnosis (Years)	0.95	0.88 – 1.04	0.287
Grade G3 vs. G1-G2	2.38	0.59 – 9.63	0.224
Stage IC vs. IA-IB	3.60	0.86 – 15.1	0.080
Adjuvant Chemotherapy Yes vs. No	0.78	0.09 – 6.98	0.822

OR, Odds ratio; C.I., Confidence interval; G1, grade 1; G2, grade 2; G3, grade 3.

Results in the context of published literature

Studies conducted between the 1970s and the 1990s suggest that patients with early-stage grade 2-3 disease should receive adjuvant chemotherapy because of their high risk of recurrence and the survival benefit after chemotherapy (3, 15–18). However, chemotherapy may cause long-term toxicities, such as secondary malignancies after etoposide exposure, bleomycin's pulmonary effects, and platinum neurotoxicity (6–11, 19). Additionally, the risk of chemotherapy-induced amenorrhea is higher with the increase in dosage and the number of therapy cycles (5), although the standard bleomycin/etoposide/cisplatin schedule seems not to impair the ovarian reserve (20).

Recently, echoing the positive experiences of surveillance in the paediatric population affected by immature teratomas (21, 22) and the established practice of avoiding adjuvant chemotherapy in some male germ cell tumours (23), some authors suggested active surveillance as an alternative to adjuvant chemotherapy in patients with post-pubertal stage I immature teratoma, reserving chemotherapy for patients with recurrent disease (5, 24–29).

An Italian multicentre study (26) found an optimal long-term prognosis in 28 patients with stage I pure immature teratomas with post-surgery surveillance and recommended chemotherapy in case of recurrence or in the presence of a yolk sac tumour component because it worsens the prognosis. Recently, Bergamini et al. (24) retrospectively analysed a large group of 108 patients with stage I pure immature teratomas who underwent surveillance or adjuvant chemotherapy after fertility-sparing surgery and were followed up at Charing Cross Hospital, London, United Kingdom, and in Italy. Stage IA, IB, and IC were respectively 66, 3, and 39 on a cohort of 108 patients. Twenty-five percent received adjuvant chemotherapy, while 75% underwent surveillance only. The recurrence rate was not different between the two groups [7.4% (2/25) vs. 11.1% (9/81), respectively (p 0.65)]. Moreover, all patients who relapsed were successfully cured at the time of recurrence, except for one who did not adhere to the recommended close follow-up procedures. Thus, they suggest surveillance as a replacement for adjuvant chemotherapy in stage I immature teratomas of any grade in the adult setting, reserving systemic treatment only for recurrent disease. Bergamini et al. (24) also found that tumour grade and complete surgical staging were the only independent prognostic factors for worse disease-free survival. In 1994, D. M. O'Connor and H. J. Norris identified the tumour grade as one of the most important risk factors for relapse in these patients (30), showing a recurrence rate of 70% in grade 3 disease and 18% in grade 2 disease. A significant association between grade and risk of recurrence is extensively reported in the literature (30–32), further confirmed by Pashankar et al. (27) and Zhao et al. (33).

Surgical staging is one of the cornerstones in the management of these patients, reported in guidelines as mandatory (4, 5, 14). Also, it's common in clinical practice to do a second-step surgery in patients not properly staged and results from Bergamini et al. (24) confirmed this crucial aspect. Indeed, a selection bias may have occurred in our population, as we retrospectively analysed only

early-stage disease, so further evidence from prospectively-collected data is warranted to clarify these findings.

Additionally, stage represents one of the most important and well-known prognostic factors for poor oncologic outcomes (34–36). However, Bergamini et al. (24) did not find a significant correlation between the substage of stage I disease and worsening outcomes. Despite our data showed that the prognostic factor most associated with relapse was the stage of disease [Odd Ratio = 3.60 (CI 95% 0.86 – 15.1)], no significance was reached. Due to the low rate of relapse in our population, a multivariate analysis appeared to be not feasible from a statistical point of view, limiting in part the statistical strength of our study, even if it would not have showed significant differences.

Finally, all patients who developed a diffuse relapse of disease, which required extensive surgery and subsequent chemotherapy, had a high-risk disease at the time of the diagnosis (grade 3 or stage IC or both - Supplementary Table 6). Therefore, we suggest carefully evaluating adjuvant treatment, discussing individual cases in multidisciplinary meetings, and adequate counselling with the patient, especially for high-risk tumours.

Strengths and limitations

The main limitations of the present study are its retrospective design, the small sample size of the population analysed, and the low number of patients with high-risk disease. The small sample size limits the study's power on reaching a real difference between the two cohorts, remarked also by the low rate of events that have limited the possibility of performing a multivariate analysis. Also, the low percentage of complete surgical staging represents a further limit. Nevertheless, this is the largest European single-centre case series reported in the literature based on a population of patients with pure ovarian immature teratoma. An advantage of being monocentric is the homogeneity of patient treatment. We found no significant differences between the postoperative treatment of patients, in terms of chemotherapy or surveillance, in the four decades considered in the study.

Implications for practice and future data

Given the limited data available on this topic, our research highlights and agrees with other Authors on the central role of surveillance in stage I immature teratomas, suggesting that adjuvant chemotherapy may be reserved for relapses. Future studies, in particular prospective collections, are required to confirm the impact of surveillance on disease recurrence.

Conclusions

Our data confirm that stage I immature teratomas are characterised by an excellent prognosis in terms of disease recurrence, as reported in the literature (2, 20, 26, 32).

As previously reported, we can conclude that adjuvant chemotherapy may be omitted in this selected population after extensive counselling, reserving it for disease relapse. However, especially for high-risk stage I tumours (stage IC and grade 3), adjuvant treatment should be discussed with the patient on an individual basis. No independent prognostic factors were found to be statistically significant in predicting relapse.

In our cohort, active surveillance resulted as a safe alternative to adjuvant chemotherapy for the postoperative management of stage I ovarian immature teratomas. Nevertheless, prospective series are needed to confirm our findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by IRB Comitato Etico Brianza, IRCCS San Gerardo dei Tintori, Monza, Italy. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GM: Investigation, Writing – original draft. TG: Investigation, Writing – original draft. EDP: Methodology, Writing – review & editing. SN: Investigation, Writing – review & editing. FT: Investigation, Writing – review & editing. DG: Conceptualization, Investigation, Writing – original draft. MDM: Investigation, Writing – review & editing. CD'O: Investigation, Validation, Writing – review & editing. DF: Investigation, Writing – review & editing. GD: Investigation, Writing – review & editing. GB: Investigation, Writing – review & editing. LM: Investigation, Writing – review & editing. CB: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. AL: Conceptualization, Supervision, Writing – review & editing. FL: Supervision, Writing – review & editing. RF: Conceptualization, Investigation, Project administration, Supervision, Writing – original draft.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1330481/full#supplementary-material

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EDITED BY
Mignon Van Gent,
Amsterdam University Medical Center,
Netherlands

REVIEWED BY
Fabian Trillsch,
LMU Munich University Hospital, Germany
Federica Perelli,
Azienda USL Toscana Centro, Italy

*CORRESPONDENCE
Giacomo Barchiesi
g.barchiesi@policlinicoumberto1.it

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High-grade serous carcinoma of unknown primary origin associated with STIC clinically presented as isolated inguinal lymphadenopathy: a case report

Paola Giancontieri[1], Camilla Turetta[2], Giacomo Barchiesi[1]*, Angelina Pernazza[1], Gemma Pignataro[1], Giuliano D'Onghia[3], Daniele Santini[1] and Federica Tomao[2]

[1]Department of Radiological, Oncological and Anatomo-Pathological Science, Policlinico Umberto I, Sapienza University of Rome, Rome, Italy, [2]Department of Maternal and Child Health and Urological Sciences, Sapienza University of Rome, Rome, Italy, [3]Department of Surgery, Sapienza University of Rome, Rome, Italy

Serous tubal intraepithelial carcinoma (STIC) is a precancerous lesion of high-grade serous ovarian carcinoma (HGSOC). Usually, it arises from the fimbrial end of the tube, and it is associated with metastatic potential. On average, the time to progress from STIC to HGSOC is 6.5 years. Therefore, whenever a STIC lesion is found, surgical staging and prophylactic salpingectomy are recommended in order to prevent ovarian cancer. We report a rare case of a 45-year-old female patient who clinically presented an isolated right inguinal lymphadenopathy. The remaining clinical examination was normal. Therefore, an excisional biopsy of the lymph node was performed. Pathological analysis revealed a high-grade serous carcinoma, most likely of gynecological origin. Due to histological evidence, a computed tomography (CT) scan was carried out. There was no CT evidence of ovarian disease, pelvic involvement, intra-abdominal lymphadenopathies, metastatic disease, or ascites. All tumor markers were negative. The patient underwent laparoscopic hysterectomy and bilateral salpingo-oophorectomy followed by surgical staging. Surprisingly, pathological examination showed a STIC lesion in the fimbria of the left fallopian tube. We aim to report the potential capability of STIC to spread particularly through lymphatic pathways rather than peritoneal dissemination.

KEYWORDS

STIC, HGSOC, ovarian, cancer, lymphadenopathy, metastasis, p53

Introduction

Ovarian carcinoma is the leading cause of death among gynecological malignancies (1). Due to the usual lack of symptoms associated with early-stage disease, most of the cases are diagnosed when the cancer has already progressed. This is one of the major contributing factors to the high mortality of this disease. The prognosis and treatment response depend on the stage, grading, and histological subtype of the tumor (1).

Ovarian cancer is known to be associated with BRCA 1 or 2 mutations. In these cases, the tumor tends to respond better to chemotherapy than a BRCA wild-type (WT) tumor at the same stage and grading, therefore showing a more favorable survival outcome (2).

High-grade serous ovarian cancer and the endometrioid subtype are sensitive to platinum-based chemotherapy, whereas low-grade serous ovarian cancer, mucinous cancer, and clear cell cancer are less sensitive to these regimens. Because of this resistance to systemic therapy, primary surgical resection has been shown to have a larger impact on reducing tumor burden in these subtypes (3, 4).

A large part of high-grade serous ovarian carcinomas (HGSOCs) seem to arise from the distal fimbrial end of the fallopian tube from a precursor lesion known as serous tubal intraepithelial carcinoma (STIC). STIC lesion happens when normal fallopian tube epithelium is substituted by atypical non-ciliated cells with immunohistochemical and morphological aspects of HGSOC with no invasion of the underlying stroma. Despite the absence of stromal invasion, the cells of STIC can exfoliate from the tissue and eventually spread, resulting in a disseminated HGSOC (5).

Lymphatic spread of ovarian carcinoma usually involves para-aortic and retroperitoneal lymph nodes (Figure 1). Isolated secluded inguinal lymph node metastasis is an uncommon manifestation of ovarian cancer. In most patients, a primary tumor was identified by either accurate imaging or a diagnostic surgical procedure (6, 7).

In this paper, we describe a rare case of a woman who presented a right inguinal lymph node metastasis of HGSOC, with unknown primary origin, that was discovered to be associated with a STIC in the fimbrial region of the left fallopian tube.

Case presentation

A 45-year-old woman was referred for an enlarged right inguinal lymph node. Her medical history consisted of Hashimoto's thyroiditis, linear scleroderma, and chronic headaches. She had only one birth (primiparous); she had a spontaneous menopause at the age of 39 years, followed by hormone replacement therapy with levonorgestrel and ethinyl estradiol. A family history of oncologic disease was negative. She did not report pain, erythema, or ulceration in the inguinal area in the previous 2 months.

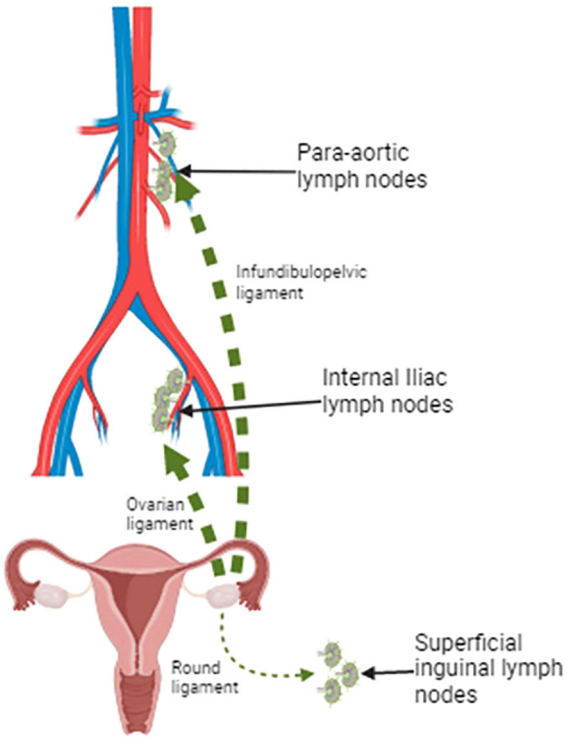

FIGURE 1
Lymphatic drainage pathways of the ovaries: the two major pathways include the lymphatic drainage via infundibulopelvic ligament toward para-aortic lymph nodes and the lymphatic drainage via the ovarian ligament to the obturator lymph nodes and the internal iliac artery. A minor drainage route goes through the round ligament and reaches the inguinal lymph node. Created with BioRender.com.

In February 2023, clinical examination confirmed the presence of an enlarged lymph node, measuring approximately 20 × 15 mm, hard consistency, and adherent to underlying tissues, suspicious for heteroplastic disease. Laboratory blood tests were normal with tumor marker levels of CA125 at 18.9 U/mL, Ca15-3 at 24 U/mL, Ca 19-9 at 8.56 U/mL, and carcinoembryonic antigen (CEA) at 1.2 ng/mL. Alpha-fetoprotein (AFP), human epididymis protein 4 (HE4), and beta-human chorionic gonadotropin (beta-HCG) were negative (respectively 2.9 ng/mL, 59.9 pmol/L, and 5.0 mUI/mL).

Therefore, an excisional biopsy of the lymph node was performed. Pathological examination revealed neoplastic cells with marked cytologic atypia, organized in solid sheets or slit-like spaces. On immunohistochemistry, the neoplastic cells were diffusely positive for CK7, PAX8, WT1, P16, and estrogen receptors (ERs) and negative for p40, GATA3, CDX2, CK20, and TTF-1; p53 exhibited an abnormal pattern expression (negative staining). Based on the morphological and immunohistochemical findings, a diagnosis of lymph node metastases from high-grade serous carcinoma most likely of tubo-ovarian origin was made.

Following the resection of the lymph node and the pathological results, a full-body computed tomography (CT) scan was requested. It showed a rounded formation (16 × 15 mm) of regular and sharp margins, with the typical density of the soft tissues, in the left retroperitoneal site between the spleen and left kidney, closely adherent to the posterior diaphragmatic profile. No other abnormalities were found. Therefore, a positron emission tomography–CT scan (PET-CT scan) was requested. No pathological uptakes were detected.

The patient underwent a primary cytoreduction, consisting of hysterosalpingo-oophorectomy, partial omentectomy, and resection of the diaphragmatic nodule.

Neither microscopic nor macroscopic neoplastic lesions were found at the pathological examination; based on hematoxylin and eosin-stained slides, the pathologist found a microscopic focus of STIC in the fimbria of the left fallopian tube (Figure 2). The epithelium of the lesion showed some degree of stratification, and its cells were characterized by irregular luminal borders; small groups of exfoliated neoplastic cells were found in the fallopian

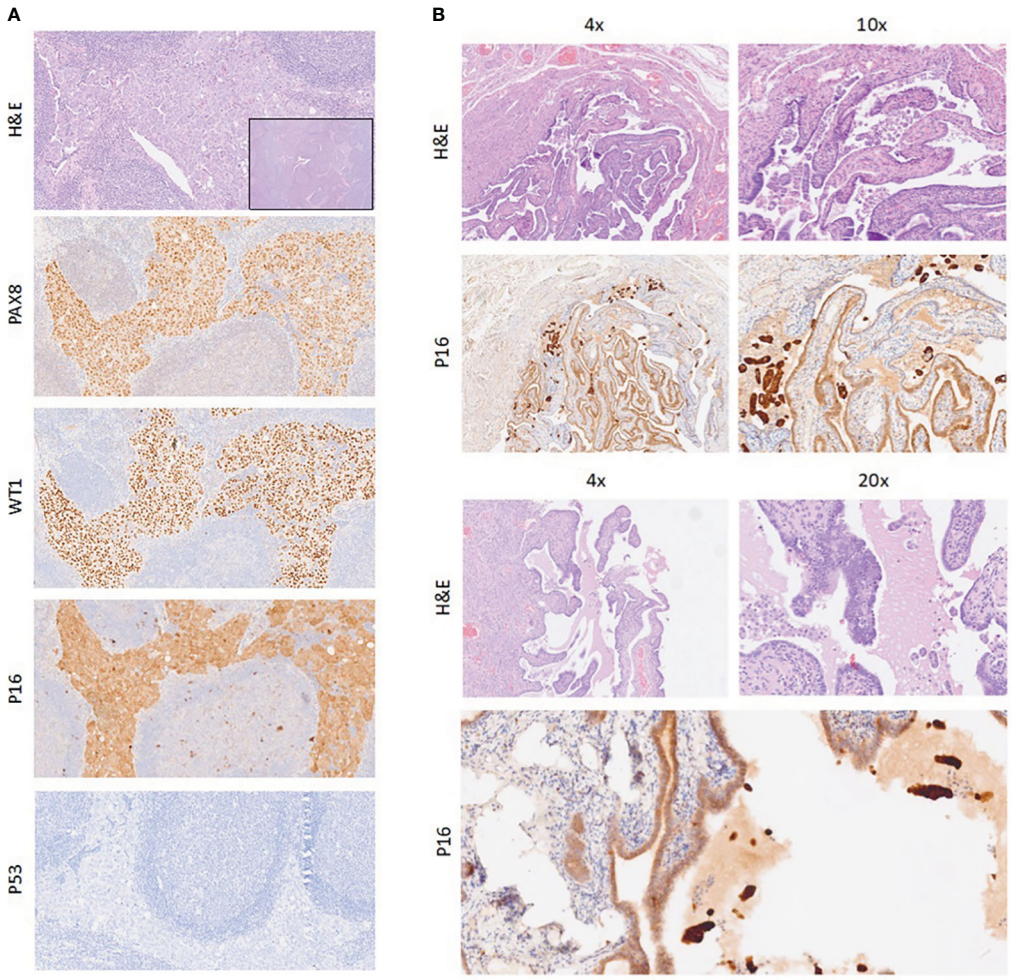

FIGURE 2
(A) Neoplastic cells in lymph node (H&E); on immunohistochemical stains, neoplastic cells are positive for PAX8, WT1, and p16 and negative for p53. Magnification, ×10. Inset: magnification, ×4. **(B)** Sections of fallopian tube with STIC and small groups of neoplastic cells exfoliated in the lumen (H&E); on immunohistochemical staining, neoplastic cells are positive for p16. Magnification, ×4, ×10, and ×20. STIC, serous tubal intraepithelial carcinoma.

tube lumen, near the STIC focus. Multiple sections were made to exclude stromal invasion. The immunohistochemical features were similar to those observed in the lymph node metastases. No other sites of disease were found.

Finally, the case was considered as an occult non-invasive tubal carcinoma (STIC) presenting with a distant inguinal lymph node metastasis. Additional investigation showed no BRCA 1 or 2 mutations. Homologous recombination deficiency (HRD) status assessed by SOPHiA DDM Dx HRD Solution® was undetermined.

According to the decision of the gynecological oncology multidisciplinary team, the patient was scheduled for six cycles of weekly carboplatin–paclitaxel-based chemotherapy every 3 weeks (Figure 3).

Discussion

This case shows a HGSOC diagnosis clinically presented with a single inguinal lymphadenopathy in the absence of heteroplastic lesions in the uterus and ovaries. The rarity of this case is the singular presence of a STIC lesion in the fimbria of the left fallopian tube with no other concomitant lesions. Patients with ovarian cancer often present with metastatic disease (8). HGSOC appears to arise from either the ovarian surface epithelium or the fallopian tube epithelium. To establish the site of origin, extensive examination of the adnexa is required (ovaries, fallopian tubes, and their fimbriae) (9).

Isolated inguinal lymph node metastasis is an uncommon manifestation of ovarian carcinoma. Only a few cases in which inguinal lymphadenopathy was the clinical manifestation of an epithelial ovarian tumor were described in the medical literature (7, 10–15).

However, lymphatic involvement is usual in ovarian carcinoma; it is reported in approximately 14%–70% of patients and mostly in the pelvic and para-aortic areas (6) (see Figure 1).

According to Kleppe et al. work (10), the possibility of a rare inguinal nodal involvement from ovarian carcinoma is based on two lymphatic drainage pathways. The two major pathways include the lymphatic drainage via the infundibulopelvic ligament toward para-aortic lymph nodes and the lymphatic drainage via the ovarian ligament to the obturator lymph nodes and the internal iliac artery. A minor drainage route goes through the round ligament and reaches the inguinal lymph node; this could explain the inguinal lymph node involvement in the absence of para-aortic or pelvic lymphadenopathies.

It has been postulated that previous abdominal surgery may lead to anatomical modifications that could favor the spread of the tumor to the groin region (11, 12). It could be assumed that in these patients, previous surgery could have a role in the tumor spread. However, in our case, the patient did not undergo intestinal or gynecological surgery before diagnosis.

About the case we report, the only finding at the histological examination after surgery was a STIC focus in the fimbria of the left fallopian tube. We suggest that this may be the precursor of HGSOC metastases in the inguinal lymph node.

Clinical evidence supported the hypothesis that STICs can arise from epithelial cells of the fallopian tube and transform into HGSOC by rapidly disseminating to involve the ovarian and peritoneal areas (16).

STIC are lesions with p53 mutations and increased proliferative capacity, and they are observed in at least 60% of women with HGSOC of the ovary and/or peritoneum (16). p53 aberrant expression may be defined by three different patterns: 1) strong and diffuse staining in at least 80% of cells, 2) no expression (with an intact internal control), and 3) cytoplasmic staining with weak nuclear staining (rare) (17).

Clinical evidence supports the hypothesis that STICs can arise from epithelial cells of the fallopian tube and transform into HGSOC by rapidly disseminating to involve the ovarian and peritoneal areas (16). Given the ability of STIC to spread beyond the fallopian tube without invasion of underlying stroma, the term carcinoma *in situ* should be abandoned, as it implies that there is no potential for metastasis. Histologically, STIC is the earliest morphologically recognizable form of tubal carcinoma. STIC is characterized, as previously stated, by the absence of invasion of underlying fallopian tube stroma and by the presence of cytologic abnormalities, which give the involved epithelium a darker

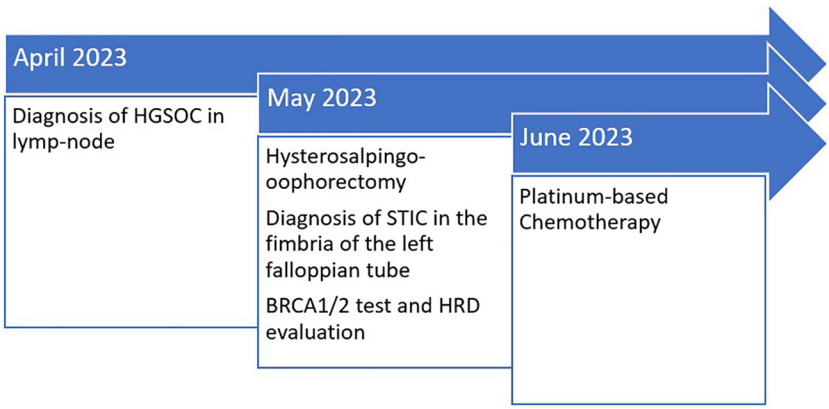

FIGURE 3
Timeline table.

appearance at low-power magnification compared with the adjacent normal epithelium. In cases with invasive carcinoma in the same tube, STIC may be found directly adjacent to the invasion.

A meta-analysis of 3,121 patients with BRCA 1/2 pathogenic variant who underwent risk-reducing salpingo-oophorectomy (RRSO) showed that the 5- and 10-year risk to develop peritoneal carcinomatosis (PC) was 10.5% and 27.5%, respectively, in patients with STIC, whereas the corresponding risk was 0.3% and 0.9%, respectively, for women without STIC at RRSO (18). Among them, 11 women with STIC who received chemotherapy did not develop PC. Four patients received chemotherapy because of positive peritoneal washing; the other seven patients received chemotherapy depending on a subjectively increased risk of PC, age at RRSO, or the histologic features of the STIC itself. Considering that chemotherapy has serious adverse effects, additional prospective evidence should be provided before this treatment is recommended, evaluating its benefits by each case and the data of the results this treatment had on PC risk throughout follow-up (18).

Przybycin et al. study (19) documented STIC in 61% of sporadic advanced HGSOC submitted for histologic examination through a specific protocol for sectioning and extensively examining the fimbrial end of the fallopian tube (SEE-FIM protocol).

The clonal relationship between STIC and concurrent HGSOC may be investigated by genomic analysis. Mutational evaluation of pelvic HGSOC with concomitant STIC has revealed that both lesions had identical TP53 mutations in most cases (20, 21). TP53 gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. Approximately 97% of extrauterine HGSOCs exhibit TP53 mutation (22).

Since finding the same mutation that occurs simultaneously in different sites is extremely rare, matching the TP53 mutation in different locations is clear evidence of clonal identity (20–22).

According to Singh N. et al., most extrauterine high-grade serous cancers arise in the distal fallopian tubes rather than the ovary, developing from STIC, a small precursor lesion (23).

A retrospective study of 231 patients detected STIC in 68.4% of all HGSOCs. Specifically, only two of them (1.26%) were affected by pelvic and para-aortic nodal metastases without any other intra-abdominal involvement, while in the majority of women, peritoneal spread was present (13.9%) (24).

Only three cases of HGSOC presenting with isolated inguinal lymph nodes with unknown primary origin have been published (Table 1).

Carrabin et al. (13) supported the possibility of the presence of ectopic ovarian tissue because the tumor was completely surrounded by normal ovarian tissue at the final histological examination. Dam et al. (14) described a patient with a history of hysterectomy for benign pathology, with an enlarged inguinal right lymph node; after surgery, a diagnosis of a nodal metastasis of a serous high-grade papillary cancer, most likely with ovarian origin, was made. Bilateral salpingo-oophorectomy was performed, but pathological examination could not identify a primary tumor. Restaino et al. (15) described a case of a 78-year-old woman who was brought to the physicians' attention because of an enlarged right inguinal lymph node. The diagnosis of metastasis from HGSOC was made. The patient underwent bilateral salpingo-oophorectomy and peritoneal biopsies. The final pathology examination did not reveal any evidence of disease. The patient received six cycles of carboplatin and paclitaxel. After 1 year, the patient developed a left inguinal enlarged bulky node of 4 cm recurrence.

To explain the absence of a primary site cancer, some authors suggest that the immune defense mechanisms of the host destroyed the primary tumor without affecting the lymphatic metastasis (25).

Finally, diffuse and strong p16 expression may be expressed in STIC lesions with similar patterns in many HGSOCs.

In the present case report, both STIC and lymph nodal lesions had the same p53 aberrant pattern (consisting of loss of expression —p53 null mutation) and p16 positive staining.

These pathological findings support the hypothesis of the origin of the metastatic lymph node from the STIC as reported in this paper. Notably, considering the exfoliation process and the non-invasive attitude of STIC, we expected a peritoneal dissemination rather than a lymphatic spread.

Conclusions

According to the literature, very few cases reported inguinal metastasis of HGSOC with unknown primary origin. Isolated ovarian metastases of inguinal lymph nodes remain rare.

Reviewing the literature on this topic, the hallmark of this case is the presence of concomitant STIC lesion and distal nodal disease in the absence of pathological adnexal or peritoneal neoplastic involvement.

Moreover, immunohistochemistry and morphological features suggested that STIC and HGSOC are not linked to a primary tumor, raising the hypothesis that STIC might be considered not just a

TABLE 1 Reported cases of inguinal lymph node metastasis of HGSOC with unknown primary site origin.

Author	Age	First diagnosis	Side	Histology	CA125	Previous abdomen surgery
Carrabin et al. (13)	59	Inguinal lymph node mtx	Right	Borderline tumor	/	Appendicectomy
Dam et al. (14)	62	Inguinal lymph node mtx	Right	HGSOC	3,628	Hysterectomy
Restaino et al. (15)	78	Inguinal lymph node mtx	Right	HGSOC	/	Hysterectomy

HGSOC, high-grade serous ovarian carcinoma; mtx, metastasis.
The symbol "/" means "not reported".

simple precursor of ovarian cancer but a carcinoma with a capacity for metastasizing.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not contain any research analysis on humans or animals.

Author contributions

PG: Conceptualization, Investigation, Writing – original draft. CT: Data curation, Writing – review & editing. GB: Conceptualization, Validation, Writing – review & editing. AP: Data curation, Writing – review & editing. GP: Data curation, Writing – review & editing. GD: Resources, Writing – review & editing. DS: Supervision, Writing – review & editing, Validation. FT: Formal analysis, Methodology, Writing – review & editing.

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EDITED BY
Mignon Van Gent,
Amsterdam University Medical Center,
Netherlands

REVIEWED BY
Federica Perelli,
Azienda USL Toscana Centro, Italy
Wuliang Wang,
Second Affiliated Hospital of Zhengzhou
University, China

*CORRESPONDENCE
Tianmin Xu
xutianmin@126.com

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New insights about endometriosis-associated ovarian cancer: pathogenesis, risk factors, prediction and diagnosis and treatment

Biqing Chen, Liping Zhao, Rulin Yang and Tianmin Xu*

Department of Obstetrics and Gynecology, The Second Hospital of Jilin University, Changchun, China

Previous studies have shown that the risk of malignant transformation of endometriosis in premenopausal women is approximately 1%, significantly impacting the overall well-being and quality of life of affected women. Presently, the diagnostic gold standard for endometriosis-associated ovarian cancer (EAOC) continues to be invasive laparoscopy followed by histological examination. However, the application of this technique is limited due to its high cost, highlighting the importance of identifying a non-invasive diagnostic approach. Therefore, there is a critical need to explore non-invasive diagnostic methods to improve diagnostic precision and optimize clinical outcomes for patients. This review presents a comprehensive survey of the current progress in comprehending the pathogenesis of malignant transformation in endometriosis. Furthermore, it examines the most recent research discoveries concerning the diagnosis of EAOC and emphasizes potential targets for therapeutic intervention. The ultimate objective is to improve prevention, early detection, precise diagnosis, and treatment approaches, thereby optimizing the clinical outcomes for patients.

KEYWORDS

EAOC, ovarian cancer, pathogenesis, diagnosis, treatment

1 Introduction

Endometriosis is a persistent, non-malignant inflammatory ailment that is subject to estrogenic influence and frequently manifests in conjunction with chronic pelvic pain, dysmenorrhea, and infertility. It is estimated to impact around 5-15% of women in their reproductive years (1). Although endometriosis is typically categorized as a benign condition, it exhibits biological characteristics akin to malignant tumors, including rapid growth, extensive proliferation, angiogenesis. A previous cohort study has shown that the prevalence of ovarian cancer in women with endometriosis is 1.37 times higher compared to the general population (2). Furthermore, previous research has indicated that the

occurrence of malignant transformation in premenopausal women with endometriosis is approximately 1%, while the likelihood of malignant transformation in postmenopausal women ranges from 1-2.5% (3). The connection between ovarian endometriosis and EAOC is believed to be established through the development of endometrial cysts within the ovary (4). Atypical endometriosis (AE) serves as an intermediary stage in which benign lesions evolve into malignant lesions. Women who have a prolonged history of endometriosis are at a heightened risk of developing EAOC, especially if the duration of the disease surpasses 10 years after the initial diagnosis of endometriosis or if there is a frequent occurrence of ovarian endometriosis (5). It is widely acknowledged that the occurrence of EAOC is atypical in instances of ovarian endometriosis, particularly in the clear cell and endometrial subtypes (6, 7).

In 1925, Sampson first outlined the diagnostic criteria for EAOC (8). The etiology of EAOC is commonly ascribed to a variety of complex pathogenic factors, such as endocrine dysregulation, oxidative stress, immune dysregulation, and intricate changes in immune surveillance, ultimately resulting in chronic inflammation (9). The primary objective of this article is to present a thorough examination of the recent progress made in comprehending the pathogenesis of endometriosis malignant transformation. Furthermore, it will explore the most recent research pertaining to the identification of early-stage EAOC, with the ultimate aim of improving prevention, early detection, precise diagnosis, and treatment approaches. We conducted a comprehensive search of the pubmed database to identify research articles pertaining to endometriosis-associated ovarian cancer (EAOC) within the last five years. The search terms employed were “endometriosis malignant transformation” and “endometriosis-associated ovarian cancer.” Only articles presenting complete experimental data and conclusive findings were considered for inclusion, while those with ambiguous or inconclusive research outcomes were excluded.

2 Pathogenesis of EAOC

2.1 Abnormal expression of related genes

Multiple studies suggest that ARID1A may act as a tumor suppressor (10). In their study, Guan et al. made the significant finding that ARID1A operates as a tumor suppressor and engages in an interaction with the P53 protein, thereby impeding cell proliferation through the p53-dependent transcriptional regulation of CDKN1A and SMAD3. The mutations in P53 or ARID1A impede the transcription of tumor suppressors, thereby causing uncontrolled cell proliferation and ultimately resulting in EAOC (11). Recent genomic research and targeted analysis have unveiled frequent mutations in the ARID1A and PIK3CA genes in ovarian clear cell carcinoma, with moderate mutations observed in PPP2R1A and KRAS (12). Similarly, endometrial carcinoma has been discovered to manifest mutations in PTEN, CTNNB1, and KRAS (13). These findings, when amalgamated with gene expression profiling, suggest the activation of the KRAS and PI3K survival pathways and the deactivation of tumor suppressor genes PTEN and ARID1A in clear cell and endometrioid ovarian cancers. Moreover, it is noteworthy that the lack of ARID1A expression, as detected by immunohistochemical analysis, could potentially be associated with ARID1A truncating mutations (14).

Furthermore, the lack of p53 has been observed to lead to an exaggerated proliferation of endometrial glands (15). ARID1A mutations has been hypothesized that this mutation plays a pivotal role as an initial molecular event in the progression of EAOC (16). Prior research has suggested that the presence of ARID1A somatic mutation and subsequent absence of BAF250a protein do not demonstrate a correlation between endometriosis and the ovarian response to chemotherapy (6). The presence of BAF250a is highly correlated with the early stages of carcinogenesis in endometriosis. The lack of ARID1A has been associated with a higher presence of CD8+ tumor-infiltrating lymphocytes (TILs) and intratumoral CD8+ immune cells in EAOC, suggesting the potential effectiveness of targeted immunotherapy in this specific context (17). Furthermore, it has been suggested that the inclusion of supplementary driver events may be imperative for the transformation of ovarian endometriosis with ARID1A loss-of-function mutations (18).

Multiple studies have provided evidence of an increase in the copy number of the CCNE1 gene and an up-regulation of CCNE1 in ovarian clear cell carcinoma. Cyclin E1, in conjunction with the regulatory subunit cyclin-dependent kinase 2 (Cdk2), plays a crucial role in facilitating the transition of the cell cycle from the G1 phase to the S phase. While normal cells tightly regulate cyclin E1 activity, cancer cells exploit its upregulation to enhance the replication of tumor cells. This phenomenon is particularly observed in clear cell carcinomas within EAOC (19).

The frequent activation of the PI3K/AKT pathway in endometrioid and ovarian clear cell carcinomas is a result of mutations in PIK3CA, AKT, and PTEN, leading to their inactivation (20). The presence of PIK3CA mutation, which activates the PI3K/AKT pathway, and the loss of PTEN expression have been extensively documented in around 33 to 40% of ovarian clear cell carcinomas and 40% of endometrioid carcinomas (21, 22). Guan et al. demonstrated that alterations in the PI3K/PTEN/AKT pathway are necessary prerequisites for promoting tumor progression (11). In a separate publication, Gounaris et al. identified the inactivation of the PIK3CA-mTOR and RAS-RAF-MAPK pathways in the eutopic endometrium of endometriosis as a significant contributing factor to the malignant transformation associated with endometriosis (23). Previous studies have provided evidence indicating the advantageous role of Met gene amplification in promoting the malignant transformation of endometriosis. The Met/PI3K/AKT pathway signal plays a significant role in the progression of malignant transformation. Therefore, targeted inhibition of the Met pathway emerges as a potentially promising therapeutic approach for EAOC (24).

The early progression of endometriosis involves the inactivation of the tumor suppressor gene protein phosphatase and tension homologue (PTEN) at locus 10q23.3, as identified in previous research (25). This inactivation is a result of the loss of heterozygosity at locus 10q23.3 and mutation of PTEN, subsequently leading to the activation of the phosphatidylinositol 3-kinase (PI3K) -protein kinase B (AKT) -mammalian target of

rapamycin (mTOR) signaling pathway (26). In the context of endometriosis, atypical endometriosis, and EAOC, the frequent occurrence of loss of heterozygosity resulting in PTEN inactivation suggests a potential continuum between endometriosis and ovarian cancer. Moreover, the presence of somatic mutations in the PTEN gene is highly prevalent in ovarian endometrioid adenocarcinoma, but uncommon in other pathological subtypes (27). Consequently, PTEN has the potential to function as a distinctive molecular alteration in EAOC.

The upregulation of Fibroblast growth factor receptor 2 (FGFR2) expression in ovarian endometriosis demonstrates aberrant elevation during the progression towards malignancy (28). This anomalous expression can be attributed to the occurrence of alternative splicing events within the FGFR2 gene, specifically involving the epithelial FGFR2IIIb subtype (encoded by exon 8) and the mesenchymal FGFR2IIIc subtype (utilizing exon 9). Furthermore, Steele et al. have demonstrated that ligands for FGFR2IIIb have a notable impact on various phenotypes that play a critical role in the growth of epithelial ovarian cancer cells (29). Furthermore, it has been postulated that autocrine FGF7 and paracrine FGF10 signaling cascades could be involved in the augmented epithelial differentiation observed during the course of malignant transformation. Specifically, the upregulation of FGFR2 expression holds the capacity to trigger excessive FGFR2 signal transduction, potentially playing a role in the pathogenesis of endometriosis. Moreover, targeting FGFR2 may present a promising therapeutic strategy for impeding the malignant advancement of endometriosis-associated cancer (refer to Table 1).

2.2 Genetic regulation of miRNA

MicroRNAs (miRNAs) are essential regulators of gene expression. They play a crucial role in functioning as either oncogenes or tumor suppressor genes. Conserved non-coding RNAs, which serve as regulators of target mRNA expression or degradation, have been recognized as potentially influential factors in the malignant transformation of endometriosis (30). As a result, these microRNAs (miRNAs) show potential as biomarkers for both endometriosis and EAOC. The simultaneous evaluation of multiple biomarkers can greatly improve the prognostic predictive value, indicating that a panel of miRNAs may offer a more dependable indicator of disease.

The miR-200 family, particularly miR-200-a and miR-200-b, have garnered significant attention in the field of endometriosis research. Notably, Ohlsson et al. conducted a study that demonstrated a noteworthy decrease in the expression of the miR-200 family, which subsequently led to the occurrence of epithelial-mesenchymal transition, a distinctive hallmark of endometriosis (31). The reduction in ARID1A expression may play a crucial role in the advancement of EAOC in patients who display heightened levels of miR-221 and miR-222 (20). Additional research is necessary to investigate the potential of miR-222 and miR-221 as biomarkers for EAOC. Furthermore, it was observed that miR-143 exhibited upregulation in the serum of patients with EAOC, thereby correlating with heightened cell invasion and migration. This augmented expression of miR-143 consequently results in the suppression of transcription of its target gene FNDC3B, a known facilitator of cell invasion and migration (32).

The association between the cycle of endometriosis and biomarker miR-20a has been extensively studied. Research has provided evidence for the significant role of miR-20a in the pathogenesis of endometriosis, as it directly targets TGF-β and Il-8 (33). A decrease in miR-20a expression results in elevated levels of these cytokines, which may contribute to the promotion of inflammation and tissue repair. By targeting miR-20a to inhibit TGF-β and Il-8, a better understanding of the development of endometriosis lesions could potentially be achieved. It is worth mentioning that miR-20a exhibits up-regulation in ovarian tissues of individuals diagnosed with ovarian endometriosis, thereby playing a role in neovascularization (34). Furthermore, the down-regulation of several mirnas, such as miR-3613-5p, miR-6755-3p (35), let7b, miR-125a (36), and others, has been observed in EAOC tissues. The investigation has provided evidence that miR-191 plays a direct

TABLE 1 Pathogenesis of ovarian cancer associated with endometriosis: abnormal expression of related genes.

Gene	Mechanism	Results	References
ARID1A	Mutation deactivation	Inhibition of the transcription of tumor suppressor factors allows cell proliferation	(10, 11)
PIK3CA	Mutation	The activation of PI3K survival pathway	(12)
KRAS	Mutation	The activation of KRAS survival pathway	(12, 13)
PTEN	Inactivation of tumor suppressor gene mutations	Proliferation of cells	(13, 25–27)
PPP2R1A	Mutation	Proliferation of cells	(12)
CTNNB1	Mutation	Proliferation of cells	(13)
P53	Deletion	Proliferation of cells	(15)
BAF250a	Deletion	It is involved in the early carcinogenesis process	(6)
ARID1A	Deletion	Mismatch repair deficiency and increased CD8+ tumor-infiltrating lymphocytes	(17)
CCNE1	A rise in gene copy number increase and CCNE1	It is involved in cell cycle regulation	(19)
AKT	PI3K/AKT pathway activation	Proliferation of cells	(20–22)
Met	Gene amplification	Met/PI3K/AKT pathway activation	(24)
FGFR2	High expression	Autocrine FGF7 and paracrine FGF10 signal ring	(28, 29)

regulatory role in the expression of TIMP3, thereby influencing cellular proliferation and invasion. TIMP3, a pro-apoptotic protein, exhibits an inverse correlation with cell growth and invasion (37, 38).

2.3 Oxidative stress

The recurrent hemorrhaging and accumulation of heme and free iron within endometriotic lesions are hypothesized to exert a substantial influence on the initiation of ovarian cancer, primarily through the production of reactive oxygen species (ROS) (39). Yamaguchi et al. have reported the high concentration of iron in endothelial cell fluid, leading to the induction of oxidative stress (5, 40). Recent studies have underscored the importance of the interaction between oxidative stress and non-coding miRNAs in the advancement of EAOC (41). *In vitro* investigations have revealed that endometriotic cyst contents manifest an elevated production of ROS and a heightened inclination to elicit gene mutations in comparison to other cyst contents (5). Correspondingly, Sanchez et al. have observed the existence of markers denoting oxidative damage, such as strand breaks, DNA adducts, and lipid peroxidation products, in ovarian cancer tissues (42, 43). The gene expression profile obtained from microarray analysis further substantiates the correlation between oxidative stress and ovarian cancer, particularly in the context of clear cell carcinoma progression (44).

A considerable percentage of the genes displaying elevated expression levels in ovarian clear cell carcinoma are linked to redox processes, including oxidative and detoxification enzymes (45). HNF-1β, acting as a transcription factor, exerts control over target genes responsible for encoding proteins involved in vital cellular processes such as proliferation, differentiation, glucose metabolism, dysplasia, and glycogen synthesis (46). In the domain of ovarian cancer, Liu et al. conducted a study employing the cut HNF-1 beta shRNA strategy, which exhibited heightened susceptibility of ovarian cancer cells to cisplatin and paclitaxel-induced cytotoxicity, both *in vitro* and *in vivo* (47).The accumulation of excessive free radicals can lead to cellular harm and eventual cell demise, whereas the persistent exposure to sublethal ROS, combined with an improved antioxidant status, has the potential to amplify the tumorigenicity of endometriotic cells (48). In a specific study, the utilization of enzyme-linked immunosorbent assay was employed to examine cyst fluid samples collected from a total of 44 patients diagnosed with ovarian endometriosis (OE) and 14 patients diagnosed with EAOC. The expression level of HO-1 is notably reduced in the EAOC group in comparison to the benign OE group, as indicated by the diminished presence of 8-hydroxy deoxyguanosine (8-OHdG) in the fluid. In contrast, the EAOC group demonstrates heightened levels of antioxidants and heme iron in the fluid in comparison to the OE group. It is worth mentioning that HO-1 exhibits the most significant diagnostic efficacy in discerning between benign and malignant cystic fluid, indicating a robust correlation between REDOX imbalance and the malignant progression of endometriosis (49).

The isoforms of GSTM1 are essential in the process of detoxifying harmful substances. Individuals without GSTM1 may have a greater risk of malignant transformation in endometriotic lesions due to insufficient elimination of oxidative stress products (50). Hydroxy-2'-deoxyguanosine (8-OHdG) has emerged as a potential biomarker with promise for evaluating oxidative DNA damage in various disease states. Within the specific context of endometriotic tissues, the up-regulation of 8-OHdG expression has been observed in EAOC when compared to OE. Additionally, CD44, a cell surface receptor responsible for binding to hyaluronic acid, has been demonstrated a potential protective function against DNA damage induced by ROS. The increased production of reduced glutathione synthesis, is accountable for the activation of CD44, specifically the variant isoform (CD44v). In contrast to OE and EAOC endometriotic tissues, a decrease in CD44v expression is evident in EAOC tumor tissues. This decrease, coupled with alterations in CD44v and 8-OHdG, could potentially be associated with the malignant progression of endometriosis (51). The findings of previous studies have provided evidence that electron microscopic replicas of malignant endometriosis cells display mitochondrial swelling and vacuolar alterations, which suggest the possibility of endometriosis lesions growing in a hypoxic microenvironment. These observations imply that the adverse impact of hypoxia on mitochondria could potentially contribute to an increased probability of malignant transformation (52).

2.4 Abnormal gene methylation

Epigenetic modifications, including DNA methylation, histone modifications, and noncoding microRNAs, have emerged as noteworthy factors in the development of EAOC (53), exerting regulatory influence on gene expression independent of alterations in the DNA sequence. Among these modifications, DNA methylation has been extensively studied, with the DNA methyltransferase (DNMT) family playing a pivotal role. Aberrant gene expression and subsequent tumorigenesis can be facilitated by low levels of methylation in cancer gene promoter regions (54). Various studies have demonstrated the involvement of specific genes, such as E-cadherin (CDH1), p16, PTEN, and PTEN hypermethylation in the promoter region, in promoting the malignant transformation of endometriosis (55, 56).

On the other hand, the anomalous hypomethylation of the promoter regions of long interspersed element-1 (LINE-1) (57) and syncytin-1 (58) has been linked to the malignant conversion of endometriosis. The elevated methylation of the hMLH1 promoter region results in the lack of hMLH1 protein expression, a vital constituent of the DNA mismatch repair (MMR) system. This deviation is highly correlated with the malignant advancement of endometriosis (59). The combination of Methylated CpG island amplification and representative difference analysis (DDA) has enabled the discovery of nine candidate genes, namely RASSF2, SPOCK2, RUNX3, GSTZ1, CYP2A, GBGT1, NDUFS1, ADAM22, and TRIM36, that exhibit distinctive methylation patterns associated with the malignant transformation of ovarian endometriosis (60). The transcription factor Runx-related transcription factor 3 (RUNX3), a member of the Runx protein family, plays a crucial role in regulating the self-renewal,

proliferation, and differentiation mechanisms (61). Nevertheless, the current literature presents contradictory results regarding the specific function of RUNX3 in ovarian cancer. For instance, Nevadunsky et al. reported a significant upregulation of RUNX3 and its involvement in promoting the proliferation of epithelial ovarian cancer cells (62). Moreover, Barghout et al. have provided evidence of a significant association between the upregulation of RUNX3 and resistance to RBMO chemotherapy in ovarian cancer cases (63). Conversely, alternative investigations have suggested that the hypomethylation and expression of the RUNX3 gene in epithelial ovarian cancer tissue and cell lines are linked to an unfavorable prognosis (64). Furthermore, these studies have corroborated a positive correlation between elevated RUNX3 methylation and the expression of ER alpha (65). One study proposes that the hypomethylation of the estrogen receptor (ESR) β promoter may potentially contribute to the development of progesterone resistance in individuals with endometriosis (66). Another study reveals a complete absence of ESR and PGR in the EAOC organization (67). However, the present study did not detect any significant alterations in the methylation of ESR and PGR genes when subjected to analysis using MCA - RDA (68).

The tumor suppressor gene RASSF2, which has been recently identified, exerts a notable influence on the Ras signaling pathways. Otsuka et al. have reported that the dysregulated activation of Ras genes leading to the upregulation of RASSF2 may play a pivotal role in the malignant transformation of endometriosis (69, 70). Moreover, Fauvet et al. have emphasized that the activation of the K-ras gene, an oncogene implicated in the Ras signaling pathway, may potentially manifest at a subsequent phase during the progression of malignant transformation in ovarian endometriosis (71). The findings of the study reveal a noteworthy discrepancy in the prevalence of RASSF2 promoter hypermethylation between tumor tissues and ectopic endometrial tissues, with a considerably higher incidence observed in the former. The results of this study indicate that the hypermethylation of the RASSF2 promoter, leading to epigenetic inactivation, may play a crucial role in the early stages of ovarian endometriosis progressing towards malignancy (60) (refer to Table 2).

2.5 Imbalance in hormonal regulation

The absence of progesterone protection in the context of persistent estrogen stimulation presents a potential hazard for the emergence of malignancy in endometriosis (72, 73). The probability of malignant transformation was found to be elevated, as evidenced by a previous investigation conducted by Lavery and Gillmer, wherein the administration of non-antagonistic estrogen as a therapeutic intervention resulted in the malignant transformation of residual ectopic endometrial lesions (74). Moreover, endometriosis fosters a microenvironment that facilitates the excessive accumulation of estrogen via diverse mechanisms (75). Although aromatase is usually not present in endometrial tissue, research has revealed heightened levels of aromatase enzyme activity in ectopic endometrial tissue. This activity facilitates the conversion of androstenedione and testosterone from the ovaries and adrenal glands into estrone and estradiol (E2) (25). Additionally, it is important to acknowledge that ectopic endometrial tissue lacks the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD), which is typically present in eutopic endometrial tissue. This enzyme plays a pivotal role in the conversion of E2 to estrone, a less potent variant of estrogen. Conversely, 17β-HSD is responsible for the conversion of estrone to the more potent E2, and this enzyme is present in endometriotic tissues. Consequently, the presence of 17β-HSD in endometriotic tissues leads to an augmented production and diminished inactivation of locally hyperestrogenic E2, thereby intensifying its cumulative impact (76). It is noteworthy to emphasize that an excess of E2 can stimulate cell proliferation by facilitating the production of cytokines, particularly IL-8 and RANTES (77). Moreover, the activation of E2 triggers the production of PGE2, thereby promoting the proliferation of tumors. Furthermore, it potentially augments the function of aromatase, thus establishing a reinforcing cycle that sustains the continuous accumulation of estrogen in endometriosis (25). An abnormal accumulation of estrogen in the local area contributes to the progression of normal ectopic endometrium towards dysplasia or potentially malignant transformation (2).

TABLE 2 Epigenetic modifications of gene methylation that occur during malignant transformation of endometriosis.

Gene	Mechanism	Results	References
E-cadherin gene	The promoter region was hypermethylated	Promote the malignant transformation of endometriosis	(55)
p16	The promoter region was hypermethylated	Promote the malignant transformation of endometriosis	(56)
PTEN	The promoter region was hypermethylated	Promote the malignant transformation of endometriosis	(56)
LINE-1	Low methylation in the promoter region of the	Malignant transformation of endometriosis	(57)
syncytin-1	Low methylation in the promoter region of the	Malignant transformation of endometriosis	(58)
hMLH1	The promoter region was hypermethylated	Loss of hMLH1 protein expression, malignant transformation of endometriosis	(59)
RUNX3	Hypermethylation	Poor prognosis	(64)
Estrogen receptor (ESR) beta	Low methylation	Absence of ESR	(66, 68)
RASSF2	The promoter region was hypermethylated	Inactivation of genes	(60)

In the context of EAOC, the endometrioid subtype is predominantly distinguished by the presence of estrogen receptor (ER) and progesterone receptor (PR) expression, while the clear cell subtype generally lacks ER or PR expression. The occurrence of oxidative stress and inflammation due to recurrent bleeding in endometriosis contributes to DNA methylation, which is linked to reduced ER expression (78–80). Previous studies have provided evidence suggesting that the classical ERα signaling pathway experiences significant inactivity during the transition from endometriosis to EAOC, as demonstrated by the downregulation of genes. In contrast, the gene expression of estrogen-associated ovarian cancer (EAOC) in patients with endometriosis demonstrates features of estrogen resistance, as indicated by notably reduced levels of estrogen receptor alpha (ERα) and progesterone receptor (PR), and elevated levels of estrogen receptor beta (ERβ) compared to individuals with normal endometrium. ERβis widely acknowledged for its antiproliferative properties and its antagonistic impact on ERα-mediated proliferation. The impact of ERαto ERβsignaling on the progression of EAOC from endometriosis is contingent upon the specific tissue context. Furthermore, the de-repression of ERαtarget genes, including FGF18, potentially plays a role in the transformation of endometriosis into EAOC (81).

There is a proposition that progesterone exhibits anti-inflammatory attributes within the endometrium. Prior research employing mouse models has provided evidence that inhibiting ERαorβisoforms, coupled with a concurrent decrease in inflammation, effectively hinders the progression of endometriosis (82, 83). Moreover, recent studies have unveiled a noteworthy association between IL-6 and E2 in the advancement of endometriosis (38). Studies have suggested that the estrogen - DNMT1 signaling pathways potentially contribute to the upregulation of RUNX3 methylation, consequently facilitating the malignant transformation of endometriosis (84). Furthermore, there is a suggestion that hormone replacement therapy (HRT) may have the potential to induce malignant transformation in women with a history of endometriosis (85). The risk of adverse effects increases with prolonged usage of hormone replacement therapy (HRT), especially when exceeding a duration of 10 years (86). It is worth noting that available evidence suggests that the use of estrogen alone carries a higher risk of endometriosis malignant transformation compared to the combined administration of estrogen and progestin (85). In contrast, the utilization of hormone replacement therapy (HRT) did not exhibit a heightened propensity for ovarian cancer in postmenopausal women with a medical history of endometriosis or the development of endometriosis (87).

2.6 Imbalance of immune regulation and inflammation

The findings from studies conducted on both human subjects and rats have demonstrated that endometriosis sites display a greater abundance of activated inflammatory cells and cytokines in comparison to the corresponding eutopic endometrium (88). The presence of acute and chronic inflammation is a distinctive characteristic of endometriosis, evident at different stages of tumor advancement, including initiation, malignant transformation, invasion, and metastasis, thereby exerting a substantial impact. Moreover, inflammation disrupts the body's immune surveillance, resulting in the infiltration of immune cells into tumor tissue and engaging in dynamic interactions with cancer cells.

The literature has provided evidence that in individuals with endometriosis, a notable increase in the population of activated macrophages has frequently been observed in the peritoneal fluid (89). Moreover, there has been an observed elevation in the concentration of various essential cytokines and chemokines, such as TNF alpha, beta, IL-1, IL-6, IL-8, regulated upon activation, normal T cell expressed and secreted (RANTES), and monocyte chemotactic protein 1. The chemotactic agent is present in the latter three, resulting in the accumulation of macrophages (90). Additionally, the presence of ferroportin was detected in the epithelium of ovarian endometrioma and clear cell ovarian cancer, while iron-coated M2 macrophages were identified in the stroma of these conditions. The infiltration of epithelial cells into the stroma of ovarian endometrioma suggests the potential participation of iron-coated M2 macrophages in the carcinogenic process of this ailment (91). Research on the quantity and characteristics of macrophages implicated in the malignant progression of endometriosis has consistently demonstrated a reduction in the expression of the antioxidant marker HO-1 in EAOC. This suggests that a diminished presence of M2 macrophages expressing HO-1 may play a significant role in promoting malignancy (92–94).

The substantial involvement of inflammatory mediators and diverse cytokines, including TNF-α, IL-1β, and IL-6, in the initiation, proliferation, and progression of epithelial ovarian cancer, akin to the observations made in endometriosis (95). Szlosarek et al. investigate the role of TNF alpha in the advancement of ovarian cancer, encompassing serous and clear cell subtypes, and observe heightened expression levels of TNF alpha in comparison to normal ovarian tissue. Moreover, previous studies have reported a substantial upregulation of TNF-α mRNA in cultured ovarian cancer cells (96). Further analysis of the same dataset has demonstrated that this increased expression of TNF network genes within the tumor microenvironment leads to augmented signaling pathways associated with inflammation, and NOTCH signaling (97). The observed up-regulation of small inducible cytokine A2 (SICA2) and small inducible cytokine subfamily A member 14 (CCL14) in endometriosis-associated endometrioid ovarian cancer suggests a notable contribution of inflammatory factors in the pathogenesis of both endometriosis and its associated endometrioid ovarian cancer. Prostaglandin E2 (PGE2), a pivotal mediator of the inflammatory response, has also been shown to exert influence on critical mechanisms linked to tumor growth, including cell proliferation, and inhibition of apoptosis (98).

The Nod-like receptor protein structure domain related protein 3 (NLRP3) inflammatory corpuscle is a multifaceted protein implicated in the innate inflammatory immune response. This

intricate assembly encompasses the NLRP3 protein, serving as a detector for inflammasome activation, and the apoptosis-associated speck-like protein containing the CARD complex (ASC). The ASC complex recruits pro-caspase via its CARD domain, thereby facilitating subsequent cascades. The precursor form of caspases is substituted by active caspases, leading to the cleavage of proinflammatory cytokines (precursors of IL-1β and IL-18) into their active states. IL-1β and IL-18, in turn, promote the recruitment of further immune cells associated with inflammation. As a result, the activation of this cancer gene takes place. Consequently, the persistent aseptic inflammation of the NLRP3 signaling pathway potentially functions as the primary phase of carcinogenesis (99). AIM2 functions as a cytoplasmic receptor that identifies double-stranded DNA, particularly originating from viral or bacterial origins, via its carboxyl end hin200 structure domain. This recognition event initiates a series of molecular processes, including the activation of inflammatory proteins and the assembly of AIM2 inflammatory corpuscles. The activation of AIM2 inflammasomes, in conjunction with other conventional inflammasomes, ultimately culminates in inflammatory cell death. In a comparative bioinformatics analysis of endometriosis and ovarian cancer, the immunohistochemical staining analysis further substantiated a robust association between elevated AIM2 expression and heightened Ki-67 activity in clinical samples of EAOC. This discovery lends support to the hypothesis that the alteration of AIM2 and the inflammatory corpuscle in EAOC significantly contribute to the regulation of disease progression.

Anomalous humoral immunity and complement activation significantly contribute to the pathogenesis of EAOC, with cell proliferation serving as a primary mechanism (9). Recent research indicates that there are multiple complement pathways present and operating within the tumor microenvironment, directly stimulating the proliferation of tumor cells and indirectly aiding in immunosuppression and neovascularization (100, 101). The study offers evidence that the activation of Kras and Pten tumor-driven pathways leads to the up-regulation of complement in epithelial cells. The aforementioned findings establish a novel association between the initiation of tumors and immune surveillance facilitated by complement. In conjunction with alterations in immune cells and cytokines, patients with endometriosis commonly manifest heightened activation of B cells. Previous research has established that individuals who have been diagnosed with endometriosis possess the ability to produce systemic antibodies and deposit immunoglobulin G (IgG) and complement in tissues as a humoral response to various autoantigens (102). The mechanism of antibody-induced complement mediated apoptosis efficiently eradicates cells through the classical pathway, which is partially triggered by the attachment of immunoglobulin Fc to infectious agents or diverse antigens found on apoptotic cells. Furthermore, the initiation of this alternative pathway can occur via sequential low-level cleavage of C3 and can be stimulated by various microorganisms such as bacteria, viruses, fungi, and tumor cells. The third complement activation pathway, referred to as the MBL pathway, is activated in response to pathogen-associated molecular patterns (103). Presently, ongoing clinical trials are investigating the focused inhibition of complement as a pharmacological intervention, and the results of these studies will contribute to the development of personalized treatment strategies for patients.

Modifications in immune surveillance might serve as an early indication of the development of cancer in benign conditions (104). Complement signaling can induce diverse immunosuppressive mechanisms, such as the regulation of CD4+ and CD8+ T lymphocytes (105). Furthermore, *in vivo* studies have confirmed the synergistic antitumor impact achieved by combining complement component fragment 5a receptor signaling blockade with PD-L1 antibody, highlighting its reliance on CD8+ T cells (106). On the other hand, the existence of infiltrating T lymphocytes (ITLs), including CD8 + T cells, regulatory T cells, regulatory B cells type II natural killer T cells, and Th2 type CD4 + cells, has been linked to tumor remodeling and potentially aiding tumor growth through immunosuppressive mechanisms (107). These cells possess the capability to hinder the host's anti-tumor response and stimulate angiogenesis within tumors. The impairment of immune cell function and aberrant expression of suppressor T cell response are widely recognized consequences of the interaction between programmed cell death protein-1 (PD-1) and its ligand, programmed cell death ligand-1 (PD-L1), in pathological conditions such as cancer and chronic infection (107). Studies have demonstrated that individuals with endometriosis display elevated levels of PD-1/PD-L1 expression in their circulatory system (108). Moreover, previous research has provided evidence indicating that the upregulation of PD-1/PD-L1 expression occurs in both eutopic and ectopic endometrial tissues among individuals with endometriosis (109). Nevertheless, the precise impact of these immune adaptations on the development and progression of ovarian cancer remains uncertain (108).

Women who have been diagnosed with endometriosis exhibit increased levels of proinflammatory substances, such as tumor necrosis factor-a, interleukin-1b, and interleukin-6. These factors may potentially play a role in the perpetuation of chronic inflammation, thereby promoting the progression and development of EAOC (110, 111). The identification of a heightened frequency of CD8+ cytotoxic T cells has emerged as a promising prognostic determinant in diverse tumor categories, encompassing ovarian cancer (112). These discoveries augment our comprehension of inflammation and immunity as plausible molecular biomarkers for monitoring the advancement of endometriosis towards malignancy, while also offering potential avenues for therapeutic interventions in instances of EAOC. Refer to Figure 1.

3 Prediction and diagnosis of malignant transformation risk factors

3.1 Organizing cytology diagnosis methods

The integration of conventional cytogenetic methods and advanced genetic detection techniques enables the identification of numerical and structural chromosomal abnormalities. However,

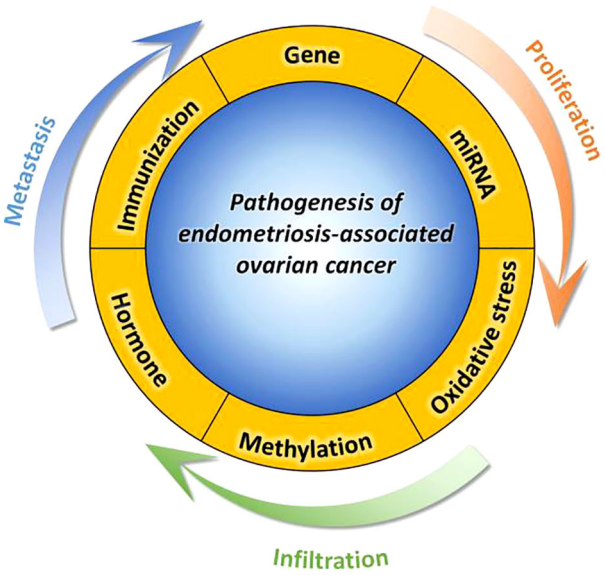

FIGURE 1
This paper aims to provide a comprehensive understanding of the molecular mechanism involved in the pathogenesis of EAOC by examining six distinct aspects, namely abnormal gene expression, miRNA genetic regulation, oxidative stress, abnormal gene methylation, hormone regulation imbalance, immune regulation imbalance, and inflammation.

current literature reports suggest that cytogenetic investigations of individuals with endometriosis often yield inconsistent results. The existing body of research indicates a widely accepted agreement that atypical endometrial hyperplasia encompasses both cellular and structural atypia. However, it is important to note that cellular atypia is more commonly observed in non-cancer patients, whereas structural atypia is more prevalent among patients diagnosed with endometrioid adenocarcinoma (113, 114).

3.2 Serological diagnosis methods

Recent retrospective studies have indicated that preoperative CA125 values are not effective in identifying the malignant transformation of endometriosis (115). Prior investigations have suggested that the assessment of CA19-9, CEA, SLX, and LDH serum levels holds promise as valuable indicators for distinguishing between ovarian tumors related to endometriosis and ovarian endometriosis itself in the preoperative evaluation (116). Arakawa et al. conducted a study in which they observed a specific elevation in serum levels of tissue factor pathway inhibitor 2 (TFPI2) in patients diagnosed with ovarian clear cell carcinoma within the subset of individuals with epithelial ovarian cancer (117). The correlation between CTNNB1 and elevated expression of the HIF1A gene suggests disease advancement, particularly during the initial phases (118). Moreover, the stimulation of AMP-activated protein kinase (AMPK) by TSPAN1 has been shown to promote the development of endometriosis and cellular proliferation (119). TSPAN1 has been recognized as a prospective gene candidate for the screening of high-risk endometriosis, thereby facilitating the advancement of therapeutic pharmaceuticals.

3.3 Imaging diagnostic methods

The ultrasonography assessment of diverse parameters indicates that the recognition of a "vascular solid component" facilitates a notably precise discrimination between benign and malignant endometrioid cysts (120, 121). The initial phase of EAOC may pose considerable diagnostic difficulties due to the lack of a mural nodule. A study has suggested that the identification of cyst wall nodules measuring over 1.5cm in height and with a maximum diameter surpassing 7.9cm could potentially serve as innovative diagnostic markers for distinguishing between EAOC and benign OE with wall nodules (122). Moreover, a retrospective case-control study revealed that several factors, including advanced age, menopause, weight loss, cyst diameter equal to or exceeding 8.33cm, and the presence of solid areas on ultrasonography, were identified as noteworthy risk factors for EAOC (123). Kobayashi et al. reported an increased vulnerability to malignancy in individuals aged 45 years or older, those undergoing menopause, and those with dimensions of 9cm or larger (124). Moreover, the existence of a solid component within the cyst increases the likelihood of developing ovarian cancer associated with endometrial cysts, in line with the findings presented by Kadan et al. (125). Notably, diagnostic studies employing MRI have demonstrated that EAOC typically manifests as a unilocular mass with a low T2WI signal within the cystic component (126). As a result, MRI shows potential as a valuable tool for distinguishing EAOC from non-EAOC and aiding in preoperative diagnoses.

3.4 The development of a diagnostic method

Yang et al. proposed a model that integrates the marker value HE4 and the ADNEX, resulting in increased the discriminatory ability and sensitivity for distinguishing benign from malignant ovarian tumors (127). The application of transvaginal near-infrared (NIR) imaging might provide diagnostic insights into the malignant advancement of endometriosis and could potentially yield further clinical ramifications, and the incorporation of MR relaxation measurements facilitates the identification of conservative therapeutic approaches (128, 129). A pioneering composite optical ultrasound system, employing near infrared guidance and transvaginal ultrasound, is proposed for the purpose of noninvasively quantifying fluid hemoglobin (Hb) levels. The results suggest that metHb is a common form of hemoglobin in benign endometriotic cysts, and the absorption ratio of cyst fluid at 620/580 nm demonstrates significant specificity and positive predictive value. Therefore, it can be utilized as a practical monitoring test for the prompt detection of malignant

transformation in endometriosis (130). Reducing the absorption rate at 620/580 nm could potentially facilitate the identification of individuals necessitating prompt monitoring and surgical intervention, thereby underscoring the significance of clinical assessment in cancer patients.

The Endometriotic Neoplasms Algorithm for risk Assessment (e-NARA) index provides a notable level of specificity in distinguishing between EAOC and benign endometriotic cysts (131). The assessment of intracytothelial iron concentration presents a valuable method for predicting and diagnosing EAOC. The application of proton transverse relaxation time (T2) and T2* (R2) and R2* and relaxation rate in magnetic resonance imaging and optical imaging, including magnetic resonance spectrometry, serves as the exclusive imaging technique (132) for the early anticipation of malignant transformation in molten iron and magnetic resonance (NMR) spectrophotometer. Regardless of age, menopausal status, and cyst size, EAOC exhibits lower R2 values and total iron levels compared to benign ovarian endometriosis cysts. The application of R2 values in distinguishing between EAOC and benign ovarian endometriosis cysts has demonstrated promising levels of accuracy, sensitivity, and specificity (133, 134). Numerous studies have retrospectively evaluated the effectiveness of the Copenhagen index (CPH-I), Risk of Ovarian Malignancy Algorithm (ROMA), and R2 prediction index in forecasting the malignant progression of OE. Notably, the CPH index has been identified as the most reliable predictor for postmenopausal patients with malignant tumors, while the R2 prediction index outperforms other indicators in distinguishing malignant tumors for premenopausal individuals (135). Machine learning algorithms have been employed for the purpose of constructing risk models with the objective of forecasting the probability of malignant transformation of endometriosis in patients (136).

4 Recent advances in EAOC related treatment

Currently, there is a dearth of established therapeutic interventions for EAOC gene mutations, whereas immunotherapy has exhibited effectiveness in the treatment of EAOC. Extensive clinical trials have been undertaken to investigate the possibility of inhibiting this pathway, encompassing inhibitors that target PI3K, AKT, and mTORC1 (137). In particular, Poly (ADP-ribose) polymerase (PARP) inhibitors have exhibited effectiveness in the treatment of ovarian cancer (138, 139). Anti-VEGF antibodies have been employed in the management of ovarian cancer, including EAOC (140). In a phase 2 clinical trial investigating the efficacy of nivolumab, an anti-PD-1 antibody, for the treatment of platinum-resistant ovarian cancer, the overall response rate was determined to be 15% (141, 142). Furthermore, Lynch syndrome, which is distinguished by germline mutations. Mutations in genes involved in mismatch repair result in a significant prevalence of microsatellite instability, which acts as a biomarker for vulnerability to immune checkpoint inhibitors (143). As a result, individuals diagnosed with clear-cell ovarian cancer associated with Lynch syndrome are more inclined to experience favorable outcomes with the administration of immune checkpoint inhibitors. Preclinical inquiries utilizing cell lines have substantiated the potential of inhibitors that target IL-6/JAK/STAT pathway as a means of therapeutic intervention (144, 145). Moreover, there have been documented reports suggesting that the administration of anti-IL-6 antibody to a mouse model of ovarian clear cell carcinoma leads to enhanced prognosis (146). Moreover, in a mouse model of ovarian clear cell carcinoma lacking the ARID1A gene, the efficacy of combination therapy comprising HDAC6 inhibitors and anti-PD-L1 antibody has been successfully demonstrated (147, 148).Reducing the generation of ROS could potentially aid in the prevention of the malignant progression of endometriosis (149). The findings of a study suggest that exploring the potential of Chk1 inhibitors as a targeted therapy may be a promising treatment approach for patients with clear-cell ovarian cancer, presenting a new opportunity for combination therapy (150).

5 Discussion

Long non-coding RNAs (lncRNAs) and post-translational modifications (PTMs), in the pathogenesis of both endometriosis and ovarian cancer has also been suggested (151). Due to the complex nature of this gynecological disorder and its strong association with tumorigenesis, the mechanisms underlying the origin and development of endometriosis are still not fully understood. Vicente Munoz et al. conducted a study in which they identified plasma metabolites in individuals diagnosed with endometriosis (152). The researchers observed heightened levels of valine, foci, choline-containing metabolites, lysine/arginine, and lipoproteins, while the concentrations of creatinine were relatively diminished compared to women without endometriosis (153). The study will contribute to our understanding of the development of malignant transformation. Additionally, it has the potential to provide a new and effective early diagnostic intervention, thereby improving the chances of successful treatment.

6 Conclusion

The specific mechanisms and strategies underlying carcinogenesis in EAOC remain unclear. Further research will contribute to a more comprehensive understanding of the progression of EAOC. Improving our understanding of the pathogenesis of EAOC will contribute to the identification of individuals most prone to the malignant transformation of endometriosis lesions. This knowledge will support the creation of efficacious preventive measures for women with endometriosis who are at the greatest risk of developing EAOC, as well as the

formulation of innovative therapeutic approaches for those diagnosed with EAOC.

Author contributions

BC: Writing – original draft. LZ: Conceptualization, Writing – review & editing. RY: Data curation, Writing – review & editing. TX: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Mihaela Carmen Cristea,
Regeneron Pharmaceuticals, Inc.,
United States

REVIEWED BY
Bojana Stefanovska,
The University of Texas Health Science Center at San Antonio, United States
Stergios Boussios,
Canterbury Christ Church University, United Kingdom
Gunjan Arora,
Yale University, United States

*CORRESPONDENCE
Nozomu Yanaihara
yanazou@jikei.ac.jp

†These authors have contributed equally to this work and share last authorship

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Bevacizumab increases the sensitivity of olaparib to homologous recombination-proficient ovarian cancer by suppressing CRY1 via PI3K/AKT pathway

Yasushi Iida[1], Nozomu Yanaihara[1*], Yuki Yoshino[2], Misato Saito[1], Ryosuke Saito[1], Junya Tabata[1], Ayako Kawabata[1], Masataka Takenaka[1], Natsuko Chiba[2†] and Aikou Okamoto[1†]

[1]Department of Obstetrics and Gynecology, The Jikei University School of Medicine, Tokyo, Japan, [2]Department of Cancer Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

PARP inhibitors have changed the management of advanced high-grade epithelial ovarian cancer (EOC), especially homologous recombinant (HR)-deficient advanced high-grade EOC. However, the effect of PARP inhibitors on HR-proficient (HRP) EOC is limited. Thus, new therapeutic strategy for HRP EOC is desired. In recent clinical study, the combination of PARP inhibitors with anti-angiogenic agents improved therapeutic efficacy, even in HRP cases. These data suggested that anti-angiogenic agents might potentiate the response to PARP inhibitors in EOC cells. Here, we demonstrated that anti-angiogenic agents, bevacizumab and cediranib, increased the sensitivity of olaparib in HRP EOC cells by suppressing HR activity. Most of the γ-H2AX foci were co-localized with RAD51 foci in control cells. However, most of the RAD51 were decreased in the bevacizumab-treated cells. RNA sequencing showed that bevacizumab decreased the expression of CRY1 under DNA damage stress. CRY1 is one of the transcriptional coregulators associated with circadian rhythm and has recently been reported to regulate the expression of genes required for HR in cancer cells. We found that the anti-angiogenic agents suppressed the increase of CRY1 expression by inhibiting VEGF/VEGFR/PI3K pathway. The suppression of CRY1 expression resulted in decrease of HR activity. In addition, CRY1 inhibition also sensitized EOC cells to olaparib. These data suggested that anti-angiogenic agents and CRY1 inhibitors will be the promising candidate in the combination therapy with PARP inhibitors in HR-proficient EOC.

KEYWORDS

ovarian cancer, olaparib, bevacizumab, homologous recombinant proficient, CRY1

Introduction

Poly-(ADP-ribose) polymerase inhibitors (PARPi) are orally active anticancer drugs causing synthetic lethality in cells with defects in homologous recombination (HR) DNA repair. PARP1 catalyzes the synthesis of poly-(ADP-ribose) (PAR) and transfers PAR to its substrates to enhance DNA single-strand break (SSB) repair (1). PARPi inhibit the catalytic activity of PARP1, resulting in delayed SSB repair, and trap PARP1 on SSBs, inducing DNA double-strand breaks (DSBs) and stalls of replication folks. The DSBs induced by PARPi would normally be repaired by HR. However, in cancer cells with HR deficient (HRD), the use of lower fidelity forms of DNA repair, such as non-homologous end-joining, significantly increases genomic instability, making repair unsustainable after multiple replications and resulting in tumor cell death (2).

The introduction of PARPi in clinical practice has greatly changed the management of patients with advanced high-grade epithelial ovarian cancer (EOC) in both first-line therapy and recurrent settings (3–8). Although PARPi are highly effective in treating EOC with HRD initially, virtually all patients develop resistance during over time (9). Additionally, EOC without HRD has primary resistance to PARPi and does not benefit from PARPi. Thus, a new strategy to overcome the resistance to PARPi is required.

A combination of PARPi and various chemotherapeutics or molecularly targeted agents has been developed to overcome the resistance to PARPi. Combinations of PARPi and chemotherapy, antiangiogenic agents, immune checkpoint inhibitors, tyrosine kinase inhibitors, and other inhibitors of DNA damage response are currently under investigation (10, 11). In particular, the combination of PARPi and antiangiogenic agents, including bevacizumab, has been extensively investigated. The phase III PAOLA-1 study of maintenance olaparib and bevacizumab in patients with newly diagnosed EOC demonstrated a substantial clinical benefit primarily in patients with HRD tumors (12). This led FDA approval of maintenance olaparib and bevacizumab only for EOC with HRD. Recent phase II clinical trials showed that the combination of PARPi and the antiangiogenic agent significantly improved progression-free survival (PFS) in patients with platinum-sensitive recurrent high-grade EOC compared with PARPi alone (13, 14). Interestingly, subgroup analyses of these trials showed that the improvement of PFS by the addition of antiangiogenic agents was independent of the HR status. These results showed that the combination of antiangiogenic agents and PARPi not only improves therapeutic efficiency in cancers with HRD but also sensitizes cancers without overt HRD to PARPi. However, the molecular mechanism of the improved therapeutic efficacy is unknown.

Antiangiogenic agents include antibodies against vascular endothelial growth factor (VEGF) or its receptor (VEGFR) and small-molecule inhibitors of VEGFR tyrosine kinase. These agents exert anticancer activity indirectly through the alteration in the endothelial function and directly by inhibiting the proliferation of signaling from VEGFR in cancer cells (15). Angiogenesis is essential for solid tumor growth and metastasis (16). VEGF and VEGFR are expressed at varying levels in EOC cells. Bevacizumab, a monoclonal antibody targeting VEGF-A, and cediranib, a small-molecule inhibitor targeting multiple factors, including VEGFRs 1–3 and c-kit, have demonstrated the antitumor activity in patients with EOC (17–20). At the time of this study, little is known about the role of the VEGF/VEGFR signaling pathway in HR. Recently, some genes and chemical agents that have not been considered to directly act on HR have been reported to affect HR activity. For example, inhibition of TTK protein kinase, which plays an important role in regulating spindle assembly checkpoint signaling, impaired HR in basal-like breast cancer cells (21), and a chemothrerapeutic agent paclitaxel, which exert its cytotoxic effect by arresting mitosis through microtubule stabilization, decease HR activity in HR-proficient (HRP) EOC cells (22). Thus, antiangiogenic agents may affect HR activity in EOC cells, which improves the sensitivity of these cells to PARPi.

This study aimed to investigate the molecular mechanism of the improvement of the antiproliferative effect by the combination of PARPi and antiangiogenic agents in EOC cell lines.

Materials and methods

Cell lines and reagents

OVSAHO, a high-grade serous ovarian cancer cell line, OVISE and OVTOKO, clear cell ovarian cancer (CCOC) cell lines, and TOV112D, an endometrioid ovarian cancer cell line, were purchased from the Japanese Collection of Research Bioresources Cell Bank, Osaka, Japan. Information on each cell line was obtained from DepMap Portal (https://depmap.org/portal/) and cBioPortal (https://www.cbioportal.org) in shown in Supplementary Table S1. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum. Bevacizumab, olaparib, and cediranib were purchased from Selleck Biotech, Houston, TX, USA. KS-15, a small-molecule inhibitor of cryptochrome circadian regulator 1 (CRY1), was purchased from MedChemExpress, Monmouth Junction, NJ, USA.

Cell viability assay

Cells were seeded in 96-well plates, incubated for 24 h, and treated with serially diluted olaparib with or without bevacizumab (20 μg/ml) or cediranib (5 μM) or KS-15 (20 μM). Cell viability was assessed after 6 days using the MTS assay. The MTS assay was performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, MTS solution was added to each of the 96-well plates and incubated for 1 h. Then, absorbance was measured at 490 nm using a microplate reader. Viability curves and the IC50 (half maximal inhibitory concentration) of each compound were calculated using GraphPad Prism 9 software (GraphPad Inc., San Diego, CA, USA). Reproducibility was confirmed by four independent experiments.

Cell proliferation assay

Cell proliferation assay was performed with olaparib (50 μM) with or without bevacizumab (20 μg/ml) or cediranib (5 μM) or KS-15 (20 μM). The cell proliferation of olaparib alone was used as a control and was compared to that of the addition of bevacizumab or cediranib or KS-15, respectively. The experiment was repeated four times.

Transfection

For the transfection of small interfering RNA (siRNA) alone and co-transfection of siRNA and plasmid, the Trans-IT X2 dynamic delivery system (Mirus BIO, Madison, WI, USA) was used according to the manufacturer's instructions. A predesigned siRNA targeting *CRY1* (Silencer Select Predesigned siRNA, Assay ID: s464) and a non-targeted control siRNA (Silencer Select Negative Control No. 1) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

HR activity assay

HR activity was analyzed using the Assay for Site-specific HR Activity (ASHRA) (23). Cells were seeded in 6-well plates, incubated for 24 h, and treated with bevacizumab (20 μg/ml), cediranib (5 μM), and siRNA targeting *CRY1*. The donor vector (Addgene ID: #169798), the expression vector for gRNA, and Cas9 (Addgene ID: #169795 and #169796) were transfected using Transporter 5 Transfection Reagent (Polysciences, Warrington, PA, USA) according to the manufacturer's instructions. After 48 h incubation, genomic DNA was extracted, and quantitative polymerase chain reaction (PCR) was performed on the StepOnePlus real-time PCR System (Applied Biosystems, Foster City, CA, USA) using Go Taq Green Master Mix (Promega). The knocked-in and control alleles were amplified with the following primer sets: 5'-GTCCTGCTGGAGTTCGTGACCG-3' and 5'-GTGCAATCAAAGTCCTCGGC-3' for the knocked-in allele and 5'-AGTTGCGTTACACCCTTTCTTG-3' and 5'-GTGCAATCAAAGTCCTCGGC-3' for the control allele. The relative quantity of the knocked-in allele was calculated using the $2^{-\Delta\Delta\ CT}$ method. Data were collected as the average values of each group and presented as mean ± standard deviation (SD). Each experiment was repeated at least three times.

Immunofluorescence staining

OVISE cells were seeded in 8-well chambered slides at a density of 5.5×10^3 cells per well, incubated for 24 h, and then treated with bevacizumab. Cells were irradiated with 2 Gy X-ray and fixed by chilled methanol 2 h after irradiation. After permeabilization by 1% TritonX-100 and blocking by a blocking solution in DNA Damage Detection Kit-gH2AX, the samples were incubated with primary antibodies diluted in a blocking solution in DNA Damage Detection Kit-gH2AX at 4°C overnight. Then, the samples were incubated with secondary antibodies diluted in a blocking solution in a DNA Damage Detection Kit-gH2AX at room temperature for 1 h with 4′,6-diamidino-2-phenylindole (DAPI, Dojindo, Kumamoto, Japan) and mounted in Vectashield. Images were observed under a fluorescence microscope (BZ-X800, Keyence, Osaka, Japan). Antibodies, including anti-RAD51 (14961-1-AP; 1:200, Proteintech, Rosemont, IL, USA), anti-γ-H2AX (in DNA Damage Detection Kit-gH2AX-Green, G265, Dojindo), and goat anti-rabbit IgG conjugated with Texas Red (4050-07; 1:200, SouthernBiotech, Birmingham, AL, USA), were used. A total of 30 cells from three random fields per sample were observed to quantify the RAD51 and gH2AX foci formation. Cells with more than five foci were considered positive, and the fraction of foci-positive cells was calculated. The average ratio of RAD51-positive cells/gH2AX-positive cells in each sample was presented with SD. Each experiment was repeated at least two times.

RNA sequencing

OVISE cells were seeded in 6 cm dishes and incubated for 24 h. Cells were treated with or without bevacizumab (20 μg/ml) and incubated for 192 h. Total RNA was extracted from these cells 2 h after treatment with 2 Gy of γ-irradiation. RNA sequencing was performed at the Kazusa DNA Research Institute. The data discussed in this study have been deposited in the NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE203044. Purified total RNA was used for RNA library preparation according to the instructions of the Quant Seq 3' mRNA-seq library preparation kit FWD for Illumina (Lexogen, Vienna, Austria). Libraries were sequenced using single-end 75 bp on a NextSeq500 instrument to an average depth of 2.8 M clusters per sample. All data analyses were performed using Strand NGS 3.4 (Strand Life Sciences Pvt. Ltd., Bengaluru, India). In addition to trimming adapters and poly-A from FASTQ files, all read sequences were trimmed by 6 bp from the 5′ ends according to the manufacturer's instructions. Reads were mapped to the human genome hg19. After DESeq normalization, a gene was considered differentially expressed if the adjusted *P*-value was <0.05 and fold-change >2 or <0.5. A Volcano plot was created using GEO2R (https://www.ncbi.nlm.nih.gov/goe/geo2r/). For enrichment analysis of the differentially expressed genes, gene set enrichment analysis was used to perform Gene Ontology analysis.

Quantitative real-time reverse transcription-PCR analysis

RT-PCR analysis was performed as described previously (24). Briefly, extracted RNAs were subjected to RT using qScript cDNA SuperMix (Quantabio, Beverly, MA, USA), followed by quantitative real-time RT-PCR using TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific). All PCR reactions were performed in 96-well plates using the StepOnePlus real-time PCR System

(Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase was used as an endogenous control, and untreated cells were set as the reference. Gene expressions were quantified using the comparative $2^{-\Delta\Delta CT}$ method.

Western blotting

Western blotting analysis was performed as described previously (24). Cells were collected 48 hours after administration of LY294002 and bevacizumab and transfection with siRNA VEFR2. Irradiation and administration of olaparib were performed 2 and 8 hours before cell collection, respectively. Total protein was resolved on gradient NuPage 4%–12% Bis-Tris gels (Thermo Fisher Scientific) and transferred to membranes using an iBlot1 Gel Transfer Device (Thermo Fisher Scientific). The membranes were incubated sequentially with primary antibodies at 4°C and horseradish peroxidase-conjugated secondary anti-rabbit or anti-mouse antibody (1:10000, Cell Signaling Technology, Beverly, MA) at room temperature with gentle agitation. Positive immunoreactions were detected using the ImmunoStar LD chemiluminescence system (Wako, Tokyo, Japan). Rabbit polyclonal antibody against CRY1 (EPR165; #ab229631; 1:1000) was purchased from Abcam (Cambridge, UK), and rabbit monoclonal antibodies against AKT (11E7; #4685; 1:1000), phosphorylated AKT (Thr308) (244F9; #4056; 1:1000), phosphorylated AKT (Ser473) (D9E; #4060; 1:2000) and β-actin (13E5; #4970; 1:4000) were purchased from Cell Signaling Technology.

Enzyme-linked immunosorbent assays

ELISAs were performed as described previously (25). Briefly, cells were seeded in 6 cm dishes and incubated for 72 h. Part of the culture supernatant was collected immediately in the control group, 2 h after irradiation in the irradiation group, 8 h after olaparib administration in the olaparib group and 48 h after bevacizumab administration and 2 h after irradiation in the irradiation and bevacizumab group, respectively. Four samples from each group were collected. ELISA analysis of VEGF concentration was performed using DVE00 for VEGF ELISA kits (R&D Systems, Minneapolis, MN, USA). The mean concentration of VEGF was compared between the control group and the irradiation and olaparib groups.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 9 software (GraphPad Inc.). Means of the control and experimental groups were compared using one-way or two-way analysis of variance, followed by Tukey's multiple comparison tests. Statistical significance was set at $P < 0.05$.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information Files). The datasets generated and analysed during the current study are available in the NCBI's Gene Expression Omnibus repository, accession number GSE203044.

Results

Combination with antiangiogenic agents enhances the effect of olaparib

The antiproliferative effect of olaparib with and without antiangiogenic agents was assessed in OVISE and OVSAHO cells. OVISE cells have no alteration in genes associated with HR, except for amplifications of unknown biological effect in the *BRCA1* and *NBN* genes, and are considered HRP. OVSAHO cells have deletions in the *BRCA2* and *CHEK1* genes that are considered to be oncogenic and are considered HRD. In OVISE cells, the IC50 of olaparib alone was 80.73 μM, which was significantly higher than that under co-treatment with bevacizumab or cediranib (51.18 μM or 39.55 μM, respectively) (Figure 1A, Supplementary Figure S1A). Similar results were seen in OVSAHO cells (Figure 1B, Supplementary Figure S1B). Co-treatment with bevacizumab or cediranib inhibited cell proliferation more than olaparib alone in both OVISE and OVSAHO cells (Figures 1C, D).

Inhibition of the VEGF signaling pathway suppressed HR activity through the downregulation of *CRY1* expression

The effect of the inhibition of the VEGF pathway on HR activity was evaluated using ASHRA to elucidate the mechanism of sensitization to olaparib by the inhibition of the VEGF pathway. ASHRA can quantify cellular HR activity, and the measured activity in ASHRA correlates linearly with sensitivity to PARP inhibitors (26). The treatment with the addition of bevacizumab or cediranib significantly suppressed the HR activity in OVISE cells (Figure 2A). The intranuclear foci formation of RAD51, a marker of functional HR, was examined after X-ray irradiation to confirm the suppression of HR activity. Most of the γ-H2AX foci, a marker of DNA damage, were co-localized with RAD51 foci in control cells. However, most of the foci formation of RAD51 was decreased in the bevacizumab-treated cells (Figures 2B, C). These findings showed that the inhibition of the VEGF pathway suppressed HR activity in these cells.

Differentially expressed genes were investigated by RNA sequencing to identify a mediator of suppression of HR activity by the inhibition of the VEGF pathway. The results showed that bevacizumab treatment decreased the expression of *CRY1* in X-ray-irradiated OVISE cells (Supplementary Figure 2A, B, Supplementary Tables S2). The decreased *CRY1* expression was

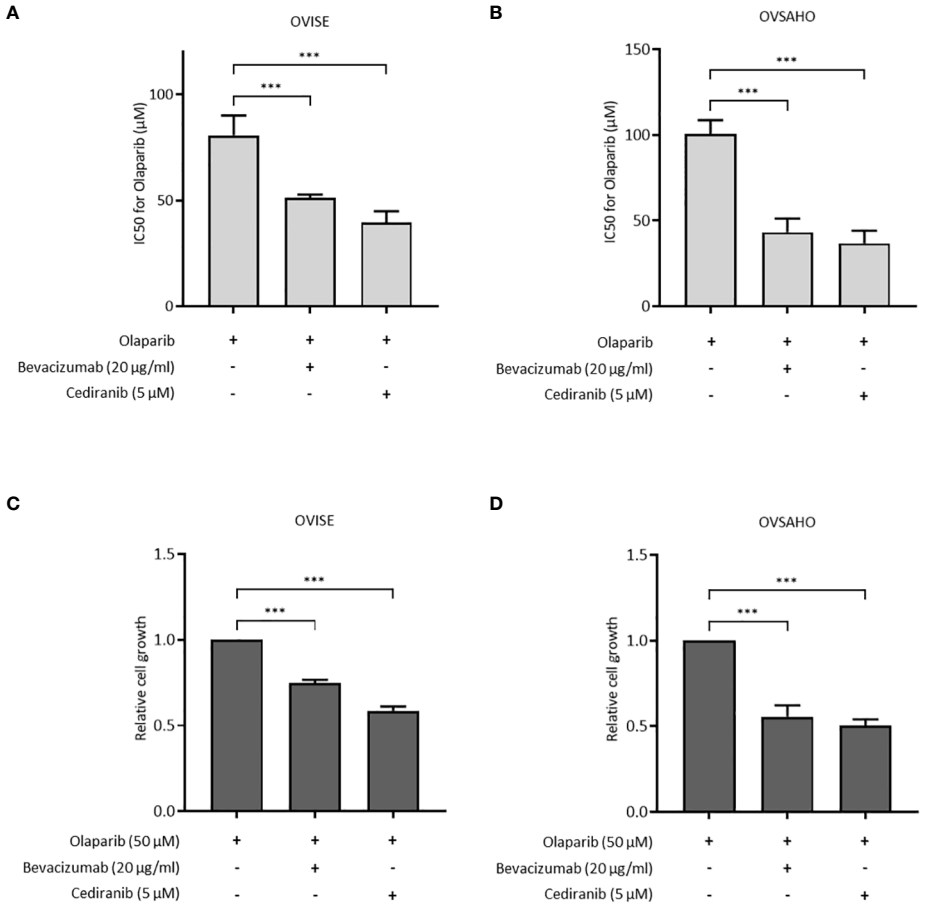

FIGURE 1
The effect of olaparib was enhanced by the addition of antiangiogenesis in homologous recombination-proficient (HRP) cells. Data are shown as mean ± standard deviation (SD). ***$P < 0.001$. **(A, B)** IC50 (half maximal inhibitory concentration) values based on the viability of OVISE and OVSAHO cells treated with olaparib with or without antiangiogenesis. **(C, D)** Growth inhibition of OVISE and OVSAHO cells treated with olaparib with or without antiangiogenesis.

confirmed by real-time PCR analysis (Figure 2D). *CRY1* is a circadian gene that regulates the expression of several genes associated with HR. Thus, the HR activity in *CRY1*-knockdown cells was evaluated. The knockdown of *CRY1* by RNAi significantly suppressed the HR activity in OVISE cells (Figure 2E). Interestingly, the exogenous expression of *CRY1* rescued the suppression of HR activity in the bevacizumab-treated cells (Figure 2F).

Bevacizumab suppressed CRY1 expression via PI3K/AKT pathway, and inhibition of CRY1 increased the effect of olaparib

Because both bevacizumab and cediranib suppressed HR activity, we speculated that VEGF might be produced in cancer cells under DNA damage stress. VEGF in the culture medium of OVISE cells was increased by X-ray irradiation and the olaparib treatment, and decreased by X-ray irradiation and bevacizumab treatment (Figure 3A). Consistent with this, the phosphorylated fraction of AKT was significantly increased by irradiation (Figure 3B). Additionally, the increase in the expression of CRY1 protein by irradiation was confirmed (Figure 3B). When X-ray-irradiated OVISE cells were treated with bevacizumab or transfected with siRNA against VEGFR2, CRY1 and the phosphorylation of AKT were significantly decreased (Figure 3B). CRY1 expression is regulated by the PI3K/AKT pathway via inhibition of the dimer formation of CLOCK and BMAL2, the upstream regulator of *CRY1* (27, 28). The cells were treated with a PI3K inhibitor LY293002 to investigate whether bevacizumab suppressed the expression of CRY1 via inhibition of the PI3K/AKT pathway and observed a similar decrease in CRY1 and the phosphorylation of AKT. The increase in CRY1 and the phosphorylation of AKT were also induced by olaparib treatment and again suppressed by the bevacizumab treatment, VEGFR2 knockdown, or PI3K inhibition (Figure 3C). Additionally, we confirmed that the olaparib treatment increased CRY1 and the phosphorylation of AKT, which was suppressed by the bevacizumab treatment, using another clear cell carcinoma cell

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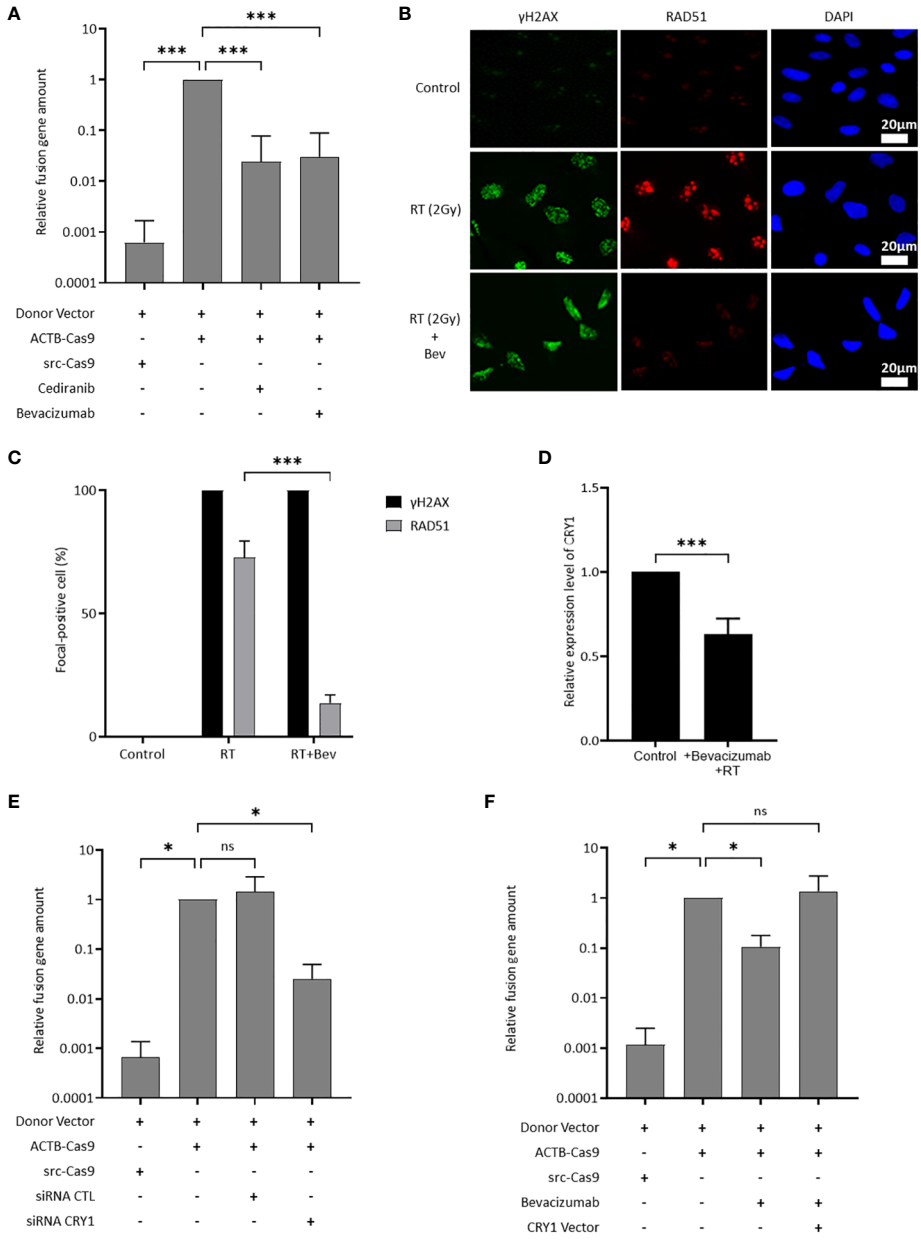

FIGURE 2
CRY1 is involved in the reduction of HR activity by the addition of antiangiogenesis. Data are shown as mean ± SD. ***$P < 0.001$; *$P < 0.05$; ns, not significant. **(A)** HR activity in OVISE cells treated with cediranib and bevacizumab was analyzed by the Assay for Site-specific HR Activity (ASHRA). **(B)** Representative images of immunofluorescence staining showing the RAD51 foci in different groups for OVISE cells. γH2AX, RAD51, and DAPI are shown in green, red, and blue, respectively. Radiation increased the expression of γH2AX and RAD51, and bevacizumab suppressed the expression of RAD51 increased by radiation. **(C)** Data were collected as the average ratio (RAD51 positive cells/gH2AX positive cells) of each group. **(D)** Relative expression levels of *CRY1* in OVISE after irradiation and the addition of bevacizumab were evaluated by reverse transcription polymerase chain reaction (RT-PCR). **(E)** HR activity in OVISE cells treated with siRNA *CRY1* was analyzed by ASHRA. **(F)** HR activity in OVISE cells treated with bevacizumab with or without *CRY1* vector was analyzed by ASHRA.

line, OVTOKO. A similar phenomenon was observed in non-clear cell carcinoma cell lines, OVSAHO and TOV-112D (Supplementary Figure S3).

The cells were treated with KS-15, a CRY1 inhibitor, concomitantly with olaparib to elucidate the importance of CRY1 in the antiproliferative effect of olaparib. The co-treatment with KS-15 significantly decreased the IC50 of OVISE cells compared with olaparib alone (Figure 3D, Supplementary Figure S4). Furthermore, similar to the co-treatment with bevacizumab, the co-treatment with KS-15 suppressed cell proliferation more than olaparib alone (Figure 3E).

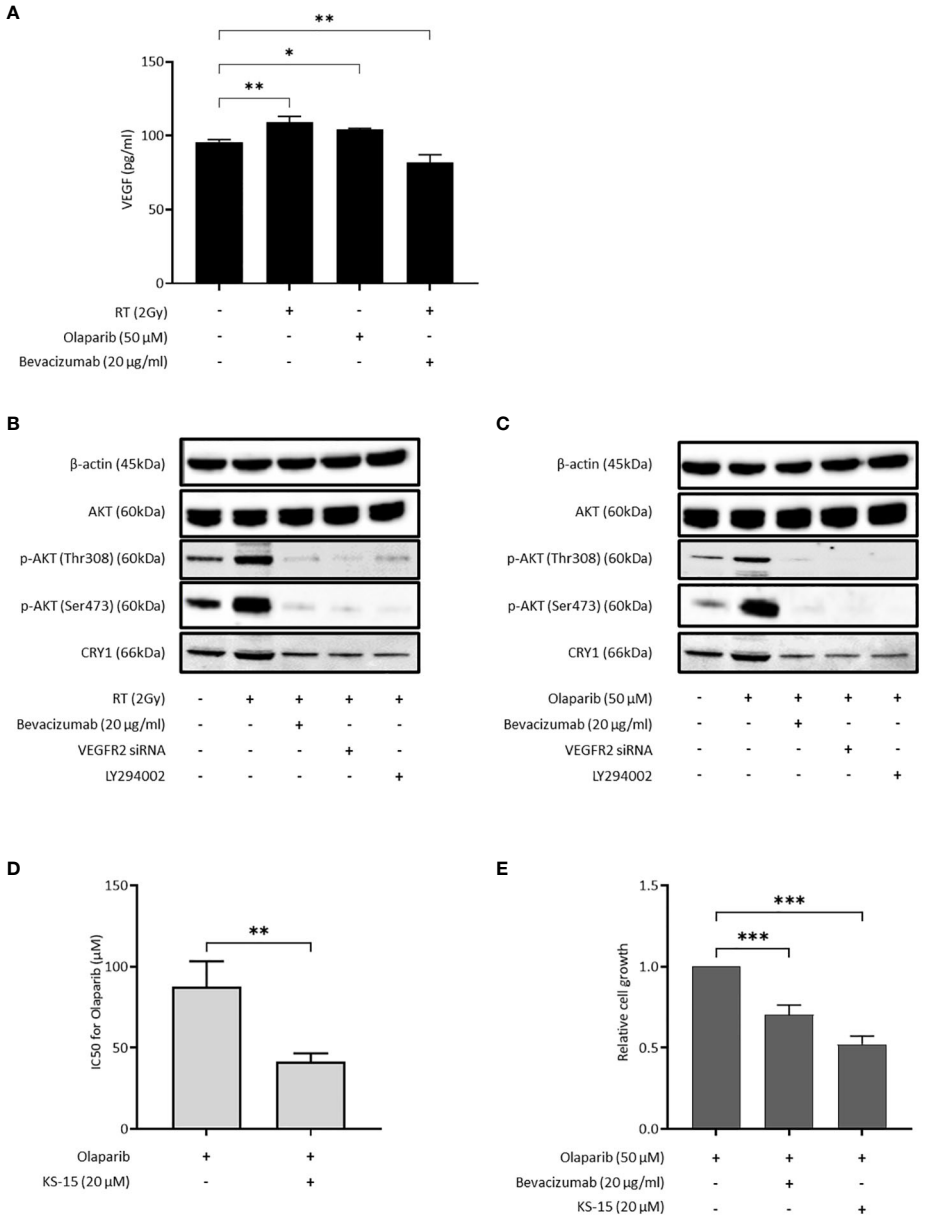

FIGURE 3
Bevacizumab suppresses CRY1 expression via the PI3K/AKT pathway. The inhibition of CRY1 enhanced the effect of olaparib in HRP cells. Data are shown as mean ± SD. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. **(A)** VEGF released by OVISE in response to irradiation and olaparib were determined by enzyme-linked immunosorbent assay (ELISA). **(B)** CRY1 expression in OVISE cells was evaluated by Western blot when bevacizumab or VEGFR2 siRNA or LY294002 was added to irradiation. Cells were collected 2 hours after irradiation. **(C)** CRY1 expression in OVISE cells was evaluated by Western blot when bevacizumab or VEGFR2 siRNA or LY294002 was added to olaparib. **(D)** IC50 values based on the viability of OVISE cells treated with olaparib with or without KS-15, an inhibitor of CRY1. **(E)** Growth inhibition of OVISE cells treated with olaparib with or without KS-15.

Discussion

In this study, we found the blockade of VEGF/VEGFR signaling suppressed the HR activity in EOC cells without obvious mutations in HR-related genes, resulting in the sensitization of the HRP EOC cells to PARPi. DNA damage stress induced by X-ray irradiation or PARPi activated the VEGF/VEGFR signaling pathway, which increased the expression of *CRY1*. *CRY1* enhanced the HR activity. Thus, antiangiogenic agents may potentiate the therapeutic effect of PARPi via inhibition of the VEGFR-PI3K/AKT-CRY1 axis (Figure 4).

The VEGF/VEGFR signaling pathway, which is directory activated by a transcription factor hypoxia-inducible factor-1, is a well-known regulator of angiogenesis (29).

Bevacizumab or cediranib, antiangiogenic agents, inhibit the VEGF/VEGFR signaling in vascular endothelial cells (30). However, the VEGF/VEGFR signaling also directly regulates cell survival, proliferation, metastasis, and sensitivity to chemotherapeutics in

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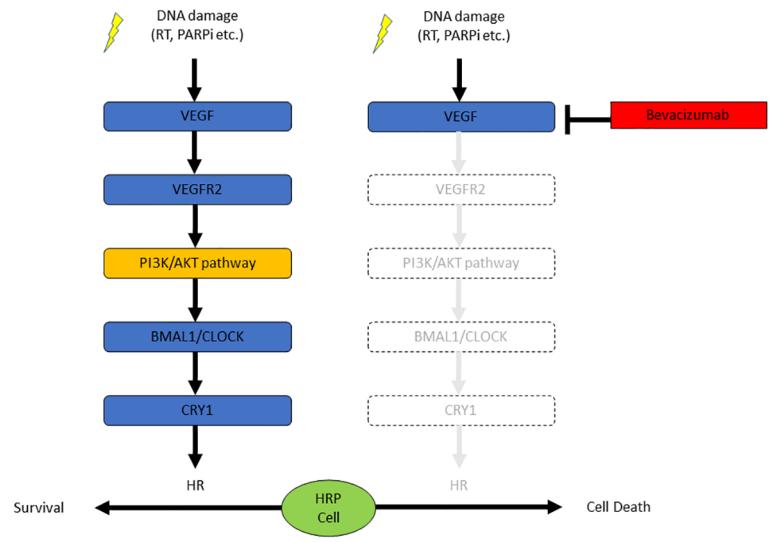

FIGURE 4
The combination of bevacizumab and olaparib is effective against HRP ovarian cancer cells due to the suppression of CRY1 via the PI3K/AKT pathway.

cancer cells (31). VEGF is produced in various cells, including cancer cells or non-neoplastic stromal cells (31). This study showed that X-ray irradiation or PARPi treatment increased the VEGF concentration in the culture media (Figure 3A). Although the precise mechanism is unknown, these data indicate that DNA damage stress stimulated VEGF production in cancer cells. The increased VEGF activated the downstream PI3K/AKT pathway, which was shown by the increase in the phosphorylated AKT fraction (Figures 3B, C, Supplementary Figure S3). The activating mutations in *PIK3CA* are common in clear cell carcinoma and relatively rare in the other histological subtypes (32). Since the increase in phosphorylated AKT by DNA damage stress was observed not only in clear cell carcinoma cell lines but also in cell lines of other histological subtypes (Figures 3B, C, Supplementary Figure S3), the activation of VEGFR-PI3K/AKT axis might be independent of *PI3KCA* mutation.

CRY1 is one of the transcriptional coregulators associated with circadian rhythm (33). Disturbance of the circadian rhythm has recently been identified as an independent risk for cancer and classified as a carcinogen (33). Furthermore, circadian rhythm affects several hallmark phenotypes of cancer, including alterations in cell proliferation, survival, DNA repair, and metabolic regulation (33). Recent research showed that DNA damage stabilized CRY1, and the stabilized CRY1 temporally regulated the expression of genes required for HR in cancer cells (34). This study reported an increase in the *CRY1* expression by DNA damage, including X-ray irradiation and PARPi treatment, and the knockdown of *CRY1* by RNAi significantly suppressed the HR activity (Figures 2D–F, 3B, C, Supplementary Figure S3). These data support the contribution of CRY1 in the enhancement of DNA damage repair by HR. Additionally, bevacizumab decreased the *CRY1* expression in irradiated cells and suppressed the HR activity (Figures 2A, D) and the suppression of HR activity by bevacizumab was restored by the exogenous expression of *CRY1* (Figure 2F). Circadian rhythm is influenced by several factors such as light and temperature (35), and the possibility that the expression CRY1 was influenced by these factors cannot be ruled out. However, the results of this study showed that bevacizumab inhibited an increase in the *CRY1* expression induced by DNA damage, resulting in the suppression of HR activity and enhancement of the PARPi effect. Furthermore, the PI3K inhibition or the knockdown of VEGFR2, which blocks the upstream signaling of PI3K decreased the CRY1 expression under DNA damage stresses as bevacizumab did (Figures 3B, C). These data indicate that inhibition of the VEGFR-PI3K/AKT-CRY1 axis may be sufficient to suppress HR activation by the increase in *CRY1*. Several clinical trials reported that the combination of PARPi and PI3K/AKT inhibitors showed enhanced efficacy regardless of cancer type and HR status (36–38). These results are consistent with those of our study.

KS-15, an inhibitor of CRY1, increased the sensitivity of olaparib as bevacizumab did (Figures 3D, E). Interestingly, KS-15 has different effects depending on the cell type. KS-15 exerted an antiproliferative effect and increased sensitivity to doxorubicin in the breast cancer cell line MCF7 but not in the non-transformed mammary epithelial cell line MCF10A (39). KS-15 showed a protective effect in non-neoplastic cells against cisplatin by promoting DNA repair and arresting the cell cycle (40). These results indicate that KS-15 selectively potentiates the therapeutic anticancer effect agents in transformed cells. Future studies are needed to investigate the mechanism of selective potentiation of therapeutic agents by KS-15. Interestingly, the enhancement of growth inhibition combined with olaparib was sustained for a longer period by KS-15 compared with bevacizumab (Supplementary Figure S5). The suppression of *CRY1* by bevacizumab was attenuated as time went by X-ray-irradiated cells (data not shown). This may be due to the rhythmic nature of *CRY1* expression regulation. Thus, KS-15 may be a better agent to inhibit HR activation in olaparib-treated cells and is a good candidate worth testing in combination with olaparib in clinical trials.

Preclinical studies showed that antiangiogenic agents affect HRR through various mechanisms, indicating synergy between PARPi and antiangiogenic agents. By blocking angiogenesis, antiangiogenic agents induce hypoxia in the microenvironment, and the hypoxic conditions lead to decreased expression of *BRCA1/2* and *RAD51* (41–43). Furthermore, VEGFR3 inhibition downregulates *BRCA* genes, and cediranib directly represses *BRCA1/2* and *RAD51* gene expression (41, 44). In this study, the antiangiogenic agents, bevacizumab or cediranib, enhanced the effect of olaparib in HRP EOC cells through a mechanism that is not associated with hypoxia induced by antiangiogenic agents reported to date.

Although the underlying mechanisms of these combinations are still not fully understood, clinical trials have been conducted to evaluate the combination of PARPi and antiangiogenic agents. In two phase II studies on patients with platinum-sensitive recurrent EOC, the combination of PARPi and antiangiogenic agents significantly improved PFS compared with PARPi alone (13, 14). A phase III study on patients with recurrent platinum-sensitive EOC, which compared the combination of cediranib and olaparib or olaparib alone with standard platinum-based chemotherapy, demonstrated that the median PFS was 10.4, 8.2, and 10.3 months for the combination, olaparib alone, and chemotherapy, respectively, and the results were similar in patients without germline *BRCA* (g*BRCA*) mutation (45). Another phase II study on heavily pre-treated patients with platinum-resistant recurrent EOC, which compared the combination of olaparib and cediranib or olaparib alone with weekly paclitaxel, demonstrated that the median PFS was 5.7, 3.8, and 3.1 months for the combination, olaparib alone, and weekly paclitaxel, respectively, and no significant difference in PFS was observed between the combination and weekly paclitaxel, and in the subgroup analysis of patients with wild-type g*BRCA*, the median PFS was 5.8, 3.8, and 2.1 months for the combination, olaparib alone, and weekly paclitaxel, respectively, indicating that the combination therapy showed a promising trend toward improved PFS compared with weekly paclitaxel (46). These results indicate that the combination of PARPi and antiangiogenic agents prolongs PFS compared with PARPi alone, but its efficacy has not been shown to be superior to standard platinum-based chemotherapy regardless of the g*BRCA* mutation status. Therefore, the combination of PARPi and antiangiogenic agents may be a viable alternative to chemotherapy for patients with recurrent EOC, particularly platinum-resistant recurrent EOC patients with wild-type g*BRCA*. The combination of PARPi and antiangiogenic agents was first evaluated in the phase III PAOLA-1 study as a maintenance treatment in the first-line setting, which reported a statistically significant improvement in the median PFS for olaparib and bevacizumab compared with placebo and bevacizumab in the overall population, and in the subgroup analysis, a substantial PFS benefit was observed with the combination treatment compared with bevacizumab alone in the HRD population but not in the HRP population (12). The lack of an olaparib alone arm makes it difficult to determine whether the combination has synergistic effects.

In conclusion, VEGF/VEGFR/PI3K signaling enhanced HR activity through the increase in the expression of *CRY1*. The study findings indicate that the antiangiogenic agents and the CRY1 inhibitors are promising combination partners to overcome primary resistance to PARPi by turning HRP cells into HRD cells. Furthermore, antiangiogenic agents and CRY1 inhibitors may concur with the secondary resistance to PARPi due to the activation of HR. These data provide an important molecular basis for the development of new therapeutic strategies for EOC.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

YI: Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft. NY: Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft. YY: Methodology, Writing – original draft. MS: Methodology, Visualization, Writing – review & editing. RS: Methodology, Writing – review & editing. JT: Methodology, Writing – review & editing. AK: Methodology, Writing – review & editing. MT: Methodology, Writing – review & editing. NC: Conceptualization, Writing – review & editing. AO: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1302850/full#supplementary-material

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Stergios Boussios,
Canterbury Christ Church University, United Kingdom
Sarah Taylor,
University of Edinburgh, United Kingdom

*CORRESPONDENCE
San-Gang Wu
wusg@xmu.edu.cn
Juan Zhou
zhoujuan@xmu.edu.cn

†These authors have contributed equally to this work

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Trends in survival of ovarian clear cell carcinoma patients from 2000 to 2015

Bing-Qian Tian[1†], Shu-Wen Wang[2†], Jing-Ying Xu[2], San-Gang Wu[3*] and Juan Zhou[2*]

[1]School of Medicine, Xiamen University, Xiamen, China, [2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China, [3]Department of Radiation Oncology, Xiamen Cancer Center, Xiamen Key Laboratory of Radiation Oncology, The First Affiliated Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China

Purpose: To analyze changes in survival outcomes in patients with ovarian clear cell carcinoma (OCCC) treated consecutively over a 16-year period using a population-based cohort.

Methods: We conducted a retrospective analysis of OCCC from 2000 to 2015 using data from the Surveillance, Epidemiology, and End Results (SEER) program. The ovarian cancer-specific survival (OCSS) and overall survival (OS) were analyzed according to the year of diagnosis. Joinpoint Regression Program, Kaplan-Meier analysis, and multivariate Cox regression analyses were used for statistical analysis.

Results: We included 4257 patients in the analysis. The analysis of annual percentage change in OCSS (P=0.014) and OS (P=0.006) showed that patients diagnosed in later years had significantly better outcomes compared to those diagnosed in early years. The results of the multivariate Cox regression analyses showed that the year of diagnosis was the independent prognostic factor associated with OCSS (P=0.004) and had a borderline effect on OS (P=0.060). Regarding the SEER staging, the OCSS (P=0.017) and OS (P=0.004) of patients with distant stage showed a significant trend toward increased, while no significant trends were found in the survival of patients with localized or regional stage diseases. Similar trends were found in those aged <65 years or those treated with surgery and chemotherapy. However, no statistically significant changes in the survival rate were found in those aged ≥65 years or those receiving surgery alone regardless of SEER stage during the study period.

Conclusions: Our study observed a significant increase in the survival outcomes in OCCC from 2000 to 2015, and patients aged <65 years and those with distant stage experienced a greater improvement in survival.

KEYWORDS

ovarian cancer, survival trend, clear cell carcinoma, annual percentage change, SEER

Introduction

Epithelial ovarian cancer (EOC) is the gynecological tumor with the highest mortality rate (1). Due to the relatively insidious onset of this disease, approximately 70% of patients were diagnosed with advanced-stage disease (1). BRCA1/2 germline mutations are the strongest known genetic risk factors for EOCs and are found in 6-15% of women diagnosed with that disease. BRCA1/2 carriers with EOCs respond better than non-carriers to platinum-based chemotherapies. This yields greater survival, even though the disease is generally diagnosed at a later stage and higher grade (2). According to the WHO classification of tumors, there are five main histological subtypes of EOC, including high-grade serous, low-grade serous, mucinous, endometrioid, and clear cell carcinoma of the ovary (3). Another rare and highly aggressive type of EOC is ovarian carcinosarcoma, which accounts for less than 5% of ovarian cancer (3). Each of the identified histotypes has distinct clinicopathological and molecular features, and different developmental origins (4). Due to the complexity of histological classification, there are significant differences in the availability and accessibility of treatment options for each subtype, resulting in varying patient outcomes (5). Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway is frequently upregulated in EOC and plays an important role in chemoresistance and preservation of genomic stability, as it is implicated in many processes of DNA replication and cell cycle regulation. The inhibition of the PI3K may lead to genomic instability and mitotic catastrophe through a decrease of the activity of the spindle assembly checkpoint protein Aurora kinase B and consequently increase the occurrence of lagging chromosomes during prometaphase (6).

Ovarian clear cell carcinoma (OCCC) is a rare and unique malignancy of the EOC and has an incidence of 0.6/100,000 (1). The incidence of OCCC in East Asian populations has been increasing, accounting for nearly 30% of EOC (7), while OCCC only accounts for 5-10% in the United States (US) population (8), suggesting that there may be some geographical and ethnic variation in the incidence of OCCC. OCCC is characterized by the presence of clear cells with a hobnail appearance and is often associated with endometriosis (9–11). Moreover, OCCC is known to have distinct clinicopathologic features, genetic alterations, and prognosis compared to other subtypes of EOC (5). OCCC has a unique genetic profile with a lower p53 mutation rate and a lower BRCA1/2 mutation rate but higher mutation rates in AT-rich interaction domain 1A (ARID1A), PIK3CA, and PTEN compared to high-grade serous EOC (12).

Generally, the overall survival (OS) rates for advanced OCCC have been reported to be lower compared to other histological subtypes of EOC (13–15). Despite a lower rate of responses due to intrinsic chemoresistance, the treatment strategy for OCCC is the same used for high-grade serous EOC, which includes aggressive cytoreductive surgery and platinum-based adjuvant chemotherapy. Over the past few decades, there have been significant efforts to improve early detection and develop targeted therapies for EOC (5). Several biological agents have been investigated in patients with newly diagnosed, persistent, or recurrent OCCC, and bevacizumab combined with platinum-taxane chemotherapy had a response rate of 63.6% and one-year progression-free survival was 50.5%, suggesting that the addition of bevacizumab to chemotherapy for OCCC could be an important treatment strategy (16, 17). The response rate in those treated with bevacizumab was higher than other biological agents and bevacizumab was approved for the treatment of EOC starting in 2007 (17–19). Survival trends are crucial in assessing the effectiveness of treatment strategies and advancements in medical care for OCCC. However, it is still unclear whether the advancement of treatment strategies will bring survival improvement to OCCC. This study aimed to investigate the changes in ovarian cancer-specific survival (OCSS) and OS of OCCC patients treated consecutively over a 16-year period using a population-based cohort.

Materials and methods

Patients

Patients diagnosed with OCCC between 2000 and 2015 were included retrospectively from the Surveillance, Epidemiology, and End Results (SEER) database (20). We identified patients who met the following inclusion criteria: 1) diagnosed with OCCC (International Classification of Diseases for Oncology, 3rd ed. [ICD-O-3], primary site: C56.9-ovary) (ICD-O-3 codes 8290/3, 8310/3, 8313/3, 8443/3, and 8444/3); 2) available SEER staging; 3) received surgery with or without chemotherapy. The patient selection flowchart has listed in Figure 1. We excluded patients with non-positive pathological diagnoses in this study. Institutional review board approval was not required for our study as the SEER database contains de-identified information.

Variables

We included the following variables in the analysis: year of diagnosis, age, race, tumor grade, SEER stage, CA125 status, and treatment receipt. The classification of the years of diagnosis was 2000-2007 and 2008-2015, which was due to the approval of bevacizumab for the treatment of EOC starting in 2007 (18, 19). SEER stage is defined by the derived SEER Summary Stage 2000

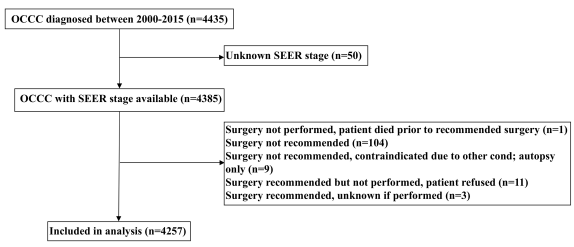

FIGURE 1
The flowchart of the cohort selection.

variable (21). It utilizes the Collaborative Staging algorithm to merge clinical and pathologic information regarding the extent of disease and assign a stage for diagnoses made in 2004 and beyond. The SEER staging system corresponds to the commonly used International Federation of Gynecology and Obstetrics (FIGO) staging system in the following way: localized (FIGO I-A, I-B, I-not otherwise specified [NOS]), regional (FIGO I-C, II-A, II-B, II-C, II-NOS), and distant stage (FIGO III-A, III-B, III-C, III-NOS, IV) (21). Elevated CA125 was defined as the level of CA125 >35 ug/ml. The primary outcomes of this study were OCSS and OS. OCSS was defined as the time period from the diagnosis of OCCC to death specifically caused by ovarian cancer. OS was defined as the duration from the diagnosis of OCCC to death from any cause.

Statistical analysis

Statistical significances in categorical variables by year of diagnosis were compared using chi-square tests. We utilized the Joinpoint Regression Program, version 4.9.1.0 (National Cancer Institute) to analyze the time trends in survival outcomes. We also explored the impact of variables such as age at diagnosis, SEER staging, and treatment receipt on changes in patient survival, and the annual percentage change (APC) metric was chosen to describe the average percentage change in survival in a given period for one year relative to survival in the previous year. Kaplan-Meier method to depict the survival curves and differences in survival were compared using the log-rank tests. Multivariate Cox regression analyses were performed to determine the independent prognostic factors associated with OCSS and OS. IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was also used in the analysis. We used a significance level of $P < 0.05$, and all tests were two-tailed.

Results

Patient characteristic

A total of 4257 OCCC patients were included between 2000 and 2015 in this study (Table 1). Of these patients, 1965 (46.2%) and 2292 (53.8%) were diagnosed in 2000-2007 and 2008-2015, respectively. A total of 3334 (78.3%), 167 (3.9%), and 690 (16.2%) patients were White, Black and Asian Americans, respectively. Patients with Asian Americans (P<0.001) or poorly/undifferentiated (P<0.001) were more likely to be diagnosed in later years. Moreover, the number of patients diagnosed with regional stage gradually increases over time, while those diagnosed with localized and distant stage gradually decrease over time (P<0.001). Regarding treatment, 3214 (73.4%) patients were treated with chemotherapy and the number of patients receiving chemotherapy gradually increased over time (P<0.001). A similar distribution of age (P=0.349) or CA125 level before treatment (P=0.107) were found over the study period. A total of 2524 patients were available data for CA125 status, including 1865 (73.9%) who had CA125 ≥35ug/ml. There were 520 (62.7%), 719 (70.5%), and 626 (92.9%) patients who had CA125 ≥35ug/ml in localized, regional, and distant stage diseases, respectively (P<0.001).

Prognostic analysis

The median follow-up was 67 months (range, 0-227 months). The results of the multivariate Cox regression analyses showed that the year of diagnosis was the independent prognostic factor associated with OCSS and had a borderline effect on OS (Table 2). Those diagnosed between 2008-2015 had a significantly higher OCSS (hazard ratio [HR] 0.846, 95% confidence interval [CI] 0.754-0.949, P=0.004) compared to those diagnosed between 2000-2007. Similar OS was found between those diagnosed between 2008-2015 and 2000-2007 (HR 0.905, 95%CI 0.816-1.004, P=0.060). Age, race, SEER stage, CA125 status, and chemotherapy receipt were also the independent prognostic factors associated with survival outcomes (Table 2).

Survival trends of OCCC from 2000 to 2015

To clarify the trend in survival of OCCC patients during the study period, we counted the trends of 3-year OCSS and 3-year OS of OCCC patients from 2000 to 2015. The 3-year OCSS rate for patients increased slightly from 2000 (3-year OCSS 76%) to 2015 (3-year OCSS 78%), with an APC value of 0.65 (P=0.014). The trend in 3-year OS was more significant than the change in OCSS over the study period (3-year OS 72% in 2000 and 74% in 2015), with an APC value of 0.75 (P=0.006). Figure 2 shows the APC in 3-year OCSS and OS over the study period. The survival curves between those diagnosed between 2000-2007 and 2008-2015 have listed in Figure 3, which also showed a better OCSS and OS in those diagnosed in later years.

Survival trends according to SEER staging from 2000 to 2015

Figure 4 shows the survival trends according to the SEER staging of the OCCC. The survival of patients with distant stage showed a significant trend toward increased, with an APC value of 2.47 in OCSS (3-year OCSS 42% in 2000 and 47% in 2015) (P=0.017) and an APC value of 2.18 in OS (3-year OS 37% in 2000 and 42% in 2015) (P=0.014). However, no significant trends were found in the survival of patients with localized or regional stage diseases. The survival curves between those diagnosed between 2000-2007 and 2008-2015 after stratification by SEER staging have listed in Figure 5. Regarding distant stage, those diagnosed between 2008-2015 had a significantly better OCSS (P=0.017) and OS (P=0.032) compared to those diagnosed between 2000-2007. However, similar OCSS and OS were found between those diagnosed between 2000-2007 and 2008-2015 in the localized or regional stage diseases. Similar findings were observed using multivariate Cox regression analyses (Table 3).

TABLE 1 Descriptive demographic and clinical characteristics of patients according to year of diagnosis (n=4257).

Variables	n	2000-2007 (%)	2008-2015 (%)	P
Age (years)				
<65	3311	1541 (78.4)	1770 (77.2)	0.349
≥65	946	424 (21.6)	522 (22.8)	
Race				
White	3334	1603 (81.6)	1731 (75.5)	<0.001
Black	167	64 (3.3)	103 (4.5)	
Asian	690	272 (13.8)	418 (18.2)	
Other	66	26 (1.3)	40 (1.7)	
Grade				
Well differentiated	53	29 (2.7)	24 (1.5)	<0.001
Moderately differentiated	377	214 (19.9)	163 (10.4)	
Poorly/undifferentiated	2218	834 (77.4)	1384 (88.1)	
Unknown	1609	–	–	
SEER stage				
Localized	1521	727 (37.0)	794 (34.6)	<0.001
Regional	1630	671 (34.1)	959 (41.8)	
Distant	1106	567 (28.9)	539 (23.5)	
CA125 level (ug/ml)				
<35	659	188 (24.0)	471 (27.1)	0.107
≥35	1865	595 (76.0)	1270 (72.9)	
Unknown	1733	–	–	
Treatment				
Surgery	1133	631 (32.1)	502 (21.9)	<0.001
Surgery + chemotherapy	3124	1334 (67.9)	1790 (78.1)	

SEER, Surveillance, Epidemiology, and End Results. '-' Indicates as none available.

Survival trends according to age groups from 2000 to 2015

Figure 6 shows the APC in 3-year OCSS and OS according to age at diagnosis. Patients aged <65 years showed a significant increase in survival from 2000 to 2015, with an APC value of 0.82 for OCSS (3-year OCSS 75% in 2000 and 80% in 2015) (P=0.007) and an APC value of 0.60 for OS (3-year OS 72% in 2000 and 76% in 2015) (P=0.012). However, the survival trends could not observed for patients aged ≥65 years. Similar findings were observed using multivariate Cox regression analyses (Table 3). The survival curves between those diagnosed between 2000-2007 and 2008-2015 in the aged <65 years and aged ≥65 years groups have listed in Figure 6. We found a significant effect on OCSS (P=0.028) and a borderline effect on OS (P=0.064) in those diagnosed between 2008-2015 compared to those diagnosed between 2000-2007 in patients aged <65 years using the Kaplan-Meier analysis (Figure 7).

The sensitivity analyses were performed to investigate the effect of SEER staging on APC according to age at diagnosis. Figure 7 shows trends in survival in those aged <65 years according to the SEER staging. The significant increase in survival for patients aged <65 years was largely due to the increase in survival for patients with distant stage (3-year OCSS 39% in 2000 and 46% in 2015, P=0.004; 3-year OS 37% in 2000 and 43% in 2015, P=0.004). With an APC value of 3.36 for 3-year OCSS and an APC value of 3.04 for 3-year OS. However, there was no statistically significant change in the survival rate of patients aged <65 years with localized and regional stage diseases over time. In addition, there was also no statistically significant change in the survival rate of patients aged ≥65 years with localized, regional, or distant stage diseases over time (Figure 8). Similar findings were observed using multivariate Cox regression analyses (Table 3).

Survival trends by treatment receipt from 2000 to 2015

We analyzed to examine the impact of different treatments on survival rates. Specifically, we focused on patients who underwent

TABLE 2 Multivariate Cox regression analyses of the independent prognostic factors associated with ovarian cancer-specific survival and overall survival.

Variables	OCSS			OS		
	HR	95%CI	P	HR	95%CI	P
Age (years)						
<65	1			1		
≥65	1.066	1.002-1.134	0.045	1.269	1.206-1.335	<0.001
Race						
White	1			1		
Black	1.422	1.131-1.798	0.003	1.464	1.193-1.797	<0.001
Asian and other races	0.855	0.736-0.993	0.040	0.885	0.776-1.009	0.067
Grade						
Well differentiated	1			1		
Moderately differentiated	1.137	0.638-2.027	0.663	1.159	0.709-1.894	0.556
Poorly/undifferentiated	1.198	0.691-2.075	0.520	1.207	0.756-1.926	0.431
Unknown	1.193	0.687-2.071	0.531	1.238	0.774-1.978	0.373
SEER stage						
Localized	1			1		
Regional	2.140	1.799-2.545	<0.001	1.673	1.463-1.914	<0.001
Distant	10.234	8.697-12.043	<0.001	6.888	6.049-7.843	<0.001
CA125 level (ug/ml)						
<35	1			1		
≥35	1.561	1.273-1.915	<0.001	1.508	1.268-1.794	<0.001
Unknown	1.252	1.013-1.548	0.038	1.275	1.065-1.525	0.008
Treatment						
Surgery	1			1		
Surgery + chemotherapy	0.921	0.805-1.054	0.231	0.817	0.732-0.913	<0.001
Years of diagnosis						
2000-2007	1			1		
2008-2015	0.846	0.754-0.949	0.004	0.905	0.816-1.004	0.060

SEER, Surveillance, Epidemiology, and End Results; OCSS, ovarian cancer-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

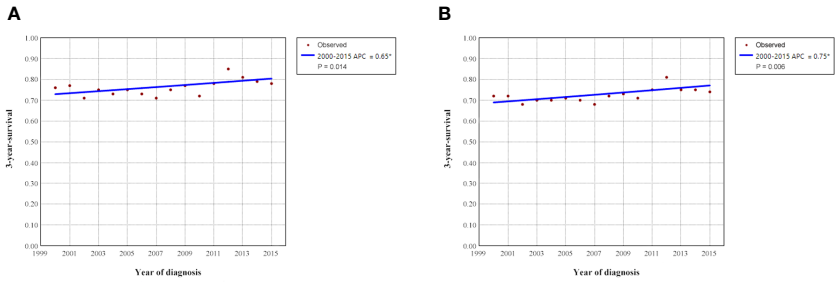

FIGURE 2
Annual percent change (APC) in 3-year ovarian cancer-specific survival **(A)** and overall survival **(B)** from 2000 to 2015.

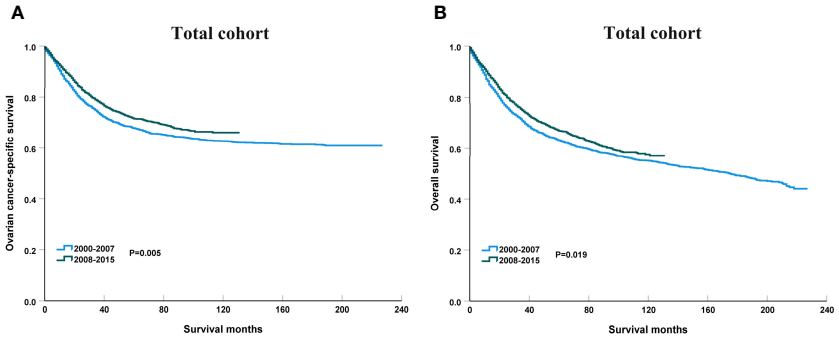

FIGURE 3
The impact of the years of diagnosis on ovarian cancer-specific survival **(A)** and overall survival **(B)** in the entire cohort.

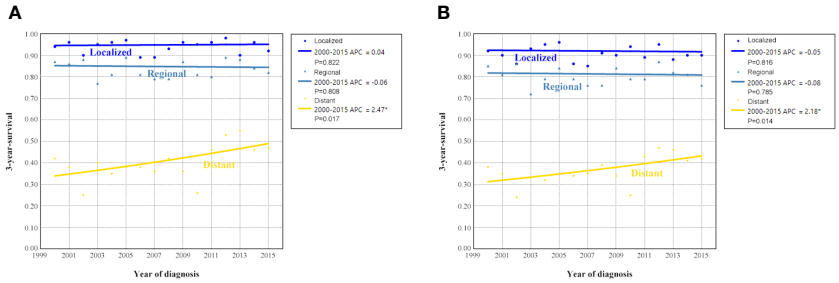

FIGURE 4
Annual percent change (APC) in 3-year ovarian cancer-specific survival **(A)** and overall survival **(B)** according to SEER staging from 2000 to 2015.

surgery alone or a combination of surgery and chemotherapy. Figure 9 shows the 3-year survival according to treatment. A significant increase in 3-year OCSS was observed for patients treated with surgery combined with chemotherapy, with an APC value of 0.92 (3-year OCSS 74% in 2000 and 79% in 2015) (P=0.004), as well as a trend toward a significant improvement in OS, with an APC value of 0.93 (3-year OS 71% in 2000 and 75% in 2015) (P=0.001), whereas there was no significant change in survival for patients treated with surgery alone. Similar findings were observed using Kaplan-Meier analysis and multivariate Cox regression analyses (Figure 10 and Table 3).

The sensitivity analyses were performed to investigate the effect of SEER staging on APC according to treatment receipt. The significant increase in survival for patients treated with surgery combined with chemotherapy was largely due to the increase in survival for patients with distant stage (3-year OCSS 46% in 2000 and 51% in 2015, P=0.024; 3-year OS 43% in 2000 and 46% in 2015, P=0.035) (Figure 10). In patients with localized or regional stage diseases, there was no statistically significant change in the survival rate of patients who received surgery and chemotherapy over time. Moreover, there was also no statistically significant change in the survival rate of patients with localized, regional, or distant stage diseases over time in those who received surgery alone (Figure 11). Similar findings were observed using the multivariate Cox regression analyses (Table 3).

Discussion

OCCC is a distinct type of cancer that has unique features in its occurrence, development, treatment, and prognosis. OCCC has a unique genetic profile with a lower p53 mutation rate (25%) and a lower BRCA1/2 mutation rate (6.3%) but higher mutation rates in ARID1A, PIK3CA, and PTEN compared to high-grade serous EOC. Since inflammatory and epigenetic processes seem to play a predominant role in the pathogenesis of OCCC, immune checkpoint inhibitors, and epigenetic treatment approaches may play an important role in the treatment of these tumor entities (12). In the past, it has not received much attention due to its rarity. However, in recent years, there has been increased interest in researching OCCC, primarily because of its specific clinical characteristics and the varying survival rates observed in early and late-stage patients. In this study, we utilized the SEER database to analyze data of OCCC patients between 2000 and 2015. We aimed to identify any changes in survival trends among OCCC patients over the past decade and explore the influence of different factors on these trends. Our study will enhance the understanding of the disease and provide valuable insights and evidence for future research on treatment modalities.

In this study, we found an increasing trend in the number of OCCC diagnoses between 2000 and 2015 in Asian Americans. Several studies have found that the incidence rate of OCCC in the

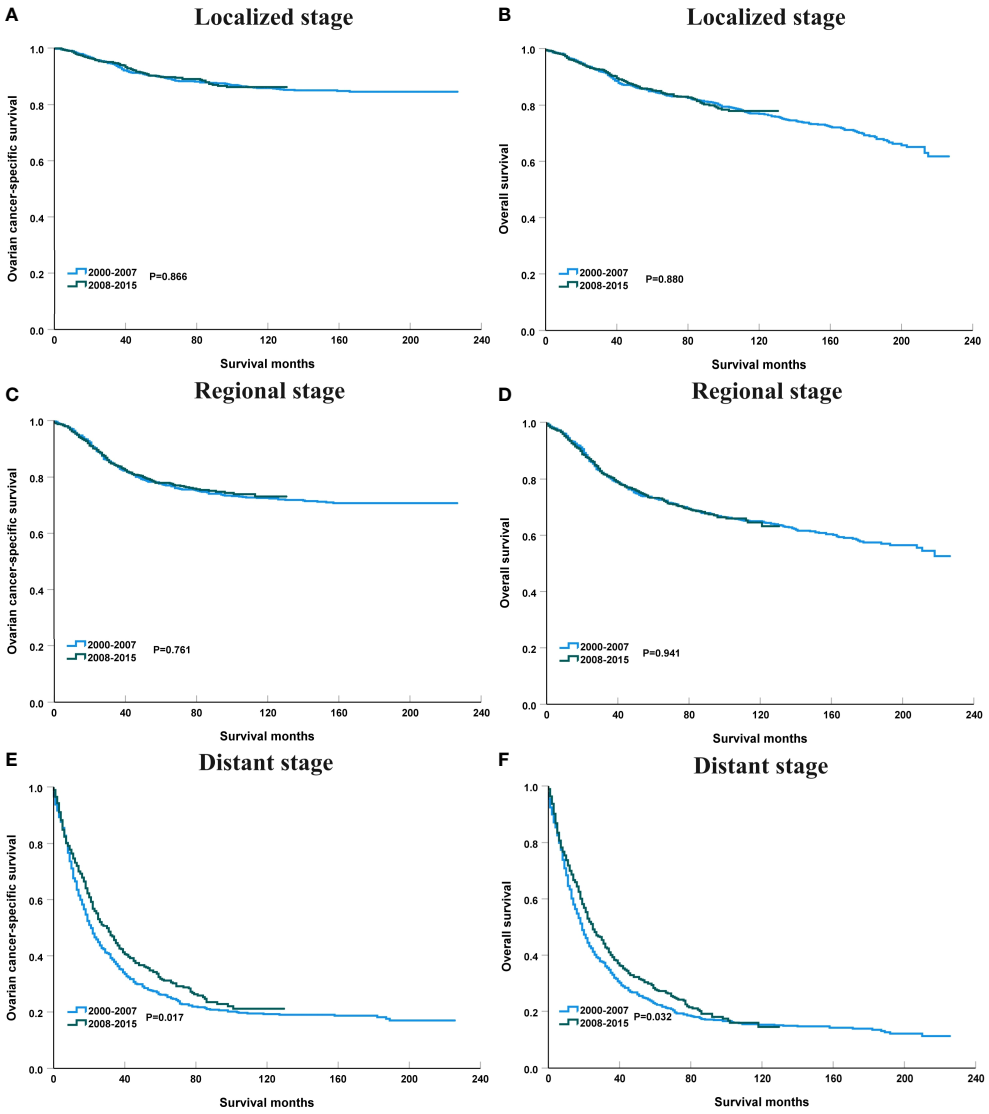

FIGURE 5
The impact of the years of diagnosis on ovarian cancer-specific survival and overall survival in patients with localized [**(A)**, ovarian cancer-specific survival; **(B)**, overall survival], regional [**(C)**, ovarian cancer-specific survival; **(D)**, overall survival], and distant stage [**(E)**, ovarian cancer-specific survival; **(F)**, overall survival].

Asian population is higher than that in the US (22, 23). We should note that those Asian Americans were first-generation immigrants or descendants of immigrants. A previous study conducted in the US identified an increased risk of OCCC among individuals of Asian Pacific Islander ethnicity. However, the study also found that the risk did not significantly vary based on place of birth, indicating that factors such as acculturation or environmental exposure may not strongly influence the association (24). These findings imply that the development of OCCC involves a complex interplay of external and intrinsic factors. The elevated risk observed in Asian Americans may be attributed to genetic predisposition, making it more difficult to modify or mitigate.

Considerable efforts have been made to implement screening programs for early diagnosis of EOC in the general population, but currently, there is no approved strategy (25). This is also reflected economically and cost-effective strategies for early detection and prevention of ovarian cancer have been investigated over the last decade. The cost of treatment per patient with ovarian cancer remains the highest among all cancer types. As an example, the average initial cost in the first year can amount to around US dollar 80,000, whereas the final year cost may increase to US dollar 100,000 (26). The combination of CA125 and transvaginal ultrasound has been explored, but there is limited evidence demonstrating its effectiveness in reducing EOC mortality (27). The number of asymptomatic ovarian masses has increased with the use of prenatal ultrasonography. Among ovarian tumors that complicate pregnancies, approximately 5% are malignant. Currently, surgical intervention is indicated for an ovarian mass over 6 cm in diameter or when symptomatic (28). A recent study also did not support effective screening in average-risk women (29). In our study, we found that approximately 70% of patients had an elevation of CA125, and patients with advanced stage had a higher

risk of elevation of CA125, which was similar to the previous studies (30, 31). However, we found a downward trend in patients with distant stage and an upward trend in regional stage. In addition, the overall trend of patients in localized stage was decreasing. There is currently no effective screening strategy for OCCC. Several studies have indicated that the rise in the proportion of OCCC is attributed to increased estrogen exposure and the subsequent rise in rates of endometriosis (9–11). Therefore, further exploration should be conducted to determine whether screening for long-term estrogen exposure and patients with endometriosis can further improve the early diagnosis of OCCC.

Adjuvant chemotherapy using carboplatin and paclitaxel is currently recommended for those with stage IC2 and above (32). However, the role of adjuvant chemotherapy in patients with stage IA to IC disease remains uncertain. The consensus from the European Society for Medical Oncology-European Society of

TABLE 3 Sensitivity analyses of the impact of the year of diagnosis on ovarian cancer-specific survival and overall survival.

Variables	OCSS			OS		
	HR	95%CI	P	HR	95%CI	P
Localized stage						
2008-2015 vs. 2000-2007	0.947	0.688-1.302	0.737	0.990	0.771-1.270	0.935
Regional stage						
2008-2015 vs. 2000-2007	0.908	0.729-1.132	0.390	0.986	0.813-1.196	0.887
Distant stage						
2008-2015 vs. 2000-2007	0.823	0.707-0.958	0.012	0.851	0.737-0.982	0.027
Aged <65 years						
2008-2015 vs. 2000-2007	0.854	0.749-0.974	0.019	0.882	0.781-0.996	0.043
Aged <65 years (localized stage)						
2008-2015 vs. 2000-2007	0.931	0.640-1.352	0.706	0.975	0.709-1.339	0.874
Aged <65 years (regional stage)						
2008-2015 vs. 2000-2007	0.951	0.743-1.216	0.688	0.977	0.781-1.220	0.835
Aged <65 years (distant stage)						
2008-2015 vs. 2000-2007	0.809	0.680-0.962	0.017	0.832	0.705-0.981	0.029
Aged ≥65 years						
2008-2015 vs. 2000-2007	0.858	0.672-1.095	0.217	0.977	0.800-1.193	0.819
Aged ≥65 years (localized stage)						
2008-2015 vs. 2000-2007	1.063	0.576-1.960	0.846	1.029	0.687-1.542	0.888
Aged ≥65 years (regional stage)						
2008-2015 vs. 2000-2007	0.772	0.471-1.265	0.304	1.060	0.722-1.557	0.765
Aged ≥65 years (distant stage)						
2008-2015 vs. 2000-2007	0.838	0.609-1.153	0.279	0.903	0.675-1.209	0.493
Surgery alone						
2008-2015 vs. 2000-2007	1.074	0.836-1.380	0.575	1.182	0.962-1.453	0.112
Surgery alone (localized stage)						
2008-2015 vs. 2000-2007	1.125	0.660-1.918	0.664	1.308	0.899-1.902	0.160
Surgery alone (regional stage)						
2008-2015 vs. 2000-2007	0.995	0.624-1.589	0.985	1.162	0.790-1.709	0.444
Surgery alone (distant stage)						
2008-2015 vs. 2000-2007	1.048	0.728-1.509	0.800	1.051	0.754-1.466	0.768

(Continued)

TABLE 3 Continued

Variables	OCSS			OS		
	HR	95%CI	P	HR	95%CI	P
Surgery + chemotherapy						
2008-2015 vs. 2000-2007	0.804	0.706-0.917	0.001	0.838	0.743-0.945	0.004
Surgery + chemotherapy (localized stage)						
2008-2015 vs. 2000-2007	0.911	0.612-1.356	0.645	0.843	0.604-1.175	0.313
Surgery + chemotherapy (regional stage)						
2008-2015 vs. 2000-2007	0.883	0.690-1.131	0.324	0.936	0.751-1.166	0.554
Surgery + chemotherapy (distant stage)						
2008-2015 vs. 2000-2007	0.782	0.660-0.926	0.004	0.815	0.694-0.957	0.013

OCSS, ovarian cancer-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

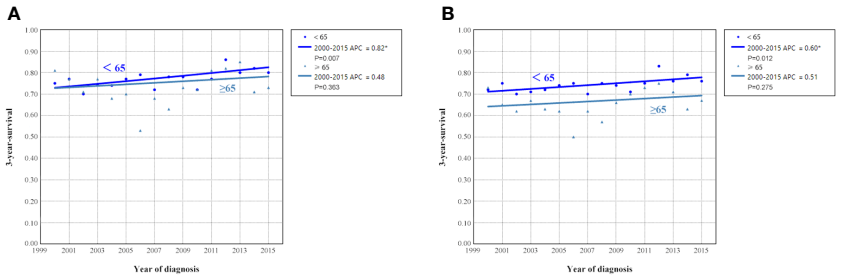

FIGURE 6
Annual percent change (APC) in 3-year ovarian cancer-specific survival **(A)** and overall survival **(B)** according to age at diagnosis from 2000 to 2015.

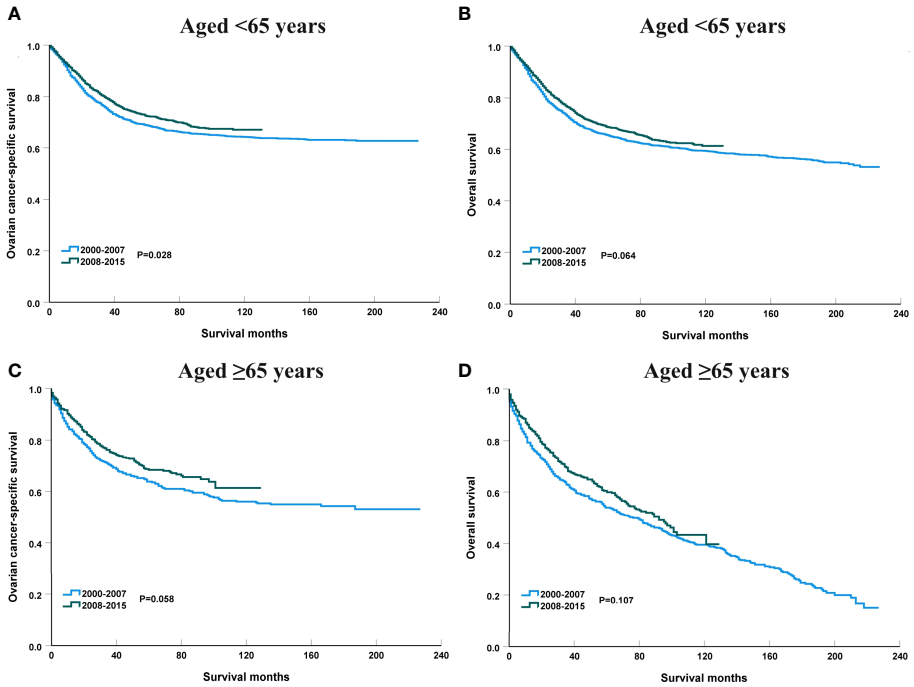

FIGURE 7
The impact of the years of diagnosis on ovarian cancer-specific survival and overall survival in patients aged <65 years [**(A)**, ovarian cancer-specific survival; **(B)**, overall survival] and those aged ≥65 years [**(C)**, ovarian cancer-specific survival; **(D)**, overall survival].

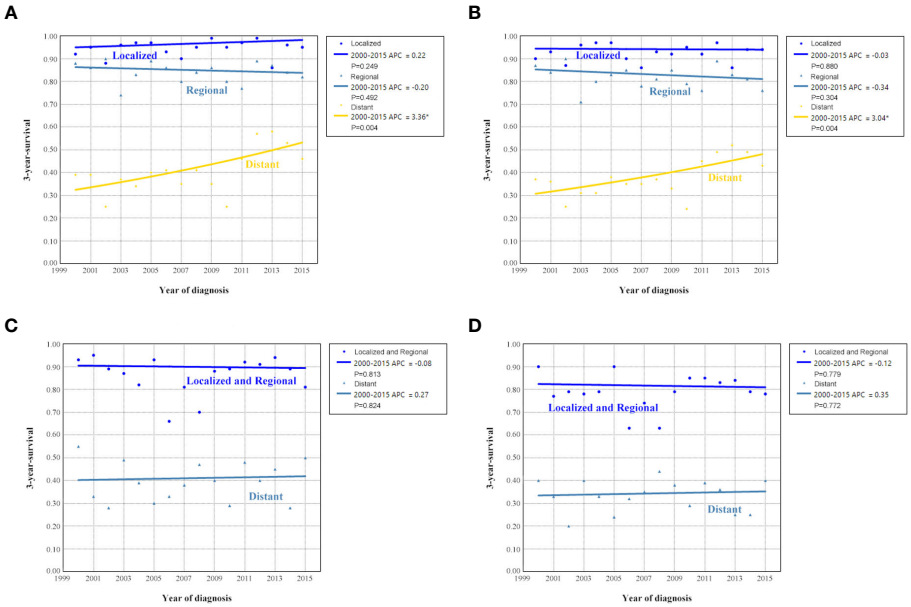

FIGURE 8
Annual percent change (APC) in 3-year ovarian cancer-specific survival and overall survival according to SEER staging in patients aged <65 years [**(A)**, ovarian cancer-specific survival; **(B)**, overall survival] and aged ≥65 years [**(C)**, ovarian cancer-specific survival; **(D)**, overall survival] from 2000 to 2015.

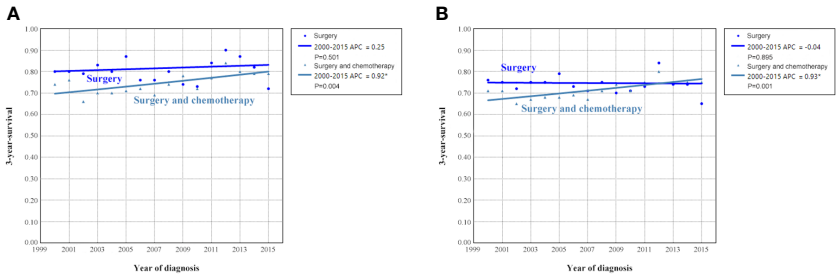

FIGURE 9
Annual percent change (APC) in 3-year ovarian cancer-specific survival **(A)** and overall survival **(B)** according to treatment receipt from 2000 to 2015.

Gynaecological Oncology indicates that adjuvant chemotherapy is not recommended for stage IA, IB, or IC1 OCCC with complete surgical staging (33). A recent SEER study showed that there was no OS benefit for patients with stage IC OCCC receiving adjuvant chemotherapy (5-year OS, 83% vs. 80%, P=0.62) (34). Several small sample studies also found that chemotherapy did not improve the survival of stage I-II OCCC (35, 36). Moreover, a previous study conducted at two tertiary centers in Toronto showed a potential benefit of adjuvant chemotherapy in reducing disease recurrence, although this did not result in an improved OS in stage I-II OCCC (37). In our study, we observed that chemotherapy did not enhance the survival of patients in the localized and regional stages, but the use of chemotherapy improved the survival of patients with distant stage. However, a cohort study conducted using the National Cancer Database demonstrated a benefit in OS for patients with stage I OCCC who received adjuvant chemotherapy (38). Considering the limited conclusive evidence regarding its efficacy in this specific subgroup, the decision to proceed with adjuvant chemotherapy or opt for observation should be personalized after thorough patient counseling.

Several studies have shown that OCCC is considered to be relatively insensitive to chemotherapy compared to other subtypes of EOC. In a study of 27 patients with stage III/IV OCCC and residual disease after surgery, the response rate to platinum-based chemotherapy was only 11.1% (39). Additionally, the response rate to chemotherapy for OCCC patients with recurrent disease was reported to be as low as 6-8% (40). A previous study has found a high probability of ARID1A gene mutation in OCCC (49%), and there is a significant correlation between ARID1A gene mutation and platinum resistance of patients (10). There is also a relationship with the specific tumor microenvironment of OCCC (41–43). Our study found that patients with distant stage receiving surgery and chemotherapy had survival improvement over the years, which may be related to the improvement of chemotherapy regimen methods and exploration of targeted drugs in patients with distant stage OCCC, including the use of bevacizumab in distant stage OCCC.

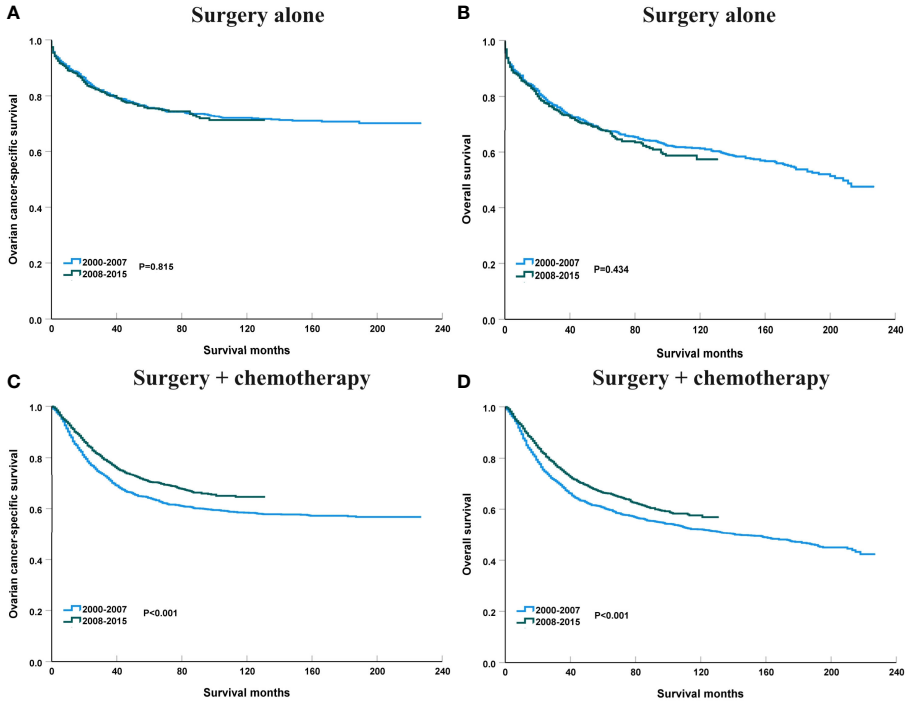

FIGURE 10
The impact of the years of diagnosis on ovarian cancer-specific survival and overall survival in patients treated with surgery alone [**(A)**, ovarian cancer-specific survival; **(B)**, overall survival] and surgery + chemotherapy [**(C)**, ovarian cancer-specific survival; **(D)**, overall survival].

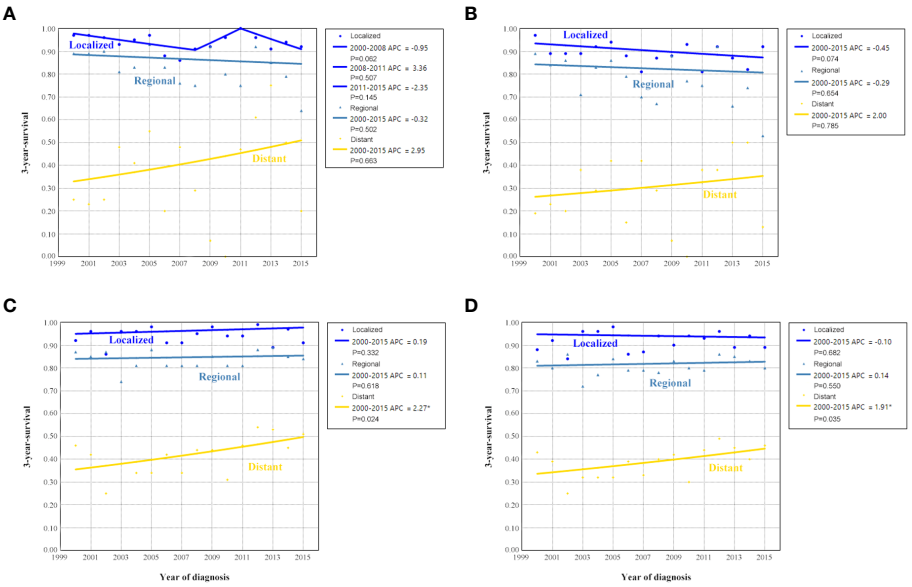

FIGURE 11
Annual percent change (APC) in 3-year ovarian cancer-specific survival and overall survival according to SEER stage in patients treated with surgery alone [**(A)**, ovarian cancer-specific survival; **(B)**, overall survival] and surgery + chemotherapy [**(C)**, ovarian cancer-specific survival; **(D)**, overall survival].

Bevacizumab was approved for the treatment of EOC starting in 2007 and several studies have found that the use of bevacizumab was associated with a higher response rate and better survival outcomes in relapsed or metastatic OCCC (17–19, 44). New chemotherapy regimens, including docetaxel and irinotecan (45), and gemcitabine (46, 47), may improve the treatment sensitivity of platinum-resistant patients. Moreover, the advent of new targeted therapies may further improve patient survival in the future (48).

While OCCC is not as chemosensitive as the more common high-grade serous EOC, there is very limited data regarding the

actual clinical benefit of chemotherapy in OCCC patients. Therefore, it is crucial to emphasize the need for novel targeted treatments for the management of OCCC. Several studies have found that OCCC had promising responses to immune checkpoint inhibitors (49–51). Moreover, the combination of immune checkpoint inhibitors and targeting angiogenesis including bevacizumab or lenvatinib also showed clinical benefit in OCCC (52–54). Notch and VEGF are essential in ovarian cancer angiogenesis and Notch has also been related to chemoresistance. Thus, Notch targeting, and mainly dual targeting of Notch and VEGF, is a promising strategy in ovarian cancer. The combination of Notch inhibition with chemotherapy or antiangiogenics showed interesting activity in early-phase clinical studies. Navicixizumab, a dual anti-Dll4 and anti-VEGF in combination with weekly paclitaxel showed a response rate of 43% in heavily pretreated platinum-resistant patients (55). However, we need to note that the survival improvement is not very significant, and the CSS and OS of distant stage patients indicate an improvement of 5% and 5% between 2000 and 2015, respectively. In addition, we should also note that studies on multiple innovative drugs, including cabozantinib (56), temsirolimus (57), and ENMD-2076, did not significantly improve patient survival (58). Therefore, further exploration based on molecular stratification should be needed in the future to optimize treatment strategies for OCCC patients.

Age itself is a poor prognostic factor in patients with EOC (59). Our study also showed better OCSS and OS in those aged <65 years compared to those aged ≥65 years. Our results also demonstrated a significant survival improvement in patients aged <65 years, especially for patients with distant stage. For young patients, there has been little overall change in the survival rates for localized and regional stage diseases over the years, which may be correlated with the overall stability in treatment patterns among these patients over the past years. However, in patients with distant stage, it is possible that more of them have been enrolled in clinical trials for new drugs or have received more aggressive treatments. In those aged ≥65 years, we found no survival improvement over the years, including those with distant stages. The reasons are not fully clarified. Several factors could contribute to the survival difference by different age groups, including comorbidity, more advanced stage at diagnosis, toxic effects of chemotherapy, or that elderly patients are less often treated with optimal surgery or chemotherapy (60). Moreover, in other histotypes of EOC, age is associated with differences in underlying biology. Therefore, there may also be different biological behaviors exhibited among age groups in OCCC. Further studies are needed to investigate the disparities in biological behaviors among age groups in OCCC (61–64). Finally, most clinical trials exclude elderly individuals or have a median age of only around 60 years (65–67). Due to the potential survival benefits inherent in participating in various clinical trials (68), suitable elderly populations should also participate in clinical trials to evaluate the impact of new treatment regimens on patient survival outcomes as much as possible.

Some limitations should be mentioned. First, the retrospective nature of the study, the long duration of the study period, and the use of different therapeutic approaches are inherent biases in the research design. Second, the lack of a centralized pathology review may have resulted in some misclassification of the histological types. High-grade serous EOC with clear cell change has historically been frequently misclassified as OCCC, which would account for some of the trends observed in the study, such as the decrease in distant stage disease diagnoses (69). Third, the SEER database did not record information regarding chemotherapy regimens, chemotherapy cycles, chemotherapy completion rates, targeted therapy, etc. Fourth, information about comorbidities was also not recorded in the SEER database, which might cause bias in the results. Moreover, some of the findings that showed borderline or marginal significance may benefit from long-term follow-up in order to enhance the statistical power. Finally, adjustment for multiple testing was not performed for this study.

Conclusions

In conclusion, our study observed a significant increase in the survival outcomes in OCCC from 2000 to 2015, and patients aged <65 years and those with distant stage experienced a greater improvement in survival.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

As the SEER database consists of de-identified information, the study was exempt from the approval process of the Institutional Review Boards of the First Affiliated Hospital of Xiamen University. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

B-QT: Conceptualization, Data curation, Writing – original draft. S-WW: Conceptualization, Data curation, Formal analysis, Writing – original draft. J-YX: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. S-GW: Funding acquisition, Resources, Validation, Visualization, Writing – review & editing. JZ: Conceptualization, Data curation, Investigation, Resources, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Angelo Finelli,
ULSS2 Marca Trevigiana, Italy
Jessica Lim,
Guy's and St Thomas' NHS Foundation Trust, United Kingdom

*CORRESPONDENCE
Fandou Kong
tg980927@126.com

†These authors have contributed equally to this work

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Construction and validation of log odds of positive lymph nodes (LODDS)-based nomograms for predicting overall survival and cancer-specific survival in ovarian clear cell carcinoma patients

Zesi Liu[1†], Chunli Jing[2†], Yashi Manisha Hooblal[1], Hongxia Yang[1], Ziyu Chen[1] and Fandou Kong[1*]

[1]Department of Gynecology and Obstetrics, The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China, [2]Department of Gynecology and Obstetrics, The Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China

Background: Ovarian clear cell carcinoma (OCCC) is one of the special histologic subtypes of ovarian cancer. This study aimed to construct and validate log odds of positive lymph nodes (LODDS)-based nomograms for predicting the overall survival (OS) and cancer-specific survival (CSS) in patients with OCCC.

Methods: Patients who underwent surgical treatment between 2010 and 2016 were extracted from the Surveillance Epidemiology and End Results (SEER) database and the data of OCCC patients from the First Affiliated Hospital of Dalian Medical University were used as the external validation group to test the validity of the prognostic model. The best-fitting models were selected by stepwise Cox regression analysis. Survival probability was calculated by the Kaplan–Meier method, and the differences in survival time between subgroups were compared using the log-rank test. Each nomogram's performance was assessed by the calibration plots, decision curve analysis (DCA), and receiver operating characteristics (ROC) curves.

Results: T stage, distant metastasis, marital status, and LODDS were identified as significant risk factors for OS. A model with four risk factors (age, T stage, stage, and LODDS value) was obtained for CSS. Nomograms were constructed by incorporating the prognostic factors to predict 1-, 3- and 5-year OS and CSS for OCCC patients, respectively. The area under the curve (AUC) range of our nomogram model for OS and CSS prediction ranged from 0.738-0.771 and 0.769-0.794, respectively, in the training cohort. The performance of this model was verified in the internal and external validation cohorts. Calibration plots illustrated nomograms have good prognostic reliability.

Conclusion: Predictive nomograms were constructed and validated to evaluate the OS and CSS of OCCC patients. These nomograms may provide valuable prognostic information and guide postoperative personalized care in OCCC.

KEYWORDS

LODDS, ovarian clear cell carcinoma, nomogram, overall survival, cancerspecific survival

1 Introduction

Ovarian cancer is one of the most common malignancies of the female reproductive tract, of which 90% are epithelial ovarian cancer (EOC) (1). Approximately 230,000 people are diagnosed with EOC each year, resulting in 150,000 deaths annually (2).Ovarian clear cell carcinoma (OCCC) is one of the special histologic subtypes of EOC, accounting for about 5% of EOC in western countries, and approximately 20% in Asian countries (3). Compared with EOC, OCCC is more refractory to platinum-based first-line chemotherapy, with the response rate in OCCC being 11.56% (4, 5). Although early-stage OCCC has a relatively good prognosis, with a 5-year survival rate of 90%, the median overall survival time in advanced-stage OCCC is significantly shorter than that in high-grade serous ovarian cancer (HGSOC) (6, 7). Lymph node (LN) metastasis is one of the main metastasis modes of OCCC (8). The status of regional lymph nodes (LNs) retrieved during surgery appears to be not only an independent prognostic factor but also an essential factor in assessing the risk of recurrence of patients with OCCC (9). The American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) tumor-node-metastasis (TNM) classification is widely used to predict prognosis but may lead to an underestimation of N-stage due to its calculation only based on the absolute number of positive LNs. Therefore, many novel LNs staging systems have been proposed to improve the assessment of prognosis in OCCC.

Log odds of positive lymph nodes (LODDS) comprehensively considers the effect of the number of positive lymph nodes (PLNs) and resected lymph nodes (RLNs) on the prognosis for tumor patients and has been widely proven as an effective prognosis prediction tool and a novel lymph node staging system in various malignancies (10). LODDS is calculated with the following expression:

$$\mathbf{Log}\left[(\mathbf{PLNs}+\mathbf{0.5})/(\mathbf{RNs}-\mathbf{PLNs}+\mathbf{0.5})\right]$$

In addition, compared with the AJCC N stage, LODDS showed better discrimination abilities and well-fitting in predicting survival in patients with stage IV rectal cancer (11).

Based on entropy, the Akaike Information Criterion (AIC) statistic calculates the tradeoff between overfitting and poor-fitting models and takes into account the number of parameters that the model estimates to select the more parsimonious model (12, 13). The corrected Akaike Information Criterion (AICc) is a modified version of the AIC including a correction term for small sample sizes and is calculated as following:

$$\mathbf{AICc} = \mathbf{AIC} + [\mathbf{2k}(\mathbf{k}+\mathbf{1})]/(\mathbf{n}-\mathbf{k}-\mathbf{1})$$

The k denotes the number of free parameters, and n is the number of observations (14, 15). In this study, we aimed to use AICc to build prognostic models of the overall survival (OS) and cancer-specific survival (CSS) for OCCC. Finally, nomogram is used to integrate multiple prognostic factors, which enables it to predict a patient's survival with relative accuracy (16).

2 Materials and methods

2.1 Data source and study population

The Surveillance, Epidemiology, and End Results (SEER) database is supported by the national cancer institute (NCI) of USA and has been around since 1973. The SEER database collects information on every case of cancer reported in 19 geographic regions of the U.S., accounting for about 34.6% of the U.S. population. The SEER∗Stat software (version 8.3.6, https://seer.cancer.gov/seerstat/) was used to screen eligible patients who were OCCC between 2010 and 2016. According to the International classification of Diseases for Oncology, 3rd edition (ICD-O-3) morphological code, histopathologic classification of patients was performed, and the subtypes included: 8310/3, 8313/3, 8443/3 and 8444/3. At the same time, in order to increase the reliability of the results of this trial and to minimize experimental bias, data of OCCC patients from the Department of Gynecology of the First Affiliated Hospital of Dalian Medical University from June 2011 to June 2021 were used as the external validation group to test the validity of the prognostic model (n = 50).

Exclusion criteria are as follows: (a) No histologic diagnosis; (b) Contain two or more primary malignancies; (c) Survival months less than one month; (d) Treatment by primary site surgery; (e) ≥18 years of age; (f) Complete LN data; (g) Lack of relevant demographic and clinicopathological characteristics.

2.2 Variables collected

The following variables for this study were extracted: age, race, marital status, grade (G1 is equivalent to well differentiated; G2 is equivalent to moderately differentiated; G3 is equivalent to poorly differentiated; G4 is equivalent to undifferentiated), 7th AJCC stage, 7th AJCC TNM stage, tumor size, chemotherapy record, RLNs, PLNs, organ metastasis. OS and CSS were considered the primary endpoints. The cut-off values were established by *X-tile* program (3.5.1) (17).

2.3 Statistical analysis

All OCCC patients from the SEER database were assigned as the training group, and 30% of them were selected by random sampling as the internal validation group. All 50 OCCC patients collected from the First Affiliated Hospital of Dalian Medical University were used as external validation group. Baseline differences in demographic variables between the training cohort and validation cohort were investigated using chi-square tests and independent-sample *t* tests. Survival probability was calculated by the Kaplan–Meier method, and the differences in survival time between distinct subgroups were compared using the Log-rank test. To identify significant univariate results, the univariate results were visually inspected in R software by comparing the cumulative incidence function (CIF) based on the Turnbull estimator to the cumulative incidence function based on the normal distribution. The Akaike Information Criterion, corrected for small sample size was determined; a smaller AICc means a better fit, and was penalized for being overloaded with parameters (18, 19). As a result, the best-fitting model was chosen by selecting the lowest *AICc*. Then, nomograms were constructed and used to predict 1-, 3- and 5-year OS and CSS for OCCC patients. The predictive performance of the nomogram was verified internally for discrimination and calibration through the *C*-statistics, area under the curve (AUC) and calibration curves (20, 21). Finally, by evaluating model performance by considering the clinical consequences of true positives and false positives, decision curve analysis (DCA) compares the net benefit between the nomogram model and the multivariate *Cox* regression model across a range of threshold probabilities so that we can select better predictive models for clinical decision making.

All statistical analyses were performed with R version 4.2.1 (www.R-project.org). A *P*-value of< 0.05 was considered statistically significant.

3 Results

3.1 Patient characteristics

A total of 766 patients with primary OCCC from the SEER database were enrolled in the trial, and data on 50 patients with primary OCCC were collected as an external validation group for the trial (Figure 1) and the characteristics of these patients from the SEER database are listed in Table 1. There were no significant differences between the training group and the validation group with regards to the demographic and clinicopathological characteristics, thus implying that two groups were comparable. The incidence of OCCC is higher in the elderly, with 86.5% of patients older than 45 years. The distribution of race among patients demonstrated that the largest ethnic groups were white people (72.1%). Although most patients were diagnosed at a limited stage (64.8%), 53.4% had poorly differentiated tumors, 36.6% had undifferentiated tumors and 82.1% received chemotherapy during treatment in the training cohort.

3.2 Survival analysis

In this study, the 14 variables included were analyzed by multivariate *Cox* analysis and stepwise *Cox* regression analysis. The results of multivariate *Cox* analysis indicated that Blacks (HR:2.27, 95% CI:1.03-5.00; P=0.042), AJCC stage III (HR:3.23, 95% CI:1.45-7.20; P=0.004), AJCC stage IV (HR:5.08, 95% CI:2.17-11.90; P<0.001), AJCC T3 stage (HR:2.20, 95% CI:1.12-4.30; P=0.022), distant metastasis (HR:1.69, 95% CI:1.12-2.17; P=0.014), and LODDS value (HR:1.61, 95% CI:1.00-2.60; P=0.048) were risk factors of OS. The OS was better for married OCCC patients (HR:0.79, 95% CI:0.57-0.91; P=0.043) (Supplementary Figure 1). By comparing the goodness-of-fit AICc statistics of model performance, the model with the lowest AICc value was the best-fitting model (22) (Figure 2A). As a result, AJCC T2 stage (HR:2.50, 95% CI:1.71-3.64; P<0.001), AJCC T3 stage (HR:5.17, 95% CI:3.69-7.25; P<0.001), distant metastasis (HR:1.77, 95% CI:1.12-2.81; P=0.015), marital status (HR:0.75, 95% CI:0.57-0.99; P=0.044), and LODDS (HR:1.57, 95% CI:1.26-1.95; P<0.001)

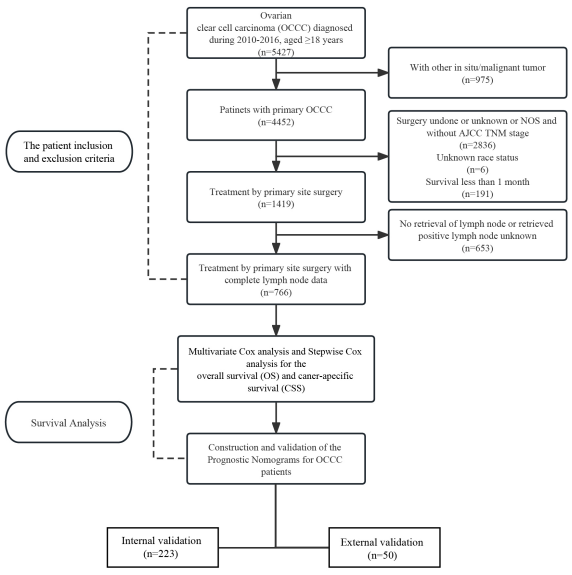

FIGURE 1
Flow diagram of patient selection and survival analysis.

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TABLE 1 Patients' demographics and clinicopathological characteristics.

Characteristic	Training cohort (n=766)	Internal validation group (n=223)	External validation group (n=50)	*P*-value
Age (years), n (%)				
Mean ± SD	55.7 ± 10.2	54.5 ± 9.7	52.9 ± 8.4	0.769
18-44	103 (13.5)	29 (12.9)	10(20.0)	0.842
45-52	197 (25.7)	58 (26.1)	16(32.0)	
>52	466 (60.8)	136 (61.0)	24(48.0)	
Race, n (%)				
White	552 (72.1)	161 (72.4)	–	0.961
Black	27 (3.5)	8 (3.7)	–	
Other/Unknown	187 (24.4)	54 (23.9)	–	
Grade, n (%)				
Well differentiated (G1)	9 (1.2)	2 (0.9)	10(20.0)	0.944
Moderately differentiated (G2)	68 (8.9%)	18 (8.2)	8(16.0)	
Poorly differentiated (G3)	409 (53.4)	121 (54.3)	20(40.0)	
Undifferentiated (G4)	280 (36.6)	82 (36.6)	12(24.0)	
AJCC T Stage, n (%)				
T1	532 (69.5)	154 (69.2)	29(58.0)	0.909
T2	110 (14.4)	31 (13.8)	11(22.0)	
T3	124 (16.2)	38 (17.0)	10(20.0)	
AJCC N Stage, n (%)				
N0	645 (85.4)	191 (85.6)	40(80.0)	0.961
N1	112 (14.6)	32 (14.4)	10(20.0)	
AJCC M Stage, n (%)				
M0	736 (96.1)	213 (95.3)	47(94.0)	0.980
M1	30 (3.9)	10 (4.7)	3(6.0)	
Stage, n (%)				
I	496 (64.8)	145 (64.7)	28(56.0)	0.948
II	89 (11.6)	24 (10.8)	11(22.0)	
III	151 (19.7)	46 (20.7)	8(16.0)	
IV	30 (3.9)	8 (3.7)	3(6.0)	
Chemotherapy, n (%)				
Yes	629 (82.1)	181 (81.2)	42(84.0)	0.713
No	137 (17.9)	42 (18.8)	8(16.0)	
Marital status, n (%)				
Married	428 (55.9)	125 (56.0)	29(58.0)	0.934
Unmarried	338 (44.1)	98 (44.0)	21(42.0)	
Tumor size (mm)				
Mean ± SD	123 ± 4.2	121 ± 3.8	124 ± 4.4	0.936
<85	209 (27.3)	60 (26.7)	14(28.0)	0.728

(Continued)

TABLE 1 Continued

Characteristic	Training cohort (n=766)	Internal validation group (n=223)	External validation group (n=50)	*P*-value
Tumor size (mm)				
85-179	411 (53.7)	119 (53.2)	27(54.0)	
≥180	146 (19.0)	44 (20.1)	9(18.0)	
CA125, n (%)				
Negative/Unknown	334 (43.6)	96 (43.1)	12(24.0)	0.871
Positive	432 (56.4)	127(56.9)	38(76.0)	
RLNs (Mean ± SD)	16.3 ± 11.9	16.6 ± 12.4	15.9 ± 12.7	0.646
PLNs (Mean ± SD)	0.56 ± 2.23	0.52 ± 2.10	0.59 ± 2.31	0.733
LODDS (Mean ± SD)	-1.26 ± 0.55)	-1.27 ± 0.54	-1.28 ± 0.52	0.727

AJCC, American Joint Committee on Cancer; RLNs, resected lymph nodes; PLNs, positive lymph nodes; LODDS, Log odds of positive lymph nodes.
£: P-value with Bonferroni adjustment.

were screened and identified as significant risk factors for OS in OCCC patients (Figure 2C).

For CSS, age<45 years old (HR:1.64, 95% CI:1.43-1.94; P=0.021), AJCC stage 3 (HR:4.23, 95% CI:2.07-8.63; P<0.001), AJCC stage 4 (HR:6.23, 95% CI:2.80-13.83; P<0.001), distant metastasis (HR:1.91, 95% CI:1.47-2.71; P=0.039) and LODDS value (HR:1.68, 95% CI:1.12-2.51; P=0.012) were identified as risk factors. Interestingly, OCCC patients with evaluative CA125 indicated better CSS (HR:0.66, 95% CI:0.43-1.00; P=0.050) (Supplementary Figure 2). Similarly, a model with the lowest AICc value (Figure 2B) included four risk factors: age<45 years old (HR:1.61, 95% CI:1.42-1.88; P=0.008), AJCC T3 stage (HR:2.07, 95% CI:1.19-3.61; P=0.010), AJCC stage III (HR:3.54, 95% CI:1.93-6.49; P<0.001), AJCC stage IV (HR:5.71, 95% CI:2.79-11.68; P<0.001) and LODDS value (HR:1.40, 95% CI:1.10-1.78; P=0.006) was screened to predict CSS (Figure 2D). The Log-rank test was also used to explore differences in survival between subgroups based on risk factors and these results were visualized using Kaplan–Meier

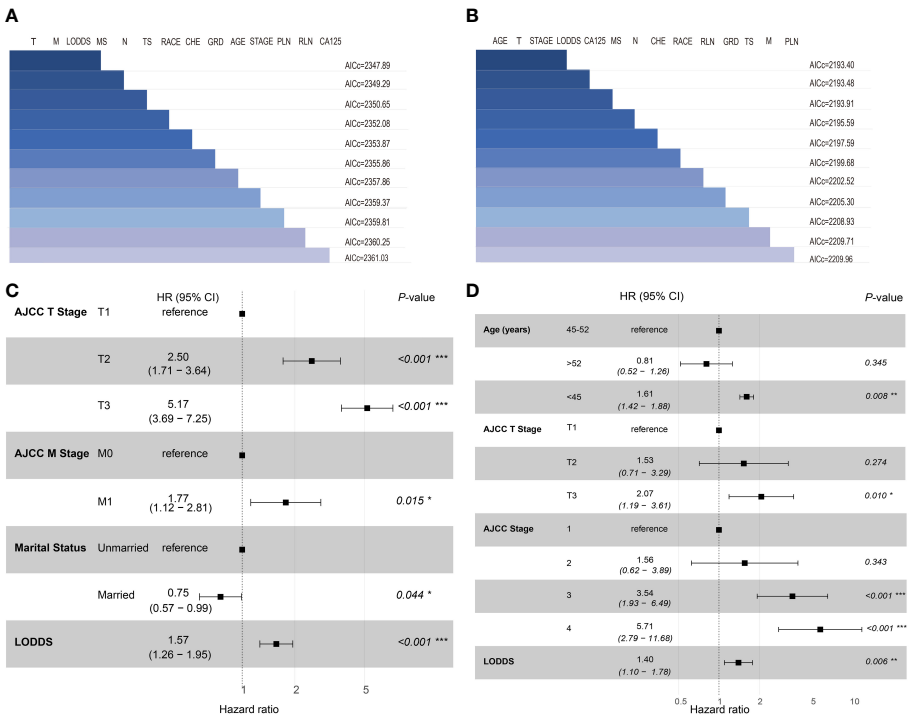

FIGURE 2
Set of models created with forward-stepwise selection for OS **(A)** and CSS **(B)**, ranked by AICc. Shaded boxes signify the factors included within the model. Forest plots of independent risk factors in stepwise Cox regression analysis of OS **(C)** and CSS **(D)**. T, 7th AJCC T Stage; M, 7th AJCC M Stage; MS, Marital status; N, 7th AJCC N Stage; TS, Tumor size; CHE, Chemotherapy; GRD, grade; PLN, positive lymph node; RLN, resected lymph node.

curves. According to the Kaplan-Meier survival curves in Figures 3A–D, there were significant differences in survival in AJCC T stage (P<0.001), organ metastasis (P<0.001), marital status (P=0.002), LODDS value (P<0.001) subgroups. In terms of competing risks, CIF curves were implemented to the risk factors according to CIF values for cancer-specific death (Supplementary Figures 3A–D).

3.3 Construction and validation of the prognostic nomograms

Nomograms were constructed by incorporating the prognostic factors to predict 1-, 3- and 5-year OS (Figure 4A) and CSS (Figure 4B) for OCCC patients. The C-statistic ranges from 0.5, which indicates the absence of discrimination, to 1.0, indicating perfect discrimination. Generally speaking, if the C-statistic value is greater than 0.7, the model has very good predictive value (23, 24). The C-statistic values of our nomogram model for OS and CSS prediction were 0.756 (95% CI: 0.728-0.764) and 0.746 (95%CI: 0.744-0.748), which denoted the good performance of the nomogram models. The actual survival rates of OCCC showed a good agreement with the optimal bootstrap predicted values, indicating good prognostic reliability (Supplementary Figures 4–7). The AUC values also indicated the nomogram had favorable sensitivity and specificity in predicting OS (Figures 5A, B) and CSS (Figures 5C, D) in OCCC patients. Additionally, the DCA curve indicated that the nomogram models had better prediction performance than the multivariate *Cox* regression model (Supplementary Figures 8, 9). Similar results were observed in the internal validation cohort. Finally, the real-world data was utilized for external validation. The 1, 3, 5-year AUC area was 0.691, 0.724 and 0.749 for OS, and the 1, 3, 5-year AUC area was 0.558, 0.667 and 0.716 for CSS, respectively (Figures 6A, B), suggesting that the prognostic model in this study could effectively predict OS and CSS in patients with OCCC.

4 Discussion

In the current study, according to stepwise Cox regression analyses, we screened out risk factors separately related to OS and CSS of OCCC patients. By comparing *AICc* scores, nomograms were constructed to assess the 1-, 3- and 5-year CSS and OS based on the identified prognostic factors (25). AUC, calibration curves and DCA curves in both training and validation sets showed favorable discrimination and calibration, indicating that our nomograms had good calibration power. Each risk factor

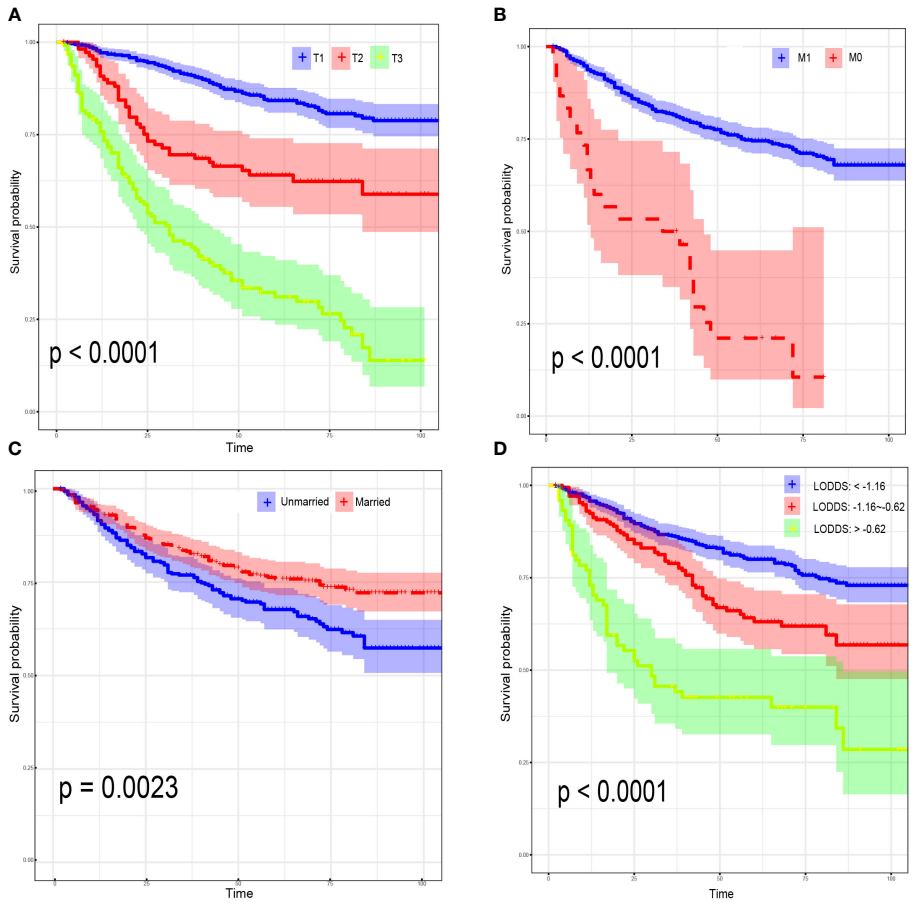

FIGURE 3
Kaplan–Meier curves for overall survival, stratified by 7th AJCC T Stage **(A)**; 7th AJCC M Stage **(B)**; marital status **(C)**; LODDS **(D)**.

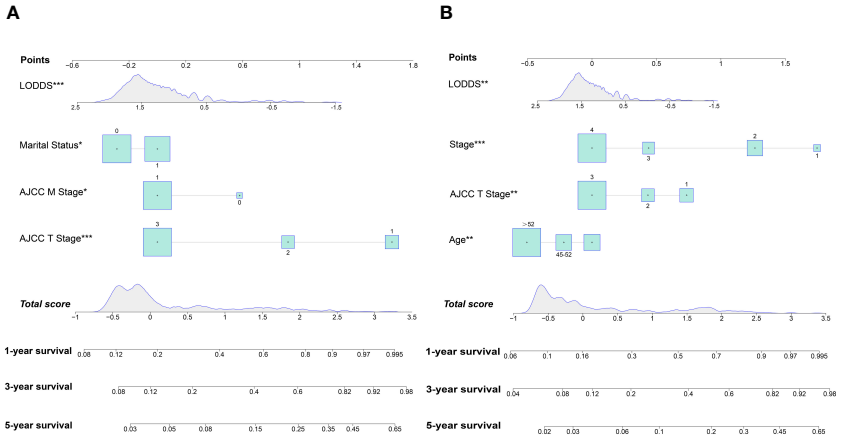

FIGURE 4
Nomogram for predicting 1-, 3- and 5-year OS **(A)**; Nomogram for predicting 1-, 3- and 5-year CSS **(B)**.

included in the nomograms was attributed a risk score and was applied to successfully build a risk stratification system for predicting the OS and CSS of OCCC patients. Generally, younger age implied a better prognosis in EOC patients due to stronger immune response and better physical fitness (26, 27). However, our result indicated that OCCC patients younger than 45 years tended to have poorer prognosis. This result was in line with those of previous studies (28), which indicated the effect of age in OCCC may be different from other EOC. Moreover, we found a significant difference in the prognosis of OCCC patients in different marital statuses. Specifically, the prognosis of unmarried OCCC patients was worse compared to those who were married, which is the same as the finding of Kravdal et al (29). In this regard we generate the following analysis. Firstly, the companionship needs of married patients are met, and previous studies have shown that patients tend to be more emotionally positive when emotional needs are met.

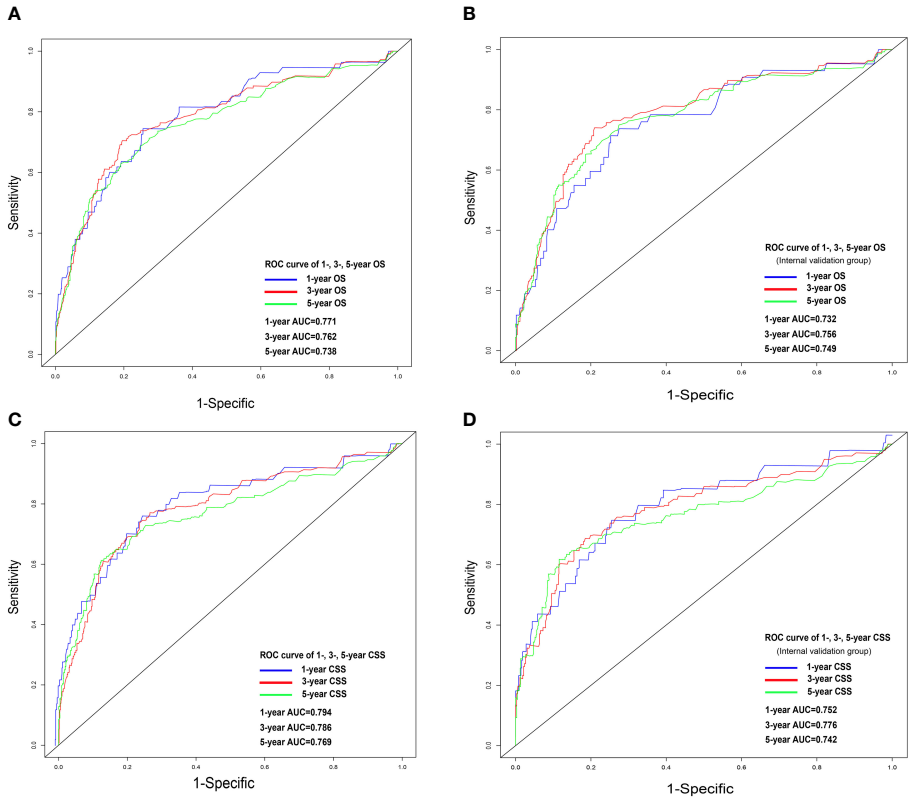

FIGURE 5
ROC analysis for OS and CSS. OS nomogram ROC curve for training cohort **(A)** and internal validation cohort **(B)**; CSS nomogram ROC curve for training cohort **(C)** and internal validation cohort **(D)**. OS, overall survival; CSS, cancer-specific survival; ROC, receiver operating characteristics.

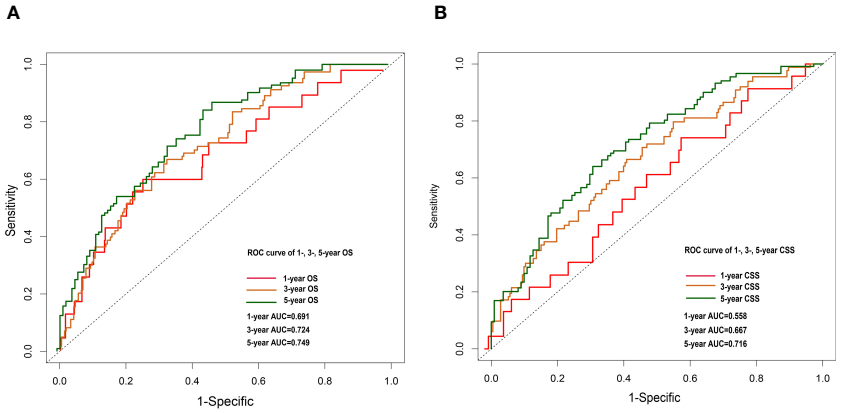

FIGURE 6
1-, 3- and 5-year OS nomogram ROC curve for external validation cohort **(A)**. 1-, 3-and 5-year CSS nomogram ROC curve for external validation cohort **(B)**.

Therefore, MS may influence the prognosis of OCCC patients through emotions (30, 31). Secondly, Nayeri and colleagues found that married individuals tend to be diagnosed with cancer at an early stage (32).

The *AJCC* N-staging, a two-category system (N0: no regional lymph node metastasis; N1: histologically confirmed retroperitoneal lymph node metastasis), is the most basic and widely used cancer staging system and plays a vital role as a key prognostic factor in the development of postoperative treatment plans as well as in follow-up (33–35). However, this LN staging system does not account for the prognostic impact of PLNs and the number of RLNs. In fact, Nie et al. found that an increase in the number of PLNs is associated with lower DFS as well as OS (36). There is increasing evidence that the extent of LN dissection is also associated with the prognosis of patients with EOC (37). Therefore, the current LN staging appears inadequate in providing physicians with sufficient valuable information. Both LNR (the ratio of PLNs/RLNs) and LODDS take into account the number of PLNs and RLNs and both are more accurate than the pN staging system in predicting prognosis in several tumors (38, 39), but it is controversial which one is more superior (40, 41). There are many drawbacks of LNR led us to choose LODDS as the LN staging tool for this study. First, when the value of LNR is 0, its applicability is limited (e.g., 1/1 vs. 30/30). As the number of RLNs increases, the risk of post-op complications such as infection, vascular/nerve injuries, lymphatic leakage and lymphoedema increases, thus affecting patient prognosis (42). Then, the prognosis of patients may be significantly different despite having the same LNR (e.g., 1/2 vs. 15/30). Third, as mentioned, the majority of OCCC patients were still in stage I at the time of diagnosis (6). The probability of LN metastasis in early OCCC is relatively low, with only 3.6% in pT1aM0 and pT2aM0, compared with 71.6% in HGSOC (43). Compared with LNR, LODDS also has a unique value in the prognostic assessment of LN-negative patients (44). The value of LODDS increases with the decrease of RLNs. Additionally, there is an active debate about systematic lymphadenectomy in early-staging OCCC (45, 46). However, considering the calculation method of LODDS mentioned above, the clinician only needs to obtain the number of RLNs and the number of PLNs respectively to achieve the accurate value of LODDS. Therefore, LODDS acquisition does not depend on systematic lymphadenectomy. This will greatly reduce the difficulty of the surgery and the postoperative complication rate.

Several studies have found that the applications of nomogram models in several tumors have a better prognostic performance than the staging systems alone (47–49). With these nomograms, doctors can calculate the risk score for each patient, allowing for individualized prognostic assessment and guides postoperative personalized treatment. The AUC of the training and validation cohorts of the nomogram developed in our study was over 0.7, with the calibration points were separated on both sides of the ideal line. This means that we can obtain a more reasonable and more accurate follow-up schedule. Based on the results of the DCA curves, we believe that our model has higher discriminatory power than the traditional multivariate Cox regression.

It should be noted that there are several limitations in this study. First, while the SEER database certainly has a larger volume of data compared to prior case-series reports, it lacks records of some key variables related to prognoses, such as specific chemotherapy protocols, preoperative comorbidities, or postoperative complications. It is worthy to note that in this study we used part of the training set as the internal validation set, which does run the risk of producing an overly optimistic assessment of the efficacy of the predictive model. Although data from the real world supported our results, we will seek to re-evaluate the efficacy of our model in the future using completely independent data sets of larger sample sizes. Then, selection bias was inevitable due to the study's retrospective nature. Fourth, statistical analyses were performed without correction for multiple testing, which may lead to potential false positives in the survival analysis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

ZL: Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. CJ: Writing – review & editing, Validation, Methodology. YH: Writing – original draft, Methodology, Investigation. HY: Writing – original draft, Software, Visualization, Validation. ZC: Writing – review & editing, Visualization, Data curation. FK: Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1370272/full#supplementary-material

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EDITED BY
Mignon Van Gent,
Amsterdam University Medical Center,
Netherlands

REVIEWED BY
Maria Magdalena Montt-Guevara,
University of Pisa, Italy
Ying-Cheng Chiang,
National Taiwan University, Taiwan

*CORRESPONDENCE
Ce Bian
terrybian@163.com

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Research progress in endometriosis-associated ovarian cancer

Ling Tang and Ce Bian*

Department of Gynecology and Obstetrics, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, West China Second Hospital, Sichuan University, Chengdu, Sichuan Province, China

Endometriosis-associated ovarian cancer (EAOC) is a unique subtype of ovarian malignant tumor originating from endometriosis (EMS) malignant transformation, which has gradually become one of the hot topics in clinical and basic research in recent years. According to clinicopathological and epidemiological findings, precancerous lesions of ovarian clear cell carcinoma (OCCC) and ovarian endometrioid carcinoma (OEC) are considered as EMS. Given the large number of patients with endometriosis and its long time window for malignant transformation, sufficient attention should be paid to EAOC. At present, the pathogenesis of EAOC has not been clarified, no reliable biomarkers have been found in the diagnosis, and there is still a lack of basis and targets for stratified management and precise treatment in the treatment. At the same time, due to the long medical history of patients, the fast growth rate of cancer cells, and the possibility of eliminating the earliest endometriosis-associated ovarian cancer, it is difficult to find the corresponding histological evidence. As a result, few patients are finally diagnosed with EAOC, which increases the difficulty of in-depth study of EAOC. This article reviews the epidemiology, pathogenesis, risk factors, clinical diagnosis, new treatment strategies and prognosis of endometriosis-associated ovarian cancer, and prospects the future direction of basic research and clinical transformation, in order to achieve stratified management and personalized treatment of ovarian cancer patients.

KEYWORDS

endometriosis, ovarian neoplasms, endometriosis-associated ovarian cancer, risk factors, diagnosis

1 Introduction

Endometriosis (EMS) is a prevalent condition that significantly impacts the quality of life and reproductive function in women. According to statistics, the prevalence of EMS among women of childbearing age ranges from 5% to 10% (1), while it can reach as high as 20% to 60% in women experiencing pelvic pain or infertility (2). Despite its benign nature, EMS shares biological characteristics with malignant tumors, showing invasive, adhesive, and metastatic potentials, with a risk of malignant transformation. As epidemiological and molecular genetic

research continues to reveal, EMS is closely related to epithelial ovarian cancer (EOC), especially ovarian clear cell carcinoma (OCCC) and ovarian endometrioid carcinoma (OEC). Therefore, ovarian cancers closely associated with endometriosis, which may arise malignantly from endometriosis, predominantly manifest as OCCC and OEC. Collectively, these are referred to as Endometriosis-associated ovarian cancer (EAOC). Early in 1925, Sampson (3) pioneered the demonstration of the correlation between EMS and ovarian cancer and subsequently proposed the pathological diagnostic criteria for EAOC. These criteria comprise: 1) the existence of cancerous tissue in proximity to endometriotic lesions, 2) exclusion of metastasis from other tumor sources, and 3) the presence of characteristic glandular epithelium surrounding endometriotic lesions. In 1953, Scott introduced an additional criterion (4): microscopic evidence of the transformation from endometriotic lesions to malignant tissue. Compared to non-EAOC patients, those with EAOC exhibit a younger age at diagnosis, an earlier onset of the disease, lower tumor grades, and lower recurrence rates (5), suggesting that EAOC represents a distinct subtype of solid tumors. At present, the diagnosis of EAOC mainly depends on surgery and pathological examination, but the rate of missed diagnosis is often increased due to the "burnout effect" of the tumor and the doctor's neglect of EMS lesions when reading the film. By comprehensively reviewing the epidemiology, pathogenesis, risk factors, clinical diagnosis, treatment modalities and prognosis of EAOC, this review aims to elucidate the distinctive characteristics of EAOC, facilitate early identification by clinicians and provide a valuable reference for enhancing the prognostic outcomes associated with EAOC.

2 Epidemiology of EAOC

In the investigation, we have noted a relatively low risk of ovarian cancer in the general population, standing at merely 1.31% (6). Nevertheless, for individuals affected by EMS, the risk of ovarian cancer undergoes a significant escalation, exhibiting a relative risk of 2.51-fold (7), with a lifetime risk reaching 2.5% (8). Despite the comparatively modest overall incidence risk, the heightened attention is warranted due to the elevated mortality rate of ovarian cancer within gynecological cancers and the prevalent and chronic nature of EMS. In recent years, substantial interest has been directed towards researching whether individuals with endometriosis face an elevated risk of cancer. Consistent findings in the research field underscore that EMS significantly elevates the risk of OCCC and OEC. A study in the Netherlands involving 131,450 patients with histologically confirmed cases of endometriosis revealed incidence rate ratios for OCCC and for OEC (9) with similar incidence rates from a Chinese study (10).

3 EAOC pathogenesis

3.1 Molecular biology

Currently, high mutation frequencies are observed in the genes ARID1A, phosphatase and tensin homolog (PTEN), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3C) in EAOC (11). The ARID1A gene, encoding a crucial component of the SWI/SNF complex, is considered a tumor suppressor gene and is frequently mutated in various cancers, with the highest mutation rates found in the two ovarian cancers associated with endometriosis (12). By using gene sequencing technology, ARID1A mutations were identified in 46% of 55 cases of OCCC, 30% of 10 cases of OEC, and none of the 76 cases of high-grade serous ovarian carcinoma (13). PTEN, located on chromosome 10, is a tumor suppressor gene involved in cell regulation,inhibiting tumor cell proliferation, adhesion, metastasis, and angiogenesis (14, 15). The PI3K/AKT/mTOR (PI3K) pathway is a classical signaling pathway that plays a crucial role in regulating cell survival, growth, and proliferation, and mutations in this pathway are common in human cancers (16).

Previous research has indicated that ARID1A gene mutations in OCCC may be associated with the abnormal activation of the PI3K-AKT pathway (17), a key player in altering tumor growth, proliferation, and metastasis. This abnormal activation enhances the invasiveness of tumors, shortening the time to cancer recurrence and death, suggesting an unfavorable prognosis (18). However, a mouse experiment revealed that the sole loss of ARID1A gene function does not induce ovarian cancer. Deleting the ARID1A gene alone does not induce ovarian cancer in mice, but when the ARID1A and PTEN genes are simultaneously knocked out, 60% of mice develop ovarian cancer with intra-abdominal dissemination, and 40% exhibit excessive proliferation of ovarian epithelium (19). Further research by Chandler et al. indicated that simultaneous deletion of the ARID1A gene and activation of the PIK3CA gene can induce OCCC in mice (20). In addition, ARID1A mutation can lead to impaired interferon (IFN) gene expression and reduce tumor response to immunotherapy (21).

A recent study involving 1,623 EAOC patients, including 1,078 cases of OEC and 545 cases of OCCC, confirmed these findings (22). Specifically, the relationship between ARID1A loss/mutation, clinical characteristics, outcomes, CD8+ tumor-infiltrating lymphocytes (CD8+TIL), and DNA mismatch repair deficiency (MMRD) revealed ARID1A gene inactivation in 42% of OCCC and 25% of OEC. However, ARID1A inactivation did not significantly impact the overall survival and progression-free survival of OCCC and OEC. Nonetheless, the continuous advancement in targeted therapeutic approaches, synthetic lethal strategies, and the investigation of the prognostic significance of ARID1A in immune modulation therapy is ongoing, indicating potential implications for prognosis (23, 24). Additional genes associated with EMS malignancy and EAOC: tumor suppressor gene p53, hepatocyte nuclear factor 1 homeobox B (HNF-1β), β-catenin gene (CTNNB1), kirsten rat sarcoma viral oncogene (KRAS), protein kinase B (KT), MicroRNA (miRN) are detailed in Table 1.

Past studies have indicated that the tumor microenvironment, particularly cancer-associated mesenchymal stem cells (CA-MSCs), plays a crucial role in the growth of ovarian cancer. Atiya et al.research report highlighted a subset of endometriosis-associated mesenchymal stem cells (enMSCs) in endometriosis (36), characterized by the loss of CD10 expression. This subset, by increasing the expression of iron export proteins, elevated intracellular iron levels in OCCC, thereby promoting OCCC

TABLE 1 Genes associated with EAOC formation.

Genes	Current research
ARID1A	The mutation rate of ARID1A gene in OCCC was 42% and in OEC was 25% (22); Mutations activation the PI3K-AKT pathway, induction of tumorigenesis and allows tumor cell proliferation (17, 18); ARID1A interacted with Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2) antagonized EZH2-mediated IFN responsiveness, shape cancer immune phenotype and immunotherapy (21)
PTEN	Mutation occurs in the early stage of tumorigenesis (25); Acts in concert with ARID1A to induction of tumorigenesis (20); Promoted metastasis and chemoresistance in ovarian cancer sell (14, 15);
PI3KCA	The mutation rate of PI3KCA gene in OCCC was 32% (26); Mutations may occur in late-stage OCCC (27);
HNF-1β	Mutations are common in OCCC, hypomethylation patterns are oncogenic (28);
CTNNB1	Mutations occurred only in OEC (29);
p53	High expression in benign endometriotic lesions next to the endometrioid or clear cell carcinoma (30, 31); Involved in tumorigenesis of malignancies (32);
KRAS	The mutation rate of KRAS gene in EAOC was 29% (33); allows tumor cell proliferation;
AKT	Activation PI3K/AKT pathway; involved in the occurrence and progression of ovarian cancer (16);
miRNA	Mirnas are involved in the regulation of angiogenesis in ovarian cancer (34); miRNA levels can predict the occurrence of early EAOC (35);

growth and enhancing resistance to chemotherapy. Significantly, CD10-enMSCs also rendered OCCC more sensitive to iron apoptosis inducers and dihydroartemisinin (DH), offering a potential intervention pathway for future OCCC treatment.

Building upon current research, Wilczyński et al. proposed the hypothesis that endometriosis stem cells might be the primary targets for the carcinogenesis of EAOC (37). They delineated the process of transformation from endometriosis stem cells to cancer stem cells and the steps involved in the evolution from endometriosis to EAOC. However, more robust evidence is needed to thoroughly elucidate the exact carcinogenic mechanisms of EAOC.

3.2 Estrogen and epigenetics

EMS, being an estrogen-dependent disease, fosters the accumulation of estrogen in the local microenvironment. Estrogen plays a crucial role in the progression of endometrial lesions to atypical hyperplasia and even malignancy (38). Understanding the changes in estrogen signaling pathway will help to reveal the mechanism of estrogen involved in the malignant transformation of EMS. Andersen et al. analyzed estrogen regulatory genes and found that inactivation of estrogen receptor ERα, decreased progesterone receptor (PR) levels, and increased estrogen receptor ERβ may be the driving factors for EMS malignant transformation (39). This transition, accompanied by the overexpression of genes induced by estrogen receptor ERα, such as nuclear receptor interacting protein 1 (NRIP1) in EAOC, and the derepression of estrogen receptor ERα target genes, like FGF18, may promote the development of lesions towards EAOC. Wang et al.found that estrogen can influence gene methylation, and the estrogen-DNMT1 signaling pathway might induce high methylation of runt-related transcription factor 3 (RUNX3) (40), thereby promoting the malignant transformation of EMS. Several studies have identified common epigenetic features between EMS and ovarian malignancies (2, 41, 42) with epigenetic modifications in EAOC involving non-coding miRNA and histone modifications. Future research should focus on the interaction between hormonal regulation and inflammatory responses during the transformation process to gain a more comprehensive understanding of the mechanisms underlying the development of EMS into EAOC.

3.3 Iron related oxidative stress

Elevated iron levels are considered a risk factor for cancer development, and patients with EMS often exhibit iron overload, which may be one of the factors contributing to EAOC. Iron is associated with cancer through a variety of mechanisms, including cancer metabolism, genome stability, and tumor microenvironment (43). Iron and its metabolites produce a large number of Reactive Oxygen Species (ROS) through Fenton reaction (44) and hemoglobin autooxidation (45), leading to DNA damage and acting as carcinogenic inducers in the process of EAOC. The body's macrophage and other antioxidant defense systems are also activated, leading to the "two-step theory" of oxidative stress (Figure 1): The enhanced antioxidant capacity can protect cells from death or apoptosis, but at the same time, it also leads to DNA damage, genomic instability and mutation accumulation, thereby promoting the occurrence of tumors (46). In addition, iron-related oxidative stress can lead to the destruction of peritoneal mesothelial, which is conducive to the adhesion and metastasis of ectopic endometrial cells and tumor cells. Therefore, oxidative stress is a "double-edged sword" in the occurrence of EAOC (47).

3.4 Inflammatory response and immunodysregulation

EMS as a chronic inflammatory disease, creates a microenvironment in ovarian EMS that promotes inflammation, and sustained chronic inflammation may be a driving factor in inducing EAOC. Galectin, an important regulator of inflammation, shows high expression in EMS. Studies have found correlations between galectin-1, -3, and -9 and EAOC (48). In cancer cells,

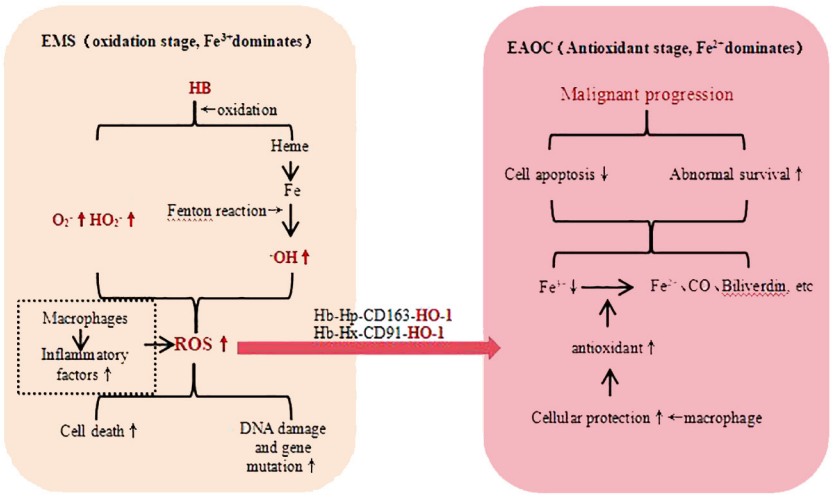

FIGURE 1
EMT Malignant Transformation - Iron Related Oxidative Stress. hemoglobin (HB), haptoglobin (HP), heme-binding glycoprotein (Hx), heme oxygenase-1 (OH-1), reactive oxygen species (ROS), carbon monoxide (CO), low-density lipoprotein receptor-related protein 1 (CD91), macrophage-specific protein (CD163), superoxide ($O2^-$), perhydroxy ($HO2^-$).

galectin is associated with the regulation of oncogenic signaling pathways, apoptosis, and changes in proliferation rates, making it a potential target for future cancer therapy (49).

The high-level expression of inflammasome complex genes (NLRP3, AIM2, PYCARD and NAIP) and inflammasome-related pathway genes (TLR1, TLR7, TOLLIP, NFKBIA and TNF) demonstrated their role in the progression of EMS and EAOC (50). However, there is still a lack of detailed analysis of the relevant immune components in the malignant transformation of EMS (51), and the exact immune pathways and cellular processes are still unclear, which is worthy of further research in the future.

4 EAOC risk factors

4.1 High estrogen state

A high estrogen state is considered a significant risk factor for the malignant transformation of EMS (52). Factors such as early menarche, infertility, or low parity keep patients in a prolonged state of endogenous high estrogen levels, increasing not only the likelihood of EMS but also the risk of EAOC. A stratified study on 66,450 women investigating 12 risk factors for epithelial ovarian cancer found that the risk of OEC gradually increases with earlier age at menarche and later age at menopause (53). Recent research exploring hormone replacement therapy (HRT) in postmenopausal women with a history of EMS found that, except for HRT using estrogen alone, other HRT regimens do not increase the risk of ovarian cancer in postmenopausal women with a history of endometriosis (54). This reflects the potential increased risk of EAOC with exogenous estrogen, highlighting different pathways in the role of endogenous and exogenous estrogen in the association between EMS and EAOC, deepening our understanding of this complex relationship.

4.2 Menopause

In a retrospective case-control study, Udomsinkul et al. identified menopause as a significant independent risk factor for EAOC (55). In postmenopausal women, ovarian function declines, leading to a significant decrease in estrogen levels. It is generally believed that postmenopausal patients may experience relief from symptoms of EMS due to the decline in estrogen levels. However, Giannella et al. reported an incidence of endometriosis in menopausal women to be 2-4% (56), highlighting the importance of special attention to this group. The decreased likelihood of physiological cysts and the increased risk of malignant transformation of ovarian masses in postmenopausal women make it a noteworthy consideration.

4.3 Age and the course of endometriosis

Current research indicates that age and the long-term development of EMS are important risk factors for EAOC patients. It is noteworthy that EAOC patients are diagnosed at a younger age, with the average diagnosis age being 48.65 years compared to 54.39 years for non-EAOC patients (57). In a study the longest duration of ovarian endometriotic cysts in EAOC patients was 23 years, with an average duration of 10 years (58). The study suggests that the long-term development of ovarian endometriotic cysts increases the risk of malignant transformation. Murakami et al.analyzed the medical history of EAOC patients and found that the median time from the diagnosis of endometriotic cysts to the diagnosis of EAOC was 36 months, with approximately 75% of patients progressing to EAOC within 60 months (59). Given the low incidence of EAOC, the phenomenon of endometriotic cysts rapidly progressing to cancer in a short period suggests that EAOC may occur in earlier, less detectable

stages, highlighting the occult nature of EAOC and emphasizing the importance of identifying the risk in early-stage EAOC patients.

4.4 Hysterectomy

The relationship between hysterectomy and ovarian cancer is intricate. Previous studies suggest that hysterectomy may impede retrograde menstrual flow and the transfer of carcinogenic substances (60), thereby reducing the risk of ovarian cancer. Khoja et al. after accounting for confounding factors such as estrogen and estrogen-progestin use, as well as a history of EMS, found that the risk of ovarian cancer decreases only in women with a combination of hysterectomy and EMS (61), while there is no correlation in women without EMS. Ring et al. research also confirms that, although hysterectomy is not generally associated with the risk of ovarian epithelial cancer (62), it significantly reduces the risk of ovarian clear cell carcinoma.

In patients with endometriosis, the infrequent use of oral contraceptives, comorbid depression, or pelvic inflammation may elevate the risk of ovarian cancer (63). However, for patients with EAOC, there is currently a lack of well-designed studies providing conclusive evidence regarding these risk factors.

5 Clinical diagnosis of EAOC

5.1 Clinical symptoms and signs

Clinical symptoms and signs of EAOC are atypical, lacking specific diagnostic criteria. According to the "dualistic model of ovarian cancer", researchers suggest that EAOC often belongs to Type I ovarian cancer, characterized by relative indolence, typically lower invasiveness, and less propensity for widespread dissemination (64). Symptoms of EAOC are often similar to those of endometriosis, mainly presenting as pelvic masses. Clinicians should be vigilant for EAOC when endometriosis patients exhibit typical cyclical pain rhythm changes, abnormal uterine bleeding, or if the mass has a maximum diameter >10 cm or shows rapid enlargement (65).

5.2 Tumor marker

Currently, there is a lack of specific and cost-effective biomarkers to identify the occurrence of EAOC. Serum carbohydrate antigen 125 (CA125) is the most commonly used ovarian tumor marker. Previous studies suggested that malignancy is likely when CA125 is >200 U/ml. However, CA125 is not highly specific, as it can be influenced by various factors such as endometriosis, inflammation, and menstruation. Its sensitivity in early-stage EAOC is also relatively low. In other study CA125 levels showed no significant statistical difference between patients with ovarian endometriotic cysts and those with EAOC (66).

Compared to CA125, carbohydrate antigen 19.9 (CA19.9) and human epididymal protein 4 (HE4) have advantages in diagnosing EAOC. CA19.9 is a potential serum marker for diagnosing EAOC; in Magalhães et al. study, a serum CA19.9 >22.31 U/ml showed a sensitivity of 82.14% in distinguishing between ovarian endometriotic cysts and EAOC (67). HE4, highly expressed in ovarian cancer and unaffected by endometriosis, exhibits high specificity. Xu et al. found that a serum HE4 >59.7 pmol/L could diagnose EAOC, with a specificity of 99.4% when HE4 >140 pmol/L (68). For epithelial ovarian cancer, the combined detection of HE4 and CA125 demonstrates higher sensitivity than CA125 alone. Multiple studies suggest that the joint examination of various tumor markers is more effective in diagnosing ovarian epithelial cancer (69). In a comprehensive review, concluded that the combination of CA125 and HE4 is currently the most effective diagnostic approach for ovarian epithelial cancer, but its discriminative ability for EAOC requires further clinical research and analysis for validation (70).

5.3 Radiology

Ultrasound plays a crucial role in the diagnosis of epithelial ovarian cancer. Typical features include cystic and solid masses, thick septa, associated solid nodules or papillary projections, and areas of necrosis. Ovarian cancer often presents with ascites and enlarged lymph nodes, with peritoneal, mesenteric, and omental metastases. In differentiating from EAOC, ultrasound examination should focus on specific characteristics of EAOC, such as a cystic lesion diameter larger than 10 cm or showing an increasing trend, having a unilocular or multilocular solid component, and rich blood flow signals (71). The disappearance of ground glass echoes is also indicative of malignancy (72). Moreover, EAOC typically manifests as a unilateral cystic lesion with papillary projections, and ascites is less commonly observed (73).

Magnetic Resonance Imaging (MRI) with its excellent soft tissue resolution and multi-planar imaging advantages offers greater accuracy in differentiating EAOC compared to Computerized Tomography (CT). A study found that Whole-Body Diffusion-Weighted Imaging/MRI (WB-DWI/MRI) achieved an accuracy of 93% in determining the benign or malignant nature of ovarian masses, significantly higher than CT's accuracy of 82% (74). Using MRI relaxation method to measure the total iron concentration and transverse relaxation rate of cyst fluid in ovarian endometriosis cysts can predict the malignant transformation of ovarian endometriosis (75). In Zhang X et al. research, using MRI to depict the features of EAOC and non-EAOC, revealed that EAOC, especially clear cell ovarian cancer, more commonly presents as a unilocular cystic mass (76), showing statistically significant lateralization. Cystic fluid exhibits low signal intensity on T2-weighted imaging, and focal nodular growth patterns are more frequent. These findings underscore the critical role of ultrasound and MRI in the diagnosis of EAOC.

6 Progress in the management and treatment of EAOC

Most ovarian cancer patients experience recurrence within approximately three years. Advanced ovarian cancer and

recurrent cases often exhibit resistance to platinum-based drugs, leading to a deterioration in clinical prognosis (77), making ovarian cancer treatment a longstanding challenge in gynecologic oncology. Compared to the common high-grade serous ovarian carcinoma, EAOC has a lower incidence rate, but it shows better early prognosis, although the late-stage survival rate is significantly lower than high-grade serous ovarian carcinoma. EAOC patients generally exhibit poorer response to platinum-based chemotherapy compared to non-EAOC cases (78, 79). Current experience in EAOC treatment primarily stems from studies on epithelial ovarian cancer. The initial standard treatment for EAOC includes surgery followed by platinum-based chemotherapy (77). Early-stage EAOC patients should undergo comprehensive staging surgery, while for intermediate to late-stage EAOC patients, consideration should be given to primary debulking surgery (PDS) upon preoperative or intraoperative assessment of extra-ovarian metastasis. Surgery should aim to remove all macroscopically visible tumors to reduce tumor burden, enhance chemotherapy efficacy, and improve prognosis.

6.1 Lymphadenectomy

Lymph nodes serve as crucial pathways for solid tumor metastasis. Systematic lymph node dissection in early-stage ovarian cancer patients is valuable for determining tumor staging, however it is not known whether it is beneficial for prognosis. EAOC as a specific subtype of ovarian epithelial cancer, is often diagnosed in its early stages. Recent evidence from a multicenter retrospective study suggests that early-stage and low-grade endometrioid ovarian cancer patients who undergo lymph node dissection have superior 5-year disease-free survival and overall survival rates compared to those who do not undergo lymph node dissection (80), with rates of 92.0% vs. 85.6% (p=0.016) and 97.7% vs. 92.8% (p=0.013), respectively. Another prospective, multicenter, randomized phase III clinical trial designed by Deng et al. in 2023 is ongoing. By comparing the progression-free survival (PFS) and overall survival (OS) outcomes of patients with stage IA-IIB epithelial ovarian cancer who undergo lymph node dissection surgery versus those who do not (81), this study aims to provide more precise evidence regarding the efficacy and safety of early lymph node surgery. The benefits and drawbacks of performing lymph node dissection in advanced ovarian cancer patients have been elucidated by high-quality evidence. A multicenter, phase III randomized controlled trial published in the New England Journal of Medicine in 2019 demonstrated that systematic pelvic and para-aortic lymph node dissection did not prolong patients' OS or PFS and was associated with a higher incidence of postoperative complications (82). Subsequently, the National Comprehensive Cancer Network (NCCN) guidelines adjusted the indications for lymph node dissection surgery.

6.2 Intraperitoneal chemotherapy

A small proportion of EAOC is diagnosed in advanced stages, where achieving complete resection through surgery is challenging. Researchers have long attempted to enhance drug efficacy through intraperitoneal chemotherapy, particularly for advanced ovarian cancer. Early clinical trials conducted by the Gynecologic Oncology Group (GOG), including GOG-104, GOG-114, GOG-172, and GOG-252, failed to establish intraperitoneal chemotherapy as a first-line treatment due to design flaws, insufficient statistical evidence, and a higher likelihood of adverse reactions. Hyperthermic intraperitoneal chemotherapy (HIPEC), which combines thermal therapy and intraperitoneal perfusion treatment with intraperitoneal chemotherapy, has become a hot topic in debulking surgery for advanced ovarian cancer in recent years. In 2018, Van Driel et al. demonstrated that adding HIPEC to stage III epithelial ovarian cancer patients led to longer recurrence-free survival and overall survival without increasing the incidence of side effects (83). The clinical trial OVHIPEC-1 reported by Aronson et al. in 2023 confirmed a 10-year survival benefit of HIPEC in primary stage III epithelial ovarian cancer patients undergoing interval cytoreduction surgery (84). The efficacy of HIPEC in patients suitable for initial cytoreduction surgery remains uncertain. The OVHIPEC-2 trial, initiated in January 2020, is expected to provide results in this regard (85). However, the statistical results of the HIPECOVA trial conducted by Villarejo Campos et al. in 2024 failed to demonstrate a significant improvement in the prognosis of ovarian cancer patients with HIPEC (86). Therefore, HIPEC treatment remains experimental rather than standard therapy.

6.3 Drug chemotherapy

Currently, the standard first-line treatment regimen for EOC and EAOC is platinum-based combination chemotherapy, specifically carboplatin plus intravenous paclitaxel administered every 3 weeks for a total of 6 cycles. The JGOG 3016 trial previously reported significant improvements in progression-free survival and overall survival with a weekly dose-dense paclitaxel regimen and a 3-weekly carboplatin regimen, whereas the ICON8 trial did not observe this benefit. These trials have different strengths and weaknesses, and the differences may be related to pharmacogenomics or other factors such as dose intensity. The findings of Clamp et al. in 2022 confirmed that weekly dose-dense first-line chemotherapy did not improve overall survival or progression-free survival compared to standard 3-weekly chemotherapy (87). Therefore, the 3-weekly regimen chemotherapy remains the first-line approach.

Late-stage EAOC carries a poor prognosis, warranting in-depth research into targeted therapy and immunotherapy. Currently, molecular targeted therapies for ovarian cancer, such as poly ADP-ribose polymerase (PARP) inhibitors and the anti-angiogenic agent bevacizumab, have shown favorable outcomes in maintenance therapy for epithelial ovarian cancer patients with BRCA mutations, thereby extending the survival of ovarian cancer patients to some extent (88). However, even with satisfactory tumor reduction achieved through surgery and standardized chemotherapy and maintenance therapy, cancer patients may still experience treatment failure due to platinum resistance or tumor recurrence, highlighting the need to enhance drug efficacy and

prolong recurrence-free survival. Mirvetuximab soravtansine (MIRV), an antibody-drug conjugate targeting folate receptor (FR) alpha, has shown promising efficacy when combined with bevacizumab in platinum-resistant recurrent ovarian cancer patients. Mirvetuximab soravtansine (MIRV) is a folate receptor (FR)-targeting antibody-drug conjugate (DC). In 2020, researchers found that MIRV combined with bevacizumab demonstrated good efficacy in treating platinum-resistant recurrent ovarian cancer patients. The confirmed objective response rate (ORR) was 39%, with a particularly effective response observed in the subset of platinum-resistant ovarian cancer patients with high FRα expression, achieving an ORR of 56%. The median duration of response was 12 months, and the PFS was 9.9 months (89). Phase 2 clinical studies of MIRV in epithelial ovarian cancer patients reported in 2023 further demonstrated its anti-tumor activity, along with good tolerability and safety, providing encouraging results (90). EAOC is highly likely to originate from endometriosis-associated ovarian cysts, which are often considered complex immune-related diseases. Immunotherapy has shown great potential in the treatment of EOC and EAOC. However, previous large phase III studies exploring the addition of immunotherapy to standard first-line treatment regimens have been disappointing, including the IMagyn050/GOG 3015/ENGOT-OV39 (91) and JAVELIN Ovarian 100 (92) studies. A turning point in immunotherapy emerged in 2023 with the release of interim data from the global multicenter phase III DUO-O study, showing promising clinical efficacy, warranting continued attention.

Recently, based on the establishment of animal models of endometriosis, successful reports of establishing EAOC mouse models have also emerged (93). By simulating tumor characteristics and reproducing the biological properties of tumors, these models can provide important reference for clinical precision treatment research, which is crucial for the study and development of precision treatment for EAOC.

7 Conclusion

The intricate relationship between EMS and ovarian cancer warrants in-depth investigation. Early identification of high-risk individuals for cancer among endometriosis patients is of paramount importance, necessitating the development of early detection methods and close monitoring. Future research directions in understanding the mechanisms and molecular genetics of EAOC may involve the utilization of advanced technologies, such as next-generation sequencing and whole transcriptome sequencing, as personalized diagnostic tools. The objective is to identify and confirm the driver mutations and candidate genes associated with the malignant transformation of EMS. These efforts hold the potential to provide more precise targeted therapies and immunotherapies for ovarian cancer, thereby improving patient prognosis and survival outcomes.

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EDITED BY
Robert Fruscio,
University of Milano Bicocca, Italy

REVIEWED BY
Basilio Pecorino,
Cannizzaro Hospital, Italy
Giuseppe Scibilia,
gynecology and obstetrics department, Italy

*CORRESPONDENCE
Robert L. Hollis
robb.hollis@ed.ac.uk

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Ovarian carcinosarcoma is highly aggressive compared to other ovarian cancer histotypes

Iona McFarlane[1], Joanna M. Porter[1], Elizabeth Brownsell[1], Nidal Ghaoui[2], Kathryn C. Connolly[3], C. Simon Herrington[1] and Robert L. Hollis[1*]

[1]The Nicola Murray Centre for Ovarian Cancer Research, Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom, [2]The Simpson Centre for Reproductive Health, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom, [3]Edinburgh Cancer Centre, Western General Hospital, NHS Lothian, Edinburgh, United Kingdom

Background: Ovarian carcinosarcoma (OCS) is an unusual ovarian cancer type characterized by distinct carcinomatous and sarcomatous components. OCS has been excluded from many of the pan-histotype studies of ovarian carcinoma, limiting our understanding of its behavior.

Methods: We performed a multi-cohort cross-sectional study of characteristics and outcomes in ovarian cancer patients from Scotland (n=2082) and the Surveillance, Epidemiology and End Results Program (SEER, n=44946) diagnosed with OCS or one of the other major histotypes: high grade serous (HGSOC), endometrioid (EnOC), clear cell (CCOC), mucinous (MOC) or low grade serous ovarian carcinoma (LGSOC). Differences in overall survival were quantified using Cox regression models to calculate hazard ratios (HR).

Results: Across both cohorts, OCS patients were significantly older at diagnosis compared to all other histotypes (median age at diagnosis 69 and 67 in Scottish and SEER cohorts) and demonstrated the shortest survival time upon univariable analysis. Within the Scottish cohort, 59.3% and 16.9% of OCS patients presented with FIGO stage III and IV disease, respectively; this was significantly higher than in EnOC, CCOC or MOC (P<0.0001 for all), but lower than in HGSOC (P=0.004). Multivariable analysis accounting for other prognostic factors identified OCS as independently associated with significantly shorter survival time compared to HGSOC, EnOC, LGSOC and MOC in both the Scottish (multivariable HR vs OCS: HGSOC 0.45, EnOC 0.39, LGSOC 0.26, MOC 0.43) and SEER cohorts (multivariable HR vs OCS: HGSOC 0.59, EnOC 0.34, LGSOC 0.30, MOC 0.81). Within the SEER cohort, OCS also demonstrated shorter survival compared to CCOC (multivariable HR 0.63, 95% CI 0.58-0.68), but this was not replicated within the Scottish cohort (multivariable HR for CCOC: 1.05, 95% CI 0.74-1.51). Within early-stage disease specifically (FIGO I-II or SEER localized stage), OCS was associated with the poorest survival of all histotypes across both cohorts. In

the context of late-stage disease (FIGO III-IV or SEER distant stage), OCS, MOC and CCOC represented the histotypes with poorest survival.

Conclusion: OCS is a unique ovarian cancer type that affects older women and is associated with exceptionally poor outcome, even when diagnosed at earlier stage. New therapeutic options are urgently required to improve outcomes.

KEYWORDS

ovarian cancer, carcinosarcoma, malignant mixed mullerian tumour, survival, ovarian carcinoma

Introduction

Ovarian carcinosarcoma (OCS) is an uncommon form of ovarian cancer, accounting for approximately 3% of diagnoses, and is distinguished by the presence of both carcinomatous and sarcomatous malignant cell populations (1–3). This biphasic histology led to the hypothesis that OCS may represent collisions of two separately originating tumors; however, the consensus has shifted over the last decade to recognize OCS as metaplastic carcinomas, with the sarcomatous population formed through complete epithelial-to-mesenchymal transition (1, 4). The unique history of OCS has resulted in its exclusion from many pan-histotype studies of ovarian carcinoma, leading to a paucity of research on OCS when compared to other uncommon histotypes (2).

Several studies have examined retrospective cohorts of OCS cases to identify factors associated with patient outcomes (5–11). These studies report a median survival time of 12-24 months across the broader OCS patient population. Earlier FIGO stage at diagnosis and achievement of complete macroscopic resection are both associated with more favorable prognosis, but recurrence and mortality rates appear high even in patients diagnosed with early-stage disease (11–13).

As most OCS cases have carcinomatous components of high grade serous type, some have conceptualized OCS as a rare variant of high grade serous ovarian carcinoma (HGSOC), the most common ovarian cancer histotype (2). However, a significant proportion have carcinomatous components of endometrioid type (3, 11), and limited comparisons of OCS and HGSOC have suggested significant differences in the behavior of these two histotypes (11, 13). Compared to HGSOC, OCS demonstrates greater levels of intrinsic chemoresistance (objective response rate between 30-60%) and is associated with an overall poorer prognosis (11, 14).

While efforts at characterizing the clinical behavior of OCS have improved our understanding of prognostic factors within OCS patients, and limited comparisons have been made against HGSOC (11, 15), there has been little comparison of OCS versus other ovarian carcinoma histotypes. Here, we compare OCS against all major epithelial ovarian carcinoma histotypes using two independent cohorts to improve our understanding of the clinical behavior of these uncommon tumors.

Methods

Scottish ovarian cancer patient cohort

A cohort of ovarian cancer (ovarian, fallopian tube or primary peritoneal cancer) patients was identified using the Edinburgh Ovarian Cancer Database (16), wherein the diagnostic, treatment and outcome details of pathologically-confirmed ovarian cancer cases treated at the Edinburgh Cancer Centre (tertiary oncology centre for South-East Scotland) are prospectively recorded as part of routine care (16). Between 2000-2019, 2573 ovarian cancer diagnoses were documented, of which 2124 were carcinomas of serous (HGSOC or LGSOC), mucinous, carcinosarcoma, endometrioid or clear cell histology (Figure 1A). Older cases documented as poorly differentiated serous carcinoma and moderately differentiated serous carcinoma were included alongside contemporary diagnoses of HGSOC. Similarly, well differentiated serous carcinomas were included alongside contemporary diagnoses of LGSOC. Serous cases of unknown grade were excluded (n=37). 5 further cases were excluded due to unknown survival time, leaving a Scottish study cohort of 2082 cases (Figure 1A). Formal pathology review was not performed for the present study; however, 77% of cases recently underwent pathology review as part of tumour molecular profiling studies (4, 11, 17–26) or represented contemporary diagnoses (2010 onwards).

Institutional review board approval for the Scottish cohort was received from the South East Scotland Cancer Information Research Governance Committee (Caldicott guardian reference CG/DF/E164, study reference CIR21087).

SEER ovarian cancer patient cohort

A cohort of ovarian cancer patients from the publicly available US Surveillance, Epidemiology, and End Results (SEER) program

was identified using SEERstat version 8.4.2 (Figure 1B). 143407 cases of ovarian (C56.9), fallopian tube (C57.0) or peritoneal cancers (C48.0, C48.1, C48.2, C48.8) were retrieved in a case listing session (November 2022 SEER incidence research data: 2000-2020, 17 registries; selected for malignant behavior and primary site listed as C48.0, C48.1, C48.2, C48.8, C56.9 or C57.0). These cases were extracted, and the following exclusion criteria applied: diagnosis prior to 2010 (n=66410) or after 2019 (n=6624), carcinoma *in situ* (n=4), unspecified histology (n=12562), mixed histologies (n=2063), granulosa cell tumors (n=852), liposarcomas (n=555), leiomyosarcomas (n=877), teratomas (n=545), and other histologies beyond serous, endometrioid, clear cell, mucinous and carcinosarcoma (n=7153). A further 27 cases were excluded due to unknown survival time, leaving a SEER study cohort of 44946 cases (Figure 1B).

Stage was defined using combined SEER summary stage 2004+ data, identifying cases with localized-, regional-, or distant-stage disease. ICD-0-3 morphology codes were used to categories the SEER cohort into the following histotypes: endometrioid (ICD.O.3 8380, 8381, 8382, 8383 or 8570), mucinous (ICD.O.3 8470, 8471, 8472, 8480, 8481), clear cell (ICD.O.3 8310, 8313, 8443, 8444), carcinosarcoma (ICD.O.3 8575, 8950, 8951, 8980, 8981) and serous (ICD.O.3 8441, 8460, 8461, 8462). Serous cases annotated as well differentiated, grade 1 or low grade were classified as LGSOC; all other serious cases were included as HGSOC.

Statistical analysis

All statistical analyses were performed using R version 4.2.2 within R Studio 2022.07.2 + 576. Comparisons of categorical variables were made using the Chi-squared test. Comparisons of continuous variables were made using the Mann-Whitney U test. For the Scottish cohort, overall survival was calculated from date of pathologically confirmed diagnosis. Cox proportional hazards regression models were used to compare survival across groups. Within the Scottish cohort, multivariable analysis accounted for age at diagnosis, FIGO stage at diagnosis, diagnosis period (5-year intervals) and residual disease status following first-line debulking surgery. For the SEER cohort, multivariable analysis accounted for disease stage, patient age and diagnosis period (5-year intervals). Results are visualized using the Kaplan-Meier method and survival differences are presented as hazard ratios (HRs) with respective 95% confidence intervals (CIs). The reverse Kaplan-Meier method was used to calculate median follow-up time. Statistical significance was defined as $P<0.05$.

Results

Scottish cohort characteristics

The Scottish cohort comprised 2082 patients with a pathologically-confirmed ovarian, fallopian tube or primary peritoneal cancer diagnosed between 2000-2019 (Figure 1A). 63 cases (3.0%) were OCS (Table 1). 1376 (66.1%), 231 (11.1%), 185 (8.9%), 146 (7.0%) and 81 (3.9%) were HGSOC, EnOC, CCOC, MOC and LGSOC, respectively, broadly reflecting previously reported histotype distributions in unselected ovarian carcinoma cohorts (4). The majority of cases presented with advanced stage disease (50.6% FIGO III, 972 of 1920 evaluable cases; 19.1% FIGO IV 366 of 1920). The median follow-up time across the cohort was 7.2 years; the survival event rate was 65.6% (Table 1).

Comparison of histotypes with carcinosarcoma

The median survival time of OCS patients was 17 months (Figure 2A). Univariable survival analysis identified OCS as the

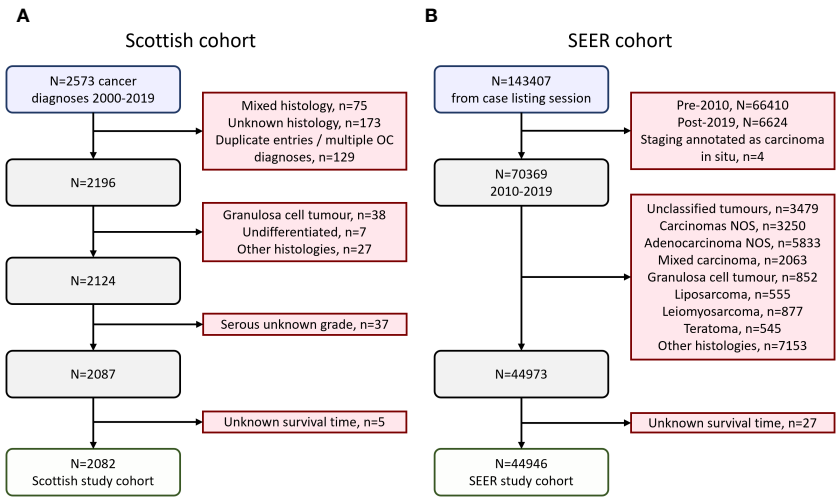

FIGURE 1
Flow diagrams of cohort identification. **(A)** Scottish ovarian cancer patient cohort. **(B)** SEER ovarian cancer patient cohort.

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TABLE 1 Characteristics of the Scottish ovarian cancer patient cohort.

		Overall		OCS		HGSOC		EnOC		CCOC		MOC		LGSOC	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cohort	N	2082		63		1376		231		185		146		81	
Age	Median	64	IQR 55-72	69	IQR 63-76	67	IQR 58-74	60	IQR 50-68	60	IQR 52-69	53	IQR 41-65	60	IQR 43-68
FIGO stage	I	365	19.0%	8	13.6%	59	4.7%	107	49.8%	71	40.6%	107	76.4%	13	16.9%
	II	217	11.3%	6	10.2%	74	5.9%	56	26.0%	52	29.7%	17	12.1%	12	15.6%
	III	972	50.6%	35	59.3%	810	64.6%	38	17.7%	34	19.4%	13	9.3%	42	54.5%
	IV	366	19.1%	10	16.9%	311	24.8%	14	6.5%	18	10.3%	3	2.1%	10	13.0%
	NA	162	–	4	–	122	–	16	–	10	–	6	–	4	–
Residual disease status	CMR	873	45.9%	29	48.3%	396	31.6%	166	76.9%	126	75.4%	119	89.5%	37	51.4%
	macroRD	1028	54.1%	31	51.7%	857	68.4%	50	23.1%	41	24.6%	14	10.5%	35	48.6%
	NA	181	–	3	–	123	–	15	–	18	–	13	–	9	–
ECOG performance status	0	372	26.7%	12	24.0%	215	22.7%	48	37.2%	49	38.6%	33	57.9%	15	32.6%
	1	604	43.4%	18	36.0%	434	45.8%	56	43.4%	53	41.7%	16	28.1%	27	58.7%
	2	287	20.6%	9	18.0%	232	24.5%	18	14.0%	19	15.0%	5	8.8%	4	8.7%
	3-4	130	9.3%	11	22.0%	103	10.9%	7	5.4%	6	4.7%	3	5.3%	0	0.0%
	NA	689	–	13	–	392	–	102	–	58	–	89	–	35	–
Progression status	Progressed	1276	61.3%	49	77.8%	1004	73.0%	64	27.7%	93	50.3%	26	17.8%	40	49.4%
	Stable	806	38.7%	14	22.2%	372	27.0%	167	72.3%	92	49.7%	120	82.2%	41	50.6%
Vital status	Alive	716	34.4%	7	11.1%	340	24.7%	146	63.2%	75	40.5%	107	73.3%	41	50.6%
	Deceased	1366	65.6%	56	88.9%	1036	75.3%	85	36.8%	110	48.1%	39	26.7%	40	49.4%
Follow-up	Median	7.2 years		8.4 years		7.0 years		7.9 years		6.4 years		7.5 years		8.5 years	

OCS, ovarian carcinosarcoma; HGSOC, high grade serous ovarian carcinoma; EnOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low grade serous ovarian carcinoma; CMR, complete macroscopic resection after primary cytoreduction; macroRD, macroscopic residual disease after primary cytoreduction; ECOG, Eastern Cooperative Oncology Group.
"-", not calculated.

histotype associated with the poorest survival outcomes (HR vs OCS: HGSOC 0.55, 95% CI 0.42-0.72; CCOC 0.36, 95% CI 0.26-0.50; LGSOC 0.21, 95% CI 0.14-0.32; EnOC 0.15, 95% CI 0.11-0.21; MOC 0.10, 95% CI 0.07-0.15) (Figure 2A). However, clinicopathological features varied significantly between histotypes; patients with OCS were significantly older at diagnosis compared to all other histotypes (median 69 years in OCS vs 67, 60, 60, 53 and 60 in HGSOC, EnOC, CCOC, MOC and LGSOC, respectively) (Figure 2D) with corresponding higher Eastern Cooperative Oncology Group (ECOG) Performance Status scores (Figure 2E). Stage distribution was also markedly different between histotypes: OCS cases had a higher frequency of early-stage (FIGO I/II) diagnosis compared to HGSOC (23.7%, 14/59 evaluable OCS vs 10.1%, 133/1254 evaluable HGSOC; P=0.004), but a higher frequency of advanced stage (FIGO III/IV) at diagnosis compared to MOC (P<0.0001), EnOC (P<0.0001) and CCOC (P<0.0001) (Figure 2F). Corresponding differences in frequency of achieving complete macroscopic resection (CMR, zero residual disease/R0) at first-line surgery were also apparent (Figure 2G). Together, these data highlight the need for multivariable analysis.

Multivariable analysis of survival accounting for patient age, stage at diagnosis, residual disease status and diagnosis period identified OCS as a histotype associated with significantly poorer outcome compared to HGSOC (multivariable HR [mHR] for HGSOC vs OCS 0.45, 95% CI 0.34-0.60), EnOC (mHR vs OCS: 0.39, 95% CI 0.27-0.56), MOC (mHR vs OCS 0.43, 95% CI 0.27-0.68) and LGSOC (mHR vs OCS: 0.26, 95% CI 0.17-0.40) (Figure 3A). There was no significant difference in survival of CCOC patients vs OCS patients in this multivariable analysis (mHR for CCOC vs OCS: 1.05, 95% CI 0.74-1.51).

Outcome in early- and late-stage disease

Survival analysis of patients diagnosed at early-stage (FIGO I-II) identified OCS as a patient group with markedly poor outcome (Figure 2B). OCS was associated with significantly shorter survival than all other histotypes in a multivariable analysis accounting for age, stage (I vs II), RD status and diagnosis period; this included significantly shorter survival in early-stage OCS versus early-stage

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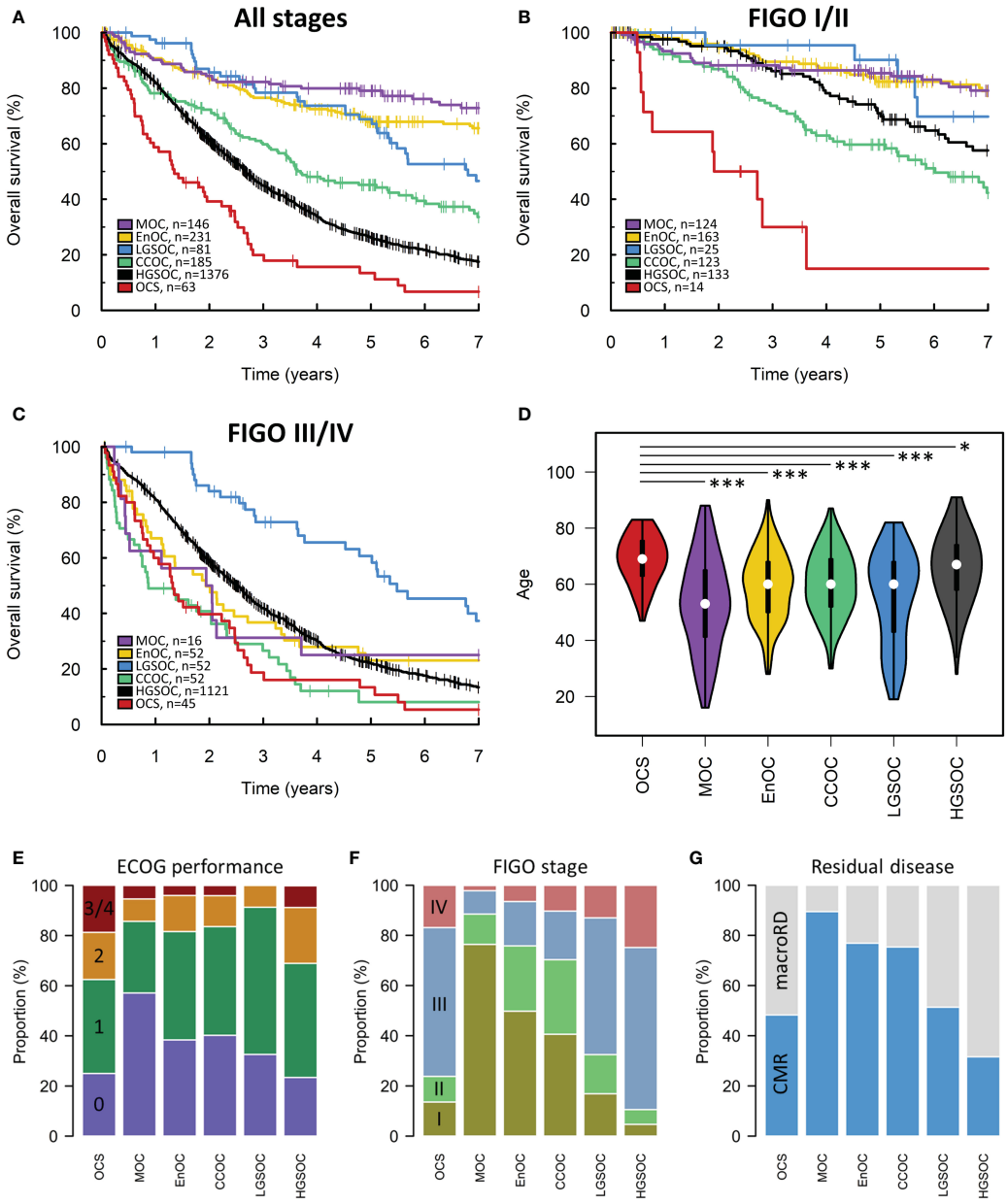

FIGURE 2
Scottish ovarian cancer patient cohort. **(A)** Survival of patient cohort according to histotype. **(B)** Survival analysis of early-stage patients (FIGO I-II). **(C)** Survival of late-stage patients (FIGO III-IV). **(D)** Age at diagnosis of patients according to histotype. *denotes P<0.05, ***denotes P<0.0001. **(E)** ECOG performance status according to histotype. Chi-squared P-values for comparison of ECOG PS (≤1 vs ≥2) in histotypes against OCS: MOC P=0.0125, EnOC P=0.0145, CCOC P=0.0056, LGSOC P=0.0023, HGSOC P=0.4322. **(F)** FIGO stage at diagnosis. Chi-squared P-values for comparison of stage distribution in histotypes against OCS: MOC P<0.0001, EnOC P<0.0001, CCOC P<0.0001, LGSOC P=0.7459, HGSOC P=0.0140. **(G)** Frequency of achieving complete macroscopic resection (CMR) versus macroscopic residual disease (macroRD) according to histotype. OCS, ovarian carcinosarcoma; HGSOC, high grade serous ovarian carcinoma; EnOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low grade serous ovarian carcinoma.

CCOC (mHR for CCOC vs OCS: 0.51, 95% CI 0.26-0.98) (Figure 2B).

A corresponding analysis of advanced stage patients (FIGO III/IV) showed late-stage OCS was associated with shorter survival compared to late-stage HGSOC, EnOC and LGSOC (Figures 2C, 3B); differences between late-stage OCS and MOC (mHR vs OCS: 0.67, 95% CI 0.34-1.32) and CCOC (HR vs OCS: 1.33, 95% CI 0.85-2.07) were not statistically significant.

SEER cohort characteristics

A second cohort of 44946 ovarian cancer patients was identified from the SEER database (Figure 1B). 2030 (4.5%), 30706 (68.3%), 5336 (11.9%), 3088 (6.9%), 2846 (6.3%) and 940 (2.1%) cases were OCS, HGSOC, EnOC, CCOC, MOC and LGSOC, respectively (Table 2). The median follow-up time for the SEER cohort was 5.6 years, with a survival event rate of 48.9%.

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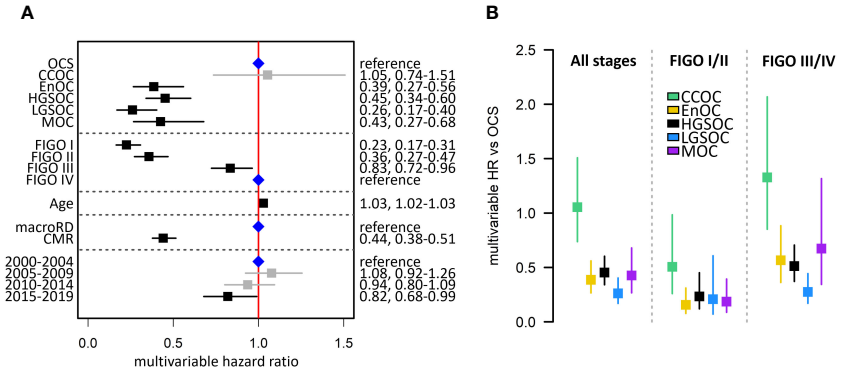

FIGURE 3
Summary of multivariable survival analysis model in the Scottish study cohort. **(A)** Multivariable model across all stages. Blue points denote the reference groups for each level; black points denote statistically significant differences; grey denotes estimates not statistically significantly different from the reference population. **(B)** Summary of multivariable hazard ratios across the whole cohort, early-stage-specific analysis and late-stage-specific analysis. OCS, ovarian carcinosarcoma; HGSOC, high grade serous ovarian carcinoma; EnOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low grade serous ovarian carcinoma; CMR, complete macroscopic resection after primary cytoreduction; macroRD, macroscopic residual disease after primary cytoreduction; HR, hazard ratio.

Comparison of histotypes in the SEER cohort

The median survival time of OCS patients within the SEER cohort was 21 months (Figures 4A–C). Within the SEER cohort, OCS demonstrated the shortest survival time upon univariable analysis (Figure 4A) and was associated with significantly older age at diagnosis compared to other histotypes (median 67, 65, 55, 57, 55 and 58 in OCS, HGSOC, EnOC, CCOC, MOC and LGSOC, respectively; P<0.0001 for all comparisons against OCS) (Figure 4D). Multivariable analysis identified significantly shorter survival in OCS patients compared to all other histotypes (mHR vs OCS: HGSOC 0.59, 95% CI 0.56-0.63; EnOC 0.34, 95% CI 0.31-0.37; CCOC 0.63, 95% CI 0.58-0.68; MOC 0.81, 95% CI 0.74-0.88; LGSOC 0.30, 95% CI 0.26-0.34) (Figure 4E).

Within the earliest SEER disease stage (Localized disease), OCS demonstrated the shortest survival of all histotypes (Figure 3B), though the difference between OCS and HGSOC did not reach statistical significance (mHR for HGSOC vs OCS: 0.79, 95% CI 0.57-1.09) (Figure 4F). Within the most advanced SEER stage (Distant disease), OCS was associated with poorer survival than HGSOC (mHR for HGSOC vs OCS: 0.60, 95% CI 0.56-0.63), EnOC (mHR for EnOC vs OCS: 0.45, 95% CI 0.41-0.51) and LGSOC (mHR for LGSOC vs OCS: 0.29, 95% CI 0.24-0.34) (Figure 3B). The outcome of late-stage OCS and CCOC was similar (mHR for CCOC vs OCS: 0.95, 95% CI 0.86-1.06), while late-stage MOC

TABLE 2 Characteristics of the SEER ovarian cancer patient cohort.

		Overall		OCS		HGSOC		EnOC		CCOC		MOC		LGSOC	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cohort	N	44946		2030		30706		5336		3088		2846		940	
Age	Median	63	IQR 54-72	67	IQR 59-75	65	IQR 56-73	55	IQR 47-65	57	IQR 50-65	55	IQR 43-65	58	IQR 45-67
SEER Stage	Localized	7411	16.8%	108	5.4%	2196	7.3%	2297	43.5%	1125	36.9%	1498	53.9%	187	20.2%
	Regional	10432	23.6%	461	23.2%	5770	19.1%	2124	40.3%	1161	38.1%	665	23.9%	251	27.1%
	Distant	26330	59.6%	1414	71.3%	22192	73.6%	856	16.2%	763	25.0%	618	22.2%	487	52.6%
	NA	773	–	47	–	548	–	59	–	39	–	65	–	15	–
Vital status	Alive	22980	51.1%	602	29.7%	17356	56.5%	4292	80.4%	2071	67.1%	1943	68.3%	722	76.8%
	Deceased	21966	48.9%	1428	70.3%	13350	43.5%	1044	19.6%	1017	32.9%	903	31.7%	218	23.2%
Follow-up	Median	5.6 years		5.8 years		5.6 years		5.6 years		5.3 years		5.6 years		4.6 years	

OCS, ovarian carcinosarcoma; HGSOC, high grade serous ovarian carcinoma; EnOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low grade serous ovarian carcinoma.
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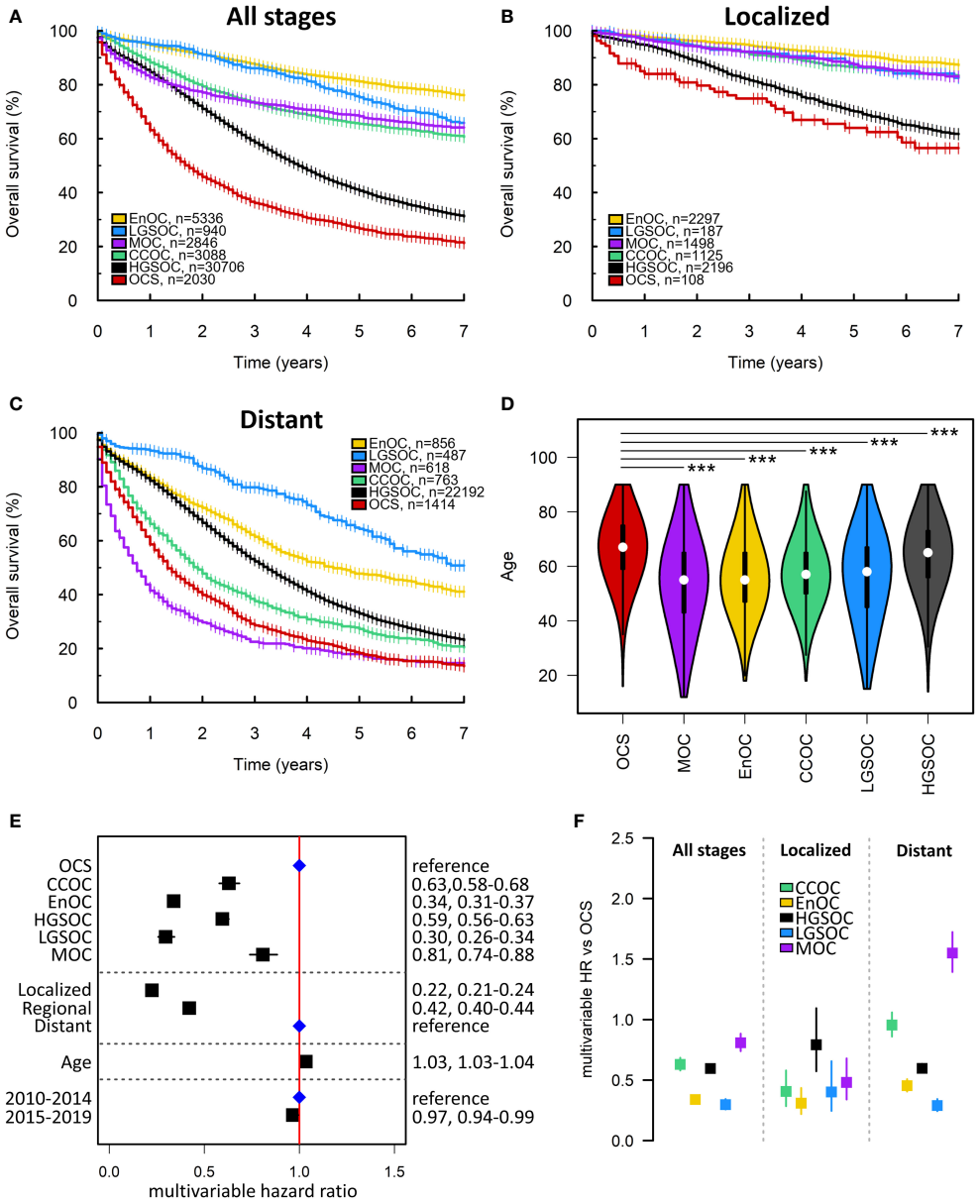

FIGURE 4
SEER ovarian cancer patient cohort. **(A)** Survival of the whole SEER cohort (Early, Regional and Distant stage) according to histotype. **(B)** Survival analysis of early-stage patients (Localized disease). **(C)** Survival of late-stage patients (Distant disease). **(D)** Age at diagnosis of patients according to histotype. ***denotes P<0.0001. **(E)** Multivariable survival analysis across all stages. Blue points denote the reference groups for each level; black points denote statistically significant differences. **(F)** Summary of multivariable hazard ratios across the whole cohort, early-stage-specific analysis and late-stage-specific analysis. OCS, ovarian carcinosarcoma. HGSOC, high grade serous ovarian carcinoma; EnOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low grade serous ovarian carcinoma; HR, hazard ratio.

demonstrated the poorest outcome of all histotypes at late-stage (mHR for MOC vs OCS: 1.56, 95% CI 1.40-1.73) (Figure 4F).

Discussion

OCS is now recognized as a histotype of ovarian carcinoma, but has received relatively little research attention to date (2). Limited comparisons have been made against HGSOC (11, 13), the most common histotype, but there is a paucity of data comparing these unusual tumors against the spectrum of major ovarian cancer histotypes. Here, we utilize two independent cohorts of ovarian cancer patients to comprehensively characterize the clinical behavior of OCS.

Our findings highlight several distinct features of OCS compared to other ovarian carcinoma histotypes. Firstly, OCS presents in women at an older age compared to other histotypes: the median age at diagnosis in OCS was 69 years in the Scottish cohort and 67 years in the SEER cohort, and this was statistically significantly older than all other histotypes across both cohorts.

Within HGSOC, the other histotype with a median diagnosis age of over 60 years, copy number gain of *CCNE1* has been associated with older age at diagnosis (17). OCS have recently been reported to commonly demonstrate *CCNE1* gain (4), and their older age at diagnosis may be linked to the frequency of this defect; however, direct comparison of *CCNE1* status and age of OCS diagnosis has not been reported.

OCS also appears to have a distinct stage distribution; the majority of OCS present at FIGO stage III-IV – unlike MOC, EnOC and CCOC – but around 25% are FIGO stage I/II at diagnosis, and this is significantly more than in HGSOC. As OCS frequently present with advanced stage disease, many patients undergo neoadjuvant chemotherapy prior to cytoreductive surgery; this approach is widely considered safe and effective for HGSOC (27, 28), but neoadjuvant chemotherapy versus primary debulking surgery has not been specifically compared for OCS. Given reports of higher levels of intrinsic chemoresistance in OCS (objective response rate 25-60%) (11–13), neoadjuvant chemotherapy may feasibly represent a less effective management strategy. Indeed, neoadjuvant chemotherapy is not the preferred approach for other histotypes with high levels of intrinsic chemoresistance (29). However, challenges in identifying OCS on diagnostic biopsies – where the sarcomatous component may not be sampled, leading to a diagnosis of more common carcinoma hisotypes – may interfere with the ability to tailor early first-line management decisions for OCS patients.

Univariable analysis identified OCS as the histotype associated with poorest survival across both cohorts. Within the Scottish cohort, multivariable analysis demonstrated that this was independent of other prognostic factors for comparisons of OCS against all other histotypes, with the exception of CCOC. The poorer outcome of OCS compared to HGSOC, EnOC, LGSOC and MOC was confirmed in the SEER cohort; this cohort also identified OCS as having significantly shorter survival than CCOC. The difference in comparisons with CCOC between cohorts may be underpinned by greater statistical power in the SEER cohort, though less detailed clinical annotation prevented inclusion of residual disease status in the SEER cohort model, likely contributing to this discrepancy. Together, these data suggest that the overall OCS population represents the highest risk histotype across ovarian carcinomas.

In an analysis specifically of earlier stage patients (FIGO I-II) in the Scottish cohort, OCS was associated with markedly shorter survival than all other histotypes, including CCOC. These findings were replicated when investigating SEER cohort patients with localized disease, though the comparison with HGSOC was not statistically significant (mHR for localized HGSOC vs localized OCS 0.80, 95% CI 0.58-1.11). This discrepancy may be due to the difference in staging between cohort; SEER localized stage equates to the very earliest FIGO stages (IA, IB and stage I not otherwise specified). These data have important implications for decisions around omission of chemotherapy for early-stage disease. Many ovarian cancer cases diagnosed at the earliest stages do not require chemotherapy (28); however, the aggressive nature of early-stage OCS suggests that chemotherapy omission may not be advisable for this group. Similarly, fertility-sparing surgery may not be feasible in this context, though most OCS patients present after reproductive age. In late-stage disease (FIGO III-IV), the Scottish cohort demonstrated that OCS was associated with significantly shorter survival compared to HGSOC, LGSOC and EnOC, but was not associated with significantly poorer outcome than CCOC or MOC. These findings were confirmed in the SEER cohort, where distant stage CCOC demonstrated similar survival to distant stage OCS, and late-stage MOC demonstrated the worst survival of all histotypes in this context. Together, these stage-specific analyses highlight OCS as highly aggressive even when diagnosed at early-stage, while in the context of late-stage disease, OCS, CCOC and MOC represent the histotypes with poorest survival. This is consistent with reports highlighting CCOC and MOC as highly chemoresistant malignancies with exceptionally poor prognosis when diagnosed at advanced stage (2). While OCS is most commonly diagnosed at advanced stage, late-stage diagnosis of CCOC and MOC is relatively uncommon, underscoring treatment of late-stage OCS as a major clinical challenge.

Major strengths of this study include the detailed clinical annotation available for the Scottish cohort, extensive follow-up time and the utilization of multivariable analysis to assess associations of histotype with outcome independent of other prognostic factors. The use of two independent cohorts from distinct geographical locations is also a notable strength; SEER is a pan-cancer database curated across a large number of centres in the US, while the Edinburgh Ovarian Cancer Database is a disease-specific resource curated centrally at a single site. A limitation of the present study is that all cases did not undergo centralized pathology review, though over 75% of the Scottish cohort has either undergone pathology review as part of recent molecular profiling studies or represented contemporary diagnoses (2010 onwards), limiting the potential for histotype misclassification. As it was not possible to perform pathology review of any cases in the SEER cohort, we utilized only recent diagnoses from the SEER database (2010-2019) to minimize potential histotype misclassification. Though we were able to include a relatively large number of cases with uncommon histotypes - and the number of these cases exceeded that in many reported cohorts of these less common diagnoses - power was still limited for some analyses. In particular, the number of advanced stage MOC and early-stage OCS or LGSOC cases was modest, though the large effect sizes detected between analyses of these groups bolstered power.

Our findings highlight the urgent need for additional treatment options for OCS patients. Molecular profiling studies have the potential to identify targeted approaches that may improve OCS patient survival; however, relatively few OCS samples have undergone genomic, transcriptomic or other molecular characterization to date. Limited available data suggest a paucity of targetable oncogenic driver mutations from the genomes of OCS tumors (4, 30), with *TP53* mutation representing one of the few recurrent molecular events. A proportion of OCS demonstrate genomic evidence of homologous recombination repair deficiency (31), and these cases may be expected to benefit from poly(ADP-ribose) polymerase (PARP) inhibitors. Case reports of OCS patients deriving clinical benefit from PARP inhibition are available in the literature, but this evidence base is extremely limited (32, 33). The

frequency of germline or somatic *BRCA1/2* mutation is poorly characterized in OCS; case reports of *BRCA1/2*-mutant OCS are available, but current data from OCS cohorts suggest the frequency is low (0/12 in (4) and 0/13 in (34)). There is also a lack of data quantifying the extent of homologous recombination deficiency with robustly established techniques due to a lack of whole genome sequencing (35).

Recent data suggest that the sarcomatous compartment of OCS is less well engaged by the host anti-tumour immune response compared to the carcinomatous component (4); immunotherapeutic drugs may therefore represent agents worthy of investigation in the hope of reinvigorating the anti-tumour immune response. In particular, immune checkpoint inhibitors targeting PD1, PDL1 and CTLA4 are of interest. Case reports of responses to such inhibitors in OCS patients provide anecdotal evidence of their potential utility in the wider population (36, 37). However, as with other candidate targeted approaches, there is a marked absence of trial data at any phase. Overexpression of HER2 and VEGF in some OCS has suggested trastuzumab and anti-angiogenics as further potential treatment strategies for investigation, alongside inhibitors of mTOR (38). Recently established preclinical models of OCS have identified eribulin as a candidate therapeutic strategy targeting epithelial-to-mesenchymal transition in OCS (39), and we eagerly await the results from initial clinical evaluations of this strategy (NCT05619913). The relative rarity of OCS is likely to hinder progress of histotype-specific trials for this tumour type; international collaborative efforts have led to successful disease-specific trials in other uncommon ovarian cancer types (40), and it is likely that similar international collaboration will be required to drive advances in the standard of care for OCS patients.

As with other uncommon ovarian cancer histotypes, a multidisciplinary approach is key for determining optimal management for individuals with OCS.

Conclusion

Together, our findings identify OCS as an exceptionally aggressive histotype of ovarian carcinoma. OCS patients represent an older patient group that are frequently diagnosed at advanced stage. Despite its aggressive behavior, OCS is a relatively under-researched tumour type, hindering progress toward new treatment options which are urgently required to improve outcomes.

Data availability statement

The Scottish dataset presented in this article is not readily available because sharing of patient data is only possible within the constraints of our local ethics framework, which means line-by-line data cannot be shared without seeking an associated ethical approval. Such approval can be sought via contact with the corresponding author (robb.hollis@ed.ac.uk). Data from the SEER database can be accessed through the SEER program website.

Ethics statement

Institutional review board approval for the Scottish cohort was received from the South East Scotland Cancer Information Research Governance Committee (Caldicott guardian reference CG/DF/E164, study reference CIR21087). The studies were conducted in accordance with the local legislation and institutional requirements. The need for informed consent for every participant was waived due to the retrospective nature of the study.

Author contributions

IM: Data curation, Formal analysis, Visualization, Methodology, Writing – original draft. JMP: Data curation, Writing – review and editing. EB: Data curation, Writing – review and editing. NG: Resources, Writing – review and editing. KCC: Resources, Writing – review and editing. CSH: Resources, Writing – review and editing. RLH: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft.

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Conflict of interest

RH: consultancy fees from GlaxoSmithKline and DeciBio.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Tricia Roxburgh,
University of Glasgow, United Kingdom
Christianne Lok,
The Netherlands Cancer Institute (NKI), Netherlands
Ailsa Oswald,
University of Edinburgh, United Kingdom

*CORRESPONDENCE
Tibor A. Zwimpfer
tibor.zwimpfer@unibas.ch

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Homologous recombination proficient subtypes of high-grade serous ovarian cancer: treatment options for a poor prognosis group

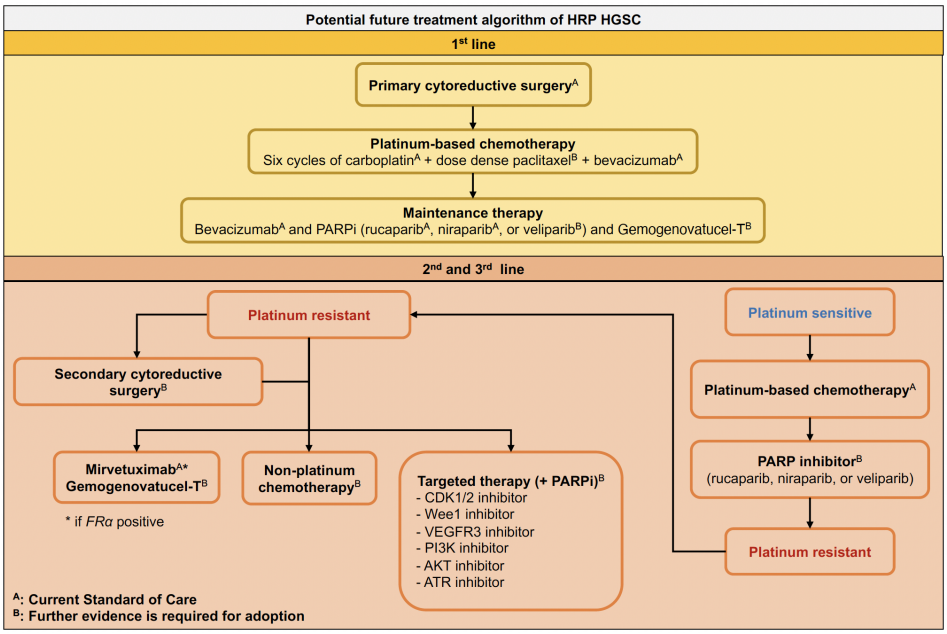
Nadja Stiegeler[1], Dale W. Garsed[2,3], George Au-Yeung[2,3], David D. L. Bowtell[2,3], Viola Heinzelmann-Schwarz[4] and Tibor A. Zwimpfer[2,4]*

[1]Medical Faculty, University of Basel, Basel, Switzerland, [2]Cancer Research, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, [3]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC, Australia, [4]Department of Gynecological Oncology, University Hospital Basel, Basel, Switzerland

Approximately 50% of tubo-ovarian high-grade serous carcinomas (HGSCs) have functional homologous recombination-mediated (HR) DNA repair, so-called HR-proficient tumors, which are often associated with primary platinum resistance (relapse within six months after completion of first-line therapy), minimal benefit from poly(ADP-ribose) polymerase (PARP) inhibitors, and shorter survival. HR-proficient tumors comprise multiple molecular subtypes including cases with *CCNE1* amplification, *AKT2* amplification or *CDK12* alteration, and are often characterized as "cold" tumors with fewer infiltrating lymphocytes and decreased expression of PD-1/PD-L1. Several new treatment approaches aim to manipulate these negative prognostic features and render HR-proficient tumors more susceptible to treatment. Alterations in multiple different molecules and pathways in the DNA damage response are driving new drug development to target HR-proficient cancer cells, such as inhibitors of the CDK or P13K/AKT pathways, as well as ATR inhibitors. Treatment combinations with chemotherapy or PARP inhibitors and agents targeting DNA replication stress have shown promising preclinical and clinical results. New approaches in immunotherapy are also being explored, including vaccines or antibody drug conjugates. Many approaches are still in the early stages of development and further clinical trials will determine their clinical relevance. There is a need to include HR-proficient tumors in ovarian cancer trials and to analyze them in a more targeted manner to provide further evidence for their specific therapy, as this will be crucial in improving the overall prognosis of HGSC and ovarian cancer in general.

KEYWORDS

ovarian cancer, homologous recombination proficiency, treatment resistance, PARP inhibitor, CDK inhibitor, PI3K inhibitor, antibody drug conjugate (ADC), vaccine



GRAPHICAL ABSTRACT

Introduction

Advanced tubo-ovarian high-grade serous carcinoma (HGSC) accounts for a majority of the disease burden and deaths from ovarian cancer (70–80%) due to its typical late presentation and high 5-year recurrence rate of 75% (1–3). Primary cytoreductive surgery followed by platinum- and taxane-based chemotherapy or neoadjuvant platinum- and taxane-based chemotherapy (NACT) followed by interval cytoreductive surgery is the standard treatment for HGSC (1–9). Most HGSC initially respond well to chemotherapy. However, the majority of patients will experience relapse with treatment resistant disease, particularly those without *BRCA* mutations and without homologous recombination deficiency (HRD) (10–13). Although there has been limited improvement in the 5-year survival rate of most patients over the past three decades (4, 8, 14–16), the introduction of poly(ADP-ribose) polymerase inhibitors (PARPis) as maintenance therapy in HGSC has had a profound impact leading to significant improvements in progression-free survival (PFS) and demonstrating a trend towards improved overall survival (OS), particularly in patients with *BRCA1* or *BRCA2 (BRCA)* mutations and HRD (1, 3, 13, 17–29).

HRD refers to a loss of homologous recombination-mediated DNA repair (HRR), which is a pathway responsible for the high-fidelity repair of double-stranded DNA breaks that restores the original DNA sequence at the site of damage. HRD contributes to genomic instability and consequently intact HRR plays a role in preventing malignant transformation (30, 31). HRD is caused by inherited or somatic loss of function genetic alterations in well-known driver genes such as *BRCA1* and *BRCA2*, but also by mutations or methylation of other HRR related genes and potentially other currently undefined mechanisms (32). Patients with HRD HGSC are more likely to benefit from a favorable chemotherapy response, maintenance treatment with PARPis and consequently a longer OS (1, 33–38). However, ~50% of HGSC are HR-proficient (HRP), an established poor prognostic marker associated with primary platinum and PARPi resistance and shorter survival times (36, 39, 40). Platinum-resistant ovarian cancer is defined as disease that relapses within six months of completing first-line treatment, and the probability of a response to platinum re-treatment is less than 10% (33, 41, 42). In fact, HGSC can also progress from HRD to become at least partially HRP by reversion of HR gene alterations through secondary genetic or epigenetic events (43–45). This acquired HR-proficiency is one of the most well described mechanisms of acquired treatment resistance and consequently a major clinical challenge.

HRD status in ovarian cancer is usually inferred by measurement of *BRCA* mutation status and/or the extent of cancer genome scarring associated with loss of HRR genes. Methodologies that assess HRD typically measure the extent of telomeric allelic imbalance, loss of heterozygosity, and large-scale transitions (31, 46). However, these scores are based on permanent genomic scars, thus failing to reflect the current HRD status in the case of HRR restoration (47). An alternative is a dynamic assessment of HR status using functional assays in *ex-vivo* cultures (46). Immunofluorescence microscopy can be used to measure the presence of RAD51 formed molecular complexes which accumulate at sites of double-stranded DNA breaks in HRP cells. By contrast, HRD cells are unable to form RAD51 formed molecular complexes and their absence thus provides a functional indication of a defect in the HR pathway (48, 49). However, such RAD51 assays are yet to be clinically validated.

Additionally, resistance to PARPis may be driven by RAD51-independent mechanisms and consequently cannot be detected by RAD51 assays (50, 51). Current HRD tests vary in the number and type of mutational features assessed, and the optimal thresholds to classify samples as HRD or HRP are not yet well defined. Variation in assays should be considered when evaluating the overall value of such assays in providing prognostic and predictive information.

The heterogeneity of HGSC, including multiple molecular subtypes even within the HRP subgroup, poses a substantial challenge to proper prognostication and clinical management (3, 33, 36, 43, 52, 53). Treatment options for patients with platinum-resistant, non-HRD HGSC are scarce, and the goal of treatment is strongly focused on symptom control and palliation, delaying time to symptomatic progression and improving quality of life (33, 53–57). To date, apart from the antibody-drug conjugate (ADC) mirvetuximab (Elahere®), few treatments in addition to cytoreductive surgery and platinum- and taxane-based chemotherapy have shown a survival benefit in this poor prognosis group (3, 33, 42, 53–57).

Recent novel approaches to treat ovarian cancer has largely benefitted patients with HRD HGSC, with or without *BRCA*-alterations (1, 3, 13, 17–29, 58). Further progress in the treatment of HGSC requires approaches that benefit patients with HRP disease, who currently have limited treatment options other than surgery. Here we summarize recent clinical and molecular findings in HRP HGSC and provide an insight into ongoing trials of new potential treatment options.

TABLE 1 Clinicopathological characteristics for Non-HRD/HRP versus HRD HGSC.

	Non-HRD/HRP	HRD
Median age (years) (10, 11, 20, 36)	63–64	Germline *BRCA*+: 54–58.5 Somatic *BRCA*/HRD+: 58–62
Frequency (%) (10, 11, 36)	~50%	~50%
Non-serous histology subtypes (11)	20%	Germline *BRCA*+: 6% Somatic *BRCA*/HRD+: 0%
Molecular characteristics (59–61)	*CCNE1*-amplification *AKT2*-amplification Whole genome duplication	*BRCA1* and *BRCA2* or other HR genes (*BRIP1*, *PALB2*, *RAD51C*, *RAD51D*)
Median NACT cycles required (10, 11)	4	Germline *BRCA*+: 3 Somatic *BRCA*/HRD+: 3
Rate of complete gross resection (11)	60%	Germline *BRCA*+: 83% Somatic *BRCA*/HRD+: 77%
Median progression-free survival (months) (10–13)	5.4–16.9	Germline *BRCA*+: 23.5–25 Somatic *BRCA*/HRD+: 20.2–25.2
Median overall survival (months) (11, 17)	40.4–42.3	Germline *BRCA*+: 68.8 Somatic *BRCA*/HRD+: 69.2

Adapted from (10–13, 17, 20, 36, 59–61).
NACT, Neoadjuvant Chemotherapy; HRP, Homologous recombination proficient; HRD, Homologous recombination deficien; HR, Homologous recombination.

Characteristics of patients with HR-proficient HGSC

Clinicopathological

Variation in outcomes between patients with HGSC is in part determined by the molecular characteristics of the tumor, with HR-status as one of the important determinants (Table 1). Patients with HRP tumors have an older median age at diagnosis compared to patients with HRD tumors (10, 11, 20, 36). A retrospective analysis of 352 patients showed that HRP tumors required a higher number of cycles of NACT to be considered for interval cytoreductive surgery compared to those with germline *BRCA* mutations and other defects conferring HRD, and less complete gross resection (R0) could be achieved (11). While complete resection in primary and interval cytoreductive surgery remains one of the strongest prognostic features in ovarian cancer (2, 3, 62, 63), the higher number of chemotherapy cycles and lower R0 rate also reflect an inherently resistant tumor (18, 22–24, 26, 33).

Genomic characteristics

Extensive genomic and transcriptomic characterization has provided insight into HGSC with HRR pathway inactivation, most commonly caused by genetic or epigenetic alterations in the *BRCA* genes and alterations in other genes, including *BRIP1*, *PALB2*, *RAD51C, or RAD51D*, which encode proteins that are also involved in HR DNA repair (59). By contrast, the molecular drivers of HGSC that have no apparent defects in HR are less well defined (2).

HRP ovarian cancer cells are often characterized by genetic alterations in signaling pathways that contribute to cell cycle dysregulation, such as cyclin E1 (encoded by *CCNE1*) and cyclin dependent kinase (*CDK*) genes (44). Cyclin E1 is an important factor in the G1/S cell cycle transition through its activation of cyclin-dependent kinase 2 (CDK2), allowing the cell to enter the S-phase (64). Besides other cellular mechanisms, limiting the supply of cyclin E1 ensures that the cell remains in the G1 phase by keeping CDK2 inactive until mitogenic signals intervene (65). *CCNE1* expression is dependent on E2F transcription factors that are bound to the retinoblastoma protein (Rb) in an inactivated state when cells are at rest. E2F is released through mitogenic stimuli such as c-MYC which increases the expression of D-type cyclins that in turn combine with CDK4 and CDK6 to phosphorylate and inactivate Rb (65). Furthermore, once activated, the cyclin E1/CDK2 complex is able to phosphorylate Rb and thus upregulate its own expression in the form of a positive feedback loop through the continued release of E2F, independent of mitogenic stimuli (65). Additionally, the cyclin E1/CDK2 complex is an essential component of the chromatin remodeling process required for

DNA replication. Overexpression of cyclin E1 increases the speed at which cancer cells transition from G1 to the S phase (66). This can lead to replicative stress, whole genome duplication, and further promote the dysregulation of genes responsible for proliferation and cell survival, which are also associated with resistance to cytotoxic and targeted therapies (67, 68).

CCNE1 amplification is currently the best characterized driver of HGSC with HR-proficiency. It is important to note, however, that cyclin E1 protein overexpression itself has not been shown to be a predictive biomarker for chemotherapy resistance in epithelial ovarian cancer (EOC), so methods to detect amplification of a gene (e.g. whole-genome sequencing, fluorescence *in situ* hybridization, polymerase chain reaction, single nucleotide polymorphism arrays) are required to identify the *CCNE1* amplified subgroup (69). Approximately 40% of HRP HGSC show an *CCNE1* amplification, which has been shown to be an early event in their development (43, 64). HR pathway gene mutations and *CCNE1* amplification have been shown to be mutually exclusive (44, 60, 65). This suggests that the pathogenesis of HGSC follows at least two distinct pathways, and that *CCNE1*-amplified tumors with cyclin E1 protein overexpression are more likely to be resistant to platinum-based chemotherapy and PARPi due to HR-proficiency (65).

AKT2 amplification is also a poor prognostic marker in EOC (34, 70, 71) and is associated with *CCNE1* amplification (70). The co-amplification of the serine/threonine-protein kinase *AKT2* and *CCNE1* appears to be explained in part by their proximity on chromosome 19q. Pathway analysis indicates that *CCNE1*-amplified cell lines are dependent on multiple genes within the CDK and AKT pathways, suggesting a specific dependence of *CCNE1*-amplified tumors on AKT activity (70). Consequently, combined CDK2 and AKT inhibition may have synergistic anti-tumor activity against *CCNE1*-amplified tumors and hold promise for clinical development (70). It should be noted that although CDK4/6 inhibitors have been investigated in ovarian cancer (72), it is the CDK2 inhibitor which is likely to be effective (73–76).

CDK12-altered HGSC represent a unique subgroup that appear to be HR competent (36). Despite lacking the typical HRD genomic scarring, *CDK12*-altered tumors have a distinct tandem duplication signature and may be more susceptible to chemotherapy and PARPis than other HRP tumors (77). Aside from alterations in *CCNE1, AKT2* and *CDK12*, the majority of HRP HGSC remain poorly defined, and integration of genomic, immune, proteomic and functional data is needed for their complete characterization (78–81).

Immune profile

Tumor-infiltrating lymphocytes (TILs) are an established prognostic factor in ovarian cancer, regardless of the extent of surgical cytoreduction and chemotherapy (82–84). The presence of CD8+ TILs in the tumor microenvironment is associated with slower tumor progression, prolonged survival and may be essential for immunotherapy response (84–86). HRD tumors have a significantly increased CD8+/CD4+ ratio of TILs and a higher number of peritumoral T cells (44). This is likely due to HRD cells accumulating a high number of somatic mutations, which is predicted to result in the expression of more tumor neoantigens that elicit an adaptive immune response and cytotoxic T cell infiltration. These cells are capable of killing cancer cells (84), and in addition to a more favorable response to chemotherapy, explains the improved survival of patients with *BRCA*-mutated ovarian cancer.

By contrast, HRP tumors are characterized by a non-inflamed or "cold" immune phenotype, with fewer CD3+ and CD8+ TILs as well as decreased expression of PD-1 and PD-L1 (87–89). HRP tumors generally have a lower tumor mutational burden due to having intact DNA repair, which, together with a low TIL density, would predict a poor response to immune checkpoint blockade (84). Therefore, HRP tumors may be poor candidates for targeted immunotherapy with PD-1 and PD-L1 inhibitors as recently shown (90–96). Recent approaches to immunotherapy for cold tumors have focused on restoring inflammation by reprogramming myeloid cells, stromal cells, and vascular epithelial cells (97). Additionally, PARPi, low-dose radiotherapy, epigenetic drugs and anti-angiogenesis therapy may enhance T cell infiltration, suggesting their use in combination with vaccines and redirected T-cells using chimeric antigen receptors or bispecific antibodies (84, 98). However, it should be noted that while T cell infiltration and the expression of PD-L1 and other immune checkpoint markers increases following chemotherapy, unlike primary disease, the extent of infiltration does not correlate with patient survival (99, 100).

Treatment options for patients with HRP HGSC

Chemotherapy

Neoadjuvant chemotherapy with interval cytoreductive surgery is currently an alternative for patients with ovarian cancer who have a low chance of initial complete resection and chemosensitive histologic subtypes, or poor health status (1). However, there is a strong correlation between HR-status and response to platinum-based chemotherapy in HGSC; patients with HRP tumors have severely limited responses to chemotherapy, with reported median PFS ranging from 5.4 to 16.9 months (Table 1) (10–13). The chemoresistant nature of HRP tumors highlights the potential benefit of favoring the currently recommended option in HGSC (1) of primary debulking surgery followed by adjuvant platinum and taxane-based chemotherapy in these patients.

An ancillary data analysis of the VELIA/GOG-3005 trial focused on paclitaxel dosing schedule and *BRCA* mutation and HR-status (101). Dose-dense (weekly) paclitaxel was compared to a schedule of every three weeks showing an improved PFS with dose-dense paclitaxel in HRP but not in *BRCA*-mutation or HRD tumors. Previous clinical trials of shorter versus longer paclitaxel intervals in ovarian cancer did not evaluate HR status and therefore further studies are needed to confirm this finding (102, 103). Interestingly, it has been shown that paclitaxel suppresses *CDK1* expression via

decreased *BRCA1* phosphorylation, thereby reducing HR activity in response to DNA damage and increasing sensitivity to PARPis (104), so this combination represents a potential new treatment strategy that needs to be further investigated in HRP HGSC.

HGSC typically involves extensive peritoneal spread and therefore intraperitoneal chemotherapy and hyperthermic intraperitoneal chemotherapy (HIPEC) have been evaluated in multiple clinical trials. The goal of intraperitoneal chemotherapy is to increase local exposure to the chemotherapeutic agent, and in the case of HIPEC, heated chemotherapy has an additional cytotoxic effect and increases sensitivity to platinum compounds by inducing a transient state of HRD (105). Koole et al. analyzed the effect of HIPEC among patients with ovarian cancer previously enrolled in the phase III OVHIPEC1 trial (105) stratified by *BRCA*-like (HRD) versus non *BRCA*-like (HRP) (106) or *BRCA* mutation status. Although patients with HRD/*BRCA*-wildtype showed a strong benefit in terms of recurrence-free survival (RFS) and a promising trend in OS from HIPEC, this was non-significant in HRP/*BRCA*-wildtype patients and absent in patients with pathogenic *BRCA* mutations, both in terms of RFS and OS (58). It appears that HRP tumors remain resistant to chemotherapy despite hyperthermia. However, there is a lack of long-term survival data for HIPEC, and thus the benefit of this treatment modality remains unclear. The importance of tumor HR status in predicting response and survival following HIPEC may be addressed in ongoing studies (107).

Poly (ADP-ribose) polymerase inhibitors

Maintenance PARPi therapy after first-line treatment and in the platinum sensitive recurrent setting have become standard treatment options in patients with *BRCA*-mutated and HRD EOC (1, 3). PARP is an enzyme that helps repair DNA damage and PARP inhibition causes an accumulation of single- and double-stranded DNA breaks (108). HRD cells are unable to effectively repair the DNA damage, resulting in an accumulation of chromosomal aberrations and cell death (109). As a maintenance therapy PARPi have led to improved PFS and shown a promising trend towards improved OS in EOC, particularly in patients with *BRCA* mutant and/or HRD tumors (17, 18, 20, 21, 23, 24). While the greatest benefit is seen in HRD cancers, an exploratory analysis of the Phase III PRIMA trial showed improvements in PFS with niraparib versus placebo as first-line maintenance monotherapy, regardless of *BRCA* and HR-status (20). Patients with *BRCA*-wildtype/HRP tumors treated with niraparib who responded to first-line chemotherapy had a median PFS of 8.1 months versus 5.4 months for placebo, with an estimated probability of survival at 24 months of 81% in the niraparib group versus 59% in the placebo group. Therefore, niraparib is clinically approved for use in patients with HRP HGSC, with beneficial effects and a manageable tolerability profile (110, 111).

An exploratory analysis of the VELIA/GOG-3005 trial (27) showed that some patients with HRP ovarian cancer and also poor chemosensitivity may have gained a transient, but non-significant benefit from the addition of the PARPi veliparib to carboplatin-paclitaxel (median PFS 14.7 vs median 6.7 months, HR 0.62, 95% CI 0.37–1.05) (112). The authors of the study hypothesized that veliparib may have induced a chemosensitizing effect on HRP tumors (112, 113). In addition, the Phase III ATHENA-MONO trial demonstrated improved PFS with rucaparib monotherapy compared to placebo in first-line maintenance in patients with newly diagnosed EOC without evidence of HRD (12.1 vs 9.1 months, HR 0.65, 95% CI 0.45–0.95) (13). As a result of such findings, the ESGO-ESMO-ESP consensus guidelines state that niraparib or rucaparib maintenance therapy may be used for patients with HRP HGSC if they have had a complete or partial response to first line chemotherapy or no evidence of disease (1).

Antiangiogenic treatment

Vascular endothelial growth factor (VEGF) promotes increased vascularity and angiogenesis in response to hypoxic conditions and is a key promoter of tumor growth (114). The anti-angiogenic VEGF monoclonal antibody bevacizumab was the first targeted agent to be approved for use in stage III and IV EOC, showing an improved PFS when used in combination with chemotherapy and as maintenance therapy in the first-line setting, however without OS benefit (115, 116). According to the ESGO-ESMO-ESP consensus guidelines, patients with HRP HGSC may receive platinum-based chemotherapy with bevacizumab followed by bevacizumab maintenance as an alternative to the option of maintenance with rucaparib or niraparib (1). Among other mechanisms of action, bevacizumab exposure may trigger HRD by inducing a hypoxic cellular state that can downregulate HR-related genes such as *BRCA1/2* and *RAD51* (117). In addition, the relative benefit of bevacizumab in EOC has been shown to increase as the disease becomes more platinum resistant (118). A retrospective analysis of 124 patients with platinum-sensitive recurrent ovarian cancer showed extended PFS with bevacizumab in patients with cyclin E1 overexpression (median 16.3 vs 7.1 months, P=0.010) (118).

Tumor VEGF secretion has been shown to be at least partially responsible for the development and maintenance of ascites, and the AURELIA trial demonstrated that the addition of bevacizumab to chemotherapy improved ascites control. This beneficial effect is certainly relevant for the HRP group as they are more frequently associated with suboptimal debulking, earlier recurrence and ascites (54). Furthermore, the combination of niraparib and bevacizumab evaluated in the pre-specified subgroup analysis of the AVANOVA trial showed a significant improvement in PFS compared to niraparib alone in the HRP population (HR 0.40, 95% CI 0.19–0.85) (119). The Phase III GOG-218 trial also showed prolonged PFS in patients with no HRR gene mutations who received bevacizumab in addition to standard chemotherapy with carboplatin and paclitaxel (HR 0.71, 95% CI 0.60–0.85, P = 0.0001). This benefit was not observed in patients with HRR gene mutations (HR 0.95; 95% CI 0.71–1.26) (120). Therefore, the ESMO guidelines recommend that the decision on bevacizumab versus niraparib maintenance in the HRP population should be based on the patient's disease and clinical characteristics, the toxicity profile of the two drug classes, the availability of each drug, and national

guidelines (3, 121). The ongoing Phase I/II MITO 25 trial (NCT03462212) may provide clearer evidence about potential therapy options by comparing whether the carboplatin-paclitaxel-bevacizumab-rucaparib or carboplatin-paclitaxel-rucaparib arms improve PFS compared to standard carboplatin-paclitaxel-bevacizumab in patients with HRP HGSC.

The inhibition of VEGF receptor-3 (VEGFR3) has been shown to decrease *BRCA1* and *BRCA2* expression in ovarian cancer cells and resulted in increased chemosensitivity (122). The randomized Phase II trial (NCT01116648) showed that the combination of olaparib plus cediranib, a VEGF receptor 1/2/3 inhibitor, significantly improved PFS in relapsed platinum-sensitive EOC compared to olaparib alone (median 17.7 months vs 9 months, *P*=0.005), with the greatest benefit in *BRCA*-wildtype patients (HR 0.32, *P*=0.008) (123). These results suggest that there may be greater synergism between the two agents in HRP tumors, with the response to olaparib in HRP tumors being enhanced by diminished HRR due to VEGFR3 inhibition. However, experimental *in vivo* efficacy data showed that the combination exhibited broad anti-tumor activity independent of HRR and that the combination effect was largely driven by influencing independent mechanisms affecting tumor cells and the tumor microenvironment (124). Clinically, the combination of cediranib and olaparib also showed some activity in the CONVERTO trial, a single-arm Phase IIb study of the two compounds in heavily pretreated, platinum-resistant, non-germline *BRCA*-mutated patients. However, the target objective response rate (ORR) of 20% was not reached (15.6%) and the overall benefit was unclear (OS 13.2 months, 95% CI 9.4–16.4; PFS 5.1 months, 95% CI 3.5–5.5) given it was a single arm study in a disease setting where most patients are expected to progress or die within 12 months (125). A Phase III trial [NCT02446600] in patients with relapsed platinum-sensitive ovarian cancer found that neither the combination of olaparib and cediranib nor olaparib monotherapy improved PFS compared to standard chemotherapy (126). An ongoing Phase II/III trial (NCT02502266) is evaluating cediranib plus olaparib compared to their monotherapies and standard chemotherapy. It remains to be determined if there is a clinical benefit of VEGF receptor inhibitors in treating EOC, particularly in chemoresistant HRP tumors. Future research efforts must focus on identifying other predictive biomarkers for anti-angiogenic therapy, as not all observed responses can be explained by *BRCA* mutation or HR-status.

Secondary cytoreductive surgery

There have been significant advances in the surgical management of HGSC with improved PFS and OS due to intensification of surgical efforts (62, 127, 128). A multicenter, open-label, randomized, controlled Phase III trial SOC-1 (NCT01611766) demonstrated in 357 patients with platinum-sensitive relapsed ovarian cancer that secondary cytoreductive surgery (SCS) followed by chemotherapy was associated with significantly longer PFS than with chemotherapy alone (median 17.4 vs 11.9 months, HR 0.58, 95% CI 0.45–0.74, *P*<0.0001) (129). Furthermore, the DESKTOP III trial (NCT01166737) analyzed 407 patients with platinum-sensitive recurrent ovarian cancer and showed that SCS followed by chemotherapy leads to a longer OS than chemotherapy alone (median 53.7 vs 46.0 months, HR for death 0.75, 95% CI 0.59–0.96, *P*=0.02). Patients with a complete resection had the most favorable outcome (130). In addition to these two positive studies, in the GOG-0213 trial, which also included patients with platinum-sensitive, recurrent ovarian cancer, SCS followed by chemotherapy did not result in a longer OS than chemotherapy alone (131). There are some differences between the trials that may explain the inconsistent results, such as the additional use of bevacizumab in the DESKTOP III trial (NCT01166737) or the process of selecting patients and centers (130). Therefore, it is important that patients are appropriately counseled about the option of SCS.

The role of surgery in patients with platinum-resistant disease has received increasing attention (132). In fact, patients with HRP tumors may benefit from SCS, similar to patients with low-grade serous ovarian cancer (133). To our knowledge, only three retrospective studies have been published analyzing the role of SCS in patients with platinum-resistant recurrent ovarian cancer. Both Petrillo et al. and Musella et al. showed a prolonged OS after recurrence when SCS was combined with chemotherapy instead of chemotherapy alone (median 32 months vs 8 months, *P*=0.002 and 67 months vs 24 months, *P*=0.035) (134, 135). However, when evaluating these two studies, it is important to consider that they were carried out before the PARPi era and therefore their conclusions must be put into perspective with current treatment options. A recent multicenter retrospective series by Tuninetti et al. in 50 heavily pretreated platinum-resistant ovarian cancer patients showed a statistically significant longer OS in the group of patients who received complete cytoreduction after SCS compared to the very low survival of patients with residual disease (meidan 33 months vs 5 months, HR 4.21, 95% CI 2.07–8.60, *P*=0.001) (136). These retrospective studies did not include stratification by *BRCA* mutation or HR-status, and any discussion of the extent of surgical clearance should also consider how residual disease may be a marker of biology that drives outcome. However, in a recent multicenter retrospective study investigating platinum sensitive recurrent ovarian cancer, SCS was shown to be effective in *BRCA*-wildtype patients, with an improvement in post-recurrence survival (PRS) when complete resection was performed (5-year PRS of 54% vs 42%, *P*=0.048), whereas in *BRCA*-mutated patients, prognosis appears to be related to molecular tumor characteristics rather than tumor resectability (137). A current prospective randomized controlled trial (NCT05633199) is now comparing SCS in platinum-resistant recurrent ovarian cancer and is expected to provide further information on whether and to what extent SCS can be used in the "platinum-resistant" HRP HGSC subgroup. Another advantage of SCS is to opportunistically obtain more comprehensive information on the pathological and molecular characteristics of HRP HGSC and how this may affect tumor evolution and clinical outcome (127). SCS in HGSC warrants further investigation in prospective trials, with particular attention paid to patient *BRCA* and HR-status.

Immunotherapy and antibody-drug conjugates

Immunotherapy for HGSC has fallen short of expectations, with immune checkpoint inhibitors so far showing limited benefit in ovarian cancer (138–142). However, there are new, potentially promising approaches, including ADCs that deliver a toxic 'payload' of chemotherapy directly to cancer cells via a linker attached to an antibody that binds to a specific surface antigen expressed on cancer cells (143). Mirvetuximab is a first-in-class ADC targeting folate receptor α (FRα), a cell surface protein that is commonly overexpressed on ovarian cancer (80–100%) and minimally expressed on normal tissue (144–146). This ADC incorporates the maytansinoid DM4 payload, a potent tubulin-targeting antimitotic agent, and is the first novel agent to demonstrate an OS benefit when used as a single agent compared to chemotherapy alone in platinum-resistant ovarian cancer, as shown in the MIRASOL Phase III clinical trial (NCT04209855) (144). Patients with platinum-resistant, FRα-positive ovarian cancer treated with mirvetuximab (n=227) experienced an OS of 16.46 months (95% CI, 4.46–24.57) vs 12.75 months (95% CI, 10.91–14.36) for the chemotherapy arm (HR 0.67, 95% CI 0.50–0.89, *P*=0.005) and showed fewer Grade 3 or higher adverse events with mirvetuximab than with chemotherapy (41.7% vs 54.1%).

Another promising immunotherapy approach is Gemogenovatucel-T (Vigil, formerly known as FANG®), the first immunotherapy to demonstrate specific efficacy in the frontline maintenance setting for the HRP population. Vigil is a vaccine composed of autologous tumor cells derived from malignant tissue removed during cytoreductive surgery (147) (Figure 1). Tumor cells are transfected with a plasmid containing GM-CSF and bi-shRNA to reduce furin activity, which subsequently downregulates the expression of the immunosuppressive proteins TGF-β1 and TGF-β2 (transforming growth factor *β*). This is important because the expression of furin and the resulting immunosuppressive TGF-β isoforms are increased in ovarian tumors compared to normal ovarian tissue (148). Long-term safety of Vigil and evidence of patient benefit have been demonstrated in multiple solid tumors, including advanced ovarian cancer (149, 150). The ongoing Phase IIb VITAL trial (NCT02346747) evaluated the efficacy of Vigil in patients with stage III/IV ovarian cancer. RFS was 11.5 months for patients treated with Vigil versus 8.4 months for patients treated with placebo (HR 0.69, 90% CI 0.44–1.07, *P*=0.078) with an acceptable toxicity profile (151). Although the primary endpoint of RFS was not met, a small subgroup analysis (n=45) showed that RFS and OS was significantly improved with Vigil compared to placebo in HRP patients (HR 0.38 and 0.34, 90% CI 0.2–0.75 and 0.14–0.83, *P*=0.007 and *P*=0.019), while no difference was seen in patients with *BRCA*-mutated disease (151, 152). Vigil increases the expression of cancer-associated neoantigens by upregulating MHC-II and processing by dendritic cells, which enhances the afferent immune response, the initial phase of immune activation characterized by antigen presentation and recognition, resulting in a systemic anti-tumor immune response including CD3+/CD8+ T cell circulation (152). T cells showed to preferentially recognize clonal neoantigens over subclonal neoantigens to target the tumor in lung adenocarcinoma and melanoma (153). HRP tumors are associated with higher clonal neoantigen expression compared to HRD tumors, which therefore contain higher proportions of subclonal neoantigen subpopulations, which may explain why

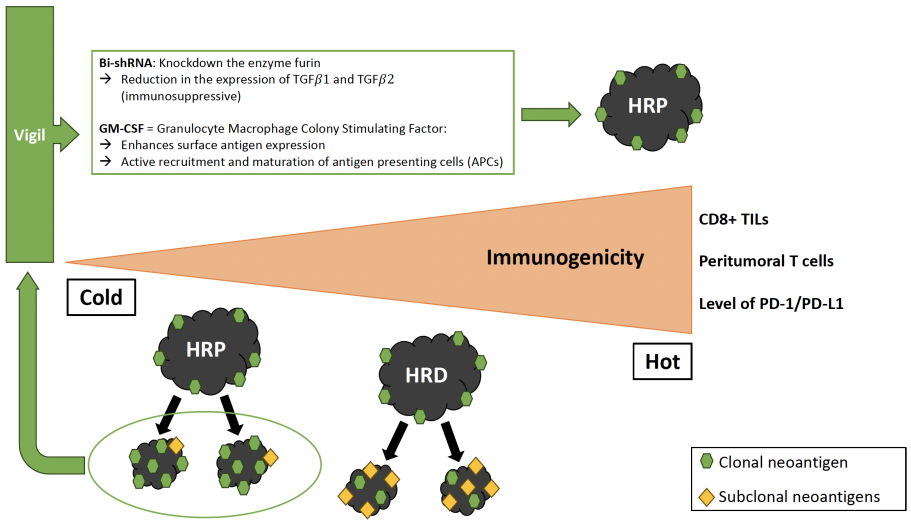


FIGURE 1
Immune profile of HRP vs. HRD tumors and effect of Vigil. HRP tumors show reduced immunophenotypic markers compared to HRD tumors. Gemogenovatucel-T (Vigil) is a vaccine composed of autologous tumor cells transfected with a plasmid containing GM-CSF and bi-shRNA resulting in a systemic anti-tumor immune response including CD8+ T cell circulation. HRP tumors have a higher proportion of clonal neoantigen expression, which explains the better effect of Vigil on HRP tumors compared to HRD. HRP, Homologous recombination proficient; HRD, Homologous recombination deficient; TILs, Tumor-infiltrating lymphocytes; Bi-shRNA, Bifunctional short hairpin RNA; GM-CSF, Granulocyte/Macrophage Colony Stimulating Factor; TGF, Transforming growth factor; APC, Antigen presenting cell.

Vigil is more effective on HRP tumors (152). A Phase III trial is planned to validate the efficacy of Vigil compared to bevacizumab and niraparib in the HRP ovarian cancer population (152). It has been suggested that the increased expression of clonal tumor neoantigens and reduced tumor suppressive effect of TGF-β may synergistically enhance the activity of checkpoint inhibitor treatment (84, 154, 155). A prospective, randomized Phase I trial of Vigil plus the immune checkpoint inhibitor atezolizumab in patients with recurrent ovarian cancer explored this approach and showed that the combination was safe, supporting further investigation of this combination, particularly in *BRCA*-wildtype patients (155).

Adoptive cell therapy is another emerging personalized form of immunotherapy in which patients are treated with their *ex vivo* expanded natural TILs, genetically engineered T lymphocytes (CAR T cells) or T-cell receptor (TCR)-engineered T cells, which could offer a potential therapeutic option for patients with cold tumors. To date, CAR T cells that have been tested in clinical trials for HGSC have not yet demonstrated clear benefit (84, 156). While this technology is promising, further development is required to investigate the full potential of T cell engineering and other novel immunotherapy approaches to address the problem of immunologically cold tumors (84).

Combined targeted therapies

Rational drug combinations are a potential strategy to prevent or delay the development of resistance and offer the opportunity to improve the therapeutic window by potentially reducing the required drug doses, resulting in fewer side effects (70). Several strategies to selectively disrupt HRR in cancer cells with drugs have been investigated both preclinically and in clinical trials in HGSC or EOC in general, including HRP tumors, and have provided the rationale for new potential therapeutic approaches (Figure 2, Table 2). Here we review the most promising approaches for HRP tumors that have been or are being investigated in ovarian cancer, including targeting the CDK, P13K/AKT or CHK pathways.

CDK pathway

Approximately 40% of HGSC with HR-proficiency have an amplification of *CCNE1* (64). Cyclins are typically regulatory proteins that modulate the activity of CDKs (65). The CDK pathway offers attractive targets for the treatment of *CCNE1*-amplified tumors due to its role as the kinase partner of cyclin E1 in the activated cyclin E1/CDK complex (65, 163) (Figure 3). Cyclin E1 is primarily regulated by *CDK2* in *CCNE1*-amplified tumors, which are selectively dependent on *CDK2* activity (73). Combination therapy with the multi-CDK inhibitor dinaciclib (targets *CDK1/2/5/9*) has shown positive preclinical responses in *CCNE1*-amplified HGSC (164–166), and there is currently an active but not recruiting Phase I trial [NCT01434316] evaluating dinaciclib in combination with the PARPi veliparib in advanced solid tumors. However, a disadvantage of broad-spectrum CDK inhibitors is their high toxicity (167). Recently, more selective CDK2 inhibitors have been investigated (74–76), including promising preclinical results using INX-315, a novel, potent and highly selective CDK2 inhibitor. INX-315 treatment resulted in tumor growth inhibition of *CCNE1*-amplified tumors by promoting retinoblastoma protein hypophosphorylation, inducing cell cycle arrest and delaying the onset of CDK4/6 inhibitor resistance in breast cancer (74). In addition, a recent first-in-human Phase I/IIa study (NCT04553133) of a novel and potent selective CDK2i (PF-07104091) found that it was well tolerated and showed antitumor activity in heavily pretreated metastatic breast cancer patients who had progressed on prior CDK4/6 inhibitors (75). Further development of selective CDK2 inhibitors in Phase I/II clinical trials are ongoing and may be of major importance for HRP HGSC.

Another strategy is to target Weel-like kinase (WEE1), which is highly upregulated in HGSC (108). Its inhibition causes activation of *CDK1* and *CDK2*, resulting in cell cycle acceleration with an early mitotic entry and mitotic catastrophe leading to irreparable DNA damage (121). The multicenter Phase II IGNITE trial [ACTRN12619001185156P] is a non-comparative trial evaluating the WEE1-inhibitor adavosertib in two cohorts of platinum resistant recurrent HGSC (cyclin E1 overexpressed/*CCNE1*

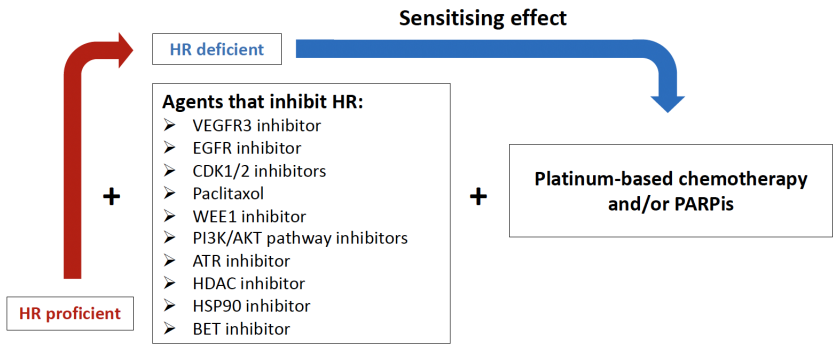


FIGURE 2
Potential combination therapies to induce homologous recombination deficiency (HRD). VEGFR3i, Vascular endothelial growth factor receptor 3 inhibitor; EGFRi, Epidermal growth factor receptor inhibitor; CDKi, Cyclin-Dependent Kinases inhibitor; WEE1i, Weel-like kinase 1 inhibitor; PI3K, Phosphatidylinositol-3-Kinase and AKT, Serine/threonine protein kinases; ATRi, Ataxia teleangiectasia Rad3-related inhibitor; HDACi, Histone deacetylase inhibitor; HSP90i, Heat shock protein 90 inhibitor; BETi, Brodomain and extraterminal protein family inhibitor.

TABLE 2 Clinical trials of potential therapy options for HRP HGSC.

Combinations	Mode of action/possible mechanism of HR suppression	Drug	Phase	Status	Indication	No. of patients	Clinical notes & No. of HRP patients and their evaluation/ biomarkers	Study title and references	Clinical-trials.gov
Chemotherapy + PARPi	Veliparib may induce a chemo-sensitizing effect.	Carboplatin/ Paclitaxel + Veliparib	III	Completed	Newly diagnosed Stage III or IV, high-grade serous, epithelial ovarian, fallopian tube, or primary peritoneal cancer	1140	Improved PFS in HRP tumors (HR 0.76) 372 HRP tumor patients determined by myChoice® assay	VELIA: A Phase III Placebo-Controlled Study of Carboplatin/ Paclitaxel With or Without Concurrent and Continuation Maintenance Veliparib in Subjects With Previously Untreated Stages III or IV High-Grade Serous Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer (27, 101, 112, 113)	NCT02470585
PARPi		Niraparib	III	Active, not recruiting	First-line maintenance therapy of advanced ovarian cancer Stage III or IV in complete or partial response to platinum-based chemotherapy	733	Significantly increased PFS (13.8 vs 8.2 months; HR 0.62, $p < 0.001$) regardless of the presence or absence of HRD, in HRP tumors 8.1 vs 5.4 months (HR 0.68) 249 HRP tumor patients determined by myChoice® assay	PRIMA: A Phase 3, Randomized, Double-Blind-Placebo-Controlled Multicenter Study of Niraparib Maintenance Treatment in Patients With Advanced Ovarian Cancer Following Response on Front-Line Platinum-Based Chemotherapy (20)	NCT02655016
PARPi + VEGFR3 inhibitor	Downregulation of *BRCA1/2* gene expression	Olaparib + Cediranib	II/III	Active, not recruiting	Recurrent platinum-resistant or refractory ovarian, fallopian tube or primary peritoneal cancer	562	Cediranib is a VEGFR1, VEGFR2 and VEGFR3 inhibitor Retrospective assessment of HRP tumor patients determined by BROCA HR assay	A Randomized Phase II/III Study of the Combination of Cediranib and Olaparib Compared to Cediranib or Olaparib Alone, or Standard of Care Chemotherapy in Women With Recurrent Platinum-Resistant or Refractory Ovarian, Fallopian Tube, or Primary Peritoneal Cancer (COCOS)	NCT02502266
PARPi + EGFR inhibitor	Using synthetic lethality to increase DNA damage	Niraparib + Neratinib	I	Recruiting	Platinum resistant ovarian cancer and other advanced solid tumors	45		iNNOVATE: Phase I/Ib Clinical Trial of Niraparib and Neratinib in Advanced Solid Tumors With an Expansion Cohort in Platinum-resistant Ovarian Cancer	NCT04502602
Vaccine	Enhanced expression of clonal tumor neoantigen and reduced tumor	Gemogenovatucel-T (Vigil)	II	Active, not recruiting	Stage IIIb, IIIc or IV high-grade papillary serous/clear cell/endometrioid ovarian,	92	A companion clinical Phase II study investigated the combination of Atezolizumab and Vigil in Patients with	VITAL: A Randomized, Double-Blind, Placebo-Controlled Phase 2 Trial of Vigil Engineered Autologous Tumor Cell	NCT02346747

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TABLE 2 Continued

Combinations	Mode of action/possible mechanism of HR suppression	Drug	Phase	Status	Indication	No. of patients	Clinical notes & No. of HRP patients and their evaluation/ biomarkers	Study title and references	Clinical-trials.gov
	suppressor activity of TGF-β				fallopian tube or primary peritoneal cancer		advanced gynecological cancers (155) 45 HRP tumor patients, retrospective analyzed determined by myChoice® assay	immunotherapy in Subjects With Stage IIIb-IV Ovarian Cancer in Clinical Complete Response Following Surgery and Primary Chemotherapy (151)	
PARPi + immune checkpoint inhibition (+/- bevacizumab)	Synergistic activity by PARPis activating immune responses	Rucaparib + Nivolumab	III	Active, not recruiting	Advanced high-grade epithelial ovarian, primary peritoneal or fallopian tube cancer who achieved response after cytoreductive surgery and initial platinum-based chemotherapy	1000	Primary outcome is PFS. Primary results of ATHENA-MONO (rucaparib monotherapy) demonstrated improved PFS also in HR proficient patients (12.1 vs 9.1 months; HR 0.65) (13). 44.2% HRP tumor patients, determined by FoundationOne CDx	ATHENA: A multicenter, randomized, double-blind, placebo-controlled Phase III Study in ovarian cancer Patients Evaluating Rucaparib and Nivolumab as Maintenance Treatment Following Response to Front-Line Platinum-Based Chemotherapy (25)	NCT03522246
		Olaparib + Durvalumab	I/II	Active, not recruiting	Patients with advanced solid tumors	264	Preliminary results of the combination in addition with bevacizumab showed an ORR of 75% in HRP patients (157) HRD status determined by FoundationOne CDx	A Phase I/II Study of MEDI4736 (Anti-PD-L1 antibody) in Combination With Olaparib (PARP inhibitor) in Patients With Advanced Solid Tumors	NCT02734004
		Durvalumab + Chemotherapy + Bevacizumab + Olaparib	III	Active, not recruiting	Newly diagnosed advanced ovarian cancer	1407	The HRP subgroup had a consistent PFS effect (HR 0.68, 95%, 0.34–0.86), safety was generally consistent (158) 55% HRP tumor patients determined by myChoice® assay	A Phase III, Double-Blind, Placebo-Controlled, Multicenter Study of Durvalumab in Combination With Chemotherapy and Bevacizumab, Followed by Maintenance Durvalumab, Bevacizumab and Olaparib in Newly Diagnosed Advanced ovarian cancer patients (DUO-O)	NCT03737643
Selective CDK2 inhibitor	Rb hypophosphorylation and reduction of CDK2 results in durable control of	INX-315	I/II	Recruiting	Advanced cancer, including ovarian cancer with *CCNE1*-ampl.	81	INX-315 is a selective inhibitor of CDK2 Biomarker for HRP tumor patients: *CCNE1*-amplification	A Phase I/II, Open-Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Efficacy of INX-315	NCT05735080

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TABLE 2 Continued

Combinations	Mode of action/possible mechanism of HR suppression	Drug	Phase	Status	Indication	No. of patients	Clinical notes & No. of HRP patients and their evaluation/ biomarkers	Study title and references	Clinical-trials.gov
	tumor growth in *CCNE1*-amplified tumors	BLU-222	I/II	Recruiting	Advanced cancer, including ovarian cancer with *CCNE1*-amplification	366	BLU-222 is a selective inhibitor of CDK2 Biomarker for HRP tumor patients: *CCNE1*-amplification	A Phase I/II Study to Evaluate the Safety, Pharmacokinetics, and Efficacy of BLU-222 as a Single Agent and in Combination for Patients With Advanced Solid Tumors	NCT0525416
		PF-07104091	I/II	Recruiting	Advanced or metastatic small cell lung, breast and ovarian cancer	320	PF-07104091 is a selective inhibitor of CDK2, already tested successfully clinically in metastatic breast cancer (75)	Phase 1/IIa Dose Escalation and Expansion Study of PF-07104091 As a Single Agent And in Combination Therapy	NCT04553133
PARPi + CDK inhibitor	Inhibition of phosphorylation of *BRCA1*	Veliparib + Dinaciclib	I	Active, not recruiting	Histologically confirmed diagnosis of a solid tumor for which no curative therapy exists	118	Dinaciclib is a multi-CDK inhibitor targeting CDK 1/2/5/9/12	Phase I Trial of ABT-888 and SCH727965 in Patients With Advanced Solid Tumors (159)	NCT01434316
PARPi + WEE1 inhibitor	Activation of *CDK1* resulting in cell cycle acceleration and mitotic catastrophe, leading to DNA damage	Olaparib + Adovesertib	II	Recruiting	Ovarian cancer progressed during PARPi therapy	104	Combination shows greater CBR, but the ORR is similar. Better ORR in BRCA wildtype vs BRCA-mutated (39% vs 19%)	EFFORT: Efficacy of AZD1775 in PARP Resistance; a Randomized 2-Arm, Non-Comparative Phase II Study of AZD1775 Alone or AZD1775 and Olaparib in Women With Ovarian Cancer Who Have Progressed During PARP Inhibition (160)	NCT03579316
PKMYT1 inhibitor + ATR inhibitor	Downregulation of Myt1 kinase, whose primary role is the negative regulation of *CDK1* in *CCNE1* amplified cells	RP-6306 as monotherapy or with RP-3500	I	Recruiting	Locally advanced or metastatic resistant or refractory solid tumors with next generation sequencing report obtained demonstrating eligible tumor biomarker	180		Phase I of the Safety, Pharmacokinetics, Pharmacodynamics and Preliminary Clinical Activity of RP-6306 Alone or in Combination With RP-3500 in Patients With Advanced Solid Tumors	NCT04855656
Chemotherapy + PKMYT1 inhibitor		Gemcitabine + RP-6306	I	Active, not recruiting	Advanced solid tumors	104	Gemcitabine treatment enhances cyclin E-driven DNA replication stress leading to sensitization of cells and tumors to RP-6306	Phase I Study of the PKMYT1 inhibitor RP-6306 in Combination With Gemcitabine for the Treatment of Advanced Solid Tumors (MAGNETIC Study)	NCT05147272

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TABLE 2 Continued

Combinations	Mode of action/possible mechanism of HR suppression	Drug	Phase	Status	Indication	No. of patients	Clinical notes & No. of HRP patients and their evaluation/ biomarkers	Study title and references	Clinical-trials.gov
PARPi + PI3K/ AKT pathway inhibitors	ERK activation/ phosphorylation, increased activation of ETS1, and suppression of *BRCA1/2* expression	Olaparib + Alpelisib	II	Active, not recruiting	HGSC with no germline *BCRA* mutation detected	358	Alpelisib and Olaparib versus chemotherapy of physician's choice	EPIK-O: A Phase III, Multi-center, Randomized (1:1), Open-label, Active-controlled, Study to Assess the Efficacy and Safety of Alpelisib (BYL719) in Combination With Olaparib as Compared to Single Agent Cytotoxic Chemotherapy, in Participants With no Germline BRCA Mutation Detected, Platinum-resistant or Refractory, High-grade Serous Ovarian Cancer (161)	NCT04729387
		Olaparib + Capivasertib	IB/II	Active, not recruiting	Recurrent endometrial, triple-negative breast, and ovarian cancer	159		A Phase Ib Study of the Oral PARP Inhibitor Olaparib With the Oral mTORC1/2 Inhibitor AZD2014 or the Oral AKT Inhibitor AZD5363 for Recurrent Endometrial, Triple Negative Breast, and Ovarian, Primary Peritoneal, or Fallopian Tube Cancer	NCT02208375
PARPi + ATR inhibitor	Restriction of the CHK1 pathway and proteins of the HRR pathway	Olaparib + Ceralasertib	II	Recruiting	Recurrent EOC	86		Combination ATR and PARP Inhibitor (CAPRI) trial With AZD 6738 and Olaparib in Recurrent Ovarian Cancer	NCT03462342
PARPi + HDAC inhibitor	Downregulation of HR pathway genes	Talazoparib + Belinostat	I	**Recruiting**	**Metastatic breast cancer, metastatic castration resistant prostate cancer, and metastatic ovarian cancer**	25		A Phase I Dose-Escalation Trial of Talazoparib in Combination With Belinostat for Metastatic Breast Cancer, Castration Resistant Prostate Cancer and Ovarian Cancer	NCT04703920
PARPi + HSP90 inhibitor	*BRCA1* and other essential HR pathway genes are HSP90 client proteins	Olaparib + HSP90 inhibitor (AT13387)	I	**Completed**	**Solid tumors that are metastatic or cannot be removed by surgery or recurrent ovarian, fallopian tube, primary peritoneal, or triple-negative breast cancer**	28	No unexpected toxicities, prolonged disease stabilization, but no further development of the combination planned	A Phase I Study of PARP Inhibitor Olaparib and HSP90 Inhibitor AT13387 for Treatment of Advanced Solid Tumors With Expansion in Patients With Recurrent Epithelial Ovarian, Fallopian Tube, Peritoneal Cancer	NCT02898207

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TABLE 2 Continued

Combinations	Mode of action/possible mechanism of HR suppression	Drug	Phase	Status	Indication	No. of patients	Clinical notes & No. of HRP patients and their evaluation/ biomarkers	Study title and references	Clinical-trials.gov
								or Recurrent Triple-Negative Breast Cancer (162)	
PARPi + BET inhibitor	Induction of DNA damage resulting in HRD phenotype	Talazoparib + ZEN003694	II	Recruiting	Recurrent ovarian, fallopian tube or primary peritoneal cancer	33		Phase II Study of a BET Inhibitor, ZEN003694, Combined With a PARP Inhibitor, Talazoparib, in Patients With Recurrent Ovarian Cancer	NCT05071937

PARPi, Poly ADP -ribose polymerase inhibitors; N, Number of patients; HR, Hazard ratio; HRP, Homologous recombination proficiency; HRD, Homologous recombination deficiency; PFS, Progression-free survival; OS, Overall survival; RB, Retinoblastoma protein; EOC, Epithelial ovarian cancer; ORR, Objective response rate; CBR, Clinical benefit rate.

amplified and cyclin E1 overexpressed/*CCNE1* non-amplified) and demonstrated an ORR of 53% and a clinical benefit of 61% in an interim analysis of 32 patients in the cyclin E1 overexpressed/*CCNE1* non-amplified cohort (168). *CDK1* is a key cell-cycle regulator and phosphorylates *BRCA1*, which is required for DNA damage-induced checkpoint control through the formation of *BRCA1*-containing foci (169); consequently, inhibition of *CDK1* impairs the ability of cells to functionally repair DNA by HRR (165). Therefore, depletion or inhibition of *CDK1* creates a state of "BRCAness" in transformed cells (170). Results from preclinical studies in other cancer modalities support the effect of WEE1 inhibition on HR, and thus the assumption that WEE1 inhibitors, in combination with a DNA damaging agent, specifically render HRP cell lines more susceptible to treatment (171, 172).

An ongoing Phase II trial (NCT03579316) in recurrent PARPi-resistant EOC (including 98% HGSC) is evaluating the efficacy of the WEE1 inhibitor adavosertib with or without olaparib. The combination showed to have a greater clinical benefit rate than adavosertib alone (89% vs 63%), but the ORR was similar between the two arms (160). Interestingly, exploratory analyses showed a larger benefit of the combination in the *BRCA*-wildtype subgroup compared to the *BRCA*-mutated subgroup (39% vs 19% ORR). Translational analyses are underway to further explore potential predictive biomarkers (160). However, adavosertib use requires consideration of single agent toxicity as well as interactions when used as a drug combination. For example, the use of adavosertib in combination with carboplatin showed an increased incidence of bone marrow suppression, diarrhea, vomiting and fatigue (168, 173). Additionally, adavosertib is metabolized via the enzyme cytochrome P450 3A4 (CYP3A4), which means that patients receiving any co-medications that are strong CYP3A4 inhibitors (for example, antibacterials such as clarithromycin and erythromycin, anticancer agents such as tamoxifen and irinotecan, anti-HIV agents such as ritonavir and delavirdine, or antihypertensives such as dihydro-dralazine and verapamil) (174) would be excluded from clinical trials.

Another promising therapeutic target of the CDK pathway specifically for *CCNE1*-amplified HGSC is *PKMYT1* (68). *PKMYT1* is a kinase encoding the pro-protein kinase Myt1, a negative regulator of *CDK1*, and was identified in a genetic screen of cellular dependencies in *CCNE1* amplified HGSC (68). Inhibition of PKMYT1 results in activation of CDK1, causing unscheduled mitotic entry and genome instability. In contrast, the WEE1inhibitor showed no selectivity towards *CCNE1*-amplified cell lines (175, 176). Ongoing first-in-human clinical trials are evaluating the PKMYT1 inhibitor lunresertib (RP-6306) as monotherapy or in combination with the ataxia-telangiectasia Rad3-related (ATR) inhibitor RP-3500 (NCT04855656) and in combination with gemcitabine (NCT05147272) in advanced solid tumors.

PI3K/AKT pathway

Phosphatidylinositol 3-kinase (PI3K) activity is stimulated by a wide range of oncogenes and growth factor receptors (177) and the

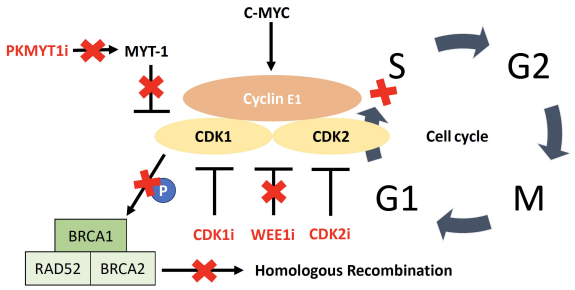

FIGURE 3
CDK/cyclin E1 complex including targets for therapy. Activation of CDK2 by cyclin E1, allowing the cell to enter the S phase. Overexpression of cyclin E1 increases the rate at which cancer cells transition from G1 to S phase, leading to replicative stress and genomic instability. The WEE1 kinase is involved in regulating cell cycle progression by inhibiting CDK1 and CDK2 and WEE1 inhibition leads to cell cycle acceleration, with early mitotic entry and consequent mitotic catastrophe leading to irreparable DNA damage. CDK1 phosphorylates BRCA1 and CDK1 inhibition impairs the ability of cells to carry out functional DNA repair through homologous recombination. PKMYT1 encodes the protein kinase Myt1, a negative regulator of CDK1.

activity of the PI3K pathway is important to the development of drug resistance in a variety of cancer types and treatment settings (178). Inhibition of the PI3K pathway also results in PI3K-mediated downregulation of *BRCA*, accompanied by extracellular signal-regulated kinase (ERK) phosphorylation and subsequent abrogation of HRR (179). Preclinical work in ovarian cancer patient-derived xenograft models has shown that the PI3K inhibitor alpelisib (BYL719) inhibits HRR and consequently sensitizes ovarian cancer models with *de novo* or acquired HR-proficiency to olaparib (180). A Phase I study in 28 patients with EOC (75% HGSC) provided preliminary clinical evidence of the efficacy of the combination of olaparib and alpelisib. An ORR of 33% was seen in patients with *BRCA*-wildtype platinum-resistant EOC compared to an ORR of 3–10% with olaparib or other PARPi monotherapy in the same setting, and with acceptable toxicity (121, 181). Importantly, objective responses to this combination of agents occurred regardless of HR status, as measured by targeted DNA sequencing (181). Further evidence will be provided by the ongoing Phase III EPIK-O/ENGOT-OV61 trial (NCT04729387), which is evaluating the efficacy and safety of alpelisib/olaparib compared to single-agent cytotoxic chemotherapy in patients with platinum-resistant or refractory *BRCA*-wildtype HGSC (161).

The AKT serine/threonine protein kinases (*AKT1*, *AKT2*, *AKT3*) are key downstream mediators of PI3K signaling (182, 183) and in particular, *AKT2* has emerged as a poor prognostic marker and potential target in EOC (34, 70, 71). Drugs targeting AKT have shown activity in breast, endometrial, and ovarian cancer and are currently being investigated in Phase I/II/III trials (183, 184). An active Phase Ib/II trial (NCT02208375) is evaluating the combination of olaparib and the AKT inhibitor capivasertib (AZD5363) in a heavily pretreated cohort of 159 patients, with encouraging clinical activity regardless of the presence of a *BRCA* mutation and despite platinum resistance (183). Further studies are needed to explore the potential of AKT and PI3K inhibitors in combination with PARPi or as monotherapy in HRP HGSC and ovarian cancer in general.

ATR inhibitors

ATR has a major role in the CHK1 (checkpoint kinase 1) pathway of DNA repair and is a regulator of several proteins in the HRR pathway, including activation of BRCA1, PALB2 and RAD51 (108). The potential for mechanistic synergism between ATR inhibitors (ATRi) and PARPis has been demonstrated in HRD and HRP ovarian cancer cells in preclinical models (108, 185, 186). Acquisition of PARPi resistance was shown to be associated with increased ATR-CHK1 activity, further supporting the potential benefit of combining of PARPis with ATR inhibitors (185). Patient-derived xenograft models of *BRCA*-wildtype and *CCNE1*-amplified platinum-resistant ovarian cancer, which are associated with increased baseline activation of ATR/CHK1, demonstrated tumor reduction and a significant increase in OS when treated with the combination of PARPi and ATRi (185). Based on these preclinical data, an ongoing Phase II clinical trial of ceralasertib (AZD6738) in combination with olaparib was developed and initial results demonstrated the potential of ATRi to overcome PARPi resistance in an HRD HGSC patient population (187).

ATRis are also being investigated as potential monotherapy, and preliminary anti-tumor activity has been demonstrated in heavily pretreated tumors across a range of histologic types and gene alterations (188). Initial results from TRESR, a phase I trial of ATRi monotherapy with camonsertib, support preclinical findings that ATRi may be clinically active in other patient populations beyond those with loss of function of ataxia telangiectasia mutated (ATM) kinase, including those with other gene alterations (e.g., *ARID1A*, *CCNE1*, and *MYC*) or phenotypic (replication) markers (188, 189). The functional assessment of replication stress biomarkers is thought to be a better predictive biomarker for ATRi response than single aberrant genes in ovarian cancer (190). This statement can also be applied to the selective CHK1/2 inhibitor prexasertib, which showed an increased sensitivity to platinum and olaparib in mouse tumor transplantation models and monotherapy efficacy in *BRCA*-wildtype platinum-resistant ovarian cancer (191, 192). To date, however, there is limited data on the safety and anti-tumor activity of CHK inhibitors, and a phase II trial of prexasertib was recently terminated prematurely due to COVID-19 and a shortage of investigational drug supplies (193).

HDAC inhibitors

The altered expression of HDACs (histone deacetylases) has been associated with resistance to platinum-based chemotherapy and poor prognosis (194) and HDAC inhibition leads to impaired HRR in cancer cells through reduced expression of critical genes such as *BRCA1* and *RAD51* (195, 196). Konstantinopoulos et al. provided a preclinical rationale for the use of HDAC inhibitors (HDACi) to reduce HRR in HRP ovarian cancer, including *CCNE1*-amplified tumors, as a means to enhance PARPi activity (197). This approach

has been confirmed by further preclinical studies showing that HDACi such as suberoylanilide hydroamic acid (SAHA), romidepsin, panobinostat and entinostat are synergistic with PARPi in HRP ovarian cancer cells (197, 198). HDACi downregulate genes in the cyclin E/CDK and HR signaling pathways and thus show a synergistic cytotoxic effect in combination with a PARPi (198–200). Based on these preclinical results, there is an ongoing Phase I dose-escalation trial (NCT04703920) of the combination of the PARPi talazoparib and the HDACi belinostat in metastatic ovarian, breast and prostate cancer.

HSP90

Another attempt to extend the benefit of PARPis to HRP patients is their combination with the heat shock protein 90 (HSP90) inhibitors. HSP90 mediates the maturation, stability and activation of several key proteins involved in DNA repair and HRR, such as CDK1, BRCA1 and BRCA2 (201). Due to its abundant expression, its dependence on adenosine ATP (adenosine triphosphate), and its massive protein interactome, it is an ideal target for pharmacological inhibition (201). Inhibition of HSP90 by ganetespib (STA-9090), a second-generation HSP90 inhibitor, sensitized HRP HGSC cells to talazoparib (201). HSP90 inhibition resulted in downregulation of *BRCA1* and *RAD51*, HRR impairment and increased DNA damage (202). A recent Phase I dose-escalation study showed that the combination of the HSP90i onalespib and olaparib resulted in prolonged disease stabilization, without dose limiting toxicities, in a heavily pretreated patient population with advanced solid tumors (162). Due to limited efficacy as a monotherapy and in other combination studies, further development of onalespib was discontinued (162). However, preclinical and clinical data may support future evaluation of novel combinations of PARPis with other HSP90 inhibitors, such as pimitespib (203). While HSP90 inhibition has the potential to sensitize HRP HGSC to PARPi and other DNA-damaging agents, further clinical research is needed.

BET inhibitors

The BET (bromodomain and extraterminal) protein family includes BRD4, an epigenetic transcription modulator involved in the expression of proteins that regulate the cell cycle and DNA repair (204). BRD4 has been shown to be a necessary factor for the proliferation and survival of HGSC cells (205). In addition, *BRD4* amplification is mutually exclusive with *BRCA1* and *BRCA2* mutations and tends to co-occur with *CCNE1* amplification in HGSC, so BET inhibition may be particularly promising in the HRP group (38, 206–208). Preclinical studies have shown that BET inhibitors (BETis) suppress the expression of WEE1 and TOPBP1 (DNA Topoisomerase II Binding Protein 1) (209, 210). WEE1 and TOPBP1 play critical roles in cellular processes related to DNA damage response and cell cycle regulation. WEE1 is a protein kinase that regulates the G2/M checkpoint in the cell cycle, controlling entry into mitosis and allowing time for DNA repair (173, 176, 211, 212). TOPBP1 acts as a scaffold protein that coordinates the activation of ATR kinase in response to DNA damage, thereby initiating signaling cascades essential for DNA repair and cell cycle arrest (213). Dysfunction or dysregulation of these proteins can lead to genomic instability and contribute to the development of diseases such as cancer. Additionally, increased BRD4 expression has been identified as a factor contributing to PARPi resistance in HGSC (210). The specific BRD4 inhibitor INCB054329 was able to directly decrease the activity of both *BRCA1* and *RAD51* and induce an HRD phenotype (108, 209). Consequently, in combination with PARPis, a synergistic effect is observed with decreased HR activity, increased DNA damage, and consequently increased tumor cytotoxicity (108, 214). Unfortunately, initial clinical studies involving single agent use of BET inhibitors in various tumor types were disappointing, as preclinical results could not be replicated and resistance to therapy occurred rapidly in some cases (215). Specific evidence in ovarian cancer will be provided by an ongoing Phase II clinical trial (NCT05071937) of the BETi ZEN003694 in combination with the PARPi talazoparib in patients with recurrent ovarian cancer who have progressed on prior PARPi therapy.

Summary

The HRP HGSC subgroup exhibits complex molecular heterogeneity combined with an immune depleted microenvironment, and these are associated with therapy resistance and a poor prognosis. A subset of these cancers are driven by *CCNE1* amplification and PI3K/AKT alterations that contribute to cell cycle dysregulation and thus these pathways represent promising targets for novel therapeutic approaches. However, a significant subset of HRP HGSC lack *CCNE1* amplification, and the molecular drivers of these cancers are still being defined. Additional studies, including the use of cell lines and potentially the use of existing data from systematic knockdown and knockout genetic screens (216, 217) in the HRP non-*CCNE1* amplified subgroup may define critical dependencies.

A large proportion of HRP HGSC are relatively immune depleted, likely in part due to a reduced mutational burden associated with intact DNA repair. The development of novel immunotherapies to boost the anti-tumor immune response remains a key area of focus for HRP tumors, including personalized approaches to enhance T-cell infiltration with therapeutic vaccines or adoptive cell therapy. Several new combination treatments are under investigation, which aim to sensitize HRP cancers to existing therapies, such as platinum and PARPis, by targeting the HRR pathway and impairing the ability of cells to functionally repair DNA. Antibody drug conjugates also represent a promising class of therapies to increase the potency and specificity of highly potent cytotoxic agents, while reducing toxicity.

These new approaches offer the opportunity to expand the otherwise very limited treatment options for patients with HRP HGSC. Importantly, explicit identification and enrollment of patients with HGSC tumors known to have intact HRR in clinical trials is crucial for the development of effective therapies for this medically underserved group.

Author contributions

NS: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. DG: Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition. GA-Y: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology. DB: Writing – review & editing, Supervision, Resources, Project administration, Methodology. VH-S: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Conceptualization. TZ: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Conflict of interest

DB reports research support grants from AstraZeneca, Roche-Genentech and BeiGene paid to institution outside the submitted work; also personal consulting fees from Exo Therapeutics, that are outside the submitted work. GA-Y reports research support grants from AstraZeneca and Roche-Genentech paid to institution outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1387281/full#supplementary-material

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EDITED BY
Robb Hollis,
University of Edinburgh, United Kingdom

REVIEWED BY
Alessandro Inserra,
Bambino Gesù Children's Hospital (IRCCS), Italy
Dan Wang,
Peking Union Medical College Hospital (CAMS), China
Federica Perelli,
Azienda USL Toscana Centro, Italy

*CORRESPONDENCE
Tingting Li
aki-yo@163.com

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Growing teratoma syndrome of the ovary: a case report and literature review

Jiaying Tao[1], Zhixian Shi[2], Mulan Li[1] and Tingting Li[1]*

[1]Department of Obstetrics and Gynecology, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China,
[2]West China Second University Hospital, Sichuan University, Chengdu, China

Growing teratoma syndrome (GTS) is a rare condition that arises secondary to malignant germ cell tumors. It is characterized by an enlarging abdominal mass during or after chemotherapy, normal tumor markers, and histopathological indications of mature teratoma components. Awareness of GTS is limited, and it is often mistaken for disease progression or recurrence. This misdiagnosis can lead to delayed treatment and increased risk of complications. Therefore, early identification of GTS is crucial to avoid unnecessary systemic treatments and reduce financial burden. GTS is unresponsive to chemotherapy or radiotherapy and complete surgical resection is the sole therapeutic strategy. In this report, we present a case of GTS in a 20-year-old female following treatment for immature teratoma, alongside a review of the relevant literature aimed at enriching our insight into the clinical manifestations of GTS.

KEYWORDS

growing teratoma syndrome (GTS), ovarian immature teratoma, ovarian cancer, chemoradiotherapy resistance, early diagnosis

1 Introduction

Ovarian immature teratoma (IMT) is one of the most common histological subtypes of malignant ovarian germ cell tumors, comprising tissues from all three germ layers as well as immature neural elements, and accounting for approximately one-third of cases Smith et al. (1). IMT predominantly occurs in young women, and given its incidence at a relatively young age, the primary treatment approach focuses on preserving fertility. This is typically achieved through fertility-sparing unilateral salpingo-oophorectomy, followed by adjuvant chemotherapy with the BEP (Bleomycin, Etoposide, and Cisplatin) regimen.

Growing teratoma syndrome (GTS) represents an extremely rare metastatic complication arising from malignant germ cell tumors Amsalem et al. (2). The phenomenon was first delineated by DiSAIA et al. (3), who observed a 'chemotherapy-induced transformation' in three female patients with ovarian immature teratoma, where post-chemotherapy, immature tumor components evolved into mature elements. Subsequently, Logothetis et al. (4) reported six cases of testicular malignant germ cell

tumors that recurred as mature teratomas following successful chemotherapy and coined the term GTS to describe these occurrences. Specifically, GTS is defined by three specific criteria: 1) Continuously enlarging abdominal mass during or after chemotherapy; 2) Previously elevated serum tumor markers are now within normal limits; 3) Pathological examination of the resected tumor reveals only mature teratoma components Logothetis et al. (4). In 2004, Amsalem et al.'s research suggested that 'chemotherapy-induced transformation' and 'GTS' appear to be the same phenomenon Amsalem et al. (2). Here, we present a case of a 20-year-old female with ovarian immature teratoma who developed GTS following treatment. Additionally, we conducted a retrospective literature review to enhance our understanding of this unique syndrome.

2 Case presentation

In December 2022, a 20-year-old woman presented to a local hospital with symptoms of abdominal pain and distension lasting for 10 days. An ultrasound examination revealed a solid-cystic mixed echoic mass in the pelvic cavity, with indistinct borders and irregular morphology. Multiple small hypoechoic areas were observed within the mass, and abundant blood flow signals were detected. Additionally, a fluid-filled dark area was visible in both the abdominal and pelvic cavity. She was then admitted to Chengdu Women's and Children's Central Hospital. Computed Tomography (CT) scans revealed a huge solid-cystic mass occupying the lower abdomen and pelvic cavity where the mass contained scattered punctate calcifications and fat density shadows. Multiple nodular shadows were observed in the peritoneum and greater omentum (Figures 1A, B). Serum tumor markers were elevated: α-fetoprotein (AFP) = 833.1ng/ml, $CA125$ = 422.1U/ml, $CA19{-}9$ = 81.81U/ml, β-human chorionic gonadotropin (HCG) was negative.During the surgical exploration, a significant amount of yellow ascites was observed. An irregular mass was found attached to the left ovary, and multiple nodules were observed in the omentum. No obvious masses were observed on either ovary or on the peritoneal surfaces, and no enlarged retroperitoneal lymph nodes were palpable. The mass and a portion of the omentum were resected, and random peritoneal biopsies were taken from the pelvis, paracolic gutters, and undersurfaces of the diaphragm. The mass was completely resected without rupture, and there was no residual gross lesion after the operation. Cytological examination of the ascites did not detect malignant cells. Histopathological analysis of the mass and omentum revealed a stage IIIc high-grade immature teratoma (grade 3), with abundant immature intestinal epithelium present within the tumor, and no distinct yolk sac tumor components were identified (Figure 2A). The patient was then administered 3 cycles of BEP (bleomycin, etoposide, cisplatin, bleomycin 30 units IV per week plus etoposide 100 mg/m2 IV daily on days 1–5 plus cisplatin 20 mg/m2 IV daily on days 1–5, repeated every 21 days) as adjuvant chemotherapy. After 3 cycles of chemotherapy, the tumor marker (AFP) returned to normal, and an MRI showed no masses in the pelvic or abdominal cavity. She achieved a complete clinical response and was recommended for regular follow-up.

In May 2023, three months after the last cycle of chemotherapy, the patient was followed up at a local hospital where a positron emission tomography (PET)/CT scan revealed an abdominal mass measuring approximately 10 cm, with normal tumor markers. She was then admitted to West China Second University Hospital where she received two cycles of BEP regimen chemotherapy. Despite this, an abdominal MRI examination showed no significant reduction in the size of the mass (Figures 3A, B). Given the limited effectiveness of chemotherapy, the patient was subsequently administered 3 cycles of chemotherapy with 'paclitaxel, ifosfamide, and cisplatin'. However, the mass continued to grow during this chemotherapy (Figures 3C, D). Consequently, the patient was admitted to The West China University Hospital for surgery. Preoperative CT results showed multiple masses in the pelvic and abdominal cavities fused into a huge irregular continuous mass (Figure 1C). Intraoperatively, a huge cystic-solid mass measuring approximately 20x15x13 cm was found deep in the left upper abdomen, behind the left lobe of the liver and above the lesser curvature of the stomach,

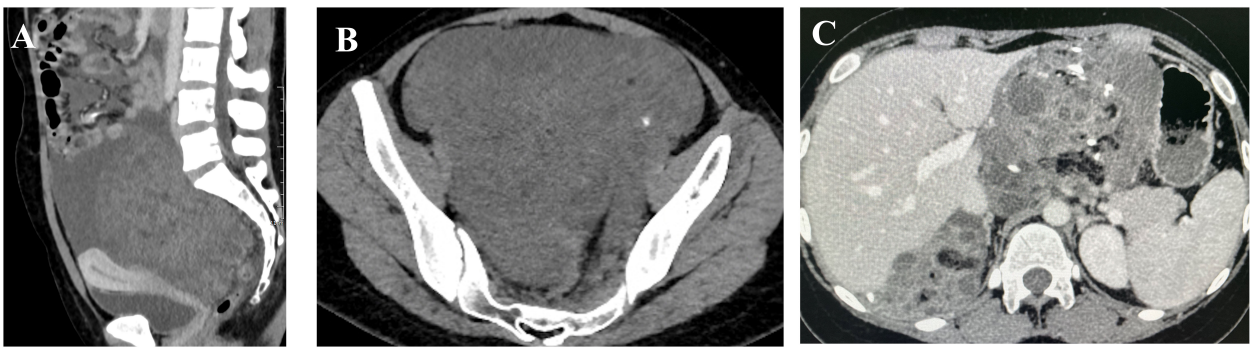

FIGURE 1
Images **(A, B)** (taken on 2022–12-14) reveal a large solid-cystic mass occupying the lower abdomen and pelvic cavity, which contained scattered punctate calcifications and shadows with fat density; image **(C)** (taken on 2023–11-27) demonstrates multiple masses in the pelvic and abdominal cavities fused into a huge irregular continuous mass.

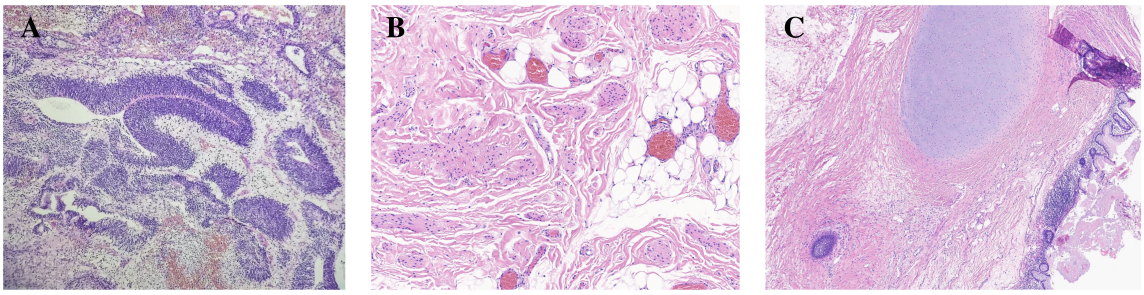

FIGURE 2
Representative histology of immature and mature teratoma. In image **(A)** (HE x100), the resected specimen of original ovarian immature teratoma showing primitive neural tube. Mature teratoma after chemotherapy in the same patient composed of various tissue components such as fat and cartilage (image **B**, HE x100), as well as neuroglia and intestinal epithelium (image **C**, HE x40).

along with multiple cystic-solid masses fused in the right liver posterior space and diaphragm, measuring approximately 6x5x7 cm. Multiple cystic-solid masses were also found in the pelvic peritoneum, as well as multiple nodules in the mesentery and greater omentum ranging in size from 0.2 cm to 2 cm. A fertility-preserving surgery was conducted, which involved the resection of the posterior peritoneal tumor located behind the left lobe of the liver, the diaphragm tumor, and the greater omentum, along with the excision of the pelvic tumor. Postoperatively, no macroscopic residual lesions were observed. Postoperative pathological examination revealed various differentiated tissues, including cartilage, neural tissue, glandular and squamous epithelium, sebaceous glands, along with keratinization, necrosis, and foam cell accumulation. No immature components were detected (Figures 2B, C). Based on the clinical progression of an enlarging abdominal mass post-chemotherapy, normalized serum tumor markers, and pathological evidence of mature teratoma, the patient was diagnosed with 'ovarian GTS'. Following NCCN guidelines Armstrong et al. (5), we outline the follow-up plan for this patient: 1) In the first year, perform physical exams and serum tumor marker tests every 2 months, with chest/abdominal/pelvic (C/A/P) CT scans every 3–4 months. 2) In the second year, continue with physical exams and serum tumor marker tests every 2 months, and C/A/P CT scans every 4–6 months. 3) During the third year, continue with physical exams and serum tumor marker tests every 4–6 months, and A/P CT scans every 6–12 months. 4) In the fourth and fifth years, continue with physical exams and serum tumor marker tests every 6 months, and A/P CT scans every 6–12 months. 5) After five years, continue with annual physical exams and serum tumor marker tests, and perform CT scans as needed based on clinical symptoms. Three months after surgery for GTS, this patient is alive with no evidence of disease.

3 Discussion

GTS manifests with an incidence of 1.9%–7.6% in male non-seminomatous germ cell tumors of the testis Lee et al. (6). In females, its prevalence is rarer and the exact incidence is somewhat nebulous, though some reports suggest a rate of approximately 12% Zagamé et al. (7). For patients with immature teratomas, the potential progression to GTS is estimated around ~20% Wang et al. (8). A review of 101 cases of ovarian GTS found that GTS mostly occurs in adolescents and young adults, with a median onset age of 22 years at the time of primary diagnosis of immature teratoma Li et al. (9).

The etiology of GTS remains elusive, with two prevailing theories cited in the literature to elucidate its pathogenesis. 1) Chemotherapy

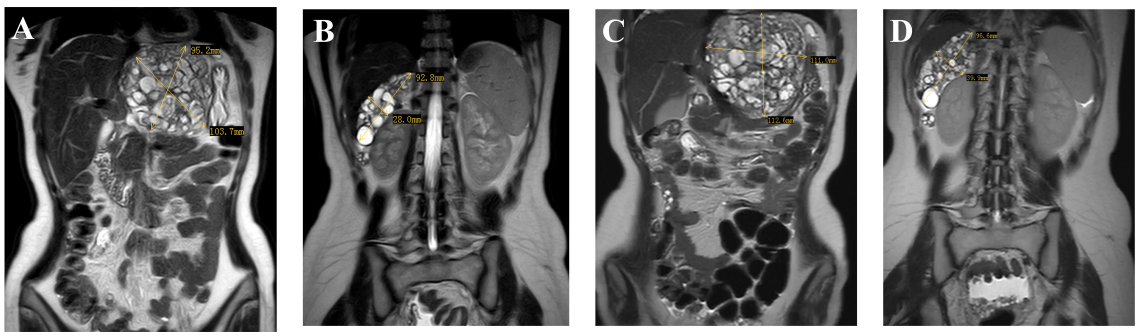

FIGURE 3
Images **(A, B)** (taken on 2023–07-19) indicate multiple masses in the pelvic and abdominal, with the largest located in the hepatogastric space; images **(C, D)** (taken on 2023–09-24) show an enlargement of the pelvic and abdominal masses.

eliminates the chemotherapy-sensitive immature components, while chemotherapy-insensitive mature components continue to grow, leading to GTS as the disease progresses André et al. (10). 2) Chemotherapy alters cellular dynamics, causing pluripotent malignant germ cells to transform into mature teratomas, acquiring a benign phenotype insensitive to chemotherapy, and proliferating autonomously DiSAIA et al. (3). Additionally, Hong et al. (11) have proposed a hypothesis suggesting that malignant cells may inherently or spontaneously differentiate into benign tissue, with this process potentially being extended by therapeutic intervention as part of the natural progression.

The risk factors that precipitate GTS remain unclear. In male patients, mature teratoma components within the primary tumor has been identified as a predictive factor for GTS André et al. (10). Research indicates that residual disease post-initial surgery and peritoneal gliomatosis are independent risk factors for the occurrence of GTS Wang et al. (8). Additionally, André et al. (10) suggested that the presence of mature teratoma components within the primary tumor, inadequate initial surgery, and metastatic disease unresponsive to chemotherapy contribute to an increased risk of GTS. Moreover, Tangjitgamol et al. (12) showed that tumor rupture during surgery might be associated with the occurrence of GTS.

The retroperitoneum is recognized as the most frequent locus for the occurrence of GTS. Additional sites of manifestation have been documented, encompassing the lungs, neck, supraclavicular region, inguinal lymph nodes, mediastinum, forearm, mesentery, and liver Zagamé et al. (7). Generally, most GTS nodules following ovarian germ cell tumors are localized to the pelvis, abdomen, and retroperitoneum, rather than exhibiting distant systemic spread Wang et al. (8) Djordjevic et al. (13). However, the exact mechanisms of disease dissemination in GTS are not fully understood. Research by Shibata et al. (14) highlighted a case of ovarian GTS that demonstrated three concurrent pathways of metastatic spread: direct extension, lymphatic dissemination, and hematogenous routes. This diversity in potential spread underscores the complex behavior of GTS and highlights the need for further investigation to better understand its pathophysiology.

Diagnosing GTS requires a collaborative approach involving the patient's medical history, treatment details, and coordination among gynecology, ultrasonography, radiology, and pathology departments. GTS is often misidentified as either disease progression or recurrence. If pelvic or abdominal growth of masses is found during or after the chemotherapy, the IOTA ADNEX model and tumor marker levels can help diagnosis to some extent. Elevated tumor markers generally signify a recurrence of IMT, whereas GTS tends to correspond with normal or slightly elevated tumor marker levels (only a few studies have noted slight elevation in tumor markers) Lorusso et al. (15). Ultrasound assessment by an expert, or the use of the IOTA ADNEX model in conjunction with the tumor marker profiles, can often indicate the specific subtype of malignancy Timmerman et al. (16). However, the clinical behaviors of recurrence in IMT (true recurrence and mature recurrence) are not fully understood. Surgery should be performed to evaluate the nature of the relapse, determining whether it is an IMT requiring further adjuvant chemotherapy or mature elements needing no further management Wang et al. (17).

The clinical manifestations of GTS are related to the location of tumor growth, which can lead to compression of surrounding organs and evoke an array of clinical symptoms such as pain, intestinal obstruction, renal failure due to ureteral compression, thrombophlebitis, and tissue necrosis. According to Li et al. (9), the median size of the primary tumor at diagnosis was reported to be 18.7 cm (range: 6–45 cm), median subsequent tumor size was 8.6 cm (range: 1–25 cm). The median tumor growth rate during the interval between primary treatment and the diagnosis of ovarian GTS was 0.94 cm/month (range: 0.3–4.3 cm/month). Furthermore, the median duration leading to the diagnosis of GTS post-treatment was 26.6 months (range: 1–264 months).

The imaging diagnosis of GTS predominantly relies on CT scans. The maturation characteristics discernible via CT include an increased density within the mass, well-delineated margins, and the emergence of internal calcifications, alongside the presence of fatty deposits and cystic alterations Moskovic et al. (18). PET/CT scans are regarded as furnishing more comprehensive diagnostic insights than CT alone, owing to their enhanced capability to differentiate between active disease and benign processes, thereby offering a more detailed evaluation of the syndrome Kikawa et al. (19).

GTS is known for its resistance to both chemotherapy and radiotherapy, rendering surgical intervention the sole method of treatment André et al. (10). According to the second pathogenic mechanism, if GTS occurs as a result of chemotherapy reversal, it seems logical that it retains a high level of histological type and malignant potential. Complete surgical resection achieving R0 status significantly improves prognosis, with a 5-year survival rate of 89%–90% Wang et al. (8). The risk of recurrence after complete resection is very low, ranging from 0%*to*4%. In contrast, the recurrence rates post-partial resection are notably higher, ranging from 72% to 83% Spiess et al. (20).

However, surgery is challenging and often involves partial organ resection and reconstruction procedures. Despite these challenges, complete resection is advocated even when vital organs are involved. Given that GTS tumors frequently involve multiple organs and multiple metastases, the final management should be determined within a multidisciplinary team or experienced centers, taking into account both the diagnostic findings and the overall patient profile Timmerman et al. (16) Bentivegna et al. (21)Pashankar et al. (22). In scenarios where surgery is unfeasible or complete resection cannot be accomplished, alternative treatments such as Pazopanib have been reported in the literature as therapeutic options for GTS Schultz et al. (23). The overall prognosis for patients with GTS is generally positive.

GTS has a risk of malignant transformation, where the teratoma may evolve into more aggressive forms of cancer such as sarcoma, squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma. The incidence rate of such malignant transformations is reported to be between 3% and 5.4% André et al. (10) Shigeta et al. (24). Considering this risk, it is crucial to maintain regular follow-up through imaging studies and serum tumor marker evaluations to promptly detect any signs of malignant change, ensuring timely intervention and management.

Ovarian GTS predominantly occurs in young women, spotlighting fertility preservation as a critical concern. For patients with stage II to IV immature teratomas, postoperative chemotherapy is recommended, though it may permanently impair reproductive functions. Cryopreservation of ovarian tissue is the primary option to preserve fertility. Fertility-sparing surgery should be considered for those who wish to retain their fertility Perelli et al. (25, 26). In an extensive review of 101 cases involving patients with ovarian GTS, Li et al. (9) reported that 5 patients conceived in the period between the primary diagnosis and the diagnosis of GTS, with 1 patient achieving pregnancy post-diagnosis of ovarian GTS. In the study by Bentivegna et al, among 38 patients with Ovarian GTS, 20 underwent fertility-sparing surgery, of which 4 out of 6 patients who planned to conceive became pregnant naturally, and 1 successfully conceived using assisted reproductive technology Bentivegna et al. (21). These findings underscore the feasibility and significance of fertility-sparing surgical approaches in managing Ovarian GTS, with both studies advocating for such interventions 'when possible'. However, the ambiguity surrounding the criteria to determine when fertility-sparing surgery is feasible highlights a gap in the existing literature. This lack of clarity calls for additional research to establish definitive guidelines that can aid in making informed decisions about preserving fertility in patients with Ovarian GTS.

4 Conclusion

GTS is a rare condition. When an abdominal mass enlarges during or after chemotherapy for immature teratoma, with normal serum tumor markers, GTS should be considered. A definitive diagnosis typically hinges on pathological examination after surgical resection. Complete surgical resection is crucial for a good prognosis and is essential in GTS management. Given the potential for malignancy, stringent and continuous follow-up is essential to monitor for any signs of progression or transformation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Chengdu Women and Children's Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JT: Writing – original draft, Conceptualization. ZS: Data curation, Writing – review & editing. ML: Investigation, Writing – review & editing. TL: Validation, Writing – review & editing.

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Conflict of interest

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Clinton Chichester,
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Giuseppe Vizzielli,
University of Udine, Italy
Dana Marie Roque,
University of Maryland, United States
Federica Perelli,
Azienda USL Toscana Centro, Italy

*CORRESPONDENCE
Thanh Hue Dellinger
tdellinger@coh.org

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Pressurized intraperitoneal aerosolized chemotherapy (PIPAC) experience in patients with recurrent low grade serous ovarian carcinoma (LGSOC): sub-cohort report of phase 1 clinical trial

Brad Nakamura[1], Rosemary Senguttuvan[1], Nora H. Ruel[2], Paul H. Frankel[2], Susan E. Yost[3], Sarah Cole[4], Sue Chang[5], Alexander Jung[6], Melissa Eng[1], Raechelle Tinsley[1], Daphne Stewart[3], Edward Wang[3], Joshua Cohen[1], Jeannine Villella[7], Richard L. Whelan[7], Amit Merchea[8], Danielle K. DePeralta[7], Mihaela Cristea[9], Mark T. Wakabayashi[9], Mustafa Raoof[1] and Thanh Hue Dellinger[1]*

[1]Department of Surgery, City of Hope National Medical Center, Duarte, CA, United States, [2]Department of Computation and Quantitative Medicine, City of Hope National Medical Center, Duarte, CA, United States, [3]Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, CA, United States, [4]Clinical Protocol Development, City of Hope National Medical Center, Duarte, CA, United States, [5]Department of Pathology, City of Hope National Medical Center, Duarte, CA, United States, [6]Department of Diagnostic Radiology, City of Hope National Medical Center, Duarte, CA, United States, [7]Department of Surgery, Northwell Health, New York, NY, United States, [8]Department of Surgery, Mayo Clinic Florida, Jacksonville, FL, United States, [9]Regeneron Pharmaceuticals, Tarrytown, NY, United States

Introduction: Low grade serous ovarian carcinoma (LGSOC) is a rare subtype of ovarian cancer (OC) that is challenging to treat due to its relative chemoresistance. Given that LGSOC patients often recur in the peritoneal cavity, novel intraperitoneal (IP) chemotherapy should be explored. Pressurized intraperitoneal aerosolized chemotherapy (PIPAC) is a method that has demonstrated peritoneal disease control in cancers with peritoneal metastases.

Methods: NCT04329494 is a US multicenter phase 1 trial evaluating the safety of PIPAC in recurrent ovarian, uterine, and GI cancers with peritoneal metastases. This analysis describes the outcomes of a sub-cohort of four LGSOC patients treated with IP cisplatin 10.5 mg/m^2, doxorubicin 2.1 mg/m^2 PIPAC q4-6 weeks. Primary endpoints included dose-limiting toxicities (DLT) and incidence of adverse events (AE). Secondary endpoints were progression free survival (PFS) and treatment response based on radiographic, intraoperative, and pathological findings.

Results: Four patients with LGSOC were enrolled of which three were heavily pretreated. Median prior lines of therapy was 5 (range 2-10). Three patients had extraperitoneal metastases, and two patients had baseline partial small bowel

obstructive (SBO) symptoms. Median age of patients was 58 (38-68). PIPAC completion rate (≥2 PIPACs) was 75%. No DLTs or Clavien-Dindo surgical complications occurred. No G4/G5 AEs were observed, and one G3 abdominal pain was reported. One patient had a partial response after 3 cycles of PIPAC and completed an additional 3 cycles with compassionate use amendment. Two patients came off study after 2 cycles due to extraperitoneal progressive disease. One patient came off study after 1 cycle due to toxicity. Median decrease in peritoneal carcinomatosis index between cycles 1 and 2 was 5.0%. Ascites decreased in 2 out of 3 patients who had ≥2 PIPACs. Median PFS was 4.3 months (1.7-21.6), median overall survival was 11.6 months (5.4-30.1), and objective response rate was 25%.

Conclusion: PIPAC with cisplatin/doxorubicin is well tolerated in LGSOC patients without baseline SBO symptoms. IP response was seen in 2 out of 3 patients that completed ≥2 PIPAC cycles. Further study of PIPAC for patients with recurrent disease limited to the IP cavity and with no partial SBO symptoms should be considered.

KEYWORDS

low-grade serous ovarian carcinoma, LGSOC, pressurized intraperitoneal aerosolized chemotherapy, PIPAC, recurrent

Introduction

Low-grade serous ovarian carcinoma (LGSOC) is a rare subtype of epithelial ovarian cancer (OC). It accounts for 2-5% of all epithelial OC and 4.7% of all serous OC (1). LGSOC is rarely associated with BRCA mutations or family histories of breast or OC (2). Compared to women diagnosed with common high-grade serous ovarian carcinoma (HGSOC), women with LGSOC often have a longer disease trajectory but experience fewer disease-free intervals. Thus, LGSOC patients often receive numerous treatment regimens in a continuous fashion, while women with HGSOC may experience several intervals of time in clinical remission allowing for time off treatment. Of women with advanced-stage LGSOC, 70% will experience a disease recurrence. When possible, obtaining a commercially available somatic mutation profile may be considered to identify the best treatment targets. Multiple options exist in this setting including secondary cytoreductive surgery, chemotherapy, endocrine/hormonal therapies, targeted agents, and clinical trials (3).

The peritoneum is one of the primary sites of metastasis and recurrence, often resulting in malignant gastro-intestinal and urinary obstruction, and reduced quality of life (QoL), and significant morbidity in LGSOC patients. These peritoneal metastases are frequently unresectable and refractory to systemic therapy due to pharmacokinetic limitations, poor peritoneal drug uptake, and impaired local drug distribution (4).

Treatment options targeting the peritoneum have not been extensively studied in this population, and innovative combinations that consider tumor biology and peritoneal metastases are urgently needed. Regional therapy offers a pharmacokinetic advantage with improved peritoneal to plasma drug ratios and has proven to be effective in epithelial OC (5). IP chemotherapy has demonstrated survival advantages for OC patients with both normothermic IP chemotherapy and hyperthermic IP chemotherapy (HIPEC). As LGSOC is a rare OC subtype, limited patients with this disease were enrolled in the GOG 172 IP chemotherapy trial (5) or in the OVHIPEC-1 trial (2). Nonetheless, both IP chemotherapy and HIPEC treatments are limited to newly diagnosed OC patients during first-line therapy, and not in the recurrent setting. The role of IP chemotherapy in recurrent epithelial OC has been limited due to the need for optimal cytoreduction for both IP chemotherapy and HIPEC.

Pressurized intraperitoneal aerosolized chemotherapy (PIPAC) is a novel treatment modality that intensifies chemotherapy delivery to peritoneal metastases to improve drug distribution and penetration of peritoneal tumors (6). It does so via aerosolization of chemotherapy into gas-like microdroplets through a micropump delivered via a high-pressure injector. This chemotherapy administration occurs during the creation of temporary intra-abdominal pressure using CO_2 gas administered during laparoscopic surgery (Figure 1A), at routine pressures of 12 mm Hg applied for a 30-minute duration. The increased intra-abdominal pressure helps to overcome the interstitial pressure within the tumor, which is one of the barriers exerted by the fluid within the tumor tissue that limits the penetration of conventional chemotherapy drugs.

In comparison to HIPEC, PIPAC does not require cytoreduction, can be frequently repeated, and is well tolerated. The clinical efficacy and safety of PIPAC in OC has been studied in multiple, international phase I and phase II trials over the past decade. The need for standardization of PIPAC protocols has been highlighted with the development of recommendations based on expert panel consensus and in person courses established by the International Society for the Study of Pleura and Peritoneum (7–9). Based on this expert panel consensus meeting in 2021, an optimal dose for the combination of cisplatin 10.5 mg/m^2 and doxorubicin 2.1 mg/m^2 was established based on safety and efficacy data from prior clinical trials including 2 phase I dose-escalation studies showing no difference in local or systemic toxicities between varying doses of cisplatin (7.5-30 mg/m^2) and doxorubicin (1.5-6 mg/m^2) (10, 11). In both phase I dose-escalation trials, the maximum tolerated doses were not reached. Of note, the Robella et al., 2021 study demonstrated a much higher tolerable dose, up to cisplatin 6 mg/m^2 with doxorubicin 30 mg/m^2, however this was administered as a single dose of PIPAC in this trial (11). Two recent retrospective studies, the systemic review by Taliento et al., 2023 and the multicenter cohort study by Kefleyesus et al., 2023 demonstrated the safety and encouraging efficacy results in a select population of ovarian cancer patients using the combination of PIPAC cisplatin 7.5 mg/m^2 with doxorubicin 1.5 mg/m^2 and cisplatin 10.5 mg/m^2 with doxorubicin 2.1 mg/m^2 (12, 13). Thus, more studies are needed to establish the optimal dose of this combination of drugs used in PIPAC.

Currently, there is an ongoing, open-label, randomized phase III trial, CTRI2018/08/021223 in India, comparing PIPAC versus IV chemotherapy in platinum-resistant recurrent OC patients (14). Preliminary data of this trial comparing 3 cycles of PIPAC cisplatin 15 mg/m^2 and doxorubicin 3 mg/m^2 versus 6 cycles of single agent IV chemotherapy has shown an objective response rate (ORR) of 66.6% versus 22.5% respectively with fewer grade 3-4 adverse events, 10.0% versus 35.7% respectively (15).

This study is the first PIPAC clinical trial in the U.S. and is being conducted as an open label U.S. multicenter phase I trial (NCT04329494). As LGSOC is a rare OC subtype, limited data exists on PIPAC in this population, and clinical trials have focused on OC of all subtypes. Here, we present preliminary data of a sub-cohort of LGSOC patients from arm 1 of this ongoing clinical trial.

Materials and methods

Ethics statement

This study was conducted according to the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects or Research and the Declaration of Helsinki. All patients completed written documentation of informed consent to participate. This consent included the use of data and images for publication. This study was approved by the City of Hope Institutional Review Board (IRB) (#19184), the Northwell Health IRB (#20-0859), and the Mayo Clinic IRB (#20-010121).

Patients

Adult patients ≥ 18 years old with histologically confirmed invasive LGSOC with peritoneal carcinomatosis who had progressed on at least one prior standard chemotherapeutic regimen were included if they had Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, no contraindications to laparoscopic surgery or aerosol therapy, intraoperative laparoscopic findings showing PIPAC access is feasible, no evidence of impending bowel obstruction, ≤ 5L of ascites, and patient is not a candidate for cytoreduction and HIPEC. Exclusion criteria included prior treatment with maximum cumulative doses of anthracyclines and/or anthracenediones. See Supplementary Table 1 for complete eligibility criteria.

Study design

This is an ongoing, phase I clinical trial without dose escalation to establish the safety of cisplatin 10.5 mg/m^2 PIPAC and doxorubicin 2.1 mg/m^2 PIPAC. The rules for accrual were slot-limited to not exceed the risk of the traditional 3 + 3 phase I trial design with modifications to adapt to the patient queue to reduce the time to complete the study (16, 17). If the proposed treatment had not been well-tolerated, the plan was to amend the study. Prior to instillation of PIPAC during each procedure, ascites was suctioned and measured, visual assessment of tumor burden was recorded via Peritoneal Carcinomatosis Index (PCI), and biopsies were obtained from all 4 quadrants if accessible to assess peritoneal regression grading score (PRGS) (18). Selection of biopsy sites in each quadrant was based on surgeon evaluation of largest and most suspicious appearing tumor lesion. The PIPAC procedure was performed with IP cisplatin 10.5 mg/m^2 in 150 mL NaCl 0.9% and doxorubicin 2.1 mg/m^2 in 50 mL NaCl 0.9% delivered using a high-pressure injection (Medrad Stellant injector, Bayer Corporation) and Capnopen nebulizer (Capnomed Corporation, Tubingen, Germany and REGER Medizintechnik GmbH, Villingendorf, Germany) at a maximum of 300 psi and 30mL/min, followed by a 30-min pneumoperitoneum at 12 mmHg containing the aerosolized chemotherapy at room temperature prior to release of the pneumoperitoneum. Laparoscopic balloon occlusion ports were used for staff safety. Standardized left lower quadrant port placement was used for PIPAC delivery unless it was not safely feasible. Limited adhesiolysis was allowed, however no other surgical interventions or resection of tumors were performed. PIPAC cisplatin and doxorubicin were given every 4-6 weeks for a total of three treatments provided that no severe AE, dose-limiting toxicity (DLT), disease progression, or patient withdrawal occurred. DLTs were defined as any delay greater than 21 days; any grade 3 or higher nonhematologic toxicity excluding grade 3 nausea, vomiting, abdominal pain, or diarrhea adequately treated that returns to grade 2 or less within 48 hours; grade 3 fatigue that returns to grade 2 or less within 7 days; grade 3 laboratory/metabolic abnormalities that are not considered clinically significant and are easily correctable to grade 2 or less

within 72 hours; grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management; and grade 3 peripheral neuropathy. Additional DLTs include Clavien-Dindo grade IIIB or higher surgical complications; grade 4 thrombocytopenia or neutropenia lasting more than 7 days or associated with fever or infection. Quality of life (QOL) measures were collected via patient surveys. Patients with clinical benefit were offered additional PIPAC cycles on compassionate care.

This paper describes the data analysis up to January 2024 of this ongoing clinical trial. The last LGSOC patient in this sub-cohort was enrolled in February 2023.

Endpoints

The primary endpoints were DLTs and incidence of treatment related AEs. AEs were assessed every 4-6 weeks using Common Terminology Criteria for Adverse Events (CTCAE v5.0) for up to 18 weeks. Follow-up after treatment completion (≥2 PIPACs) was every 12 weeks. Secondary endpoints included PFS and treatment response. Treatment response was based on changes in computed tomography (CT) imaging Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, intraoperative PCI and pathologic PRGS of multiple biopsies taken each cycle.

Statistical analysis

Simple mathematical ratios, medians, and ranges are reported. Measurements of association and statistical significance were not calculated given a limited sample size.

Micropump device

The micropump used for chemotherapy delivery is a Class III, Category A nebulizer device, and an investigational new drug (IND) combination product application by City of Hope (COH). The U.S. Food and Drug Administration (FDA) approved the study (IND/IDE 147749) in 2020. In this study, high-pressure micro-injection pump (MIP) is interchangeable with nebulizer.

Results

Patient characteristics

Nine recurrent epithelial OC patients were enrolled, of which four had LGSOC (Figure 1B). The median age of LGSOC patients was 58 years (range 38-68) (Table 1). Three (75%) patients had good performance status with ECOG score 1, and one patient had ECOG score 2. LGSOC patients were heavily pretreated, with median prior lines of therapy of 5 (range 2-10). At baseline, three (75%) patients had extraperitoneal metastases, and two (50%) patients had baseline partial small bowel obstructive (SBO) symptoms. The median baseline PCI was 20 and the median PRGS was 2.75. The volume of ascites at time of first PIPAC cycle for each patient was 10 cc, 50 cc, 1500 cc, and 3000 cc. Supplementary Table 2 displays de-identified individual patient data.

Feasibility of PIPAC

There were no technical failures in completing the laparoscopy or administering the PIPAC. Three (75%) patients completed two

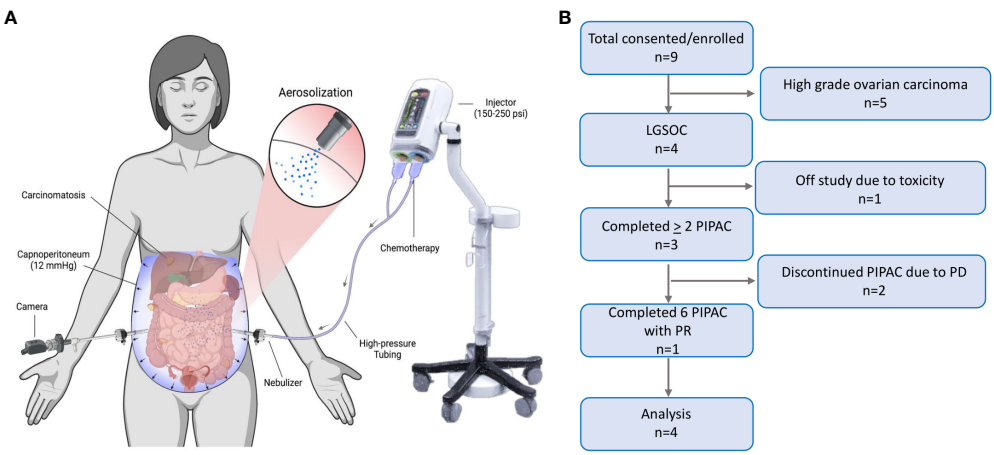

FIGURE 1
Pressurized Intraperitoneal Aerosolized Chemotherapy. **(A)** PIPAC is a laparoscopic chemotherapy delivery method for superior drug delivery to peritoneal metastases. It improves drug distribution through aerosolization of chemotherapy in the abdominal cavity, via a nebulizer. It improves drug tissue absorption through pressurization of the drug via a 12 mmHg capnoperitoneum induced by a high-pressure injector (BioRender.com). **(B)** Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the progression of patients through the trial, including consent, enrollment, treatment completed, follow-up, and analysis. LGSOC, low grade serous ovarian carcinoma; PIPAC, pressurized intraperitoneal aerosolized chemotherapy; PD, progressive disease; PR, partial response.

TABLE 1 LGSOC patient characteristics, response, and survival.

Characteristic	N=4
Age, years[1]	58 (38-68)
Race/Ethnicity	
Non-Hispanic White	4 (100%)
Hispanic White	0 (0%)
ECOG	
1	3 (75%)
2	1 (25%)
Prior lines of therapy[1]	5 (2-10)
Baseline metastatic sites	
IP only	1 (25%)
Extraperitoneal and IP	3 (75%)
Patients with ≥2 PIPAC cycles	3 (75%)
Baseline PCI[1]	20 (20-33)
Baseline PRGS[1]	2.75 (1.75-3.50)
Baseline ascites volume	
Large volume (≥500cc)	2 (50%)
Small volume (<500cc)	2 (50%)
Not present	0 (0%)
Best response per RECIST	
PR	1 (25%)
SD	1 (25%)
PD	1 (25%)
Unknown[2]	1 (25%)
Percent change in PCI from cycle 1 to 2 for patients receiving ≥2 cycles[1]	-5% (-30% - +15%)
PFS, months[1]	4.3 (1.7-21.6)
OS, months[1]	11.6 (5.4-30.1)
Off treatment reason	
Progression	2 (50%)
Toxicity	1 (25%)
Treatment complete	1 (25%)
Progression type	
IP only	1 (25%)
Extraperitoneal and IP	2 (50%)
Unknown	1 (25%)

[1]Median (range); [2]No follow-up imaging after 1 cycle of PIPAC; ECOG, Eastern Cooperative Oncology Group; IP, intraperitoneal; PIPAC, pressurized intraperitoneal aerosolized chemotherapy; PCI, peritoneal carcinomatosis index; PRGS, peritoneal regression grading system; RECIST, Response Evaluation Criteria in Solid Tumors; PFS, progression free survival; OS, overall survival

or more cycles of PIPAC, including 1 (25%) patient that completed six cycles, of which the last 3 cycles were given as compassionate use. Median follow-up was 11.5 months (range 5.4-30.1). One (25%) patient had a prolonged recovery time after the first PIPAC cycle leading to study withdrawal. Two (50%) patients had disease progression following the second PIPAC treatment.

Safety of PIPAC

There were no Clavien-Dindo surgical complications or DLT. There were ten grade 2 or higher toxicities (one grade 3, nine grade 2) recorded for this cohort of 4 patients, attributable to the treatment (possible/probably/definite). The most common toxicity was abdominal pain (Supplementary Table 3). Following PIPAC cycle 1, one patient had grade 2 toxicity and one patient had grade 3 toxicity. The grade 3 abdominal pain toxicity was associated with "Patient 1" who discontinued treatment due to prolonged recovery after her first cycle of PIPAC; her discontinuation of treatment was noted as toxicity. Of note, she had chronic partial SBO symptoms. No grade 4/5 AEs occurred. There were no port-site complications. There was no difference in QOL measures between patients over time. Daily step counts available from 3 patients followed similar patterns with a decrease immediately after surgery and gradually increasing over time until next cycle of PIPAC (Supplementary Figure 1).

Efficacy of PIPAC

Response to PIPAC treatment was assessed in three ways: CT imaging by RECIST, intraoperative PCI, and pathologic PRGS. Following the first PIPAC cycle, two (50%) patients had a decrease in PCI (Figure 2). After two PIPAC cycles by RECIST, "Patient 4" (25%) had a partial response (PR) (Figures 2A, B) and "Patient 3" (25%) had progressive disease (PD) based on progression of extraperitoneal and liver parenchymal lesions, but partial response was seen in the peritoneum based on PCI (Figures 2C, D).

Response

ORR was 25% based on measurable intraperitoneal disease at trial entry. Figure 3A shows swimmer plot reporting the best response of each patient to treatment measured by CT imaging using RECIST. The change in laparoscopic PCI over each PIPAC cycle by best response via RECIST is shown in Figure 3B, with the blue line representing "Patient 3" with PD, green line representing "Patient 4" with PR, and purple line representing "Patient 2" with stable disease (SD). The change in histologic response by mean PRGS over each PIPAC cycle using best response via RECIST is reported in Figure 3C. "Patient 4" (25%) shown in Figures 3D–F had a decrease in PRGS following three cycles of PIPAC. "Patient 2 and 3" (50%) came off study after two cycles due to

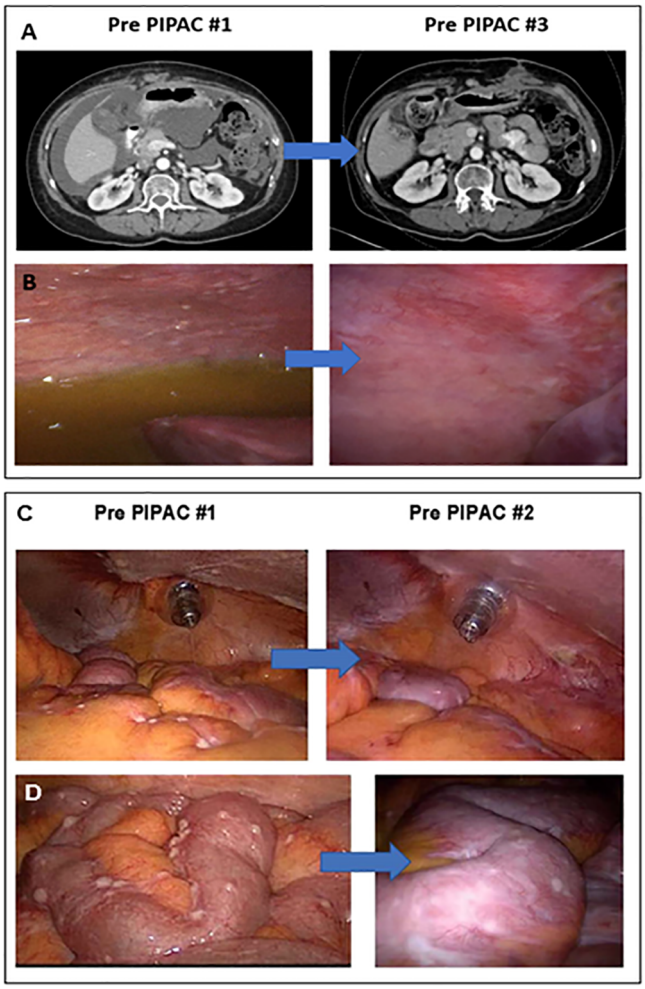

FIGURE 2
PIPAC treatment in two LGSOC patients. **(A)** "Patient 4" CT scan imaging after two cycles demonstrated a subtotal resolution of ascites with a moderate decrease in peritoneal carcinomatosis; **(B)** "Patient 4" had significant flattening of peritoneal and diaphragmatic nodules, as well as a total resolution of ascites, seen on the laparoscopic assessment performed during PIPAC cycle #3; **(C)** "Patient 3" showed a decrease in the number of nodules evident in the bowel mesentery on the laparoscopic evaluation performed before PIPAC cycle #2 compared to PIPAC cycle #1; **(D)** "Patient 3" showed a post-treatment flattening effect was noted in bowel surface nodules on the laparoscopic evaluation conducted before PIPAC cycle #2 compared to PIPAC cycle #1.

PD; "Patient 3" had increase in RECIST and "Patient 2" with best response SD by RECIST but had clinical signs of PD with increasing, symptomatic ascites. In "Patients 2, 3, and 4" who completed at least 2 PIPAC cycles, there was a 5% median decrease in PCI between cycle 1 and 2. Among these three patients, ascites decreased in "Patients 3 and 4" (67%).

Survival

The median PFS was 4.3 months (range 1.7-21.6). "Patients 2 and 3" who came off study after cycle 2 at 3.2 months and 1.7 months respectively had areas of IP and extraperitoneal disease progression. Of note, both patients had baseline IP and extraperitoneal metastatic disease. "Patient 3" had received 10 prior lines of therapy and had pre-existing thoracic and liver parenchymal metastases. She had significant peritoneal regression (Figures 2C, D) as observed by PCI reduction of 20 to 14. However, due to PD of her extraperitoneal and liver parenchymal metastases, she was taken off trial and restarted on IV chemotherapy. "Patient 2" progressed at 3.2 months had received 6 prior lines of therapy and had pre-existing thoracic, breast, and flank metastases. She was noted to have overall SD by RECIST (mixed imaging response in IP region and minimal increase in extraperitoneal metastases), but developed recurrent, worsening ascites, requiring paracentesis treatment, and elected to withdraw from the trial to restart IV chemotherapy. "Patient 1" withdrew from the study for toxicity after cycle 1 had received only 2 prior lines of therapy including letrozole and trametinib, however she was intolerant of this MEK inhibitor. She also had baseline poor ECOG performance status (ECOG=2), chronic partial SBO symptoms, and IP and extraperitoneal metastatic disease. Following withdrawal from the trial, she transferred her care to another provider out of state and no further CT imaging data was available to assess disease status. She

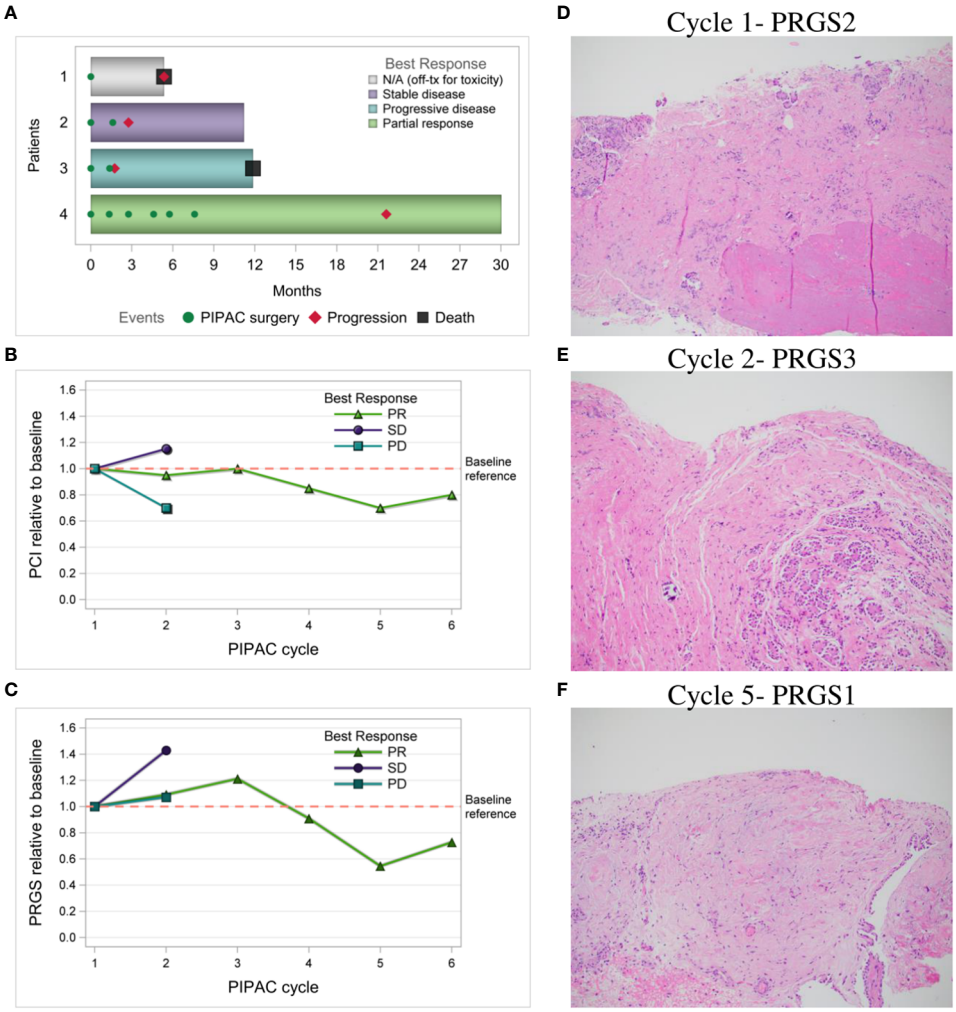

FIGURE 3
Response to PIPAC treatments. **(A)** Swimmer plot of each patient and best response to treatment measured by CT imaging using RECIST; **(B)** Laparoscopic PCI relative to baseline over PIPAC cycles by best response via RECIST; **(C)** Histologic response relative to baseline by mean PRGS over PIPAC cycles by best response via RECIST; **(D–F)** "Patient 4" PRGS in the left upper quadrant over multiple PIPAC cycles, H&E stained FFPE slides, resolution 10x; **(D)** PIPAC cycle #1 PRGS2 shows infarct-like necrosis (bottom of photo) and dense fibrosis with occasional calcifications (middle), and a small number of nests of viable carcinoma near the surface (top of photo); **(E)** PIPAC cycle #2 PRGS3 about half viable carcinoma (right side of photo) and half treatment-associated dense fibrosis (left side); and **(F)** PIPAC cycle #5 PRGS1 shows only fibrosis with some hemorrhagic areas, and no viable tumor nests or single tumor cells. RECIST, Response Evaluation Criteria in Solid Tumors; PCI, peritoneal carcinomatosis index; PRGS, peritoneal regression grading score; PD, progressive disease; SD, stable disease, PR, partial response; H&E, hematoxylin and eosin; FFPE, formalin-fixed paraffin-embedded.

died at 5.4 months after starting treatment and this was noted as her date of disease progression for statistical purposes. "Patient 4" had only baseline IP disease without partial SBO symptoms and was noted to have partial response after 3 cycles with reduction in RECIST, resolution of large volume ascites, and normalization of CA 125 (367 to 32), with stable PCI 20. Given her excellent response to therapy, a compassionate use extension was applied, and she received an additional 3 cycles of PIPAC treatment, for a total of 6 cycles of PIPAC. She had further reduction in disease evidenced by reduction in mean PRGS (3.33 to 2.00), PCI (20 to 16), and RECIST over her last 3 cycles. Her DFI was 21.6 months, including 14.0 months following completion of PIPAC treatment.

The median OS was 11.6 months (range 5.4-30.1). "Patients 2 and 4" remain alive and their follow-up time to date is 11.2 and 30.1 months, respectively. Overall survival for "Patient 1" and "Patient 3" were 5.4 and 11.9 months from initiation of PIPAC, respectively.

Discussion

Treatment options for LGSOC patients are limited, and clinical trials including patients with LGSOC histology are uncommon. Our trial evaluated the role of PIPAC, a novel intraperitoneal chemotherapy method, for regional recurrent disease in LGSOC patients who are not candidates for cytoreductive surgery. This is one of the strengths of this study as few have focused primarily on LGSOC.

Based on safety data from this phase I trial, PIPAC with cisplatin 10.5 mg/m^2 and doxorubicin 2.1 mg/m^2 appears to be

safe and well tolerated in LGSOC patients without baseline partial SBO symptoms. In our study, no G4/G5 AEs were observed. Overall, our rate of severe AEs (grade 3 or higher) was 25% with one patient having G3 abdominal pain. While we excluded patients with small bowel obstruction, we allowed entry of two patients with partial small bowel obstruction (SBO) who were on limited liquid diet or had chronic nausea and emesis. Unfortunately, these two patients did not tolerate more than 1 or 2 cycles of PIPAC, suggesting a limited role of PIPAC for those patients with partial SBO. Thus, for patients with malignant SBO symptoms, PIPAC may not be well tolerated, likely due to bulkier intraabdominal disease, causing obstruction and poor treatment effect.

Although no other current PIPAC trials have focused on recurrent LGSOC, our observed PFS of 4.3 months and OS of 11.6 months were similar in comparison to the outcomes seen in the PIPAC-OV1 trial, a Phase II trial of platinum resistant recurrent OC patients treated with PIPAC cisplatin/doxorubicin, in which PFS of 4.7 months, and OS of 10.9 months were reported. This trial similarly included a heavily pre-treated population with median lines of therapy 3 (range 2-8) (19). One key difference in patient characteristics was that their trial excluded patients with extraperitoneal disease except for pleural effusion, while our trial included patients with extraperitoneal disease, including lung and liver metastases.

While ORR was measured with RECIST criteria, other measures of peritoneal response were evaluated in our trial, including PCI. Our study demonstrated a decrease in PCI in 66.7% of evaluable patients, which is similar to the decrease seen in the PIPAC-OV1 study, where 76% of patients demonstrated a decreased PCI (19).

In most PIPAC studies to date, histologic regression has been evaluated with a peritoneal regression score called PRGS (18). This grading system was explored in the two Phase II PIPAC OC studies published to date, PIPAC-OV1 and PARROT. However, in PIPAC-OV1, the histologic grading system was based on a neoadjuvant chemotherapy response score rather than PRGS used in in the PARROT and other PIPAC trials (20). The regression rate of 33% in our trial was similar to the PRGS histologic regression of 29.6% in PARROT (21). This contrasts with a histologic regression score of 62% in PIPAC-OV1, where PRGS was not used (19). While PRGS has been used as an endpoint in PIPAC trials, its utility as a primary endpoint has not been universally accepted. Potential bias in PRGS may be introduced by the subjective biopsy selection of surgeons intraoperatively. Additionally, the gross differentiation of normal versus tumor tissue in fibrotic peritoneum can be challenging, contributing to the variability of histologic regression as a reliable measure and universally accepted primary endpoint.

Per the National Comprehensive Cancer Network guidelines and expert consensus report, treatment options for patients with recurrent LGSOC who are not candidates for cytoreductive surgery determined either by imaging or laparoscopic evaluation, include MEK inhibitors, combination MEK and BRAF inhibitors, hormonal therapy, and systemic chemotherapy based on platinum status (3, 22, 23). As the response rate of LGSOC to cytotoxic chemotherapy is <5% in the recurrent setting (24), more effective therapies for these patients are urgently needed. Despite recent advances with MEK inhibitors shown in GOG 281 (ORR 26% trametinib vs 6% standard of care chemotherapy) and MILO/ENGOT-ov11 (16% binimetinib vs 13% physician's choice chemotherapy), poor tolerance of these drugs limits their role in most LGSOC patients (22, 25). In the NCI-MATCH Trial Subprotocol H looking at BRAF V600E mutated tumors, which included 5 LGSOC patients, the combination of the BRAF inhibitor, dabrafenib with trametinib demonstrated an ORR of 37.9% (26). Anti-estrogen therapy is another alternative to chemotherapy treatment with aromatase inhibitors, tamoxifen, and leuprolide acetate having shown some benefit with ORR 9-14% in the recurrent setting (3). Preliminary data from GOG 3026 combining letrozole with the CDK4/6 inhibitor ribociclib has shown an ORR of 24% (27). However, most of these patients will eventually progress on hormonal therapy. Given the preponderance of peritoneal metastatic disease, regional intraperitoneal therapy may represent a promising novel treatment for LGSOC patients. Our study was limited by a small sample size of four patients, given the rare nature of the disease. Nonetheless, in a heavily pretreated group, a significant intraperitoneal response was demonstrated in two out of three patients who completed PIPAC. Thus, for patients with recurrent disease limited to the IP cavity, and no partial SBO symptoms, further study of PIPAC use in this patient population should be explored. Furthermore, multimodal therapy with systemic chemotherapy in combination with PIPAC could be explored in the future, especially for recurrent LGSOC patients with extraperitoneal and parenchymal tumors. Thus, consideration should be given to future trials which include a combination approach of PIPAC with systemic therapy to improve peritoneal and systemic response in this population of recurrent LGSOC patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by City of Hope IRB (#19184) Northwell health IRB (#20-0859) Mayo Clinic IRB (#20-010121). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

BN: Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. RS: Data curation, Writing – review & editing. NR: Formal analysis, Visualization, Writing – review & editing. PF: Formal analysis, Writing – review & editing. SY: Writing – original draft, Writing – review & editing. SCo: Writing – review & editing. SCh: Methodology, Visualization, Writing – review & editing. AJ: Methodology, Writing – review & editing. ME:

Writing – review & editing. RT: Writing – review & editing. DS: Methodology, Writing – review & editing. EW: Methodology, Writing – review & editing. JC: Writing – review & editing. JV: Methodology, Writing – review & editing. RW: Methodology, Writing – review & editing. AM: Methodology, Writing – review & editing. DD: Methodology, Writing – review & editing. MC: Methodology, Writing – review & editing. MW: Methodology, Writing – review & editing. MR: Conceptualization, Methodology, Project administration, Writing – review & editing. TD: Conceptualization, Methodology, Project administration, Writing – review & editing.

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Conflict of interest

MC and MW are employed by Regeneron and have Regeneron stock options. JC served on advisory boards for AstraZeneca and Immunogen.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1404936/full#supplementary-material

SUPPLEMENTARY TABLE 1
Eligibility Criteria.

SUPPLEMENTARY TABLE 2
Patient Data.

SUPPLEMENTARY TABLE 3
Adverse Events.

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EDITED BY
Mihaela Carmen Cristea,
Regeneron Pharmaceuticals, Inc.,
United States

REVIEWED BY
Giuseppe Marino,
University of Milano-Bicocca, Italy
Stergios Boussios,
Canterbury Christ Church University,
United Kingdom
Sarah Taylor,
University of Edinburgh, United Kingdom
Angelo Finelli,
ULSS2 Marca Trevigiana, Italy

*CORRESPONDENCE
Mark S. Carey
mark.carey@ubc.ca

†These authors have contributed equally to this work and share senior authorship

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Ovarian carcinosarcomas: p53 status defines two distinct patterns of oncogenesis and outcomes

Gurdial Dhillon[1], Marta Llaurado-Fernandez[1], Basile Tessier-Cloutier[2], Keiyan Sy[3], Dina Bassiouny[4], Guangming Han[5], Nelson K. Y. Wong[6], Kathryn McRae[1], Mary Kinloch[7], Jennifer Pors[8], Laura Hopkins[9], Allan Covens[10], Martin Köbel[11], Cheng-Han Lee[12†] and Mark S. Carey[1,13*†]

[1]Department of Obstetrics & Gynaecology, University of British Columbia, Vancouver, BC, Canada, [2]Department of Pathology, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC, Canada, [3]Department of Pathology, University of Toronto, Toronto, ON, Canada, [4]Department of Pathology, Sunnybrook Health Science Centre, Toronto, ON, Canada, [5]Department of Pathology and Laboratory Medicine, Surrey Memorial Hospital, Surrey, BC, Canada, [6]Department of Experimental Therapeutics, BC Cancer, Vancouver, BC, Canada, [7]Department of Pathology and Laboratory Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [8]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [9]Division of Gynaecologic Oncology, Saskatoon Cancer Centre, Saskatoon, SK, Canada, [10]Division of Gynaecologic Oncology, Sunnybrook Health Science Centre, University of Toronto, Toronto, ON, Canada, [11]Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada, [12]Department of Pathology and Laboratory Medicine, University of University of Alberta, Edmonton, AB, Canada, [13]Department of Clinical Research, BC Cancer, Vancouver, BC, Canada

Objectives: Ovarian carcinosarcoma (OCS) is a rare and lethal type of ovarian cancer. Despite its incredibly poor prognosis, it has received little research attention. In this study, we aim to evaluate the molecular features of OCS and elucidate their clinical significance.

Study methods: We examined 30 OCS by immunohistochemistry (IHC) and targeted panel sequencing collected from a single institution (2003–2013) as the initial molecularly characterized cohort (Cohort A). From November 2016 to April 2023, we collected an additional 67 OCS cases from three institutions across British Columbia and Alberta as the contemporary cohort (Cohort B) for clinical correlation. The Kaplan–Meier method was used to estimate overall and progression-free survival, and differences in survival rates were compared using the log-rank test. All tests were two-sided. A *p*-value of less than 0.05 was considered statistically significant.

Results: The majority of OCS (82%) in the initial Cohort A were p53-mutated, and the carcinomatous component displayed the histological and molecular features of a high-grade tubo-ovarian serous carcinoma (HGSC-like). In a minority of OCS, the epithelial components were characteristics of endometrioid or clear cell carcinomas, and IHC staining was wild type for p53. In the contemporary Cohort B, we observed the same histological findings related to the p53 IHC staining pattern. The median overall survival of the p53-mutated HGSC-like OCS (47 patients) was significantly higher (43.5 months) compared with that of the p53 wild-type OCS (10 patients, 8.8 months; $P < 0.01$). Pathogenic *BRCA1/2* germline/

somatic mutations were observed in 7 patients (17.5%) of HGSC-like OCS, and all these patients were alive at 3 years from diagnosis compared to a 51% 3-year survival among the patients with *BRCA1/2* wild-type HGSC-like OCS (33 patients) (p = 0.022). Majority of patients (6/7) with *BRCA1/2*-mutated OCS received poly (ADP-ribose) polymerase inhibitor as maintenance therapy in this cohort.

Conclusions: Most OCSs have a morphologic and molecular profile resembling HGSC; however, some OCSs display a molecular profile that suggests origin through non-serous oncogenic pathways. This molecular distinction has both prognostic and treatment (predictive) implications. These findings underscore the importance of routine p53 IHC testing on all OCS and *BRCA1/2* testing on p53-mutated OCS.

KEYWORDS

ovarian cancer, ovarian carcinosarcoma, MMMT, immunohistochemistry, p53 IHC, *BRCA*, PARPi

Introduction

Ovarian carcinosarcoma (OCS) is a rare ovarian malignancy comprising only 1%–4% of all ovarian cancers (1–4). For some time, it was thought that OCS was a distinct sarcoma type within ovarian malignancies, unrelated to the more common epithelial ovarian cancers. We now appreciate that gynecologic carcinosarcomas (CSs) represent an epithelial metaplastic carcinoma with sarcomatous transdifferentiation. This was originally demonstrated using ultrastructural and immunohistochemical studies on these cancers; however, subsequent genomic analyses have revealed that the carcinomatous and sarcomatous components are clonally related and their mutational profiles more closely resemble the usual types of epithelial carcinomas arising from the ovary (5–12). Applying The Cancer Genome Atlas (TCGA) endometrial cancer molecular classification, Gotoh et al. recently examined 109 gynecologic CSs that included 17 OCSs and found that the majority (88%) exhibited a copy number–high molecular profile that was enriched by the presence of *TP53* mutation, whereas the rest exhibited a copy number–low molecular profile (13). None of the OCSs examined were *POLE* ultramutated or microsatellite unstable. These findings suggest underlying heterogeneity in the oncogenesis of OCS. More recent studies in OCS have also shown that approximately 80% were *TP53*-mutated and displayed WT-1 expression, which is characteristic for high-grade tubo-ovarian serous carcinomas (HGSCs) (4, 9, 13, 14). Some also occur in the presence of serous tubal intraepithelial carcinoma lesions or recur as CS after initially presenting as HGSC (15–17). Despite these molecular similarities, OCSs often have a more aggressive clinical course, with a significantly worse 5-year survival compared to HGSC (2–4, 18). This survival difference may be related to adverse prognostic factors such as advanced stage, suboptimal surgical cytoreduction, presence of heterologous sarcomatoid features on histopathology, increased expression of vascular endothelial growth factor, as well as differences in treatment response as OCSs typically respond poorly to platinum and taxane-based chemotherapy (19–22). Furthermore, poly (ADP-ribose) polymerase inhibitors (PARPis) are now routinely used for treating homologous recombination–deficient (HRD) HGSC (23, 24). This may also contribute to the discrepant outcomes between HGSC and OCS recognizing that PARPi may not be used to treat OCS. We know that, within HGSC, *BRCA1/2* mutation status remains a significant prognostic biomarker for overall survival (OS) (25). There are few reports characterizing *BRCA1/2* mutation status in OCS although a recent study demonstrated pathogenic *BRCA1/2* mutations in 5 of the 49 (10.2%) patients (26). Moreover, aside from a few case reports, PARPi response data and patient outcomes information in *BRCA1/2*-mutated OCS are lacking.

The goal of our study was to examine a series of OCS and evaluate the prognostic and therapeutic significance of p53 immunohistochemistry (IHC) and *BRCA1/2* status in OCS.

Methods

Cohort A: initial cohort for molecular characterization

Study samples

We examined an initial cohort of 30 OCS cases collected from 2003 to 2013 at Sunnybrook Hospital (Toronto, Canada). Each case was reviewed to confirm the diagnosis by an expert gynecologic pathologist. A tissue microarray was constructed with duplicate 1-mm tissue cores from the carcinomatous and sarcomatous components, respectively, for each of the 30 cases. Ethical approval for the study was obtained from the institutional research board.

Immunohistochemistry

IHC was performed on the tissue microarray. The primary antibodies used were as follows: Paired Box 8 (PAX 8) (clone BC12/ACI 438, 1:100, Biocare Medical Concord, California, USA), Wilms Tumor 1 (WT1) (clone 6F-H2, ready-to-use, Dako, Burlington, Ontario, Canada), Estrogen Receptor (ER) (clone SP1, RM-9101, 1:25, Thermo Fisher Scientific, Ottawa, Ontario, Canada), Tumor protein P53 (p53) (clone DO-7, 1:800, M7001, Dako, Burlington, Ontario, Canada), DNA mismatch repair protein Mlh1 (MLH 1) (clone ES05, 1:100, Dako, Burlington, Ontario, Canada), DNA mismatch repair protein Msh2 (MSH2) (clone 25D12, prediluted, NCL), MSH6 (clone 44/MSH6, 1:2000, BD Biosciences), and DNA mismatch repair endonuclease postmeiotic segregation increased 2 (PMS2) (clone A16-4, 1:100, BD Biosciences). The unstained slides were processed using the Ventana Discovery XT and the Ventana Benchmark XT automated system (Ventana Medical Systems, Tucson, Arizona, USA) as per the manufacturer's protocol with proprietary reagents. Heat-induced antigen retrieval method was used in the Cell Conditioning Solution (CC1-Tris–based EDTA buffer, pH 8.0, Ventana). The Ventana Universal Secondary Antibody was used for 32 min at 37°C. The detection system used was the Ventana DABMap kit and the Ventana OptiView DAB kit.

For PAX8, ER, WT1, MLH1, PMS2, MSH2, and MSH6, only nuclear staining was considered and evaluated; the carcinomatous and sarcomatous components were evaluated separately. PAX8 and ER immunostains were scored as positive if greater than 10% of the cells exhibited moderate to strong positive (definite) nuclear staining. p53 expression was interpreted in both the carcinomatous and sarcomatous components using established published criteria (27). Staining was considered to be mutation-type/aberrant/abnormal if the tumor showed: (i) diffuse moderate to strong uniform nuclear staining in ≥80% of the tumor cells (p53 overexpression mutation pattern); (ii) diffuse complete absence of nuclear staining in the tumor cells in the presence of focal nuclear staining of the stromal cells as an internal positive control (p53 absent expression mutation pattern); or (iii) diffuse cytoplasmic staining (p53 cytoplasmic mutation pattern). p53 expression was classified as wild type in cases with nuclear staining involving <80% of the tumor cells, displaying variable intensity.

DNA extraction and targeted sequencing

For each case, paraffin scrolls (3 μm × 20 μm) from a tumor-rich tumor block (greater than 50% tumor content) containing both the carcinomatous and sarcomatous components were obtained. DNA was extracted from the paraffin scrolls using the Qiagen formalin-fixed paraffin-embedded tissue DNA extraction kit based on the manufacturer's protocols. We performed sequencing analysis to detect mutations in 26 genes that have been previously found to be recurrently mutated in carcinomas of the gynecologic tract as described previously (28). These included the full coding regions of *AKT1*, *ARID1A*, *FBXW7*, *FGFR2*, *JAK1*, *KRAS*, *MLH1*, *MSH2*, *MSH6*, *NRAS*, *PIK3CA*, *PIK3R1*, *PIK3R2*, *PMS2*, *POLE*, *PPP2R1A*, *PTEN*, *RNF43*, *RPL22*, *SMARCA4*, *STK11*, *SPOP*, and *TP53* in selected exon in *CTNNB1* (exon 3). The Illumina custom TruSeq amplicon panel was designed using Illumina's DesignStudio and included 1,173 amplicons (175 bp) that covers 98% of the exons and untranslated regions of these 26 genes. Custom amplicon libraries were prepared starting with 250 ng of DNA as per the Ilumina's Custom TruSeq Library Preparation protocol. Before pooling, normalization was performed by quantifying individual libraries using the Qubit fluorometer and then pooled on the basis of equal concentrations. Library pools were then quantitated for amplifiable libraries using the Kapa Biosystems FAST qPCR SYBR quantification kit on the basis of the manufacturer's protocols. Pooled TruSeq libraries were sequenced using the Illumina MiSeq using 300 cycle V2 kits. Analysis was performed using the MiSeq Reporter and somatic variant caller 3.2.3.0. Only non-synonymous mutations passing quality filter with at least 10% variant allele frequency were further evaluated. These mutations were manually checked in bam files using Integrated Genome Viewer.

Cohort B: contemporary cohort for clinical correlation

We then collected contemporary OCS cases from three institutions [BC Cancer Agency (Vancouver, BC, Canada), University of Alberta Cancer Center (Edmonton, AB, Canada), and University of Calgary (Calgary, AB, Canada)] from November 2016 to April 2023. This population-based contemporary cohort was assembled to address questions related to tumor type, p53 status, *BRCA1/2* mutation status, and clinical outcome in the PARPi era. Each case was reviewed by a subspecialty pathologist in gynecologic pathology who verified the presence of the carcinomatous and sarcomatous components. *BRCA1/2* mutation status (if performed as part of the routine clinical care), treatment, and clinical outcome data were collected. The study was approved by institutional research boards. Participant consent was waived because of the minimal risk and the retrospective nature of the study. OS was calculated as the time from the date of pathologically confirmed diagnosis till death or date of last known follow-up. Progression-free survival (PFS) was reported as the time from date of diagnosis to the time of progression, recurrence, or death. Majority of patients (6/7) with *BRCA1/2*-mutated OCS received PARPi as part of their therapy.

Statistical analysis

Demographics and baseline characteristics were summarized using descriptive statistics (N, median, and range) for continuous variables and N (%) for discrete variables. The Student's t-test was used to compare means between two groups. The Kaplan–Meier method was used to estimate the OS, and the stratified log-rank test was used to assess survival differences. All tests were two-sided. A *p*-value of less than 0.05 was considered statistically significant.

Results

Molecular analysis of study Cohort A demonstrates heterogeneity in OCS

The results of the molecular analysis (DNA sequencing panel and IHC panel) are summarized in Table 1, and additional IHC results

TABLE 1 Summary of immunohistochemistry and targeted sequencing results of 30 ovarian carcinosarcomas (OCS) cases in Cohort A.

Case	TP53 mutation	P53 IHC (CA)	P53 IHC (SA)	Other mutations	MMR	PAX8 (CA)	PAX8 (SA)	WT1 (CA)	WT1 (SA)
1	R141H	Mutated (OE)	Mutated (OE)	*PIK3CA* (*Y644C*)	Normal	Pos	Neg	Pos	Neg
2	R43H	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Pos	Neg
3	f.s.	Mutated (AE)	Mutated (AE)	*FBXW7* (*RS9Q*), *PIK3R2* (*R101H*)	Normal	Neg	Neg	Pos	Neg
4	f.s.	Mutated (AE)	Mutated (AE)		Normal	Pos	Neg	Pos	Neg
5	G134R	Mutated (OE)	Mutated (OE)	*KRAS* (*G12D*), *PIK3CA* (*ES45K*), *FBXW7*	Normal	Pos	Neg	Neg	Neg
6	No SNV/indel	Wild-type	Wild-type	*RPL22* (*f.s.*), *ARID1A* (*f.s.*), *PIK3CA* (*R524K*), *MSH6* (*f.s.*), *POLE* (*Q1625X*)	Normal	N/A	Neg	Neg	Neg
7	R81X	Mutated (AE)	Mutated (AE)	*BRCA1* (*D401V*)	Normal	Pos	Neg	Pos	Neg
8	C124X	Mutated (AE)	Mutated (AE)		Normal	Pos	Neg	Pos	Neg
9	H61R	Mutated (OE)	Mutated (OE)	*FGFR2* (*N615I*)	Normal	N/A	Neg	Neg	Neg
10	No SNV/indel	Wild-type	Wild-type	*AKT* (*E17K*), *PIK3CA* (*RS24K*), *CTNNB1* (*537C*)	Normal	Pos	Neg	Neg	Neg
11	R1 75H	Mutated (OE)	Mutated (OE)	*PIK3R2* (*L127F*)	Normal	Pos	Neg	Pos	Neg
12	f.s.	Mutated (AE)	Mutated (AE)	*POLE* (*f.s.*)	Normal	Pos	Neg	Pos	Neg
13	R1 17T	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Pos	Neg
14	f.s. R210X	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Pos	Neg
15	R81X	Mutated (AE)	Mutated (AE)	*MSH6* (*N742S*)	Normal	Pos	Neg	Pos	Neg
16	I63T	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Pos	Neg
17	No SNV/indel	Mutated (AE)	Mutated (AE)		Normal	Pos	Neg	Pos	Neg
18	I63T	Mutated (OE)	Mutated (OE)		Normal	Neg	Neg	Pos	Neg
19	R4 3H	Mutated (OE)	Mutated (OE)		Normal	Neg	Neg	Neg	Neg
20	G113D	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Neg	Neg
21	f.s.	Mutated (AE)	Mutated (AE)		Normal	Pos	Neg	Pos	Neg
22	C44Y	Mutated (OE)	Mutated (OE)	*PIK3CA* (*H1047R*), *MSH2* (*Q374H*)	Normal	Pos	Neg	Pos	Neg
23	R1 75H	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Neg	Neg
24	f.s.	Mutated (AE)	Mutated (AE)		Normal	Neg	Neg	Pos	Neg

(Continued)

TABLE 1 Continued

Case	TP53 mutation	P53 IHC (CA)	P53 IHC (SA)	Other mutations	MMR	PAX8 (CA)	PAX8 (SA)	WT1 (CA)	WT1 (SA)
25	No SNV/indel	Wild-type	Wild-type	*KRAS* (*G12A*), *MSH2* (*L279V*)	Normal	Pos	Neg	Neg	Neg
26	V142G	Mutated (OE)	Mutated (OE)	*SPOP* (*D291G*)	Normal	Pos	Neg	Pos	Neg
27	C1 43Y	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Pos	Neg
28	No SNV/indel	Wild-type	Wild-type	*PIK3CA* (*E545G*)	Normal	Pos	Neg	Neg	Neg
29	No SNV/indel	Mutated (AE)	Mutated (AE)	*POLE* (*R47W*)	Normal	Pos	Neg	Pos	Neg
30	V142F	Mutated (OE)	Mutated (OE)		Normal	Pos	Neg	Neg	Neg

CA, carcinoma component; SA, sarcoma component; MMR, mismatch repair protein status by immunohistochemistry; OE, overexpression of p53 (mutation pattern); AE, absent expression of p53 (mutation pattern); f.s., frameshift mutation; SNV, single-nucleotide variation; Indel, small insertion or deletion.

and clinical information are shown in Supplementary Table 1. Of the 30 OCSs studied, 26 (86.7%) demonstrated genetic and immunohistochemical (IHC) evidence of a *TP53* mutation. There were 24 tumors that harbored *TP53* mutations, 15 tumors that harbored missense mutations, five tumors that harbored frameshift mutations, three tumors with non-sense mutations, and one tumor that had both a non-sense and a frameshift mutation. By p53 IHC, 26 tumors exhibited mutation staining patterns, with 16 tumors showing overexpression mutation pattern and 10 tumors showing absent expression mutation pattern. Of note, all eight tumors harboring either a missense or a frameshift *TP53* mutation exhibited absent expression mutation-pattern p53 staining, which suggests that there was likely concurrent loss of heterozygosity in *TP53*. The single tumor that harbored both a frameshift and a nonsense (R210X) *TP53* mutations exhibited diffuse expression p53 mutation pattern. There were two OCSs without demonstrable single-nucleotide variation or small insertion/deletion (indel) by targeted sequencing and both exhibited absent expression mutation pattern by p53 IHC. In all cases with mutation-pattern p53 staining, the carcinomatous and sarcomatous components showed concordant p53 staining result and pattern. All 26 OCSs that demonstrated genetic and/or immunohistochemical evidence of TP53 mutation were DNA mismatch repair (MMR)-intact, with 20 tumors (77%) exhibiting WT1 nuclear expression and 17 tumors (65%) exhibiting ER expression in the carcinomatous component by IHC. Four of the 30 (13%) OCSs lacked evidence of *TP53* mutation by genetic and IHC analyses, and three of the four tumors harbored mutations involving *KRAS* (one G12A and one G12D), *RPL22* (one frameshift), *ARID1A* (one frameshift), and/or *CTNNB1* (one with S37C) that are often seen in non-HGSC ovarian carcinomas. These four tumors also lacked WT1 expression and were MMR-intact; two of the four tumors were ER-positive. In terms of PIK3CA pathway alterations, five tumors harbored exon 9 or 20 hotspot activating *PIK3CA* mutations (including three of the four TP53 wild-type CSs). None of the OCS examined harbored pathogenic *POLE* exonuclease domain mutations, although one tumor was found to have a non-sense mutation (Q1625X) outside of exonuclease domain. None of the tumors showed human epidermal growth factor receptor 2 (HER2) overexpression by IHC and the sarcomatous component in all 30 CSs consistently lacked nuclear expression of PAX8, ER, and WT1, including cases where the corresponding carcinomatous component showed expression for these proteins. All tumors showed intact expression of ARID1A except for the one tumor with wild-type *TP53*. This cancer had a frameshift *ARID1A* mutation. Overall, the findings from Cohort A confirms the molecular heterogeneity of OCS, with the majority showing a HGSC-like p53-mutated profile in the carcinomatous component and a minority (cases 6, 10, 25, and 28) showing a p53 wild-type non-HGSC profile in the carcinomatous component.

High-grade serous-like OCSs in Cohort B harbor high rates of mutations in high-penetrance homologous recombination–deficient genes, including *BRCA1/2*

The clinical and molecular features of study Cohort B (67 patients) are summarized in Table 2. P53 IHC was performed as part of the pathology diagnostic work-up in 57 of the 67 patients (85%) (Figure 1). The great majority (82.5%, 47 of 57) were p53-mutated with a carcinomatous component that displayed histologic features of HGSC. Ten cases showed wild-type p53 expression, and the carcinomatous component in nine of these 10 cases displayed endometroid-type histologic features, with one showing mismatch repair–deficient immunostaining pattern from a patient with known Lynch syndrome. Seven of the 10 wild-type p53 cases had *BRCA1/2* germline/or somatic testing, and none showed and pathogenic *BRCA1/2* mutations. These findings are in keeping with the observation made in Cohort A that the majority of OCSs belong to a HGSC-like group (p53-mutated) with a minority in the non–HGSC-like group characterized by wild-type p53.

We then further examined the 47 HGSC-like OCSs to see if they had tumor or germline *BRCA1/2* testing performed. Among the 40 cases with BReast CAncer gene 1 and 2 (BRCA 1/2) testing, seven (17.5%) harbored pathogenic *BRCA1/2* mutation (three cases

TABLE 2 Baseline patient characteristics of Cohort B.

OCS cases		Total	BRCA wild type	BRCA mutated	p53 mutated	p53 wild type
Number of cases		67	45	7	47	10
Age (years)						
	Median Range	67 43–88	65 43–88	69 56–81	68* 43–88	52* 44–76
Stage						
	I II III IV	8 (12) 13 (19) 34 (51) 12 (18)	3 (6) 9 (20) 26 (58) 7 (16)	1 (14) 1 (14) 4 (57) 1 (14)	4 (9) 7 (15) 26 (55) 10 (21)	2 (20) 4 (40) 3 (30) 1 (10)
Neoadjuvant treatment						
	Yes No	15 (22) 52 (78)	14 (31) 31 (69)	0 (0) 7 (100)	13 (28) 34 (72)	1 (10) 9 (90)
First-line treatment						
	Platinum-based chemotherapy PARPi maintenance No systematic therapy	60 (90) 16 (24) 7 (10)	43 (95) 10 (25) 2 (5)	7 (100) 6 (86) 0 (0)	45 (96) 14 (30) 2 (4)	9 (90) 1 (10) 1 (10)
Residual disease						
	Microscopic or less than 1 cm Greater than 1 cm No surgery	41 (61) 25 (37) 1 (2)	27 (60) 18 (40) 0 (0)	6 (86) 1 (14) 0 (0)	29 (62) 18 (38) 0 (0)	5 (50) 4 (40) 1 (10)
Disease status at last follow-up						
	No evidence of disease Alive with disease Died of disease or other cause	16 (24) 9 (13) 42 (63)	9 (20) 5 (11) 31 (69)	2 (29) 4 (57) 1 (14)	12 (26) 9 (19) 26 (55)	1 (10) 0 (0) 9 (90)

OCS, ovarian carcinosarcoma; PARPi, poly (ADP-ribose) polymerase inhibitor.
*There is a statistically significant difference in mean age at diagnosis between the p53 mutated and the p53 wild-type OCS.

germline). Additionally, within the remaining HGSC-like OCS, two patients with wild-type germline *BRCA1/2* carried germline moderate penetrance pathogenic mutation in other HRD genes: one with *RAD51C* c.404G>C mutation and the other with *BRIP1* c.1018C>T mutation. All patients harboring pathogenic germline HRD gene mutation had been referred to hereditary medicine for further counseling. For the 10 non–HGSC-like OCS (wild-type p53), seven had tumor and/or germline *BRCA1/2* testing with no pathogenic mutations involving *BRCA1/2* or other HRD genes such as *PALB2*, *RAD51D/C*, or *BRIP1* identified.

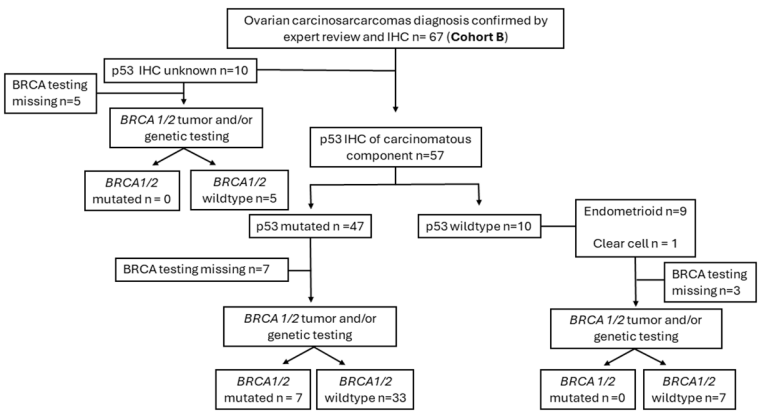

FIGURE 1
Diagrammatic overview of the ovarian carcinosarcoma in contemporary Cohort B. IHC, immunohistochemistry; BRCA, *BRCA1* and *BRCA2*.

P53 status and *BRCA1/2* mutation status confer prognostic significance

We subsequently evaluated the clinical outcome of the contemporary Cohort B in relation to tumor molecular groups (p53-mutated HGSC-like or p53 wild-type non–HGSC-like). Patients with p53 wild-type (non–HGSC-like) OCS had significantly shorter median OS (8.8 months) compared with patients with p53-mutated HGSC-like OCS (43.5 months) ($P < 0.01$) (Figure 2). There was also a statistical difference in PFS between p53 wild-type (non–HGSC-like) and p53-mutated HGSC-like OCS ($P < 0.001$) (Supplementary Figure 1). There were no apparent confounding clinical features that accounted for the observed difference in survival between the p53 mutant versus the p53 wild-type OCS. Patients were younger at diagnosis in the p53 wild-type group ($p = 0.02$); however, there were no significant differences in stage, use of neoadjuvant chemotherapy, or residual disease between the two groups (Table 2).

In the p53-mutated HGSC-like OCS cases, all patients with *BRCA1/2* mutation were alive at 3 years compared to 51% of patients with wild-type *BRCA1/2* status ($p = 0.022$) (Figure 3). Once again, there were no apparent differences in clinical factors (age, stage, use of neoadjuvant chemotherapy, and residual disease), between the BRCA1/2-mutated and BRCA1/2 wild-type groups that would account for the observed difference in survival (Table 2). As expected, PFS was longer in the BRCA-mutant cases compared to wild-type p53; however, this difference did not reach statistical significance ($p = 0.12$) (Supplementary Figure 2).

Discussion

OCS is an uncommon but highly aggressive histotype of ovarian carcinoma and is believed to arise through sarcomatous transformation (epithelial mesenchymal transition) of the epithelium. Its uncommon nature has limited our understanding of this cancer. The primary treatment strategy for OCS remains a combination of primary cytoreductive surgery and platinum-based chemotherapy, with emerging potential seen with immunotherapy and targeted therapies (29). The utilization of comprehensive molecular testing could improve outcomes by facilitating tailored treatments for particular patient cohorts. Here, we molecularly characterized a series of OCS and confirm the presence of molecular heterogeneity within OCS. We have shown that the majority of OCSs examined have mutation and immunophenotypic features that resemble high-grade serous carcinomas of tubo-ovarian origin (HGSC-like OCS). This is in keeping with the notion of OCS representing a type of metaplastic carcinoma and suggests that many have evolved through a HGSC oncogenic pathway. Conversely, a small subset of OCS exhibits a mutation and immunophenotypic profile that are not compatible with an origin from HGSC (non–HGSC-like OCS). The profiles in these cases more closely resemble ovarian endometrioid or clear cell-type carcinoma, and all are p53 wild type. This suggests that that a minor subset of OCS can arise through endometrioid/clear cell carcinoma oncogenic pathways as previously suggested (30–35). Our findings challenge the notion that all OCSs are variant of HGSC but perhaps represent a distinct metaplastic subtype that likely evolved through serous type or non-serous type oncogenic pathways.

When looking at the clinical outcomes of Cohort B, we found that separating OCS into HGSC-like and non–HGSC-like groups based on TP53 status have clinical implications with regard to survival. Here, we observed that HGSC-like OCS (p53-mutated) and non–HGSC-like OCS (p53 wild type) have different survival outcomes. Although histologic subtyping of the carcinomatous component alone has not been associated with differential survival outcomes in the past, it is worth noting that the use of

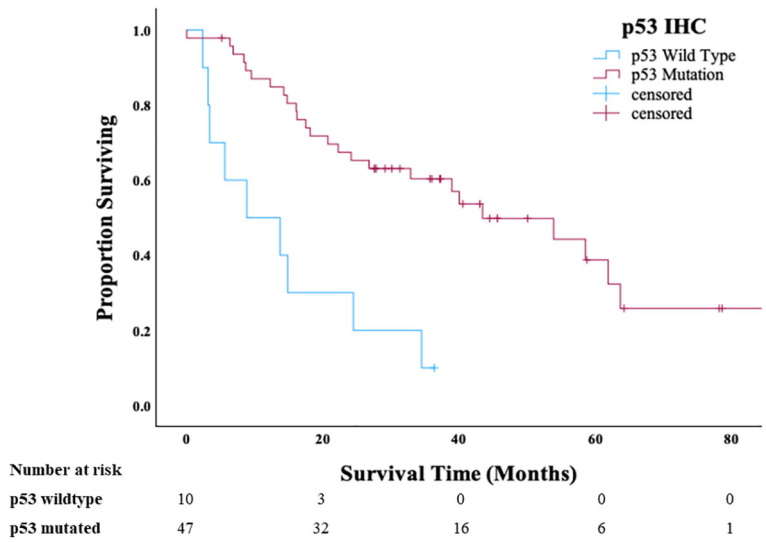

FIGURE 2
Overall survival of patients with ovarian carcinosarcoma in Cohort B stratified by p53 status.

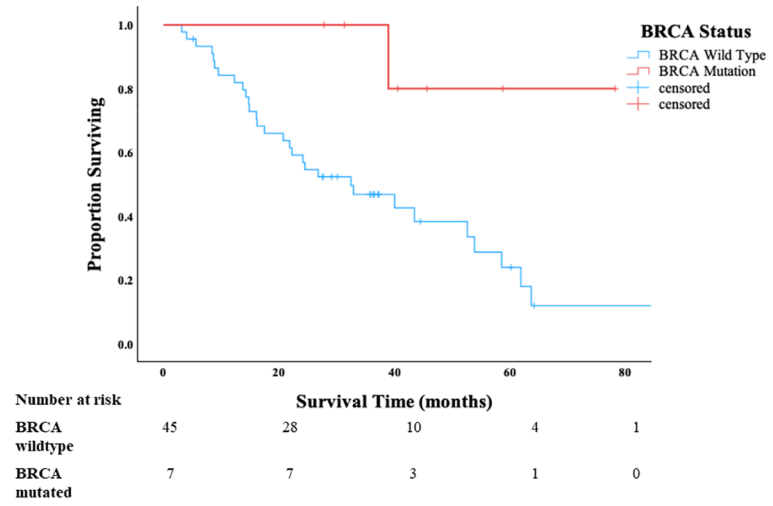

FIGURE 3
Overall survival of patients with p53-mutated HGSC-like ovarian carcinosarcoma in Cohort B stratified by *BRCA1/2* status.

TP53 IHC provides a more objective and accurate method of subtyping OCS into a HGSC-like and non–HGSC-like groups. Furthermore, the difference in survival observed in this contemporary cohort may also be partially attributed to access to PARPi that may have increased survival in this group. PARPis have changed the treatment paradigm for ovarian cancer patients and have remarkable efficacy, particularly in HRD ovarian carcinomas. Based on our results, we advocate for the routine use of TP53 IHC analysis to subtype OCS into HGSC-like and non–HGSC-like groups. Furthermore, all HGSC-like OCSs should be sent for *BRCA1/2* testing to identify patients eligible for PARPi therapy.

A contemporary review of endometrial CS recently suggested that p53 wild-type CS may, in fact, represent misclassified endometrioid carcinomas with reactive stroma or spindle cell growth, and they found that all endometrial CS in their study were p53 abnormal (36). Hence, it is possible that our p53 normal OCS were misclassified ovarian endometrioid or clear cell carcinomas with desmoplastic stroma or spindle cell growth. While there is no objective gold standard, all our cases underwent expert pathology review. Furthermore, the shorter survival of p53 wild-type OCS compared to p53 mutant OCS argues against misclassification because patients with ovarian endometrioid carcinomas have a longer survival compared to HGSC (3). Nevertheless, we support the recommendation that all p53 wild-type gynecologic CSs warrant pathology review to exclude mimics (36).

Another important finding in this study relates to the poor prognosis of patients with p53 wild-type OCS. In both Cohorts A and B, the adenocarcinoma component of these OCSs was usually endometrioid/clear cell histology. These OCSs frequently contain mutations in *KRAS* or *PIK3CA*, resulting in upregulation of their respective pathways. Upregulated phosphatidylinositol 3-kinase (PI3K) pathway can play an important role in chemoresistance and preservation of genomic stability (37). Alternate therapies for these patients represent an urgent unmet need, and novel agents targeting *KRAS* or *PIK3CA* mutations should be evaluated (38).

In the contemporary Cohort B, it should be noted that there was only one MMR-deficient OCS in a patient with a known Lynch Syndrome. Although uncommon, MMR deficiency in OCS may represent another opportunity for tumor-agnostic therapy, as there have been two landmark studies showing a remarkable survival benefit using checkpoint inhibition in MMR-deficient endometrial cancer (39, 40). Therefore, another consideration is to perform MMR IHC or microsatellite instability testing in non–HGSC-like p53 wild-type OCS.

POLE exonuclease domain mutations were not identified in the current molecular cohort (Cohort A) of OCS. This is not unexpected as the great majority of OCS appears to arise through HGSC-like pathway in our molecular cohort and pathogenic *POLE* mutations are never seen in serous tubo-ovarian carcinoma. Evidence of *POLE* exonuclease domain mutations in p53 wild-type OCS does not exist outside of the case reports of sarcomatous transformation of *POLE*-mutated endometrioid endometrial carcinomas (41). Because these cases are associated with ultra-mutated profiles and indolent behavior, designating them as CS does not reflect their true biology because *POLE*-mutated endometrioid carcinomas often show areas of low-grade atypia inconsistent with the definition of a CS (42, 43).

Strengths and limitations

The main strength of our study includes expert pathology review of our OCS cases along with detailed clinical annotation and outcomes data for a contemporary cohort of patients. Our study is limited by a relatively small sample size (for both the molecular analysis Cohort A and contemporary Cohort B) limiting the ability to perform multivariable analyses. Thus, our findings require further validation in other contemporary cohorts. The

evolving management of OCS, particularly with the advent of PARPi was addressed through the analysis of a contemporary cohort, as the initial molecular cohort analysis predated the clinical use of PARPi.

Conclusions

Our results show that, based on histological and molecular profiles, OCS can be divided into p53-mutated (HGSC-like) and p53 wild-type (non–HGSC-like) molecular subtypes. Because this molecular distinction suggests different oncogenic pathways and differences in survival and response to therapy, we recommend routine p53 IHC in all OCSs. All p53-mutated cases should be referred for somatic and germline *BRCA1/2* testing due to high percentage (approximately 20%) of these cases harboring pathogenic *BRCA1/2* mutations. P53 wild-type OCSs should be confirmed by gynecological pathology subspecialty review and then undergo MMR IHC and *POLE* genetic testing, if feasible.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Human Ethics Boards (H18-00280) at University of British Columbia, University of Edmonton, University of Calgary and University of Toronto. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

GD: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. ML: Writing – review & editing, Project administration, Investigation. BT: Writing – review & editing, Data curation. KS: Writing – review & editing, Validation, Data curation. DB: Validation, Writing – review & editing. GH: Writing – review & editing. NW: Writing – review & editing. KM: Writing – review & editing. MK: Writing – review & editing. JP: Writing – review & editing. LH: Writing – review & editing. AC: Writing – review & editing. MK: Writing – review & editing, Methodology, Investigation, Formal analysis. CL: Writing – original draft, Resources, Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. MC: Investigation, Writing – original draft, Writing – review & editing, Validation, Supervision, Software, Resources, Methodology, Formal analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1408196/full#supplementary-material

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