Gynecologic Oncology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Prognostic impact of interleukin-6 expression in stage I ovarian clear cell carcinoma

Ayako Kawabata [a,1], Nozomu Yanaihara [a,*,1], Chie Nagata [a,b], Misato Saito [a], Daito Noguchi [c], Masataka Takenaka [a], Yasushi Iida [a], Hirokuni Takano [c], Kyosuke Yamada [a], Masami Iwamoto [d], Takako Kiyokawa [d,2], Aikou Okamoto [a,2]

[a] Department of Obstetrics and Gynecology, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan
[b] Department of Education for Clinical Research, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
[c] Department of Obstetrics and Gynecology, The Jikei University Kashiwa Hospital, 163-1 Kashiwashita, Kashiwa-shi, Chiba 277-0004, Japan
[d] Department of Pathology, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan

HIGHLIGHTS

- Stage I OCCC prognosis differs depending on the substage.
- Substage and IL-6 expression could be the predictive biomarkers in stage I OCCC.
- Loss of ARID1A expression did not correlate with poor prognosis in stage I OCCC.
- IL-6 molecular stratification may optimize therapeutic strategies for stage I OCCC.

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 12 June 2017
Accepted 20 June 2017
Available online xxxx

Keywords:
ARID1A
Interleukin-6
Ovarian clear cell carcinoma
Prognosis
Stage I
Substage

ABSTRACT

Objective. Ovarian clear cell carcinoma (OCCC) frequently presents at an early stage. In stage I OCCC, the prognosis differs according to substage. In particular, predictive biomarkers and new treatment strategies are needed for stage IC2/IC3 disease. We investigated tumor biology and prognostic factors for stage I OCCC from a clinicopathological perspective, including the expression of ARID1A and IL-6, which are considered critical for OCCC carcinogenesis.

Methods. A retrospective cohort study of 192 patients with stage I OCCC treated at a single institution was performed. We calculated overall survival (OS) with respect to 12 clinicopathological parameters that included the unique and diverse histological features of OCCC.

Results. The estimated 5-year OS rate in patients with all stage I OCCC was 88.9% during a median of 91 months of follow-up. The multivariate analysis indicated that substage classification and IL-6 expression status were associated with poor OS ($p = 0.010$ and $p = 0.027$, respectively). Loss of ARID1A expression had no impact on survival; however, it was associated with substage ($p = 0.001$), capsule rupture status ($p = 0.011$), and ascites cytology ($p = 0.016$). No clear association was found between ARID1A and IL-6 expressions. Histological findings, including the presence of endometriosis, adenofibroma, architectural pattern, and tumor cell type, showed no prognostic effects.

Conclusions. Both substage classification and IL-6 expression status may be independent prognostic factors in stage I OCCC. Therefore, IL-6 molecular stratification may be crucial in optimizing therapeutic strategies for early stage OCCC to improve survival.

1. Introduction

Ovarian clear cell carcinoma (OCCC) has unique clinicopathological and biological features [1,2]. The tumor is commonly associated with endometriosis, paraneoplastic hypercalcemia, thromboembolism, and higher incidence in Eastern Asian women. The histopathology of OCCC includes a diverse range of architectural patterns and tumor cell types

* Corresponding author at: Department of Obstetrics and Gynecology, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan.
E-mail address: yanazou@jikei.ac.jp (N. Yanaihara).
[1] These authors contributed equally to this paper.
[2] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.ygyno.2017.06.027
0090-8258/

[3–5]. More than half of women with OCCC present at stage I, and the 5-year survival rate is relatively good at approximately 90% [6–9]. In contrast, advanced-stage cases are resistant to conventional platinum-based chemotherapy and can be complicated by thromboembolism, thus resulting in worse prognoses than those for high-grade serous carcinoma [10,11]. Notably, prognosis in stage I OCCC differs depending on substage [6,7,9,12,13]; patients with stage IA and IC1 (surgical spill) disease have favorable outcomes, whereas a statistically poorer prognosis has been shown in patients with stage IC2 (capsule rupture before surgery or tumor on ovarian surface) and IC3 (malignant cells in the ascites or peritoneal washings) disease. Therefore, substage stratification may be crucial in optimizing the treatment paradigm for stage I OCCC patients. In addition, defining prognostic markers other than International Federation of Gynecology and Obstetrics (FIGO) staging would likely improve the survival of these patients.

The molecular characteristics of OCCC known till date include genetic mutations of AT-rich interactive domain 1A (*ARID1A*) and phosphatidylinositol-45-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) and highly activated signals including phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin, hypoxia-inducible factor 1α/vascular endothelial growth factor, hepatocyte nuclear factor 1β, interleukin 6 (IL-6)/signal transducer and activator of transcription 3 (STAT3), and MET pathways [14]. *ARID1A* encodes BAF250a, a key configuration factor of the SWItch/sucrose non-fermenter chromatin remodeling complex, and is mutated in approximately half of OCCC patients [15]. In addition, *ARID1A* mutation is considered an early event in the development of OCCC from endometriosis [16]. Although several earlier studies showed that the loss of ARID1A expression was associated with unfavorable prognosis in OCCC [17,18], some studies showed no association and consensus about the relationship between ARID1A expression and patient outcome is lacking [19–22]. *ARID1A* and *PIK3CA* mutations frequently coexist in OCCC [21], and although tumor formation is not observed with a single mutation, OCCC develops in *ARID1A-PIK3CA* co-mutated transgenic mice through sustained IL-6 overproduction, which suggests that both of these mutations may contribute to initial OCCC carcinogenesis converging on pro-tumorigenic cytokine signaling [23]. Moreover, elevated IL-6/IL-6R/STAT3 pathway activity has been shown to enhance invasion capability and chemoresistance on OCCC cells, and high IL-6R expression is significantly associated with reduced progression-free survival and overall survival (OS) in OCCC [24,25]. Therefore, the IL-6/IL-6R/STAT3 signaling pathway plays a key role in OCCC pathogenesis and may be a promising therapeutic target for OCCC.

In this study, we investigated the prognostic factors for stage I OCCC from a clinicopathological perspective, including ARID1A expression, IL-6 expression, and various histological features, to identify predictive biomarkers to improve understanding of OCCC molecular carcinogenesis, which would, in turn, further develop individualized treatment strategies for stage I OCCC.

2. Materials and methods

2.1. Patients and clinical samples

This is a retrospective cohort study of 192 patients with stage I OCCC who were treated with primary surgery between 2000 and 2012 at the Department of Obstetrics and Gynecology, The Jikei University School of Medicine. The Ethics Review Committee approved this study protocol [28-212 (8455)], and informed consent was obtained from each patient. All available hematoxylin and eosin-stained slides of the ovarian cancer were reviewed by two gynecological pathologists (TK and MI) to confirm the histological tumor type based on the World Health Organization classification (2014) and the presence of endometriosis and/or adenofibroma associated with carcinoma. The predominant histological features such as architectural patterns and tumor cell types were also evaluated. Tumors were staged in accordance with the FIGO system (2014). OCCC in all patients was classified as FIGO stage I, and the substages were defined as follows: IA (capsule intact, no tumor on ovarian surface, negative washings), IC1 (surgical spill), IC2 (capsule rupture before surgery or tumor on ovarian surface), or IC3 (malignant cells in the ascites or peritoneal washings).

We also retrospectively investigated 12 clinicopathological parameters: age at diagnosis, substage, capsule rupture status, ascites or peritoneal washings cytology, surgical method, chemotherapy administration, immunohistochemistry for ARID1A and IL-6, the presence of endometriosis and/or adenofibroma, architectural pattern, tumor cell type, and patient OS. Optimal surgery for OCCC includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, subtotal omentectomy, and pelvic and para-aortic lymphadenectomy, and suboptimal surgery provides less than optimal staging except as a fertility-sparing surgery.

2.2. Immunohistochemistry

To examine ARID1A and IL-6 expression in tumor tissues, we conducted immunohistochemistry using polyclonal antibodies against ARID1A (NBP1-88932; Novus Biologicals) at a dilution of 1:200 and IL-6 (21865-1-AP; Proteintech) at a dilution of 1:400. A BenchMark XT autostainer (Ventana Medical Systems, Tucson, AZ, USA) was used for immunostaining. According to the current immunohistochemistry standard protocol, formalin-fixed, paraffin-embedded tissue sections (4 μm thick) were deparaffinized, and antigen retrieval was performed by incubating the sections in CC1 standard solutions (Cell Conditioning 1; citrate buffer pH 8.5, Ventana Medical Systems) at 100 °C for 60 min.

Immunostaining slides were evaluated in a blinded manner by two independent gynecological pathologists without any clinical information. Standardization of scoring was achieved by comparison of scores between the observers, and discrepancies were resolved by consensus obtained by reevaluating the slides using a multi-head microscope. A complete lack of nuclear immunoreactivity in the tumors was considered a loss of ARID1A expression, and definite nuclear staining was considered retained expression [16]. Immunoreactivity for IL-6 in the cytoplasm of tumors was categorized as none/focal (0%–50%) or diffuse (60%–100%).

2.3. Statistical analysis

Hazard ratios in univariate and multivariate analyses were calculated with 95% confidence intervals using a Cox proportional hazard model. Patient survival curves were estimated with the Kaplan–Meier method, and the resulting curves were compared using the log-rank test. The associations between immunoreactivity for ARID1A/IL-6 and clinicopathological parameters were analyzed using the chi-square test. The SAS software program (version 9.4; SAS Institute Inc., Cary, NC, USA) was used to analyze the data. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics in stage I OCCC

The clinicopathological characteristics of the stage I OCCC patients are summarized in Table 1. The median follow-up period was 91 months (range, 6–170 months). Patient age ranged between 27 and 84 years (median: 54 years). Patient substages were as follows: IA, 46; IC1, 89; IC2, 10; and IC3, 47. Of 192 OCCC patients, 94 patients were optimally staged and underwent systematic pelvic and para-aortic lymphadenectomy, whereas 88 and 10 patients underwent suboptimal and fertility-sparing surgery, respectively. One hundred and fifty-four patients received more than 3 cycles of platinum-based adjuvant chemotherapy. One hundred and twenty-six patients (65.6%) were associated with endometriosis alone, 8 (4.2%) with adenofibroma alone, 16 (8.3%) with

Please cite this article as: A. Kawabata, et al., Prognostic impact of interleukin-6 expression in stage I ovarian clear cell carcinoma, Gynecol Oncol (2016), http://dx.doi.org/10.1016/j.ygyno.2017.06.027