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# Prognostic impact of Dynamin related protein 1 (Drp1) in epithelial ovarian cancer

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## Abstract

**Background:** The mitochondrial fission protein, Dynamin related protein 1 (Drp1), and its upstream protein calcium/calmodulin-dependent protein kinase I (CaMKI) play a critical role in chemoresistance in ovarian cancer (OVCA). Thus, we examined the expression of Drp1, CaMKI and their phosphorylated forms and their prognostic impact in epithelial OVCA patients.

**Methods:** Expression analysis was performed by immunohistochemistry (IHC) of paraffin-embedded tumor samples from 49 patients with epithelial OVCA. Staining intensity and the percentage of positively stained tumor cells were used to calculate an immunoreactive score (IRS) of 0–12. The expression scores calculated were correlated with clinicopathological parameters and patient survival.

**Results:** High immunoreactivity of phospho-Drp1<sup>Ser637</sup> was significantly correlated with high-grade serous carcinoma (HGSC) ( $p = 0.034$ ), residual postoperative tumor of  $> 1$  cm ( $p = 0.006$ ), and non-responders to adjuvant chemotherapy ( $p = 0.007$ ), whereas high expression of CaMKI was significantly correlated with stage III/IV [International Federation of Gynecologists and Obstetricians (FIGO)] ( $p = 0.011$ ) and platinum-resistant recurrence ( $p = 0.030$ ). ROC curve analysis showed that Drp1, phospho-Drp1<sup>Ser637</sup> and CaMKI could significantly detect tumor progression with 0.710, 0.779, and 0.686 of area under the curve (AUC), respectively. The Kaplan-Meier survival curve showed that patients with high Drp1, phospho-Drp1<sup>Ser637</sup> and CaMKI levels had significantly poorer progression free survival (PFS) ( $p = 0.003$ ,  $p < 0.001$  and  $p = 0.017$ , respectively). Using multivariate analyses, phospho-Drp1<sup>Ser637</sup> was significantly associated with PFS [ $p = 0.043$ , hazard ratio (HR) 3.151, 95% confidence interval (CI) 1.039–9.561].

**Conclusions:** Drp1 and CaMKI are novel potential candidates for the detection and prognosis of epithelial OVCA and as such further studies should be performed to exploit their therapeutic significance.

**Keywords:** Epithelial ovarian cancer, Drp1, Phospho-Drp1<sup>Ser637</sup>, CaMKI, Prognostic biomarker

## Background

Ovarian cancer (OVCA) is the most lethal gynecological malignancy, and ranks fifth as the cause of cancer death among women. The standard treatment is cytoreductive surgery coupled with the treatment of first-line chemotherapy with paclitaxel and carboplatin [1]. Regardless,

more than half of the patients treated experience disease recurrence within 2 years, irrespective of the effectiveness of first-line chemotherapy and is associated with poor prognosis. Reliable prognostic biomarkers are therefore needed to aid in patient differential diagnosis and tailored therapeutic alternatives to improve patient survival.

Plasma tumor markers such as carbohydrate antigen 125 (CA125) is widely used for differential diagnosis of ovarian tumor and prognosis, tumor recurrence and the

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prediction of treatment response [2, 3]. However, CA125 is unstable and their levels are affected by histological subtypes, FIGO stage or physiological conditions; thus, making their utilization questionable [4]. Circulating plasma gelsolin (pGSN) has recently been shown to be effective in detecting early stage OVCA and predicting residual disease compared with CA125; however, a large patient cohort is needed to substantiate these findings [5]. The combination of pGSN and CA125 provided a 100% sensitivity in detecting early stage OVCA [5] thus, providing an evidence that combining multiple tumor markers on a panel could increase OVCA diagnosis and revolutionize treatment. In terms of the prediction of treatment response or prognosis, various genomic, transcriptomic and proteomic biomarkers have been reported [6]. Moreover, the usefulness of imaging modalities such as  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography ( $^{18}\text{F}$ -FDG/PET) which reflect cellular glycolytic metabolism has also been reported, and is believed to be a more accurate prediction tool of chemotherapeutic response than CA-125 [7]. However, the identification of reliable biomarkers applied for all patient is urgently needed.

Mitochondria are highly dynamic organelles, and their fission and fusion fulfill mitochondrial function, including respiration, calcium buffering, apoptosis, and autophagy. Dynamin-related protein 1 (Drp1) is the master regulator of mitochondrial fission. Drp1 is mainly present in the cytoplasm but is translocated into the mitochondrial outer membrane and binds to its partner fission proteins such as mitochondrial fission factor (MFF) or mitochondrial fission 1 protein (Fis1) during mitochondrial fission [8]. Drp1 controls the balance between fission and fusion by their phosphorylation at two distinct serine moieties. Phosphorylation of Ser616 activates Drp1 and induces mitochondrial fission, whereas Drp1 is inactivated via Ser637 phosphorylation, resulting in mitochondrial fusion [9]. The role of Drp1-dependent mitochondrial fission and fusion in apoptotic progression and chemoresistance has been reported in different cancer studies [10]. Few studies have reported the relationship between Drp1 and chemoresistance in OVCA although most of them were in vitro studies using OVCA cells with no reports on their clinical relevance [11–16].

We have previously reported that OVCA cells expressed phospho-Drp1<sup>Ser637</sup> and are prone to form highly interconnected networks [17]. A calcium mobilizing agent, Saikosaponin-d, suppresses phospho-Drp1<sup>Ser637</sup> content and calcium/calmodulin-dependent protein kinase I (CaMKI) phosphorylation - which has also been reported to up-regulate Drp1 - leading to mitochondrial fission and subsequently apoptosis [17].

Extending from these in vitro findings, we have examined in this current study the clinical relevance and prognostic impact of Drp1, CaMKI and their phosphorylated forms in epithelial OVCA. Our results could assist in the development of targeted treatment options related to mitochondrial dynamics and calcium signaling in epithelial OVCA patients.

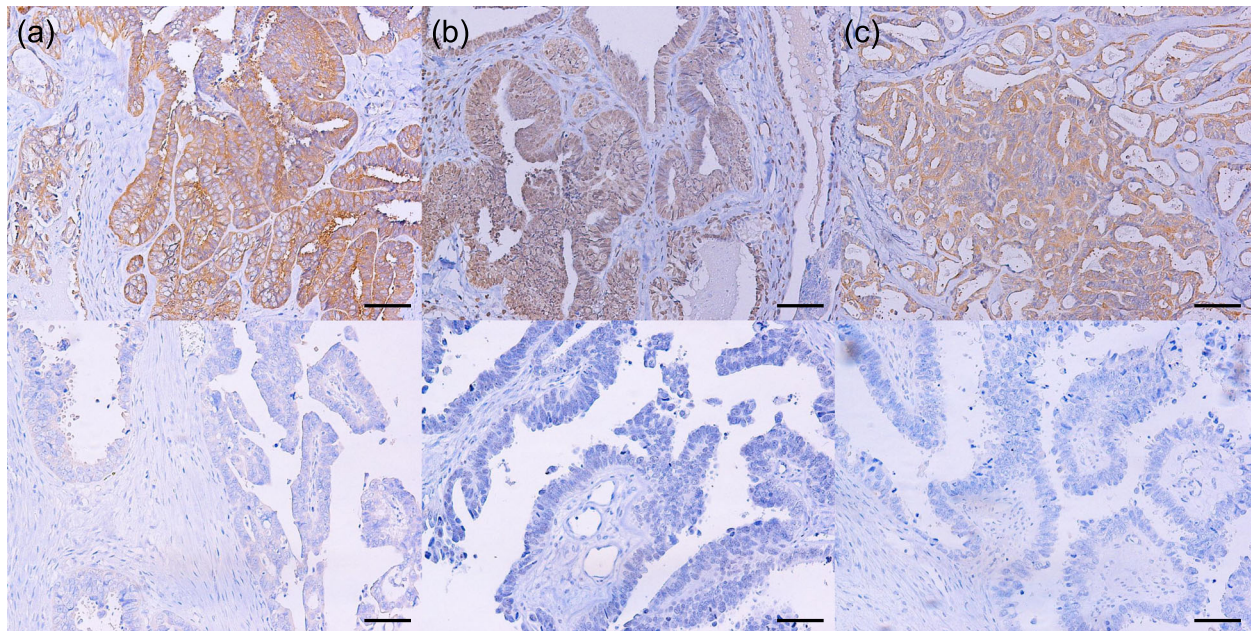
## Methods

### Patients and treatment

This study included 49 patients with primary epithelial OVCA treated between 2012 and 2017 at the Department of Obstetrics and Gynecology, University of Fukui. Formalin-fixed, paraffin-embedded tissue samples for all patients were obtained and analyzed retrospectively. Clinical and pathological factors were evaluated by reviewing medical charts and pathology records. The patients with histologically confirmed epithelial OVCA were included and the definitive histopathological diagnosis was performed by 2 certified pathologists based on the World Health Organization (WHO) classification. The patients' treatment included a combination of debulking surgery and adjuvant chemotherapy according to the clinical guidelines of the Japan Society of Gynecologic Oncology. Patients were followed-up for at least 24 months after the date of their first visit or until death. The study protocol has been approved by the institutional review board of the University of Fukui Hospital (IRB Number:20180150).

### Tissue samples and immunohistochemistry

Formalin-fixed, paraffin-embedded, 2.5  $\mu\text{m}$  sections were obtained from the samples collected. IHC staining was performed with the avidin-biotin-peroxidase complex method as previously described [18]. Antibodies used in IHC staining are shown in supplementary Table 1. To determine the stain intensity, the stroma was used as internal negative control and the vascular endothelium cells used as internal positive control (supplementary figure 1) [19–21]. The intensity and distribution of the Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI, and phospho-CaMKI<sup>Thr177</sup> immunohistochemical staining reaction was evaluated using a semi-quantitative method (IRS-score) as described previously [18]. IRS-score was calculated as follows:  $\text{IRS} = \text{SI} \times \text{PP}$ , where SI is the optical stain intensity graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining, and PP is the degree of positively stained cells defined as 0 = no staining, 1 = < 10%, 2 = 11–50%, 3 = 51–80%, and 4 = > 81%. Immunohistochemistry staining was scored by 2 independent observers. Representative images of immunostaining are shown in Fig. 1.



**Fig. 1** Representative high-grade serous ovarian cancer showing immunostaining for Drp1 (a), phospho-Drp1<sup>Ser637</sup> (b), and CaMKI (c) (upper) and each negative or weak staining (bottom) (magnification,  $\times 200$ ). Scale bar is 50  $\mu\text{m}$

### Statistical analysis

The outcome measures were progression-free survival (PFS) and overall survival (OS). PFS was assessed from the date of debulking surgery, whereas OS was from the date of first visit. Tumor progression was confirmed by either tissue biopsy or serial imaging showing evidence of progressive disease. The sample size calculation was performed by the statistical software EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [22] based on the results of CA125 as the predictive marker for PFS [23]. The Mann-Whitney U test was used to analyze relationships between clinical characteristics and IRS-score of each protein. Receiver operating characteristic (ROC) curve analysis was performed to determine optimal cut-off values for discrimination with high accuracy based on the area under the curve (AUC) for each protein. The Kaplan-Meier curve was used to assess the relationship between tissue markers and PFS and OS; log-rank test was used to calculate the statistical significance. The correlative studies using Pearson were used to determine the correlation among proteins or clinical characteristics. Cox proportional hazards regression modeling was used for univariate and multivariate analyses. Significance was defined as  $p < 0.05$  (2-sided testing). All statistical analyses were performed using SPSS Statistics version 24 (IBM, Armonk, NY).

### Results

#### Patient characteristics

Clinical information of the 49 patients with their FIGO stages (I, 19; II, 3; III, 20; IV, 7) is summarized in Table 1.

The median age at diagnosis was 57.6 years (range, 31–82 years). Twenty patients were  $< 55$  years old and 29 patients were  $\geq 55$  years old. Histopathological subtypes included high-grade serous carcinoma (HGSC) ( $n = 21$ ), clear cell carcinoma ( $n = 13$ ), endometrioid carcinoma ( $n = 6$ ), mucinous carcinoma ( $n = 6$ ), low-grade serous carcinoma (LGSC) ( $n = 2$ ) and malignant Brenner tumor ( $n = 1$ ). Twenty-nine patients received complete surgery (no postoperative residual tumor), 10 patients received optimal surgery (postoperative residual tumor of less than 1 cm), and 10 patients received suboptimal surgery (postoperative residual tumor of greater than to equal to 1 cm). Forty-three patients (87.8%) received adjuvant chemotherapy with paclitaxel and carboplatin. If the patients had severe neurotoxicity by paclitaxel, docetaxel was used instead of paclitaxel. Six patients (12.2%) did not receive adjuvant chemotherapy because of the early stage of disease or the patients' demand. Based on the findings of computed tomography after last cycle of adjuvant chemotherapy, patients were evaluated for treatment response, which was classified into four categories: complete response when there was resolution of all evidence of disease for at least 1 month; partial response when there was a decrease of  $\geq 50\%$  in the product of the diameters (maximum and minimum) of all measurable lesions without the development of new lesions for at least 1 month; stable disease if there was a decrease of  $< 50\%$  or an increase of  $< 25\%$  in the product of the diameters of all measurable lesion; and progressive disease if there was an increase of  $\geq 25\%$  in the product of the diameters of all measurable

**Table 1** Patient and tumor characteristics

Characteristics	n	%
Total number of patients	49	
Age		
< 55	20	40.8
≥ 55	29	59.2
FIGO stage		
I	19	38.8
II	3	6.1
III	20	40.8
IV	7	14.3
Histology		
High-grade serous carcinoma	21	42.9
Non- high-grade serous carcinoma		
Clear	13	26.5
Endometrioid	6	12.2
Mucinous	6	12.2
Low-grade serous carcinoma	2	4.1
Brenner	1	2.0
Completeness of surgical reduction		
Complete	29	59.2
Optimal	10	20.4
Suboptimal	10	20.4
Treatment response to adjuvant chemotherapy		
Responder	32	74.4
Non-responder	11	25.6
Tumor progression	22	44.9
Platinum-sensitive	8	16.3
Platinum-resistant	14	28.6
Death	10	20.4

lesions or the development of new lesions. In the present study, 32 (74.4%) patients who had complete response were considered as responders, whereas 11 (25.6%) patients in the other three categories (partial response, stable disease, and progressive disease) were considered as non-responders [24, 25]. The median follow-up period was 43.5 months (range, 11.7–80.7 months). Twenty-two patients (44.9%) had tumor progression including platinum-sensitive (greater than to equal to 6 months) (16.3%) and –resistant (less than 6 months) (28.6%) during the follow-up period, and 10 patients (20.4%) died.

#### Correlation between Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI, and phospho-CaMKI<sup>Thr177</sup> expression and clinicopathological parameters

To determine the clinical and prognostic impact of Drp1, CaMKI and their activated forms, their IRS-scores were calculated after IHC staining and

correlated with clinicopathological parameters. Their expression was mainly observed in the cancerous lesions compared with the healthy ovarian tissues or the other adjacent organs (supplementary figure 2). The mean IRS-score of Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI, and phospho-CaMKI<sup>Thr177</sup> were  $10.71 \pm 0.30$  (range, 6–12),  $5.76 \pm 0.44$  (range, 1–12),  $9.76 \pm 0.35$  (range, 6–12), and  $0.96 \pm 0.16$  (range, 0–6), respectively. No significant correlations were seen between Drp1 or phospho-CaMKI<sup>Thr177</sup> and clinical parameters (Table 2). Significant correlations were identified between high expression of phospho-Drp1<sup>Ser637</sup> and HGSC ( $p = 0.034$ ), suboptimal surgery ( $p = 0.006$ ), and non-responders ( $p = 0.007$ ) (Table 2). Significant correlations were also identified between high expression of CaMKI and both FIGO stage III-IV ( $p = 0.011$ ) and platinum-resistant recurrence ( $p = 0.030$ ). Furthermore, CA125 showed significant correlations with FIGO stage ( $p < 0.001$ ) and HGSC ( $p = 0.003$ ) (Table 2).

#### Clinical performances of Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI, and phospho-CaMKI<sup>Thr177</sup> expressions

Using Fisher's test to determine optimal IRS-score cut-offs, ROC curve analysis was used to test and compare the performances of tissues markers under investigation. With a cut-off of 10.5, Drp1 could significantly detect tumor progression, but not overall survival (OS) (area under the curve (AUC), 0.710, 95.5% sensitivity, 48.1% specificity for tumor progression; AUC, 0.604, 90.0% sensitivity, 33.3% specificity for OS). A phospho-Drp1<sup>Ser637</sup> cut-off of 7.0 was observed to significantly detect both tumor progression and OS (AUC, 0.779, 63.6% sensitivity, 88.9% specificity for tumor progression; AUC, 0.715, 60.0% sensitivity, 71.8% specificity for OS). With a cut-off of 10.5, CaMKI significantly detected tumor progression and demonstrated a tendency for OS (AUC, 0.686, 68.2% sensitivity, 63.0% specificity for tumor progression; AUC, 0.697, 80.0% sensitivity, 56.4% specificity for OS). At a cut-off of 0.5, phospho-CaMKI<sup>Thr177</sup> demonstrated no significant detection for tumor progression and OS (AUC, 0.513, 72.7% sensitivity, 37.0% specificity for tumor progression; AUC, 0.454, 70.0% sensitivity, 33.3% specificity for OS), (Fig. 2).

#### Prognostic effect of Drp1, phospho- Drp1<sup>Ser637</sup>, and CaMKI expression

We excluded phospho-CaMKI<sup>Thr177</sup> in the subsequent analysis because ROC curve analysis failed to show any significant effect on the tumor progression and survival. Kaplan-Meier survival curves showed that the patients with high expression of Drp1, phospho-Drp1<sup>Ser637</sup>, and CaMKI showed significantly poor PFS ( $p = 0.003$ ,  $p < 0.001$  and  $p = 0.017$ , respectively) compared with patients with low expression. Moreover, patients with high

**Table 2** Immunoreactive Score of Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI, phospho-CaMKI<sup>Thr177</sup> of the tumor and CA125 in relation to clinical factors of patients with ovarian cancer

Variable	Number of patients	Drp1		phospho-Drp1 <sup>Ser637</sup>		CaMKI		phospho-CaMKI <sup>Thr177</sup>		CA125	
		mean ± SE	<i>p</i>	mean ± SE	<i>P</i>	mean ± SE	<i>p</i>	mean ± SE	<i>p</i>	mean ± SE	<i>p</i>
Age											
< 55	20	10.05 ± 0.52	0.054	4.75 ± 0.66	0.053	9.25 ± 0.55	0.231	1.10 ± 0.24	0.311	792.3 ± 376.3	0.127
≥55	29	11.17 ± 0.35		6.45 ± 0.57		10.10 ± 0.44		0.86 ± 0.21		2141.0 ± 921.6	
FIGO stage											
I-II	22	10.14 ± 0.46	0.050	5.00 ± 0.58	0.133	8.77 ± 0.52	0.011*	0.73 ± 0.19	0.122	340.8 ± 195.6	< 0.001*
III-IV	27	11.19 ± 0.39		6.37 ± 0.63		10.56 ± 0.42		1.15 ± 0.24		2608.7 ± 987.8	
Histology											
High-grade serous carcinoma	21	11.24 ± 0.43	0.098	6.62 ± 0.61	0.034*	9.71 ± 0.52	0.974	1.05 ± 0.26	0.502	2832.5 ± 1243.6	0.003*
Non- high-grade serous carcinoma	28	10.32 ± 0.42		5.11 ± 0.61		9.79 ± 0.48		0.89 ± 0.20		658.9 ± 278.7	
Completeness of surgical reduction											
Complete or optimal	39	10.39 ± 0.36	0.085	5.15 ± 0.46	0.006*	9.46 ± 0.40	0.116	1.03 ± 0.19	0.669	1753.4 ± 712.7	0.264
Suboptimal	10	12.00 ± 0.00		8.10 ± 0.94		10.90 ± 0.57		0.70 ± 0.15		955.0 ± 281.5	
Treatment response											
Responders	32	10.34 ± 0.40	0.209	5.00 ± 0.47	0.007*	9.25 ± 0.44	0.252	1.00 ± 0.23	0.732	1118 ± 381.5	0.117
Non-responders	11	11.45 ± 0.55		7.82 ± 0.86		10.36 ± 0.70		0.82 ± 0.12		3183 ± 2231	
Tumor progression											
No progression or platinum-sensitive	35	10.37 ± 0.38	0.056	5.40 ± 0.52	0.160	9.29 ± 0.43	0.030*	0.89 ± 0.16	0.633	1007.7 ± 300.8	0.101
Platinum-resistant	14	11.57 ± 0.43		6.64 ± 0.80		10.93 ± 0.47		1.14 ± 0.39		3047.4 ± 1839	

\* *p* < 0.05

expression of CaMKI showed significantly poorer OS (*p* = 0.030) than those with low expressions (Fig. 3).

Univariate analysis showed that high IRS-score of Drp1, phospho-Drp1<sup>Ser637</sup>, and CaMKI were significantly associated with poor PFS (*p* = 0.018, 0.001, and 0.022, respectively). ≥55 years old, FIGO stage III-IV, HGSC, suboptimal surgery, non-responders, platinum-resistant recurrence, and high values of CA125 were also significantly associated with poor PFS (*p* = 0.041, *p* < 0.001, *p* = 0.013, *p* < 0.001, *p* < 0.001, and *p* = 0.021, respectively; Table 3). In multivariate analysis, since Drp1 and phospho-Drp1<sup>Ser637</sup> were related variables using Pearson correlative studies (*p* = 0.001), two different models including Drp1 and phospho-Drp1<sup>Ser637</sup> separately were used. FIGO stage, the completeness of surgical reduction, treatment response to adjuvant chemotherapy, and tumor progression were also related variables (*p* = 0.001). Therefore, FIGO stage was used for multivariate analysis. FIGO stage III-IV (*p* = 0.009) and phospho-Drp1<sup>Ser637</sup> (*p* = 0.043) were significantly associated with poor PFS and independent prognostic factors for PFS (Table 3).

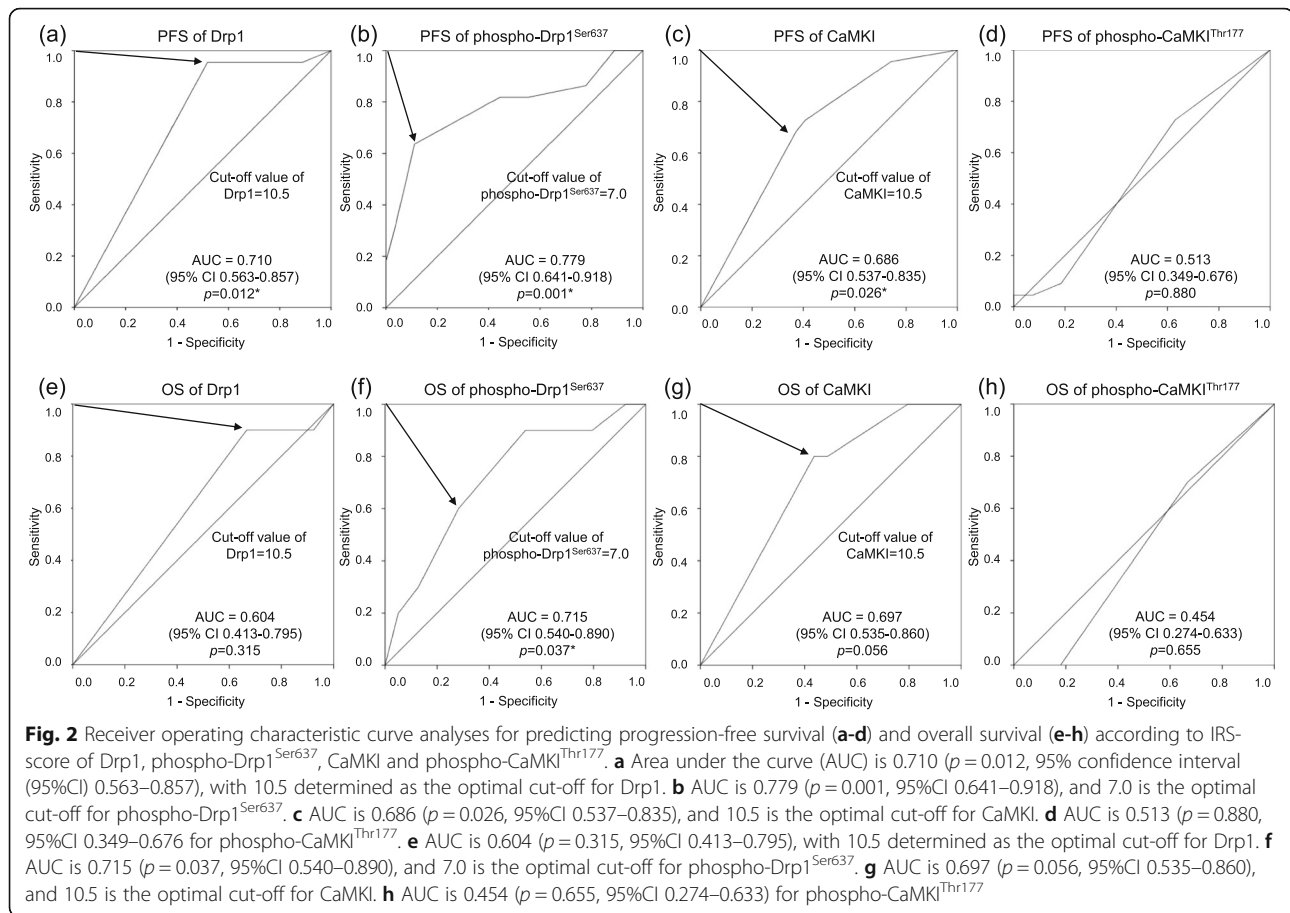
Univariate analysis showed that suboptimal surgery, non-responders, platinum-resistant recurrence, and

CaMKI were significantly associated with poor OS (*p* = 0.015, 0.003, 0.002, and 0.048, respectively). In multivariate analysis, no independent prognostic factor for OS was identified other than suboptimal surgery (*p* = 0.047) (Table 4).

## Discussion

In the present study, we have demonstrated that the expressions of Drp1 and CaMKI had the most significant prognostic correlations in the patients with epithelial OVCA. Combined with our previous in vitro studies, both Drp1 and CaMKI could serve as potential target proteins for therapeutic purposes as well as possible prognostic biomarkers.

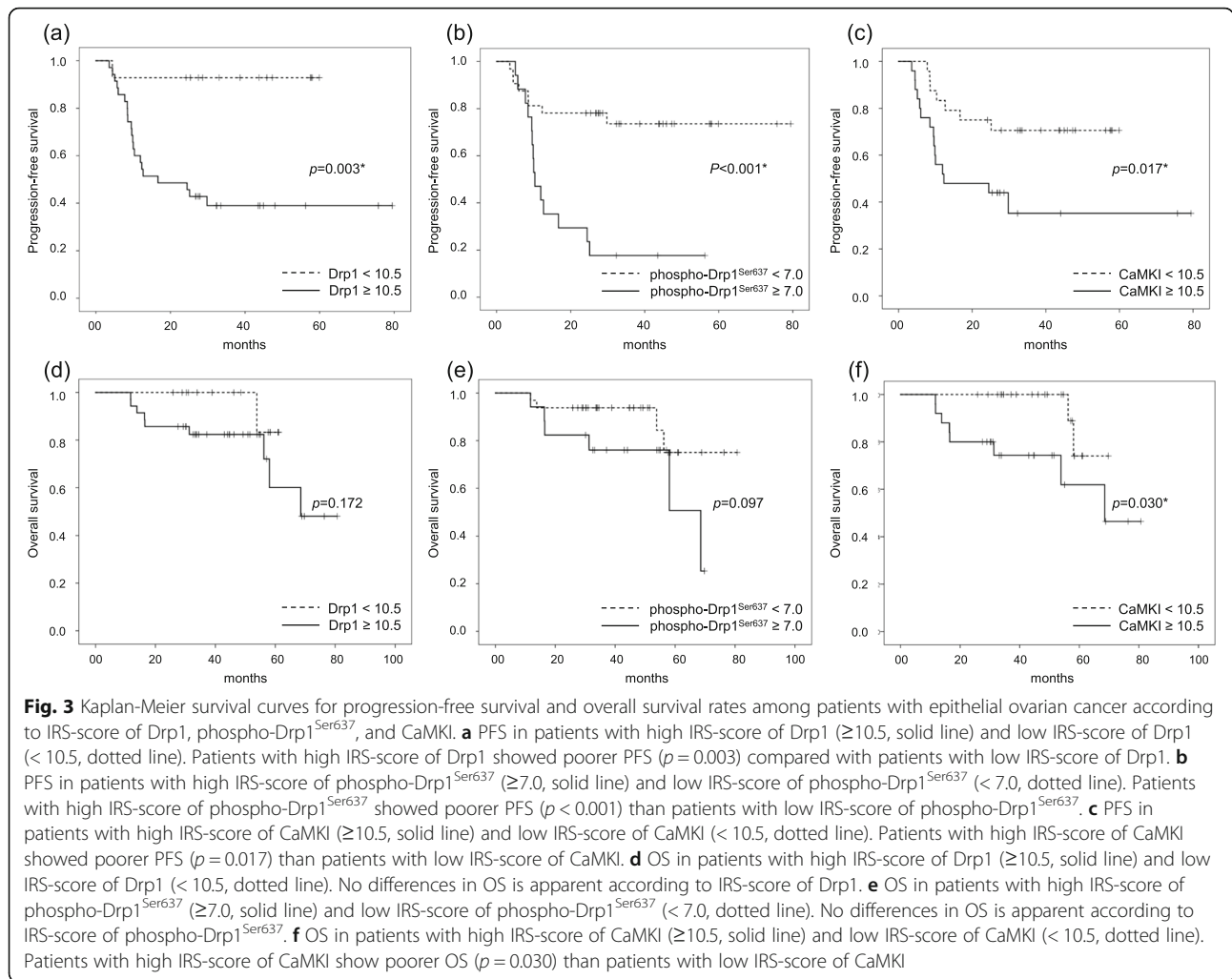
Mitochondria fusion and fission are essential to maintaining healthy mitochondrial function cells and important for many physiological functions, including energy generation, metabolism, calcium signaling and cell death. Drp1 is a member of the dynamin family of guanosine triphosphatases (GTPases) and plays a critical role in the mitochondrial dynamics. Drp1 has also been reported to be associated with the development of cancers by regulating various cellular processes such as cell death, metabolic reprogramming or cell cycle. Although many



in vitro studies with various cancer cells on the possible involvement of Drp1 in cancer development and progression have been reported, very few involve an assessment of tissue samples in the context of the clinical outcome on the cancer patients [9, 10]. Rehman et al. have demonstrated that Drp1 was highly expressed in adenocarcinoma lesions compared with healthy lungs in tissue samples from patients with lung cancer [26]. Zhao et al. also observed in breast cancer patients that the expression of Drp1 is proportional to the degree of invasiveness and metastasis [27]. In the present studies, the expression of Drp1 is mainly observed in the cancerous lesions compared with the healthy ovarian tissues; this also correlated with tumor progression suggesting that Drp1 plays a pivotal role in the progression of various cancers including ovarian cancer.

Drp1 controls the balance between fission and fusion by phosphorylation at two distinct serine moieties. Phosphorylation of Ser616 activates Drp1 and induces mitochondrial fission whereas Drp1 is inactivated via Ser637 phosphorylation, resulting in mitochondrial fusion [9]. In some cancers such as lung cancer, breast cancer or melanoma, increased levels of phospho-Drp1<sup>Ser616</sup> and mitochondrial fission are associated with cancer

progression [26–28]. Han Y et al. also reported in ovarian cancer cells that hypoxia promoted mitochondrial fission and cisplatin resistance through down-regulation of phospho-Drp1<sup>Ser616</sup> [11]. In contrast, we observed that chemoresistant uterine cervical and ovarian cancer cells exhibit highly interconnected mitochondrial networks and that mitochondrial fusion may contribute to chemoresistance [16, 17, 29]. Moreover, we have previously shown that the calcium mobilizing agent Saikosaponin-d suppresses phospho-Drp1<sup>Ser637</sup> content and CaMKI phosphorylation, leading to mitochondrial fission and subsequent apoptosis [17]. These results are consistent with the study by Yu Y et al. which reported that the inhibitor of anti-apoptotic BCL2 family protein increased the levels of Drp1, mitochondrial fission and apoptosis in cisplatin resistant ovarian cancer cells [13]. Thus, Drp1-dependent mitochondrial dynamics may confer chemosensitivity or resistance depending on the cancer type and in a cell line-specific manner. Therefore, we explored the expression of Drp1, CaMKI and their phosphorylated-form; these were related with clinical and prognostic effects using immunohistochemical analysis of tissue samples collected from patients with epithelial OVCA.



**Table 3** Prognostic factors for progression-free survival with ovarian cancer selected by Cox’s uni- and multivariate analysis

Variables	Univariate analysis		Multivariate analysis			
	Hazard ratio (95%CI)	<i>P</i>	Hazard ratio (95%CI)	<i>p</i>	Hazard ratio (95%CI)	<i>p</i>
Age ( $\geq 55$ )	2.830 (1.043–7.682)	0.041*	1.045 (0.234–4.666)	0.954	0.781 (0.163–3.731)	0.757
FIGO stage (III-IV)	13.605 (3.157–58.631)	$< 0.001^*$	7.299 (1.492–35.721)	0.014*	9.094 (1.723–48.010)	0.009*
Histopathologic type (High-grade serous carcinoma)	3.032 (1.268–7.249)	0.013*	1.885 (0.505–7.031)	0.345	1.937 (0.523–7.171)	0.322
Completeness of surgical reduction (Suboptimal)	8.007 (3.239–19.795)	$< 0.001^*$				
Treatment response (non-responders)	16.067 (5.362–48.143)	$< 0.001^*$				
Tumor progression (platinum-resistant)	38.183 (10.233–142.469)	$< 0.001^*$				
CA125 ( $\geq 399.3$ )	2.730 (1.161–6.421)	0.021*	1.176 (0.413–3.350)	0.762	0.686 (0.222–2.114)	0.511
Drp1 ( $\geq 10.5$ )	11.338 (1.521–84.495)	0.018*	4.568 (0.551–37.879)	0.159		
phospho-Drp1 <sup>Ser637</sup> ( $\geq 7.0$ )	4.632 (1.912–11.220)	0.001*			3.151 (1.039–9.561)	0.043*
CaMKI ( $\geq 10.5$ )	2.864 (1.161–7.065)	0.022*	1.809 (0.690–4.744)	0.228	2.740 (0.997–7.532)	0.051

\*  $p < 0.05$

**Table 4** Prognostic factors for overall survival with ovarian cancer selected by Cox's uni- and multivariate analysis

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	<i>p</i>	Hazard ratio (95%CI)	<i>p</i>
Age ( $\geq 55$ )	1.094 (0.270–4.441)	0.900		
FIGO stage (III-IV)	7.256 (0.913–57.650)	0.061		
Histopathologic type (High-grade serous carcinoma)	1.487 (0.423–5.223)	0.536		
Completeness of surgical reduction (Suboptimal)	4.691 (1.346–16.343)	0.015*	3.610 (1.020–12.779)	0.047*
Treatment response (non-responders)	11.701 (2.291–59.775)	0.003*		
Tumor progression (platinum-resistant)	12.210 (2.583–57.716)	0.002*		
CA125 ( $\geq 461.35$ )	2.272 (0.651–7.925)	0.198		
Drp1 ( $\geq 10.5$ )	3.847 (0.479–30.881)	0.205		
phospho-Drp1 <sup>Ser637</sup> ( $\geq 7.0$ )	2.797 (0.787–9.942)	0.112		
CaMKI ( $\geq 10.5$ )	4.877 (1.011–23.519)	0.048*	3.844 (0.772–19.145)	0.100

\*  $p < 0.05$ 

We have explored the correlation of Drp1, phospho-Drp1<sup>Ser637</sup>, CaMKI and phospho-CaMKI<sup>Thr177</sup> expressions as well as serum CA125 levels with clinicopathological parameters. Age was not correlated with any parameter although most ovarian cancers develop after menopause. High levels of CA125 was significantly associated with advanced FIGO stage and HGSC and these results are consistent with previous report [4]. High expression of phospho-Drp1<sup>Ser637</sup> but not Drp1 was associated with HGSC, suboptimal surgery and non-responders for adjuvant chemotherapy. High expression of CaMKI was significantly associated with advanced FIGO stage and platinum-resistant recurrence whereas the expression of phospho-CaMKI<sup>Thr177</sup> was not observed in the present study. In our previous in vitro study, CaMKI was phosphorylated by calcium mobilizing agent, leading to mitochondrial fission. Thus, phospho-CaMKI<sup>Thr177</sup> was not expressed because the patients were newly diagnosed without any exposure to treatment. Interestingly, phospho-Drp1<sup>Ser637</sup> and CaMKI were more associated with clinicopathological parameters than Drp1, suggesting that these patients could be stratified and selected for targeted therapy such as mobilizing agents as previously described [17]. However, our results are not consistent with the interactive open-access database which was published in 2017 [19] where CaMKI was not expressed in OVCA tissues. The reason (s) for this apparent difference is not known, it is possible that this could partly be due to the differences of antibodies used for the immunohistological analysis as we used monoclonal antibody for CaMKI detection against their polyclonal antibody. Thus, further studies are needed to investigate the expression patterns of these tissue markers using more reliable antibodies. In terms of patients' outcome, the

expression of Drp1, phospho-Drp1<sup>Ser637</sup> and CaMKI as well as CA125 were significantly associated with PFS. In particular, phospho-Drp1<sup>Ser637</sup> emerged as an independent prognostic factor for PFS but not OS. PFS provides insight into the time frame for tumor recurrence and thus plays a key role in chemoresistance. These results are conceivable since high expression of phospho-Drp1<sup>Ser637</sup> was also associated with suboptimal surgery and non-responders for adjuvant chemotherapy. These findings are consistent with that of Meshach A-W et al, where pGSN mRNA expression was associated with PFS but not OS in ovarian cancer patients [30]. Conversely, none of these proteins presented as independent prognostic factors for OS, a phenomena consistent with other reports [19]. Thus, further investigations are needed to explore novel biomarkers predictive of patient survival.

Although the findings from this study are promising, we also acknowledge some associated limitations. Our study is retrospective and monocentric. Therefore, further prospective and multicenter studies are needed to determine the prognostic value of Drp1, CaMKI and their phosphorylated-forms. We also look forward to validating these findings in larger patient cohorts with diverse histological subtypes.

## Conclusion

We have for the first time provided new insight into the clinical prognostic impact of Drp1 and CaMKI in epithelial OVCA patients. Phospho-Drp1<sup>Ser637</sup> emerged as an independent prognostic factor for PFS which serves as the first report to explain the role of Drp1 and its related proteins in comprehensive patients' cohort using immunohistochemistry. These findings are promising and provide important insights into developing novel prognostic marker and targeted therapy in the patients with epithelial OVCA.



## Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12885-020-06965-4>.

**Additional file 1: Supplementary figure 1.** Representative high-grade serous ovarian cancer showing immunostaining for the vascular endothelium cells as internal positive control (arrows) and the stroma as internal negative control (magnification,  $\times 200$ ). Scale bar is 50  $\mu\text{m}$ . **Supplementary figure 2.** Representative ovary, corpus uteri, uterine cervix, and omentum showing immunostaining for Drp1, phospho-Drp1<sup>Ser637</sup>, and CaMKI (magnification,  $\times 200$ ). In the ovary, all of them were expressed in the granulosa and theca cells whereas none of them were expressed in the epithelial cells as well as weakly expressed in the primordial follicle. In corpus uteri, Drp1 and CaMKI were strongly expressed in the endometrial glands, whereas phospho-Drp1<sup>Ser637</sup> were moderately expressed. In uterine cervix, Drp1 and CaMKI were strongly expressed in the cervical glands and squamous epithelium cells, whereas phospho-Drp1<sup>Ser637</sup> were moderately expressed. In omentum, none of them were expressed except for the vascular endothelium cells. Scale bar is 50  $\mu\text{m}$ .

**Additional file 2: Supplementary Table 1.** Primary antibodies and dilutions used for immunohistochemical analysis.

### Abbreviations

Drp1: Dynamin related protein 1; CaMKI: Calcium/calmodulin-dependent protein kinase I; OVCA: Ovarian cancer; IHC: Immunohistochemistry; IRS: Immunoreactive score; HGSC: High-grade serous carcinoma; FIGO: International Federation of Gynecologists and Obstetricians; ROC: Receiver operating characteristic; AUC: Area under the curve; PFS: Progression free survival; HR: Hazard ratio; CI: Confidence interval; CA125: Carbohydrate antigen 125; pGSN: Plasma gelsolin; <sup>18</sup>F-FDG/PET: <sup>18</sup>F-fluorodeoxyglucose positron emission tomography; MFF: Mitochondrial fission factor; Fis1: Mitochondrial fission 1 protein; WHO: World Health Organization; IRB: Institutional review board; OS: Overall survival; LGSC: Low-grade serous carcinoma; GTPases: Guanosine triphosphatases

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### Authors' contributions

HT, and MO designed experiments. HT, and YF were involved in acquisition, analysis, and interpretation of data. HT wrote the manuscript. MAW, BKT, and YY proofread and revised the manuscript. YY supervised study. All authors read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

### Ethics approval and consent to participate

The study protocol has been approved by the institutional review board of the University of Fukui Hospital (IRB Number:20180150). This study is a retrospective study. Patients provided written informed consent to participate in the study and anonymous clinical data were used. Patients provided written consent for the use of residual samples and were offered an Opt-out option as disclosed on the website ([https://www.u-fukui.ac.jp/cont\\_about/disclosure/privacy/](https://www.u-fukui.ac.jp/cont_about/disclosure/privacy/)).

### Consent for publication

All samples used in this study were de-identified. No personal information was exposed.

### Competing interests

The authors have no conflict of interest.

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