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# Whole-genome sequencing revealed novel prognostic biomarkers and promising targets for therapy of ovarian clear cell carcinoma

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**Background:** Ovarian clear cell carcinoma (OCCC) is mostly resistant to standard chemotherapy that results in poor patient survival. To understand the genetic background of these tumours, we performed whole-genome sequencing of OCCC tumours.

**Methods:** Tumour tissue samples and matched blood samples were obtained from 55 Japanese women diagnosed with OCCC. Whole-genome sequencing was performed using the Illumina HiSeq platform according to standard protocols.

**Results:** Alterations to the switch/sucrose non-fermentable (SWI/SNF) subunit, the phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway, and the receptor tyrosine kinase (RTK)/Ras signalling pathway were found in 51%, 42%, and 29% of OCCC tumours, respectively. The 3-year overall survival (OS) rate for patients with an activated PI3K/Akt signalling pathway was significantly higher than that for those with inactive pathway (91 vs 40%, hazard ratio 0.24 (95% confidence interval (CI) 0.10–0.56),  $P=0.0010$ ). Similarly, the OS was significantly higher in patients with the activated RTK/Ras signalling pathway than in those with the inactive pathway (91 vs 53%, hazard ratio 0.35 (95% CI 0.13–0.94),  $P=0.0373$ ). Multivariable analysis revealed that activation of the PI3K/Akt and RTK/Ras signalling pathways was an independent prognostic factor for patients with OCCC.

**Conclusions:** The PI3K/Akt and RTK/Ras signalling pathways may be potential prognostic biomarkers for OCCC patients. Furthermore, our whole-genome sequencing data highlight important pathways for molecular and biological characterisations and potential therapeutic targeting in OCCC.

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Ovarian clear cell carcinoma (OCCC) is recognised in the World Health Organisation classification of ovarian tumours as a distinct histological entity that demonstrates a markedly unique clinical behaviour from the other epithelial ovarian cancers (Scully, 1975; Itamochi *et al*, 2008). The OCCC constitutes ~4–12% of all epithelial ovarian cancers in western countries and >20% in Japan (Itamochi *et al*, 2008). The OCCC was diagnosed twice as frequently (11.1%) among Asian women living in the United States that that among Caucasians (4.8%) (Chan *et al*, 2008). However, the reason for the ethnic differences of OCCC prevalence remains unknown. The poor prognosis of patients with advanced disease (median survival time 12.7 months) may reflect the resistance of CCC to conventional platinum- and taxane-based chemotherapy (Sugiyama *et al*, 2000; Itamochi *et al*, 2008). Recently, randomised phase III clinical trial of irinotecan plus cisplatin (CPT-P) compared with paclitaxel plus carboplatin (TC) in treating patients with CCC was conducted by the Japanese Gynecologic Oncology Group (JGOG) in collaboration with the Gynecologic Cancer Intergroup (GCIG; JGOG3017/GCIG Trial) (Sugiyama *et al*, 2016). However, no significant survival benefit was observed for CPT-P. Therefore, effective and novel treatment strategies (e.g., incorporating molecular targeted agents) are required to improve outcomes for women with advanced OCCC.

Previous molecular studies have shown that CCC had a variety of genetic alterations, such as frequent mutations of the *AT-rich interactive domain 1A* (*ARID1A*, 40–57%) and *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit  $\alpha$*  (*PIK3CA*, 33–50%) genes, amplifications of *AKT2* (14%) and *protein phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup> dependent 1D* (*PPM1D*, 10%) genes, and loss of mismatch repair genes (7–18%) (Wilson and Roberts, 2011; Tan *et al*, 2013; Itamochi *et al*, 2015; Friedlander *et al*, 2016). Loss of phosphatase and tensin homolog (*PTEN*, 40–51%) expression and amplification and overexpression of *Erb-b2 receptor tyrosine kinase 2* (*ERBB2*, 9.3–14%) has also been reported (Tan *et al*, 2013; Friedlander *et al*, 2016). Most of these studies have analysed only targeted genes, although ovarian cancers, including OCCC, have heterogeneous gene alterations (Bast *et al*, 2009; Tan *et al*, 2011).

Recently, high-throughput sequencing of DNA has been successfully applied to several cancers, enabling the discovery of cancer genes and network-attacking mutations that can possibly translate into advances in cancer diagnosis and treatment (Garraway and Lander, 2013; Creixell *et al*, 2015). In the present study, whole-genome sequencing of 55 fresh-frozen surgically resected OCCC tumours was performed to identify novel molecular drivers and molecular features that may be predictive of clinical behaviour and enable the development of novel therapeutic strategies for individual patients. Here we provide novel evidence that OCCC tumours have a high incidence of mutations of neuroblastoma breakpoint family (NBPF) member genes. We also found that activations of the phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway and the receptor tyrosine kinase (RTK)/Ras signalling pathway were independent prognostic factors for patients with OCCC.

## MATERIALS AND METHODS

**Clinical samples.** Ovarian tumour tissue samples and matched blood samples were obtained from 55 Japanese women diagnosed with OCCC and treated surgically at Iwate Medical University, Tottori University, Niigata University, Tokai University, Tohoku University, Juntendo University, Shikoku Cancer Center, Kurume University, Hokkaido University, Keio University, Osaka University, Kagoshima City, or Hirosaki University Hospitals from 2003 to 2012. The study protocol was approved by the institutional review boards of each institution and all patients submitted written

informed consent before collection of specimens, in accordance with institutional guidelines. These patients underwent complete surgical staging, including intraperitoneal cytology, bilateral salpingo-oophorectomy, hysterectomy, omentectomy, pelvic-/paraortic lymphadenectomy, and aggressive cytoreductive surgery, followed by platinum-based chemotherapy. Thirty-five (64%) patients received postoperative chemotherapy, consisting of paclitaxel at 175 mg m<sup>-2</sup> plus an area under the curve of 6.0 mg ml<sup>-1</sup> of carboplatin on day 1, every 3 weeks for up to six cycles. The remaining 20 (36%) patients received 60 mg m<sup>-2</sup> of irinotecan on days 1, 8, and 15, plus 60 mg m<sup>-2</sup> of cisplatin on day 1, every 4 weeks for up to six cycles. There were 28 patients with stage I disease, 5 with stage II, 19 with stage III, and 3 with stage IV according to the surgicopathological staging guidelines of the International Federation of Gynaecology and Obstetrics (FIGO).

Tumour tissue samples (~1 g each) were collected during surgery and then immediately frozen in liquid nitrogen and stored at -80 °C until assayed. Blood was withdrawn and fractionated as a pretreatment to separate the buffy coat, comprising white blood cells and platelets, from erythrocytes and plasma. The buffy coat was cryopreserved and was used for normal genomic DNA matching to the tumour specimens. Before whole-exome sequencing, germline single-nucleotide polymorphism analysis was performed on tumour-blood pairs to confirm identities, showing matching of all tumour samples to the respective blood samples.

**Massively parallel sequencing.** Genomic DNA from tumour and blood was extracted using the DNeasy Blood & Tissue DNA kit (QIAGEN, Hilden, Germany). Exome capture was performed using the Agilent SureSelect BTA XT AUTO Human All Exon V5 + lncRNA Platform (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Whole-genome sequencing was performed on the Illumina HiSeq platform (Illumina, Inc., San Diego, CA, USA) according to standard protocols (Imielinski *et al*, 2012). Base calling and quality scoring was conducted using Real-Time Analysis Software, then demultiplexing and generation of FASTQ files were performed using the CASAVA package provided by Illumina (Illumina, Inc.). The data set analysed here (the data set ID: JGAS00000000076) is available at the National Bioscience Database Center (NBDC) website (<https://humandbs.biosciencedbc.jp/en/hum0067-v1>) in controlled access.

**Detection of somatic single-nucleotide variants (SNVs) and short insertion and deletion (INDEL) variants.** Mapping of genomes of raw sequence data (FASTQ files) was performed using the Burrows–Wheeler alignment algorithm (Li and Durbin, 2009). Then, realignment, recalibration, and generation of binary sequence alignment/map files were performed using the GATK toolkit and SAMtools (Li *et al*, 2009; McKenna *et al*, 2010). The SNVs and INDELS were detected by comparing sequence data from tumour-blood pairs using the MuTect, VarScan, and SomaticIndelDetector GATK algorithms (Koboldt *et al*, 2012; Cibulskis *et al*, 2013). Then, the ANNOVAR tool was used to annotate all SNVs and INDELS (Wang *et al*, 2010).

**Somatic copy number variation (CNV) detection.** Exome CNV and Control-FREEC were used to analyse the copy number (CN) in sequence data from tumour-blood pairs (Sathirapongsasuti *et al*, 2011; Boeva *et al*, 2012). A CN of  $\geq 4$  was considered as a CN gain and the size of regions of  $\leq 3$  Mb were defined as focal amplifications.

**Statistical analyses.** Statistical analyses were performed with JMP, version 12, software (SAS Institute Inc., Cary, NC, USA) and GraphPad PRISM, version 7, software (GraphPad Software, Inc., La Jolla, CA, USA). The  $\chi^2$  test, Fisher's exact test, and unpaired *t*-test were used for statistical analysis. Cluster analyses were performed by hierarchical clustering with Ward's minimum

variance method. Survival distributions were calculated using the Kaplan–Meier method and the significance of apparent differences in survival distribution between groups was tested using the log-rank test. In addition, the Cox proportional hazards model for multivariable analysis was applied. A probability ( $P$ ) value of  $<0.05$  was considered statistically significant.

## RESULTS

**Identification of gene mutations.** A total of 4792 genomic alterations were identified in the 55 tumours with a median of 62 alterations per tumour (range 25–2676), although no germline mutations and CNVs were correlated with these genomic alterations. Of these, 37 genes were found to be mutated in at least 10 (18%) of the tumours, with the most common alterations being *NBPF* members 20 (67% of tumours), 10 (60%), and 14 (60%) (Supplementary Table 1). A total of 51 (93%) cases had a mutation in *NBPF20*, 10, and 14 genes, and these mutations were all nonsynonymous SNVs. The relationship between nonsynonymous SNVs of these genes and clinicopathological factors and prognostic significance in OCCC were not observed (Supplementary Table 2). Mutations of *ARID1A* were found in 23 (42%) tumours. Univariate analysis revealed that mutations in *PIK3CA* (35% of tumours), major *histocompatibility complex, class II, DR  $\beta$ 1* (*HLA-DRB1*) (25%), *mu*cin 4, *cell surface associated* (*MUC4*) (22%), *zinc finger protein 717* (*ZNF717*) (22%), or *ARID1B* (18%) of these 37 genes were correlated with better overall survival (OS) of patients with OCCC. In contrast, mutations of *TBC1 domain family member 3* (*TBC1D3*) (24%) or

*ataxin 1* (*ATXN1*) (18%) were correlated with poor OS of these patients.

Unsupervised hierarchical clustering by these 37 genes grouped the tumours into four clusters (Figure 1A). Cluster 1 tumours had frequent mutations of *NBPF20* (88% of these tumours) and *NBPF10* (88%). Cluster 2 tumours were mostly lacking mutations of *ARID1A* (0.5% of these tumours), *PIK3CA* (0.5%), and *ARID1B* (0%). Patients in clusters 2 and 4 tended to have worse OS compared with those in clusters 1 and 3, with the 3-year survival rates being 52 and 75% (hazard ratio 2.24 (95% confidence interval (CI) 0.96–5.24),  $P=0.0623$ ), respectively (Figure 1B).

**Pathways influencing patient's outcome.** Although the relationship between molecular alterations of genes and clinicopathological variables and prognostic significance in several cancers has been reported recently, it is unclear whether gene alterations affect the clinical behaviour of OCCC. Therefore, we investigated whether the alteration status could be a prognostic biomarker for this disease.

Somatic mutations of *ARID1A*, which encodes a member of the switch/sucrose non-fermentable (SWI/SNF) family protein, were identified in approximately half of OCCC cases (Wilson and Roberts, 2011; Itamochi *et al.*, 2015). We therefore examined mutations of the SWI/SNF subunit genes. A total of 28 (51%) cases had a mutation in *ARID1A* (42%), *ARID1B* (18%), *B-cell CLL/Lymphoma 11A* (*BCL11A*) (2%), *double PHD fingers 1* (*DPF1*) (2%), *SWI/SNF related, matrix associated, actin dependent regulator of chromatin* (*SMARCA1*) (2%), *SMARCA2* (2%), *SMARCA4* (5%), or *SMARCC1* (2%) (Figure 2A). These mutations occurred more frequently in patients with FIGO stage I/II disease

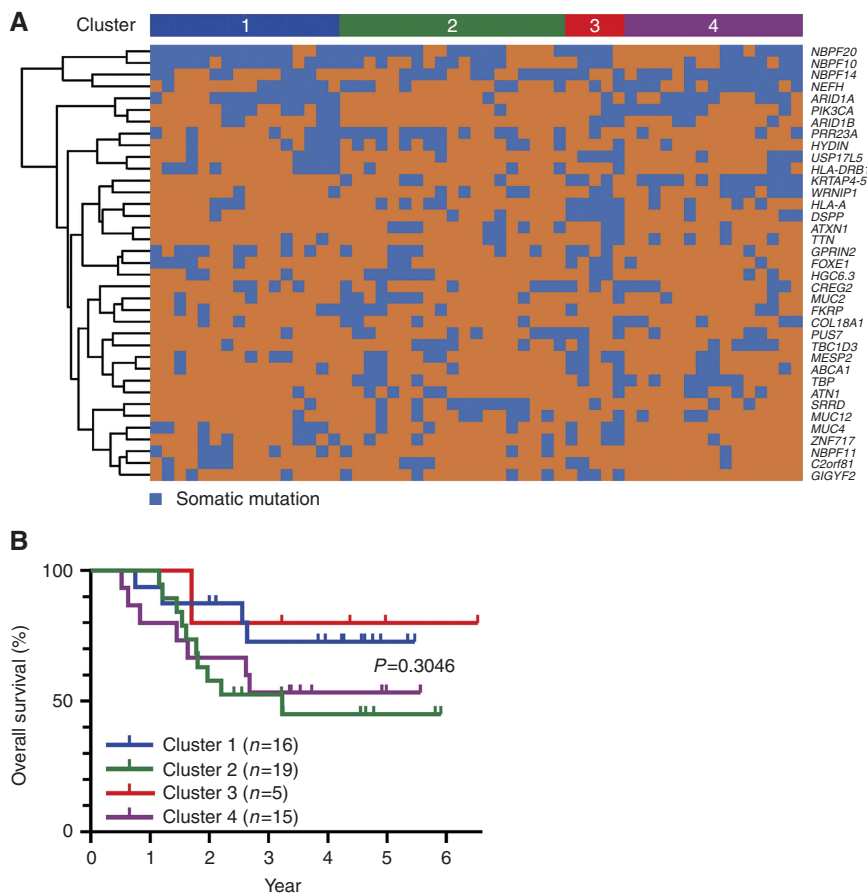


Figure 1. Somatic mutations in ovarian clear cell carcinomas. (A) Tumours were hierarchically clustered into four groups based on somatic mutations. The heat map shows somatic mutations in each tumour (horizontal axis) plotted by genes (vertical axis). (B) Kaplan–Meier curves of overall survival (OS) for each mutation cluster. There was no significant difference in OS between patients in each cluster.

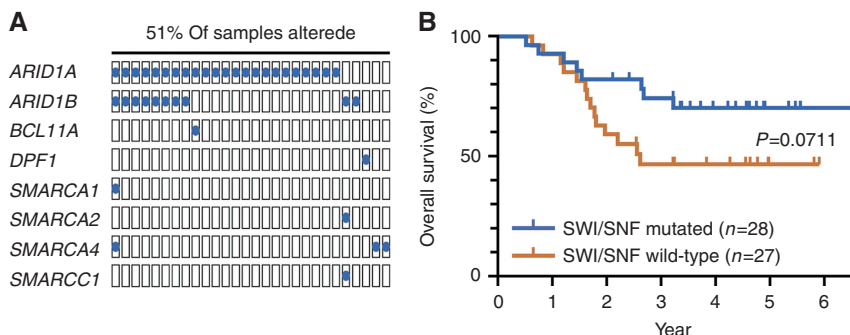


Figure 2. Mutations of the switch/sucrose non-fermentable (SWI/SNF) family subunit genes and outcome prediction in ovarian clear cell carcinoma of the ovary (OCCC). (A) The patterns of gene mutations of the SWI/SNF family subunit. Mutations of the SWI/SNF family subunit gene were observed in 51% of cases. (B) Kaplan–Meier analysis of OS for mutation status of SWI/SNF family subunit genes. No significant difference in OS was observed according to the mutation status of these genes.

Table 1. Comparison of clinicopathological factors between gene alterations in ovarian clear cell carcinoma

	SWI/SNF mutation		P-value	PI3K/Akt activation		P-value	RTK/RAS activation		P-value
	Yes	No		Yes	No		Yes	No	
N	28	27		22	33		11	44	
Age (years)			0.7261			0.2981			0.5253
Range	36–67	36–86		36–67	36–86		46–68	36–86	
Median	56	53		53.5	55		50	55.5	
FIGO stage			0.0208 <sup>a</sup>			0.0493 <sup>a</sup>			>0.9999 <sup>a</sup>
I	17	11		14	14		7	21	
II	4	1		3	2		0	5	
III	6	13		5	14		4	15	
IV	1	2		0	3		0	3	
Regimens			0.0111			0.0013			0.2932
TC	13	22		8	27		9	26	
CPT-P	15	5		14	6		2	18	
Deaths	8	14	0.1022	3	19	0.0017	1	21	0.0356

Abbreviations: CPT-P = irinotecan (60 mg m<sup>-2</sup>) on days 1, 8, and 15 + cisplatin (60 mg m<sup>-2</sup>) on day 1, every 4 weeks; FIGO = International Federation of Gynaecology and Obstetrics; PI3K = phosphatidylinositol-3-kinase; RTK = receptor tyrosine kinase; SWI/SNF = switch/sucrose non-fermentable; TC = paclitaxel (175 mg m<sup>-2</sup>) + carboplatin (an area under the curve 6.0 mg ml<sup>-1</sup>) on day 1, every 3 weeks.  
<sup>a</sup>FIGO stage I/II vs III/IV.

compared with those with stage III/IV (Table 1). Univariate analysis demonstrated that mutations of the SWI/SNF subunit genes tend to correlate with better OS among patients with OCCC, with the 3-year survival rates being 74 and 47% (hazard ratio 0.46 (95% CI 0.20–1.07),  $P = 0.0711$ ), respectively (Figure 2B). However, the OS rate was not significantly higher in patients with FIGO stage I or II OCCC with mutations of the SWI/SNF subunit genes, compared with those with the wild-type phenotype of these genes, with 3-year survival rates of 85 and 67% (hazard ratio 0.37 (95% CI 0.08–1.75),  $P = 0.2082$ ), respectively. Similarly, these mutations did not influence OS of patients with FIGO stage III or IV OCCC (hazard ratio 1.12 (95% CI 0.37–3.34),  $P = 0.8464$ ).

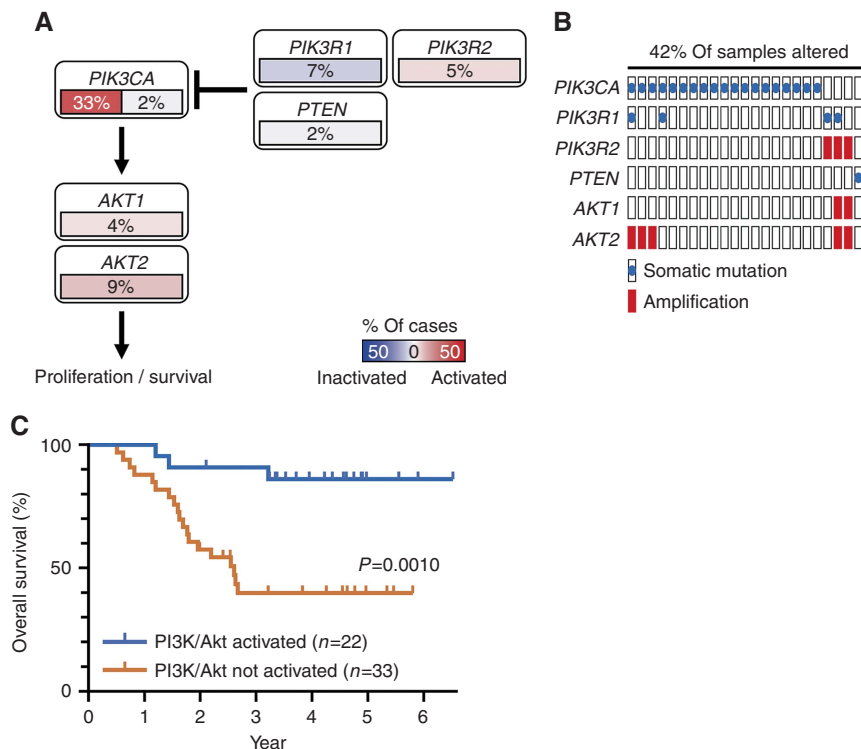
Next we analysed alterations to PI3K/Akt signalling genes. A total of 23 (42%) cases had alteration in these genes, including mutations of *PIK3CA* (35%), *PIK3R1* (7%), and *PTEN* (2%), and amplifications of *PIK3R2* (5%), *AKT1* (4%), and *AKT2* (9%) (Figure 3A and B). These gene alterations were frequently found in patients with FIGO stage I/II OCCC, compared with those with stage III/IV (Table 1), and were significantly more common in tumours with mutations of the SWI/SNF subunit genes (82%,  $P < 0.0001$ ). Univariate analysis demonstrated that activation of the PI3K/Akt signalling pathway was correlated with better OS of patients with OCCC, with 3-year survival rates of 91% and 40% (hazard ratio 0.24 (95% CI 0.10–0.56),

$P = 0.0010$ ), respectively (Figure 3C). After adjustment for patient age, FIGO stage, and treatment regimen, activation of this pathway was also correlated with better OS of patients with OCCC (hazard ratio 0.27 (95% CI 0.06–0.86),  $P = 0.0249$ ). Interestingly, the OS rate was also significantly higher in patients with FIGO stage I or II OCCC with activated pathway compared with not activated, with 3-year survival rates of 94% and 61% (hazard ratio 0.19 (95% CI 0.04–0.83),  $P = 0.0276$ ), respectively.

Alterations of the RTK/Ras signalling pathways genes were found in a total of 16 (29%) cases, including amplifications of *ERBB2* (11%) and *ERBB3* (5%), and mutations of *ERBB2* (4%), *ERBB3* (7%), *KRAS* (9%), and *BRAF* (2%) (Figure 4A and B). Of these alterations, the RTK/Ras signalling pathway was activated in 11 (20%) cases. Univariate analysis revealed that activation of this signalling pathway was correlated with better OS of patients with OCCC, with 3-year survival rates of 91% and 53% (hazard ratio 0.35 (95% CI 0.13–0.94),  $P = 0.0373$ ), respectively (Figure 4C). After adjustment for patient age, FIGO stage, and treatment regimen, activation of this pathway was also correlated with better OS of patients with OCCC (hazard ratio 0.11 (95% CI 0.01–0.54),  $P = 0.0034$ ).

Multivariable analysis of age, FIGO stage, and genes alterations was performed that found that FIGO stage, activation of PI3K/Akt





**Figure 3.** Alterations to the phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway and outcome prediction in ovarian clear cell carcinoma (OCCC). **(A)** The PI3K/Akt signalling pathway is altered through several mechanisms. Alteration frequencies are expressed as the percentage of all cases. Activated genes are shown in red and inactivated genes in blue. **(B)** Patterns of gene alterations in the PI3K/Akt signalling pathway. Alterations to the PI3K/Akt signalling pathway genes were observed in 42% of cases. **(C)** Kaplan–Meier analysis of overall survival (OS) for the activation status of the PI3K/Akt signalling pathway. Overall survival was significantly higher in patients with activation of the PI3K/Akt signalling pathway compared with those with no activation of this pathway.

signalling, and activation of RTK/Ras signalling were independent prognostic factors of OCCC (Table 2).

## DISCUSSION

Analysis of whole-genome sequencing data from 55 OCCC tumours revealed that the *ARID1A* and *PIK3CA* genes, which are known to be altered in OCCC, were frequently altered in this study. We also found frequent genetic alterations in NBPF member genes. To our knowledge, this is the first study to report the comprehensive landscape of somatic genome alterations in OCCC.

The *ARID1A* encodes a member of the SWI/SNF family protein BAF250a (*ARID1A*) and is the most frequently mutated of the SWI/SNF subunit genes in solid cancers, including OCCC (Wilson and Roberts, 2011). The SWI/SNF family proteins, which form SWI/SNF complexes, are involved in the regulation of many cellular processes, including development, differentiation, proliferation, DNA repair, and tumour suppression. Indeed, we found mutations in the SWI/SNF subunit genes in 51% (28/55) of cases and the most frequently mutated genes were *ARID1A* (42%) followed by *ARID1B* (18%) and *SMARCA4* (5%). Eight of the 10 cases (80%) with an *ARID1B* mutation also had an *ARID1A* mutation. In contrast, 2 of the 3 cases (67%) with an *SMARCA4* mutation harboured no mutations in *ARID1A* or *ARID1B*. Similarly, a *SMARCA4* mutation was observed in 1 of 8 OCCC cases (13%) that did not have mutations of *ARID1A* (5 of these tumours) or *ARID1B* (one) (Shain and Pollack, 2013). Both the *ARID1A* and *ARID1B* subunits contain an ARID domain that binds DNA in a sequence-nonspecific manner, whereas the *SMARCA4* protein, also known as BRG1, is a catalytic ATPase subunit of the SWI/SNF complex (Wilsker *et al*, 2004; Wilson and

Roberts, 2011). These observations suggest that one of these mutations may be sufficient for deterioration of the tumour suppressor role of this complex in OCCC.

The impact of *ARID1A* status on the treatment outcome of patients with OCCC has been evaluated. Several studies failed to find a correlation between negative expression of the *ARID1A* protein or mutations of the gene and OS of patients with OCCC (Itamochi *et al*, 2015). However, we previously found that the 5-year OS rate for FIGO stage I or II OCCC patients with negative tumour expression of *ARID1A* was lower than that of patients with positive tumour expression of *ARID1A* (74% vs 91% respectively). Another study showed that the loss of *ARID1A* expression was significantly correlated with shorter progression-free survival for patients with OCCC, but not OS (Katagiri *et al*, 2012). In contrast, Abou-Taleb *et al* (2016) reported that the loss of expression of one or multiple SWI/SNF subunit proteins demonstrated aggressive behaviour and poor prognosis of OCCC. In this study however, we observed no significant difference in OS between patients with mutations of the SWI/SNF subunit genes and those with the wild-type phenotypes of these genes, regardless of FIGO stage. Therefore, further studies are needed to elucidate the prognostic significance of SWI/SNF subunit alterations in OCCC.

The relationship between the loss of *ARID1A* expression and activation of the PI3K/Akt pathway has been reported in various cancers, including OCCC, suggesting a collaboration in tumourigenesis (Yamamoto *et al*, 2012; Zang *et al*, 2012; Bosse *et al*, 2013; Huang *et al*, 2014). Yamamoto *et al* (2012) reported that mutations of *PIK3CA* (which encodes the catalytic subunit p110 $\alpha$  of PI3K) were detected in 40% (17/42) of OCCC tumours and a majority (71%) of these were found in *ARID1A*-deficient carcinomas. Another study also showed that the loss of *ARID1A* was more frequent in OCCC tumours with an activated PI3K/Akt pathway

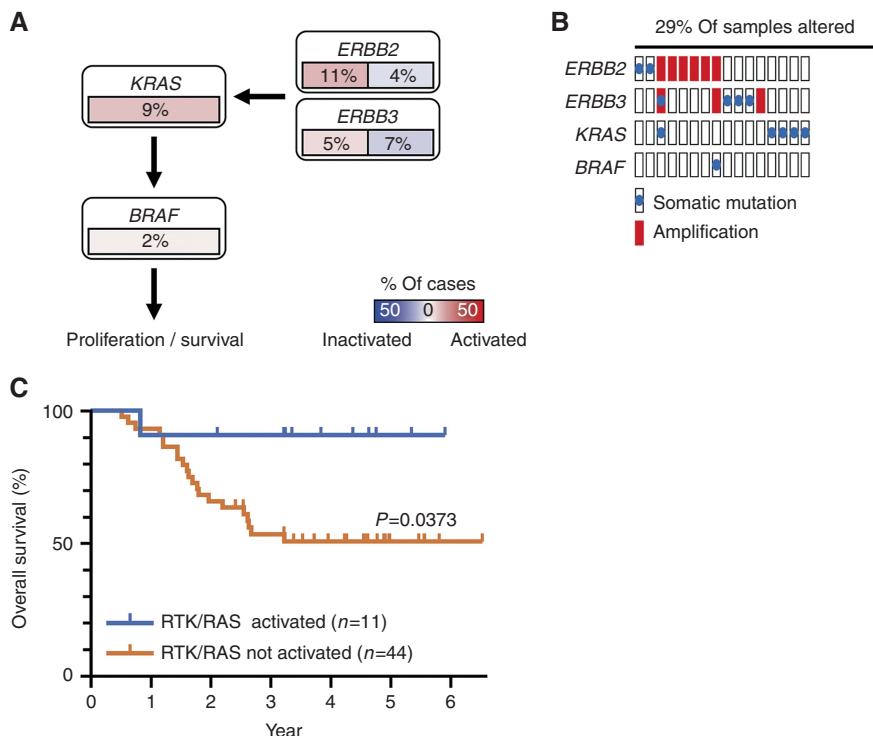


Figure 4. Pathway alterations of receptor tyrosine kinase (RTK)/Ras signalling and outcome prediction in ovarian clear cell carcinoma (OCCC). (A) The RTK/Ras signalling pathway is altered through several mechanisms. Alteration frequencies are expressed as the percentage of all cases. Activated genes are shown in red and inactivated genes in blue. (B) Patterns of gene alterations to the RTK/Ras signalling pathway. Alterations to the RTK/Ras signalling pathway genes were observed in 29% of cases. (C) Kaplan–Meier analysis of overall survival (OS) for the activation status of the RTK/Ras signalling pathway. Overall survival was significantly higher in patients with activation of the RTK/Ras signalling pathway compared with those with no activation of this pathway.

**Table 2. A multivariable Cox proportional hazards model of overall survival in ovarian clear cell carcinoma**

	Relative risk (95% CI)	P-value
Age	0.9658 (0.9272–1.0018)	0.0628
FIGO stage I/II vs III/IV	3.5219 (1.4317–9.5893)	0.0057
PI3K/Akt alteration Yes vs no	0.2562 (0.0575–0.8168)	0.0195
RTK/RAS activation Yes vs no	0.1780 (0.0099–0.8723)	0.0298

Abbreviations: CI = confidence interval; FIGO = International Federation of Gynaecology and Obstetrics; PI3K = phosphatidylinositol-3-kinase; RTK = receptor tyrosine kinase; SWI/SNF = switch/sucrose non-fermentable.

(*PIK3CA* mutations or loss of *PTEN* expression) (54%) than those without alterations to the PI3K/Akt pathway (30%) (Huang *et al*, 2014). Indeed, in the present study, 82% of tumours with activation of the PI3K/Akt pathway were observed in tumours with mutations of the SWI/SNIF subunit genes. Interestingly, clustering analysis revealed that tumours in cluster 2 were almost lacking mutations of *ARID1A*, *PIK3CA*, and *ARID1B*, and patients in this cluster tended to have worse OS compared with those in clusters 1 and 3. Furthermore, univariate and multivariable analyses revealed that activation of the PI3K/Akt signalling pathway, but not mutations of the SWI/SNIF subunit genes, was correlated with better OS of patients with OCCC. Several studies have shown that *PIK3CA* mutations or overexpression were correlated with improved OS of patients with OCCC (Rahman *et al*, 2012; Abe *et al*, 2013). Other studies, however, reported that *PIK3CA* status was not a prognostic factor for these patients (Huang *et al*, 2014; Ye *et al*,

2016). Although the prognostic significance of *PIK3CA* alterations remains controversial, these novel findings suggest that activation of the PI3K/Akt signalling pathway may have some effect on the treatment outcome of patients with OCCC.

Amplification and overexpression of *ERBB2*, also known as human epidermal growth factor receptor 2 (HER2), and mutations of *KRAS* have been reported in 9.3–14% and 4.7–13% of OCCC cases respectively (Tan *et al*, 2011, 2013; Friedlander *et al*, 2016; Zannoni *et al*, 2016). Overexpression of HER2 is associated with poor sensitivity to conventional anticancer agents and poor prognosis in several types of tumours, including ovarian cancer (Kim *et al*, 1998; Rolitsky *et al*, 1999; Itamochi *et al*, 2008). On the contrary, Nodin *et al* (2013) reported that a *KRAS* mutation was associated with a significant improvement in cancer-specific survival in patients with ovarian endometrioid carcinoma. However, the prognostic significance of these signalling pathways for OCCC remain largely unknown. Here, we found that the activation of the RTK/Ras signalling pathway was observed in 20% of tumours and was a favourable prognostic factor for OCCC. Moreover, the RTK/Ras pathway and its downstream signalling pathway are thought to be potential targets for cancer therapy (Mandal *et al*, 2016). We previously reported that inhibition of mitogen-activated protein kinase (MEK) 1/2, which is the downstream signalling cascade of RTK/Ras, by selumetinib reduced growth of OCCC cell lines and suppressed tumour growth in a OCCC xenograft model (Bartholomeusz *et al*, 2012). These findings suggest that the RTK/Ras signalling pathway may be an important prognostic biomarker for patients with OCCC and a potential therapeutic target for OCCC.

The genomic alterations have also been examined in high-grade serous ovarian cancer (HGS-OvCa), which is the most common

histological subtype, by the Cancer Genome Atlas (TCGA) study (Cancer Genome Atlas Research Network, 2011). These TCGA analyses reveal that the *BRCA1* and *BRCA2* mutations in 20% HGS-OvCa samples triggered aberrations in DNA damage repair by homologous recombination. Patients with germline *BRCA1/2* mutation are thought to have clinical benefit from using PARP (poly ADP-ribose polymerase) inhibitors, such as olaparib (Sato and Itamochi, 2015). However, in this study of OCCC, only 3 cases (5%) had somatic *BRCA2* mutations (2 nonsynonymous SNV and 1 frameshift insertion) (data not shown). Similarly, almost all HGS-OvCa tumours (96%) had mutations in *TP53*, but 3 OCCC tumours (5%) had mutations (1 stopgain, 1 frameshift deletion, and 1 nonsynonymous SNV) in this gene (data not shown). On the contrary, *ARID1A* and *PIK3CA* mutations were more common in OCCC cases compared with that in HGS-OvCa. These differences between ovarian cancer subtypes suggested that subtype-specific treatment strategies might be needed to improve ovarian cancer outcomes.

The limitations of this study are that we have analysed only DNA sequencing data in a relatively small number of OCCC samples, and it lacks independent validation of the prognostic association. Therefore, the sequencing of larger OCCC cohorts will be necessary to determine a more comprehensive genomic landscape for OCCC. Furthermore, precise prevalence frequency and prognostic significance for the mutated genes detected in our analysis also need to be validated by DNA sequencing as well as by multiple-assay platform. Recently, Friedlander *et al* (2016) examined the results of a multiplatform profiling panel, such as DNA sequencing, immunohistochemistry, fluorescent or chromogenic *in situ* hybridisation, and RNA fragment analysis, in OCCC to identify the potential therapeutic targets. Consistent with our results, they confirmed that the PIK3CA/Akt/mTOR pathway was altered in 61% OCCC tumours.

In summary, our data showed that mutations of *ARID1A* and *PIK3CA* were frequently observed in OCCC tumours. We also found that the activation of PI3K/Akt and RTK/Ras signalling pathways may be a favourable prognostic marker for patients with OCCC. We believe that these whole-genome sequencing data will be valuable and useful for further analysis of the molecular and biological characteristics of OCCC, and may lead to the establishment of novel treatment strategies to improve survival of patients with OCCC.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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