Clinica Chimica Acta 494 (2019) 1–7

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Targeted sequencing of a specific gene panel detects a high frequency of *ARID1A* and *PIK3CA* mutations in ovarian clear cell carcinoma

Yu-Fa Su[a,1], Eing-Mei Tsai[a,b,c,1], Chih-Chieh Chen[d], Chun-Chieh Wu[e], Tze-Kiong Er[f,g,h,*]

[a] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[c] Headquarters of the Research Center, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung, Taiwan
[e] Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[f] Division of Laboratory Medicine, Asia University Hospital, Asia University, Taichung, Taiwan
[g] Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan
[h] Department of Biotechnology, Asia University, Taichung, Taiwan

ARTICLE INFO

Keywords:
Ovarian cancer
Ovarian clear cell carcinoma
Endometrioid ovarian carcinoma
Targeted sequencing
Somatic mutations

ABSTRACT

Background: The objective of this study was to assess the mutational profile in epithelial ovarian cancer using formalin-fixed, paraffin-embedded (FFPE) tumor specimens from a Taiwanese population by performing targeted sequencing of 9 cancer-associated genes.

Methods: Targeted sequencing was performed on 32 formalin-fixed, paraffin embedded (FFPE) tumor specimens, consisting of matched samples from 16 epithelial ovarian cancer patients. Genetic alterations in the 9 cancer-associated genes were detected using a deep sequencing (> 1000×) approach.

Results: *ARID1A* and *PIK3CA* were the most frequently mutated genes. Specifically, *ARID1A* mutations and *PIK3CA* mutations were detected in 77.8% and 66.7% of ovarian clear cell carcinoma patients, respectively. Mutations in other genes, including *MLH1* (6.3%) and *CREBBP* (6.3%), were detected in the Taiwanese population. We also identified coexisting *ARID1A-PIK3CA* mutations (43.8%) and *ARID1A-KRAS* mutations (12.5%) in tumors. It should also be noted that we identified the presence of three coexisting mutations, the *ARID1A-KRAS-PIK3CA* mutations and the *ARID1A-CREBBP-PIK3CA* mutations.

Conclusions: In summary, we identified novel genetic alterations in patients with epithelial ovarian carcinoma (EOC) in a Taiwanese populations. Further studies are needed to elucidate the mechanism of chromatin remodeling to examine the role of the PI3K/AKT pathway, to determine the critical roles of these mechanisms in tumor development and the progression of ovarian malignancy and to investigate new targeted therapies. Overall, our findings were reliable and are worthy of further study.

1. Introduction

Ovarian cancer especially epithelial ovarian carcinoma (EOC), continues to be a leading cause of gynecological cancer-related mortality worldwide, and EOC is the fifth leading cause of cancer-related deaths among women in the United States [1]. Recently, it has been reported that there were an estimated 22,440 new cases of EOC and 14,080 EOC-related deaths in 2017 [2]. Ovarian cancer is the eighth most common cause of cancer-related death in Taiwanese women [3].

The classification of EOC is usually based on the origin of the tumor [4,5]. Approximately 90% of primary ovarian cancers are EOCs, which are divided into four major histological subgroups, including the serous, mucinous, endometrioid, and clear cell groups [6,7]. EOC is classified into two categories: type I includes low-grade serous, endometrioid, clear cell, mucinous, and transitional cell carcinomas, and type II includes high-grade serous carcinomas, undifferentiated carcinomas, and carcinosarcomas [8].

Next-generation sequencing (NGS) has been widely implemented for sequencing analysis, including whole-genome sequencing (WGS), whole-exome sequencing (WES), and the sequencing of the transcriptome, targeted regions, and epigenetic modifications. There is great potential for the application of NGS in disease management,

* Corresponding author at: Division of Laboratory Medicine, Asia University Hospital, Asia University, Taichung 41354, Taiwan.
E-mail addresses: tzekiong92@gmail.com, T00016@auh.org.tw (T.-K. Er).
[1] Yu-Fa Su and Eing-Mei Tsai contribute equally to this article.

https://doi.org/10.1016/j.cca.2019.03.003
Received 14 January 2019; Received in revised form 5 March 2019; Accepted 5 March 2019
Available online 06 March 2019
0009-8981/

disease treatment, genetic counseling, and risk assessment [9]. NGS technology is a feasible and reliable method with which to detect novel and rare somatic mutations. NGS has been applied for the simultaneous identification of base substitutions, small deletions, insertions, copy-number alterations, and structural alterations with much greater sensitivity and cost effectiveness than traditional Sanger sequencing for screening a large number of genes. Furthermore, NGS has the potential to substantially improve the detection rates of a wide spectrum of mutations in EOC patients compared with that of *BRCA1/2* testing alone [10]. Recently, Kim et al. [11] revealed that significantly altered pathways were associated with cell proliferation and survival in 87% of patients with endometriosis-associated ovarian clear cell carcinoma (OCCC) and with chromatin remodeling in 47% of patients with OCCCs, based on WES in patients of Korean ethnicity. Shibuya et al. [12] demonstrated that the most frequently mutated genes are *ARID1A*, *PIK3CA*, *PPP2R1A*, and *KRAS* in OCCCs in the Japanese population using an NGS approach. Our previous study showed that the most frequently mutated genes were *PIK3CA* and *ARID1A* in a Taiwanese population with endometriosis-associated ovarian cancer using targeted NGS [13]. However, Dicks Ed et al. [14] indicated that WES data may have clinical implications for risk prediction and prevention approaches for high-grade serous ovarian cancer. In brief, NGS studies may lead to significant advances in our understanding of the genome of EOC, and current efforts are aimed toward bringing sequencing discoveries into the clinic in the form of biomarkers and biomarker-based clinical trials designs.

Nine cancer-related genes (including *ARID1A*, *ARID2*, *CREBBP*, *NUMA1*, *PPP2R1A*, *NCOA2*, *MLH1*, *PIK3CA*, and *KRAS*) were analyzed in the formalin-fixed, paraffin-embedded (FFPE) materials by targeted NGS. In the present study, targeted NGS was undertaken to characterize the gene alterations in EOC patients of Taiwan ethnicity.

2. Materials and methods

2.1. Patients

Between October 2008 and July 2013, patients with EOC were recruited from the Department of Obstetrics and Gynecology at Kaohsiung Medical University Hospital in Southern Taiwan. All participants signed the written informed consent forms, and the protocols were written and conducted in accordance with the institutional guidelines and the Declaration of Helsinki and were approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-G(I)-20150040). The clinical and pathological characteristics of the 16 patients are summarized as Table 1. In our study population, patient 1 had recurrence after two years with multiple metastases and died. Patients 3 and 12 had lymph node hyperplasia in the pelvis. Patients 6, 10 and 15 had carcinomas in bilateral ovaries. Notably, patient 10 had adenocarcinoma metastasis to the right fallopian tube, peritoneum, omentum, ascending colon, appendix and lymph nodes. Additionally, patient 15 experienced after four years with peritoneum, omentum and right fallopian tube metastasis and died. Intriguingly, we found that patient 13 had both endometrium and ovarian cancers simultaneously.

2.2. Samples

Thirty-two FFPE specimens from EOC patients were included in this study. Patients with EOC were confirmed using clinical and histological data. Each patient's clinical cancer TNM (tumor–node–metastasis) stage was determined according to the 1992 criteria of the American Joint Cancer Committee/Union International Cancer Control (AJCC/UICC). Sufficient and good-quality DNA from FFPE samples was extracted from 16 pairs of matched normal and EOC samples for targeted sequencing. Representative histological features of normal endometrium, endometroid carcinoma, and clear cell carcinoma were shown in Fig. S1.

Table 1
The clinical and pathological characteristics of the 16 EOC patients.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Age of onset	50	NA	59	NA	NA	43	NA	50	NA	58	NA	59	44	54	51	80
Histology	Clear cell	Clear cell	Clear cell	Clear cell	Clear cell	Clear cell	Clear cell	Clear cell	Clear cell	Endometrioid	Endometrioid	Endometrioid	Endometrioid	Endometrioid	Endometrioid	Endometrioid
Tumor site	Left	NA	Left	NA	NA	Bilateral	NA	Left	NA	Bilateral	NA	Left	Left	Left	Bilateral	Left
Stage	IA	NA	I	IIIb	NA	IB	NA	IC	IA	IIIc	NA	IIIC	IA	IC	IIIc	IIA
Grade	NA	NA	II	II	NA	III	NA	II	II	III	NA	NA	I	II	III	III
TNM stage	pT1aN0	NA	pTaN0	NA	NA	pT1N0	NA	pTaN0	NA	pT3cN1M1	NA	NA	pT1aN0	pT1cN0	pT3cN0	cT2aN0
Lymph node status (pelvic)	Benign	NA	Bilateral hyperplasia	NA	NA	Benign	NA	Benign	NA	Right adenocarcinoma meta	NA	Bilateral hyperplasia	Benign	Benign	Benign	Bilateral adenocarcinoma meta

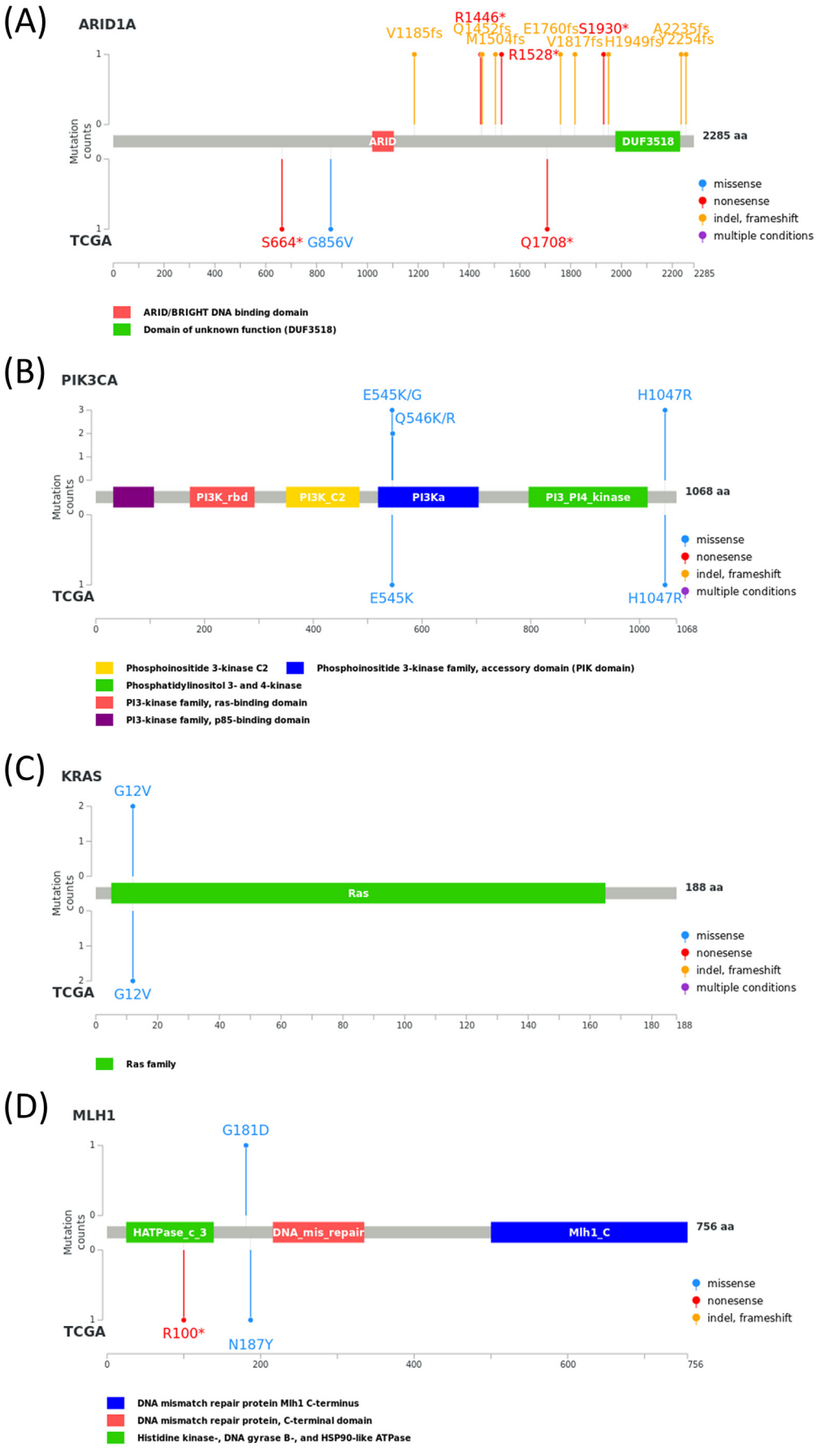

Fig. 1. Distribution of genetic alterations detected in EOC. Schematic representation of protein structures of (A) ARID1A (B) PIK3CA (C) KRAS and (D) MLH1. The upper panel indicates the genetic alterations detected in EOC. The lower panel indicates the mutation spectrum in The Cancer Genome Atlas (TCGA) ovarian cancer dataset. The left scale indicates the number of cases. The blue, yellow, red, and purple circles indicate missense mutations, insertions/deletions, nonsense mutations, and multiple types of mutations, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

List of mutations detected by targeted deep sequencing after applying the relevant filters in sixteen patients with matched carcinoma and normal endometrium specimens.

Chromosome coordinate					1		2		4		5		6		7		8		9		13		14	
Gene	Chr	EXON	cDNA change	protein chan	T	U	T	U	T	U	T	U	T	U	T	U	T	U	T	U	T	U	T	U
ARID1A	1	3/20	c.1756C>T	p.Q586*																				
ARID1A	1	14/20	c.3545_3546insCAATT	p.V1185fs					60.8%															
ARID1A	1	18/20	c.4336C>T	p.R1446*											37.6%									
ARID1A	1	18/20	c.4350delC	p.Q1452fs									27.6%											
ARID1A	1	18/20	c.4512_4525delGACCTATAATTA	p.M1504fs																			81.2%	
ARID1A	1	18/20	c.4582C>T	p.R1528*	33.5%																			
ARID1A	1	20/20	c.5276_5277insG	p.E1760fs											36.1%									
ARID1A	1	20/20	c.5449delG	p.V1817fs							72.0%													
ARID1A	1	20/20	c.5789C>A	p.S1930*													32.5%							
ARID1A	1	20/20	c.5846delA	p.H1949fs													35.3%							
ARID1A	1	20/20	c.6700_6706delGCTGCCC	p.A2235fs			34.4%																	
ARID1A	1	20/20	c.6758_6759delTG	p.Y2254fs																	31.5%			
CREBBP	16	-	c.3061-3A>G	-																			10.8%	
KRAS	12	2/5	c.35G>T	p.G12V	45.8%						66.8%													
MLH1	3	6/19	c.542_543delGCinsAT	p.G181D									44.1%	9.6%										
PIK3CA	3	10/21	c.1633G>A	p.E545K					64.6%		52.7%													
PIK3CA	3	10/21	c.1634A>G	p.E545G																			26.8%	
PIK3CA	3	10/21	c.1636C>A	p.Q546K			32.4%																	
PIK3CA	3	10/21	c.1637A>G	p.Q546R											34.5%									
PIK3CA	3	21/21	c.3140A>G	p.H1047R													32.7%		22.0%		62.1%			

Fig. 2. The OncoPrint provides an overview of genetic alterations in particular genes (rows) affecting particular individual EOC samples (columns).

2.3. DNA extraction

Representative sections were stained with hematoxylin and eosin (H &E) and reviewed by pathologists before DNA extraction. Five 10 μm FFPE tissue sections from each tissue block were deparaffinized, and DNA was extracted using a QIAamp DNA mini kit (Qiagen, Heidelberg, Germany) following the manufacturer's protocol. The quality and quantity of the nucleic acids were analyzed using a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA) and Nano200 Nucleic Acid Analyzer, respectively. The quantity and integrity of the extracted gDNA were determined by using a Qubit (Invitrogen) and Fragment Analyzer™ (Advanced Analytical Technologies, Inc), respectively.

2.4. Customized panel sequencing

A total of 9 cancer-associated genes were analyzed, namely, *ARID1A*, *ARID2*, *CREBBP*, *NUMA1*, *PPP2R1A*, *NCOA2*, *MLH1*, *PIK3CA*, and *KRAS*. Sixty nanograms of genomic DNA were amplified using three pools of 523 primer pairs (Ion AmpliSeq Comprehensive Cancer Panel, Life Technologies) to target all coding exons of 9 cancer-related genes. Amplicons were ligated with barcoded adaptors using the Ion Amplicon Library Kit (Life Technologies). Barcoded libraries were subsequently conjugated with sequencing beads by emulsion PCR and enriched using IonChef (Life Technologies) according to the Ion Torrent protocol (Life Technologies). Quality and quantity of amplified library were determined using the fragment analyzer (AATI) and Qubit (Invitrogen). Sequencing was performed on the Ion Proton sequencer using the Ion PI chip (Life Technologies) according to the manufacturer's protocol.

2.5. Data analysis

Raw reads generated by the sequencer were mapped to the hg19 reference genome using the Ion Torrent Suite (v. 4.2). Coverage depth was calculated using the Torrent Coverage Analysis plug-in. Single nucleotide variants (SNVs) and short insertion/deletions (INDELs) were identified using the Torrent Variant Caller plug-in (v. 4.2). The Variant Effect Predictor (VEP) was used to annotate every variant with a database from COSMIC: v.70; dbSNP 138 and 1000 Genomes: phase 1. Variants with a coverage lower than 25 or a frequency lower than 5% were filtered out. We also performed in silico analyses with SIFT [15] and Polyphen2 [16] to predict whether the observed mutations were likely to be deleterious or not.

3. Results

3.1. Mutation status of the customized panel in EOC

DNA was extracted from 32 FFPE blocks of 16 patients diagnosed with endometrioid ovarian cancer (ENOC) and OCCC. Nine patients were histologically defined as OCCC, and the other seven patients were defined as ENOC. We sequenced each sample on the Ion Torrent™ Customized Panel to an average sequencing depth of 4000×. Sequencing results to a median depth > 4000× in tumors and matched normal endometrium DNA was used to establish a baseline of pathogenic mutations. A total of 27 nonsynonymous somatic mutations, including frameshift, stop-gain, splice -site, and missense mutations, with an allele frequency ≥ 10% were detected in the 16 tumor DNA samples. The predicted functional impact of all the amino acid-substituting mutations was analyzed using SIFT and PolyPhen2 and the results are shown in Table S1.

Missense mutations accounted for the majority (51.9%) of the identified variants, followed by frameshift (29.6%), stop-gain (14.8%), and splice-site (3.7%) mutations.

Mutation of the *ARID1A* gene was detected in 56% (9/16) of EOC specimens, with mutations at p.Q586*, p.V1185 fs, p.R1446*, p.Q1452fs, p.M1504 fs, p.R1528*, p.E1760fs, p.V1817 fs, p.S1930*, p.H1949fs, p.A2235fs and p.Y2254fs identified in our study populations. Mutations in the *PIK3CA* gene were detected in 50% (8/16) of EOC specimens, with mutations at p.E545K, p.E545G, p.Q546K, p.Q546R and p.H1047R in our study populations. Other genes, such as *KRAS* (12%), *CREBBP* (6%) and *MLH1* (6%), were also identified in the current study. Lollipop plot highlighting genomic alterations of select genes, including *ARID1A*, *PIK3CA*, *KRAS*, and *MLH1*, was generated (Fig. 1A–D).

3.2. Mutational landscape in the targeted genes

The 27 nonsynonymous somatic mutations identified in the present study were located in 5 genes. The most frequently mutated genes were *ARID1A* (44.5%; 12/27), *PIK3CA* (37%; 10/27), *KRAS*, *MLH1* (7.4%; 2/27 each), and *CREBBP* (3.7%; 1/27). Notably, we revealed that *ARID1A* mutations coexists with *PIK3CA*, *KRAS*, *MLH1*, and *CREBBP* gene mutations in the current study. As shown in Fig. 2, *ARID1A* mutations coexist with *PIK3CA* gene mutations in patients 2, 4, and 13. *ARID1A* mutations coexist with the *KRAS* mutation in patient 1. *ARID1A* mutations coexisted with the *MLH1* mutation in patient 6. Additionally, we identified triple mutations in patients 5, 7, 8, and 14.

4. Discussion

Here, we provide a survey of the mutational landscape of EOC in Taiwanese populations using a customized panel. *ARID1A* and *PIK3CA* were the most frequently mutated genes identified in this study. Specifically, *ARID1A* mutations and *PIK3CA* mutations were identified in 77.8% and 66.7% of OCCC patients, respectively.

Previously, Friedlander et al. [17] conducted a large study with 521 patients, confirming that the PIK3CA/AKT/mTOR pathway is

commonly altered in OCCC. These researchers revealed that *PIK3CA* mutations were observed in 50% of pure OCCCs and in 18.1% of mixed OCCCs. In addition, Rahman et al. [18] revealed that *PIK3CA* mutations were found in 28.6% (16/56) of OCCCs in Japanese women. The authors suggested a significant association of *PIK3CA* mutations with a favorable prognosis. In another study, Kuo et al. [19] indicated that *PIK3CA* is the most frequently mutated gene, occurring in approximately 33% of patients with OCCC. Intriguingly, the authors found that the *PIK3CA* mutations occurred in 50% in the Taiwanese population. In the present study, we showed that 66.7% of OCCC patients harbor *PIK3CA* mutations. Similarly, Jones et al. [20] demonstrated that *PIK3CA* mutations were observed in 40% OCCC patients based on PCR amplification and Sanger sequencing. Most recently, Shibuya et al. [12] indicated that the frequently mutated genes included *ARID1A*, *PIK3CA*, *PPP2R1A*, and *KRAS* in OCCC based on NGS. Similarly, Murakami et al. [21] showed that *ARID1A* and *PIK3CA* were frequently mutated in OCCC using whole exome sequencing. On the other hand, Maru et al. [22] also demonstrated that the most frequently mutated gene in Japanese OCCC patients was the *PIK3CA* gene. These researchers found a *PIK3CA* mutation in 28% of OCCC patients. Zannoni et al. [23] revealed that the *PIK3CA* mutations were present in 32% (20/63) of primary OCCC patients based on pyrosequencing analysis. In the current study, we demonstrated that *PIK3CA* mutations, including mutations in p.E545K, p.Q546K/R, and p.H1047R, occurred in 66.7% (6/9) of patients with OCCC. The crystal structure of human p110α (p110α; encoded by the PIK3CA gene) was obtained from the protein data bank (PDB ID: 2RD0). As shown in Fig. 3, the PIK3-hotspot mutation sites E545 and Q546 are located in the helical domain (yellow), and H1047R is located in the kinase domain (red). Notably, we found that only 1 patient (14.3%, 1/7) with ENOC harbored the *PIK3CA* mutation, p.H1047R. As shown in Table 2, the *PIK3CA* mutation frequency ranged from 28.6% to 51% in OCCC. Overall, our findings showed that the frequency of the *PIK3CA* mutations in OCCC appears to be higher in Taiwanese population and provide further evidence that *PIK3CA* is the most frequently mutated gene in OCCC.

Our previous study [13] indicated that the most frequently mutated genes in 10 Taiwanese EAOC patients were *PIK3CA* and *ARID1A* based on deep sequencing. Very recently, Kim et al. [11] showed that the frequencies of *ARID1A*, *PIK3CA*, and *TP53* in Korean OCCC patients were 40%, 40%, and 13%, respectively, using whole exome sequencing. Similarly, a study showed that *ARID1A* somatic mutations were observed in 62% of the OCCC samples based on whole-exome sequencing from 39 OCCC patients [21]. In addition, multiple studies showed that *ARID1A* was the most frequently mutated gene in OCCC, occurring in at least 57% of patients with OCCC [12,20]. As shown in Table 2, the *ARID1A* mutation frequency has ranged from 40% to 66.7% in OCCCs. It should be noted that the frequency of *ARID1A* mutations was 77.8% (7/9) in patients with OCCC and 14.3% (1/7) in patients with ENOC in the present study. Overall, our findings indicate that the frequency of *ARID1A* mutations in OCCC appears to be higher in Taiwanese population. Taken together, our findings are consistent with those of previous studies showing that *ARID1A* was the most frequently mutated gene in OCCC.

Recent studies have shown that *ARID1A* and *PIK3CA* mutations might cooperate to promote tumor growth [24,25]. Previously, a study showed that the loss of ARID1A protein expression occurs as a very early event in CCC development and frequently coexists with *PIK3CA* mutations [26]. In this study, we identified one patient with *ARID1A* double mutations and a *PIK3CA* mutation. Notably, we also revealed that four patients had two mutations each in *ARID1A/PIK3CA*. Two patients had two mutations each in *ARID1A/KRAS* or *ARID1A/MLH1*. Two patients harbored triple mutations in *ARID1A/CREBBP/PIK3CA* or *ARID1A/KRAS/PIK3CA*. To the best of our knowledge, this study is the first to show the occurrence of either double mutations or triple mutations in these genes in Taiwanese patients with EOC. However, the impact of patients with EOC harboring double or triple mutations in clinical practice and the underlying mechanisms need to be elucidated in future studies.

According to previous studies, the incidence of *KRAS* mutations is approximately 15%~39% [27,28]. In fact, a higher incidence of *KRAS* mutations was identified in mucinous epithelial ovarian cancer than in other types of ovarian cancer. Indeed, Mueller et al. [29] showed that the frequency of *KRAS* mutation was 71% in mucinous ovarian cancer based on massively parallel sequencing. Similarly, Dobrzycka et al. [30] detected *KRAS* mutations in 23% of mucinous epithelial ovarian cancers. Although the *KRAS* mutation appears more frequently in mucinous epithelial ovarian cancer, *KRAS* mutations were identified in 20% of Korean ovarian clear cell carcinoma patients [11]. Meanwhile, Zannoni et al. [23] showed that *KRAS* mutations were present in 13% of OCCC patients, especially codon 12 mutations. In the current study, we revealed *KRAS* mutations in 22.2% (2/9) of patients with OCCC. Among these patients, we revealed one patient with a rare coexistence of *KRAS* and *ARID1A* mutations and another patient with rare coexistence of *KRAS*, *ARID1A*, and *PIK3CA* mutations.

Taken together, our results are consistent with those of other studies and suggest that *ARID1A* and *PIK3CA* were frequently mutated in

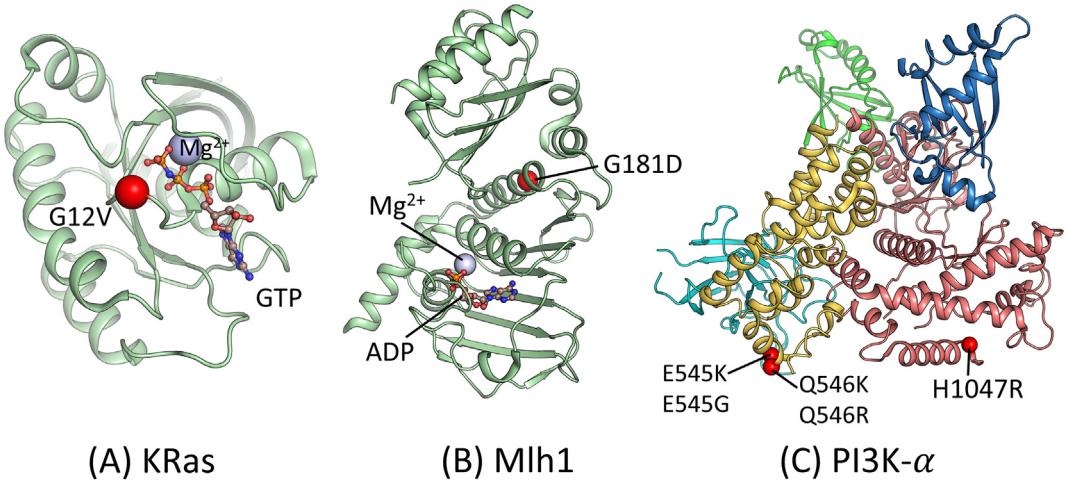

Fig. 3. Ribbon diagram of the protein structures of (A) KRas (PDB ID: 3GFT), (B) Mlh1 (PDB ID: 4P7A), and (C) PI3K- (PDB ID: 2RD0). The most frequent hotspot mutation sites are labeled and shown as red spheres. The KRas hotspot mutation G12V is located at the P-loop. The Mlh1 hotspot mutation G181D is located at the 6-helix. The PIK3 hotspot mutation sites E545 and Q546 are located in the helical domain (yellow) and H1047R is located in the kinase domain (red). The figure was generated using PyMOL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Studies that investigated *ARID1A* and *PIK3CA* mutations in ovarian cancer.

Authors, year of publication	Ovarian carcinoma subtypes	*ARID1A* mutations status	*PIK3CA* mutations status	Ref
Friedlander ML et al., 2016	521 clear cell ovarian cancers	–	*PIK3CA* mutations in 52 of 104 pure clear cell ovarian cancers samples (50%).	[17]
Rahman M et al., 2012	56 ovarian clear cell carcinomas	–	*PIK3CA* mutations in 16 of 56 ovarian clear cell carcinomas (28.6%).	[18]
Kuo KT et al., 2009	97 ovarian clear cell carcinomas (including 10 ovarian clear cell carcinomas cell lines)	–	*PIK3CA* mutations in 32 of 97 cases of ovarian clear cell carcinomas (including 10 ovarian clear cell carcinomas cell lines) (33%).	[19]
Jones S et al., 2010	42 ovarian clear cell carcinomas	*ARID1A* mutations in 24 of 42 ovarian clear cell carcinomas (57%).	*PIK3CA* mutations in 17 of 42 ovarian clear cell carcinomas (40%).	[20]
Shibuya Y et al., 2018	48 ovarian clear cell carcinomas	*ARID1A* mutations in 32 of 48 ovarian clear cell carcinomas (66.7%).	*PIK3CA* mutations in 24 of 48 ovarian clear cell carcinomas (50%).	[12]
Murakami R et al., 2017	39 ovarian clear cell carcinomas	*ARID1A* mutations in 24 of 39 ovarian clear cell carcinomas (62%).	*PIK3CA* mutations in 20 of 39 ovarian clear cell carcinomas (51%).	[21]
Maru Y et al., 2017	18 ovarian clear cell carcinomas	–	*PIK3CA* mutations in 5 of 18 ovarian clear cell carcinomas (28%).	[22]
Zannoni GF et al., 2016	63 ovarian clear cell carcinomas	–	*PIK3CA* mutations in 20 of 63 ovarian clear cell carcinomas (32%).	[23]
Kim SI et al., 2018	15 ovarian clear cell carcinomas	*ARID1A* mutations in 6 of 15 ovarian clear cell carcinomas (40%).	*PIK3CA* mutations in 6 of 15 ovarian clear cell carcinomas (40%).	[11]

OCCC. The current study has some limitations that must be noted. First, the size of the study was relatively small (n = 16). Second, there is a lack of comprehensive clinical information related to therapies or responses, prognosis, and survival rate. Further work needs to be done to collaborate with research centers throughout Taiwan to understand the molecular alterations in EOC and to better identify therapeutic targets for patients with EOC.

In summary, we have successfully developed a custom NGS-based panel including 9 cancer-associated genes, namely, *ARID1A*, *ARID2*, *CREBBP*, *NUMA1*, *PPP2R1A*, *NCOA2*, *MLH1*, *PIK3CA*, and *KRAS*. We also successfully characterized the genomic landscape of 16 patients with EOC. In the current study, we identified 27 nonsynonymous somatic mutations located in 5 genes. Our study may provide an opportunity for future studies to investigate therapeutic targets for the treatment of EOC.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.03.003.

Author contributions

Concept and design of the experiments: YFS, EMT, CCC, CCW, and TKE. Performance of the experiments: YFS, CCC, and TKE. Data analysis and discussion: YFS, EMT, CCC, CCW, and TK. Contribution of reagents/materials/analysis tools: EMT and TKE. Clinical information: EMT and CCW. Manuscript preparation: YFS, EMT, CCC, CCW, and TKE. All authors read and approved the final manuscript.

Conflict of interest

The authors report no conflict of interest.

Acknowledgements

We thank ACT Genomics Co., Ltd. for their assistance with bioinformatics analysis. We are also grateful to Dr. Shu-Jen Chen and Dr. Yen-Jung Lu for their expertise in the fields of NGS and bioinformatics. This study was supported by grants from Asia University and China Medical University Hospital (ASIA-105-CMUH-07, ASIA-106-CMUH-14, ASIA-106-51009, and ASIA-107-51009).

References

[1] L.E. Dockery, W.P. Tew, K. Ding, K.N. Moore, Tolerance and toxicity of the PARP inhibitor olaparib in older women with epithelial ovarian cancer, Gynecol. Oncol. 147 (3) (2017) 509–513.
[2] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, CA Cancer J. Clin. 67 (1) (2017) 7–30.
[3] A. Chao, T.C. Chang, N. Lapke, S.M. Jung, P. Chi, C.H. Chen, L.Y. Yang, C.T. Lin, H.J. Huang, H.H. Chou, J.D. Liou, S.J. Chen, T.H. Wang, C.H. Lai, Prevalence and clinical significance of BRCA1/2 germline and somatic mutations in Taiwanese patients with ovarian cancer, Oncotarget 7 (51) (2016) 85529–85541.
[4] S.F. Serov, R.E. Scully, L.H. Sobin, Histological typing of ovarian tumours, International Histological Classification of Tumours, vol. 9, World Health Organization, Geneva, 1973, pp. 19–20.
[5] A. Desai, J. Xu, K. Aysola, Y. Qin, C. Okoli, R. Hariprasad, U. Chinemerem, C. Gates, A. Reddy, O. Danner, G. Franklin, A. Ngozi, G. Cantuaria, K. Singh, W. Grizzle, C. Landen, E.E. Partridge, V.M. Rice, E.S. Reddy, V.N. Rao, Epithelial ovarian cancer: an overview, World J. Transl. Med. 3 (1) (2014) 1–8.
[6] J.D. Seidman, I. Horkayne-Szakaly, M. Haiba, C.R. Boice, R.J. Kurman, B.M. Ronnett, The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin, Int. J. Gynecol. Pathol. 23 (1) (2004) 41–44.
[7] M.E. Schaner, D.T. Ross, G. Ciaravino, T. Sorlie, O. Troyanskaya, M. Diehn, Y.C. Wang, G.E. Duran, T.L. Sikic, S. Caldeira, H. Skomedal, I.P. Tu, T. Hernandez-Boussard, S.W. Johnson, P.J. O'Dwyer, M.J. Fero, G.B. Kristensen, A.L. Borresen-Dale, T. Hastie, R. Tibshirani, M. van de Rijn, N.N. Teng, T.A. Longacre, D. Botstein, P.O. Brown, B.I. Sikic, Gene expression patterns in ovarian carcinomas, Mol. Biol. Cell 14 (11) (2003) 4376–4386.
[8] M. Koshiyama, N. Matsumura, I. Konishi, Recent concepts of ovarian carcinogenesis: type I and type II, Biomed. Res. Int. 2014 (2014) 934261.
[9] C. Meldrum, M.A. Doyle, R.W. Tothill, Next-generation sequencing for cancer diagnostics: a practical perspective, Clin. Biochem. Rev. 32 (4) (2011) 177–195.
[10] K.J. Eoh, J.E. Kim, H.S. Park, S.T. Lee, J.S. Park, J.W. Han, J.Y. Lee, S. Kim, S.W. Kim, J.H. Kim, Y.T. Kim, E.J. Nam, Detection of germline mutations in patients

with epithelial ovarian cancer using multi-gene panels: beyond BRCA1/2, Cancer Res. Treat. 50 (3) (2018) 917–925.
[11] S.I. Kim, J.W. Lee, M. Lee, H.S. Kim, H.H. Chung, J.W. Kim, N.H. Park, Y.S. Song, J.S. Seo, Genomic landscape of ovarian clear cell carcinoma via whole exome sequencing, Gynecol. Oncol. 148 (2) (2018) 375–382.
[12] Y. Shibuya, H. Tokunaga, S. Saito, K. Shimokawa, F. Katsuoka, L. Bin, K. Kojima, M. Nagasaki, M. Yamamoto, N. Yaegashi, J. Yasuda, Identification of somatic genetic alterations in ovarian clear cell carcinoma with next generation sequencing, Genes Chromosom. Cancer 57 (2) (2018) 51–60.
[13] T.K. Er, Y.F. Su, C.C. Wu, C.C. Chen, J. Wang, T.H. Hsieh, M. Herreros-Villanueva, W.T. Chen, Y.T. Chen, T.C. Liu, H.S. Chen, E.M. Tsai, Targeted next-generation sequencing for molecular diagnosis of endometriosis-associated ovarian cancer, J. Mol. Med. (Berl.) 94 (7) (2016) 835–847.
[14] E. Dicks, H.L. Song, S.J. Ramus, E. Van Oudenhove, J.P. Tyrer, M.P. Intermaggio, S. Kar, P. Harrington, D.D. Bowtell, M.S. Cicek, J.M. Cunningham, B.L. Fridley, J. Alsop, M. Jimenez-Linan, A. Piskorz, T. Goranova, E. Kent, N. Siddiqui, J. Paul, R. Crawford, S. Poblete, S. Lele, L. Sucheston-Campbell, K.B. Moysich, W. Sieh, V. McGuire, J. Lester, K. Odunsi, A.S. Whittemore, N. Bogdanova, M. Durst, P. Hillemanns, B.Y. Karlan, A. Gentry-Maharaj, U. Menon, M. Tischkowitz, D. Levine, J.D. Brenton, T. Dork, E.L. Goode, S.A. Gayther, P.D.P. Pharoah, A.S. Grp, Germline whole exome sequencing and large-scale replication identifies FANCM as a likely high grade serous ovarian cancer susceptibility gene, Oncotarget 8 (31) (2017) 50930–50940.
[15] N.L. Sim, P. Kumar, J. Hu, S. Henikoff, G. Schneider, P.C. Ng, SIFT web server: predicting effects of amino acid substitutions on proteins, Nucleic Acids Res. 40 (2012) W452–W457 Web Server issue.
[16] I.A. Adzhubei, S. Schmidt, L. Peshkin, V.E. Ramensky, A. Gerasimova, P. Bork, A.S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods 7 (4) (2010) 248–249.
[17] M.L. Friedlander, K. Russell, S. Millis, Z. Gatalica, R. Bender, A. Voss, Molecular profiling of clear cell ovarian cancers: identifying potential treatment targets for clinical trials, Int. J. Gynecol. Cancer 26 (4) (2016) 648–654.
[18] M. Rahman, K. Nakayama, M.T. Rahman, N. Nakayama, M. Ishikawa, A. Katagiri, K. Iida, S. Nakayama, Y. Otsuki, M. Shih Ie, K. Miyazaki, Clinicopathologic and biological analysis of PIK3CA mutation in ovarian clear cell carcinoma, Hum. Pathol. 43 (12) (2012) 2197–2206.
[19] K.T. Kuo, T.L. Mao, S. Jones, E. Veras, A. Ayhan, T.L. Wang, R. Glas, D. Slamon, V.E. Velculescu, R.J. Kuman, M. Shih Ie, Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma, Am. J. Pathol. 174 (5) (2009) 1597–1601.
[20] S. Jones, T.L. Wang, M. Shih Ie, T.L. Mao, K. Nakayama, R. Roden, R. Glas, D. Slamon, L.A. Diaz Jr., B. Vogelstein, K.W. Kinzler, V.E. Velculescu, N. Papadopoulos, Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma, Science 330 (6001) (2010) 228–231.
[21] R. Murakami, N. Matsumura, J.B. Brown, K. Higasa, T. Tsutsumi, M. Kamada, H. Abou-Taleb, Y. Hosoe, S. Kitamura, K. Yamaguchi, K. Abiko, J. Hamanishi, T. Baba, M. Koshiyama, Y. Okuno, R. Yamada, F. Matsuda, I. Konishi, M. Mandai, Exome sequencing landscape analysis in ovarian clear cell carcinoma shed light on key chromosomal regions and mutation gene networks, Am. J. Pathol. 187 (10) (2017) 2246–2258.
[22] Y. Maru, N. Tanaka, M. Ohira, M. Itami, Y. Hippo, H. Nagase, Identification of novel mutations in Japanese ovarian clear cell carcinoma patients using optimized targeted NGS for clinical diagnosis, Gynecol. Oncol. 144 (2) (2017) 377–383.
[23] G.F. Zannoni, G. Improta, A. Pettinato, C. Brunelli, G. Troncone, G. Scambia, F. Fraggetta, Molecular status of PI3KCA, KRAS and BRAF in ovarian clear cell carcinoma: an analysis of 63 patients, J. Clin. Pathol. 69 (12) (2016) 1088–1092.
[24] R.L. Chandler, J.S. Damrauer, J.R. Raab, J.C. Schisler, M.D. Wilkerson, J.P. Didion, J. Starmer, D. Serber, D. Yee, J. Xiong, D.B. Darr, F.P.M. de Villena, W.Y. Kim, T. Magnuson, Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling, Nat. Commun. 6 (2015).
[25] B.G. Bitler, N. Fatkhutdinov, R. Zhang, Potential therapeutic targets in ARID1A-mutated cancers, Expert Opin. Ther. Targets 19 (11) (2015) 1419–1422.
[26] S. Yamamoto, H. Tsuda, M. Takano, S. Tamai, O. Matsubara, Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations, Mod. Pathol. 25 (4) (2012) 615–624.
[27] A.E. Karnoub, R.A. Weinberg, Ras oncogenes: split personalities, Nat. Rev. Mol. Cell Biol. 9 (7) (2008) 517–531.
[28] J. Tammela, K. Odunsi, Gene expression and prognostic significance in ovarian cancer, Minerva Ginecol. 56 (6) (2004) 495–502.
[29] J.J. Mueller, B.A. Schlappe, R. Kumar, N. Olvera, F. Dao, N. Abu-Rustum, C. Aghajanian, D. DeLair, Y.R. Hussein, R.A. Soslow, D.A. Levine, B. Weigelt, Massively parallel sequencing analysis of mucinous ovarian carcinomas: genomic profiling and differential diagnoses, Gynecol. Oncol. 150 (1) (2018) 127–135.
[30] B. Dobrzycka, S.J. Terlikowski, O. Kowalczuk, W. Niklinska, L. Chyczewski, M. Kulikowski, Mutations in the KRAS gene in ovarian tumors, Folia Histochem. Cytobiol. 47 (2) (2009) 221–224.