Profiling of actionable gene alterations in ovarian cancer by targeted deep sequencing

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Abstract. To construct a profile of therapeutically actionable gene alterations in the major histological types of ovarian cancer, 72 Japanese patients with surgically resected ovarian cancers were selected from an original cohort consisting of 267 patients who had not received pre-treatment before surgery. Somatic mutations and copy number alterations at 740 hotspots in 46 cancer-related genes were detected by deep sequencing of genomic DNAs obtained from snap-frozen tumor tissues using a next generation sequencer. The alterations were verified by Sanger sequencing and quantitative genomic PCR. Mutations and/or copy number aberrations which will make tumors respond to molecular targeting drugs were detected in nine genes of 35/72 (48.6%) patients; *PIK3CA* (25.0%), *KRAS* (13.9%), *ERBB2* (4.3%), *PTEN* (2.8%), *RB1* (2.8%), *CDKN2A* (2.8%), *AKT1* (1.4%), *CTNNB1* (1.4%) and *NRAS* (1.4%). These mutations tended to occur in a mutually exclusive manner. Non-serous histological type tumors showed such actionable gene alterations frequently (32/47; 68.1%). Therefore, ovarian cancers, particularly of non-serous types, frequently carry gene aberrations that link to therapy using molecular targeting drugs.

Introduction

Ovarian cancer is a leading cause of cancer mortality from gynecological malignancies worldwide and, in the United States and Japan, accounts for ~14,000 and 4,600 deaths annually (1,2). Ovarian cancer predominantly consists of four major histological types: serous, clear cell, endometrioid and mucinous adenocarcinomas. The incidence of each of these subtypes varies geographically: serous carcinoma is the most common type in Western and Asian countries; clear cell adenocarcinoma is prevalent (the second-most common) in Japan, but not in most of the other Asian countries, and is not prevalent in European countries (2,3). More than 70% of ovarian cancers are diagnosed as advanced stage cancers (3). The majority of these patients with advanced ovarian cancer show an initial response to platinum-based chemotherapy; however, most of these patients relapse (3). Consequently, the 5-year overall survival rate for ovarian cancer patients remains <50% (3). Personalized therapy using molecular targeting drugs based on gene aberrations in tumor cells is a promising option to improve the therapeutic efficacy of the treatment for advanced ovarian cancer (4).

Recent genome-wide analysis has revealed alterations of ovarian cancer genomes (5-8). The most frequent alterations found are inactivating mutations in tumor suppressor genes, such as *TP53, PTEN, BRCA1, BRCA2,* and *RB1*, and in a SWI/SNF chromatin remodeling gene, *ARID1A*. Other studies have detected activating mutations in the oncogenes *KRAS, BRAF, PIK3CA* and *ERBB2*, indicating that a subset of patients with ovarian cancer could benefit from therapy using existing molecular targeting drugs (5-7,9-11). However, the prevalence and specificity of such oncogene aberrations by clinicopathological factors, such as histological subtype, and whether the aberrations are present in a mutually exclusive manner, have not been fully examined in a defined population. Thus, we constructed a profile of actionable aberrations of 46 cancer-related genes in a cohort of 72 Japanese ovarian cancer patients. The cohort was chosen from 267 consecutive patients who had received surgery for ovarian cancer. Of these patients, 72 patients with ovarian cancer were surgically treated without prior chemotherapy, and the carcinomas in this cohort included all four histological tumor types and tumors at various stages.

Materials and methods

Patient cohort. Seventy-two patients with ovarian cancer (study cohort subjects) were selected from 267 consecutive patients with ovarian cancer (original cohort) who received surgery for ovarian cancer in the Department of Obstetrics and Gynecology, Jikei University School of Medicine, Tokyo,

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Japan, between 2000 and 2009. The 72 subjects were surgically treated patients with ovarian cancer who had not had prior chemotherapy. The selection procedure ensured that all four major histological types and stages of tumor were included in proportions similar to those found in the original cohort and were also representative of the proportions found in all Japanese ovarian cancer patients (Fig. 1) (2,3). Written informed consent was obtained from all patients. This study was approved by the Institutional Review Board of the contributing institutions.

The tumors and the adjacent non-cancerous tissues were macro-dissected and flash-frozen after surgery. All tumor tissues were resected from solid components without necrotic tissue in each tumor. Several tumor tissues were randomly selected for making paraffin sections and their cellularity was confirmed as being >80%.

Clinical information for each patient, including age, stage, histology, grade, residual tumor, treatment information, and survival time from primary surgery, was collected retrospectively. Tumors were staged in accordance with the International Federation on Gynecology and Obstetrics (FIGO) system. For each patient, the size of the residual tumor was recorded at the end of surgery. Tumors resistant to platinum-containing adjuvant chemotherapy (i.e., platinum resistance) were defined as those in patients who exhibited progression-free survival for <6 months after the completion of chemotherapy.

Cell lines. Fourteen ovarian cancer cell lines were used in this study. JHOC-5, JHOC-7, JHOC-8, and JHOC-9 were obtained from Riken BioResource Center (Tsukuba, Japan). HAC-2 was provided by Dr M. Nishida (Tsukuba University, Tsukuba, Japan). RMG-I and RMG-II were provided by Dr D. Aoki (Keio University, Tokyo, Japan). A2780 (undifferentiated carcinoma) was provided by Dr E. Reed (NCI, Bethesda, MD, USA) and 2008 was provided by Dr S.B. Howell (UCSD, San Diego, CA, USA). SKOV3, MCAS, TYK-nu, Ov-1063, and SW626 were obtained from ATCC (Rockville, MD, USA).

Deep sequencing of 46 cancer-related genes. Genomic DNA was extracted using a QIAamp DNA mini kit according to the manufacturer's instructions (Qiagen, Limburg, The Netherlands). Purified genomic DNA obtained from tumor tissues and cell lines (10 ng) was used for the library construction using the Ion AmpliSeq Cancer primer pool (cat. no. 4471262, Life Technologies, Rockville, MD, USA) that targets 739 mutational hotspot regions of 46 cancer-related genes and, additionally, a set of custom primers for the E17K mutation hotspot in the *AKT1* gene. Sequencing was run on the Ion Proton/PGM platform (Life Technologies). The median depth of coverage for aligned reads was 3,024 x (2,010-35,534) by map quality ≥20. Data analysis, including the hg19 human reference genome and variant calling, was carried out using the Torrent Suite Software v3.2 (Life Technologies).

Sanger sequencing. Genomic DNA (10 ng) was amplified by PCR using KAPA Taq DNA Polymerase (KAPA Biosystems, Woburn, MA, USA). PCR products were directly sequenced in both directions using the BigDye Termination kit and an ABI 3130xl DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Real-time genomic PCR. Copy number variations suggested by deep sequencing analysis were validated by real-time genomic PCR using a TaqMan Copy Number Assay and the ABI 7900HT real-time PCR system (Applied Biosystems). All TaqMan probes were purchased from Thermo Applied Biosystems: *ERBB2* (ID Hs01932585_cn), *PTEN* (ID Hs05128032_cn), *RB1* (ID Hs00331762_cn and a set of custom primers), *TP53* (ID Hs06424630_cn), and *FGFR1* (ID Hs02422066_cn) with *RPPH1* (cat. no. 4403328) as a reference. Data were analyzed using ABI PRISM 7900HT Sequence Detection Software v2.3 for copy number analysis.

Statistical analysis. Statistical analyses were performed using JMP software (SAS Institute, New York, NY, USA). Associations of the gene alterations with clinicopathological factors were evaluated using Fisher's exact test. For survival analysis, the Cox proportional hazard model was used for the univariate and multivariate analyses.

Results

Profiling of aberrations in 46 cancer-related genes in 72 ovarian cancers. Clinical and histological characteristics of the study subjects are provided in Table I. The frequency of clear cell adenocarcinoma in this cohort was higher than that found in other countries, reflecting known prevalence of ovarian cancer in Japan (2,3).

We sequenced genomic DNAs from 72 ovarian cancer tissues, with a mean sequencing depth >2,000 in all cases, followed by Sanger sequencing validation (representative results in Fig. 2A). The results revealed 115 single-nucleotide variations (SNVs), but no insertions/deletions, at 740 hotspot sites in 46 cancer-related genes. The 115 SNVs were of 50 distinct types, and included 64 SNVs (43 types) that were deduced as somatic according to data from the Catalogue of Somatic Mutation in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). The somatic nature of these mutations was verified in some samples by Sanger sequencing of DNAs from corresponding non-cancerous tissues (Table II). In addition, another SNV detected in a single case, *PIK3CA*-N345H, caused an amino acid change in the PIK3CA protein, which is recurrently mutated in human cancers. Therefore, these 65 SNVs (44 types) were considered to represent somatic mutations. Furthermore, Sanger sequencing of DNAs from non-cancerous tissue revealed that the remaining 50 SNVs (six types) consisted of two missense mutations and 48 single-nucleotide polymorphisms (four types). Thus, in total, 67 somatic missense mutations (46 types) were detected in the study cohort (Table II).

TP53 (38.9%), *PIK3CA* (25.0%), and *KRAS* (13.9%) were the three most frequently mutated genes (Table III). The other genes in which mutations were found were *PTEN, FGFR2, CDKN2A, AKT1, CTNNB1, NRAS, MET* and *KIT*. The frequency of the *TP53, PIK3CA* and *KRAS* mutations were different in each histological subtype. *TP53* was more frequently mutated in serous carcinomas (56.0%) than in the other subtypes (P=0.042 by Fisher's exact test, compared with non-serous patients with ovarian cancer; 29.8%); *PIK3CA* was more frequently mutated in clear cell carcinomas (48.1%) than in the other subtypes

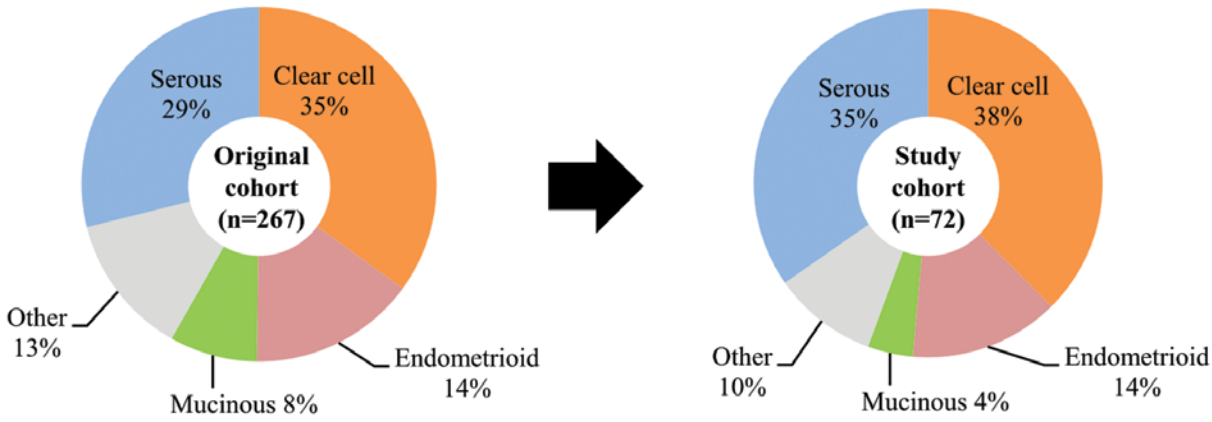

Figure 1. Preparation of the study cohort. The study cohort included all four representative histological types of ovarian carcinoma in proportions similar to those of the original cohort.

Table I. Characteristics of 72 Japanese patients with ovarian cancer.

Clinicopathological variables	No. of patients with ovarian cancer	Frequency (%)
Age		
<60	49	68
≥60	23	32
Stage		
I	31	43
II	7	10
III	27	37
IV	7	10
Histology		
Clear cell	27	37
Endometrioid	10	14
Mucinous	3	4
Other	7	10
Serous	25	35
Grade		
G1	11	15
G2	15	21
G3	11	15
Unknown/not graded	35	49
Residual tumor (cm)		
≤1	55	76
>1	17	24
Adjuvant chemotherapy		
Platinum	1	1
Platinum + Taxane	45	63
Platinum + Irinotecan	18	25
None	8	11
Platinum resistance		
Sensitive	52	72
Resistant	11	15
Not evaluated	9	13

(P<0.001 by Fisher's exact test, compared with non-clear cell carcinoma patients; 11.1%), as previously indicated (7,10,12-14). KRAS was more frequently mutated in clear cell carcinomas (25.9%) than in the other subtypes, consistent with previous reports (P=0.034 by Fisher's exact test, compared with non-clear cell carcinoma patients; 6.7%) (9,10,15-17).

Copy number aberrations (CNAs) in the 46 genes were deduced by calculating the ratios of the sequence read fraction in each tumor compared to the sequence read fraction of a single non-cancerous tissue subjected to sequencing. Loci that were potentially affected were selected using the criteria of >2-times gains and <1/4-times losses (suggesting homozygous deletion), followed by verification with quantitative genomic PCR analysis (Fig. 2B). Ten CNAs in *PTEN* (1 case), *RB1* (4 cases), *TP53* (1 case), *ERBB2* (3 cases) and *FGFR1* (1 case) were suggested in 10 tumors, and six of them were confirmed by quantitative genomic PCR analysis; gains of the *ERBB2* gene in three patients (4.2%); and homozygous deletions of the *RB1* gene in two patients (2.8%) and of the *PTEN* gene in a patient (1.4%) (Table III).

Profile of cancer-related gene actionable alterations in the histological subtypes. A profile of therapeutically actionable alterations was next constructed (Fig. 2C). Genetic alterations, possibly affecting the gene function and sensitivity to existing therapeutic drugs or strategies, were selected here as actionable alterations. *ERBB2* amplification is a well-known actionable alteration (18-20). All the SNVs detected in the *AKT1, CTNNB1, KRAS, PIK3CA* and *NRAS* genes (31 SNVs in total) affected hotspot amino acids, and were considered actionable as targets for existing protein kinase inhibitors (21-25). In addition, the SNVs found in the *CDKN2A* and *PTEN* genes, and the *RB* and *PTEN* homozygous deletions, were considered actionable as they are linked to responsiveness to several inhibitors (26-28).

In total, 41 actionable gene alterations were detected in 35 of 72 (48.6%) patients (Fig. 3A). These mutations tended to exist in a mutually exclusive manner (Fig. 2C). In 30 of the 35 (85.7%) patients there was only one actionable alteration, while five patients had multiple alterations: one patient had mutations in three genes and four patients had mutations in two genes. In these five patients, fractions of mutant alleles

Table II. Somatic SNVs detected in 72 patients with ovarian cancer.

Gene	Position (ch: bp)	Nucleotide change	AA change	COSMIC ID	Variant rate (%)	SNV type	Validated as somatic change	Mutated sample
TP53	17: 7,579,882	G31C	E11Q	COSM11606	52.9	Non-synonymous	C	T12
	17: 7,579,358	G329C	R110P	COSM11250	48.6	Non-synonymous	C	T55
	17: 7,578,535	A395G	K132R	COSM11582	32.9	Non-synonymous	C	T3
	17: 7,578,534	G396C	K132N	COSM43963	83.9	Non-synonymous	C, S	T44
	17: 7,578,526	G404A	C135Y	COSM10801	70.5	Non-synonymous	C	T8
	17: 7,578,461	G469T	V157F	COSM10670	64.4	Non-synonymous	C, S	T22
	17: 7,578,454	C476T	A159V	COSM11148	59.0	Non-synonymous	C, S	T33
	17: 7,578,406	G524A	R175H	COSM10648	32.1-76.4	Non-synonymous	C, S	T23, T50, T57, T59
	17: 7,578,271	A578T	H193L	COSM11066	58.5-73.2	Non-synonymous	C, S	T16, T39
	17: 7,578,269	C580T	L194F	COSM10995	67.3	Non-synonymous	C	T65
	17: 7,578,263	C586T	R196X	COSM10705	65.6	Stop-gain	C, S	T48
	17: 7,578,257	G592T	E198X	COSM44241	50.8	Stop-gain	C, S	T30
	17: 7,578,212	C637T	R213X	COSM10654	52.3	Stop-gain	C, S	T21
	17: 7,578,203	G646A	V216M	COSM10667	71.8	Non-synonymous	C	T13
	17: 7,578,203	G646T	V216L	COSM11210	81.3	Non-synonymous	C, S	T67
	17: 7,578,196	T653G	V218G	COSM44198	71.5	Non-synonymous	C	T43
	17: 7,577,538	G743A	R248Q	COSM10662	76.1	Non-synonymous	C	T46
	17: 7,577,120	G818A	R273H	COSM10660	51.1-67.1	Non-synonymous	C	T49, T51
	17: 7,577,114	G824T	C275F	COSM10701	59.5	Non-synonymous	C, S	T69
	17: 7,577,106	C832A	P278T	COSM43697	22.1	Non-synonymous	C	T36
	17: 7,577,094	C844T	R282W	COSM10704	94.3	Non-synonymous	C, S	T25
	17: 7,577,022	C916T	R306X	COSM10663	55.1	Stop-gain	C	T58
	17: 7,574,003	C1024T	R342X	COSM11073	83.1	Stop-gain	C	T68
PIK3CA[a]	3: 178,916,876	G263A	R88Q	COSM746	32.5	Non-synonymous	C	T14
	3: 178,921,551	A1033C	N345H		65.6	Non-synonymous		T45
	3: 178,936,074	C1616G	P539R	COSM759	45.5	Non-synonymous	C, S	T71
	3: 178,936,082	G1624A	E542K	COSM760	10.1-22.2	Non-synonymous	C, S	T6, T42, T57, T63
	3: 178,936,091	G1633A	E545K	COSM763	18.8-71.6	Non-synonymous	C	T53, T60
	3: 178,936,094	C1636A	Q546K	COSM766	92.8-93.4	Non-synonymous	C	T27, T28
	3: 178,952,085	A3140T	H1047L	COSM776	31.6	Non-synonymous	C	T20
	3: 178,952,085	A3140G	H1047R	COSM775	12.5-42.4	Non-synonymous	C, S	T4, T7, T11, T12, T35, T36
KRAS[a]	12: 25,398,284	G35C	G12A	COSM522	6.8-22.1	Non-synonymous	C	T15, T63
	12: 25,398,284	G35A	G12D	COSM521	29.1-95.7	Non-synonymous	C, S	T10, T54, T72
	12: 25,398,284	G35T	G12V	COSM520	6.4-58.3	Non-synonymous	C, S	T5, T29, T37, T58
	12: 25,398,285	G34C	G12R	COSM518	46.1	Non-synonymous	C, S	T4
CDKN2A[a]	9: 21,971,161	A197G	H66R	COSM14253	36.5	Non-synonymous	C	T50
	9: 21,971,203	T155G	M52R	COSM608436	50.0	Non-synonymous	C	T51
FGFR2	10: 123,279,677	C755G	S252W	COSM36903	21.3	Non-synonymous	C	T36
	10: 123,258,036	A1645C	N549H	COSM250083	38.7	Non-synonymous	C, S	T14
PTEN[a]	10: 89,711,902	T520G	Y174D	COSM28897	73.6	Non-synonymous	C	T20
	10: 89,720,799	T950G	V317G		41.9	Non-synonymous	S	T44
AKT1[a]	14: 105,246,551	G49A	E17K	COSM33765	23.0	Non-synonymous	C	T11
CTNNB1[a]	3: 41,266,103	G100C	G34R	COSM5684	16.8	Non-synonymous	C	T31
KIT	4: 55,593,464	A1621C	M541L	COSM28026	56.2	Non-synonymous	C	T2
MET	7: 116,411,966	T3005C	V1002A		52.2	Non-synonymous	S	T65
NRAS[a]	1: 115,258,747	G35A	G12D	COSM564	60.4	Non-synonymous	C	T20

[a]Actionable gene. S, somatic mutations confirmed by Sanger sequencing. C, somatic mutations validated in the COSMIC database.

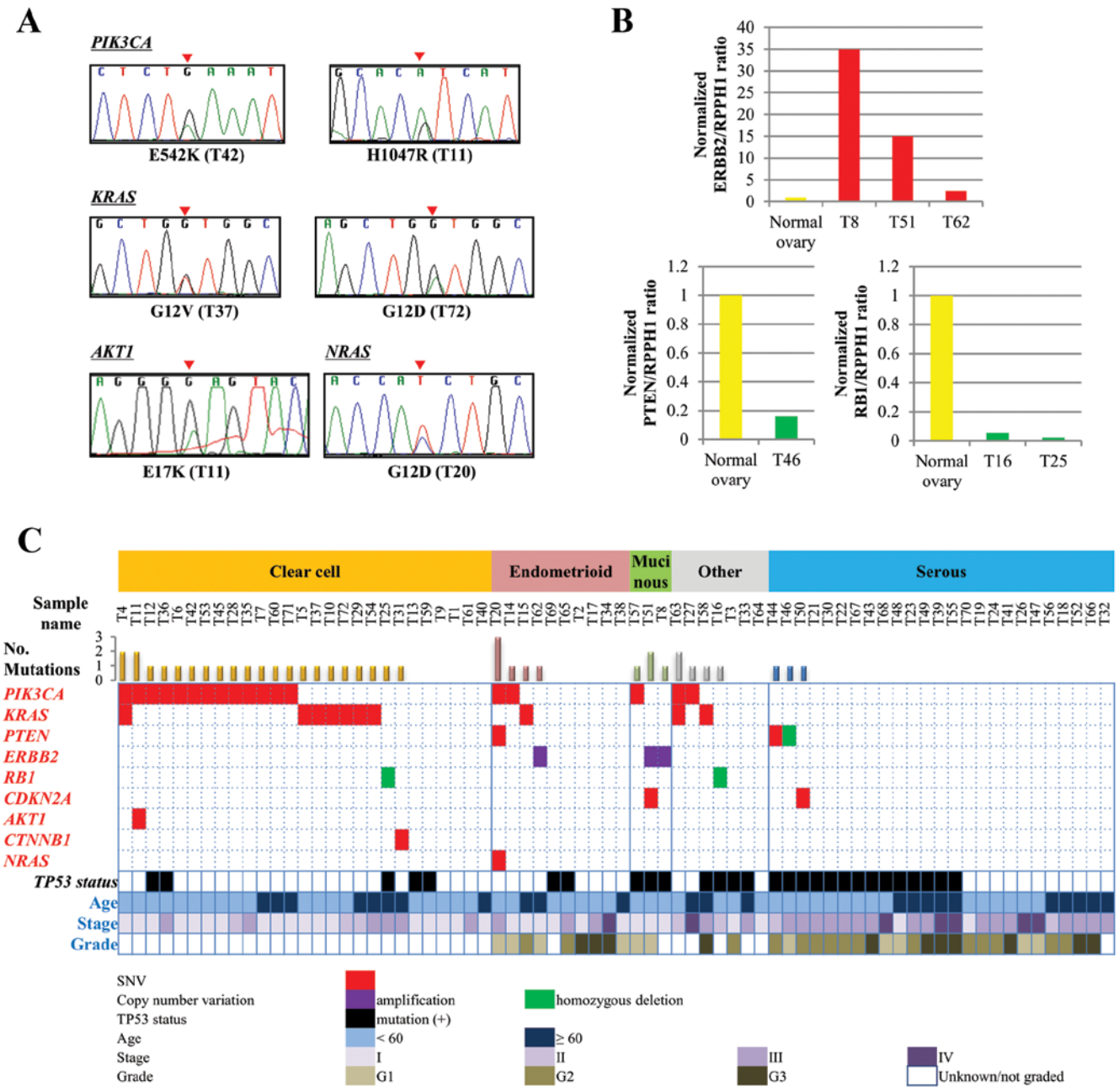

Figure 2. Validation of the somatic mutation and copy number validation, and profiling of actionable gene alterations. (A) Representative hotspot mutations validated by Sanger DNA sequencing. (B) Results of real-time genomic PCR analysis for *ERBB2* amplification and the homozygous deletions of *PTEN* and *RB1*. (C) Actionable gene alterations in 72 Japanese ovarian carcinomas. Cases are arranged from left to right by histological type: clear cell, endometrioid, mucinous, other, and serous. The total number of actionable genetic alterations is indicated by vertical bars.

were not evidently different between mutated genes, therefore, these mutations were likely to have occurred in similar fractions of cancer cells in each tissue. Of the different histological subtypes, clear cell carcinomas showed the highest frequency of actionable alterations (21/27; 77.8%), whereas serous carcinomas had the lowest frequency (3/25; 12.0%; Fig. 3B and C). This was largely due to the differential occurrence of *PIK3CA* mutations in the different histological subtypes (Fig. 2C). In non-serous carcinomas, the majority (32/47; 68.1%) had at least one actionable mutation (P<0.0001 by Fisher's exact test, compared with serous carcinomas).

We proceeded to investigate the associations between the actionable alterations and clinicopathological factors (Fig. 4). However, we identified no significant associations between any of the actionable alterations and age, stage, differentiation grade, presence/absence of residual tumor, or therapeutic response to platinum therapy. Actionable gene alterations were not significantly associated with prognosis, either among all cases (data not shown) or among non-serous carcinomas in particular.

Discussion

We constructed an actionable gene alteration profile of ovarian cancer of a Japanese population using the deep genome sequencing method. The majority (48.6%) of ovarian cancers, in particular non-serous carcinomas (68.1%), were found to

Table III. SNVs and CNAs detected in 72 ovarian cancers.

Type of alteration	Gene/alteration	No (%) Total (n=72)	Clear cell (n=27)	Serous (n=25)	Endometrioid (n=10)	Mucinous (n=3)	Others (n=7)
SNV	*TP53*	28 (38.9)	5 (18.5)	14 (56.0)	2 (7.1)	3 (100)	4 (14.3)
	PIK3CA[a]	18 (25.0)	13 (48.1)	0	2 (20.0)	1 (33.3)	2 (28.6)
	KRAS[a]	10 (13.9)	7 (25.9)	0	1 (10.0)	0	2 (28.6)
	PTEN[a]	2 (2.8)	0	1 (4.0)	1 (10.0)	0	0
	FGFR2	2 (2.8)	1 (3.7)	0	1 (10.0)	0	0
	CDKN2A[a]	2 (2.8)	0	1 (4.0)	0	1 (33.3)	0
	AKT1[a]	1 (1.4)	1 (3.7)	0	0	0	0
	CTNNB1[a]	1 (1.4)	1 (3.7)	0	0	0	0
	NRAS[a]	1 (1.4)	0	0	1 (10.0)	0	0
	MET	1 (1.4)	0	0	1 (10.0)	0	0
	KIT	1 (1.4)	0	0	1 (10.0)	0	0
	Total	67	28	16	10	5	8
CNAs	*ERBB2*[a]/gain	3 (4.3)	0	0	1 (10.0)	2 (67.7)	0
	RB[a]/homozygous deletion	2 (2.8)	1 (3.7)	0	0	0	1 (14.3)
	PTEN[a]/homozygous deletion	1 (1.4)	0	1 (4.0)	0	0	0
	Total	6	1	1	1	2	1

[a]Actionable gene.

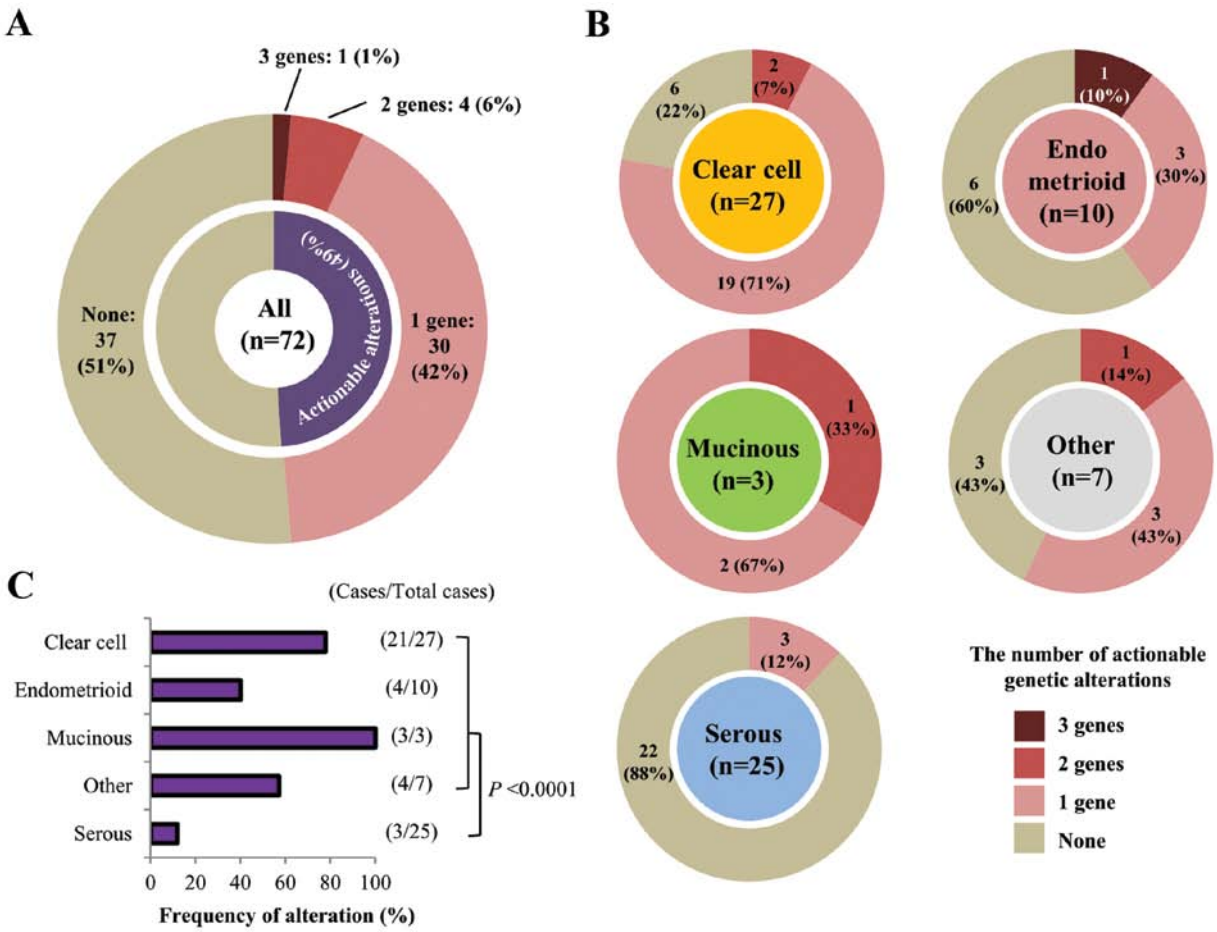

Figure 3. Distribution of actionable gene alterations in 72 ovarian carcinomas. (A) The number of genes with actionable alterations detected in 72 patients with ovarian cancer. The center circle represents the fraction of patients with ovarian cancer with at least one actionable event. (B) The number of actionable alterations for each histological subtype. (C) Frequencies of actionable gene alterations according to histological subtype. P-value by Fisher's exact test is shown.

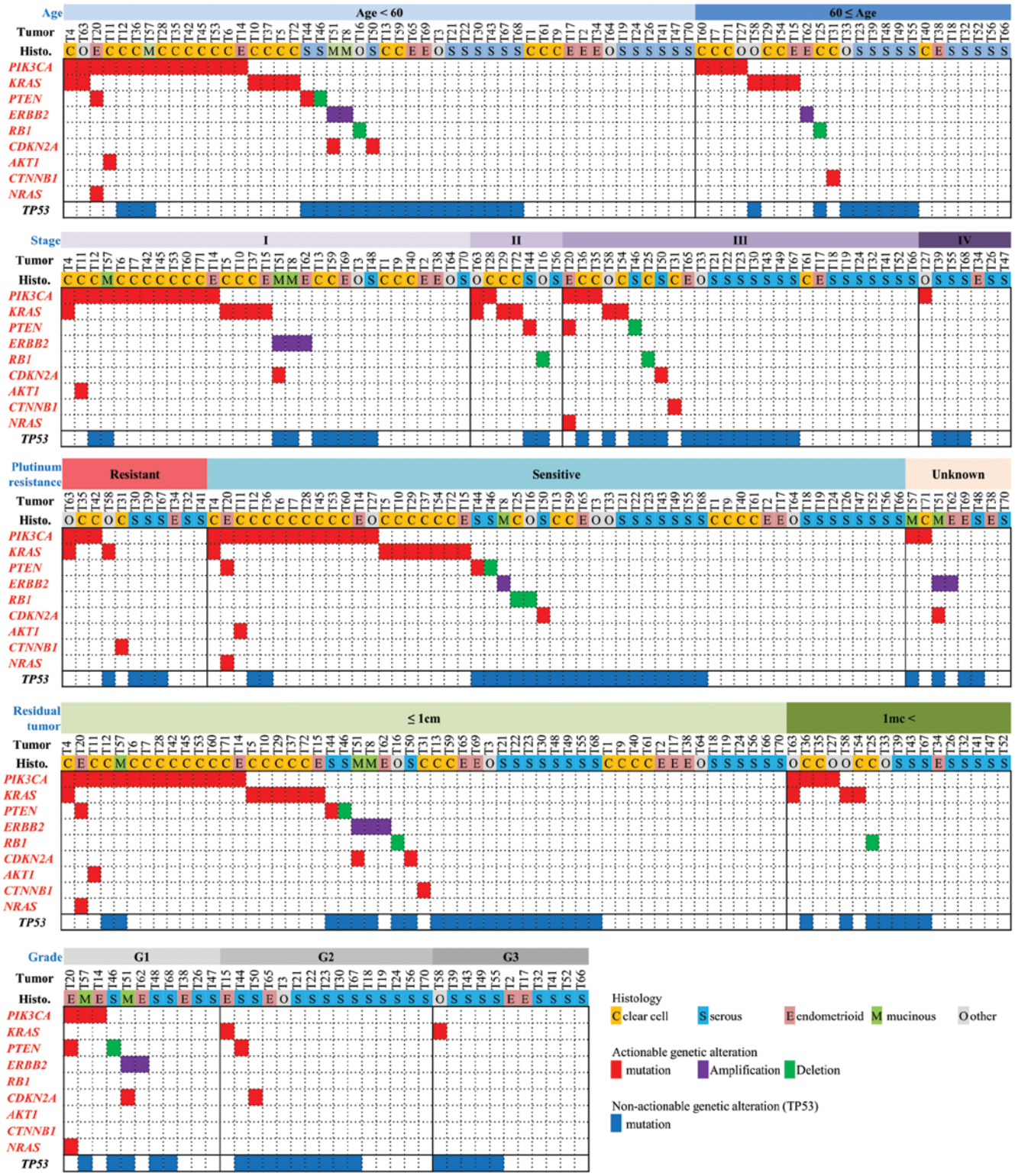

Figure 4. Distributions of actionable genetic alterations stratified by age, stage, platinum resistance, residual tumors and grade. Sample name, histological subtype and *TP53* mutations are shown.

carry at least one actionable alteration in the 46 cancer-related genes examined. The *TP53, PIK3CA* and *KRAS* genes were top three mutation genes. Consistent with previous reports (13,29), *TP53* and *PIK3CA* were preferentially mutated in serous and clear cell carcinoma, respectively, while *KRAS* was preferentially mutated in clear cell carcinoma (Table III). Distinct molecular features of Japanese ovarian cancers were suggested. Frequency of *KRAS* mutation in clear cell carcinoma (25.9%) was higher than that in cases described previously (7%) (13), while frequency of hotspot *TP53* mutations (57.8%) in high-grade serous carcinoma were less than that in Caucasian cases (>80%) (7,12). These results provide basic information for the understanding of ovarian carcinogenesis by different ethnicity.

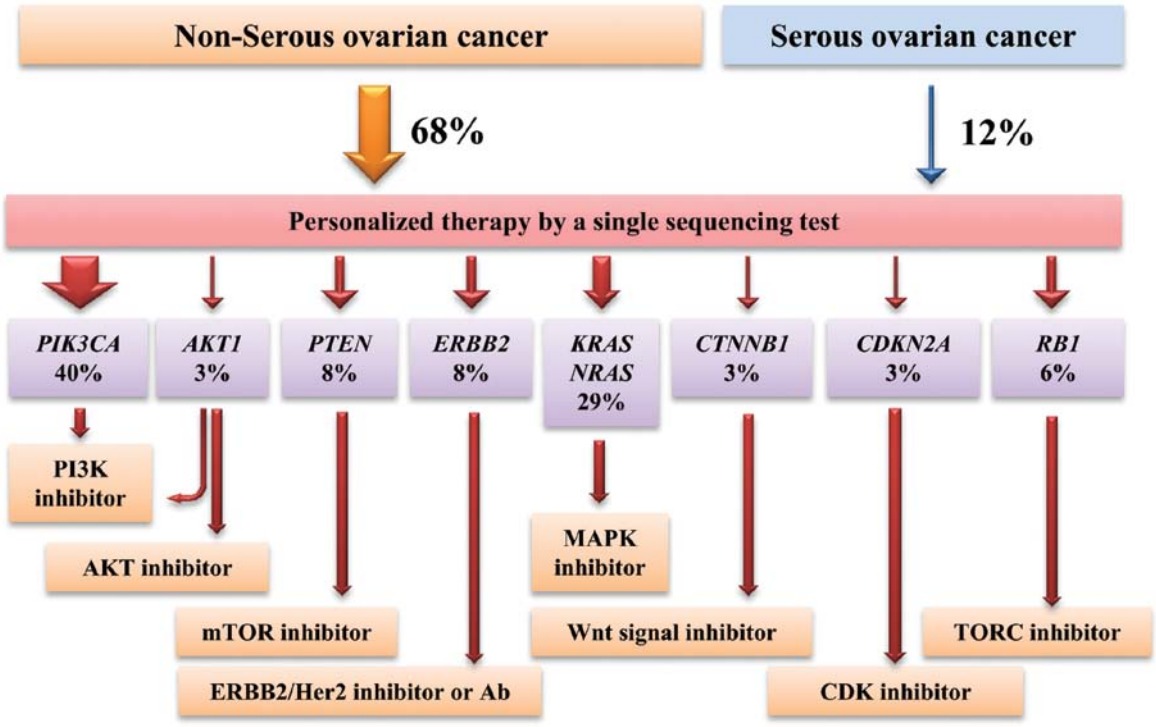

Figure 5. Potential personalized therapy for ovarian cancer based on actionable gene alterations. Percentages used in the upper tier, 68 and 12%, are those of non-serous and serous tumors, respectively, with at least one actionable gene alteration. The percentages of the above cases with actionable alterations in the indicated genes are shown in the lower tier. Candidate inhibitory drugs for each actionable gene alteration are shown at the bottom.

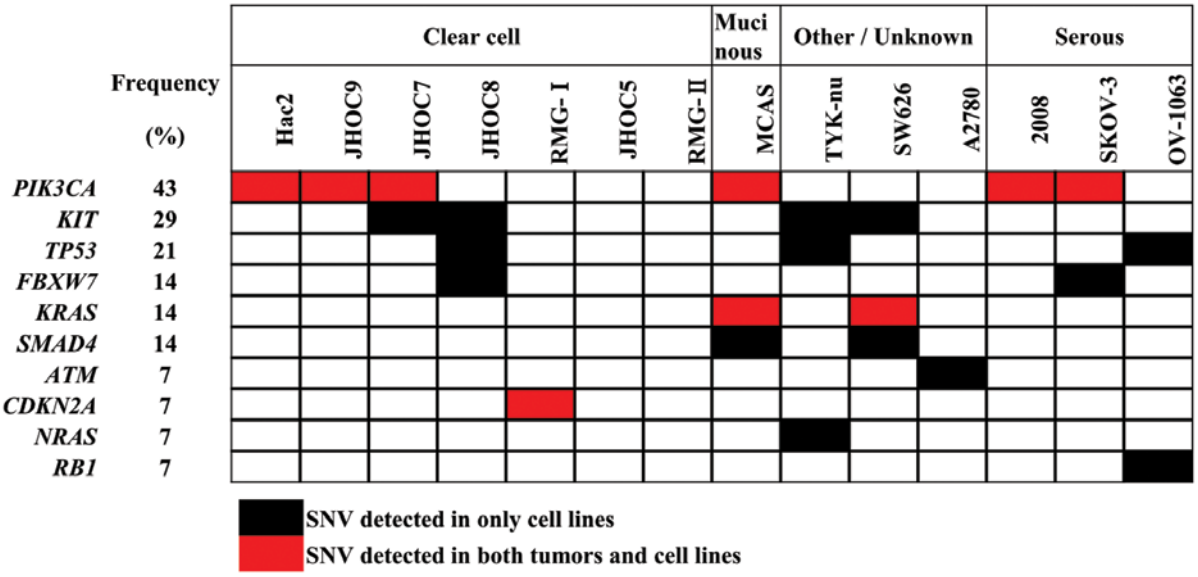

Figure 6. Mutational profile of 14 ovarian cancer cell lines. Mutations detected only in cell lines are marked in black and those detected in both surgical specimens and cell lines are marked in red.

According to this profile, therapeutic strategy using molecularly targeted drugs can be considered (Fig. 5). Tumors with *PIK3CA, AKT1* and PTEN mutations, which cause activation of the PI3K-AKT-mTOR pathway, are targetable by PI3K/AKT/mTOR inhibitors (21,24,27). Indeed, the results of clinical trials have demonstrated that ovarian tumors with *PIK3CA* mutations exhibit a high response rate to these inhibitors (30). Tumors with *ERBB2* amplification are targetable by ERBB2 inhibitors or antibodies, as evidenced by observations that ovarian cancer cases with *ERBB2* amplification exhibit high response rates to an anti-ERBB2 antibody drug (20,31,32).

Tumors with *KRAS* and *NRAS* mutations can be targeted by MAPK inhibitors, although in many cancers therapeutic responses are less than expected based on clinical trials (33). Sorafenib, which targets RAF and other kinases and inhibits the RAS-RAF-ERK pathway, has been shown to be an effective treatment for two Japanese patients with recurrent ovarian clear cell carcinoma (34). Selumetinib, a MEK1/MEK2 inhib-

Table IV. SNVs detected in 14 ovarian cancer cell lines.

Sample name	Gene	Position (ch: bp)	Nucleotide change	AA change	Variant rate (%)	SNV type
2008	*PIK3CA*	3: 178,936,091	G1633A	E545K	47.9	Non-synonymous
A2780	*ATM*	11: 108,123,551	C1810T	P604S	25.7	Non-synonymous
Hac2	*PIK3CA*	3: 178,952,085	A3140G	H1047R	70.8	Non-synonymous
JHOC7	*KIT*	4: 55,593,461	G1606C	V540L	58.5	Non-synonymous
JHOC7	*PIK3CA*	3: 178,936,082	G1624A	E542K	35.8	Non-synonymous
JHOC8	*FBXW7*	4: 153,247,366	G1196A	R399Q	75.3	Non-synonymous
JHOC8	*KIT*	4: 55,593,464	A1621C	M541L	99.8	Non-synonymous
JHOC8	*TP53*	17: 7,578,406	G524A	R175H	99.6	Non-synonymous
JHOC9	*PIK3CA*	3: 178,936,082	G1624A	E542K	96.1	Non-synonymous
MCAS	*KRAS*	12: 25,398,284	G35A	G12D	80.7	Non-synonymous
MCAS	*PIK3CA*	3: 178,952,085	A3140G	H1047R	48.2	Non-synonymous
MCAS	*SMAD4*	18: 48,604,690	T1512A	S504R	95.1	Non-synonymous
OV-1063	*RB1*	13: 49,037,903	A2143T	K715X	99.2	Stop-gain
OV-1063	*TP53*	17: 7,578,181	C272T	P91L	22.0	Non-synonymous
OV-1063	*TP53*	17: 7,577,118	G820T	V274F	59.0	Non-synonymous
RMG-1	*CDKN2A*	9: 21,971,184	A217C	S73R	100.0	Non-synonymous
SKOV-3	*FBXW7*	4: 153,247,288	G1274T	R425L	44.8	Non-synonymous
SKOV-3	*PIK3CA*	3: 178,952,085	A3140G	H1047R	46.0	Non-synonymous
SW626	*KIT*	4: 55,593,464	A1621C	M541L	48.3	Non-synonymous
SW626	*KRAS*	12: 25,398,284	G35T	G12V	51.0	Non-synonymous
SW626	*SMAD4*	18: 48,591,888	G1051C	D351H	99.6	Non-synonymous
TYK-nu	*KIT*	4: 55,593,464	A1621C	M541L	99.6	Stop-gain
TYK-nu	*NRAS*	1: 115,258,747	G35A	G12D	33.7	Non-synonymous
TYK-nu	*NRAS*	1: 115,256,530	C181A	Q61K	63.5	Non-synonymous
TYK-nu	*TP53*	17: 7,578,406	G524A	R175H	99.3	Non-synonymous

itor, significantly suppressed the growth of a mouse xenograft of a human ovarian clear cell adenocarcinoma (35). In addition, molecular targeted therapies for tumors with *CTNNB1* mutations and *CDKN2A* inactivation have been indicated (22,28), while tumors with *RB1* mutations are treatable by TORC inhibition based on synthetic lethality (28).

Importantly, the ability to prescribe a personalized therapy based on genetic alterations, guided by the single sequencing test described here, would be useful in clinical settings. Ovarian cancers in Japan include more non-serous cases than in other countries due to a higher fraction of clear cell cancers. Our study indicates that frequencies of the actionable alterations do not differ significantly by clinicopathological factors, therefore, analysis of all non-serous ovarian cancers at progressive stages will be an effective way to perform precision medicine of ovarian cancers based on actionable gene aberrations. In the strategy above, patients with serous ovarian cancers will not benefit from the therapy. However, recent studies have also suggested therapeutic approaches targeting p53 mutant proteins (36). Such approaches will benefit ovarian cancer patients with serous type ovarian cancer due to frequent *TP53* mutations.

The present study has limitations. First, the utility of the above inhibitors has not been biologically proved. Gene aberration profiles for the same 46 genes were also obtained for 14 commonly used ovarian cancer cell lines (Table IV and Fig. 6). The results are consistent with those deposited in the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). *PIK3CA* and *KRAS* mutations are present in a subset of ovarian cancer cell lines, and consistent *in vitro* therapeutic responses by PI3K and MAPK inhibitors have been reported in a few cell lines (35-37). However, cell lines with other infrequent alterations were not detected in these cell lines. Thus, more sets of cultured ovarian cancer cells are needed to investigate the therapeutic significance of such gene alterations. Second, tumor suppressor and chromatin remodeling genes that lack mutation hotspots but are actionable for synthetic lethality therapy were not examined in the present study: *BRCA1, BRCA2* and *ARID1A* are examples (5,6,9). Tumors with *BRCA1* and *BRCA2* inactivation are susceptible to PARP1 inhibitors, while therapeutic strategies against tumors with *ARID1A* inactivation are also proposed (38,39). A more comprehensiveprofiling study including these genes are ongoing in our laboratory.

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