Published in final edited form as:

Gynecol Oncol. 2013 September ; 130(3): 565–569. doi:10.1016/j.ygyno.2013.06.016.

Poor survival with wild-type *TP53* ovarian cancer?

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Abstract

Objective—To investigate whether wild-type *TP53* status in high-grade serous ovarian carcinoma is associated with poorer survival.

Methods—Clinical and genomic data of 316 sequenced samples from The Cancer Genome Atlas (TCGA) ovarian high-grade serous carcinoma (HGOSC) study were downloaded from TCGA data portal. Association between wild-type *TP53* and survival was analyzed with Kaplan Meier method and Cox regression. The diagnosis of HGOSC was evaluated by reviewing pathological reports and high-resolution hematoxylin and eosin (H&E) images from frozen sections. The authenticity of wild-type *TP53* in these tumor samples was assessed by analyzing SNP array data with ASCAT algorithm, reverse phase protein array (RPPA) data and RNAseq data.

Results—Fifteen patients with HGOSCs were identified to have wild-type *TP53*, which had significantly shorter survival and higher chemoresistance than those with mutated *TP53*. The authenticity of wild-type *TP53* status in these fifteen patients was supported by SNP array, RPPA, and RNAseq data. Except four cases with mixed histology, the classification as high grade serous carcinomas was supported by pathological reports and H&E images. Using RNAseq data, it was found that *EDA2R* gene, a direct target of wild-type *TP53*, was highly up-regulated in samples with wild-type *TP53* in comparison to samples with either nonsense or missense *TP53* mutations.

Conclusion—Patients with wild-type *TP53* high grade ovarian serous carcinomas appeared to have a poorer survival and were more chemoresistant than those with mutated *TP53*. Differentially

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Conflict of interest statement

The authors declare that there are no conflicts of interest

expressed genes in these *TP53* wild-type tumors may provide insight in the molecular mechanism in chemotherapy resistance.

Keywords

Ovarian Cancer; p53 mutation; wild-type p53; *EDA2R*; Survival Statistics; Collagen VI

Introduction

Many researchers have tried to correlate *TP53* mutations with clinical outcomes for patients with ovarian cancer. The results have been conflicting [1–4], likely due to methodological issues, small sample sizes and mixed histological subtypes/grades. The Cancer Genome Atlas consortium has performed whole-genome massively parallel sequencing of selected tumor and nontumor pairs to identify somatic mutations in cancer cells. Recent exome sequencing analysis of 316 high-grade ovarian serous carcinomas (HGOSCs) identified mutations in the tumor repressor protein 53 (*TP53*) gene in 96% of all cases [5], which supports the idea that *TP53* is a driver mutation in ovarian cancer [6]. The use of whole-exome sequencing and subsequent independent validation of *TP53* mutations in The Cancer Genome Atlas (TCGA) ovarian cancer samples provide the most reliable data to date. However, it will be stimulating to investigate at the comprehensive TCGA data that are freely available for the 15 cases of HGOSCs that apparently had no detectable *TP53* mutation. Here, we interrogated the genomic, pathological and clinical data of the 15 cases with wild-type *TP53*. The result indicated that these were tumors with authentic wild-type *TP53*. More interestingly, patients with wild-type *TP53* ovarian cancer appeared to have a poorer survival and were more chemoresistant than those with mutated *TP53*.

Methods

Survival analysis

Clinical data from 316 newly diagnosed patients with ovarian serous adenocarcinoma who had received no prior treatment was retrieved from the supplemental data of the TCGA Nature paper [5] and integrated with *TP53* mutation data from the TCGA data portal (S1). Kaplan-Meier survival analysis was performed with SPSS version 19.0 software (IBM, Armonk, New York); Significance was estimated with the log-rank test. The Cox proportional hazards regression model was used to evaluate the relationship between survival time with age and cytoreduction status. Overall survival was defined as the interval from the date of initial surgical resection to the date of last known contact or death. Progression free survival was defined as the interval from the date of initial surgical resection to the date of progression, date of recurrence, or date of last known contact if the patient was alive and has not recurred. The definition of platinum free interval is similar to that of progress free survival but was from the date of last primary platinum treatment. Chemoresistance status was defined as sensitive when the platinum free interval was six months or greater, and there was no evidence of progression or recurrence. Chemoresistance status was defined as resistant when the platinum free interval was less than six months and the patient had progressed or recurred. Chemoresistance status was defined as refractory if

the patient had progressed or recurred during the platinum treatment or right after the last primary platinum treatment.

Review of pathological data of wild-type TP53 samples

Pathology Reports were downloaded from The Cancer Genome Atlas data portal control-access directory. Quality control Hematoxylin and Eosin (H&E) images from frozen sections were visualized at TCGA BioSig website hosted at Lawrence Berkeley National by our gynecologic pathologist (Michael Deavers) [7].

Analysis of The Cancer Genome Atlas Data for high-grade ovarian serous cystadenocarcinoma

Level 3 somatic mutations derived from Next Generation exome sequencing and RPPA data were retrieved from The Cancer Genome Atlas data portal open-access directory [8]. Affymetrix SNP6.0 level 1 data was downloaded from controlled-access directory. DNA copy number aberrations were analyzed using level 1 Affymetrix SNP6.0 data processed by Nexus 6.0 software with ASCAT (allele-specific copy number analysis of tumors) algorithm [9]. With the matched normal control DNA, ASCAT can accurately dissect the allele-specific copy number of solid tumors, and simultaneously estimate and adjust for both tumor ploidy and percentage of normal stromal cell contamination. RNAseq data was available from 420 samples and transcript expression level in terms of RPKM (reads per kilobase per million mapped reads), which has been normalized by total count and gene length, were compiled for differential gene expression analysis (S2). RPPA level 3 data for 177 samples were also consolidated for statistical analysis (S3). RPPA data from the TCGA data portal were from MD Anderson Cancer Center. Tumor lysates were two-fold serial diluted for 5 dilutions and arrayed on nitrocellulose coated slides, which were probed with antibodies. Relative protein levels for each sample were determined by interpolation of each dilution curves from the standard curve ("SuperCurve") as described previously [10]. All the data points were normalized for protein loading and transformed to linear value. Normalized data for RNAseq and RPPA were uploaded to dChip software for identification of differentially expressed genes/proteins by student t-test [11]. The false discovery rate (q-value) was computed using software SAM by randomly permuting the sample labels 200 times [12].

Results

Patients with wild-type TP53 had poorer survival than those with mutated TP53

Patients with wild-type *TP53* had significantly poorer survival than those with mutated *TP53* (Figure 1). The median overall survival for the patients with wild-type *TP53* was 27 months, while the median survival for patients with mutated *TP53* was 45 months. The mean follow-up time for patients with *TP53* wild-type was 57 months and for *TP53* mutation was 63 months (p value = 0.272, not significantly different). The median follow-up for patients with *TP53* mutation was 62 months which is much longer than the median survival time for patients with *TP53* mutation. After adjustment for age and debulking status by Cox regression analysis, the difference in both overall and progression-free survival between mutated *TP53* and wild-type *TP53* was still significant (p=0.02 and 0.01, respectively). Moreover, seven patients with wild-type *TP53* appeared to be resistant to

standard chemotherapy (carboplatin/taxane). Four patients had recurrent disease in less than six months and the other three patients had tumors that were refractory to treatment with progressive disease (Table 1).

Pathological characteristics of high-grade ovarian serous cystadenocarcinoma with wild-type TP53

Pathology reports were available for seven samples with wild-type *TP53*. All seven cases had a diagnosis as HGOSC except for TCGA-24-2038, which was diagnosed as serous borderline tumor and was excluded in the survival analysis. Another two cases (TCGA-10-0933 and TCGA-24-1544) had mixed components with serous and mucinous neoplastic cells. High resolution H&E Images from frozen sections were available for all 15 cases from the Biosig website and were retrieved for detail review. In general, the histology images agreed with the available pathology reports. For the eight cases without a pathology report, four had high-grade serous features, one had features of malignant mixed mullerian tumor, and the remaining three had areas of low-grade papillary serous features. The pathological and clinical features were listed in Table 1 and two examples of H&E staining with low-grade features were shown in supplemental data (S4).

Mutational profiles of high-grade ovarian serous cystadenocarcinoma with wild-type TP53

Using somatic mutation level 3 TCGA data (validated mutations) for these 15 tumor samples with wild-type *TP53*, we identified 354 genes that were mutated (S5). This list of genes was analyzed through the use of IPA (Ingenuity® Systems, www.ingenuity.com). The top 3 biological functions associated with these mutated genes were identified (Table 2). Out of these 354 genes, 88 genes are involved in the pathogenesis of cancer and seven genes in the estrogen receptor signaling, which may be involved in the pathogenesis of these tumors. With the exception of *KRAS* mutations in two samples (TCGA-24-2038 and TCGA-25-1316), no *BRAF*, *CTNNB1*, *PIK3CA*, or *ARID1A* mutations were found in any of these wild-type *TP53* samples. Since these mutations were more frequently found in epithelial ovarian cancers of other histological subtypes – *BRAF* in serous borderline tumors and low-grade serous carcinomas, *CTNNB1* in endometrioid ovarian carcinomas, and *PIK3CA* and *ARID1A* in clear cell carcinomas, misclassification of these wild-type *TP53* tumors from other histological subtypes as high grade ovarian serous cystadenocarcinomas was less likely.

Reverse-Phase Protein Array, RNAseq, and SNP Array Data Analysis

Reverse-phase protein array data for TP53 protein was available for thirteen wild-type *TP53* samples and 112 samples with missense *TP53* mutations. Wild-type *TP53* samples had a significant 1.85-fold ($p < 0.005$) lower TP53 protein level than those with missense mutated *TP53* but 1.22-fold ($p=0.0439$) higher than that of nonsense mutated *TP53* samples (Figure 3). This result is in agreement with the fact that mutated TP53 protein is frequently more stable than wild-type TP53 protein is. Since mammalian cells has an RNA surveillance system (nonsense mediated mRNA decay) to eliminate faulty mRNA transcripts with nonsense mutations [13], we would expect that samples with nonsense *TP53* mutation would have lower expression level of *TP53* transcript and protein. Using the RNAseq data, we

found that the mean *TP53* transcript level (41.49 RPKM) of the thirteen wild-type *TP53* samples was 4.15 fold higher than the mean *TP53* transcript level (9.99 RPKM) of the 35 samples with nonsense *TP53* mutation ($p = 0.001397$; S4). More interestingly, we also identified that *EDA2R* gene, a direct target of *TP53* [14], was highly up-regulated (>5-fold) in wild-type *TP53* samples (S6 and S7). From the RPPA proteomic data, wild-type *TP53* samples had a higher expression of collagen VI but lower expressions of B-Raf, Chk2, cyclin E1, GAB2, and Syk (S8 and S9). To determine if the wild-type phenotype could be due to the contamination of stromal component, we applied the ASCAT algorithm on the SNP array data with paired normal tumor samples to determine the percentage of normal stromal contamination and copy number aberrations. The median percentage of normal stromal contamination was 22%, which was not significantly different from the rest of the samples (Table 3). The SNP array data also indicated that these wild-type *TP53* samples were genomically more stable with less DNA copy number aberrations than that of missense or nonsense *TP53* mutated samples (Table 3, S10). These data support that these tumor samples have authentic wild-type *TP53* tumor cells.

Discussion

In this study, we found that the fifteen patients with wild-type *TP53* in the published TCGA high grade ovarian serous carcinoma study (316 patients) had a poor survival than those patients with mutated *TP53*. To exclude the possibility of pathological misclassification, for these 15 samples we assessed for other gene mutations, reviewed available pathology reports, and reviewed all the images from H& E stained slides with a gynecologic pathologist. Furthermore, the protein and mRNA expression level of *TP53* in these wild-type *TP53* samples was significantly higher than that of samples with nonsense *TP53* mutation. Moreover, there was a significant difference in copy number aberrations between wild-type and missense samples. The differences may be directly related to the *TP53* mutation. When the function of *TP53* is lost, genomic instability could result from checkpoint loss that leads to aneuploidy, gene amplifications, and other chromosomal aberrations [15]. All these data support that these samples have wild-type *TP53*.

The existence of HGOSC with wild-type *TP53* have previously been demonstrated by sequencing analysis of DNA extracted from purified tumor cells [16] as well as by immunostaining of p21 (a downstream target of wild-type *TP53*) from these cells [17]. The existence of low-grade features in three of the supposedly HGOSC is interesting since low-grade serous carcinomas are usually more chemoresistant [18]. Although the progression of low-grade serous carcinoma to high-grade serous carcinoma is very rare, Vang et al found no *TP53* mutations in a series of six ovarian serous carcinomas with both high-grade and low-grade components [19]. Although no *TP53* mutation was detected in these samples, mutations were detected in 354 genes in all these samples together (Table 2, S5). Out of these 354 genes with mutations, 88 genes are involved in the pathogenesis of cancer, seven genes were involved in estrogen receptor signaling (Table 2), and six genes (*DSCAMLI*, *KIAA1012*, *TNN*, *MUC16*, *FBXW7*, and *KRAS*) occurred in more than one wild-type *TP53* samples (S5). Some of these genes may be involved in the pathogenesis of these tumors. Among these recurrent mutated genes, *FBXW7* is a p53-dependent tumor suppressor [20] and down-regulation of *FBXW7* expression correlates with poor survival in gastric cancer

[21]. In a recent study, *FBXW7* mutation was found to be significantly correlated with positive lymph nodes in endometrial cancer [22]. By querying the TCGA uterine cancer study using the cBio Cancer Genomics Portal [23], we found that *FBXW7* is more frequently mutated in more aggressive uterine serous carcinomas (33%) than that of uterine endometrioid cancer (12%).

Recently, it was shown that wild-type TP53 promoted ovarian cancer cell survival and invasion by up-regulating genes involved in cell proliferation and metastasis in a mouse model [24]. Wild-type TP53 can also impair the effectiveness of chemotherapy against breast cancer [25]. However, the underlying molecular mechanism is not clear. From the RNAseq data, we found that *EDA2R* is significantly up-regulated in wild-type *TP53* samples. *EDA2R* is a member of the tumor necrosis factor receptor family, which plays a major role in the activation of the NF-kappaB and JNK pathways [26]. Activation of NF-kappaB can enhance the chemoresistance of ovarian cancer cells through the regulation of MAPK and apoptosis [27]. From the RPPA data, collagen VI was found to be highly up-regulated in wild-type TP53 tumors (S7). Overexpression of collagen VI has previously been shown to contribute to cisplatin resistance in ovarian cancer cells [28]. Thus, the poorer survival of patients with wild-type *TP53* tumors may be partly due to their tumors being more chemoresistant than mutated *TP53* tumors are. In fact, seven of the ten cases of wild-type *TP53* tumors with chemoresponse data were chemoresistant (Table 1). Thus, it may be beneficial to check HGOSC for *TP53* mutation status by both DNA sequencing analysis and immunohistochemistry for stratification of patients for future clinical trials since both techniques will provide complementary validation of the *TP53* mutational status [29]. Determination of TP53 mutation status will become more important for personalized therapy especially when promising drugs selectively targeting wild-type TP53 or mutant TP53 are emerging [30]. In a recent study, when an ovarian cancer cell line with wild-type *TP53* was treated with Nutlin-3a, an MDM2 antagonist, cell cycle arrest and apoptosis were induced through up-regulation of p21 [24]. Nutlin-3a has been shown to have anti-proliferative activity in a number of malignancies with wild-type TP53 [31]. Thus, Nutlin-3a or similar agent could be a possible therapeutic strategy for these rare HGOSC patients with wild-type *TP53*. This study represents a detailed integrated analysis of 15 wild-type TP53 high grade serous carcinoma samples including RPPA data which was not available in the initial TCGA study. While the TCGA data set is the largest data set available at the moment, it will be important to have another dataset for validation. Since only H&E images from frozen sections for each of the samples were available for pathological review in this study and not all pathology reports are available, we could not eliminate the possibility of misdiagnosis in a few cases. Further investigation of the role of wild-type *TP53* in chemoresistance is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The results published here are in whole based upon data generated by The Cancer Genome Atlas pilot project established by the NCI and NHGRI. Information about TCGA and the investigators and institutes who constitute

the TCGA research network can be found at <http://cancergenome.nih.gov>. Supported in part by grants from the National Institutes of Health, including The University of Texas MD Anderson Cancer Center Specialized Program of Research Excellence in Ovarian Cancer (P50 CA08369), grant CA133057, and MD Anderson's Cancer Center Support Grant (CA016672); and the Blanton-Davis Ovarian Cancer Research Program; the Sara Brown Musselman Fund for Serous Ovarian Cancer Research. E.R.K. is supported by the National Cancer Institute–Department of Health and Human Services–National Institutes of Health Training of Academic Oncologists Grant (T32 CA101642).

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Figure 1.

Overall (A) and progression-free survival (B) of patients with ovarian tumors with mutated *TP53* or wild-type (wt) *TP53*.

Figure 2.

The protein expression level (normalized linear value) of TP53 protein from TCGA samples with wild-type *TP53*, *TP53* missense mutations, and *TP53* nonsense mutations. Data were retrieved from TCGA reverse-phase protein assay data. The box is bounded by the 25th and 75th percentile with the median depicted by the line in the box. Outlying values are drawn individually.

Table 1Clinical and Pathological characteristics of TCGA ovarian tumors with wild-type *TP53*.

Sample ID	H&E images Characteristics	Pathology Report	Vital status in months (chemoresistance, platinum free interval)
TCGA-9-2056	High-grade serous	High-grade serous	Living, 12.43 (sensitive, 7.6)
TCGA-10-0933	Poor histology	High-grade with mixed components (serous, endometrioid, mucinous, and sarcomatoid)	Deceased, 14.46 (sensitive, 7.3)
TCGA-13-0727	High-grade serous	N/A	Deceased, 15.15 (resistant, 4.3)
TCGA-13-0755	High-grade serous	N/A	Deceased, 2.46 (refractory, 0)
TCGA-13-1408	High-grade serous	N/A	Living, 5.9 (N/A)
TCGA-13-1477	High grade serous	N/A	Deceased, 53.67 (resistant, 1)
TCGA-24-1544	High-grade serous, with clusters of mucinous neoplastic cells	High-grade serous with minor clear cell component and clusters of mucinous neoplastic cells	Deceased, 26.89 (sensitive, 14.5)
TCGA-24-1565	High-grade serous	High-grade serous	Deceased, 64.1 (N/A)
TCGA-24-2038	Serous borderline tumor (with KRAS mutation)	Serous borderline tumor	Deceased, 44.36 (N/A)
TCGA-24-2293	High-grade serous	Suggestive of primary peritoneal high-grade serous	Deceased, 16.56 (refractory, 0)
TCGA-25-1316	MMMT (with KRAS mutation)	N/A	Deceased, 41.93 (resistant, 1.9)
TCGA-25-1328	Low-grade serous	N/A	Diseased, 68.57 (resistant, 1)
TCGA-25-2042	High-grade serous	N/A	Deceased, 12.98 (refractory, 0)
TCGA-25-2408	Low-grade serous	N/A	Deceased, 30.89 (N/A)
TCGA-61-2095	Low-grade serous	Moderately differentiated serous with focal prominent psammoma body	Deceased, 61.54 (N/A)

* H&E images were visualized at TCGA BioSig website hosted at Lawrence Berkeley National Laboratory. Clinical data was extracted from the supplemental data in the published TCGA ovarian paper in Nature Journal. MMT, malignant mixed Mullerian tumor; N/A: information not available.

Table 2

Top biological functions associated with mutated genes in ovarian high grade serous carcinomas with wild-type TP53

Top Biological Functions	p-value	Number of Genes
Top 3 Diseases and Disorders		
Cancer	0.00015	88
Hematological Disease	0.00024	26
Immunological Disease	0.00024	12
Top 3 Molecular and Cellular Functions		
Cellular Development	0.00015	15
Cell Cycle	0.00015	25
Cell Morphology	0.00015	48
Top 3 Canonical Pathways		
Gα12/13 Signaling	0.00271	8
Glioblastoma Multiforme Signaling	0.00313	8
Estrogen Receptor Signaling	0.00417	7

Table 3
Estimation of total DNA copy number aberrations and percentage of normal contamination in TCGA high grade ovarian serous carcinomas by ASCAT algorithm using SNP array data.

TP53 status	Total DNA Copy Number Aberrations					Percentage of normal contamination				
	Mean	Std. Deviation	Range	Median	t-test	Mean	Std. Deviation	Range	Median	t-test
wild-type (n=11)	132	84	31–276	91		34	21	16–80	22	
Frame_Shift (n=18)	193	112	65–560	162	p=0.111	33	15	5–58	35	p=0.940
Missense_Mutation (n=109)	199	122	60–670	176	p=0.030	31	13	8–77	28	p=0.633
Nonsense_Mutation (n=17)	204	91	74–374	208	p=0.043	34	13	14–62	32	p=0.950
Splice_Site (n=17)	214	122	80–556	173	p=0.046	23	7	12–36	23	p=0.135