Published in final edited form as:

Gynecol Oncol. 2015 July ; 138(1): 159–164. doi:10.1016/j.ygyno.2015.04.039.

***TP53* hot spot mutations in ovarian cancer: Selective resistance to microtubule stabilizers *in vitro* and differential survival outcomes from The Cancer Genome Atlas**

Brandon-Luke L. Seagle^{a,1}, Chia-Ping Huang Yang^{b,c,1}, Kevin H. Eng^d, Monica Dandapani^a, Oluwatosin Odunsi-Akanji^a, Gary L. Goldberg^c, Kunle Odunsi^e, Susan Band Horwitz^b, and Shohreh Shahabi^{f,*}

^aDepartment of Obstetrics, Gynecology and Reproductive Sciences, Western Connecticut Health Network, 24 Hospital Avenue, Danbury, CT 06810, USA

^bDepartment of Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

^cDepartment of Obstetrics and Gynecology and Women's Health, Division of Gynecologic Oncology, Albert Einstein College of Medicine and Montefiore Medical Center, 1300 Morris Park Avenue, Bronx, NY 10461, USA

^dDepartment of Biostatistics and Bioinformatics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA

^eDepartment of Gynecologic Oncology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA

^fDivision of Gynecologic Oncology, Department of Obstetrics and Gynecology, Prentice Women's Hospital, Northwestern University Feinberg School of Medicine, 250 E Superior Street, Suite 05-2168, Chicago, IL 60611, USA

Abstract

Objective—To test if *TP53* hot spot mutations (HSMs) confer differential chemotherapy resistance or survival outcomes, the effects of microtubule stabilizers on human ovarian carcinoma cells (OCCs) expressing *TP53* HSMs were studied *in vitro*. Survival outcomes of patients with high grade serous epithelial ovarian carcinoma (HGS EOC) expressing matched HSMs were compared using The Cancer Genome Atlas (TCGA) data.

Methods—Growth inhibition of OCCs transfected with a HSM (m175, m248 or m273) was measured during treatment with paclitaxel, epothilone B (epoB), or ixabepilone. Effects of epoB on p53 expression, phosphorylation, and acetylation, as well as p53-regulated expression of p21 and mdm2 proteins, were determined by Western blot analysis. Expression of p53 target genes

*Corresponding author at: Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Prentice Women's Hospital, Northwestern University Feinberg School of Medicine, 250 E Superior Street, Suite 05-2168, Chicago, IL 60611, USA. Tel.: +1 312 472 4684; fax: +1 312 472 4688. Shahabi.shohreh@northwestern.edu (S. Shahabi).

¹These authors contributed equally to this work.

Conflict of interest statement

The study authors have no conflicts of interest to declare.

P21, *GADD45*, *BAX*, *PIDD*, *NF- κ B2*, *PAI-1*, and *MDR1* was measured by RT-PCR. cBioPortal.org identified patients with codon R175, R248 or R273 HSMs from TCGA data. Survival outcomes were characterized.

Results—p53-m248 confers chemoresistance and is not acetylated during epoB treatment. m273 demonstrated high *MDR1* expression and resistance to paclitaxel. *P21*, *GADD45* and *PAI-1* expression were down-regulated in mutant OCCs. Optimally cytoreduced patients with codon R273 (n = 17), R248 (n = 13), R175 (n = 7) HSMs, or any other *TP53* mutation demonstrated median 14.9, 17.6, 17.8 and 16.9 months (p = 0.806) progression free survival and 84.1, 33.6, 62.1 and 44.5 months (p = 0.040) overall survival, respectively.

Conclusions—Human OCCs harboring different *TP53* HSMs were selectively resistant to microtubule stabilizers. Patients with different HSMs had significantly different overall survival. Both *in vitro* data and clinical experience support further studying the outcomes of particular *TP53* HSMs.

Keywords

TP53 genes; Ovarian neoplasms; Drug therapy; Survival

1. Introduction

Ovarian cancer is the deadliest gynecologic malignancy, with a 44.6% 5-year overall survival (OS) rate and 1% lifetime mortality risk among American women [1]. High grade serous epithelial ovarian carcinoma (HGS EOC) is most often diagnosed at stage III or IV, associated with 34% and 18% 5-year OS, respectively [1]. Management of HGS EOC is by surgical staging and cytoreduction followed by adjuvant platinum–taxane chemotherapy [2]. Advanced stage at presentation and chemotherapy resistance causes high mortality among HGS EOC patients.

TP53 is the most frequently mutated tumor suppressor in human cancers [3], and is mutated in 96% of HGS EOC [4]. The most common oncogenic p53 mutations among HGS EOCs are missense hot spot mutations (HSMs) at codons R273, R248 and R175 that comprise 19.7% of all p53 mutations in HGS EOC [5,6]. These three *TP53* mutations convert p53 from a tumor suppressor to an oncogene [6]. The distinct effects of R273, R248 and R175 *TP53* HSMs on tumor progression and chemoresistance in various *in vitro* and *in vivo* models were recently reviewed [6]. To determine if these oncogenic *TP53* HSMs confer resistance to the microtubule stabilizing agents (MSAs) paclitaxel, epothilone B (epoB), and ixabepilone, we studied p53 wild-type OCC A2780 stably transfected with a HSM at codon R273, R248 or R175 [7]. We also performed survival analysis of HGS EOC patients from The Cancer Genome Atlas (TCGA) to determine if HSMs are associated with differential survival outcomes [4].

Additionally, p53 signaling in OCCs with HSMs was studied during treatment with epoB. Like paclitaxel, epoB stabilizes microtubules by binding β -tubulin, thereby decreasing microtubule dynamic instability and inhibiting mitosis [8]. EpoB causes cell cycle arrest at the G2-M phase leading to apoptosis [9] and demonstrates activity in paclitaxel-resistant cell

lines and tumors [10,11]. Ixabepilone is an epoB analog, FDA approved for treatment of taxane-resistant metastatic breast cancer [12–14].

2. Materials and methods

2.1. Evaluation of R273, R248 and R175 HSMs from TCGA HGS EOC patients

TCGA HGS EOC cases (N = 316) with whole exome sequencing, DNA copy number and mRNA expression data were searched for cases with *TP53* HSMs at codons R273, R175, and R248 using cBioPortal [15,16]. TCGA patients underwent surgical staging followed by platinum (100%) and taxane (94%) chemotherapy [4]. cBioPortal was used to obtain *TP53* mutation location and type, total number of mutations in each tumor, *TP53* gene copy number, *TP53* MRA expression level (expressed as a percentile relative to the mean), surgical stage, extent of residual disease after staging, platinum sensitivity status, progression free survival (PFS), and OS. Staging was considered optimal if residual disease was ≤ 1.0 cm. The R platform for statistical computing was used for survival analysis and statistical testing [17,18].

2.2. Materials and cells

EpoB and paclitaxel were obtained as described [19]. Ixabepilone was from Bristol-Myers Squibb. Monoclonal anti-p53 (DO-1, sc-126) was purchased from Santa Cruz Biotechnology. Anti-phosphorylated-p53 (Ser-15, #9284S) and anti-acetylated-p53 (Lys-382, #2525S) antibodies were from Cell Signaling. Monoclonal anti-human MDM2 antibody (556353) was purchased from BD Biosciences. Polyclonal anti-PARP p85 fragment (G734A) and monoclonal anti-p21 (HJ21) antibodies were from Promega and Neomarkers, respectively. The human ovarian wild-type p53 cancer cell line A2780 and p53 mutants derived from A2780 (m175, m248 and m273) were from Dr. Elisabeth de Vries, MD/PhD, and were grown in RPMI 1640 containing 10% fetal bovine serum and 0.25 mg/ml G418 [7]. OCCs with *TP53* HSMs m175, m248 and m273 harbor an Arg to His mutation at residue 175, an Arg to Trp mutation at residue 248 or an Arg to His mutation at residue 273, respectively.

2.3. Preparation of cell lysates and immunoblot analysis

Denatured cell lysates were prepared as described [20]. Equal amounts of proteins in the lysates were resolved by SDS-PAGE and the expression of p53, phosphorylated-p53 (P-p53), acetylated-p53 (Ac-p53), PARP p85 and p21 were determined by Western blot analysis using specific antibodies.

2.4. Cytotoxicity assays

Wild-type and mutant p53 cells were plated in 96-well culture dishes at 1000 cells/well and treated with increasing concentrations of epoB, ixabepilone and paclitaxel. SRB cytotoxicity assays were performed after 72 h as described [21]. Alternatively, 1.2×10^4 cells were cultured in 12-well dishes and 1, 10 and 100 nM epoB were added 16 h after plating. Cell numbers in each well were counted 24, 48 and 72 h after drug treatment using a Coulter Counter.

2.5. Quantitative real-time PCR (RT-PCR)

RNA was prepared using RNeasy Protect Mini Kit from Qiagen and RT-PCR was done using gene specific primers for *P21*, *PIDD*, *BAX*, *GADD45*, *NF- κ B* and *PAI-1*. Gene expression levels were normalized to the loading control (cyclophilin B) and expressed as a fold change relative to that of untreated cells, using the comparative C_T method.

3. Results

3.1. TP53 HSMs were associated with significant differences in overall survival

TP53 somatic mutations were present in 299/316 (94.6%) sequenced tumors. Median OS was 45.0 months among cases with any *TP53* mutation. *TP53* HSMs at codons R273, R248 and R175 were identified in 21, 17, and 8 patients, respectively. Two patients had a R273 and R248 HSM and were included in survival analyses of both the R273 and R248 groups when cases were compared by HSM. In total, 44/316 (13.9%) of TCGA HGS EOCs had one of the three most common HSMs. 255 patients had another *TP53* mutation. Of these 255 patients, 254 and 207 had reported OS and PFS, respectively. All patients with a HSM had grade 3 disease except for one case without a reported grade and one grade 2 case. Age was similar among groups (Table 1).

Patients with a R273 HSM had increased median OS compared to patients with a R248 HSM (84.1 vs 36.1 months, log rank $p = 0.031$) (Table 1). All other pairwise comparisons for OS or PFS were insignificant. By Cox proportional hazards regression, normalized *TP53* expression level ($p = 0.89$) and the total number of tumor mutations (across all genes studied by whole-exome tumor sequencing) ($p = 0.31$) did not influence OS of cases with a HSM. Two cases with both R273 and R248 HSMs were both stage IIIC, optimally cytoreduced and given platinum–taxane combination adjuvant chemotherapy. One case of platinum refractory disease demonstrated a PFS and OS of 3.6 and 6.7 months, respectively. The second case was platinum sensitive, with 19.1 months PFS and 38.3 months OS. Platinum sensitivity was 55.2% among cases with HSMs who survived longer than 6 months from finishing adjuvant chemotherapy and was not significantly different between cases grouped by HSMs (Table 1). Differences in surgical staging between HSM groups were not significant (Table 1). Grouping cases with HSMs by *TP53* gene copy number differences, *TP53* mRNA expression level, or surgical stage rather than by observed HSMs did not produce groups with significant differences in PFS or OS (Table 1). Optimal *versus* suboptimal cytoreduction was associated with increased OS (52.0 vs 24.2 months, $p = 0.037$) and PFS (14.0 vs 12.3 months, $p = 0.056$). Therefore, optimally cytoreduced patients were analyzed separately (Fig. 1 and Table 1). OS but not PFS was significantly different between optimally cytoreduced patients with a HSM or another *TP53* mutation (non-HSM) (Table 1). Median OS was 33.6, 44.5, 62.1, and 84.1 months in the R248, non-HSM, R175 and R273 groups, respectively ($p = 0.040$). R273 HSM was associated with significantly increased OS compared with non-HSM (hazard ratio for death (HR) 0.48, 95% confidence interval (CI) 0.27–0.84) or a R248 HSM (HR 0.29, 95% CI 0.10–0.80).

3.2. EpoB induced p53 accumulation, phosphorylation and acetylation in A2780 cells

EpoB induced dose-dependent p53 activation as indicated by p53, P-p53 and Ac-p53 accumulation in p53 wild-type A2780 cells, with maximal induction at 20–50 nM (Fig. 2A and B). These results are consistent with previous findings that low concentrations of paclitaxel induce p53 and p21 activation and arrest cells at G1 and G2 of the cell cycle, whereas higher concentrations of paclitaxel cause mitotic arrest [22,23].

3.3. Mutant OCCs express high levels of p53 HSM proteins and have different chemosensitivities

Mutant cells contained 4–5 fold more mutant p53 than wild-type p53 (Fig. 2C). Arg to Trp substitution at p53 residue 248 decreased p53 electrophoretic mobility (Fig. 2C). Doubling times of mutant OCCs was similar to wild-type, with m248 growing slower (Fig. 2D). m248 cells were 1.9-fold and 1.6-fold resistant to epoB and ixabepilone, respectively (Table 2). m273 cells were approximately 2-fold resistant to paclitaxel (Table 2). Unlike other mutant OCCs, m273 expresses multidrug resistant gene 1 (*MDR1*) (Fig. S1).

3.4. p53-m248 was not activated when exposed to epoB, and activated p53-m273 did not induce p21

To explore epoB, a microtubule stabilizer which is known to overcome paclitaxel resistance, the effects of epoB on the expression of p53, P-p53, Ac-p53, mdm2 and p21 were analyzed in mutant OCCs (Fig. 3). EpoB did not alter the expression of mutant p53 in mutant OCCs (Fig. 3). Mutant P-p53 and Ac-p53 levels were reduced when m175 and m273 cells were treated with increasing concentrations of epoB. In contrast, mutant P-p53 levels were increased when m248 cells were treated with epoB. EpoB did not induce Ac-p53 in m248 cells, indicating a defect in p53 activation. Mdm2 expression was decreased with increasing concentrations of epoB for m175 and m273 cells. For CMV and m248 cells, mdm2 expression was increased at 10–20 nM epoB, but decreased at higher concentrations of epoB. In CMV and m273 OCCs, a 57 kDa mdm2 cleavage product was observed at 50–200 nM epoB. The induction of p21 expression in CMV, m175 and m248 was similar, but no induction of p21 was observed in m273 cells.

3.5. m248 cells had decreased epoB-induced apoptosis

PARP is cleaved to PARP p85 early during apoptosis [24]. EpoB caused increased PARP p85 levels in A2780, CMV, m175 and m273 cells, but not in m248 cells, indicating a defect in epoB-induced apoptosis in m248 cells (Fig. 4). m248 cells also exhibited greater cell growth than other HSM OCCs during treatment with epoB (Fig. S2).

3.6. EpoB-induced expression of p53 target genes P21, GADD45 and PAI-1 was reduced in HSM OCCs

EpoB increased *P21*, *PAI-1*, and *GADD45* mRNA expression in A2780 cells (Fig. S3). EpoB-induced expression of *P21*, *PAI-1* and *GADD45* was reduced in the HSM OCCs (Fig. S4). EpoB-induced *P21* mRNA expression in m273 cells was less than other HSM OCCs (Fig. S4), consistent with the p21 protein results (Fig. 3).

4. Discussion

Consistent with previous reports [6], codon R273 is the most frequently mutated hot spot among TCGA HGS EOC patients. *TP53* mutation at R273 conferred cisplatin resistance in lung and bladder carcinoma cells [25,26]. We showed that m273 OCCs were resistance to paclitaxel, likely due to *MDR1* expression. Inhibition of *MDR1* reverses paclitaxel resistance in human OCCs and knockdown of *MDR1* sensitizes breast cancer cells to paclitaxel [27,28]. Mutant p53-m273 protein exerted a dominant-negative effect over wild-type p53 in preventing epoB-induced p21 mRNA and protein expression. This decreased expression of p21 in m273 cells would be expected to result in elevated p53-m273 protein expression given decreased negative feedback of activated p21 on p53 expression [29]. In contrast to our *in vitro* data, TCGA reported clinical experience demonstrated that patients with a R273 mutation had increased OS compared to patients with a R248 or a non-hot spot mutation. Further studies may characterize *MDR1* expression levels in R273 mutant HGS EOC tumors.

R248 is the second most common HSM among TCGA cases. R248 mutation conferred doxorubicin and paclitaxel resistance in hepatocellular carcinoma cells, and was associated with induction of *MDR1* [30]. We did not observe increased *MDR1* expression in m248 cells. Also, R248 mutation conferred cisplatin and paclitaxel resistance in ovarian cancer cell lines [31]. We did not observe paclitaxel resistance in m248 OCCs with an A2780 background, but we did observe epoB resistance likely due to a defect in epoB-induced apoptosis in m248 cells. Consistent with multiple *in vitro* studies reporting m248 OCCs being relatively chemoresistant to commonly used platinum and taxane agents, TCGA cases with a R248 mutation had decreased OS. Case numbers were small, and the decreased OS was only significant compared to the longest surviving group, patients with a R273 mutation.

Third in frequency, mutations at R175 have been shown to mediate resistance to etoposide and cisplatin in lung carcinoma cells and to paclitaxel in endometrial carcinoma cells [25,32]. We did not observe any significant *in vitro* results related to R175, and survival of TCGA patients with R175 mutations was not significantly different than the general cohort of patients with a *TP53* mutation.

Similar to previous reports that *TP53* HSMs stabilize the p53 protein leading to accumulation of mutant p53 protein in human ovarian cancers [6], mutant p53 proteins were over-expressed in the HSM OCCs. Expression levels were unaffected by increasing concentrations of epoB and by mdm2 expression (Fig. 3). These results suggest that mdm2 expression is not a significant determinant of p53 accumulation in mutant OCCs regardless of *TP53* mutation status. Mdm2 is an ubiquitin ligase that targets p53 for degradation [33]. Mdm2-catalyzed ubiquitination of mutant p53 is inefficient compared to wild-type p53 ubiquitination, and ubiquitination of mutant p53 is generally low in cancer cells [33].

P21 arrests the cell cycle at the G1/S checkpoint in response to p53 activation after cellular or genomic stress, allowing an opportunity for DNA repair that is associated with an anti-apoptotic effect [29]. EpoB induced transcription of mRNAs of p53 target genes involved in

cell cycle arrest, namely *P21*, *GADD45* and *PAI-1*, in wild-type p53 A2780 and CMV cells (Fig. S3 and Fig. S4), with less mRNA induction of these genes seen in the HSM OCCs (Fig. S4). Less transcription of *P21*, *GADD45* and *PAI-1* in HSM OCCs may result in decreased replicative senescence and decreased apoptosis of OCCs in response to cellular or genomic stress [29,34,35].

Associations with increased mutant p53 expression and differential survival outcomes are inconsistently reported in populations composed of epithelial ovarian cancers with various histologic subtypes [36,37]. A meta-analysis of clinical studies evaluating *TP53* mutation status and chemotherapy resistance found no significant correlations between *TP53* mutation status and chemoresistance, and also demonstrated that few studies met high quality inclusion criteria including full exome sequencing of the *TP53* gene and distinguishing between different *TP53* mutations [38]. Patients with a gain-of-function *TP53* HSM, including the three HSMs evaluated in this study, did not demonstrate survival differences compared to patients with a *TP53* mutation that is not believed to result in gain-of-function p53 activity [39]. Another study reported significantly increased PFS (HR 1.60 (1.09–2.33), $p = 0.015$) among patients with gain-of-function p53 mutations [31]. This analysis did not report outcomes of patients grouped by individual HSM according to codon location of each HSM [31]. All three HSMs evaluated in our study are gain-of-function mutations.

Limitations of this study include that findings from an *in vitro* system may not necessarily reflect *in vivo* effects, especially given the high genetic instability and heterogeneity of HGS EOCs *in vivo*. The transfected HSM OCCs also expressed lower levels of wild-type p53, which complicates interpretation of studies comparing p53 signaling in control and mutant OCCs because observed differences, or lack thereof, may not be strictly related to mutant p53 proteins. Additionally, the number of patients with individual HSMs available in HGS EOC TCGA datasets is small given the low occurrence frequency of even the more common on-cogenic *TP53* mutations. Finally, observed OS differences could be related to differences in post-recurrence treatments which were not analyzed.

We present a combination of *in vitro* studies in cultured human OCCs transfected with one of three common *TP53* mutations and *in silico* analysis of cases with matched *TP53* mutations from publically-accessible TCGA Research Network datasets. Together these studies provide evidence and clinical experience (reflected in TCGA cases) suggesting that the particular *TP53* mutation of a high grade serous ovarian cancer may have an influence regarding chemotherapy sensitivity and overall survival outcomes. Further characterization of clinical outcomes associated with different *TP53* mutations is needed to determine if knowing the particular *TP53* mutation of an ovarian cancer tumor may have utility for management in clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The results published here are in part based upon the data generated by the TCGA Research Network: <http://cancergenome.nih.gov/>. We thank Dr. Elisabeth de Vries, MD/PhD, Professor of Medical Oncology, University Medical Center Groningen, Netherlands, for providing the cell lines used in this study, and Dr. Gerda Hofstetter, MD for performing the RT-PCR experiments. This study is funded in part by the National Foundation for Cancer Research (SBH), Roswell Park Cancer Institute Alliance Foundation Award (KO), National Cancer Institute Cancer Center Support Grant NIH 2P30 CA016056-36 (KO), NIH 1R01CA158318-01A1 (KO), and RPCI-UPCI Ovarian Cancer SPORE NIH P50CA159981-01A1 (KO), NIH K01LM012100 (KE), and NIH P30CA016056 (RPCI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2015.04.039>.

References

1. American Cancer Society. <http://www.Cancer.Org/Cancer/Ovariancancer/Detailedguide/Index>. (accessed August, 2014)
2. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer. 2014 (Version 1.2014).
3. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. *Nature*. 2013; 502:333–9. [PubMed: 24132290]
4. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011; 474:609–15. [PubMed: 21720365]
5. Muller PAJ, Vousdan KH. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell*. 2014; 25:304–17. [PubMed: 24651012]
6. Brachova P, Thiel KW, Leslie KW. The consequence of oncomorphic TP53 mutations in ovarian cancer. *Int J Mol Sci*. 2013; 14:19257–75. [PubMed: 24065105]
7. Skeijfer S, Le TK, de Jong S, Timmer-Bosscha H, Withoff S, Mulder NH. Combined cytotoxic effects of tumor necrosis factor-alpha with various cytotoxic agents in tumor cell lines that are drug resistant due to mutated p53. *J Immunother*. 1999; 22:48–53. [PubMed: 9924699]
8. Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, et al. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res*. 1995; 55:2325–33. [PubMed: 7757983]
9. Donovan D, Vahdat LT. Epothilones: clinical update and future directions. *Oncology (Williston Park)*. 2008; 22:408–16. [PubMed: 18472615]
10. Kowalski RJ, Giannakakou P, Hamel E. Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel. *J Biol Chem*. 1997; 272:2534–41. [PubMed: 8999970]
11. Harrison M, Swanton C. Epothilones and new analogues of the microtubule modulators in taxane-resistant disease. *Expert Opin Investig Drugs*. 2008; 17:523–46.
12. Fournier MN. Ixabepilone, first in a new class of antineoplastic agents: the natural epothilones and their analogues. *Clin Breast Cancer*. 2007; 7:757–63. [PubMed: 18021476]
13. Goodin S. Ixabepilone: a novel microtubule-stabilizing agent for the treatment of metastatic breast cancer. *Am J Health Syst Pharm*. 2008; 65:2017–26. [PubMed: 18945860]
14. McDaid HM, Mani S, Shen HJ, Muggia F, Sonnichsen D, Horwitz SB. Validation of the pharmacodynamics of BMS-247550, an analogue of epothilone B, during a phase I clinical study. *Clin Cancer Res*. 2002; 8:2035–43. [PubMed: 12114401]
15. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013; 6:p11. [PubMed: 23550210]

16. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2012; 2:401–4. [PubMed: 22588877]
17. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. (<http://www.R-project.org>)
18. Therneau, R. A package for survival analysis in S. R package version 2.37-7. 2014. <http://CRAN.R-project.org/package=survival>;
19. He L, Yang CPH, Horwitz SB. Mutations in β -tubulin map to domains involved in regulation of microtubule stability in epothilone-resistant cell lines. *Mol Cancer Ther.* 2001; 1:3–10. [PubMed: 12467233]
20. Yang CPH, Horwitz SB. Taxol mediates serine phosphorylation of the 66-kDa Shc iso-form. *Cancer Res.* 2000; 60:5171–8. [PubMed: 11016645]
21. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.* 1990; 82:1107–12. [PubMed: 2359136]
22. Giannakakou P, Robey R, Fojo T, Blagosklonny M. Low concentrations of paclitaxel induce cell type-dependent p53, p21 and G1/G2 arrest instead of mitotic arrest: molecular determinants of paclitaxel-induced cytotoxicity. *Oncogene.* 2001; 20:3806–13. [PubMed: 11439344]
23. Torres K, Horwitz SB. Mechanisms of taxol-induced cell death are concentration dependent. *Cancer Res.* 1998; 58:3620–6. [PubMed: 9721870]
24. Duriez PJ, Shah GM. Cleavage of poly(ADP-ribose) polymerase: a sensitive parameter to study cell death. *Biochem Cell Biol.* 1997; 75:337–49. [PubMed: 9493956]
25. Blandino G, Levine AJ, Oren M. Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. *Oncogene.* 1999; 88:477–85.
26. Chang FL, Lai MD. Various forms of mutant p53 confer sensitivity to cisplatin and doxorubicin in bladder cancer cells. *J Urol.* 2001; 166:304–10. [PubMed: 11435891]
27. Duan Z, Brakora KA, Seiden MV. Inhibition of ABCB1 (MDMR1) and ABCB4 (MDR3) expression by small interfering RNA and reversal of paclitaxel resistance in human ovarian cancer cells. *Mol Cancer Ther.* 2004; 3:833–8. [PubMed: 15252144]
28. Shi JF, Yang N, Ding HJ, Zhang JH, Hu ML, Leng Y, et al. ERalpha directly activated MDR1 transcription to increase paclitaxel-resistance of ERalpha-positive breast cancer cells *in vitro* and *in vivo*. *Int J Biochem Cell Biol.* 2014; 53:35–45. [PubMed: 24786296]
29. Mirzayans R, Andrias B, Scott A, Murray D. New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. *J Biomed Biotechnol.* 2012; 2012:170325. [PubMed: 22911014]
30. Chan KT, Lung ML. Mutant p53 expression enhances drug resistance in a hepatocellular carcinoma cell line. *Cancer Chemother Pharmacol.* 2004; 53:519–26. [PubMed: 15004724]
31. Brachova P, Meuting SR, Carlson MJ, Goodheart MJ, Button AM, Mott SL, et al. *TP53* oncomorphic mutations predict resistance to platinum- and taxane-based standard chemotherapy in patients diagnosed with advanced serous ovarian carcinoma. *Int J Oncol.* 2015; 46:607–8. [PubMed: 25385265]
32. Meng X, Laidler LL, Kosmacek EA, Yang S, Xiong Z, Zhu D, et al. Induction of mitotic cell death by overriding G2/M checkpoint in endometrial cancer cells with nonfunctional p53. *Gynecol Oncol.* 2012; 128:461–9. [PubMed: 23146687]
33. Grossman SR, Frum RA. Mechanisms of mutant p53 stabilization in cancer. *Subcell Biochem.* 2014; 85:187–97. [PubMed: 25201195]
34. Tamura RE, de Vasconcellos JF, Sarkar D, Libermann TA, Fisher PB, Zerbini LF. GADD45 proteins: central players in tumorigenesis. *Curr Mol Med.* 2012; 12:634–51. [PubMed: 22515981]
35. Kortlever RM, Higgins PJ, Bernards R. Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol.* 2006; 8:877–84. [PubMed: 16862142]
36. Havrilesky L, Darcy KM, Hamdan H, Priore RL, Leon J, Bell J, et al. Prognostic significance of p53 mutation and p53 overexpression in advanced epithelial ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol.* 2003; 21:3814–25. [PubMed: 14551300]

37. Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, et al. Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. *Clin Cancer Res.* 2001; 7:2884–97.
38. Brown R, Paul J, Hall J. Critical evaluation of p53 as a prognostic marker in ovarian cancer. *Expert Rev Mol Med.* 2004; 6:1–20.
39. Kang HJ, Chun SM, Kim KR, Sohn I, Sung CO. Clinical relevance of gain-of-function mutations of p53 in high-grade serous ovarian carcinoma. *PLoS ONE.* 2013; 8:e72609. [PubMed: 23967324]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HIGHLIGHTS

- Mutant *TP53* ovarian cancer cells show selective microtubule stabilizer resistance.
- R248 mutant ovarian cancer cells have reduced epothilone B induced cell death.
- Patients with different *TP53* mutations may have different overall survival.

Fig. 1. Kaplan-Meier (KM) overall survival curves of optimally cytoreduced HGS EOCs with a *TP53* HSM or another *TP53* mutation (non-HSM). Inset: KM curves of the same cases with data analysis truncated at 60 months.

Fig. 2.

EpoB induces p53 accumulation, phosphorylation and acetylation. (A) A2780 cells were treated with 2.5–200 nM epoB for 16 h. Total denatured cell lysates were prepared and the levels of p53, p53 phosphorylated at Ser-15 (P-p53), and p53 acetylated at Lys-382 (Ac-p53) were determined by Western blot analyses using antibodies against p53, P-p53 and Ac-p53. C: Untreated, control cells. (B) Expression levels of p53, P-p53 and Ac-p53 were quantified using ImageJ and normalized to the level of GAPDH. (C) p53 protein levels in the lysate prepared from A2780 cells which was stably transfected with plasmids carrying no p53 mutation (CMV), or p53 mutated at codon 175 (m175), 248 (m248), or 273 (m273). (D) Doubling time of the above five cell lines is shown.

Fig. 3.

EpoB differentially induces the expression of p53, phosphorylated-p53 (P-p53), and acetylated-p53 (Ac-p53), mdm2 and p21 in the mutants harboring hot spot mutation of p53. Different p53 mutants were treated with 2.5–200 nM epoB for 16 h and total denatured cell lysates were prepared. Expression levels of p53, P-p53, Ac-p53, mdm2 and p21 were determined by Western blot analyses. C: Untreated, control cells.

Fig. 4.

PARP p85 fragment was much less induced in the p53 mutant m248, compared to other p53 mutants. A2780 cells and different p53 mutants were treated with 1 and 10 nM epoB for 24, 48 and 72 h. Cell lysates were prepared and PARP p85 fragment levels were determined by Western blot analysis using polyclonal antibody against PARP p85. C: Untreated, control cells.

Table 1Survival analysis of *TP53* hot spot mutations.

	Number:	OS (months) Median (95% CI)	PFS (months) Median (95% CI)
<i>All cases with a TP53 mutation</i>			
R175	8	62.1 (35.0–62.1)	14.7 (6.08–40.5)
R248	17	36.1 (22.3–51.9)	12.3 (5.00–21.9)
R273	21	84.1 (35.3–119.0)	14.5 (10.2–21.3)
Non-HSM	254 OS/207 PFS	45.0 (41.0–49.3) p = 0.097 ^a	16.8 (15.3–18.7) p = 0.716 ^a
<i>Optimally cytoreduced cases with a TP53 mutation</i>			
R175	7	62.1 (35.0–62.1)	17.8 (6.08–40.5)
R248	13	33.6 (22.3–59.1)	17.6 (5.00–25.1)
R273	17	84.1 (35.3–119.0)	14.9 (10.8–34.0)
Non-HSM	163 OS/139 PFS	44.5 (41.0–48.7) p = 0.040 ^a	16.6 (14.7–18.2) p = 0.806 ^a
<i>Survival by copy number alteration of the TP53 gene among HSM cases</i>			
Heterozygous	33	41.5 (35.0–84.1)	14.1 (10.8–19.1)
Diploid	7	51.9 (6.67–52.0)	10.2 (3.61–25.1)
Gain	6	24.2 (19.5–36.1) p = 0.818 ^b	14.5 (8.97–40.5) p = 0.890 ^b
<i>Survival by relative TP53 mRNA expression levels among HSM cases^c</i>			
90%	10	59.1 (51.9–84.1)	17.8 (14.5–40.5)
<90%	36	39.1 (33.9–62.1) p = 0.250 ^d	12.3 (9.23–19.1) p = 0.199 ^d
<i>Survival by surgical stage among HSM cases</i>			
IIA	1	NC	NC
IIIB	4	84.1 (36.1–NC)	34.0 (21.9–34.0)
IIIC	32	39.1 (33.6–62.1)	12.3 (9.2–19.1)
IV	9	41.5 (26.9–59.1) p = 0.748 ^a	13.9 (9.6–17.4)p = 0.198 ^a
<i>Comparison of survival among HSM cases by cytoreduction status</i>			
Optimal	37	52.0 (35.3–84.1)	14.9 (10.0–21.9)
Suboptimal	9	24.2 (20.0–41.5) p = 0.037 ^d	12.3 (8.0–14.5) p = 0.056 ^d
<i>Platinum resistance among HSM cases that survived at least 6 months after adjuvant chemotherapy administration</i>			
R273	7/16 (43.8%)		
R248	5/14 (35.7%)		
R175	5/8 (62.5%) p = 0.91 ^e		
<i>Surgical stage among optimally cytoreduced HSM cases</i>			
R273	III (15/17), IV (2/17)		
R248	III (9/13), IV (4/13)		
R175	II (1/7), III (6/7)		

	Number:	OS (months)	PFS (months)
		Median (95% CI)	Median (95% CI)
p = 0.571 ^f			
<i>Median age of optimally cytoreduced groups characterized by KM survival analysis</i>			
Non-HSM	58.28		
R273	59.02		
R248	61.58		
R175	59.12		
p = 0.499 ^f			

NC: not calculated.

^aLog rank (four groups).

^bLog rank (three groups).

^cHSM cases were also grouped and compared by *TP53* mRNA expression level quartiles or tertiles and no significant differences in OS or PFS were observed.

^dLog rank (two groups).

^eFisher exact test.

^fKruskal–Wallis test.

Table 2Cytotoxicity of MSAs against ovarian carcinoma cells with *TP53* hot spot mutations.

	IC₅₀^a (±SE)			
	CMV	m175	m248	m273
Epothilone B	0.20 ± 0.03 (1.0)	0.30 ± 0.04 (1.5)	0.38 ± 0.02 (1.9)	0.22 ± 0.01 (1.1)
Ixabepilone	2.87 ± 0.39 (1.0)	3.28 ± 0.40 (1.1)	4.59 ± 0.31 (1.6)	3.81 ± 0.13 (1.3)
Paclitaxel	1.51 ± 0.23 (1.0)	1.80 ± 0.16 (1.2)	1.98 ± 0.31 (1.3)	3.06 ± 0.63 (2.0)

^aIC₅₀ equals the concentration (nM) of drug that inhibits 50% cell growth. 2000 cells were added to each well of a 96-well plate. Increasing concentrations of the indicated drugs were added 18 h after plating. IC₅₀ values were determined after 72 h of incubation at 37 °C using the sulforhodamine B (SRB) method [21]. Data are expressed as mean ± standard error (n = 4–6).