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Expression of Poly (Adenosine Diphosphate-Ribose) Polymerase and p53 in Epithelial Ovarian Cancer and Their Role in Prognosis and Disease Outcome

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Summary

PARP, poly (adenosine diphosphate-ribose) polymerase, is a damage-sensing protein, which is essential for the repair of DNA single-strand breaks. PARP and p53 function synergistically in repairing DNA damage and suppressing chromosomal rearrangements. The aim of this study was to determine the expression of PARP and p53 in epithelial ovarian cancer (EOC) and to correlate their expression with clinicopathologic characteristics. PARP and p53 were evaluated using immunohistochemistry applied on a tissue microarray of 189 EOC and their expressions were correlated to clinicopathologic variables, including the age of diagnosis, stage, grade, histologic type, optimal debulking, progression-free survival, and overall survival (OS). PARP and p53 expressions were shown in 61% and 54% of cases, respectively. PARP-positive tumors are more likely to have higher grade ($P = 0.03$) and complete response to initial first-line chemotherapy ($P = 0.009$). Patients with positive p53 staining are more likely to be at the advanced stage disease ($P = 0.004$). Finally, there were no significant associations between PARP and p53 expression and no differences in progression-free survival and OS for PARP or p53 expressions. The overexpression of PARP and p53 in high grade, and advanced stage tumors indicated that these 2 markers might serve as an indicator of aggressive disease behavior. Additional studies are warranted to evaluate the role of PARP and PARP inhibitors in the setting of adjuvant chemotherapy.

Keywords

PARP; p53; Immunoexpression; Epithelial ovarian cancer; Outcome

Epithelial ovarian cancer (EOC) is the leading cause of death in the United States in women diagnosed with gynecologic malignancies, with 21,550 new cases and 14,600 women estimated to have died of ovarian cancer in 2009 (1). Overall survival (OS) rates remain disappointing, notwithstanding improvements in response rates, disease-free interval, and median survival associated with the “standard therapy,” including staging laparotomy with cytoreduction followed by platinum/taxane-based chemotherapy (2). The clinical course of remission and relapse is commonly seen in patients undergoing therapy for ovarian cancer.

However, most patients will eventually die of chemotherapy-resistant disease (3,4). Thus, improved and novel therapies such as targeted therapy among others including inhibition of angiogenesis, and immune-based therapy are needed for better patient management (5).

The most common means of targeting the cell cycle is to magnify the effect of DNA-damaging drugs. DNA damages induce cell-cycle arrest and cell death either directly or after DNA replication during the S-phase of the cell cycle. However, the toxicity of DNA-damaging drugs can be reduced by the activities of DNA repair pathways that repair the damaged area and prevent cell cycle arrest or apoptosis.

PARP, poly (adenosine diphosphate-ribose) polymerase, is a damage-sensing nuclear enzyme, which helps in the repair of DNA. PARP is essential for the repair of DNA single-strand breaks by the base excision repair pathway (6,7). Specifically, PARP-1 contains DNA-binding domains, which function to localize the sites of DNA damage. On identification of DNA damage, PARP-1 promotes the transfer of ADP-ribose units from intracellular nicotinamide adenine dinucleotide to nuclear acceptor proteins, contributing to the confirmation of the bound ADP-ribose polymers. This mechanism is thought to be instrumental in the repair of DNA caused by chemotherapy and radiation agents (8,9). Increased PARP-1 activity has been shown in several tumor cell lines, including colorectal carcinoma, breast cancer, hepatocellular carcinoma, and non-Hodgkin lymphoma. This increased level of PARP-1 activity may enable tumor cells to withstand genotoxic stressors and potentially become resistant to chemotherapeutic agents (10–12). The inhibition of PARP with Olaparib (AZD2281) has recently been shown to be beneficial in showing antitumor activity with BRCA1 and BRCA-positive breast cancers (13).

P53 protein plays an instrumental role in the regulation of the cell cycle and suppression of the development of tumors. p53 and PARP play an important role in a cell's response to genotoxic stressors. The exact mechanism of interaction between p53 and PARP has not been identified. However, a balance between DNA repair and apoptosis is important in considering the response to treatment, disease-free survival, and OS.

The aims of this study are to determine the expression of PARP and p53 in EOC and to correlate their expression with clinicopathologic characteristics.

METHODS

Patients and Specimens

We searched the archives for patients with EOC who underwent debulking surgery for their EOC during a 10-year period from 1995 to 2005. All tissue specimens were collected under an approved protocol from the Institutional Review Board (IRB). The medical records of the patients were also retrospectively reviewed under an approved IRB protocol. The review included outpatient and inpatient treatment, including surgery and chemotherapy. Study outcomes included OS and time to progression, each measured from the time of definitive surgery. Progression was defined as objective evidence of recurrence because all therapies were given in the adjuvant setting. The duration of OS was the interval between definitive surgery and death. Observation time was the interval between definitive surgery and last

contact (death or last follow-up). Data were censored at the last follow-up for patients with no evidence of recurrence, progression, or death.

Hematoxylin and eosin (H&E)-stained slides were available for histologic review. The tumor subtypes and grade were re-reviewed for confirmation by 1 experienced pathologist (P.M-F.). Histologic subtypes were based on World Health Organization (14). The histologic grade was determined according to the criteria used by the Silverberg grading system (15). In this grading scheme, a score from 1 to 3 was given for each of the predominant architectural pattern, cytologic atypia, and mitotic rate per 10 high power fields. A total score of 3 to 5 was classified as grade 1, 6 or 7 as grade 2, and 8 or 9 as grade 3.

Immunohistochemistry

Paraffin-embedded tissues from 189 patients' samples were included in this study. A tissue microarray was constructed as described earlier by Kononen et al. (16,17). Briefly, after carefully choosing the morphologically representative region from the H&E section, 0.6-mm cores were punched from the individual paraffin-embedded blocks (donor blocks), and transferred to the receiver paraffin-embedded block (receiver block). To overcome tumor heterogeneity, core biopsies were performed from 3 different areas of each tumor. One section was stained with H&E to confirm the presence of the tumor by light microscopy.

Four-micrometer thick sections were deparaffinized and pretreated in citrate buffer (pH 6.0) for 20 minutes using a steamer. Sections were cooled for 20 minutes and were incubated for 10 minutes with 3% hydrogen peroxide to quench endogenous peroxidase activity. Blocking was done using the serum-free protein block, Dakocytomation (Carpenteria, CA) for 30 minutes. Sections were incubated with PARP and p53 antibodies as follows: PARP (monoclonal; 1:25, Abcam, Cambridge, MA) and p53 (monoclonal; 1:50, NovaCastra, Newcastle, UK) for 60 minutes at room temperature. Diaminobenzidine tetrahydrochloride was then added for development for 10 minutes, followed by counterstaining with hematoxylin solution. Negative control slides omitting the primary antibody were included in all the assays. The evaluation of the IHC slides was done semiquantitatively by 1 pathologist (P.M-F.). At first, the staining was scored based on intensity, 0 (negative), 1 + (weak), 2 + (moderate), and 3 + (strong). The pathologists were blinded to the original histologic diagnosis. The IHC evaluation was done twice and each review was separated by a 6-week interval, and the scores were compared. Whenever a discrepancy occurred between the first and the second readings, the pathologist made the final scoring. Disagreement was not very frequent between the first and the second reading and occurred in approximately 5% of the specimens. The staining patterns were nuclear for both PARP and p53. However, for the statistical analysis, the cases were categorized in 2 groups, 0 and 1 + as group 1, and moderate and strong as group 2.

Statistical Analysis

Significance of the gene expression association with the survival outcome of interest was assessed using the type 3 Wald *P* value. If the expression was significant, then the parameter estimates and hazard ratios for that model were shown for further information. Kaplan-Meier plots were also developed to provide a visual comparison of the survival distribution

across gene expression level. Log rank *P* values were included on the plots. Associations between gene expression and outcomes of clinical response or disease recurrence were tested using logistic regression methods, following the logic similar to the survival analysis. Odds ratios and confidence intervals were estimated if the association of interest was significant.

RESULTS

Study Population

The characteristics of the study population are presented in Table 1. The mean age of the study population was 62 years (range: 34–86 yr), and the median duration of follow-up was 40 months (range: 0.5–165 mo). The majority of patients presented with grade 3 tumors (81.5%), advanced stage III (77%), and serous subtype (83%). A complete response to therapy was reached in 91 patients (48%). The median survival for all patients was 40 months (0.5–165 mo).

Expression of p53 and PARP in EOC

Positive staining for PARP was observed in 61% (115/189) of overall tumors. On the basis of the subtypes, PARP was positive in 94/158 (59%) papillary serous carcinoma, 6/9 (67%) clear cell carcinoma, 9/10 (90%) endometrioid carcinoma, 4/4 (100%) carcinosarcoma, 2/5 (40%) mucinous carcinoma, and 0/4 (0%) undifferentiated and other type carcinomas. p53 was positive in 52% (99/189) of overall cases. On the basis of the histologic subtype, p53 was positive in 91/158 (57%) papillary serous carcinoma; 3/9 (33%) clear cell carcinoma, 2/10 (20%) endometrioid carcinoma, 2/4 (50%) carcinosarcoma, 1/4 (2%) mucinous carcinoma, and 0/4 (0%) undifferentiated and other type carcinomas. Both PARP and p53 were expressed in the same tumor in 66/189 (35%) of tumors (Fig. 1 for PARP and p53 staining).

Correlation of PARP and p53 Expression and Clinical Outcomes

PARP and p53 expressions were shown in 61% and 54% of cases, respectively. PARP-positive tumors are more likely to be of higher grade ($P = 0.03$) and an initial complete response to treatment ($P = 0.009$). Patients with positive p53 staining are more likely to be having an advanced stage tumor ($P = 0.004$). Patients with tumors expressing p53+/PARP– showed a trend of increased likelihood of disease progression (odds ratio: 2.25; confidence interval: 0.389–13), but this finding did not reach statistical significance. However, there were no significant associations between PARP and p53 expressions. Finally, there was no significant difference in progression-free survival or OS for PARP and/or p53 expression (Fig. 2).

DISCUSSION

PARP and p53 plays an integral role in the regulation of DNA repair and apoptosis. They also play an important role in tumor development, progression, treatment, and in the overall prognosis. p53 and PARP are both involved in the cell's response to genotoxic stressors.

However, the mechanism of action of these 2 enzymes functioning synergistically has yet to be defined.

Our results showed that PARP and p53 were expressed in more than half of our series of cases, 61% of cases of the former and 54% of the latter. In addition, PARP was overexpressed in high-grade tumors and p53 was overexpressed in the advanced stage disease. These findings led us to believe that PARP and p53 might be used as biomarkers for aggressive disease behavior and as predictors of poor OS. Furthermore, our findings are consistent with other studies in which p53 overexpression and mutation in ovarian carcinoma was found to be positively associated with higher grade and advanced stage at diagnosis translating into overall worse prognosis (18,19). Even though numerous studies of PARP expression in different tumors have been documented, the role of PARP in tumor progression and angiogenesis in EOC has been explored by (19–21) few.

Brustmann showed strong PARP expression in 76% of serous carcinomas and this strong expression of PARP was associated with the tumor stage and grade (based on Malpica et al. (22) grading scheme). Applying the grading system based on Shimizu et al's. (15) grading scheme, we found similar results in which strong expression of PARP was associated with the tumor grade but not with the stage. In addition, Brustmann's study showed a trend between PARP immunoexpression and poor outcome. Our study failed to show similar findings or any value of PARP in patient OS and DFS. These differences in the results might be due to numerous reasons; Brustmann's study evaluated a small number of cases (n = 50), only serous subtypes were considered and high number of cases were stage I tumors.

Both p53 and PARP play a vital role in maintaining the integrity of the genome. Both are intricately involved in DNA repair, however, the exact mechanism by which these 2 genomic guardians interact has yet to be identified. In our study, we did not find a significant association between PARP and p53 protein expression.

The role of DNA repair mechanisms in cancer cells has become a target for translational research. Mutations in DNA repair mechanisms leads to an increased risk of malignant transformation and resistance to chemotherapies and radiation treatment. Research has typically focused on protecting cells from DNA damage. However, in recent years there has been a paradigm shift toward the concept of "synthetic lethality," promoting the susceptibility of cancer cells when 1 DNA repair mechanism has been lost, by targeting a second pathway to ensure cell death (23).

Elaborating on the concept of synthetic lethality, PARP inhibitors may be a therapy for the treatment of women with hereditary BRCA1/2-associated EOC (24,25). It has been published earlier that tumors that have lost BRCA1 or BRCA2, a component of the DNA repair pathway, are particularly sensitive to PARP inhibition, and treatment of these specific breast and ovarian cancers with PARP inhibitors may affect OS (13,26–28). However, BRCA-associated EOC is only associated with 10% of all ovarian cancers. Identifying a subset of EOC that are similar to BRCA1 and BRCA2-mutated tumors will be helpful in identifying a group of patients that may respond to PARP inhibition therapy. There are currently many PARP inhibitors in clinical trial development. Both in-vitro and in-vivo

evidence suggests that PARP inhibitors could be used not only as chemo/radiotherapy sensitizers, but also as single agents to selectively kill cancer cells that are defective in DNA repair.

In summary, as seen in our study, the frequent and overexpression of PARP in EOC might be of value as in cancer therapy. In addition, high PARP expression maybe involved in cisplatin drug resistance and identifying those patients with high PARP expression would be of great benefit when selecting patient therapy. Still, additional studies are warranted to evaluate the role of PARP inhibitors in the setting of adjuvant therapy for EOC patients especially those associated with both BRCA1 and BRCA2 mutations.

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FIG. 1.
(A–D) Immunohistochemical staining of poly (adenosine diphosphate-ribose) polymerase (PARP) and p53, both are in nuclear patterns. All figures are 40× magnification. (A) PARP weak staining. (B) PARP strong staining. (C) p53 weak staining. (D) p53 strong staining.

FIG. 2.
Overall survival.

TABLE 1

Patient and histologic characteristics

Evaluable patients	189
Age median (range) (yr)	62 (86–34)
Follow-up median (mo)	40 (0.5–165)
FIGO stage	
I	8 (4%)
II	9 (5%)
III	147 (78%)
IV	24 (13%)
Unknown	2 (0.1%)
Histology	
Papillary serous	158 (84%)
Clear cell	9 (5%)
Endometrioid	10 (5%)
Mucinous	5 (2%)
Undifferentiated	2 (1%)
Carinosarcoma	4 (2%)
Other	2 (1%)
Grade	
1	10 (6%)
2	16 (8%)
3	163 (86%)
Residual tumor	
< 1 cm	105 (56%)
> 1 cm	84 (44%)
Response to frontline therapy	
Complete response	91 (48%)
Persistent disease	2 (1%)
Progression	93 (49%)
Unknown	3 (2%)
Current status	
Alive, NED	22 (12%)
Alive with disease	11 (6%)
Dead of disease	154 (81%)
Unknown	2 (1%)
PARP status	
PARP positive	115 (61%)
PARP negative	74 (39%)
p53 status	
p53 positive	99 (52%)
p53 negative	90 (48%)

FIGO indicates International Federation of Gynecology; PARP, poly (adenosine diphosphate-ribose) polymerase.

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