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Logan M. Lawrence
Marshall University Joan C. Edwards School of Medicine

Rebecca Russell
Cabell Huntington Hospital

Claire E. Denning
Marshall University Joan C. Edwards School of Medicine

Nadim Bou Zgheib
Marshall University Joan C. Edwards School of Medicine

Travis Salisbury
Marshall University Joan C. Edwards School of Medicine

See next page for additional authors

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Original article

Expression of poly-ADP-ribose polymerase (PARP) in endometrial adenocarcinoma: Prognostic potential

Logan M. Lawrence[a], Rebecca Russell[b], Claire E. Denning[a], Nadim Bou Zgheib[c], Travis Salisbury[d], Seth T. Lirette[e], Jagan Valluri[f], Pier Paolo Claudio[g,*], Krista L. Denning[a]

[a] Cabell Huntington Hospital Laboratory, Department of Pathology, Marshall University Joan C. Edwards School of Medicine, Huntington, WV, 25701, USA
[b] Cabell Huntington Hospital, Edwards Comprehensive Cancer, Tissue Procurement, Huntington, WV, 25701, USA
[c] Marshall University Joan C. Edwards School of Medicine, Department of Obstetrics and Gynecology, Edwards Comprehensive Cancer Center, Huntington, WV, 25701, USA
[d] Marshall University Joan C. Edwards School of Medicine, Department of Biomedical Sciences, Huntington, WV, 25701, USA
[e] Department of Data Science, University of Mississippi Medical Center Jackson, MS, 39216, USA
[f] Marshall University, College of Science, Department of Biological Sciences, Huntington, WV, 25701, USA
[g] Department of BioMolecular Sciences, National Center for Natural Products Research, and Department of Radiation Oncology, University of Mississippi Medical Center, Cancer Center and Research Institute, Jackson, MS, 39216, USA

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ABSTRACT

Background: In the United States endometrial carcinoma is the most common female gynecologic malignancy. An average of more than 60,000 new cases of endometrial carcinomas have been diagnosed yearly over the past 5 years, with a higher incidence occurring in the central Appalachian states of Ohio and West Virginia. In the U.S., the national average of newly diagnosed endometrial carcinomas is 26.8 in every 100,000 women, while in the states of Ohio and West Virginia the average is 30.5 and 31.1 in every 100,000 women, respectively. This notable increase in the incidence of endometrial carcinomas may be due a variety of elevated risk factors including but not limited to: tobacco use, obesity, and genetic predisposition of the predominant demographic. The American Cancer Society estimates that approximately 55,000 new cases of endometrial carcinoma will be diagnosed in 2020 yet, this disease is widely considered understudied and under-represented in mainstream cancer research circles.

Methods: The aim of this study was to quantitate the co-expression of two DNA repair proteins poly-ADP-ribose polymerase 1 and 2 (Parp-1 and Parp-2) by enzyme- linked immuno-sorbent assay (ELISA) in 60 endometrioid endometrial tumor samples and compare their expression to matched non-malignant endometrial tissue from the same corresponding donors from central Appalachia.

Results: We found that Parp-1 was significantly overexpressed in endometrial carcinoma relative to corresponding normal tissue. This overexpression implicates Parp inhibition therapy as a possible treatment for the disease. Our results also found a protective effect of native Parp-2 expression in non-malignant endometrial tissue with each 1 ng/mL increase in PARP-2 concentration in normal tissue was associated with a 10 % reduction in the hazard of tumor progression (HR = 0.90; p = 0.039) and a 21 % reduction in the hazard of death (HR = 0.79; p = 0.044).

Conclusions: This study demonstrated the over-expression of the druggable target Parp-1 in endometrial adenocarcinoma and observed a strong negative correlation of native Parp-2 expression and disease progression via the quantification of the Parp proteins using enzyme- linked immuno-sorbent assay (ELISA) assays.

1. Background

Parp-1 and Parp-2 enzymes are DNA repair proteins in the PARP family of nuclear enzymes involved in a number of cellular processes including DNA-repair, genetic stability, and programmed cell-death [1,2]. The primary role of Parp-1 and Parp-2 within the cell is to

Abbreviations: PARP, poly-ADP-ribose polymerase; HR, hazard ratio; FIGO, federation of gynecology and obstetrics; SSB, single-strand; (DSB) DNA, double-strand; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered saline; TGF-β, transforming growth factor beta-1

* Corresponding author at: University of Mississippi Medical Center, Cancer Center and Research Institute, Jackson, MS, 39126, USA.
E-mail address: pclaudio@olemiss.edu (P.P. Claudio).

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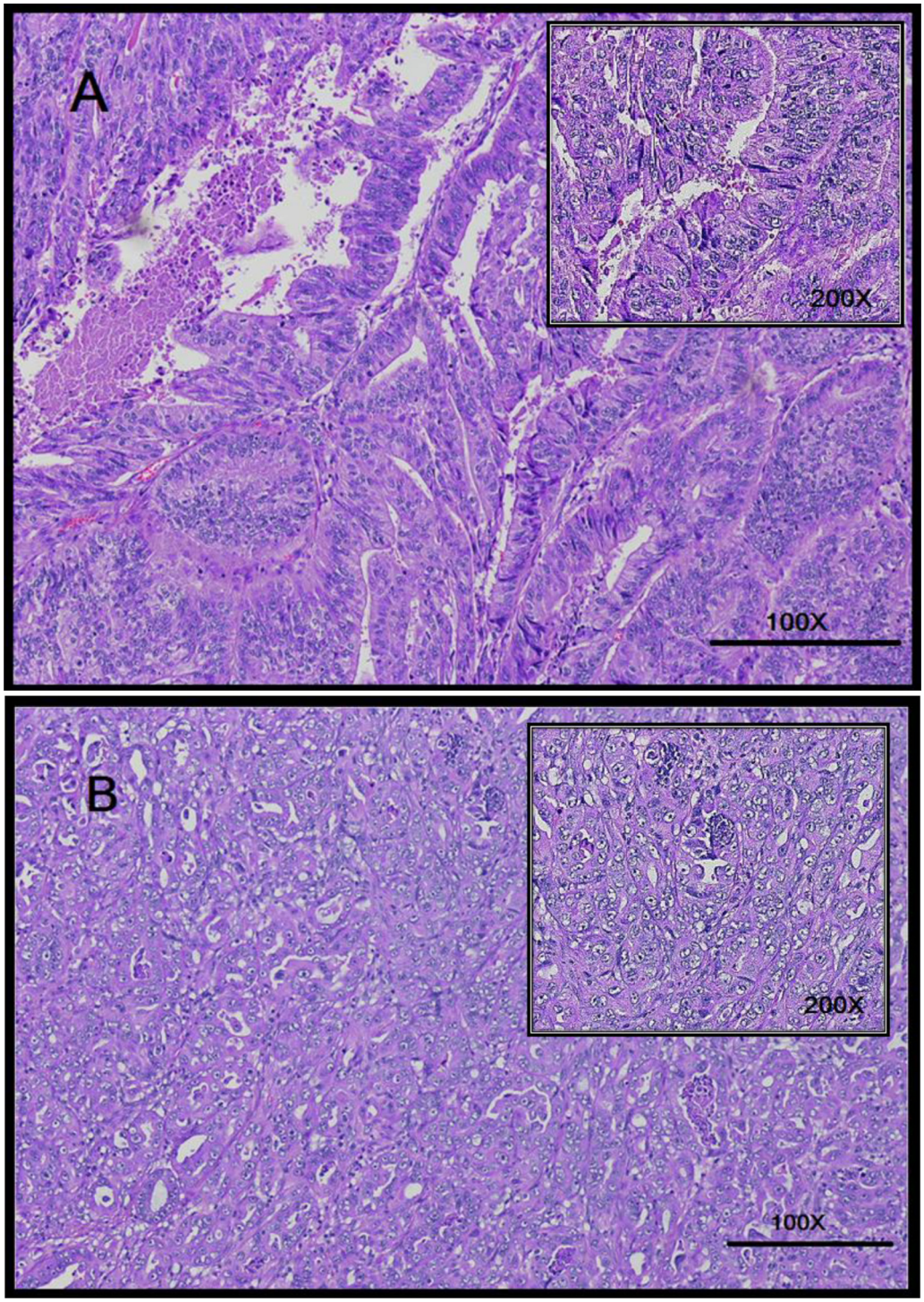

Fig. 1. Hematoxylin & eosin staining of a grade -2 and -3 endometrial carcinoma.
A) 100 X magnification of a grade-2 endometrial carcinoma showing a 6 %–50 % non-squamous solid growth pattern. Insert shows a 200 X magnification.
B) 100 X magnification of a grade-3 endometrial carcinoma showing a > 50 % non-squamous solid growth pattern. Insert shows a 200 X magnification.

identify and initiate a repair response to single-strand (SSB) and double-strand (DSB) DNA breaks during homologous recombination [1–8]. There is, however, a growing body of evidence to suggest that the Parp family of proteins, specifically Parp-2 may play a more diverse role within the cell and could possibly serve as prognostic markers in the cancer setting [9–11].

The roles of the Parp-1 and Parp-2 proteins in the repair process of damaged DNA are by far the most characterized functions of the family of Parp proteins and have been targets of recently developed anticancer therapies for the treatment of ovarian cancer [7,12,13]. These therapies are now being examined for their utility in a variety of other malignancies with and without radiation or other chemotherapeutic agents in ovarian, breast, prostate, rectal, lung, pancreatic, peritoneal, head and neck, brain, squamous cell carcinomas and sarcomas, to list a few [14–16]. Clinical trials investigating Parp inhibitors for the treatment of endometrial cancer are also underway (NCT03586661, NCT01237067, NCT03572478, and NCT03951415).

Both Parp-1 and Parp-2 recognize SSB and DSB DNA breaks via their

Table 1
Participant Characteristics.

Median Age	64
PT1A stage	32 (53 %)
PT1B stage	17 (28 %)
PT2 stage	6 (10 %)
PT3A stage	2 (3 %)
PT3B stage	2 (3 %)
PT4 stage	1 (2%)
Grade 2	40 (67 %)
Grade 3	20 (33 %)
Reg. Lymph Node	3 (5 %)
Cerv. Stroma Invasion	9 (15 %)
Myometrial Invasion %	40.54 ± 33.49
PARP-1 Tumor	19.96 ± 8.5
PARP-1 Normal	9.64 ± 6.14
PARP-2 Tumor	7.63 ± 5.23
PARP-2 Normal	8.93 ± 6.24
Death	6 (11 %)
Progression	13 (22 %)

Shown are the mean for continuous variables and count (percentage) for categorical variables.

zinc-finger DNA binding domains [8,17,18]. After Parp-1 directly binds to the altered DNA, Parp-1 increases its catalytic activity and uses NAD + to create polymers of poly(ADP-ribose) (PAR) and transfers them to acceptor proteins, including additional Parp. The accumulation of the resulting (PAR) polymers recruits various DNA repair proteins to the site of damage. Subsequent to the PAR accumulation DNA polymerases alpha and beta are recruited to the damaged DNA and facilitate DNA repair. In somatic tissues, this function of Parp-mediated DNA repair serves to limit the accumulation of genetic alterations resulting from replication and oxidative damage [19]. In the cancer setting however, malignant cells have been shown to utilize the Parp family of proteins to promote chemotherapy and radiotherapy resistance. To defeat this Parp-mediated therapy resistance, Parp inhibitors have been developed. In conjunction with radiation therapy and the classic platinum based chemotherapeutic agents Cisplatin, Oxaliplatin, and Carboplatin, Parp inhibitors are used to sensitize malignant cells to DNA damage induced by chemotherapy [6,7].

When PARP inhibitors are present, PARP-dependent repair systems are not activated due to catalytic inhibition and/or direct trapping. This results in replication fork stalling during DNA replication, and creation of DSBs. In cells where homologous recombination (HR) is not impaired (such as in the case of wild-type BRCA), DSBs are repaired and replication may restart, resulting in cell survival. However, in BRCA-deficient cells where homologous recombination is impaired, DSB cannot be efficiently repaired and DSB accumulates, resulting in cell death [20].

Parp inhibitors Olaparib, Niraparib, Rucaparib, and Talazoparib have been developed and approved for the treatment of a variety of malignancies [21–28]. These inhibitors directly bind to the Parp molecules and prevent their interaction with damaged DNA. Although Parp inhibition therapies have been approved for the treatment of ovarian cancer, fallopian tube cancer, and primary peritoneal cancer; to date their utility has not been widely investigated for endometrial cancer.

In addition to their DNA repair function, Parp proteins have been shown to regulate a number of essential processes that directly influence the growth and maintenance of tumors such as metabolic, angiogenetic, and proliferation pathways [18,29–31]. All this emerging literature on the multifaceted nature and function of Parp proteins within cancer opens the opportunity to use Parp immunostaining expression as a potential prognostic assay in the clinical setting. Parp-2 over-expression in particular may demonstrate clinical significance in predicting the virulence of a malignancy.

Parp-2 has been shown to be involved in dysregulated malignant processes including, but not limited to, proliferative signaling, angiogenesis, and metabolic shift. All of these cellular processes are positively correlated to higher-grade of solid malignant tumors, including high-grade endometrial cancer. In the light of all this, the intent of our study was to help filling the knowledge gap on the expression of Parp-1 and Parp-2, for their potential use as druggable targets in the treatment of endometrioid endometrial cancer. We examined 60 patients from southern Ohio and southwestern West Virginia diagnosed with Endometrioid Endometrial Adenocarcinoma with an International Federation of Gynecology and Obstetrics (FIGO) score grade-2 or above over the course of 3 years. The FIGO grading system is based on the architectural features of the endometroid endometrial carcinoma [32]. Grade-2 endometrioid endometrial carcinomas show a 6 %–50 % non-squamous solid growth pattern, while grade-3 endometrioid endometrial carcinomas show greater than 50 % non-squamous solid growth pattern. Nuclear atypia, which is more that what would be expected for architectural grade, increases the FIGO grade by one [32] (Fig. 1). The aims of this investigation were to establish the protein expression profiles of Parp-1 (Poly-ADP-ribose polymerase-1) and Parp-2 (Poly-ADP-ribose polymerase-2), two cancer-associated DNA repair proteins, in tumor tissue compared with matched non-malignant endometrial tissue and stratify this data based on the grade of the malignancy.

2. Methods

2.1. Patient samples

De-identified research samples were obtained from the non-profit, open access biorepository bank at the Edwards Comprehensive Cancer Center at Cabell Huntington Hospital, Huntington, WV. Endometrioid Endometrial Cancers with a FIGO score of equal or greater than 2 were obtained from the tumor bank along with their corresponding normal tissue samples. A sample cohort of 40 grade-2 endometrioid endometrial adenocarcinomas and 20 grade-3 endometrioid endometrial carcinomas were collected (Table 1). Each of the patient samples were from total hysterectomy specimens. The tumor samples and their matched normal tissue counterparts were collected on ice in the surgical pathology department with no more than 15 min of time elapsed from surgery. Tumor tissue and corresponding normal tissue were identified based upon gross examination by either the pathologist on call or by the attending pathology assistant. Once the tissue identifications were established, representative sections were cut using aseptic technique from both the malignant tissue and the grossly normal tissue. Blade changes were made between cutting of the tumor tissue and the non-tumor tissue. After collection, the samples were immediately flash frozen in liquid nitrogen and transferred to liquid nitrogen vapor phase storage for not more than 3 years.

2.2. ELISA assay

Our study utilized Parp-1 and Parp-2 enzyme- linked immuno-sorbent assay (ELISA) kits purchased from Aviva Systems Biology®. After screening and sample selection was complete, the samples were thawed at room temperature. After this initial thaw, the samples were rinsed with sterile 1X PBS (Phosphate Buffered Saline, Thermo Fisher). The samples were mechanically homogenized with a sterile glass rod in sterile 15 mL centrifuge tubes in 5 mL of 1X PBS without added protease inhibitors. Additional 1X PBS was then added to the tissue homogenates to achieve a volume of 10 mL. These homogenates were then subjected to an additional freeze/thaw cycle at −20 °C for 24 h followed by thawing at room temperature to further lyse the cell membranes. After the final thaw at room temperature, the samples were vortexed and subsequently centrifuged for 5 min at 5000 × g at 4 °C as per assay specifications. The supernatant was removed and protein sample concentrations were assayed. Equal amounts of diluted protein

supernatant were then plated on the ELISA plates supplied with the purchased kits and the assay protocol was followed. The ELISA assay resulted in various absorbance values corresponding to levels of target protein in each sample. The absorbance values that were obtained were then transferred to an in-house data analysis software developed using the Windows Microsoft Excel platform to calculate the standard deviations for each group and compared the average absorbance for each group to the standardized controls. Each test group for each of the proteins of interest consisted of four replicates. The assay specific controls were run with each plate to ensure optimal accuracy of the results.

2.3. Statistical analyses

Descriptive statistics were compiled where appropriate and univariate comparisons of PARP concentrations between tissue types were made with Mann-Whitney U tests and stratified by grade. To visually depict these results, plots were constructed to display underlying data and mean PARP concentrations along with 95 % confidence intervals. Hazard ratios for death and progression were calculated via Cox proportional hazard models. Ordinary least squares regression was used to assess the relationship between myometrial invasion percentage and PARP, while logistic models were used for PARP and the regional lymph node involvement or cervical stroma invasion, the latter two being combined into one group due to small percentages. All models were age adjusted, and all analyses were performed with Stata v15.1 (StataCorp, College Station, TX).

3. Results

The success of PARP inhibitors in the treatment of ovarian cancer serves as a model for the appropriate use of these targeted therapies in a variety of malignancies. We have studied the expression of Parp-1 (Poly-ADP-ribose polymerase-1) and Parp-2 (Poly-ADP-ribose polymerase-2) on 60 patients from southern Ohio and southwestern West Virginia diagnosed with Endometrioid Endometrial Adenocarcinoma with an International Federation of Gynecology and Obstetrics (FIGO) score grade-2 or above over the course of 3 years. Fig. 1 shows a hematoxylin and eosin staining of a grade-2 endometrioid endometrial carcinomas showing 6–50 % non-squamous solid growth pattern, while grade-3 endometrioid endometrial carcinomas show greater than 50 % non-squamous solid growth pattern.

The mean participant age was 64 years. Most patients presented with stage 1 primary tumors (PT1A plus PT1B = 49/60 cases; 81 %). Two thirds (40/60 cases; 67 %) of the tumors were grade 2. Three participants (5 %) had regional lymph node involvement, while 9 (15 %) had cervical stroma invasion and the mean myometrial invasion percentage was 40.5. Thirteen (22 %) participants had documented disease progression, while 6 (11 %) died during the course of the study (Table 1).

In this investigation, we aimed at establishing the protein expression profiles of PARP-1 and -2 cancer associated DNA repair proteins in tumor tissue compared with matched non-malignant endometrial tissue and to stratify this data based on the grade of the malignancy. Figs. 2 and 3 combined with Table 2 display the results of this aim. PARP-1 concentration was significantly higher in tumor tissue compared to normal for both grades 2 and 3 (both $p < 0.001$). Interestingly, PARP-2 concentration was significantly higher for tumor tissue in grade 3 ($p < 0.001$), but was significantly higher for normal tissue in grade 2 ($p = 0.001$).

Further, age-adjusted survival analysis results demonstrated that PARP-1 concentration was not associated with neither death nor progression (death PARP-1 tumor $p = 0.632$; progression PARP-1 tumor $p = 0.332$; death PARP-1 normal $p = 0.552$; progression PARP-1 normal $p = 0.652$). PARP-2 tumor concentration trended in a hazardous direction for both death and progression (death HR = 1.09; progression HR = 1.10, per 1 ng/mL increase), although the study was underpowered to garner statistical support (death $p = 0.267$, progression $p = 0.067$). PARP-2 concentration in normal tissue, however, did have statistical support for a protective effect. Each 1 ng/mL increase in PARP-2 concentration in normal tissue was associated with a 10 % reduction in the hazard of progression (HR = 0.90; $p = 0.039$) and a 21 % reduction in the hazard of death (HR = 0.79; $p = 0.044$). See Table 3.

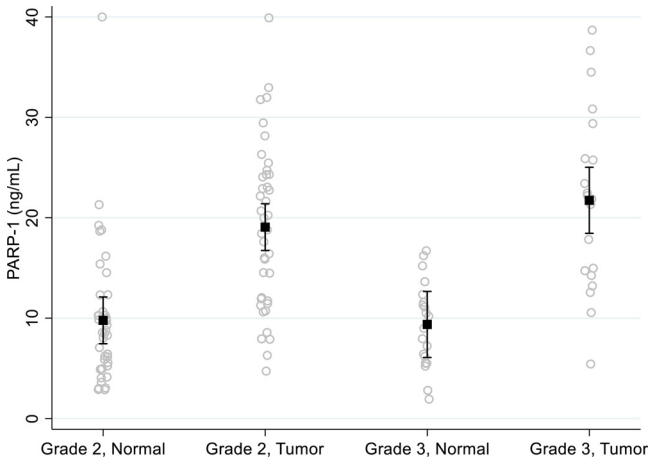

Fig. 2. PARP-1 expression (ng/mL) in extracts from matched normal endometrial tissue compared to grade-2 and grade-3 endometrial tumors measured by ELISA assay.

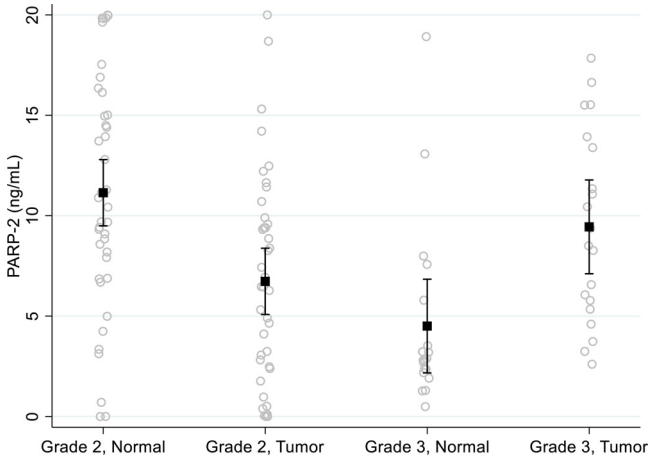

Fig. 3. PARP-2 expression (ng/mL) in extracts from matched normal endometrial tissue compared to grade-2 and grade-3 endometrial tumors measured by ELISA assay.

As an exploratory aim, we were interested in investigating if a relationship exists between PARP concentration and myometrial invasion percentage. We did not find any associations supporting this (PARP-1 tumor $p = 0.481$; PARP-1 normal $p = 0.995$; PARP-2 tumor $p = 0.592$; PARP-2 normal $p = 0.939$). Also, no associations were found between PARP and probability of regional lymph node involvement or cervical stroma invasion (PARP-1 tumor $p = 0.472$; PARP-1 normal $p = 0.627$; PARP-2 tumor $p = 0.858$; PARP-2 normal $p = 0.343$).

These results strongly suggest that more studies should be conducted toward understanding the clinical utility of Parp-1 and -2 inhibition therapy for the treatment and care management of endometrial cancer.

4. Discussion

We report here an evaluation of PARP-1 and -2 concentrations

Table 2
Comparisons of PARP proteins expression between tissue samples.

		Normal	Tumor	p-value
Grade 2	PARP-1	9.78 (6.95) ; 8.42 [5.28–11.5]	19.1 (8.19) ; 19.3 [11.7–24.3]	< 0.001
	PARP-2	11.1 (5.85) ; 10.7 [7.39–15.4]	6.73 (5.28) ; 6.39 [2.45–9.62]	0.001
Grade 3	PARP-1	9.37 (4.25) ; 9.62 [6.00–11.9]	21.7 (9.07) ; 21.9 [14.6–27.6]	< 0.001
	PARP-2	4.50 (4.43) ; 3.04 [2.27–4.56]	9.44 (4.75) ; 8.88 [5.45–13.6]	< 0.001

Statistics within each cell are mean (standard deviation); median [1st quartile – 3rd quartile]. p-value derived from Mann-Whitney *U* test.

Table 3
Age-adjusted hazard of progression or death per one unit increase in PARP expression.

	Progression	Death
PARP-1 Tumor	HR = 0.97; p = 0.332 (0.90–1.04)	HR = 1.02; p = 0.632 (0.93–1.12)
PARP-1 Normal	HR = 0.97; p = 0.652 (0.86–1.10)	HR = 0.94; p = 0.552 (0.80–1.13)
PARP-2 Tumor	HR = 1.10; p = 0.067 (0.99–1.23)	HR = 1.09; p = 0.267 (0.94–1.27)
PARP-2 Normal	HR = 0.90; p = 0.039 (0.81 – 0.99)	HR = 0.79; p = 0.044 (0.62 – 0.99)

relative to grade of the disease and to clinical progression and survival in an endometrioid endometrial adenocarcinoma patient cohort. Our study examined 60 patients from southern Ohio and southwestern West Virginia diagnosed with Endometrioid Endometrial Adenocarcinoma with a FIGO score grade-2 or above over the course of 3 years (Table 1). We studied the expression profiles of Parp-1 and -2 in tumor tissue compared with matched non-malignant endometrial tissue and stratified this data based on the grade of the malignancy.

The Parp-1 and Parp-2 enzymes are DNA repair proteins in the PARP family of nuclear enzymes involved in a number of cellular processes including DNA-repair, genetic stability, and programmed cell-death [1,2].

In addition to their DNA repair function, Parp proteins have been shown to regulate a number of essential processes that directly influence the growth and maintenance of tumors such as metabolic, angiogenetic, and proliferation pathways [18,29–31]. In fact, both Parp-1 and Parp-2 have upstream regulatory effects on cellular oxidative metabolic processes via the Parp/SIRT1 protein interaction [3,33,34]. Parp-2 has been shown to directly bind to the SIRT1 gene promoter where it blocks SIRT1 transcription. This results in significant impediments to mitochondrial biogenesis with a shift of cellular processes towards glycolytic metabolism, which is one of well-known hallmarks of the carcinogenic process (shift from traditional oxidative respiration to aerobic glycolysis) [4,35]. This is known as the “Warburg effect” and this effect is seen in high-grade neoplasms including grade-3 endometrial carcinomas. Our data showed that there is a statistically significant increased expression in grade-2 and -3 tumors compared to their normal matched tissues (Table 2 and Figs. 2 and 3). Interestingly, PARP-2 concentration was significantly higher for tumor tissue in grade 3 ($p < 0.001$), but was significantly higher for normal tissue in grade 2 ($p = 0.001$).

Parp proteins have been found to promote tumor angiogenesis by SIRT1 inhibition. Angiogenesis is a crucial function in the development, growth, and maintenance of healthy tissues [36–39]. New blood vessel formation serves to oxygenate and nourish healthy and growing tissues, however this process is also utilized by tumors. Just as in healthy tissues, malignant tumors rely on angiogenesis to nourish the rapidly growing neoplastic mass of cells [40]. In addition to allowing a tumor to rapidly increase in size, the new blood vessels formed by tumor angiogenesis provide a route for tumor metastasis and migration to other locations within the body [36,41]. The Parp-1 protein has a well-documented angiogenic effect; however, the specific molecular pathways involved have yet to be determined. Our data on the expression levels of Parp-1 and -2 in grade-2 and -3 endometrioid endometrial adenocarcinoma is in agreement with the idea that tumors need increased angiogenesis for their growth and homeostasis in comparison to normal tissues.

Recently, Parp-2 has also been found to promote proliferative signaling via the suppression of Transforming Growth Factor Beta-1 Pathway Regulation [37]. TGF-β is a polypeptide protein involved in a variety of cellular functions including cell cycle regulation, cell growth, cell proliferation, cell differentiation and apoptosis [42]. The TGF-β pathway is an important tumor suppressor pathway that promotes tissue homeostasis and cell differentiation particularly of endothelial cells. TGF-β is suppressed in the carcinogenesis of a variety of cancers including endometrial cancer [42]. Our data that PARP-2 is more expressed in higher-grade endometrioid endometrial cancer is also in agreement with the fact that more aggressive tumors may proliferate more abundantly, and could display a suppressed TGF-β pathway.

From a therapy point of view, it has been widely shown that malignant cells utilize the Parp family of proteins to promote chemotherapy and radiotherapy resistance. To defeat this Parp-mediated therapy resistance, Parp inhibitors have been developed. Parp inhibition therapies have been developed and approved for the treatment of a variety of malignancies [21–28]. These inhibitors directly bind to the Parp molecules and prevent their interaction with damaged DNA. This effectively silences the Parp DNA repair activities, which promote apoptosis of cells in which chemo-radiation therapies determined DNA damage that cannot be repaired. In fact, in conjunction with radiation therapy and the classic platinum based chemotherapeutic agents Cisplatin, Oxaliplatin, and Carboplatin, Parp inhibitors have been used in the clinics to sensitize malignant cells to DNA damage induced by chemotherapy [6,7]. Platinum based chemotherapeutic drugs affect rapidly dividing cancer cells by cross-linking DNA at adjacent N-7 positions on guanine bases. The crosslinks result in inhibited DNA repair leading to DNA single-strand (SSB) and double-strand breaks (DSB). On detection of a SSB or DSB, the recruitment and activation of PARP leads to SSB and DSB repair via polymers of ADP-ribosylation of histones and chromatin-remodeling enzymes, autoPARylation of PARP and recruitment of PARP-dependent DNA repair proteins. Repaired DNA can subsequently undergo replication, resulting in the survival of the cell [43]. Radiation therapy also targets the DNA of rapidly dividing neoplastic cells and induces similar SSBs and DSBs which are repaired by the Parp proteins [44]. From this knowledge, Parp inhibitors have been developed to decrease the ability of cancer cells to repair DNA damage induced by chemoradiation therapy.

Although Parp inhibition therapies have been studied and approved for the treatment of ovarian cancer, fallopian tube cancer, and primary peritoneal cancer; to date their utility has not been widely investigated for endometrial cancer. To our knowledge, this is the first time that PARP-1 and -2 expression have been measured in endometrial adenocarcinoma samples. Our findings that PARP-1 and -2 are expressed at higher levels than matched normal tissue in patients affected by endometroid endometrial carcinomas paves the way to further investigating the potential use of PARP-inhibitors in the treatment of endometrial cancer. More studies are needed to determine if PARP-1 and -2 expression could be considered in the future as biomarkers to

stratify the response of endometrial carcinoma patients to therapy with Parp inhibitors.

5. Conclusions

This study to our knowledge demonstrated for the first time in endometrial adenocarcinoma the over-expression of druggable targets via the quantification of the Parp proteins that paves the way to the idea that PARP inhibitors could be useful for the treatment of this endometrial disease.

Ethics approval and consent to participate

Ethical approval was obtained from Marshall University Institutional Review Board (IRB) to use de-identified research samples acquired from the non-profit, open access biorepository bank at the Edwards Comprehensive Cancer Center, Cabell Huntington Hospital, Huntington, WV.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Logan M. Lawrence carried out the experiments and helped interpreting the data and writing the manuscript.

Rebecca Russell provided the specimens and helped carrying out some experiments.

Claire E. Denning helped carrying out some experiments and helped interpreting the data and writing the manuscript.

Nadim Bou Zgheib provided the specimens and helped interpreting the data and writing the manuscript .

Travis Salisbury helped interpreting the data and writing the manuscript.

Seth T. Lirette helped interpreting the data and writing the manuscript.

Jagan Valluri helped interpreting the data and writing the manuscript.

Pier Paolo Claudio helped interpreting the data and writing the manuscript.

Krista L. Denning carried out some experiments, supervised the project, and helped interpreting the data and writing the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Not applicable.

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