P R A C E O R Y G I N A L N E
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PARP-1 activity in normal and cancerous human endometrium and its relationship with quantity of abasic sites (AP)

Aktywność PARP-1 w prawidłowym i nowotworowym *endometrium* w relacji do liczby miejsc apurynowych/ apirymidynowych

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Abstract

Objectives: *Poly (ADP-ribose) polymerase (PARP-1) is involved in the processes of DNA repair, contributing to the maintenance of genomic stability. Recent data suggest that polymerase is involved in the development of endometrial adenocarcinomas and more advanced tumors displaying lowest enzyme protein expression. Data on PARP-1 activity regarding carcinogenesis in human endometrium are scarce. That was the reason why the authors of the present work wished to investigate the enzyme activity in human uterine hormone-dependent cancer and to compare the results with those obtained for normal endometrial tissue. The next aim was to check whether enzyme activity in normal and cancerous endometrium depends on the number of AP sites, which are widely known as oxidative stress DNA damage markers and PARP-1 activity stimulators.*

Material and methods: *Universal Colorimetric PARP Assay Kit was used to estimate the enzyme activity in units/ mg protein. Apurinic sites/105 base pairs (bp) were measured by Oxidative DNA Damage Kit Quantitative. Results were calculated for 47 endometrial samples and 15 uterine adenocarcinomas specimens. Finally, the PARP-1 activity was analyzed for histological and some clinical features of neoplasms.*

Results and conclusions:

1. *no differences in PARP-1 activity were found in non-cancerous types of human endometrium;*
2. *mean enzyme activity was lower in sporadic endometrial cancers than in noncancerous endometrial specimens (2.89±0.55 vs 6.39±0.06; p<0.005);*
3. *mean PARP-1 activity in lower grade neoplasms was higher than in G3 tumors and was lower in adenocarcinomas displaying deep uterine wall infiltration;*
4. *there was no relationship between PARP-1 activity and AP level.*

Key words: **PARP-1 activity** / **human endometrium** / **uterine adenocarcinoma** /

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Streszczenie

Cel pracy: *badanie aktywności polimerazy poly(ADP-ryboza) (PARP-1) w prawidłowej i w nowotworowo zmienionej błonie śluzowej macicy w relacji do liczby miejsc apurynowych/apirymidynowych (AP), uznanych za aktywatora enzymu.*

Materiał i metody: *badania wykonano w utkaniu 15 gruczolakoraków endometrium oraz 47 fragmentach prawidłowej błony śluzowej macicy. Aktywność PARP oznaczano przy użyciu Universal Colorimetric PARP Assay Kit (Trevigen) i przeliczano na miligram białka. Liczbę miejsc AP kwantyfikowano w 105 par zasad wykorzystując Oxidative DNA Damage Kit Quantitative (Kamiya). Aktywność polimerazy w utkaniu nowotworowym analizowano w relacji do niektórych histologiczno-klinicznych cech guzów gruczołowych.*

Wyniki i wnioski:

1. *nie stwierdzono różnic aktywności PARP-1 w relacji do typu histologicznego nowotworowo niezmienionej błony śluzowej macicy;*
2. *średnia aktywność enzymu była niższa w utkaniu gruczolakoraków niż w endometrium nienowotworowym (2,89±0,55 vs. 6,39±0,06; p<0,005);*
3. *średnia aktywność PARP-1 w utkaniu nowotworów wyżej zróżnicowanych histologicznie była wyższa niż odnotowano w guzach G3 i niższa w gruczolakorakach wykazujących głęboką penetrację błony mięśniowej trzonu macicy;*
4. *w utkaniu guzów gruczołowych endometrium nie stwierdzono relacji między aktywnością PARP-1 a liczbą miejsc AP.*

Słowa kluczowe: **aktywność PARP-1** / ***endometrium* u kobiet** / / **gruczolakorak błony śluzowej macicy** /

Introduction

Poly (ADP-ribose) polymerase (PARP, ADPRP, EC 2.4.2.30) is a nuclear enzyme which becomes activated in response to DNA damage, especially single-strand breaks [1, 2]. PARP accounts for about 90% of the total cellular poly(ADP-ribose) formation which is a post-translational protein modification involving the transfer of ADP-ribose groups from NAD^+ to more than 30 nuclear acceptor proteins, including histones and PARP itself [3, 4]. PARP-1 enzyme, which is the best studied member of the PARP protein family that are encoded in mammals by eighteen genes, plays the primary role in the process of poly(ADP-ribosyl) ation [5, 6]. Experimental studies have revealed that rapid increase in PARP activity is strongly connected not only with estrogen administration but also tissue proliferation, whereas prompt decrease in that enzyme activity was reported to occur in cells after treatment with pharmaceuticals that blocked proliferation [7, 8, 9, 10].

Bürkle et al. [11] suggest that higher activity of PARP protects against genome instability and probably is helpful in increasing cell longevity. Paradoxically, however, during inflammation, ischemia-reperfusion, or shock, over-activation of PARP-1 leads to necrotic cell death, because poly(ADP-ribosyl) ation reaction depletes cellular energy by overconsumption of NAD^+ that affects the size of the cellular NAD^+ pool [12].

Poly(ADP-ribosyl)ation is involved in DNA base-excision repair (BER) that allows to protect the proliferating cells from certain types of DNA damage, including those related to oxidative stress, i. e. abasic sites, and DNA single-strand breaks that are directly or indirectly generated by reactive oxygen species (ROS) [13, 14].

Recently, Piskunova et al. [15] revealed that deficiency in poly(ADP-ribose) polymerase-1 accelerates aging and spontaneous carcinogenesis in mice.

In this study the authors discovered that PARP-1 null homozygous mice exhibit a reduction of life-span along with a significant increase of aging population rate and shortening of mortality rate doubling time. Although the incidence of spontaneous tumors in this population was similar to wild type animals, the malignant neoplasms were significantly more frequent in mice devoid of PARP-1 gene.

It has also been shown that human malignant tumors display higher poly(ADP-ribosyl)ation, PARP-1 activity or PARP gene expression than was revealed in neighboring cancer-free tissue [16, 17, 18, 19, 20]. Brustmann [21] using immunocytochemistry showed that PARP-1 expression in serous ovarian cancers is not only higher than in borderline malignancy but it increases correspondingly to neoplasm advancement classified according to FIGO. He also showed that benign ovarian tumors did not display enzyme protein expression. As mentioned by Brustmann, the mechanism of PARP-1 up-regulation in human cancer is not fully understood since the enzyme, which is currently also known as 'the guardian angel', is believed to be a suppressor of tumorigenesis due to its ability to protect the genome [22, 23].

To the best of our knowledge there have been only two studies concerning PARP-1 expression in endometrial normal and cancerous tissues [23, 24], both of them limited to the topic of immunohistochemical PARP density. There has been no information on enzyme activity in endometrial tissues, including uterine cancer which is the most common malignant tumor of the female genital organs in developed countries [25, 26].

In the paper the authors present PARP-1 activity in normal and cancerous human endometrial samples. Additionally, the authors tried to discover the relationship of the enzyme activity with abasic sites level, since this oxidative stress marker is recognized during BER as a single strand lesion and thus may activate PARP-1 [27].

Material and methods

The activity of PARP-1 and AP sites level estimates were done in 15 specimens of sporadic endometrial adenocarcinomas and 47 non-cancerous endometrial samples, obtained from women who had undergone surgery of internal genital organs at the II Department of Gynecology of Medical University in Lublin, Poland. A small amount of tissue material received for our investigations due to necessity of performing the postoperative microscopic evaluation did not permit estimating both PARP-1 activity and AP sites in every collected tissue specimen. Thus, the number of cases taken for correlation analyses was lower than the total number of presented measurements. Informed consent was obtained from every woman who was enrolled into the study. Mean (±SEM) age of cancer affected women was 58.8±2.1 (range 42-79 years) whereas cancer-free endometrium was excised from wombs of younger patients removed during surgery (49.6±1.2; range 31-76 years). Pathological assessment of the neoplasms was performed according to the WHO staging system [28]. There were three (20%) well-differentiated (G1), 10 (67%) moderately-differentiated (G2) and two (13%) poorly-differentiated (G3) tumors. 2/3 cases showed less than 50% infiltration of the uterine wall thickness, whereas 5 (33%) neoplasms revealed invasion above half of the myometrium. Ten samples of endometrial adenocarcinomas were categorized into stage IB, four into stage IC and one into stage IIB based on FIGO staging scale [29].

Non-cancerous endometrium according to commonly known histological criteria was divided into: proliferative (n=19), secretory (n=12), atrophic (n=12) and hyperplastic (n=4).

After hysterectomy, the uterine corpus was gently cut from the fundus toward internal os of the cervical canal to avoid any contamination of the uterine cavity with endocervical cells. Opened uterine cavity was then washed twice with cold (4°C) physiological saline solution to remove blood clots or uterine discharge. The tissue material was then immediately collected into sterile Eppendorf tubes under binocular magnification (5x) using punch biopsy forceps. All tissue samples were quickly frozen and stored in liquid nitrogen. The Universal Colorimetric PARP Assay Kit (Trevigen) containing a histone-coated plate was used to estimate the PARP-1 activity in units/mg protein [30]. The amount of AP sites was estimated using Oxidative DNA Damage Kit Quantitative (Kamiya Biomedical Company) as described earlier [31].

Statistical calculations were done using StatSoft, Inc. (2009) STATISTICA (data analysis software system), version 9.0. www.statsoft.com.

U Mann-Whitney test was used to check statistical differences and correlation analyses were performed using Spearman's rank correlation coefficient. All data are expressed as the means ±SEM.

Results

In non-cancerous endometrium (n=43) the mean PARP-1 activity was 6.39±0.06U/mg protein (range, 0.57-16.77; median 5.3). There was a negative correlation between enzyme activity and age of women, however it was not statistically significant (R= -0.71, p=0.48). In the proliferative endometrium (n=17) the mean PARP-1 activity was 7.11±0.99 (range, 0.57-13.0) and in this group the protein activity was not related to patient age

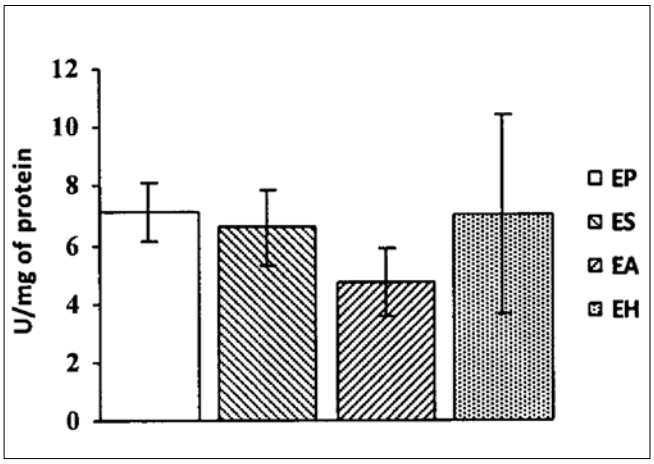

Fig. 1. The mean±SEM PARP-1 activity in proliferative (EP), secretory (ES), atrophic (EA) and hyperplastic (EH) endometrium.

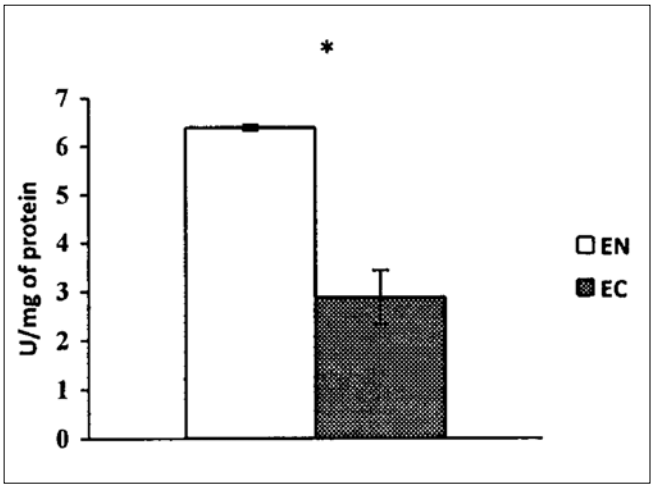

Fig. 2. The mean±SEM PARP-1 activity in non-cancerous (EN) and neoplastic (EC) endometrium, *p<0.005.

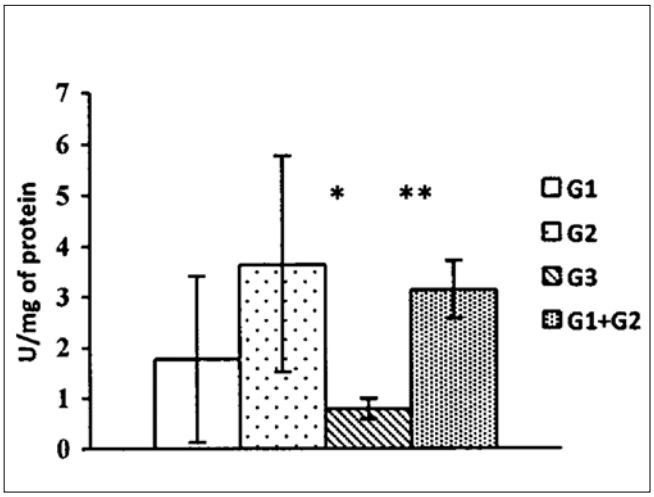

Fig. 3. The mean±SEM PARP-1 activity in endometrial carcinomas according to WHO, *p<0.04; **p<0.026.

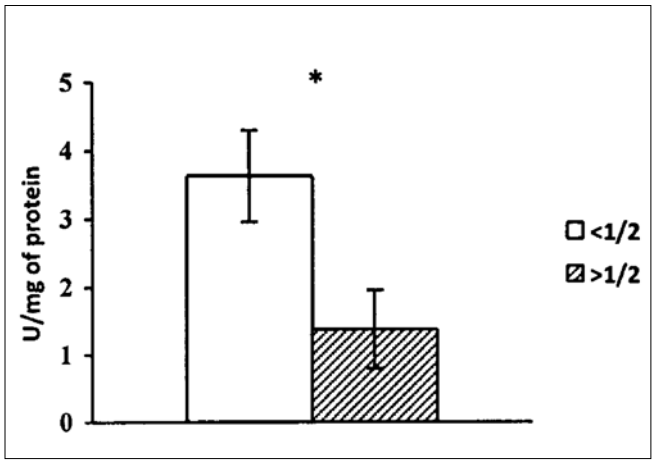

Fig. 4. The mean±SEM PARP-1 activity in uterine adenocarcinomas according to the depth of myometrial invasion, *p<0,013..

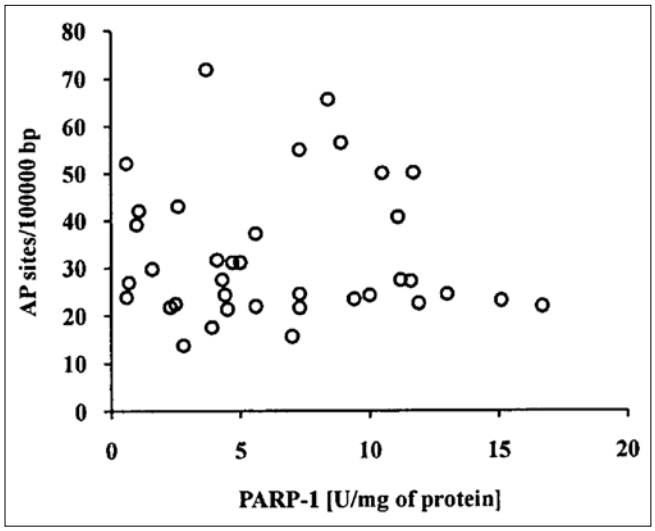

Fig. 5. Relationship between AP sites quantity and PARP-1 activity in non-cancerous human endometrium.

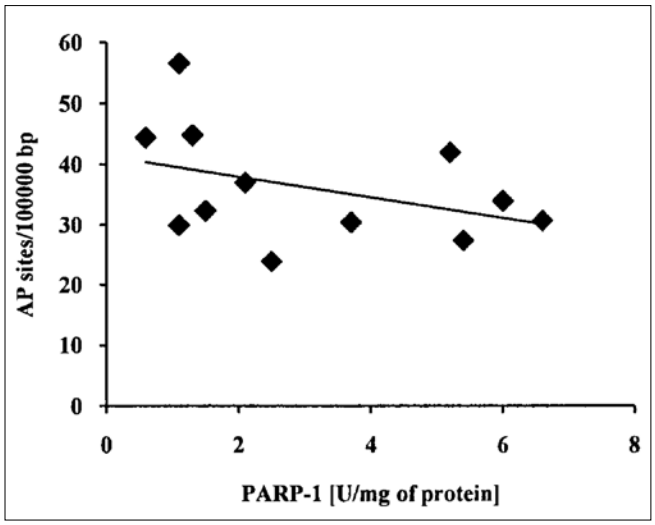

Fig. 6. Relationship between AP sites quantity and PARP-1 activity in human uterine adenocarcinomas.

(R= -0.07, p=0.99). In secretory endometrium the mean PARP-1 activity (n=12) was roughly the same as in the proliferative one (6.59±1.29; range, 1.58-15.06, median 4.73). There was no relationship between enzyme activity and age in this investigated group (R=0.03, p=0.92). Hyperplastic endometrium (n=4) displayed the mean PARP-1 activity quite similar to those recognized in proliferative tissue (7.04±3.39, range 1.08-16.77). Additionally, there was strong but non-significant negative correlation between polymerase activity and the age of the women (R= -0.63, p=0.37).

Lowest mean PARP-1 activity (4.71±1.16) was found in atrophic endometrium specimens (n=10). Similarly to proliferative tissue, there were interindividual substantial differences in the enzyme activity (0.64-11.71; median 3.9). (Fig. 1.).

The activity of poly(ADP-ribose) polymerase in adenocarcinomatous endometrium samples (n=15) was between 0.61 to 6.58 U per milligram of protein. The mean enzyme activity in those tissues (2.89±0.55) was statistically lower than in the cancer-free endometrium (p<0.005). (Fig. 2.).

In well-differentiated endometrial adenocarcinomas the mean PARP-1 activity (1.77±1.65) was almost two fold lower than in G2 (3.64±2.13) and two fold higher than in G3 cancers (0.78±0.2). However, these differences were not statistically significant.

PARP-1 activity in G3 endometrial neoplasms was significantly lower than in moderately-differentiated tumors (p< 0.04) or those recognized in cancers displaying lower histological dedifferentiation (G1+G2) (3.14±0.57; p<0.026). (Fig. 3.).

In tumors invading myometrium for more than half of its thickness the mean polymerase activity was significantly lower than in neoplasms displaying lower local invasiveness activity (1.38±0.58 vs 3.63±0.67; p<0.013). (Fig. 4.).

The amount of AP sites expressed per 100000 bp of genomic DNA in cancer-free endometrium was included between 13.8 to 71.9 (mean 33.1±2.04; median 29.7). There was no correlation between amount of this oxidative stress molecular marker and poly(ADP-ribose) polymerase-1 activity (R=-0.04; p=0.81). (Fig. 5.).

The highest mean AP sites was recognized in atrophic endometrium samples DNA (40.03±4.48). It was statistically higher (p<0.04) than in secretory endometrial tissue (25.51±2.06), slightly exceeding the mean values in proliferative and hyperplastic endometrium (34.73±3.33 vs 33.05±4.33, respectively). There was no correlation between abasic sites quantity and PARP-1 activity in our investigation, irrespectively of the histological type of cancer-free endometrium.

In uterine adenocarcinomas the mean AP sites (37.26±2.31) was non-significantly higher than in non-cancerous endometrium (p<0.09). The quantity of this stress oxidative marker in neoplasms was inversely correlated with PARP-1 activity, however it was not statistically significant (R= -0.48, p<0.086). (Fig. 6.).

Discussion

To the best of our knowledge the present study has been the first attempt at describing PARP-1 activity in human cancerous and cancer-free endometrial tissue. Information about this enzyme in endometrial tissue is scarce and covers only PARP-1 immunohistochemical expression [23, 24]. Thus, we believe our observations may constitute a starting point for further research.

Since estrogens are believed to stimulate PARP activity and proliferation, we expected to observe highest polymerase activity in proliferative and hyperplastic endometrial tissues as they reflect elevated serum estradiol levels [32, 33]. Indeed, similar mean values of PARP-1 activity in those endometrial samples that displayed increased proliferative potential were the highest, what could support the theory that estrogenic stimulation leads to the activation of poly(ADP-ribose) polymerase [24]. Mean PARP-1 activity, slightly lower than in proliferative endometrium, was noted in secretory endometrium, possibly due to progesterone influence on PARP activation machinery. Although this steroid increased enzyme activity in sheep ovarian surface epithelial cells culture correspondingly to the applied dose, in spayed rats it blocked estrone-stimulated PARP activity [33,34]. In the secretory phase of a woman's menstrual cycle, that might be characterized by secretory endometrium, relatively high estradiol level and increasing blood progesterone concentration which dramatically drops down just before menstruation, are still observed, similarly to the proliferative phase. Thus, according to the experimental data of Cummings, the secretory endometrium PARP-1 activity ought to be lower than during the proliferative phase since blood progesterone is very low at that time [33].

Assuming that hormonal regulation of PARP activity is a fact, it came as no surprise that the lowest poly(ADP-ribose) polymerase activity was recognized in the atrophic endometrium. Such endometrial tissue becomes atrophic after the menopause as a result of ovarian insufficiency. At that time of a woman's life, estrone becomes the predominant estrogen. Estrone is a weaker estrogenic compound comparing to the previously (i. e. during the reproductive period) secreted estradiol, which is believed to be the most biologically potent human estrogen [35]. Thus, the decreased enzyme activity could not only be the result of low blood estrogens, but might also depend on the kind of estrogenic compound that dominates the climacteric blood of women.

Our results of PARP-1 activity in cancer-free endometrium correspond to those published by Ghabreau et al. concerning nuclear enzyme immunoreactivity [24]. They revealed a significant decrease of PARP-1 expression toward the end of the secretory phase. Additionally, the highest immunostaining of PARP-1 was noted in the late proliferative phase, just before ovulation, when blood estradiol increases to its maximal concentration.

Brustmann using the same antibody as Ghabreau team, showed low immunoreactivity of PARP in normal proliferative endometrium, but individual immunoscores were lowest in secretory endometrial [23]. Those results, together with our findings, strongly support the experimental data that estrogens may activate poly(ADP-ribose) polymerase, thus potentiating poly(ADP ribosyl)ation.

We are also deeply convinced that hormonal changes occurring during the menstrual cycle are at least partially responsible for interindividual substantial differences in the activity of PARP-1 in endometrial tissue. They also could depend on endometrial tissue heterogeneity, since in our investigations we collected specimens using punch biopsy technique instead of tissue microdissection. However, Liotta and Petricoin believe that the most accurate picture of the in vivo state is reflected only by the analysis of cells in their tissue environment, as tissues are 'complicated three-dimensional structures that are composed of large numbers of interacting cell populations' [36]. Indeed, Cooke et al. demonstrated by means of tissue recombination experiments that estradiol induction of mouse uterine epithelial proliferation appears to be a paracrine event mediated by stromal estrogen receptors [37].

The present results show that PARP-1 activity is lower in estrogen-responsible uterine adenocarcinomas than in cancer-free endometrium. In contrast, Singh revealed that PARP-1 activity was higher in ovarian cancers that are suspected to be estrogen-dependent tumors than in surrounding disease-free tissue [19,38]. In breast cancers, other steroid-dependent neoplasms, there was higher *PARP* expression than in the control tissue specimens [20].

We suspect that, irrespectively of the underlying mechanisms, a decline in PARP-1 activity should be considered as a loss of the ability to defend against advancement of neoplastic progression. This view could be supported by the previously cited results of Piskunova et al. of animal experimental study, and by our own findings, which clearly demonstrate that more advanced tumors demonstrate lower PARP-1 activity [15]. However, Brustmann found an increase of immunostaining for PARP corresponding to FIGO stage in serous ovarian carcinomas in women [21]. Moreover, Katsuhiko et al. were able to reveal that in colorectal early stage tumors PARP-1 overexpression was significantly correlated with tumor size and histopathology [39].

On the other hand, we ought to stress that our results of PARP-1 activity in uterine adenocarcinomas, categorized according to WHO, are roughly consistent with polymerase immunoexpression demonstrated in such tumors by Ghabreau team [24]. However, in Brustmann's study there were no differences in PARP-1 immunoreactivity in combined G1 and G2 uterine tumors group in relation to the most histologically dedifferentiated neoplasms, e. g. G3 [23]. Additionally, he did not reveal any convincing correlation between poly(ADP-ribose) polymerase expression and clinicopathological variables in endometrial adenocarcinomas.

Our investigations of PARP-1 activity and the amount of abasic sites in non-cancerous and neoplastic endometrium do not allow us to draw firm conclusions about the connection between those parameters. However, in both cancer-free and neoplastic endometrium the highest values of AP sites content were associated with lower polymerase activity. Those observations, along with the highest abasic sites level in the atrophic endometrium displaying lowest mean PARP-1 activity in cancer-free tissue, and with the occurrence of a non-significant for the purpose of study correlation between poly(ADP-ribose) polymerase activity and AP sites level in cancer-negative specimens, support the view that PARP-1 can regulate the severity of oxidative stress, thus playing an active role in endometrial cancer, also in human endometrial tissue.

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