Original Article

Changes in Expression of Some Apoptotic Markers in Different Types of Human Endometrium

(apoptosis / caspase-3 / Bax / Bcl-2 / PARP / human endometrium)

D. DRIÁK¹, M. DVORSKÁ¹, I. ŠVANDOVÁ², B. SEHNAL¹, K. BENKOVA³, Z. ŠPŮRKOVÁ³, M. HALAŠKA¹

¹Department of Gynaecology and Obstetrics, First Faculty of Medicine, Charles University in Prague and University Hospital Bulovka, Prague, Czech Republic
²Department of Physiology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
³Department of Pathology, University Hospital Bulovka, Prague, Czech Republic

Abstract. The maintenance of tissue homeostasis and highly balanced counteraction of cellular proliferation and apoptosis are essential for tissue integrity. In our study, we evaluated the expression of apoptosis-regulating proteins Bcl-2, Bax and PARP, and executive apoptotic enzyme caspase-3 in normal, atrophic, hyperplastic and cancerous endometrium. Endometrial samples were obtained from patients who underwent curettage, hysteroscopy or hysterectomy. The protein levels were quantified by immunoblotting. We observed a higher level of important apoptotic enzyme pro-caspase-3 and its active form in hyperplastic and cancerous endometrium, when compared to normal endometrium. The value of Bcl-2/Bax ratio, which reflects cellular resistance to apoptosis, was determined as > 1 for cancerous, normal, and atrophic endometrium. Thus, the effort to eliminate pre-neoplastic and neoplastic cells by apoptosis indicated by high pro-caspase-3 and caspase-3 levels seems to be overcome by a greater proliferative adjustment suggested by higher Bcl-2/Bax ratios in the samples examined. The PARP levels did not vary significantly among the groups. The levels of all examined proteins were significantly lower in atrophic endometrium. Our results suggest that pre-neoplastic and neoplastic states of human endometrium are not influenced simply by changes in apoptosis, but may also be affected by cellular proliferation. A high Bcl-2/Bax ratio as observed in cancerous endometrium can point to deregulation of apoptotic programmes. Thus, the onset and progression of endometrial malignancy could be linked to increased cellular proliferation with defects in apoptotic control.

Introduction

The maintenance of tissue homeostasis and a highly balanced counteraction between cellular proliferation and cell loss are essential for tissue integrity. Apoptosis, a process morphologically and biochemically different from necrosis, plays a fundamental role in elimination of excess or dysfunctional cells (Prindull, 1995; Meier et al., 2000). Deregulation of apoptosis is known to contribute to many pathological conditions such as developmental defects, autoimmune diseases or cancer (Thompson, 1995). The normal human endometrium is one of the few tissues undergoing a cyclic process of apoptosis, proliferation and differentiation. This process is strongly influenced by the endocrine state of the organism and depends especially on oestradiol and progesterone levels (Shiozawa et al., 1996). Apoptosis is reported to be detected in late secretory and menstruating endometrium and scarce in normal proliferating endometrium or at the beginning of the secretory phase (Tao et al., 1997; Vaskivuo et al., 2002).

Apoptosis is controlled by several pro- and anti-apoptotic genes. It was demonstrated that endometrial apoptosis involves proteins of the Bcl-2 and caspase families among others (Watanabe et al., 1997; Abe et al., 2006). The Bcl-2 protein family is a part of a complex regulatory network of apoptosis and includes both the
anti-apoptotic and pro-apoptotic molecules. It can be divided into at least three groups including anti-apoptotic Bcl-2 and its relatives, as well as pro-apoptotic proteins such as Bax, Bad, Bak, Bid or Bim. Members of the Bcl-2 family can form homo- or heterodimers with each other (Oltvai et al., 1993). The effects of pro-apoptotic Bax homodimers acting on the outer mitochondrial membrane (Nomura et al., 1999; Shimizu et al., 1999) can be prevented by antagonistic Bcl-2 formation of Bcl-2/Bax heterodimers (Shimizu et al., 1999; Jin and El-Deiry, 2005). Hence, the cellular Bcl-2/Bax ratio was proposed to be a key determinant of cell resistance to apoptotic stimuli. A high Bcl-2/Bax ratio points to cells resistant to apoptotic stimuli, whereas a low one indicates cells heading to cell death (Sedlak et al., 1995; Reed et al., 1996). Bcl-2 expression was described in a variety of human tissues, including normal or hyperplastic endometrium (Avellanaira et al., 2006; Villavicencio et al., 2007) or different human neoplasms including endometrial, breast, lung, prostate, and thyroid carcinoma or melanomas (Saegusa et al., 1996; Perez et al., 1997; Eerola et al., 1999; Le et al., 1999; Soda et al., 1999; Peiró et al., 2001; Raisova et al., 2001; Vaskivuo et al., 2002; Abe et al., 2006).

Caspases are among the main initiators of apoptosis. Concerning apoptosis, they can be divided into two groups. The first group includes apoptotic initiator caspases (caspases-2, -8, -9 and -10, respectively), the second one includes apoptotic effector caspases (-3, -6, -7) with active caspase-3-derived from proenzyme pro-caspase-3 as a crucial executioner enzyme (For review see e.g. Jin and El-Deiry, 2005). Several recent studies showed caspase-3 expression in normal, hyperplastic and neoplastic endometrium (Atasoy et al., 2003; Di Paola et al., 2005; Abe et al., 2006; Villavicencio et al., 2007).

One of the proteins cleaved by caspases during apoptosis is poly(ADP-ribose) polymerase (PARP). PARP is a nuclear enzyme that binds to DNA single- and double-strand breaks and has been proposed to take part in DNA repair, regulation of transcription, cell death and genome stabilization (Beneke et al., 2000). Its expression was also described in normal, hyperplastic and cancerous endometrial tissues (Ghabreau et al., 2004; Brustmann, 2007).

In the present study, we evaluated expression of apoptosis-regulating proteins Bcl-2, Bax and PARP and executive apoptotic enzyme caspase-3 in normal, atrophic, hyperplastic and cancerous endometrium, respectively.

**Material and Methods**

**Subjects**

Endometrial samples were obtained from 25 informed patients (mean age of 58 years) who underwent probability curettage, hysteroresection or hysterectomy. The tissue specimens were classified as follows: normal endometrium obtained in proliferative phase of the menstrual cycle (NE, N = 8), atrophic endometrium (AE, N = 5), endometrium from patients with endometrial hyperplasia (HE, N = 6) and endometrium from patients with endometrial carcinoma (CE, grades I and/or II, N = 6). After removal, tissue was immediately frozen on dry ice. Histopathological investigation was carried out using routine histopathologic methods. Samples of venous blood for assessment of hormonal levels were collected from the same patients prior to operation. Clinical characteristics of the groups are shown in Table 1. Written consent was obtained from all subjects participating in the study.

**Preparation of samples**

To isolate the cytosol fraction, tissue was brought to 0 °C, cut into small pieces in homogenization buffer (20.0 mM Tris-HCl; 2.5 mM EDTA; 50.0 mM NaF; 10.0 mM Na$_2$PO$_4$; 1 % Triton X-100; pH 7.4; all the chemicals from Sigma, St. Louis, MO) containing complete protease inhibitor cocktail for mammalian tissues (AEBSF, aprotinin, leupeptin, bestatin, pepstatin A, E-64; Sigma) and homogenized in ten volumes of the buffer on ice by using a tight teflon-glass homogenizer (3 × 10 min at 2000 rpm; Brown, Melsungen, Germany). The homogenate was centrifuged at low speed at 10 000 g for 2 × 10 min at 4 °C. The supernatant was subsequently separated from the pellet, collected and snap frozen in liquid nitrogen and stored at −80 °C until use. The concentration of the proteins was determined by the Lowry method.

**Immunoblotting**

The sample containing 25 µg of protein was diluted in Laemmli buffer (50 mM Tris/HCl, pH 8.0, 6 % (w/v) dithiothreitol, 5 % (w/v) SDS, 0.005 % (w/v) bromophenol blue) and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a standard method. The proteins were transferred to nitrocellulose membranes and blocked in 1% BSA (w/v) for 1 h at room temperature. The membranes were incubated with primary antibodies (1:1000 dilution) for 2 h at room temperature and washed with TBS-T (1× TBS, 0.1 % (v/v) Triton X-100) for 3 × 10 min. The membranes were reacted with horseradish peroxidase-conjugated secondary antibody (1:4000 dilution) for 1 h at room temperature and washed with TBS-T for 3 × 10 min. The proteins were visualized using chemiluminescence reagents (ECL kit, Amersham Pharmacia Biotech). The protein bands were detected by exposing the membranes to X-ray film. The expression level of each protein was compared with the housekeeping protein GAPDH, which was used as a control for equal protein loading. The results were expressed as the ratio of the target protein to GAPDH (OD$_{target}$/OD$_{GAPDH}$).

**Table 1. Clinical characteristics of healthy women (NE), women with atrophic endometrium (AE), women with hyperplasia (HE) and women with endometrial carcinoma (CE)**

<table>
<thead>
<tr>
<th></th>
<th>NE (N = 8)</th>
<th>AE (N = 5)</th>
<th>HE (N = 6)</th>
<th>CE (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.75 ± 1.93</td>
<td>68.60 ± 3.29</td>
<td>53.00 ± 3.90</td>
<td>67.00 ± 1.94</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>0.98 ± 0.11</td>
<td>1.48 ± 0.66</td>
<td>3.10 ± 1.81</td>
<td>1.35 ± 0.43</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>1.41 ± 0.42</td>
<td>1.20 ± 0.34</td>
<td>1.17 ± 0.44</td>
<td>0.93 ± 0.09</td>
</tr>
<tr>
<td>Oestradiol (nmol/l)</td>
<td>0.21 ± 0.06</td>
<td>0.15 ± 0.03</td>
<td>0.19 ± 0.03</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>Prolactin (µg/l)</td>
<td>16.34 ± 4.13</td>
<td>11.62 ± 2.47</td>
<td>11.72 ± 2.11</td>
<td>60.78 ± 14.15</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>32.18 ± 9.95</td>
<td>32.34 ± 13.34</td>
<td>28.60 ± 8.04</td>
<td>32.70 ± 5.62</td>
</tr>
</tbody>
</table>

FSH – follicle-stimulating hormone, LH – luteinizing hormone.
nol blue). The proteins were dissolved by standard SDS-PAGE (15% gels). Electrophoresis was run at 200 V for 1 h, using a Mini Protean II gel kit (Bio-Rad, Hercules, CA). After the SDS-PAGE, proteins were transferred to nitrocellulose membrane using a wet apparatus. Blotting was run at 35–50 mA overnight. The membrane was blocked for 2 h at room temperature in appropriate primary antibody in 1% fat-free milk in PBS-T buffer (PBS containing 0.05% (v/v) Tween 20). Then the membrane was incubated for 2 h at room temperature in appropriate secondary antibody in 1% fat-free milk in PBS-T buffer. The following primary antibodies were used: against pro-caspase-3 (E-8, Santa Cruz Inc., Santa Cruz, CA, 1:500), against caspase-3 (sc-22171, Santa Cruz Inc, 1:500), against Bax (SPM336, Santa Cruz Inc., 1:500), against Bcl-2 (8C8, Santa Cruz Inc., 1:500), against PARP (F-2, Santa Cruz Inc., 1:500), against F-actin (C-11, Santa Cruz Inc., 1:1000). After the primary antibody was removed, the blot was washed three times for 10 min in PBS-T buffer. Subsequently, the membrane was incubated for 1 h at room temperature in the appropriate secondary antibody (Santa Cruz Inc., 1:10 000–20 000) in 1% fat-free milk PBS-T buffer. After removal of secondary antibody the membrane was placed three times for 10 min in PBS-T buffer and the blot was visualized by ECL (Amersham Biosciences, GE Healthcare Sciences, Piscataway, NJ).

Statistical analysis

The levels of pro-caspase-3, caspase-3, Bcl-2, Bax, PARP, actin and the Bax/Bcl-2 ratio were analysed and quantified by scanning densitometry (ImageQuant TL v 2005, Amersham Biosciences). Protein expression was normalized to band intensities observed for F-actin used as internal control. Detection of Bcl-2 and Bax was performed separately on the same membrane to determine the protein ratios in the same sample. Values of protein expression are given in arbitrary units. Statistical analysis was performed using the SigmaStat 3.5 program (Systat Software, Inc., Chicago, IL). The one-way ANOVA test was used.

Results

Pro-caspase-3 and caspase-3

The protein expression of pro-caspase-3 and caspase-3 was studied using two specific non-cross-reactive antibodies recognizing either pro-caspase-3 or the active form of the enzyme. We observed a band of 35 kDa for pro-caspase-3 and a band of 18 kDa for caspase-3 in all the tissue specimens.

We detected significantly higher levels of pro-caspase-3 and caspase-3 in CE and HE (1680% and 700% in CE for caspase-3 and 375% and 160% in HE for pro-caspase-3, respectively, Table 2 and Figs. 1 and 2) compared to NE. Expression of pro-caspase-3 and caspase-3 was significantly lower in HE compared to CE. Expression of both the inactive and active form of caspase-3 was significantly lower in AE with respect to NE, HE and CE.

Interestingly, the increase in pro-caspase-3 expression in HE a CE when compared to NE was more dramatic than the increase of caspase-3 in the same expression scheme. The expression was four times higher for caspase-3 than for the inactive enzyme form pro-caspase-3.

Bcl-2 and Bax expression

We observed a band of 25 kDa for Bcl-2 and a band of 23 kDa for Bax in all the tissue samples. The quantitative expression levels of Bcl-2 a Bax and the Bcl-2/Bax ratio are listed in Tables 3 and 4. The profile of pro-

Fig. 1. Western blotting analysis of pro-caspase-3 levels in HE, AE, CE and NE. Twenty-five µg of protein was loaded to each lane. Pro-caspase-3 bands were detected at 35 kDa. Protein intensities were normalized to intensities observed for F-actin as internal control. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M. (*P < 0.05).

Table 2. Expression of pro-caspase-3 and caspase-3 in HE, AE, CE and NE. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>HE (N = 6)</th>
<th>AE (N = 5)</th>
<th>CE (N = 6)</th>
<th>NE (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pro-caspase-3</td>
<td>R.U. 0.78 ± 0.15</td>
<td>0.03 ± 0.01</td>
<td>1.80 ± 0.23</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>caspase-3</td>
<td>R.U. 1.47 ± 0.18</td>
<td>0.04 ± 0.01</td>
<td>3.52 ± 0.27</td>
<td>0.21 ± 0.06</td>
</tr>
</tbody>
</table>
tein expression of Bcl-2 and Bax was similar in all the groups studied; however, Bax expression reached only 60–70% of Bcl-2 levels (Fig. 2). With respect to NE, Bcl-2 expression was significantly higher in CE (150% of Bcl-2 levels in NE), whilst the changes of Bax expression in CE were considered non-significant. Interestingly, a significant decrease of Bcl-2 and Bax expression was determined in HE with respect to CE. In HE, expression of Bcl-2 was determined to be 85% of the Bcl-2 level in CE. In HE, the level of Bax protein detected by Western blotting reached only 55% of Bax expression in CE. The expression of all markers was significantly lower in AE compared to NE.

The value of the Bcl-2/Bax ratio, which is also called apoptotic rheostat and indicates the ability of cells to resist apoptosis, was determined as >1.00 for CE, NE and AE, respectively. The Bcl-2/Bax ratio was 30% higher in CE and about 25% lower in HE when compared to NE (Fig. 3). The only significant difference we detected was about an 86% higher value of the Bcl-2/Bax ratio in CE in comparison with HE. Interestingly, we observed a slightly higher Bcl-2/Bax ratio in AE with respect to NE.

Table 3. Expression of Bcl-2 and Bax in HE, AE, CE and NE. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>HE (N = 6)</th>
<th>AE (N = 5)</th>
<th>CE (N = 6)</th>
<th>NE (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2</td>
<td>0.71 ± 0.05</td>
<td>0.06 ± 0.01</td>
<td>1.17 ± 0.04</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>Bax</td>
<td>0.42 ± 0.07</td>
<td>0.04 ± 0.01</td>
<td>0.77 ± 0.08</td>
<td>0.56 ± 0.70</td>
</tr>
</tbody>
</table>

Table 4. Descriptive characteristics of Bcl-2/Bax ratio in HE, AE, CE and NE. Results are in relative units (R.U.)

<table>
<thead>
<tr>
<th></th>
<th>HE (N = 6)</th>
<th>AE (N = 5)</th>
<th>CE (N = 6)</th>
<th>NE (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.90 ± 0.09</td>
<td>1.35 ± 0.20</td>
<td>1.68 ± 0.14</td>
<td>1.28 ± 0.16</td>
</tr>
<tr>
<td>Median</td>
<td>0.86</td>
<td>1.18</td>
<td>1.45</td>
<td>1.04</td>
</tr>
<tr>
<td>P25</td>
<td>0.68</td>
<td>0.76</td>
<td>1.39</td>
<td>0.89</td>
</tr>
<tr>
<td>P75</td>
<td>1.14</td>
<td>1.56</td>
<td>1.77</td>
<td>1.64</td>
</tr>
<tr>
<td>P95</td>
<td>1.54</td>
<td>2.59</td>
<td>2.76</td>
<td>2.13</td>
</tr>
</tbody>
</table>
We observed a band of 110 kDa for PARP in all the tissue specimens. The highest levels of PARP were detected in CE, in which the PARP expression reached 130% of PARP expression in NE, although the difference was considered non-significant. The only significant difference was found in AE when compared to all the other tissue specimens. The PARP expression in AE was only about 4, 5 and 3% in comparison with NE, HE and CE, respectively.

Discussion

Deregulation of tissue homeostasis results in many pathological conditions and is known to contribute to neoplastic transformation and neoplasia growth. Tissue integrity and homeostasis is substantially based on proliferation and precise balancing of apoptosis. A comprehensive effort has been made to disclose defects in apoptotic molecular mechanisms leading to onset and development of neoplastic transformation. However, literary evidence concerning endometrial tissue transformation into hyperplasias or carcinomas with respect to apoptosis still remains quite controversial and scarce. In this study, we detected expression of executive apoptotic enzymes pro-caspase-3 and its active form caspase-3 and regulatory proteins Bcl-2, Bax and PARP, respectively, in an effort to disclose possible differences between normal, atrophic, hyperplastic and cancerous endometrium.

Apoptosis is detected in late secretory and menstruating endometrium, whilst nearly never detected during the proliferative phase or in early secretory phase of the cycle (Tao et al., 1997; Vaskivuo et al., 2000; Abe et al., 2006). Thus, we chose healthy endometrium in proliferative phase as a control tissue specimen, with expected minimal expression of apoptosis-related proteins. In immunoblotting experiments, we found significantly higher expression of both pro-caspase-3 and caspase-3 in HE and CE samples, with the highest levels in cancerous endometrium. This is in agreement with findings of Atasoy et al. (2003), who immunohistochemically (IHC) showed significantly higher expression of caspase-3 in the carcinoma group than in the simple hyperplasia group of their study population. Also immunostaining results presented by Peiró et al. (2001) describe high caspase-3 levels in endometroid tumour type and lower levels of caspase-3 expression in cases of atrophy and simple and complex hyperplasia, when compared with...
caspase-3 and caspase-3, the different experimental ap

2003). As in the case of expression evaluation of pro-

Taskin et al., 1997; Kokawa et al., 2001; Mitselou et al.,

al., 1996; Niemann et al., 1996; Nakamura et al., 1997;

tributed to different methods used and should be directly

Vaskivuo et al. (2002). Conversely, the authors demon-

was also shown in immunohistochemical analyses by

In the present study, we found significantly higher levels

block with prolonged cell survival as a result, and can

and determining Bcl-2 levels in cancerous endometri-

other hand, Kokawa et al. (2001) report in their IHC

In the present study, we found significantly higher levels

bodies of Dako (Carpinteria, CA). Generally, these quite

The over-expression of Bcl-2 can cause apoptosis

The over-expression of Bcl-2 can cause apoptosis

block with prolonged cell survival as a result, and can

play an important role in carcinogenesis (Reed, 1994).

In the present study, we found significantly higher levels

of Bcl-2 in CE and only a slight decrease in Bcl-2 ex-

pression in HE when compared to NE. In the AE tissue

specimen, the Bcl-2 expression was significantly lower

in comparison with all the other tissue samples. The

lower expression of Bcl-2 in HE than in NE was also

observed by Villavicencio et al. (2007) in their Western

blot experiments. However, they did not evaluate Bcl-2

and Bax expression in cancerous endometrium. On the

other hand, Kokawa et al. (2001) report in their IHC

study stronger Bcl-2 expression in HE than in NE and

dometrioid adenocarcinoma. Very low expression of

Bcl-2 in AE and slightly lower in HE with regard to NE

was also shown in immunohistochemical analyses by

Vaskivuo et al. (2002). Conversely, the authors demon-

strated low levels of Bcl-2 in adenocarcinoma, with de-

creasing pattern correlated with higher FIGO stage of

the carcinoma, although some others suggest that Bcl-2

expression is not associated with tumour grade (Chieng

et al., 1996; Henderson et al., 1996; Peiró et al., 2001).

For that matter, other immunohistochemical studies are

also in favour of decreased Bcl-2 expression in carci-

nomas and at least in atypical hyperplasia (e.g. Chieng

et al., 1996; Niemann et al., 1996; Nakamura et al., 1997;

Taskin et al., 1997; Kokawa et al., 2001; Mitsuol et al.,

2003). As in the case of expression evaluation of pro-
caspase-3 and caspase-3, the different experimental ap-

proach can underlie the discrepancy between different

observations of Bcl-2 expression. To the authors’ best

knowledge, in the literature available there is no study

using the same or very similar experimental protocols

determining Bcl-2 levels in cancerous endometri-

um, and thus supporting or colliding with our observa-
tions on cancerous endometrium tissue specimens.

Interestingly, the same expression pattern was also

observed for Bax in the study at hand. In accordance

with our results, decreased expression of Bax in HE in

comparison to NE was shown in Western blot study by

Villavicencio et al. (2007). Peiró et al. (2001) also im-

munohistochemically described lower levels of Bax in

HE when compared with carcinoma. On the contrary,

in the IHC study of Vaskivuo et al. (2002) the expression

of Bax reached a peak in simple hyperplasia and gradu-

ally decreased to proliferative endometrium, complex

and atypical hyperplasia, atrophic endometrium, and

carcinoma in that order. On the other hand, using the

IHC technique Kokawa et al. (2001) have shown stron-
ger expression in adenocarcinoma than in hyperplasia,

which is in agreement with our results.

The Bcl-2/Bax ratio is an important factor in regula-

tion of apoptosis. A high Bcl-2/Bax ratio increases the

resistance of the cell to the apoptotic stimuli, whereas a

low ratio sways the cell to the apoptotic death. We de-

tected the Bcl-2/Bax ratio > 1 in NE and CE. Interestingly,

in the case of AE the Bcl-2/Bax ratio was also estab-

lished to be > 1. The Bcl-2/Bax ratio value < 1 observed

in our experiments for HE was also shown in their Western

blot study by Villavicencio et al. (2007). However, Vaskivuo et al. (2002) demonstrated, using the IHC technique, the only Bcl-2/Bax ratio value > 1 in proliferative endometrium, whereas in all the other end-

ometrial tissue specimens included in their study the

Bcl-2/Bax ratio was < 1. Interestingly, the authors re-

ported higher values for the Bcl-2/Bax ratio in AE than

in complex and atypical hyperplasia and CE of all the

FIGO grades; however, its value was < 1.

Literary evidence of PARP expression in endometrial

tissue is very limited. In any case, there are studies car-

ried out on other tissues specimens that indicate a sub-

stantial change of PARP expression in neoplasm estab-

lishment and progression. E.g., PARP over-expression

was found to be significant in hepatocellular carcinoma

(Nomura et al., 2000; Shimizu et al., 2004) or malignant

melanoma (Staibano et al., 2005). In the endometrial tis-

ue, using IHC staining Ghabreau et al. (2004) found

PARP-1 expression higher in the proliferative phase of

menstrual cycle than in the secretory phase. In the non-

atypical hyperplasia samples, the PARP-1 level was de-

creased in comparison with proliferative endometrium.

The authors observed gradual increase of PARP-1 ex-

pression in atypical hyperplasia, reaching its highest

level in endometrial carcinoma of grade I. PARP-1 ex-

pression then significantly decreased toward carcinomas

of grade III. These findings are in a good agreement with

our findings of higher PARP expression in CE and lower

in HE, with respect to NE, although we did not observe

statistically relevant changes. Similar data were also ob-

served in the study by Brustmann et al. (2007). In their

IHC study, PARP immunoreactivity increased signifi-

cantly from NE via non-atypical hyperplasia to the aty-

pical ones, and decreased from atypical hyperplasias to

carcinomas. The authors did not find any statistically

relevant differences between endometrioid carcinomas
of grades II and III and serous carcinomas or clear-cell carcinomas.

Despite some variances probably resulting mainly from usage of different methodological approaches, our data together with a substantial body of reports by other authors suggest that pre-neoplastic and neoplastic states of human endometrium are not defined simply by changes in apoptotic cell death, but may also be affected by changes in cell proliferation. A high Bcl-2/Bax ratio observed in our experiments for CE can point to deregulation of the endometrial tissue apoptotic programme, which should eliminate malignant cells. Thus, the onset and progress of endometrial malignancy could be linked to an increased proliferation of cells with apoptotic control defects.

In conclusion, the current results show the presence of apoptosis in normal, atrophic, hyperplastic and cancerous endometrium, respectively. We describe here higher levels of expression of important apoptotic enzyme pro-caspase-3 and its active form caspase-3 in hyperplastic and cancerous endometrium, which may be an attempt of the tissue to eliminate pre-neoplastic and neoplastic cells by apoptosis. However, at least in the case of cancerous endometrium, this effort seems to be overcome by a very strong proliferative adjustment of the tissue indicated by the highest Bcl-2/Bax ratio in the examined samples. Unlike some special situations, e. g. breast carcinoma treated by neoadjuvant chemotherapy (Pecha et al., 2011), induction of apoptosis in endometrial cancerogenesis seems to be overwhelmed by other, stronger factors such as proliferative, metabolic, vascular, etc.

Acknowledgements

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References


