Original Article

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

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

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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, hysteroresection 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

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Abbreviations: AE – atrophic endometrium, CE – endometrial carcinoma, FSH – follicle-stimulating hormone, HE – endometrial hyperplasia, IHC – immunohistochemical, LH – luteinizing hormone, NE – normal endometrium in proliferative phase, PARP – poly(ADP-ribose) polymerase.

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 (Avellaira 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 probatory 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 $Na_4P_2O_7$, 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) bromphe-

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

	NE (N = 8)	AE (N = 5)	HE (N = 6)	CE (N = 6)
Age	42.75 ± 1.93	68.60 ± 3.29	53.00 ± 3.90	67.00 ± 1.94
Testosterone (nmol/l)	0.98 ± 0.11	1.48 ± 0.66	3.10 ± 1.81	1.35 ± 0.43
Progesterone (nmol/l)	1.41 ± 0.42	1.20 ± 0.34	1.17 ± 0.44	0.93 ± 0.09
Oestradiol (nmol/l)	0.21 ± 0.06	0.15 ± 0.03	0.19 ± 0.03	0.23 ± 0.05
Prolactin (μg/l)	16.34 ± 4.13	11.62 ± 2.47	11.72 ± 2.11	60.78 ± 14.15
FSH (IU/l)	32.18 ± 9.95	32.34 ± 13.34	28.60 ± 8.04	32.70 ± 5.62
LH (IU/l)	18.66 ± 6.46	17.38 ± 7.33	21.65 ± 7.11	26.65 ± 9.28

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 1 h in 5% 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 primary 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 : 15 000–20 000) 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 Sciencies, 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-

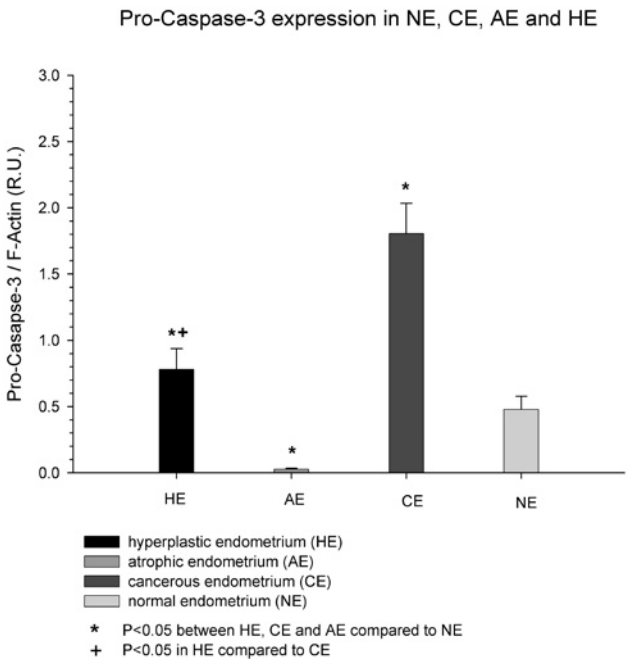

Fig. 1. Western blotting analysis of pro-caspase-3 levels in HE, AE, CE and NE. Twenty-five μg of protein was loaded to each lane. Pro-caspase-3 bands were detected at 35 kDa. Protein intensities were normalized to intensities observed for F-actin as internal control. Results are in relative units (R.U.) 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.

		HE (N = 6)	AE (N = 5)	CE (N = 6)	NE (N = 8)
pro-caspase-3	R.U.	0.78 ± 0.15	0.03 ± 0.01	1.80 ± 0.23	0.48 ± 0.10
caspase-3	R.U.	1.47 ± 0.18	0.04 ± 0.01	3.52 ± 0.27	0.21 ± 0.06

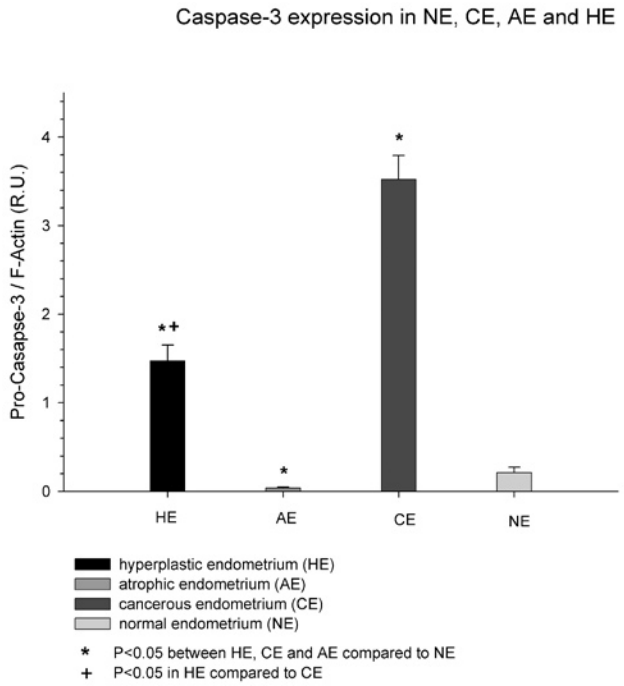

Fig. 2. Western blotting analysis of caspase-3 levels in HE, AE, CE and NE. Twenty-five μg of protein was loaded to each lane. Caspase-3 bands were detected at 18 kDa. Protein intensities were normalized to intensities observed for F-actin as internal control. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M. (*P < 0.05).

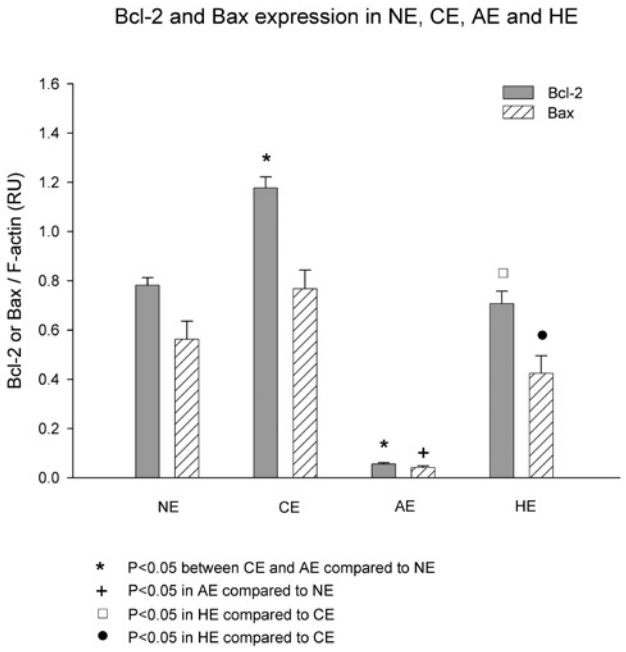

Fig. 3. Western blotting analysis of Bcl-2 and Bax levels in HE, AE, CE and NE. Twenty-five μg of protein was loaded to each lane. Bcl-2 band and Bax bands were detected at 25 and 23 kDa, respectively. Protein intensities were normalized to intensities observed for F-actin as internal control. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M. (P < 0.05).

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.

		HE (N = 6)	AE (N = 5)	CE (N = 6)	NE (N = 8)
Bcl-2	R.U.	0.71 ± 0.05	0.06 ± 0.01	1.17 ± 0.04	0.78 ± 0.03
Bax	R.U.	0.42 ± 0.07	0.04 ± 0.01	0.77 ± 0.08	0.56 ± 0.70

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

	HE (N = 6)	AE (N = 5)	CE (N = 6)	NE (N = 8)
Mean	0.90 ± 0.09	1.35 ± 0.20	1.68 ± 0.14	1.28 ± 0.16
Median	0.86	1.18	1.45	1.04
P25	0.68	0.76	1.39	0.89
P75	1.14	1.56	1.77	1.64
P95	1.54	2.59	2.76	2.13

Table 5. Expression of PARP in HE, AE, CE and NE. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M.

	HE (N = 6)	AE (N = 5)	CE (N = 6)	NE (N = 8)
R.U.	2.80 ± 0.42	0.10 ± 0.02	3.18 ± 0.30	2.49 ± 0.24

PARP expression

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

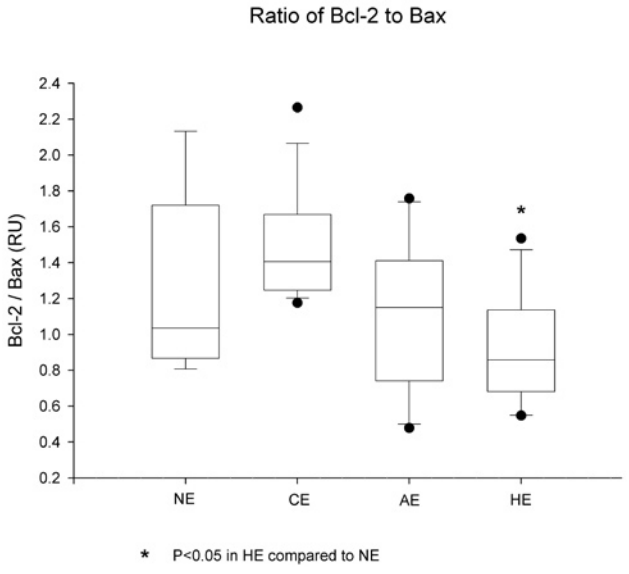

Fig. 4. Bcl-2/Bax ratio in HE, AE, CE and NE. The protein expression was determined by Western blotting, as described in Methods. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M. ($P < 0.05$).

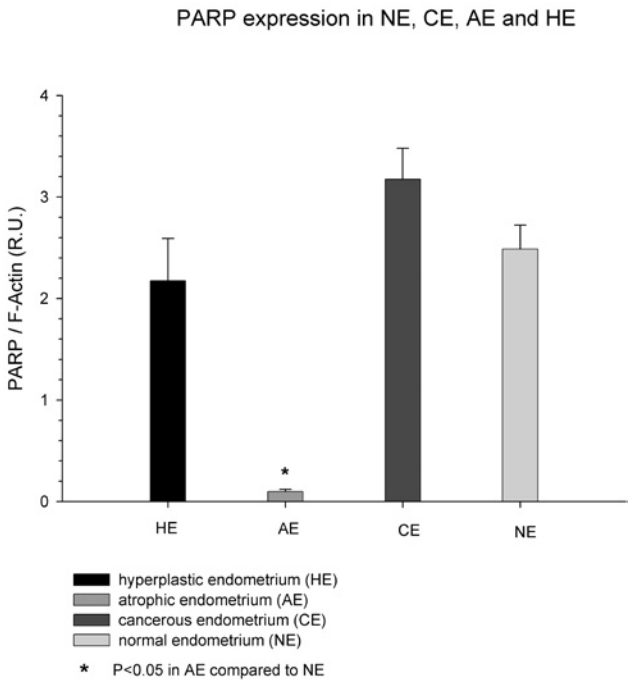

Fig. 5. Western blotting analysis of PARP in HE, AE, CE and NE. Twenty-five μg of protein was loaded to each lane. PARP bands were detected at 110 kDa. Protein intensities were normalized to intensities observed for F-actin as internal control. Results are in relative units (R.U.) and represent the mean of seven independent experiments ± S.E.M. ($P < 0.001$).

carcinoma. Based on survival analysis, the authors in addition conclude that caspase-3 levels may by a useful marker in predicting the outcome of the patients. On the other hand, in caspase-3 activity studies published by Di Paola et al. (2005), no significant differences between caspase-3 activity in normal and pathological endometrium were found. Moreover, in a Western blot study carried out by Villavicencio et al. (2007), no active caspase-3 in the tissue specimens of NE and HE was detected, whereas levels of detectable pro-caspase-3 were significantly higher in HE than in NE. The differences between the results of Villavicencio group, who used very similar protocols for tissue processing and Western blotting, and our data may reside in the different antibodies used. Unlike in the presented study using caspase-3 mouse monoclonal antibody raised against amino acids 1–2777 of full-length caspase-3 of human origin, Villavicencio et al. (2007) used polyclonal antibodies of Dako (Carpinteria, CA). Generally, these quite contradictive results in studies listed above can be attributed to different methods used and should be directly compared with caution.

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 expression 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 endometroid 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 demonstrated low levels of Bcl-2 in adenocarcinoma, with decreasing pattern correlated with higher FIGO stage of the carcinoma, although some others suggest that Bcl-2 expression is not associated with tumour grade (Chhieng 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 carcinomas and at least in atypical hyperplasia (e.g. Chhieng et al., 1996; Niemann et al., 1996; Nakamura et al., 1997; Taskin et al., 1997; Kokawa et al., 2001; Mitselou et al., 2003). As in the case of expression evaluation of procaspase-3 and caspase-3, the different experimental approach 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 and determining Bcl-2 levels in cancerous endometrium, and thus supporting or colliding with our observations 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 immunohistochemically 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 gradually 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 stronger expression in adenocarcinoma than in hyperplasia, which is in agreement with our results.

The Bcl-2/Bax ratio is an important factor in regulation 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 detected the Bcl-2/Bax ratio > 1 in NE and CE. Interestingly, in the case of AE the Bcl-2/Bax ratio was also established 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 endometrial tissue specimens included in their study the Bcl-2/Bax ratio was < 1. Interestingly, the authors reported 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 carried out on other tissues specimens that indicate a substantial change of PARP expression in neoplasm establishment 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 tissue, 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 decreased in comparison with proliferative endometrium. The authors observed gradual increase of PARP-1 expression in atypical hyperplasia, reaching its highest level in endometrial carcinoma of grade I. PARP-1 expression 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 observed in the study by Brustmann et al. (2007). In their IHC study, PARP immunoreactivity increased significantly from NE via non-atypical hyperplasia to the atypical 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.

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