Chumak Z. V., Shapoval N. V. The processes of apoptosis and telomerase activity in endometrial cells under different morphological conditions. Journal of Education, Health and Sport. 2018;8(5):235-243. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1256846
http://ojs.ukw.edu.pl/index.php/johs/article/view/5547

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 07.05.2018. Revised 15.05.2018. Accepted: 31.05.2018.

THE PROCESSES OF APOPTOSIS AND TELOMERASE ACTIVITY IN ENDOMETRIAL CELLS UNDER DIFFERENT MORPHOLOGICAL CONDITIONS

Z. V. Chumak, N. V. Shapoval

Odessa National Medical University, Odessa Municipal Center for Menopause
e-mail: chumak-doc@rambler.ru

Abstract

Aging and apoptosis are two mechanisms of a powerful anti - cancer defense. The apoptosis inhibitor is Bcl-2 gene. It expresses in transformed cells, blocks programmed death, promotes the survival of malignant ones. Under experimental conditions, when the Bcl-2 gene was transfected into the endothelial aging cells of mice, there was a decrease in mitochondrial oxidative stress, renewal of the potential of mitochondrial membranes, and improvement of angiogenesis. **The objective:** to study the expression of the Bcl-2 marker and telomerase activity in endometrial tissue with hyperplasia. **Materials and methods.** The endometrial tissue samples: 54 with hyperplastic process and 14 patients with a biphasic menstrual ovulatory cycle. Immunohistochemical reactions, real-time PCR with SYBR Green (RQ-TRAP) dye by Wege et al. In endometrial cells with complex non-atypical hyperplasia take place initial disturbances in the processes of programmed cellular death. In complex hyperplasia with atypia in endometrial cells there is inhibition of apoptosis, which can be regarded as a trigger mechanism in the development of cellular atypia. The statistically significant parallelism between the processes of telomerase reactivation in endometrial cells at complex atypical hyperplasia and the existence of telomerase activity in 85% of tumors allows to appraise it as a predictor of oncological process.

Key words: apoptosis, p53, Bcl-2, telomeres, telomerase, malignancy, senescence.

Introduction. Apoptosis is genetically controlled and energetically dependent factor [3, 9] which activity is hormone – mediated in endometrium (Amalinei C., et al., 2011), and its prognostic criteria in proliferative states and malignancy prospects [3, 4, 5] are of interest.

Replicative aging (senescence) is one of the protection against malignancy mechanisms, it manifests itself by stopping cell division in G1 stage and resistance to apoptosis. Molecular genetics recent activity showed a correlation between the cellular aging and the length of telomeres, the activity of telomerase enzyme, on the basis of which the "telomere theory of aging and immortalization" was constructed [1, 6]. The shortening of telomeres, and as a consequence their "dysfunction," is perceived by the cell as an abnormal DNA structure, which induces cellular cycle arrest [2], triggering the mechanisms of cellular senescence and apoptosis. The exception is immortal cells: genital, stem, malignant [1, 10].

From the evolutionary point of view it remains unclear why under the daily birth and death of millions of cells, there is a senescence, as the types of damage that trigger cellular aging are similar to the processes activating apoptosis [1].

Aging and apoptosis are two mechanisms of a powerful anti - cancer defense. The period from the telomeres critical shortening to apoptosis in the senescent cells can last from several months to several years [6, 7]. According to the researchers [2, 7] accumulated with age cells are resistant to apoptosis and can lead to neoplasia. At present, works demonstrating the anti - apoptotic effect of telomerase through interaction with proteins of the Bcl-2 family [8, 11] prevail in the literature.

The apoptosis inhibitor is Bcl-2 gene. It expresses in transformed cells, blocks programmed death, promotes the survival of malignant ones. Cellular senescence is accompanied by changes in the expression of this gene and an increase of oxidative stress in them [1, 2, 6]. Under experimental conditions, when the Bcl-2 gene was transfected into the endothelial aging cells of mice, there was a decrease in mitochondrial oxidative stress, renewal of the potential of mitochondrial membranes, and improvement of angiogenesis [12].

The objective: to study the expression of the Bcl-2 marker and telomerase activity in endometrial tissue with hyperplasia.

Materials and methods. The endometrial tissue samples were examined in 68 patients, of which 54, according to histological findings, had a hyperplastic process and there were 14 patients with a biphasic menstrual ovulatory cycle. The women under observation had been trained in the implementation of assisted reproductive technologies (control group).

Immunohistochemical reactions were placed on serial paraffin sections. As primary specific antibodies the ones to Bcl-2 (DAKO-Germany) were used. The results of

immunohistochemical reactions were evaluated for Bcl-2 in plusses in terms of the intensity of brown staining. Its intensity was assessed in points as follows: (0) - no staining, (+) - slight staining, (++) - moderate staining, (+++) - strong staining.

The relative activity of telomerase was determined using real-time PCR with SYBR Green (RQ-TRAP) dye by Wege et al. Analysis in the reaction mixture with 2 μl of tissue extracts, 0.1 μg of forward primer, 0.05 μg of reverse primer and 12.5 μL of Mastermix 480 SYBR Green I (Roche Applied Biosystems). To increase the accuracy of the research, in each experiment we used from 4 to 6 samples of the examined cellular material and calculated their average value.

The results of the study were processed statistically with the help of variation statistics with Student's test and standard computer systems.

The results and their discussion

Proliferative processes were differentiated according to the accepted classification (1994) and the following groups were formed: I group consisted of 17 samples of scrapings with simple endometrial hyperplasia without atypia, group II - 11 samples with complex hyperplasia without atypia, group III was presented with 12 specimens with simple hyperplasia and atypical processes in endometrium, group IV - 14 samples with complex atypical hyperplasia. Control group (V) included 14 women with morphologically unchanged endometrium (proliferation stage - 6 patients (VP), stage of secretion - 8 patients (VC)).

Clinical indications for the examination of women were the presence of echographic signs of endometrial hyperplasia in 23 cases (43.6%), and / or menstrual irregularities (meno- or metrorrhagia) in 21 cases (56.4%).

Table 1

Clinical characteristics of the patients in the survey groups (M ± m)

	Age	IMT (?)	menstrual function onset	Menstrual cycle duration
I group	47.8±0.75	29.91±0.99	13.29±0.31	28.76±0.34
II group	47.7±0.89	29.02±0.80	13.18±0.33	28.27±0.36
III group	48.3±0.97	24.43±0.74	13.50±0.40	28.17±0.34
IV group	49.1±0.72	28.05±0.58	12.86±0.31	28.36±0.25
VP group	36.8±0.60	19.72±0.34	13.07±0.48	28.33±0.21
VC group	37.63±0.50	20.04±0.34	12.75±0.31	28.01±0.33

Note: in all cases $p > 0.05$.

As a marker of apoptosis, Bcl-2 protein is actively studied. The expression of this protein in the endometrial cells are presented in Table 2 (own data).

Table 2

The level of Bcl-2 protein expression in endometrial epithelial cells (abs. p., %)

	Gr. I	Gr.II	Gr.III	Gr.IV	Gr.P	Gr.C
Bcl-2 (0)	4 (23.53%)	3 (27.27%)	0	1(7.14%)	1(16.67%)	5(62.5%)
Bcl-2 (+)	8 (47.06%)	5 (45.45%)	2(16.67%)	1(7.14%)	2(33.33%)	1(12.5%)
Bcl-2 (++)	5 (29.41%)	2(18.18%)	6(50.0%)	3(21.43%)	2(33.33%)	2(25.00%)
Bcl-2 (+++)	0	1(9.09%)	4(33.33%)	9(64.28%)	1(16.67%)	0

In the endometrial cells at the proliferation phase of the control group women, moderate and weak expression of Bcl-2 protein was detected in 2 (33.33 ± 19.24%) cases and there was 1 (16.67 ± 15.22%) case either with strong expression or its absence. Endometrial secretion phases in 5 (62.5 ± 17.12%) cases of expression were this protein free; in 1 (12.5 ± 11.69%) case the staining intensity was weak and in 2 (25.00 ± 15, 31%) cases it was moderate. These indicators can testify to apoptosis processes increase in the secretory phase against the background of relatively low levels of protein inhibition of programmed cellular death.

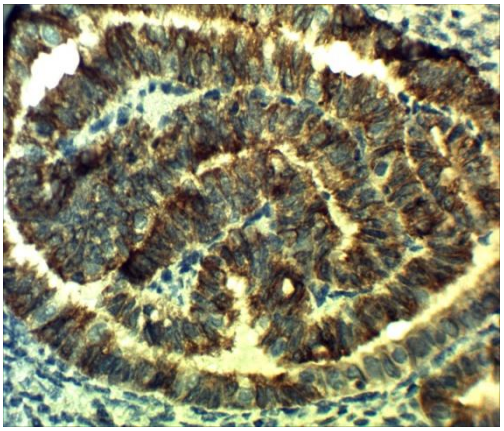

Fig.1. Complex atypical hyperplasia of edometrium. Expression of Bcl-2 (+++), x40

The expression of Bcl-2 protein in total in the V group was: in 6 (42.86 ± 13.22%) cases there was its absence, in 3 (21.43 ± 10.97%) cases weak staining took place, in 4 (28.57 ± 12.07%) cases it was moderate, and the maximum expression was found in 1 (7.14 ± 6.88%) case. In all cases, Bcl-2 protein was detected predominantly in epithelial cells, in stromal ones the protein's expression was detected in a very small percentage of cells and with a low level of staining.

In the study of endometrial specimens of the 1st group women, the maximum expression of Bcl-2 protein was not revealed. In this group 5 endometrium's specimens (29.41

± 11.05%) had a moderate intensity of staining, in 8 (47.06 ± 12.11%) specimens a weak staining was noted and in 4 (23.53 ± 10.29%) there were no staining. In group II the following pattern was revealed: in 1 (9.09 ± 8.67%) specimen there was strong staining, in 2 (18.18 ± 11.63%) - moderate, in 5 (45.45 ± 15.01%) - weak, and the absence of expression took place in 3 (27,27 ± 13,43%) cases.

When study the endometrial tissues with atypical hyperplastic processes, a shift in the expression of Bcl-2 protein towards enhanced staining was noted, and the percentage of its detection in stromal cells was slightly higher. Thus, in the endometrial specimens with simple atypical hyperplasia, a strong staining was detected in 4 (33.33 ± 13.61%) cases (Fig. 2), in 6 (50.0 ± 5.48%) cases it was moderate, in 2 (16, 67 ± 10,76%) staining there was a weak staining, the absence of expression was not detected in any case.

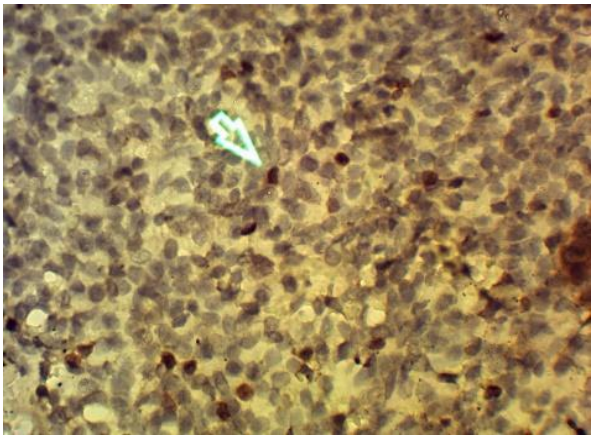

Fig. 2. Simple atypical hyperplasia of endometrium/ expression of Bcl-2 (+++), x40

When study the specimens with complex atypical hyperplasia, the strong expression of Bcl-2 protein was detected in 9 (64.28 ± 12.81%) cases (Fig. 1), in 3 (21.43 ± 10.97%) it was moderate and in 1 case (7.14 ± 6.88%) it was weak and there was no expression either.

The statistical processing of the data obtained showed that with endometrial complex atypical hyperplasia the frequency of detection of high expression of Bcl-2 protein significantly exceeds the expression levels for simple hyperplasia ($\chi 2 = 8.25$; df = 3; p = 0.04). At the same time, with complex atypical hyperplasia (IV Gr.), the level of expression of Bcl-2 in comparison with complex non-atypical hyperplasia (Gr. II) also significantly differed ($\chi 2 = 5.69$; df = 3; p = 0.02). There were no statistically significant changes between groups with simple hyperplasia with atypia and complex atypical hyperplasia ($\chi 2 = 1.39$, df = 3, p = 0.23).

The results obtained suggest that the expression of Bcl-2 protein in the endometrial tissue changes both in dependence and on the degree of the pathological process progression. Changes in protein expression are traced already with complex hyperplasia without atypia, the most significant they are at atypical complex hyperplasia and somewhat lower with adenocarcinoma with progression of increase in the intensity of staining, which suggests a significant weakening of apoptosis mechanisms in atypical complex hyperplasia.

To interpret the activity of telomerase, we used a method based on amplification of the signal by real-time PCR, which allowed us to quantify the indices.

The results of the studies are presented in Table 3.

Table 3

The activity of telomerase in endometrial tissue cells e. a. (M ± m)

Groups	Telomerase activity	P
Group I	1.22±0.10	p_I-p_{II}>o.o5 p_I-p_{III}>0.05 p_I-p_{IV}>0.05 p_I-p_V>0.05 p_I-p_{VI}>0.05
Group II	1.35±0.07	p_{II}-p_{III}>0.05 p_{II}-p_{IV}>0.05 p_{II}-p_V>0.05 p_{II}-p_{VI}>0.05
Group III	1.23±0.07	p_{III}-p_{III}>0.05 p_{III}-p_{IV}>0.05 p_{III}-p_V>0.05 p_{III}-p_{VI}>0.05
Group IV	1.54±0.05	p_{IV}-p_V>0.05 p_{IV}-p_{VI}>0.05
Group VP	1.25±0.07	p_V-p_{VI}>0.05
Group VC	1.18±0.05	

The data obtained indicate a definite tendency towards an increase in telomerase activity in the proliferative type of endometrium - 1.25 ± 0.07 e. a, and may be used for indication of its activity with respect to the proliferative processes flow as a whole. Telomerase's activity in the endometrium of secretory type reduces and is 1.18 ± 0.05 e. a. The results of our work coincide with those of other researches (Adamyan LV et al., 2006; Rainer Lehner et al., 2002), and thus, it is possible to admit that activation or inhibition of telomerase activity is reflected during proliferative processes. The second, no less important conclusion, is the presence of a corresponding parallelism between telomerase activity and cyclic manifestations of steroidogenesis corresponding to the phases of the menstrual cycle.

The established statistically significant reactivation of telomerase in endometrial cells in atypical complex hyperplasia - 1.54 ± 0.05 e. a. is an important fact.

Analysis of telomerase activity in tissues with simple hyperplasia - 1.22 ± 0.10 e. a, simple atypical hyperplasia – 1.23 ± 0.08 e. a. and complex hyperplasia without atypia - 1.35 ± 0.07 e. a. did not show statistically significant differences. Therefore, the reactivation of telomerase in endometrial cells can confirm the elongation of telomere length in proliferative stage, and thus increase the capacity for cells' division, and on the other hand, confirm the telomerase ability to control cellular division in the endometrial tissue.

The data presented in the study coincide with the results of the studies (Dong Y., et al., 2004), which determined the increase in hTERT mRNA signal in endometrial cancer and atypical hyperplasia. While to a certain extent our data contradict the results of individual researchers who found an increase in the expression of the hTERT gene in endometrial carcinoma and the absence of changes in simple and atypical endometrial hyperplasia (Mazurek et al., 2001). Perhaps these differences can be explained by the detection of telomerase activity increase only at endometrium complex atypical hyperplasia, whereas there was no observed difference in atypical hyperplasia.

According to the works published, similar results were obtained in the study of telomerase activity in adenocarcinoma, proliferative, secretory and atrophic endometrium. The authors also noted a slight telomerase activity in the proliferation phase and its increase in carcinoma.

The discrepancy between the results obtained in normal and hyperplastic endometrium can be caused by the difference in the methods used. Detailing the Telomerase activation mechanisms detaling is difficult, because the main processes are studied mainly on the cellular lines, which makes it difficult to interpret *in vivo* mechanisms [4, 6].

Cell malignancy is basically a multi-stage process that affects numerous chromosomal changes, but not every damaged cell becomes malignant. In malignified cells, more than 10 mutations can occur. In most cases, defective cells die from either apoptosis or are destroyed by the immune system cells [2, 7]. In some malignant cells telomerase can reactivates, which supports the telomere length at a certain level necessary for their functioning.

Conclusions:

In endometrial cells with complex non-atypical hyperplasia immunohistochemically manifested by weak coloration of Bcl-2 protein took place initial disturbances in the processes of programmed cellular death. In complex hyperplasia with atypia in endometrial cells, a significant increase in the intensity of Bcl-2 protein staining is found. These data indicate the

predominance of inhibition of apoptosis in the endometrium, which can be regarded as a trigger mechanism in the development of cellular atypia.

Thus, the definition of markers characterizing the disruption of the programmed death of p53 and Bcl-2 can be regarded as predictors of cancer prognosis under hyperplastic endometrial processes. These changes should be considered when choosing tactics and scope of surgery, their dynamics for assessing the effectiveness of conservative therapy.

The establishment of statistically significant parallelism between the processes of telomerase reactivation in endometrial cells at complex atypical hyperplasia and the existence of telomerase activity in 85% of tumors allows to appraise it as a predictor of oncological process. Telomerase's activity marker should be taken into consideration when choosing tactics and the scope of surgical treatment, and the dynamics of its activity should be used for assessing the effectiveness of conservative therapy.

Prospects for the further researches: to conduct comparative studies on the diagnostic accuracy of various methods, specify their use in clinical practice, and to search for complex markers that determine the risk of malignancy.

References:

1. Berestyanaya AN, Grodzinsky DM The role of telomeres in the process of cell aging // Naukovy Visnik Uzhgorodskogo yniversitetu. Ser. Biology. - 2012. - V. 33. - P. 5-16

2. Egorov EE Telomeres, telomerase, carcinogenesis and health measure // Clinical oncohematology. - 2010. - V 3. - No. 2. - P. 184 - 197

3. Expression of genes that regulate apoptosis in different types of endometrial hyperplasia and endometrial adenocarcinoma / Chernukha GE, Dumanovskaya MR, Burmenskaya OV, et al. // Obstetrics and gynecology .- 2013.- № 1. - P. 11-14.

4. Telomerase: methods for determining activity / D.A. Skvortsov, M.E. Zvereva, O. V. Shpanchenko, et al.// Acta Nature.- 2011. - V.1.- №1 (8) .- P. 51 - 72.

5. Frolova I.I. Risk factors for endometrial cancer // Questions of gynecology, obstetrics and perinatology .- 2010.- N.1. - P. 57 - 66.

6. Telomerase functions: telomere extension and not only / M.P. Rubtsova, D. P. Vasilkova, A.N. Malyavko, et al. // Acta Nature.- 2012.- V.4.- №2 (13) .- P.44-61.

7. Chumakov P.M. Protein p53 and its universal functions in a multicellular organism. // Uspekhi biologicheskoi khimii. - 2007.- V. 47.- P. 3 - 52.

8. Aberrant Telomerase Expression in the Endometrium of Infertile Women with Deep Endometriosis/ Fernanda A. Mafra, Denise M. Christofolini, Viviane Cavalcanti et al. // Archives of Medical Research.-2014.- 45.- 31-35.

9. Immunohistochemical analysis of steroid receptors, proliferation markers, apoptosid related molecules, and gelatinases in non-neoplastic and neoplastic endometrium./ Amalinei C., Cianga C., Balan R. et al.// Ann. Anat.-2011; 193(1): 43-45.

10. Pat, Monaghan. Organismal stress, telomeres and life histories// The Journal of Experimental Biology.- 2014.- 217.- P. 57-66.

11. Preoperative evaluation of P53 and Bcl-2 over expression in clinical stage 1 endometrial carcinoma and their correlation with surgical-pathological data and prognosis of patients/ Emad A. Fyallah, Reda A. Hemida, Kamal I. Anwar et al.// Open Journal of Obstetrics and Gynecology.-2011.-№1.-P.55-63.

12. The use of an immunocytochemical double-labeling staining can display the distribution of Bcl-2/Ki67 cells in endometrial adenocarcinomas as well as in normal endometrium/ Mourtzikou A., Kosmas K., Marouga A. et al.// Clin Lab.- 2012.- №58(1-2).- P.133-144.