Estrogen and progesterone receptor expression in HPV-positive and HPV-negative cervical carcinomas

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Abstract. Human papillomavirus (HPV) is widely accepted as the main cause of cervical cancer. However, the presence of HPV DNA does not inescapably lead to the development of the cancerous phenotype of the infected cell. Therefore, it is considered that the induction of full cancerous expression of HPV requires additional cofactors. The aim of this study was to assess the expression of estrogen receptor α (ERα) and progesterone receptor (PR) in archived tissue blocks of squamous cell carcinoma and adenocarcinoma of the uterine cervix and to ascertain whether expression of these receptors is associated with the presence of HPV DNA. The investigation was performed using formalin-fixed, paraffin-embedded cervical cancer specimens obtained from 250 women who underwent surgery for histologically confirmed neoplastic lesions. The control group consisted of normal cervical tissues obtained from 50 patients who underwent myomectomy. The results of this study revealed that the expression of ER and PR in planoepithelial cancers and adenocarcinomas of the cervix were decreased to undetectable levels. Only in singular cases in the pattern of staining the expression of ER and PR was noted. In stromal cells of the tested neoplasms, higher expression of both types of receptors was found. Comparison of the expression of ER and PR in the staining pattern and stroma of both squamous cell carcinoma and adenocarcioma of the cervix, showed statistically higher expression in the stromal cells. Strong expression (+1, +2, +3) of ER and PR was noted in the stromal cells irrespective of HPV infection, histopathological type of cancer, and clinical and histopathological grade.

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Introduction

Human papillomavirus (HPV), a sexually transmitted DNA virus, is widely accepted as the main cause of cervical cancer (1,2). However, the presence of HPV DNA does not inescapably lead to the development of the cancerous phenotype of the infected cell. This has been confirmed by many clinical observations, as not all HPV-positive women develop cervical cancer (3-5). Therefore, it is believed that the induction of full cancerous expression by HPV requires additional cofactors.

Studies of the genome of anogenital papilloma viruses have revealed the presence of virus sequences (LCR, long control region) (6,7), capable of binding receptors of steroid hormones. The featured sequences have a configuration of elements that respond to the glucocorticoid receptor and the progesterone receptor (PR) (8). To date, no response elements to the estrogen receptor (ER) have been found in the regulatory region of the virus. However, in the genome of HPV-16 seven different regions of high resemblance to ER sequences have been discovered. Dimers of steroid receptors are formed by combining with ligands and become active forms. After binding with DNA sequences in the promoter regulatory region, they function as transcription factors that regulate the expression of genes (3). Hence, variable plasma concentrations of hormones during their uncontrolled reception or hormone disorders can result in the increased gene expression of HPV-16 and -18 (9-11), the two HPV subtypes most commonly associated with cervical cancer (12). In addition, most cases of cervical cancer arise in the transformation zone, the most estrogen-sensitive region of the cervix (13,14).

In recent years, the results of epidemiological research, particularly those which demonstrate the higher risk of developing cervical cancer as a result of long-term oral contraceptive use are alarming (15-17). A study by Moreno *et al* (16), performed on behalf of the IRAC Word Heath Organization,

and a meta-analysis carried out by Smith *et al* (17) found that long-term oral contraceptive usage can increase the risk of the incidence of cervical cancer up to 4 times, particularly in women with persistent HPV infection (15-17). Research conducted by Salazar *et al* (18) revealed that cervical carcinomas in women using oral contraceptives, had higher levels of the ER than the those in women who had not taken estrogen-containing oral contraceptives.

The findings of molecular research investigating the interaction between estrogen, progesterone and HPV infection are less divergent than the epidemiological results. A number of investigations have been performed using *in vitro* models. Arbeit *et al* (19) found direct hormonal activation of the viral genome in K14 promoter-HPV transgenic mice, in which exogenous estrogen exposure induced multistage neoplastic progression in the squamous epithelium of the cervix and vagina in 100% of transgenic mice. Thus, estrogen appears to contribute to the persistence of HPV infections and subsequent neoplastic progression by increased viral gene expression. Women who expressed higher levels of ER transcripts were significantly more likely to have cervical HPV infection. However, a recent study showed that E7 oncoprotein and exogenous estrogen failed to promote atypical squamous metaplasia in the absence of ERα suggesting that this receptor plays a crucial role at an early stage of cervical carcinogenesis in mouse models (20).

Steroid hormones achieve their biological effect through receptors. Current research indicates the existence of two types of estrogen receptors, ERα and ERβ (21,22) and several isoforms of both receptors (23,24). ERs are coded by genes located in different chromosomes (correspondingly 6 and 11), and their expression is altered in target tissues. Usually, both types of receptors exist simultaneously, although type α is predominant in the breast, corpus and uterine cervix and in the vagina, whereas type β is prevalent in the ovaries, prostate, testis and lungs (24). Both receptors are present in the central nervous, cardiovascular and osseous systems (24,25). PR exists as two isoforms, which differ in the size of the protein molecule. PR B is 164 amino acids longer than PR A. The difference in the length of the PR isoforms is due to the site of initiation of transcription on the gene. In the case of the shorter form (PR A) the initiation of transcription starts between nucleotide 737 and 842. This difference has no influence on the relationship of the PR to its ligands. The only difference in biological activity is the presence of an additional activation function (AF-3 activation function) (26).

The aim of this study was to assess the expression of ERα and PR in archived tissue blocks of squamous cell carcinoma and adenocarcinoma of the uterine cervix which were obtained from patients who underwent tumor resection and to ascertain whether expression of these receptors is associated with the presence of HPV DNA.

Materials and methods

Materials. The investigation was performed using formalin-fixed, paraffin-embedded cervical cancer specimens obtained from 250 women who underwent surgery during the period 1998-2008 at the Department of Gynecological Surgery of Lublin County for histologically confirmed neoplastic lesions. The Local Ethics Committee of the Medical University of Lublin approved the research protocol. The study material was obtained from patients presenting with i) cervical carcinoma (histopathological finding, squamous cell carcinoma and adenocarcinoma of the uterine cervix) and ii) uterine myoma (histopathological finding, leiomyoma uteri). The control group consisted of normal cervical tissues obtained from 50 patients who underwent myomectomy.

The histopathological criteria of the World Health Organization (WHO) were used to establish the diagnosis of cervical carcinoma (27). Fig. 1 illustrates hematoxylin and eosin staining for carcinoma *in situ* of the uterine cervix. Of the squamous cell carcinoma cases, the histopathological types included 130 cases of keratinized type and 70 cases of non-keratinized type (Fig. 2). Of the 50 patients with adenocarcinoma of the cervix, 25 cases of mucinous type, 10 cases of endometrioid type, 8 cases of clear-cell type, 4 cases of serous type and 1 case of mesonephric type were noted.

According to the degree of dedifferentiation of the neoplastic cells, the cases were grouped as follows: well-differentiated (G1) carcinomas (89 squamous cell carcinomas and 15 adenocarcinomas), moderately differentiated (G2) carcinomas (78 squamous cell carcinomas and 18 adenocarcinomas), and poorly differentiated (G3) carcinomas (83 squamous cell carcinomas and 17 adenocarcinomas). According to the FIGO clinical staging (28), 150 patients were classified as having stage I and 100 as having stage IIA disease. There were no significant differences in the average age of women who underwent surgery for planoepithelial cervical cancer when compared to the control women (45.67±35.49 vs. 45.94±6.12 years).

Methods. Paraffin blocks of tissue fixed in 10% buffered formalin were cut into two or three sections (4 μm). The microtome was rinsed with alcohol before cutting each block. A new cutting blade was used for the cutting of each of the paraffin blocks. The pieces obtained in this manner were placed in a 1.5-ml testing tube with polypropylene, and the paraffin was removed using xylene at a temperature of 37˚C for 30 min. They were centrifuged twice at 6000 rpm for 3 min, rinsed twice in 1 ml of absolute alcohol for 30 min and air dried. After removal of the paraffin, the pieces were homogenized with the addition of 1 ml Hirt buffer having the following composition: 0.01 M Tris-HCI (pH 7.5), 0.01 M EDTA, 0.6% SDS.

The homogenate was incubated for 30 min at room temperature. K proteinase was then added reaching a final concentration of 50 μg/ml, and incubation was carried out for 24 h at 37˚C. After incubation, half the volume of phenol:chloroform:isoamyl alcohol (in a ratio of 25:24:1) mixture was added to the solution; it was shaken for 15 min at room temperature and centrifuged for 15 min at 3000 rpm. Half the volume of phenol, chloroform, isoamyl alcohol mixture was again added to the obtained water phase. This was shaken energetically and subsequently centrifuged. The above steps were repeated until complete purification of DNA manifested by the lack of interphase. Then, half the volume of isopropylene alcohol and 0.1 volume of 3 M acetate with pH 7.0 was added to the water phase.

The DNA samples obtained in this manner were then rinsed in 80% ethanol and dissolved in distilled water after drying. The samples with dissolved DNA were stored at -20˚C. Quantitative determination of the DNA obtained was carried out spectropho-

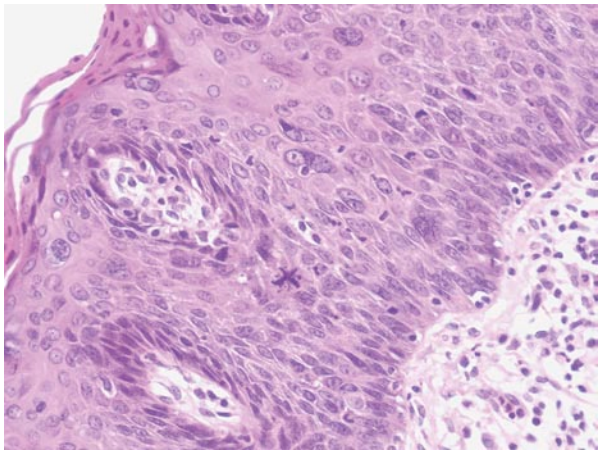

Figure 1. Carcinoma *in situ* of the uterine cervix. H&E. Magnification, x400.

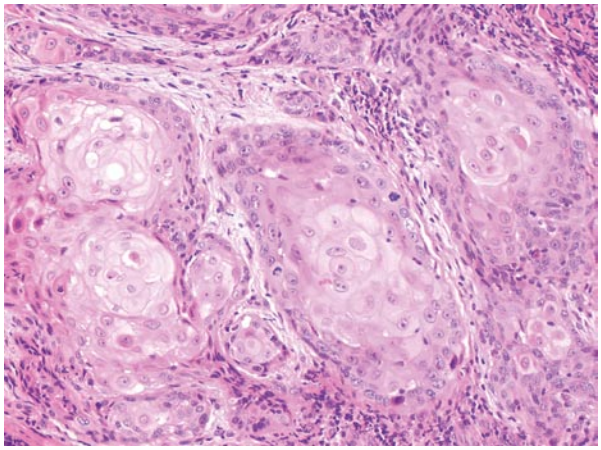

Figure 2. Squamous cell carcinoma (well differentiated) of the cervix, keratinizing type. H&E. Magnification, x400.

tometrically using an automatic spectrophotometer (Amersham Pharmacia Co.). In order to determine the amount of DNA in a given sample, 1 μl of the sample was dissolved in 69 μl of re-distilled water and, after calibration of the spectrophotometer, placed in its measuring chamber. After automatic processing of the measured data, the results were recorded in μg/ml.

HPV-PCR identification. In order to identify viral genome incorporated into cancerous DNA, polymerase chain reaction (PCR) was used to recognize HPV types 16 or 18. Identification of the HPV types was performed using consensus PCR primers for L1: MY09: 5'-CGTCCMARRGGAWACTGATC-3' and MY11: 5'-GCMCAAGGWCATAAYAATGG-3' where M=A+C, R=A+G, W=A+T, Y=C+T. This set of primers amplifies DNA of at least 33 different HPV genotypes.

Identification of HPV types 16 and/or 18 was performed using the fallowing type-specific PCR primers: HPV-16/L1A/HPV-16/L1B, 5'-GCCTGTGTAGGTGTTGAGGT-3' and 5'-TGGATTTACTCCAACATTGG-3' (product size 264 bp); HPV-18/L1A/HPV-18/L1B, 5'-GTGGACCAGCAAATACAGGA-3' and 5'-TGCAACGACCACGTGTTGGA-3' (product size 162 bp); HPV-18ME12/HPV-18ME50/E6, 5'-CACGGCGACCCTACAAGCTACCTG-3' and 5'-TGCAGCACGAATTGGCACTGGCCTC-3' (product size 404 bp).

The total volume (10 μl) of PCR mixture contained 1 μM of primers, 200 μM deoxynucleotide triphosphate, 1X PCR buffer (0.1 M Tris-HCl pH 8.8, 0.5 M KCl, 0.015 M $MgCl_2$, 1% Triton X-100), the investigated DNA (10 ng/μl) and Tag polymerase at a final concentration of 40 U/ml. After preliminary denaturation (15 min at 94˚C) samples were amplified for 31 cycles in a thermal cycler. Each cycle consisted of the following steps: denaturation at 94˚C for 30 sec, annealing at 59˚C for 30 sec followed by primer extension at 72˚C for 1 min. In the last PCR cycle, the stage of complementary DNA synthesis at 72˚C was extended to 420 sec. PCR products were analyzed using agarose gel electrophoresis in the presence of pBluescript DNA digested with *Hin*dI.

Immunohistochemical (IHC) analysis of the expression of ER and PR. The monoclonal mouse antibody IgG1 class (Novocastra) against the epitope characteristic for ERα protein was used for IHC analysis (ER, cat. no. NCL-L-ER-6F11). Prokaryotic recombinant protein corresponding to the full length ERα molecule was the antigen used for immunization.

The monoclonal mouse antibody class IgG_1 against PR protein was used for IHC study of the PR recombinant protein corresponding to the N-terminal region of the PR A form which was the antigen used for immunization.

IgG_1 antibodies (DakoCytomation; cat. no. X0931) were used as a positive and negative control standard. For deparaffinization, the rehydrated slides were placed in a thermostat at 37˚C, overnight. The next day the slides were deparaffinized in xylene and passed through graded alcohols to distilled water. The antigens were unmasked in 0.01 M citrate buffer (pH 6.0) using a microwave oven for three cycles for 5 min. After 20 min, the tissue sections were placed in Tris buffer with NaCl (pH 7.6) at room temperature. The staining was performed in an autostainer (DAKO automated immunostainer) utilizing a staining system. Sections were then washed with 0.3% solutions of hydrogen peroxidate (H_2O_2) for 5 min. After rising in TBS buffer, the slides were incubated for 1 h with the appropriate primary antibody against ER (dilution 1:50; clone 6F11, Novocastra) and against PR (dilution 1:100; clone 1A6, Novocastra). The Vectastain Elite ABC Kit was used as the detection system (Vector Laboratories, USA). The DAB Substrate kit (Vector Laboratories) was used as a chromogen. Subsequently, all sections were counterstained with Mayer's hematoxylin and mounted with Canadian balm.

IHC evaluation of the expression of the studied ER and PR proteins was performed independently by two pathomorphologists. The distribution of nuclear staining for ER and PR was evaluated for each section. The number of positive cells were counted under x200 magnification (on a field of 4x4=16 squares), which corresponded to the area of 0.5x0.5 mm = 0.25 mm^2. The results are presented in Tables II, III and IV.

Statistical analysis. The frequency of a specific feature was included in the statistical analysis. For assessing correlations between variables, the Spearman rank coefficient (R) was used. Relationships between categorized variables were assessed using the Pearson's χ^2 method. Relationships between

Table I. Prevalence of HPV DNA in the study groups.

	Analysis of HPV DNA types			
	HPV DNA 33 type[a]	HPV DNA type 16	HPV DNA type 18	
Group	n (%)	n (%)	n (%)	p-value
Squamous cell carcinoma (n=200)	180 (90.0)	140 (70.0)	60 (30.0)	<0.001[b]
Adenocarcinoma (n=50)	48 (96.0)	8 (16.0)	40 (80.0)	<0.001[c]
Control (n=50)	3 (6.0)	2 (4.0)	1 (2.0)	

[a]Universal primer. [b]Compared to the control: χ^2=20.085, p<0.001; Fisher's test p<0.001; [c]Compared to the control: χ^2 =26.421, p<0.001; Fisher's test p<0.001.

Table II. Comparison of individual clinical parameters and expression of estrogen and progesterone receptor protein in the examined groups of patients.

Group	HPV DNA (10-50% cells with expression) n (%)	ER expression		PR expression	
		Carcinoma n (%)	Stromal cells n (%)	Carcinoma n (%)	Stromal cells n (%)
Control group (n=50)	3 (6)	+	++	+	++
Case groups					
SCC (n=200)	180 (90)				
		- 90 (95)	- 97 (48.5)	- 194 (97)	- 89 (44.5)
		+ 10 (5)	+ 75 (37.5)	+ 6 (3)	+ 68 (34.0)
		++	++ 18 (9.0)	+++	++ 21(10.5)
		+++	+++ 10 (5.0)	+++	+++ 22 (11.0)
Adenocarcinoma (n=50)	48 (96)				
		- 47 (94)	- 29 (58.0)	- 48 (96)	- 30 (60.0)
		+ 3 (6)	+ 16 (32.0)	+ 2 (4)	+ 15 (30.0)
		++	++ 3 (6.0)	++	++ 3 (6.0)
		+++	+++ 2 (4.0)	+++	+++ 2 (4.0)
FIGO classification[a]					
I (n=150)	140 (93)	- 133	- 73	- 135	-
		+ 10	+ 47	+ 5	+ 50
		++	++ 18	++	++ 18
		+++	+++ 8	+++	+++ 20
II (n=100)	90 (90)	- 87	- 51	- 87	-
		+ 3	+ 44	+ 3	+ 33

For SCC cases, ER: carcinoma vs stroma cell, p=0.023; PR: carcinoma vs. stroma cell, p=0.041, χ^2 test. For adenocarcinoma cases, ER: carcinoma vs stroma cell, p=0.018; PR: carcinoma vs. stroma cell, p=0.015, χ^2 test. [a]Differentiation of the neoplastic process according to the FIGO classification.

variables were also assessed by an agglomeration algorithm, a tree-based method (city distance, full linkage). The range of significance was set at p<0.05, p>0.1, which was considered to be marginally significant.

Results

Frequency of HPV DNA. Infection of HPV types 16 and/or 18 was noted in 3 out of 50 (6%) cases in the control group. HPV DNA was found in 180 out of the 200 (90%) examined microscopic sections from the patients with squamous cell carcinoma, whereas HPV DNA was noted in 48 of 50 (96%) cases of adenocarcinoma (Table I).

Based on the clinical staging of the cervical cancer (FIGO classification) the DNA of HPV type 16 and/or 18 was detected in 140 of 150 (93%) grade I cases and in 90 out of 100 (90%) grade II cases. There was no correlation between the prevalence of DNA type 16/18 and clinical grade. Analysis

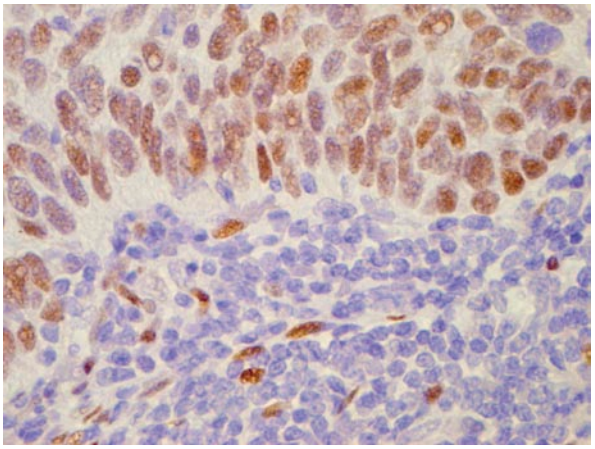

Figure 3. Immunohistochemical staining for ER. Squamous cell carcinoma showing 3+ positivity, exhibits an intense, nuclear reaction in nearly all of the tumor cells. There is strong staining of the individual stromal cells but negative staining in the lymphocytes. Magnification, x400.

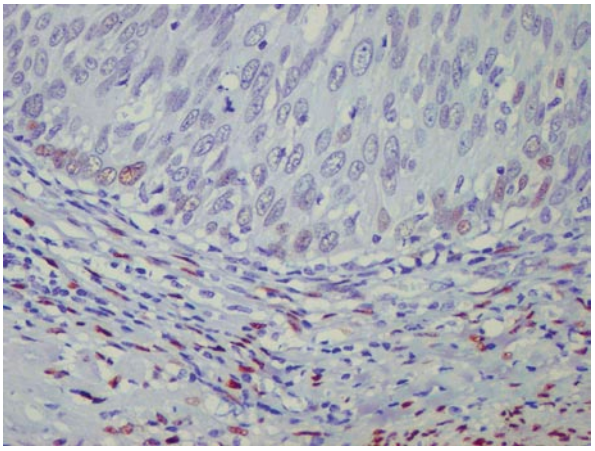

Figure 4. Squamous cell carcinoma showing 1+ positivity for ER. Note the weak staining in the basal layer of the neoplastic tissue and intense staining of the stromal cells. Magnification, x400.

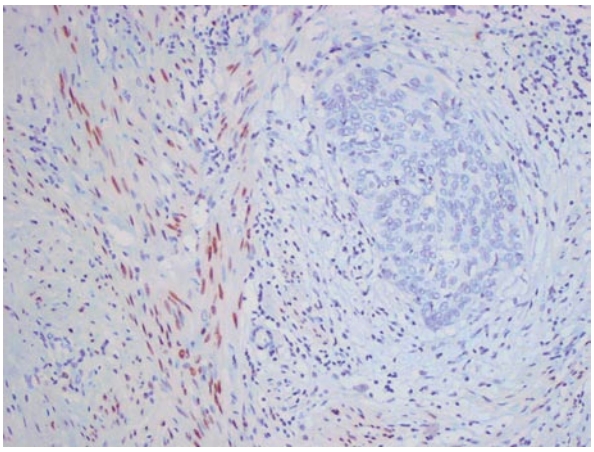

Figure 5. Immunohistochemical staining for PR. In a tumor with negative staining there is no positivity in the tumor cells although staining is present in the scattered stromal cells. Magnification, x200.

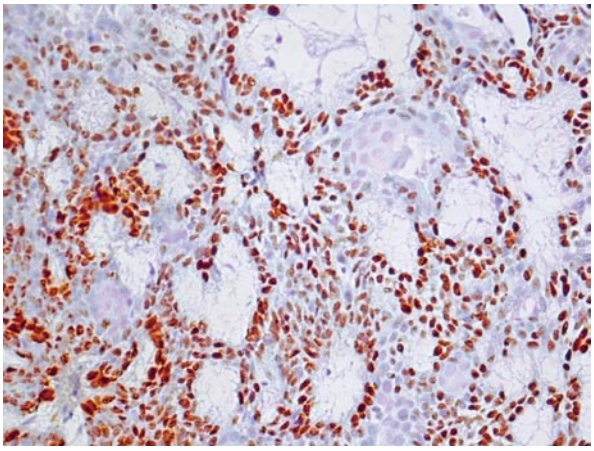

Figure 6. Adenocarcinoma of the uterine cervix with intense positive nuclear immunostaining for PR. Magnification, x200.

of the correlation between histopathological grade and presence of HPV type 16 and/or 18 DNA revealed the presence of viral DNA in 86 out of 89 (96.6%) GI cases, in 75 out of 78 (96%) G2 cases, and in 80 out of 83 (96%) G3 cases. There were no significant differences in the prevalence of HPV DNA in relation to tumor grade (Table II).

Expression of ER and PR protein. In the control group consisting of normal epithelium of the cervix, the expression of ER and PR protein was between 10 and 50% (Table III). Figs. 3-6 illustrate IHC staining for both receptors.

Squamous cell carcinoma group. In the squamous cell carcinoma cases, the expression of ER (determined according to described standard methods) was absent in 190 of the 200 (95%) cases. Expression of +1 was found in 10 out of the 200 (5%) cases, and no expression of +2 nor +3 was detected. No expression of ER protein was noted in 97 of the 200 (48.5%) stromal cell samples. ER protein expression of +1 was noted in 75 of the 200 (37.5%) cases, +2 in 18/200 (9%) and +3 in 10 out of 200 (5%).

PR protein expression was absent in 194 out of the 200 (97%) cases of squamous cell carcinoma while it was evaluated as +1 in only 6 of the 200 (3%) squamous cell carcinoma samples. However, in the stromal cells an absence of PR expression was noted in 89 of the 200 (44.5%) cases, while the expression was assessed as: +1 in 68 out of 200 (34%), +2 in 21 out of 200 (10.5%) and +3 in 22 out of 200 (11%) cases. When comparing the difference in expression of the receptors between the staining pattern and stromal cells, the expression levels were statistically significantly higher in the stroma (ER: carcinoma vs. stromal cells, p=0.023; PR: carcinoma vs. stromal cells, p=0.041 χ^2 test)

Adenocarcinoma group. Expression of ER (evaluated accordingly to described standard methods) was absent (-) in 47 out of 50 (94%) cases while expression of +1 was noted in 3 out of 50 (6%) cases. ER expression of +2 or +3 was not detected in

Table III. Number of cells showing expression of estrogen and progesterone receptor proteins.

	N	Min	Max	M	SD	10%	Me	75%
ER (no.)	250	48.00	256.00	168.57	61.85	72.00	180.00	224.00
ER (%)	250	18.75	100.00	65.85	24.16	28.13	70.31	87.50
PR1 (no.)	250	85.50	304.00	195.07	58.55	118.75	190.00	247.00
PR (%)	250	28.13	100.00	64.17	19.26	39.06	62.50	81.25

no., number of cells showing expression; %, net area of cells considered positive (+); Min, minimal number of cells showing positive reaction; Max, maximal number of cells showing positive reaction; M, mean value; SD, standard deviation; 10%, in 10% of watched preparations, expression was observed in the following number of cells; 75%, in 75% of watched preparations, expression was observed in the following number of cells; Me, median; N, number of cases; ER, estrogen receptor; PR, progesterone receptor.

Table IV. Criteria for evaluation of the reaction to estrogen and progesterone receptor protein expression.

No. of cells (+)/preparation	% of cells (+)	Reaction
Estrogen receptor		
<70	<10	-
72-180	10-50	+1
181-224	51-75	+2
>224	>75	+3
Progesterone receptor		
<70	<10	-
118-190	10-50	+1
191-247	51-75	+2
>247	>75	+3

(+), with positive expression.

Table V. Spearman rank correlation coefficient (R).

Variables	R	p-value
Grade and ER	-0.3241	0.4531
Grade and PR	-0.3278	0.3216
HPV 16/18 and ER (Ca)	-0.1987	0.3156
HPV 16/18 and PR (Ca)	0.2990	0.1050

any case. Absence of ER expression was noted in 29 out of 50 (58%) stromal cell cases while ER expression of +1 was noted in 16 out of 50 (32%), +2 in 3 out of 50 (6%) and +3 in 2 out of 50 (4%) cases. Expression of PR was absent in 48 out of 50 (96%) adenocarcinoma cases and only 2 cases were evaluated as +1 (4%). However, in the stromal cells the lack of expression was noted in 30 out of 50 (60%) cases while expression was confirmed as +1 in 15 out of 50 (30%), +2 in 3 out of 50 (6%) and +3 in 2 out of 50 (4%) cases.

The difference between the expression of the tested receptors in the pattern of staining and stromal cells was statistically significantly higher in the stroma (ER ca vs. stromal cells, p=0.018; PR ca vs. stromal cells, p=0.015). Based on the IHC results of disease stage I and II (FIGO classification) cases, ER expression was lacking in 133 out of 140 (95%) cases with stage I clinical grade and in 87 out of 100 (87%) stage II cases. Higher expression of the tested proteins was found as well in the stromal cells when comparing to the pattern of cancer.

In every stage of histological grading no expression of the studied receptors was found in the stainging patterns of planoepithelial cancer and adenocarcinoma of the uterine cervix. Only single cases of expression evaluated as +1 were noted. In the stromal cells, the lack of expression was weakly observable in G1, G2 and G3 but frequently affirmed the existence of tested receptors as +1, +2 or +3 (G1: +1, 37; +2, 10; +3, 5; G2: +1, 25; +2, 6; +3, 4; G3: +1, 10, +2, 5; +3, 3 for ER and G1: +1, 30; +2, 8; +3, 0; G2: +1, 44; +2, 6; +3, 4; G3: +1, 28, +2, 10; +3, 8 for PR) (Table IV). The analysis of correlations between individual parameters did not show any significant statistical relation for grade and HPV 16/18 status (χ^2=5.128, p=0.02351) (Table V).

Discussion

The presence of steroid hormone receptors in normal epithelium and in cervical intraepithelial neoplasia (CIN) was a focus of study in the 1970s (29). A series of reports have shown that the mean ER positivity in cervical mucosa can vary from 13 to 100%. For PRs, this variation is even wider, i.e. 0-100% of tissues from the uterine cervix has been reported to be PR positive (30-32). No statistically significant correlations have been found for the ER/PR ratio in the cervix when related to menstrual cycle, menopause, histological type or ploidy analysis (30-33).

However, there are reports claiming that the amount of ER and PR can change dynamically during persistent HPV infection (34). Higher levels of estradiol receptor were observed in low-grade CIN tissues from patients who were oral contraceptive users (18), and low expression of ER was observed in CIN II (cervical intraepithelial neoplasia gradus mediocris), while cases of CIN III and ICC (invasive carcinoma of the cervix) were negative for the receptor. Down-regulation of ER expression might be the first alteration to

occur in normal epithelium during the development of cervical dysplasia in women infected with high-risk HPV (35). A study by Fonscesca-Moutinho *et al* (36) found a significantly higher positive coexpression of ER and PR in CIN III than in CIN II of microinvasive carcinomas of the cervix (MIC). The authors hypothesized that in MIC, most ERs have no or reduced functional activity. This finding was supported by the fact that in MIC with coexpression of ER and PR, the ER staining was stronger and expressed in more cells than PR, which was not true for the CIN III group.

The prognostic significance of PR and ER levels in invasive carcinoma of the cervix (ICC) is controversial. In cervical cancer, increased bcl-2 is generally associated with a better prognosis. Co-expression of ER, PR and bcl-2 may be a useful tool in identifying CIN III lesions with low risk of progression to cervical cancer (36). Potish *et al* (37) found that premenopausal patients with PR- and ER-positive tumors had a statistically significantly greater survival probability. Nevertheless, this finding did not apply to postmenopausal women. Hunter *et al* (38), however, found no correlation between ER and PR expression and menopausal status. They also found a weak correlation (p=0.063) between the presence of PR and length of survival and no correlation between ER status and survival. Nonogaki *et al* (39) found that the cells in all cases of ICC lost their immunoreactivity to ER, apart from two cases that contained HPV types 31/33/35. This finding led them to suggest that ER expression in ICC may be related to the HPV DNA types and tumor progression.

One major explanation for these discrepancies may be related to the location of the receptors, which can be found either in the epithelium or in stromal cells, at the site of the tissue analyzed, and dependent on the method for receptor detection. In an attempt to clarify some of these issues, the present authors sought to evaluate the concentrations of ER and PR in samples from normal uterine cervix and from invasive carcinoma of the cervix: squamous cell carcinoma and adenocarcinoma of the cervix, associated or not with the presence of DNA HPV.

This study corroborates other research demonstrating an increased grade of malignancy with a decreased number of receptors (34,39,40). The expression of ERs in invasive carcinoma of the cervix can decrease to undetectable levels. The absence of expression in neoplasmic tissues in cervical cancer associated with high-risk HPV does not exclude the influence of estrogen and progesterone on HPV transcription. In recent years, it has been revealed that activated ER can have an effect on DNA, not only through the ER, but also through transcription factor AP-1 (activating protein-1). Transcription complex AP-1 is a dimer composed of factors belonged to the FOS, JUN and MAF families. It plays a crucial role in proliferation and differentiation of epithelial cells. Both types of ERs interact with the AP-1 factor, most frequently with the c-JUN:c-JUN complex in sites of binding (41). However, the influence of each individual type of receptor is dissimilar. Binding of receptor α with 17β-estradiol activates transcription under the influence of E2 in AP-1, while ERβ deactivates transcription. Anti-estrogens such as tamoxifen or raloxifen appear to act as agonists of the ER, particularly in the case of ERβ (43).

Another method of influence is interaction with the SP-1 protein. Impact of the activation of the SP-1 factor on ERα is independent of the ligand. ERα or β create complexes with SP-1 in the promoter of the retinoid acid receptor α-1 (RAR1) gene and activate its transcription. The site of binding of SP-1 is rich in sequences GC, therefore the methylation of cytosine in sequences recognized by this factor, neutralize its activated performance (43,44).

One of the biological effects of the activation of ERs is an induction of biosynthesis of PRs. Simultaneously, PR inhibits the creation of ER. The biological effect of estradiol is dependent on PRs. Estradiol induces the expression of PR and achieves its biological function through PR receptors. In the family of nuclear receptors, orphan receptors with no ligands have been discovered. However, recently it has been considered that estrogens might act as ligands (45).

The present study demonstrates that the expression of ER and PR in planoepithelial cancers and adenocarcinomas of the cervix is decreased to undetectable levels. Only in singular cases in the pattern of staining was expression of ER and PR noted. In stromal cells of the studied neoplasms, higher expression of both types of receptors was found. Strong expression (+1, +2, +3) was noted in stromal cells irrespective of the presence of HPV DNA, histopathological type of cancer, and clinical and histopathological grade. Comparison of the presence of the selected receptors (ER and PR) in the staning pattern and stroma in both squamous cell carcinoma and adenocarcioma of the cervix, showed statistically higher expression in stromal cells.

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