Increased Serum Progesterone and Estradiol Correlate to Increased COX-2 Tissue Expression in Cervical Intraepithelial Neoplasia

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Abstract. *The aim of this study was to To investigate correlations between serum progesterone and serum estradiol levels and expression of tissue tumor markers in cervical intraepithelial neoplasia (CIN) and normal epithelium. Materials and Methods: Eighty women of fertile ages with cervical biopsies ranging histologically from normal to CIN III were included. Expression of eleven tumor markers was studied. Serum levels of progesterone and estradiol were analyzed. Exclusion criterion was hormonal contraceptive use. Results: In normal epithelium, low progesterone levels correlated to expression of epidermal growth factor receptor (EGFR) and CD4+. In initial analyses of CIN, high progesterone levels correlated with expression of retinoblastoma protein, p16 and cyclooxygenase-2 (COX-2), but after adjustment for CIN grade, only correlation to COX-2 expression remained significant. Expression of COX-2 and CD4+ correlated to serum estradiol levels in CIN. Conclusion: Serum levels of progesterone and estradiol appear to correlate with increased COX-2 expression in CIN. In addition, the study shows that evaluation of expression of tumor markers must take into account the grade of CIN.*

A number of tumor markers have been studied in cervical intraepithelial neoplasia (CIN). The objectives have generally been to find tumor marker expression that could discriminate between normal cervical epithelium and CIN, or aid the histological diagnosis of CIN grade. Other studies have focused on determination of the aggressiveness of CIN, but with no conclusive results. During recent years, much attention and extensive research has been directed towards studies of p16, retinoblastoma protein (Rb), p53 and Ki-67/MIB1 (1-3).

The epidemiological correlation between long-term oral contraceptive (OC) use and cervical neoplasias is well established, and OC use has been accepted as a risk factor since the mid 1980s, when a number of studies were able to control for sexual risk behavior (4, 5). It seems plausible that there is a biological role for sex steroid hormones in the development of cervical neoplasms, but the underlying mechanisms are virtually unknown and available results are, in general, restricted to *in vitro* studies on cervical cancer cell lines (6-12).

In previous studies on invasive cervical squamous cell cancer we showed that serum estradiol and serum progesterone levels correlated with the DNA growth fraction in fertile women (13). Women with a fatal outcome had a longer survival when serum estradiol levels were high and serum progesterone levels were low (14).

The aim of the present study was to evaluate eleven tumor markers and their correlation, if any, to serum progesterone and serum estradiol levels. The women included in the study were premenopausal with regular menstruations. OC users and those with gestagen-only contraceptives were excluded. Some tumor markers based on our previous results on invasive cervical cancer (15), and those that were previously studied in cervical neoplasias which could be of possible clinical importance were analyzed. Tumor markers were chosen to represent different mechanisms in carcinogenesis.

Materials and Methods

The study population comprised 80 women. Sixty women were consecutively recruited from the Out-patient Surgery, Department of Obstetrics and Gynecology, Falun Hospital, and were to undergo a laser conization of the *cervix uteri* because of CIN or suspicious CIN; atypical squamous cells of unknown significance (ASCUS). Inclusion criteria were premenopausal status, regular menstruation, and no oral contraceptive or gestagen-only contraceptive use. Menstrual cycle phase did not influence the recruitment. After

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informed consent, a serum sample was collected and immediately frozen at –70˚C. Once paraffin embedded, the cone was stored at the Department of Pathology and Clinical Cytology.

In addition, twenty apparently healthy volunteers with similar inclusion criteria were recruited. Colposcopically directed punch biopsies were taken at the Out-Patient clinic of the Department of Obstetrics and Gynecology and were paraffin embedded. A serum sample was frozen as above. Informed consent was obtained from all study participants. Women were recruited from 2005 to 2007.

A structured questionnaire included birth date, age, last menstruation, cycle day, history of abnormal Pap smear, contraceptive use and smoking habits were given to the participants.

Serum progesterone and serum estradiol were analyzed by standard methods. Sections from the original paraffin blocks were microscopically reviewed by one of the authors (TT) and the most representative area(s) was marked for tissue microarray (TMA). One two-millimeter punch biopsy was taken from the blocks corresponding to the marked area and joined into TMA paraffin blocks, containing 24 to 30 punch biopsies on average. Each TMA block also included one control and one empty square to avoid invalid diagnoses.

Immunohistochemistry was performed as previously described (16). In brief, glass slides were deparaffinized in xylene (2×15 min), dehydrated through graded alcohols and endogenous peroxidase was blocked (using H_2O_2 in 70% ethanol). Antigen retrieval was performed using Target Retrieval Solution (TRS pH 6.0 or pH 9.0; Labvision, CA, USA) in a decloaking chamber (Biocare Medical, Walnut Creek, CA) for 4 minutes at 125˚C. Thereafter the slides were immunostained in an automated staining instrument, where slides were incubated with primary antibodies and secondary reagent (see Table I) 30 minutes at room temperature (RT). Finally, the slides were incubated with diaminobenzidine (DAB) as chromogen for 10 min and counterstained with Mayers hematoxylin (Sigma-Aldrich, St Louis, MO, USA) for 15 min. Slides were washed in distilled water for 10 min, dehydrated through graded alcohols to xylene, and mounted in Pertex organic mounting medium (Histolab, Gothenburg, Sweden).

Details of the 11 antibodies chosen for the study are given in Table I. One of the authors (GP) who was blinded for clinical details evaluated the tumor marker expressions and consensus was achieved with another pathologist (TT) in uncertain cases. All the reviewed cases were categorized as normal, CIN I, CIN II, CIN III, or as borderline (if showing some but not all of the following features: mitotic figures, vertical nuclear growth pattern, perinuclear halo, indistinct cytoplasmic border and no primitive cells in upper 1/3 of squamous layer). Frequency of immunostained cells and intensity of staining were evaluated. A four-grade semi-quantitative score was used for frequency, where 0 represented absence of biomarker expression and 1-3 represented increasing percentages of immunopositive cells (1-19%, 20-49% and ≥50%, respectively). Intensity of staining was graded in four steps: absent, weak, moderate and strong. In the analyses there was, in general, a good correlation between frequency and intensity and the best discrimatory evaluation was used for presentation in Tables.

For the analyses, the most explanatory cut-off level was used when the results were dichotomized. When there was no evidence of any correlation to serum progesterone and/or estradiol, data were dichotomized so that an equal number of patients, if possible, were included in the two groups. For adjustments according to CIN grade, the material was divided into high-grade (CIN II and CIN III) and low-grade (borderline and CIN I) squamous intraepithelial lesions (HSIL and LSIL, respectively) and logistic regression was used. For statistical analyses, the JMP statistical package (SAS Institute) was used. Student's *t*-test was used for significance testing. The study was approved by the Research Ethical Committee, Uppsala University.

Results

The mean and median age was 35.7 years and 34.5 years, respectively, with a range of 22-52 years. Twenty-four (30.0%) women were smokers. The diagnoses were distributed as 25 (31.3%) cases of normal epithelium, 10 (12.5%) borderline, 18 (22.5%) CIN I, and 27 (33.8%) CIN II-III.

The mean and median menstrual cycle day at serum sampling was day 18.3 and 19.0, respectively. The mean serum progesterone was 20.1 nmol/l, and the corresponding serum estradiol was 449.2 pmol/l.

Table II gives the results of serum progesterone levels and their correlations to tumor marker expression. Positive or negative correlations between serum progesterone levels and intensity of expression were evident in CIN for Rb, p16, and cyclooygenase-2 (COX-2) in crude analysis. After adjustment for CIN grade, only COX-2 expression remained significantly correlated with serum progesterone levels. In normal epithelium, epidermal growth factor receptor (EGFR) and $CD4^+$ expression correlated with low serum progesterone levels. In HSIL there was a higher correlation between COX-2 expression and high progesterone levels (17.5 *vs*. 6.6 nmol/l; p=0.009).

Serum estradiol levels were analyzed similarly (Table III). In normal epithelium, there were no significant correlations. In CIN, serum estradiol levels correlated weakly, but significantly with increased COX-2 expression.

Discussion

It is evident that serum estradiol and progesterone levels show associations with tumor markers that are involved in proliferation, immunological mechanisms and muliple functions (COX-2) in CIN and normal epithelium. The strongest correlation was observed between high serum progesterone levels and COX-2 expression in high grade CIN.

This is the first study of its kind and correlations between serum sex steroid hormones levels might help us to understand the mechanisms behind epidemiological findings, such as long-term OC use and parity. Both progesterone and, to some extent, estrogens have been claimed to induce cervical cancer in *in vitro* studies.

In cancer studies, the proteins that were included here are generally referred to as tumor markers. We chose to present the results of expression in normal epthelium and those with LSIL/HSIL. An interesting finding was significant correlations that vanished when adjustment was made for the

Table I. *Tumor markers included in the study and their major functions.*

Biological marker	Functions	Clone	Species	Dilution	Antigen retrieval	Source
Fragile histidine triad (FHIT)	Tumor suppressor	RB-9232	Rabbit	1:300	HIER pH6	Lab Vision/Neo Markers, Fremont, CA, USA
p53	Tumor suppressor, apoptosis	M7001	Mouse	1:1000	HIER pH6	DakoCytomation, Glostrup, Denmark
p16	Tumor suppressor	NCL-p16-432	Mouse	1:100	HIER pH9	Novocastra, Newcastle, Great Britain
Retinoblastoma protein (Rb)	Tumor suppressor	554136	Mouse	1:300	HIER pH9	Pharmingen, San Diego, USA
$CD4^+$	Immunological marker	NCL-CD4-1F6	Mouse	1:25	HIER pH9	Novocastra, Newcastle, Great Britain
Interleukin 10 (IL-10)	Immunological marker	RHCIL1000	Rat	1:100	HIER pH6	Caltag, Buckinham, Great Britain
Epidermal growth factor receptor (EGFR)	Proliferation	28-0005	Mouse	1:40	Proteinase K	Zymed, San Fransisco, CA, USA
Ki-67 (MIB-1)	Proliferation	M7240	Mouse	1:200	HIER pH9	DakoCytomation, Glostrup, Denmark
Cytokeratin10	Cytoskeleton	MS-611	Mouse	1:600	HIER pH9	Lab Vision/Neo Markers, Fremont, CA, USA
E-Cadherin	Cell cell adhesion	13-1700	Mouse	1:2500	HIER pH9	Zymed, San Fransisco, CA, USA
Cyclooxygenase-2 (COX-2)	Inflammation and multiple functions	18-7379	Mouse	1:2000	HIER pH9	Zymed, San Fransisco, CA, USA

Table II. *Serum progesterone and its correlation to tumor marker expression in CIN and normal epithelium.*

	n	Progesterone nmol/l Normal epithelium	*p*-Value	n	Progesterone nmol/l CIN		*p*-Value	*p*-Value[a]
Cyclooxygenase-2 (Cox-2): moderate/strong *vs*. no/light intensity	28	32.2 *vs*. 21.5	0.17	31	22.3 *vs*. 11.4		0.02	0.04
Retinoblastoma protein: strong *vs*. none/moderate intensity	26	32.0 *vs*. 18.3	0.06	25	21.8 *vs*. 10.6		0.03	0.66
p16: strong *vs*. none/moderate intensity	28	-*vs*. 25.3	-	27	9.8 *vs*. 20.5		0.04	0.83
Fragile histidine triad (FHIT): intensity light/strong *vs*. none	25	9.7 *vs*. 26.1	0.24	22	7.9 *vs*. 18.2		0.07	0.39
Epidermal growth factor receptor: strong *vs*. none/moderate intensity	26	15.4 *vs*. 36.5	0.002	26	14.3 *vs*. 21.0		0.25	0.41
Interleukin-10 (IL-10): frequency 2-3 *vs*. 0-1	25	21.5 *vs*. 24.2	0.84	27	10.8 *vs*. 17.9		0.14	0.85
$CD4^+$: frequency 1-3 *vs*. 0	23	22.2 *vs*. 39.8	0.048	30[b]	-	-	-	-
Cytokeratin 10 (CK10): moderate/strong *vs*. none/light intensity	26	27.6 *vs*. 24.3	0.69	27	14.2 *vs*. 16.0		0.76	0.17
p53: frequency 2-3 *vs*. 0-1	25	39.3 *vs*. 22.4	0.11	25	9.5 *vs*. 15.3		0.36	0.19
Ki-67: strong *vs*. none/moderate intensity	26	28.0 *vs*. 6.6	0.06	25	17.1 *vs*. 12.7		0.47	0.96
E-Cadherin: strong *vs*. none/moderate intensity	25	27.3 *vs*. 19.7	0.30	23	15.6 *vs*. 28.7		0.20	0.67

Hormonal contraceptive users and postmenopausal women were excluded. [a]Adjusted for LSIL *vs*. HSIL. [b]Only 1 CIN was $CD4^+$ negative. Intensity was measured as none, light, moderate and strong staining; frequency of staining was measured semiquantitatively: 0: 0%, 1: 1-19%, 2: 20-49% and, 3: ≥50% stained cells.

grade of CIN. Differential expression of tumor markers in LSIL and HSIL explain these results and must be kept in mind in any similar study.

Intense staining of EGFR correlated with low progesterone levels in normal epithelium, but not in CIN. Progesterone is a suppressor of endometrial growth, but the effect on squamous cervical epithelium has been poorly studied. A previous study did not show any correlation to CIN grade or human papillomavirus (HPV) infection (17). An increased staining intensity of EGFR was reported with CIN progression, mainly because expression extended from the basal to superficial epithelial layers (18).

The retinoblastoma pathway is closely associated with $p16^{INK4a}$ expression. The HPV E7 gene product inactivates

Table III. *Serum estradiol and correlation to tumor marker expression in CIN and normal squamous cell epithelium.*

	n	Estradiol pmol/l Normal epithelium	*p*-Value	n	Estradiol pmol/l CIN	*p*-Value	*p*-Value[a]
Cyclooxygenase-2 (COX-2):							
frequency 2-3 *vs*. 0-1	31	587.8 *vs*. 420.0	0.22	45	518.7 *vs*. 340.3	0.04	0.046
Epidermal growth factor receptor:							
frequency 1-3 *vs*. 0	30	420.1 *vs*. 785.8	0.06	39	399.1 *vs*. 569.5	0.40	0.40
Retinoblastoma protein:							
moderate/strong *vs*. none/light intensity	29	511.3 *vs*. 327.0	0.28	36	451.1 *vs*. 338.4	0.32	0.30
p16:							
light/strong *vs*. none intensity	32	430.8 *vs*. 474.3	0.80	40	391.1 *vs*. 500.8	0.45	0.68
Fragile histidine triad (FHIT):							
frequency 2-3 *vs*. 0-1	28	212.0 *vs*. 487.8	0.48	35	350.9 *vs*. 482.9	0.44	0.12
$CD4^+$:							
moderate/strong *vs*. none/light intensity	32	470.0 *vs*. 454.4	0.93	45	394.5 *vs*. 423.7	0.89	0.55
Interleukin 10 (IL10):							
frequency 1-3 *vs*. 0	29	512.1 *vs*. 446.7	0.70	40	348.3 *vs*. 464.8	0.18	0.18
Cytokeratin 10 (CK10):							
moderate/strong *vs*. none/light intensity	30	508.1 *vs*. 453.9	0.73	40	400.6 *vs*. 424.9	0.83	0.80
p53:							
frequency 3 *vs*. 0-2	29	493.0 *vs*. 455.3	0.89	38	638.0 *vs*.372.9	0.11	0.13
Ki-67:							
strong *vs*. none/moderate intensity	29	493.4 *vs*. 429.5	0.75	38	381.5 *vs*. 554.1	0.12	0.14
E-cadherin:							
strong *vs*. none/moderate intensity	29	460.24 *vs*. -	-	35	392.9 *vs*. -	-	0.76

Hormonal contraceptive users and postmenopausal women were excluded. [a]Adjusted for HSIL *vs*. LSIL. Intensity was measured as none, light, moderate and strong staining; frequency of staining was measured semiquantitatively: 0: 0%, 1: 1-19%, 2: 20-49% and 3: ≥50% stained cells.

Rb by phosphorylation, resulting in a negative feedback loop with increased $p16^{INK4a}$ expression, but as the Rb response is poor it will lead to a continuously high $p16^{INK4a}$ expression. $p16^{INK4a}$ serves as a surrogate marker for HPV infection, but its clinical significance is obscure (1-3, 19). Our finding of a diminished correlation between strong Rb and low p16 expression and very high progesterone levels when adjustment for CIN grade was made seems likely to reflect the higher frequency of HPV infection in HSIL. The inverse relationship between high $p16^{INK4a}$ expression and low Rb expression confirms the correlation between these tumor markers in HPV infections.

Expression of another two tumor suppressor proteins were included in the study, fragile histidine triad (FHIT) and p53. The *FHIT* gene is located at chromosome 3p and is frequently lost in many cancer types. The *FHIT* gene product acts as a tumor suppressor, one mechanism is *via* its proapoptotic activity. A progressive loss is seen in cervical neoplasias and invasive cancer (20, 21). In the present study, no correlation between high serum progesterone or estradiol and altered FHIT expression was found.

The last tumor suppressor included, p53, did not correlate to progesterone levels. p53, like Rb, was one of the first detected tumor suppressors and one of the major mechanisms of p53 action is cell-cycle arrest, allowing for DNA repair or apoptosis of damaged cells. In most cancer types, mutant p53 is found. Interestingly, in cervical cancer the wild-type p53 dominates. p53 is, however, degraded by the HPV E6 gene product, in analogy with the Rb protein (22). In a previous study of invasive cancer, we found significantly lower p53 expression with high serum progesterone levels (15).

COX-2 responds to a variety of mitogenic and inflammatory stimuli and is thought to be involved in a number of steps in cancer development, in itself or through prostaglandins. Among these are apoptosis, proliferation, immune suppression and neoangiogenesis. Evaluations of a clinical role for COX-2 expression indicate an association with poor prognosis in cervical cancer (23, 24). The finding in this study, a correlation between strong intensity of COX-2 expression and elevated serum progesterone levels, in particular in HSIL, suggests an adverse effect of progesterone on CIN. Similar correlations were found for serum estradiol levels.

Cytokines are released in response to infection of the uterine cervix by HPV among others. Interleukin-10 (IL-10) is a T-helper cytokine and has a suppressive effect on cell mediated immunity. Increased expression has been found to be associated with progressive cervical neoplasia and cancer (25, 26). In the cervix IL-10 facilitated establishment of HPV infections by inhibiting immune responses (26). In the present study, high progesterone levels did not correlate to IL-10 expression.

Cellular immune responses, most important by antigen-specific T lymphocytes, are considered the critical defence mechanisms against HPV-infected cells. $CD8^+$ responses are involved in the generation and clonal expansion of cytotoxic T lymphocytes. One role for $CD4^+$ T-cells is to help during the generation of $CD8^+$ T-cell responses (27). The correlation beween absence of $CD4^+$ expression and high progesterone levels in normal epithelium could not be analyzed in CIN because of almost universal expression of $CD4^+$.

Progesterone has previously been suggested as a major candidate risk hormone in cervical neoplasias, although the present study is the first to investigate molecular biological mechanisms. Estrogen has been reported to reduce susceptibility to primary HPV infection, but might be of no importance once an HPV infection has been established (28). HPV has a tendency to transfect cells with progesterone receptors. Both HPV 16 and HPV 18 contain progesterone and glucocorticoid response elements that increase expression of the HPV E6 and E7 oncogenes, considered crucial in cell transformation (6). Such a transformation has been reported to take place when progesterone or OC gestagens were added to cell cultures (8).

In an experimental study (9), an enhanced colony-forming efficiency was found in the HPV 16-DNA-integrated cervical cancer cell line, CaSki, after at least three days of progesterone treatment. The progesterone antagonist RU 486 was able to abrogate the enhancement of progesterone on cell growth. Progesterone and glucocorticoid hormones increase HPV mRNA and significantly stimulate viral replication (29). Progesterone response elements occur in the long control regions of HPV 16 in the cervical cancer cell line SiHa and are thought to mediate an increase in transcripts encoding E6 and E7 proteins (6). Estrogen-treated SiHa cells were also found to induce HPV transcripts, while there was no such effect when progesterone was added (7). An increased cell proliferation with high serum progesterone levels in invasive squamous cell cervical cancer has also been reported in a study which included more than 100 women (14).

When transgenic mice expressing HPV 16 were treated with estrogens, squamous cell carcinomas developed exclusively in the transformation zone (30). Estrogens might also have a role in increasing the levels of HPV-induced apoptosis (10) or attenuating p2X7-R-mediated apoptosis (12). Experimental studies have produced conflicting results, particularly in studies of estrogens. Experimental studies suffer from the problem of introducing correct, physiological concentrations of hormones and might be a cause for discrepant results in different studies. Sex steroid hormones might also be involved in single steps in the neoplastic progression, similarly to that of many tumor markers. Once invasive cancer has been established, high serum estrogen levels might have a positive effect on outcome, while high serum progesterone levels have been found to have a deleterious effect (14).

Some previous studies have studied sex steroid hormone interactions with HPV, *in vitro* experiments on cervical cancer cell lines, and animal experiments. This clinical study on women with normal or neoplastic epithelium indicates that serum progesterone and estradiol levels influence cellular and extracellular proteins which have been associated with neoplastic development, in normal epithelium and CIN. Future studies should involve additional biological markers of possible importance in CIN and invasive cervical cancer.

References

1 Cenci M, Pisani T, French D, Alderisio M and Vecchione A: pRb2/p130, p107 and p53 expression in precancerous lesions and squamous cell carcinoma of the uterine cervix. Anticancer Res *25*: 2187-2192, 2005.

2 Van Niekerk D, Guillaud M, Matisec J, Benedet JL, Freeberg JA, Follen M and MacAulay C: p16 and MIB1 improve the sensitivity and specificity of the diagnosis of high grade squamous intraepithelial lesions: Methodological issues in a report of 447 biopsies with consensus diagnosis and HPV HCII testing. Gynecol Oncol *107*: S233-S240, 2007.

3 Nam EJ, Kim JW, Kim SW, Kim YT, Kim JH, Yoon BS, Cho NH and Kim S: The expressions of the Rb pathway in cervical intraepithelial neoplasia; predicitive and prognostic significance. Gynecol Oncol *104*: 207-211, 2007.

4 Hellberg D, Valentin J and Nilsson S: Long-term use of oral contraceptives and cervical neoplasia: an association confounded by other risk factors. Contraception *32*: 337-346, 1985.

5 Castellsaque X and Munoz N: Cofactors in human papillomavirus carcinogenesis – role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monographs *31*: 20-28, 2003.

6 Chan W-K, Klock G and Bernard H-U: Progesterone and glucocorticoid elements occur in the long control regions of several human papillomaviruses invloved in anogenital neoplasia. J Virol *63*: 3261-3269, 1989.

7 Mitrani-Rosenbaum S, Tsivieli R and Tur-Kaspa R: Oestrogen stimulates differential transcription of human papillomavirus type 16 in SiHa cervical carcinoma cells. J Gen Virol *70*: 2227-2232, 1989.

8 Pater A, Bayatpour M and Pater MM: Oncogenic transformation by human papillomavirus type 16 deoxyribonucleic acid in the presence of progesterone or progestins from oral contraceptives. Am J Obstet Gynecol *162*: 1099-1103, 1990.

9 Yuan F, Auborn K and James C: Altered growth and viral gene expression in human papillomavirus type 16-containing cancer cell lines treated with progesterone. Cancer Invest *17*: 19-29, 1999.

10 Webster K, Taylor A and Gaston K: Oestrogen and progesterone increase the levels of apoptosis induced by the human papillomavirus type 16 E2 and E7 proteins. J Gen Virol *82*: 201-213, 2001.

11 Salazar EL, Mercado E, Sojo I and Salcedo M: Relationship between estradiol 16α-hydroxylation and human papillomavirus infection in cervical cell transformation. Gynecol Endocrinol *15*: 335-340, 2001.

12 Gorodeski GI: Estrogen attenuates p2X7-R-mediated apoptosis of uterine cervical cells by blocking calcium influx. Nucleosides Nucleotides Nucl Acids *23*: 1287-1293, 2004.

13 Hellberg D, Lindström AK and Stendahl U: Correlation between serum estradiol/progesterone ratio and survival length in invasive squamous cell cervical cancer. Anticancer Res *25*: 611-616, 2005.
14 Lindström A, Backström T, Hellberg D, Tribukait B, Strang P and Stendahl U: Correlations between serum progesterone and smoking, and the growth fraction of cervical squamous cell carcinoma. Anticancer Res *20*: 3637-3640, 2000.
15 Lindström AK, Ulf Stendahl U, Tot T and Hellberg D: Associations between ten biological tumor markers in squamous cell cervical cancer and serum estradiol, serum progesterone and smoking. Anticancer Res *27*: 1401-1406, 2007.
16 Uhlen M and Ponten F: Antibody-based proteomics for human tissue profiling. Mol Cell Proteomics *4*: 384-393, 2005.
17 Tervahautu A, Syrjänen S and Syrjänen K: Epidermal growth factor receptor, *c-erbB-2* proto-oncogene and estrogen receptor expression in human apillomavirus lesions of the uterine cervix. Int J Gynecol Pathol *13*: 234-240, 1994.
18 Boiko IV, Mitchell MF, Hu W, Pandey DK, Mathevet P, Malpica A and Hittelman WN: Epidermal growth factor receptor expression in cervical intraepithelial neoplasia and its modulation during an α-difluoromethyllornithine chemoprevention trial. Clin Cancer Res *4*: 1383-1391, 1998.
19 Murphy N, Ring M, Heffron CCBB, King B, Killalea AG, Hughes C, Martin CM, McGuinness E, Sheils O and O'Leary JJ: $p16^{INK4A}$, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. J Clin Pathol *58*: 525-534, 2005.
20 Terry G, Ho L, Londesborough P, Cross P, Lopes A, Monagham J and Cuzick: The role of human papillomavirus type 16 and the fragile histidine triad gene in the outcome of cervical neoplastic lesions. Br J Cancer *91*: 2056-2062, 2004.
21 Martin CM, Astbury CM and O'Leary JJ: Molecular profiling of cervical neoplasia. Expert Rev Mol Diagn *6*: 217-229, 2006.
22 Hofseth LJ, Hussain SP and Harris CC: p53: 25 years after its discovery. Trends Pharm Sci *25*: 177-181, 2004.
23 Young JL, Jazaeri AA, Darus CJ and Modesitt SC: Cyclooxygenase-2 in cervical neoplasia: A review. Gynecol Oncol *109*: 140-145, 2008.
24 Lindström AK, Stendahl U, Tot T, Lidström BM and Hellberg D: Predicting the outcome of squamous cell carcinoma of the uterine cervix using combinations of individual tumor marker expression. Anticancer Res *27*: 1609-1616, 2007.
25 Azar KK, Tani M, Yasuda H, Sakai A, Inoue M and Sasagawa T: Increased secretion patterns of interleukin-10 and tumor necrosis factor-alpha in cervical squamous intraepithelial lesions. Hum Pathol *35*: 1376-1384, 2004.
26 Bermudez-Morales VH, Gutierrez LX, Alcocer-Gonzalez JM, Burguete A and Madrid-Marina V: Correlation between IL-10 gene expression and HPV infection in cervical cancer: A mechanism for immune response escape. Cancer Invest *26*: 1037-1043, 2008.
27 Eiben GL, Velders MP and Kast WM: The cell-mediated immune response to human papilloma-induced cervical cancer: Implications for immunotherapy. Adv Cancer Res *86*: 113-148, 2002.
28 Brabin L: Interactions of the female hormonal environment, susceptibility to viral infections and disease progression. AIDS Patient Care *16*: 211-221, 2002.
29 De Villiers E-M: Relationship between steroid hormone contraceptives and HPV, cervical intraepithelial neoplasia and cervical carcinoma. Int J Cancer *103*: 705-708, 2003.
30 Elson DA, Riley RR, Lacey A, Thordarson G, Talamantes FJ and Arbeit JM: Sensitivity of the cervical transformation zone to estrogen-induced squamous carcinogenesis. Cancer Res *60*: 1267-1275, 2000.

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