

Review

The Biological Role of Smoking, Oral Contraceptive Use and Endogenous Sexual Steroid Hormones in Invasive Squamous Epithelial Cervical Cancer

DAN HELLBERG^{1,2,3} and ULF STENDAHL⁴

¹Center for Clinical Research Dalarna, ²Department of Obstetrics and Gynecology, Falun Hospital, Falun;

³Department of Women's and Children's Health, Uppsala University, Uppsala;

⁴Department of Gynecologic Oncology, Norrlands University Hospital, Umea, Sweden

Abstract. For at least twenty years, epidemiological studies have found a correlation between cervical neoplasia and smoking and oral contraceptives. Over the last ten years, laboratory evidence has supported epidemiological findings. One example is the addition of progesterone to human papillomavirus-transfected cervical cells, which has led to oncogenic cell transformation. Very high concentrations of tobacco constituents in cervical mucus, as compared to serum, in smokers provided evidence of a biological role for smoking. It has previously been shown that the S-phase DNA fraction (as a measure of proliferation) was correlated to smoking and serum progesterone. There has been a gap between epidemiological and laboratory findings, as the clinical importance of these correlations have rarely, if ever, been reported. Our recent finding of a positive correlation between serum estradiol/progesterone ratio and length of survival in premenopausal women with invasive cervical cancer might add further knowledge, if confirmed by larger studies. Future studies on tumor markers could elucidate these observations.

Cervical human papillomavirus (HPV) infection is now established as an etiological agent for cervical neoplasia. HPV infection is commonly referred to as a necessary but not sufficient factor for invasive cervical cancer, which is evident from the high proportion of women of fertile age that, at some time, will have a cervical HPV infection.

Correspondence to: Dr Dan Hellberg, Center for Clinical Research, Nissers väg 3, 791 82 Falun, Sweden. Tel: 0046 23 4920 00, Fax: 0046 23 490989, e-mail: dan.hellberg@ltdalarna.se

Key Words: Cervical cancer, smoking, oral contraceptives, steroid hormones, review.

Immortalization of the cervical cell is necessary for progress of cervical intraepithelial neoplasia (CIN) to invasive cancer. Integration of viral DNA to the host genome, thereby enabling expression of viral oncogenes E6 and E7, seems to be a necessary step in immortalization and probably does not occur without the presence of co-factors (1).

Among proposed risk factors for cervical neoplasia are sexual risk behavior, long-term use of oral contraceptives (OC), smoking, dietary factors and sexually transmitted infections, mainly genital chlamydial infection. Sexual risk behavior is now recognized as a surrogate for cervical HPV infection. Smoking and oral contraceptive use have been the most widely studied epidemiological co-factors in cervical neoplasia (2-4), while the role of endogenous sex hormones has not been established (5, 6).

Smoking

Smoking, previously regarded as a confounder of sexual risk-taking, was, during the 1980s, gradually accepted as an independent risk factor of cervical neoplasia, when different studies (3, 4) were able to control for sexual risk behavior. It is possible that smoking is a stronger risk factor for squamous cell, as compared to adenomatous cervical cancer (7). Smoking also seems to be an independent risk factor for cervical HPV infection (8).

The first biological evidence was the finding that levels of nicotine, and its major metabolite cotinine, were increased forty-fold and four-fold, respectively, in the cervical mucus of healthy female smokers (9) and in women with CIN (10) as compared to serum levels.

Defects in DNA repair are related to carcinogenesis, and DNA damage has been found in cervical tissue of smokers with high DNA adduct levels (11, 12). Benzo(a)pyrene has been detected in cervical tissue, and DNA adducts were

twice as common in smokers. Cell growth and DNA damage induced by benzo(a)pyrene was higher in HPV-16 immortalized cervical cells than in normal tissue (13).

Small cell lung cancer, also of squamous epithelial origin has, in addition to benzo(a)pyrene, been attributed to tobacco-specific nitrosamines, which were identified in the cervical mucus of smokers, but not of non-smokers (14). Additionally, mutations of the *p53* tumor suppressor gene, with a subsequent decreased capacity for DNA repair, is one mechanism that contributes to cancer growth (15).

Oral Contraceptive Use

The consequences of OC use in cervical neoplasia have still not been established (16). A slight but apparent increased risk with long-, but not short-term use of OCs has been found in most, but not all, studies suggesting a hormonal influence (14, 17). There is recent evidence that oral contraceptive use influences squamous cell cancer more than adenomatous cancer (7). Although HPV is present in both diseases, it is possible that the main co-factors between the two histological types differ.

It should be emphasized that, in epidemiological studies, it is notoriously difficult to control for all confounding factors, such as sexual behavior, smoking, current HPV infection and reproductive history. Results from experimental studies are needed to further elucidate the role of OCs.

Steroid Sex Hormones

There are three major routes to study the possible influence of estrogens and progestogens: i) during the menstrual cycle, characterized by high serum levels of serum estradiol during the follicular phase and progesterone during the luteal phase; ii) during pregnancy, characterized by high levels of progesterone in particular; iii) during oral contraceptive use where there is an exogenous supply of hormones. Progesterone has previously been suggested as the major candidate-hormone in cervical neoplasia due to its immunosuppressive effect and to a possible connection with HPV infection (18). In contrast, estrogen has been reported to reduce susceptibility to primary HPV infection, but might be of no importance once the HPV infection has been established (19).

Both estrogen and progesterone receptors are present in cervical neoplasia. The expression of both receptors is higher in immature squamous metaplasia of the transformation zone than in the ectocervix (20). It is doubtful whether the presence of sex hormone receptors has an influence on cervical carcinogenesis.

Progesterone-HPV interactions. 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, with gestagenic stimuli (21).

Such a transformation has been reported to take place when progesterone or oral contraceptive gestagens were added (22). It is not known if this occurs *in vivo* when serum progesterone levels are high, such as during pregnancy or in the menstrual luteal phase, or with oral contraceptive use. High serum progesterone levels, when adjusted for menstrual phase, have been correlated to a high prevalence of HPV infection (23). It has also been speculated that pregnancy facilitates the transmittance of HPV infection (24). CIN during pregnancy often disappears *post partum*, but recurred in more than 25% of the women in a long-term follow-up (25). In one study, serum progesterone levels were higher in premenopausal women with adenomatous, as compared to squamous cell cancer (7).

In an experimental study (26), 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 (27). 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 cases (5).

Estrogen-HPV interactions. In epithelium of the transformation zone, where cervical neoplasia is initiated, 16- α -hydroxylation of estradiol occurs resulting in 16- α -hydroxyestrone (28), which is linked to malignant transformation of estrogen-sensitive cells transfected by HPV. Serum estrone was elevated in patients with CIN who were HPV-positive as compared to HPV-negative women with or without CIN (29). When transgenic mice expressing HPV 16 were treated with estrogens, squamous cell carcinomas developed exclusively in the transformation zone (30).

Once invasive cancer has been established, high serum estrogen levels might have a positive effect on outcome, while high serum progesterone levels have been cited to have a deleterious effect (6). There was a significant, linear correlation to a high estradiol/progesterone ratio and longer survival in premenopausal women who eventually died, thereby adding clinical, epidemiological and experimental for an influence of female sex steroid hormones in cervical cancer.

Tumor Markers

Biological markers associated with epidemiological risk factors for cervical cancer can aid in clarifying data from epidemiological studies and provide insight into the carcinogenic process.

A number of serological and biochemical tumor markers for cancer have been evaluated (31). Some of them act as prognostic predictors, while others seemingly only reflect the presence of neoplasia. Despite promising results for different markers, few have been thoroughly evaluated and established.

The main mechanisms or modes of actions of tumor markers are related to cell-cell interaction, extracellular space proteins, membranous factors, cell proliferation, angiogenesis, tumor hypoxia, immunological factors, oncogenes, tumor suppressor genes and apoptosis. There have been few studies regarding the relationship between hormones or smoking, and biochemical markers in the context of cervical neoplasia. However, some investigations have been carried out in other squamous cell cancers, such as lung cancer.

Cell Proliferation

A mitogenic effect of nicotine on normal and HPV-16 DNA-transformed squamous cervical epithelial cell lines, but not in adenomatous cell lines, has been observed (32). Smoking has been significantly associated with cervical cancer cell proliferation, measured as the DNA S-phase fraction (5).

In addition, serum progesterone levels have been significantly associated with cancer proliferation. When the serum-estradiol/progesterone ratio was estimated among premenopausal women, a strong positive association was found not only with survival length, but also with the S-phase fraction (6).

In one study, Ki-67, often used as a marker of proliferation, was negative in cervical parabasal cells during the follicular phase, but positive during the luteal phase, thus indicating an association with progesterone levels (33).

Epithelial growth factor receptor (EGFR) was up-regulated in smokers in both HPV-positive and HPV-negative cervical cell lines (34). An increase of EGF-like activity after addition of progesterone, but not estradiol, to benign ovarian tumors has been reported (35).

The above findings suggest that both smoking and female sex steroid hormones are independent risk factors in cervical neoplasia.

DNA Damage

Telomeres are repetitive DNA sequences at the ends of chromosomes and are shortened at each cell division.

Progressive shortening of DNA leads to chromosome instability and cell death. Telomerase is activated by the *HPV 16 E6* gene, and there is a significant dose-dependent correlation between telomerase activity and smoking status in lung cancer (36). In the uterus, the proliferative phase endometrium is correlated to a high telomerase activity, but is inversely correlated to progesterone levels (37).

The *p53* tumor suppressor gene encodes the p53 protein, which is activated in response to DNA damage and causes cell cycle arrest by blocking the cell at the G1- and G2-phase prior to DNA replication and mitosis, thereby aiding the DNA repair process and preventing mutations (38). The *HPV E6* oncogenes bind p53 and direct its rapid degradation in a step thought to be important in viral DNA replication (39). Tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons seem to increase the mutations of *p53* in lung cancer (40).

Intercellular Mechanisms

E-cadherin has been shown to have an almost linear decrease from normal cervical epithelium, through stages of CIN to invasive cervical cancer (41). This transmembrane glycoprotein is probably the most important factor in the interconnection of cells. Aberrant methylation is an important pathway in silencing tumor suppressor genes, and in lung cancer the mean methylation index has been found to be significantly higher in smokers than in non-smokers (42).

Tumor metastases require degradation of extracellular matrix through proteolysis that allows for the penetration of tumor cells into the basement membrane. Different types of metalloproteinases (MMP) are the most extensively studied enzymes involved in extracellular space degradation. Some related studies have found a strongly significant, independent correlation between MMP-2 and TIMP-2 and a poor survival rate in cervical cancer (43). A dose-dependent relationship between smoking and MMP-2 polymorphism in lung cancer has also been reported (44).

Angiogenesis

The growth of tumors beyond 1-2 mm is dependent on the formation of new blood vessels, or neoangiogenesis. Vascular endothelial growth factor (VEGF) is a cytokine that serves a central function in angiogenesis and is the most studied marker of angiogenesis. It has been demonstrated, by staining cervical tissue with VEGF antibodies, that angiogenesis is an early event in cervical neoplasia. An increased VEGF expression in invasive cervical cancer as compared with CIN III has also been observed (45). In an experimental endothelial culture model, VEGF expression was increased upon addition of nicotine and its major

metabolite, cotinine, in concentrations representative of those measured in the plasma in active smokers (46).

Langerhans Cells, NK and *FHIT*

Estrogens seem to positively influence the immune response, while progestogens have a negative influence (47). Low serum levels of natural killer cells (NK) have been observed in smokers (48). In cervix uteri the most studied immune response factor is Langerhans cells, which present an antigen for the T cells and are found in lower levels in cervix uteri of smokers as compared to non-smokers (49).

Reduction of fragile histidine triad (*FHIT*) expression has been observed in various cancers. *FHIT* encompasses the common chromosomal fragile site FRA3B and is probably a tumor suppressor gene that is involved in the control of apoptosis and proliferation. A significant relationship has been found between *FHIT* gene deletion and high-risk HPV in preinvasive cervical lesions (50). A progressively lower *FHIT* expression from low-grade cervical lesions to high-grade lesions and invasive cancer has also been found in other studies (51). In lung cancer a significant association between loss of *FHIT* function and smoking has been cited (52).

Conclusion

In summary, there is solid evidence of epidemiological correlations between smoking and cervical neoplasia. In most such studies long-term oral contraceptive use is a risk factor, but as cervical neoplasia is closely connected to sexual risk behavior, the correlation is difficult to establish definitively. Experimental studies have shown that both tobacco constituents and female sexual steroid hormones influence different pathways in cervical carcinogenesis. Correlations to proliferation, as measured by the S-phase fraction, have been reported. The first evidence of clinical importance is the finding of a longer survival in premenopausal women, who eventually died, but who had high serum estradiol/progesterone ratios.

However, several obstacles prevent full elucidation of the topic at this time. Epidemiological studies may lack control for all possible involved factors. Results from animal studies might be species-specific. Furthermore, laboratory studies on cervical tissue, normal or cancerous, where sex hormones or tobacco constituents are added, might not be equivalent to the concentrations of hormones found *in vivo*. Perhaps the study of tumor markers in relation to co-factors will provide new insights.

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Received February 2, 2005

Revised June 2, 2005

Accepted June 3, 2005