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Review

Sex Steroids and Cervical Cancer

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Abstract. *During the 19th century, studies indicated that reproductive events were involved in cervical cancer. Human papillomavirus (HPV) infection is a prerequisite for development of cancer, but co-factors, among them the action of sexual steroid hormones, are necessary. Childbirth has been an important risk factor but now probably plays a minor role in the industrialized world, where parity is low. Long-term oral contraceptive use has been thoroughly studied epidemiologically, and correlates to cervical cancer in most studies. In vitro studies on cervical cell lines transfected with HPV and animal studies indicate that sex steroid hormones are capable to induce cancer. In in vivo cervical cancer tissue studies there have been observations that endogenous progesterone in serum correlates to a negative pattern of expression of cellular and extracellular proteins, tumor markers. Immune response could be another mechanism. Estradiol might be associated with a positive pattern and high estradiol and low progesterone levels increase duration of survival in cervical cancer. Studies where treatment of compounds that influence sex steroid hormones have been given are rare and have been disappointing.*

Cervical cancer is the second most common female-specific cancer after breast cancer. Approximately half a million women in the world are affected annually and the global mortality approaches 60%, and even higher in developing countries (1).

Hippocrates, the founder of modern medicine, described cervical cancer in 400 B.C. Thus, he recognized that it was an incurable disease that distroyed the uterus and invariably led to death, and concluded that it should be left untreated (2).

Gynecological mass screening was introduced into the industrialized world during the 1960s, and by discovering and treating cancer precursors, the incidence decreased to half that of previous levels (3-5). This success could not have happened without the findings of Hinselmann and Papanicolau during the 1930s and 1940s. On Hinselmann's invention of the colposcope, it became possible to identify precursor lesions in the epithelium of portio and cervix uteri, hereafter referred to simply as the cervix, and targets for biopsies and microscopical examination. The binocular colposcope usually magnifies the cervix, cleased with a weak acetic acid solution, by 8-16 times and the portio and the lower part of the cervical canal is thus studied from outside the vagina during a routine gynecological examination (6). Papanicolau discovered that after brushing the cervix and studying the smear under a microscope, normal epithelium, precursors and invasive cancer could be identified. Vaginal cytology is still often referred to as pap smear (Papanicolau smear) (7).

During the 1970s, zur Hausen found that to a high degree, cervical cancer was accompanied by human papillomavirus infection (HPV) (8). It took 20 years before the scientific community was convinced that HPV was the major etiological factor in development of cervical cancer, for which zur Hausen was rewarded a Nobel prize in 2008. Approximately 15 of more than 100 HPV types are considered as being high-risk, specifically targeting the cervix (9).

HPV infection is considered 'necessary but not sufficient' for progression of the precursor, cervical intraepithelial neoplasia (CIN), to invasive cancer (10). *In vitro*, the presence of HPV by itself leads to the development of carcinoma *in situ* from normal epithelial cells, but not to invasive cancer. Co-factors seem to be necessary for this last step. The most studied candidate co-factors are smoking and female sex steroid hormones (11, 12). Here the evidence for a biological role of endogenous and exogenous sex steroid hormones in cervical cancer is discussed. When not otherwise stated, 'cervical neoplasia' refers to invasive squamous cell cancer or CIN. It must also be stressed that in the following, biological tissue markers, and cellular and extracellular proteins, will be referred to as tumor markers, to differentiate them from proteins that have not been associated with cancer.

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Reproduction

During the 1840s, the Italian physician Rigoni-Stern published his famous articles, that indicated an association between reproductive factors and cervical cancer. Thus, he observed that the disease was common in prostitutes and almost absent from nuns. Cervical cancer became a disease of "the poor and socially deprived women" and a low socioeconomic status remains as a risk factor in many studies. Later in the 19th century, the German physician von Scazoni proposed that causal factors were "too much sex or self pollution" (13). During the 19th century, masturbation was considered to cause a large number of diseases. During the 1950s, studies appeared that confirmed the role of risky sexual behaviors as a major epidemiological risk factor in cervical cancer. Age at first intercourse was one of the first factors that was studied (14). This association was considered to be caused by a vulnerable cervix in young women. Subsequently, the number of lifetime sexual partners was found to be a stronger risk factor (15). The hypothesized biological role of a low age at first intercourse is still controversial. The more years of sexual activity, however, the higher is the statistical chance of multiple partners, and the higher risk of engagement with a male carrier of genital HPV infection.

Parity was one of the first reproductive factors studied, and Rigoni-Stern also observed that the disease was more common in married woman and widows compared to unmarried women. If sex steroid hormones are true co-factors, there is on one hand a biological rationale for multiparity as a co-factor, as serum hormone levels are high during pregnancy (16). Multiparity could, on the other hand, be a confounder for risky sexual behaviors, in particular before the introduction of modern contraceptives, but these are still not available in many parts of the world. Cervical neoplasm, CIN and cancer, are relatively often detected during pregnancy. Pregnancy is, however, a situation where women frequently have gynecological examinations and pap-smears performed, which could introduce a detection bias. A common observation is the spontaneous healing of such lesions after delivery, which could support the hormonal theory. In regard to this matter, "healing" was often found 3-6 months post partum. In a long-term study, however, recurrence of CIN was as high as 25%, and the true regression rate was similar to that for non-pregnant women with CIN (17).

In most epidemiological studies, parity has been associated with cervical cancer and CIN. Most of the major and recent studies, restricts the analyses to HPV-positive women, report an increased risk for cervical neoplasia with increasing parity (18), but the results have been conflicting, as a number of studies did not find any effect of parity. Early studies were not able to adjust for sexual behavior (19) and will not be commented on here. Even when sexual risk factors are adjusted for, several problems remain in drawing causal associations. In many parts of the industrialized world, high parity is rare (20, 21), while multiparity is common in developing countries, or those with restrictions for contraceptive use (16, 22). In countries with low parity its correlation to incidence of cervical neoplasms tends to be absent or low (21), or have poor statistical power (20). Adjustment for risky sexual behaviors is crucial when correlations between reproductive factors or hormonal contraceptive use, and cervical neoplasia are investigated (see below). The above studies that adjusted for sexual behavior (16, 20, 22) all found an increased risk for cervical neoplasia with increasing number of childbirths, but residual confounding must be considered.

Overall, epidemiological studies in general suggest a correlation between parity and cervical neoplasia. It has been suggested that the increased exposure of the cervical transformation zone, where cervical neoplasia is initiated, after pregnancy might facilitate HPV infection (18). This is in contrast to the theory that sex steroid hormones are true co-factors, *i.e.* involved in cellular molecular events, for the development of invasive cervical cancer. The results of one study did not find any correlation between parity and HPV infection, which indicates that parity is an independent co-factor (23). Experimental evidence is necessary to elucidate a role for sex steroids in cervical neoplasias.

Hormonal Contraceptives

The first report on a possible effect on genital cancer in humans by sex steroid hormones appeared in 1971 (24). A higher frequency of clear-cell adenocarcinoma in the vagina, a rare cancer type, had been diagnosed in females and in girls as young as 7 years of age, whose mothers had been exposed to diethylstilbestrol during pregnancy. Subsequent studies on cervical neoplasms were conflicting (25, 26). Diethylstilbestrol is a synthetic estrogen that was given from the 1940s to the 1960s to pregnant women considered to be at risk for spontaneous abortion.

Oral contraceptive (OC) use early emerged as an epidemiological risk factor for cervical neoplasia, but only in the early 1980s did studies with adjustment for other risk factors appear (27, 28). Studies on OC use necessitate even more careful methodology than those concerning parity. It could be expected that OC users are more sexually active than non-users. Sexual abstinence, marriage and other characteristics associated with low-risk sexual behaviors will reduce the necessity for contraceptive use. Parity could be lower among OC users and could introduce a negative bias. Smoking habits, detection bias due to frequent pap smear evaluations, and socioeconomic status are other sources of bias. Most importantly, a dose response effect should be taken into account, *i.e.* number of years of OC use. Previously, used 'high dose' OCs was common and this further complicates the interpretation of results.

Risky sexual behaviors other than multiple sexual partners and age at first intercourse must also be considered, such as sexual intercourse with unknown men and sex at first date. Anal intercourse is associated with increased prevalence of sexually transmitted infections, such as HPV infection. What is most important, but has rarely, if ever has been included in investigations, is the male partner's risky sexual behavior. It is well known that in different populations and parts of the world, women have relatively few lifetime sexual partners, in contrast to men. In one study, number of partners of husbands was more important than that of the wives. These variables have never been included in studies of hormonal contraceptive use (29).

OC increase serum levels of sex steroid hormones. In epidemiological studies, it is not possible to confirm the theory of sex steroid hormones as causal co-factors with HPV during the transition from normal epithelium to CIN and invasive cancer. The ideal study would be conservative management of CIN until regression to normal or progression to cancer related to OC use. Such a study would obviously be deeply unethical. An alternative would be to study women with CIN that were lost to follow-up and who eventually developed cervical cancer, but retrospective studies are invariably biased and these results would also be inconclusive.

When the first epidemiological studies with adjustment for risky sexual behavior that also included smoking habits were published it became clear that OC use, but only long-term use, *i.e.* more than 4-5 years, was independently correlated to cervical neoplasia, irrespective of sexual behavior and smoking (27, 28). Odds ratios were in general moderate, at 1.5-2.0, but significant. Subsequent studies have shown diverging results, but in general with a tendency towards a significant association between cervical neoplasia and OC use (30-32).

OC use, like parity, must be investigated for a possible correlation to HPV infection. OC use might merely be a bystander. If OC use is a risk factor independent of HPV infection, it might be a true biological co-factor, and not only correlated to cervical neoplasia in epidemiological studies. Several studies have investigated a possible correlation of OC use to HPV infection and adjusted the results for risky sexual behaviors (33-36). These studies found independent correlations between OC use and cervical neoplasia. Interestingly, use of high dose OCs, but not low dose OCs, was significantly associated with HPV infection (34). The cause is unclear but might suggest that the biological influence of exogenous steroid hormones, such as in cervical ectopies, might directly facilitate the entrance of HPV into the cervical epithelium. The border between the glandular endometrial epithelium and the vaginal squamous cell epithelium, the transformation zone, is the origin of cervical neoplasia, and the target for HPV. An ectopy will increase the vaginal exposure of the transformation zone and might be an easier target for HPV. In studies conducted during the last 20 years, high-dose OCs have rarely, if ever, been included.

Reviews have concluded that there is an epidemiological correlation between long-term OC use and cervical neoplasms, whether CIN or invasive cancer, independent of HPV status. An increased frequency with up to 15 years of OC use has been reported (30-32). Discrepant results have been reported but in any study, one must take into account the size of the study population. Stratification into groups by year of OC use might give limited power to achieve significant differences. As an example, analyzing 60 cases using OCs for 5-10 years and 40 cases using OCs for more than 10 years did not demonstrate any significant difference from non-users, despite a continuous increased risk for cervical cancer from 1.5 to 3.4 (odds ratio) when 1 year to more than 10 years of use were compared (37).

In postmenopausal hormone replacement therapy (HRT) natural estradiol or estrogens are given alone or in combination with a progestogen. Long-term use is relatively uncommon and studies are rare but results suggest that there is no correlation to cervical cancer (38, 39) and HRT seems to have no effect on prognosis after treatment of cervical cancer (38, 40).

In an important meta-analysis covering 24 worldwide studies, 16573 woman with cervical cancer and 35509 women without cervical cancer were analyzed. The pooled relative risk with long-term OC use (mean=11 years among cases) was 1.9, which is the most relevant risk estimate at present. As for the association of smoking and lung cancer, the risk for cervical neoplasia decreased to normal 10 years after cessation of OC use (41). Available data do not indicate that steroid hormonal contraceptives influence the prognosis for CIN or invasive cancer. In summary, OCs must be regarded as a risk factor for cervical neoplasia, independent of HPV infection and epidemiological risk factors.

Progestogenic contraceptives are most commonly administered orally or as injectable medroxyprogesterone acetate, but progestins are also released in medicated intrauterine devices and subdermal implants. Discrepant results on possible effects in cervical epithelium have been reported. In a study from South Africa, the odds ratio was 1.0 for progestogenic contraceptive users to develop invasive cervical cancer (42), while a Jamaican study on carcinoma *in situ* reported an odds ratio of 1.9, but this was non-significant (43). The WHO Collaborative Study of Neoplasia and Steroid Contraceptives found a relative risk of 2.4 with at least five years of use of injectible depo-medroxyprogesterone acetate, but did not rule out if the result was due to inappropriate adjustment for sexual factors (44).

Immunity

Sex steroid hormones modulate immune responses. Overall, progesterone is associated with immune suppression, while estradiol seems to be associated with an increased immune defense (45). Females have higher immunoglobulin levels than men, but might have a decreased cell-mediated immunity,

allowing for immunological escape of HPV infected cells (46, 47). During pregnancy, the natural killer cell activity is suppressed, indicating a decreased immunological response (48). Estrogen receptors have been identified in a number of immunocompetent cells, and progesterone increases the production of immunosuppressive factors in endometrial tissue and by interleukin-1 in monocytes (49). These are some examples of immunological effects by sex steroid hormones. The clinical role of these findings is unclear.

Experimental and Laboratory Research

As discussed above, epidemiological studies cannot provide conclusive results and causal evidence when correlations between disease and risk factors are studied. Results are strengthened when several independent studies show similar observations and when adjustment was made for known confounding factors. Still, there might be residual confounding, in particular when relative risks or odds ratios decline after adjustments.

Experimental and laboratory results will increase the biological plausibility of epidemiological results and *vice verse*. Such investigations include animal studies, human cell cultures with interventions, serological studies and molecular studies of human tissue.

Animal studies. The results of animal studies are confused by the different susceptibility to compounds in different animal species compared to humans. One example is the carcinogenic effect of estrogens in some strains of mice, but not in other strains or species (50). Transgenic mice expressing different HPV types have been studied. By treating mice with successively reduced doses of estrogens, a five-fold reduction of multistage vaginal and cervical cancer was achieved in one study (51), and finally the estrogens solely influenced the transformation zone. It confirmed that this area of the cervix is the target for estrogen-induced cervical cancer in mice (52). It also illustrates the problem of choosing relevant concentrations of the investigated compound, and this is also true in cell culture studies. It was also reported that mice transgenic for the E6 or E7 HPV oncogenes developed cervical cancer after treatment with estrogen for 6-9 months (53).

Early studies also showed epithelial abnormalities of the mouse cervix when treated by progestogens (54). Oncogenic transformation of baby rat kidney cells with integrated HPV 16 DNA was found when progesterone or progestins from OCs had been administered (55). In animals, however, estrogens have been studied more extensively than progestins.

Cervical cancer cell lines. In an early study, progesterone and glucocorticoid response elements were identified in the long region of several types of the HPV genome, and administration of progestins increased expression of the oncogenes E6 and E7, considered crucial in cell transformation (56) . In another study on HPV-positive cell lines, progesterone treatment enhanced the colony formation, while no effect was observed on HPV-negative cell lines (57). In addition, it was found that increasing concentrations of progesterone caused growth inhibition through cell-cycle arrest, but also reduced apoptosis (programmed cell death) allowing for cell growth (58). These studies on human cell lines support the notion that progesterone is the major sex steroid co-factor in cervical cancer. It was, however, also reported that estrogen treatment stimulated HPV 16 transcripts in another cell line, while progesterone did not (59). Similar results were obtained in other human cervical cancer cell lines after treatment with an estrogen (2-methoxyestradiol) (60). The authors speculated if adjunct anti-estrogen therapy were an option in the treatment in cervical cancer. These different results of cell culture studies exemplify the difficulties of drawing clinical conclusions, in particular when different cell lines, different estrogens and progestins, and different doses are used. However, cell culture studies are necessary for basic research, and generate ideas for clinical research and *vice versa*.

Finally, tumor marker expression has been increasingly studied in cell lines. Thus, p53 expression increased in HPV-infected cervical cancer cell lines after treatment with high doses of estradiol, but not with low or medium doses, a possible favorable effect in tumor suppression (61). p53 is a key cell cycle mediator causing cell cycle arrest, allowing for repair of mutant DNA or inducing apoptosis. cox-2 is a protein involved in several mechanisms of carcinogenesis, probably acting as a tumor promoter. Incubation with estradiol increased Cox-expression, while a synthetic progestin reduced expression (62). It must be stressed that carcinogenesis is so complex, and associated with such a large number of events and expression of tumor markers that influence many steps necessary in tumor development, and investigating only single markers will give inconclusive results.

Sex Steroid Serum Levels

The idea of studying cervical neoplasms by correlating clinical variables to serum hormone levels is attractive as it reflects physiological conditions. There are, however, a number of biases. Such studies should be prospective in order to obtain a reliable and relevant structured patient history. When endogenous hormone levels are evaluated, women with hormonal medications and contraceptives must be ruled out, as should surgical procedures that would influence the hormone levels and clinical tumor stage. The day of the menstrual cycle is closely related to endogenous hormone levels. According to our experience, postmenopausal women must be excluded, as circulating hormone levels in menopausal women are extremely low. When such biases are considered, however,

serological studies could provide increased clinical and experimental knowledge about cervical neoplasia.

Two studies were performed, one clinical and one laboratory, where the above biases were taken into consideration. In both studies, all pre- and postmenopausal women were analyzed together and separately. Analyses that included all participants showed no differences regarding the variables included. In the premenopausal group, one outcome was related to the S-phase fraction, *i.e.* the percentage of dividing cells in the cancer tissue, as a marker of proliferation and cancer growth. Nearly all tumors where serum progesterone levels were high had a high S-phase fraction. There were no correlations to serum estradiol levels and after adjustment for eight variables, only serum progesterone and smoking emerged as being significantly correlated to proliferation. This supports the theory that progestins are promoters of cancer growth and correlate to poor prognosis (63).

In our clinical study, mortality in invasive cervical cancer was studied and adjustments were made for a number of variables, such as clinical cancer stage, the major prognostical variable. Serum steroid levels were not useful as a prognostic tool in postmenopausal women. Serum levels of estradiol and progesterone did not correlate to overall prognosis and mortality. Premenopausal women with high serum estradiol levels, and who eventually died from their disease, showed increased survival-duration, compared to those with low serum estradiol, but this was nonsignificant. Women with high progesterone levels, on the other hand, had lower survival-duration (nonsignificant) than those with low levels. An estradiol-progesterone ratio was calculated and the combination of high estradiol and low progesterone correlated significantly to longer survival (64).

Thus, it seems that for mortality factors other than sex steroid hormone levels are involved, in particular the clinical stage at diagnosis. However, the study indicated increased tumor growth in association with low endogenous estradiol and high progesterone levels, which influenced the duration until death occurred.

CIN is graded into CIN1 (mild dysplasia), CIN2 (moderate dysplasia) and CIN3 (carcinoma *in situ*). We found no correlations between CIN grade and estradiol and progesterone levels (Hellberg D, unpublished results). These findings support the results of a study that included estradiol, progesterone, estrone, sex hormone-binding globulin and dehydroepiadrosterone. The study was limited and included 67 premenopausal and 43 postmenopausal women with either HPV-positive CIN or invasive cancer. None of the hormones measured correlated with the degree of precancerous lesions or invasive cancer stage, neither in premenopausal, nor in postmenopausal women (65). In another study, a higher incidence of HPV in pap smears in connection with higher serum progesterone levels was observed, a finding that requires confirmation (66). It has also been hypothesized that increased levels of estradiol, leading to increased conversion to estrone and eventually 16α-hydroxysterone would be a risk factor for cervical cancer due to promotion of proliferation. Increased estrone levels were found with increasing CIN grade, but the three study groups only averaged 20 women, and neither the hypothesis, nor the results have been confirmed (67).

Cervical Tissue Studies

We conducted four studies on invasive cancer and CIN and studied expression of 14 and 17 tumor markers, respectively, and correlated expression to serum levels of estradiol and progesterone. In invasive cancer, high serum progesterone levels correlated significantly to increased expression of c-MYC and decreased expression of p53, while high estradiol levels correlated to low expression of epidermal growth factor receptor (EGFR) (45). p53 is one of the most important tumor suppressors while c-MYC is a classical oncoprotein. EGFR seems to be one of the most important proliferation factors. These findings support our hypothesis that progesterone influences the development of cervical cancer and is related to poor prognosis.

Further support was found in a study on the novel tumor marker leucine-rich immunoglobulin-like repeats 1 (LRIG1). LRIG1 expression correlated to a favorable prognosis of early-stage cervical cancer and appeared to be a tumor suppressor. Expression was not associated with serum estradiol, but decreased expression was observed with increasing progesterone levels (68). Finding of lower expression of p27, a tumor suppressor, in pre- compared to postmenopausal women, gave additional evidence for a role of steroid hormones, and indicated that younger and older women should be analyzed separately before being studied as one group (69).

In CIN, a significantly higher expression of cox-2, low retinoblastoma protein (tumor suppressor) and low p16 (tumor suppressor) expression with high progesterone levels, the former were independent of CIN grade, was found (70). No correlations between serum estradiol and expression of these tumor markers were found. Expression of the remaining tumor markers also did not correlate to sex steroid levels. It could be concluded that progesterone levels in CIN and invasive cancer are associated with a negative tumor marker pattern. There are no previous studies in this area and our results need confirmation.

Steroid Receptors

Much attention has been given to the presence of estrogen (ER) and progesterone (PR) receptors in cervical neoplasia. This is partly caused by the early possibility of analyzing the expression of steroid receptors when few other biological markers were available. Thus, there were numerous articles on

the subject during the 1980s and early 1990s and few articles thereafter. The results were in general clinically disappointing (71, 72).

It is natural that steroid receptors are present of higher levels in pre- than in postmenopausal women (71), but down-regulation of ER expression has been claimed to be an early event in transition of normal epithelium to CIN (73).

Some studies reported that expression of ER and PR correlated to survival, while others found no difference. These studies, conducted during the 1980s, did generally not adjust for clinical staging. In one study, increased expression of ER and PR correlated to a higher survival rate irrespective of stage (74), and was also found when only early-stage cancer was analyzed (75). Correlations were restricted to premenopausal women. Among weaknesses was the inability to estimate serum hormone values. Other studies found no correlation between steroid receptors and prognosis, but failed to analyze pre- and postmenopausal women separately (76, 77). Serum sex steroid hormones in premenopausal women considering the day of menstrual cycle might better reflect the cellular hormonal milieu and could be a more sensitive method for evaluating tumor aggressiveness and prognosis.

Androgen receptor expression has been poorly studied, but seems to decline with increasing stage of cervical neoplasm (78). Further studies of the prognostic role should be conducted.

HPV Infection

A large number of studies have tried to elucidate the molecular links between HPV infection and sex steroid hormones, if any. HPV DNA must be integrated into the host genome for cancer development. The HPV DNA is divided into the early gene region (E) and late control region (LCR), where the latter is involved in activation of the E6 and the E7 genes. E6 and E7 are the major HPV oncogenes. E7 degrades retinoblastoma protein, while E6 degrades p53, which is why there is no dominance of mutant p53 in cervical cancer, in contrast to many other cancer types. Inactivation of both these tumor suppressors is crucial and one of the hallmarks of carcinogenesis.

Investigations of a relationship between sex steroids and the presence and activation of HPV have been clinical and experimental. As mentioned above, OC use seems to have no or little effect on prevalence or acquisition of cervical HPV infection when confounders are controlled for (79, 80). Few studies showed an increase of CIN3 compared to CIN1-CIN2 in OC users with HPV-positive lesions. Persistence of HPV infection in normal epithelium increases the risk of development into CIN, but the role of OC use, if any, is still unclear. It has been speculated that OC may play a role in the transition from CIN to invasive cancer, but such studies will not be carried out due to ethical reasons, as discussed above (23).

Experimental studies have been conducted in benign cell cultures immortalized by high-risk HPV types and with cancer cell lines with HPV DNA integrated in the host genome. Thus, when HPV type 16 was introduced in a cervical epithelial cell culture, progesterone induced HPV expression and a marked increase in viral messenger RNA. The response was inhibited by the anti-progestin RU486. The authors concluded that the HPV expression in response to progesterone was mediated by HPV glucocorticoid response elements (81). Furthermore, primary human ectocervical cells with integrated HPV type 16 were transfected with glucocorticoid response elements. Treatment with progesterone induced higher growth rates and dysplastic cells in these cells compared to these of the primary culture (82).

In HPV 18 transgenic mice, high estrogen or progesterone levels activated the early HPV promoter β-galactosidase. Ovariectomy caused suppression exclusively in the cervix and vagina, but administration of estrogen alone or in combination with progesterone restored β-galactosidase expression, which was inhibited by RU486 (83) Another mechanism might be the regulation of class I human leukocyte antigen (HLA) by progesterone, which was also blocked by RU 486. The immune suppression caused by HLA might promote integration of HPV DNA and lead to immunologic escape from cytotoxic T-cells (84). These and other studies demonstrate influences of steroid hormones on HPV in cultures of benign cells and suggest that these hormones might have a role early in HPV infections.

In cancer cell lines the focus has been to establish if administration of sex steroid hormones will enhance expression of the oncogenes E6 and E7, and the E2 gene, required for replication. Several human cervical cancer cell lines have been used in different studies, among those HeLa, C-33A, SiHa, HCE16/3 and CaSki. Studies show discrepant results and the lack of confirmatory studies using the same methods, cell lines, doses of sex steroid hormone, incubation *etc*. limits conclusive evidence.

In a study of CaSki cells, progesterone increased transcription of both HPV E6 and E7 (57), as also found in the HCE16/3 cell line (85). The latter study also estimated cell growth but did not find any effect of progesterone or by estradiol. Discrepant results were found in a study that included both CaSki and SiHa cells where no increased expression of E6 or E7 was found, after treatment with estrogens or progesterone. Progesterone increased proliferation in both cell lines, and also had an apoptotic effect, while estrogens only increased proliferation in SiHa cells (86). In a study using HeLa cells, the intention was to administer physiological levels of estradiol. No increase in E6 expression after administration of estradiol was observed (87). These divergent results stress the necessity of confirming results with similar methods and cell lines.

To complicate matters further, the rate of apoptosis has been studied in several studies. As mentioned above, one study

found that progesterone increased apoptosis in CaSKi and SiHa cell lines (86). In another study using the HeLa cell line, both estrogen and progesterone adminstration was associated with E2- and E7-induced apoptosis (88).

Studies on the mechanisms by which sex steroid hormones influence expression of HPV DNA in both benign and cancerous cells are conflicting and incompletely studied, but at least progesterone seems to be associated with proliferation as in our *in vivo* tissue studies (63). Whether the mechanisms are E6- and E7-mediated remain unclear.

Adenocarcinoma

While the ectocervix is covered by squamous epithelium as in the vagina, the endocervix has a glandular epithelium, similar to that in the uterine endometrium. The endometrium responds differently to sex steroid hormones than does squamous epthelium. Estrogens increase proliferation, while progestogens reduce glandular epithelium. One might therefore expect different effects by sex steroids in adenocarcinoma than in squamous cell cancer. Differences in tumor marker expression between these two cervical cancer histological types have been reported. In squamous cell carcinoma, there was higher expression of p53, cluster of differentiation 4 (CD4), EGFR, CD44 and stratifin than in adenocarcinoma, while one of the major oncoproteins, c-MYC, was more strongly expressed in adenocarcinoma. Furthermore, three out of 11 investigated tumor markers correlated to prognosis in squamous cell carcinoma, while none did in adenocarcinoma (89). This is also interesting, as both cervical cancer subtypes share HPV as an etiological factor.

The majority of studies of adenocarcinoma and sex steroid hormones have been epidemiological and examined the role of OC use in this cancer subtype. Expression of hormone receptors was evaluated in some studies. A longer disease-free but not overall survival with high expression of PR have been reported (90). In a small study, 30-40% of the tumors expressed PR and ER. There were no significant correlations to disease-free or overall survival (91). Other laboratory studies focusing on a hormonal role in cervical adenocarcinoma are rare. Most studies on long-term OC use have found an increased risk for adenocarcinoma of the same magnitude as for squamous cell carcinoma (92, 93). A high odds ratio (5.5) between OC use for more than 12 years and risk of adenocarcinoma has been reported (94).

Cancer treatment with sex steroid hormones

Based on laboratory findings in cell lines, hormonal or antihormonal treatment of cervical cancer has been suggested. In the study where HLA suppression was observed when progesterone was added, the authors concluded that the study provided evidence for treatment with the antiprogesterone RU486 in early stage cervical cancer (84). Estrogens are known to induce cervical cancer in mice. In a mouse model, the estradiol receptor antagonist ICI was given to mice with cervical neoplasms. ICI was claimed to effectively clear both precursors to, and invasive cancer, and the authors concluded that it could be of potential value in the treatment of humans (95). In the CaSki cell line, estradiol increased expression of HPV oncogenes. Administration of 13C, a compound with antiestrogenic activity, abrogated the expression of HPV oncogenes and the authors concluded that 13C could prevent cancer in cervical cells (96).

In at least two studies, *in vivo* treatment with sex steroids in women with CIN was evaluated. After treatment with dehydroepiandrosterone of women with CIN1 for six months, 83% of CIN had regressed. There was no control group and it is possible that regression could also have occurred without treatment. Only 12 women were included in this study and no larger randomized study has been published (97). For unclear reasons, vaginal progesterone treatment for six months of CIN1 was also used in a larger study (40 cases and 96 nonrandomized controls). Interestingly, progesterone treatment was associated with higher CIN persistence (98).

Conclusion

An association with cervical cancer and reproductive events has been observed since the 19th century. In epidemiological studies in the early 1990s, long-term OC use, irrespective of other risk factors, was reported to correlate with cervical cancer. In animal studies and in cervical cell line studies both estrogens and gestagens have transformed normal cells into cancer cells. Recently, the influence of sex steroid serum levels and of hormonal contraceptives on expression of tumor markers has been studied. Available evidence indicates that high serum progesterone levels correlate to expression of an unfavorable tumor marker pattern, while the role of estrogen is unclear, but high serum levels might correlate to a favorable prognosis in cervical cancer.

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