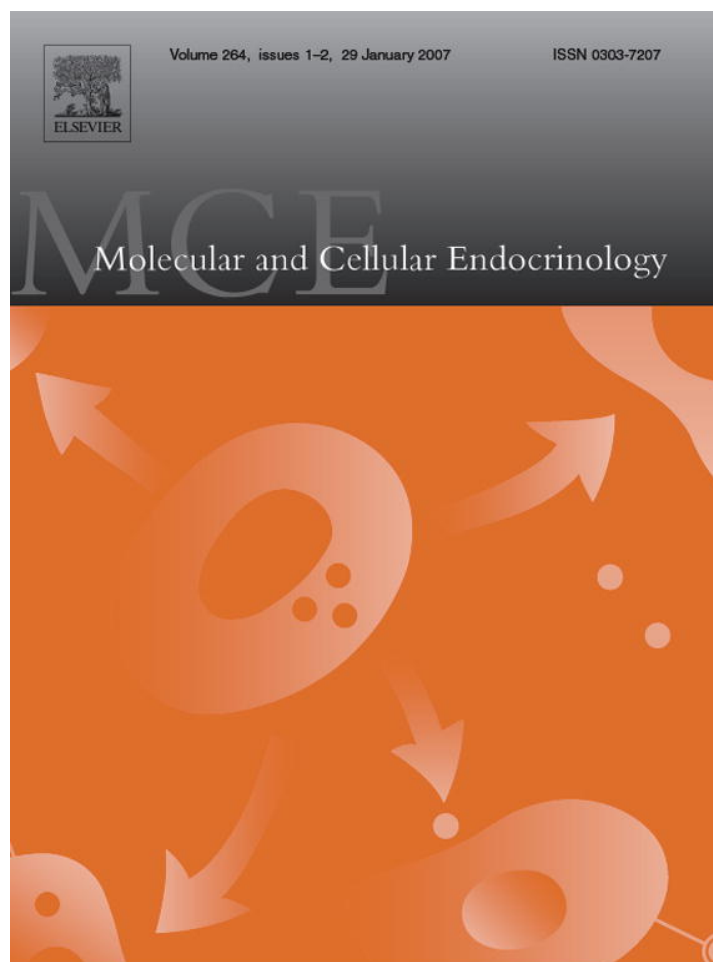


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Review

# Role of hormone cofactors in the human papillomavirus-induced carcinogenesis of the uterine cervix

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## Abstract

If human papillomavirus (HPV) is necessary for the development of (pre)neoplastic lesions of the uterine cervix, it is not sufficient. Among the cofactors involved in the malignant transformation of cells infected by HPV, sex hormones may facilitate the cervical carcinogenesis by different mechanisms, including the induction of squamous metaplasia in the transformation zone of the cervix, interactions between steroid hormones and HPV gene expression and alterations of the local immune microenvironment.

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**Keywords:** Sexual hormones; Human papillomavirus; Metaplasia; Carcinogenesis

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## 1. Introduction

Etiopathogenic and epidemiological studies have demonstrated that viruses are etiologically linked to approximately 20% of all human malignancies worldwide (Blattner, 1999). Although viruses alone are not sufficient to induce a complete neoplastic transformation, they have been shown to be necessary to promote and maintain the transformed state. With hepatitis and Epstein Barr viruses, human papillomavirus (HPV) is one of the best characterized virus associated with human cancer diseases. HPV is a major pathogen involved in the malignant transformation process of the skin and of ano-genital regions, especially in the uterine cervix. Cervical cancer is the most

common cancer in developing countries and the second most common cancer in women worldwide (Mohar and Frias-Mendivil, 2000). Specific types of human papillomaviruses are strongly implicated as causative agents in the etiology of cervical cancer and its precursors which are designated as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SILs). More than 20 HPV genotypes have been isolated from the genital mucosa but only a limited number can be considered as high-risk viruses. HPV 16 is the most frequently detected HPV type in cervical cancer (Bosch et al., 1995). Whereas mortality due to cervical cancer is highest in developing countries, morbidity is also considerable in the industrialised world, where direct and indirect costs from disease are very high.

Despite the evidence that human papillomavirus is strongly implicated as the causative agent of cervical cancer, HPV infection alone is not sufficient for tumor development. In addition to immune, microbial or chemical cofactors (de Vet and van

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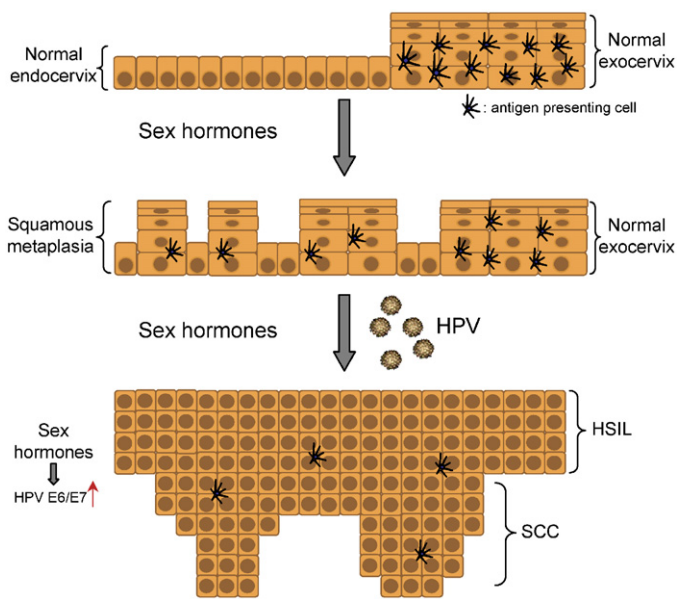


Fig. 1. Schematic representation of the potential mechanisms by which sex hormones may facilitate the cervical carcinogenesis: induction of squamous metaplasia in the endocervical epithelium; alterations of antigen presenting cells and increased HPV gene expression. HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma.

Leeuwen, 1986; Tindle, 2002), sex hormones may also play a role in the development of (pre)neoplastic lesions of the uterine cervix (Fig. 1). Indeed, the variation of hormonal status depending of age, pregnancy or contraceptive use has been shown to influence the development of cervical (pre)cancers (Brisson et al., 1994; Moreno et al., 2002; Munoz et al., 2002; Salazar et al., 2001).

## 2. Role of sex hormones in the induction of squamous metaplasia

A substantial majority of cancers and precursors lesions (squamous intraepithelial lesions; SILs) develop within a specific region of the cervix, the transformation zone (TZ) (Burghardt and Ostor, 1983), where the glandular epithelium of the endocervix is transformed progressively into a squamous epithelium during a process called metaplasia. The TZ is hypothesized to contain multipotent stem cells (or reserve cells) that can give rise to both endocervical and ectocervical cell types. Reserve cells are considered to be important in the evolution of the TZ during reproductive years. Several lines of evidence suggest that estrogen exposure is implicated in the hyperplasia and squamous differentiation of reserve cells. First, squamous metaplasia appears to be induced in the cervix by a decreased pH and epithelial acidification occurs during adolescence as a result of increased estrogen production and vaginal bacterial flora alteration. This acidosis may be a signal that contributes to a modified fate decision of reserve cells (Elson et al., 2000). Secondly, it is well known that estrogen treatment induces benign proliferation of squamous epithelial cells in the cervix and vagina presumably by signalling through the estrogen receptor (Lubahn et al., 1993). In mouse models, it has been demonstrated that chronic estrogen

treatment of mice induces the development of extensive metaplasia with squamous/columnar cell junctions evident throughout the entire cervical canal (Brake and Lambert, 2005). Moreover, administration of estrogen to 1-month-old mice results in the conversion of the reserve cells to squamous epithelium (Witkiewicz et al., 2005).

Interestingly, the topography and natural history of the human TZ are also affected by age, hormonal status and parity (Buckley, 1994). For example, the mechanism by which the original squamocolumnar junction changes location after the onset of puberty may be due to swelling of the cervical stroma in response to hormonal stimulation and pregnancy is associated with more endocervical tissue moving out onto the ectocervix (Hendrickson and Kempson, 1997). The influence of estrogen exposure in the process of squamous metaplasia has also been shown in human TZ tissues implanted in SCID mice (Tewari et al., 2000).

Estrogen exposure is involved not only in the metaplastic process but also in the particular sensitivity of the TZ to the development of (pre)neoplastic lesions. Using K14-HPV16 transgenic mice in which the expression of HPV16 E6 and E7 oncogenes is driven by the keratin 14 promoter in the basal keratinocytes of the squamous epithelium, some investigators have shown that the TZ is five-fold more sensitive to the induction of squamous cell carcinogenesis by estrogen compared to other sites of the reproductive tract (Elson et al., 2000). We also found, by using immunohistological techniques and total hysterectomy samples from young women undergoing surgery for non-cervical benign uterine disease, that TZ biopsies with immature squamous metaplasia exhibit a significantly higher density of hormone receptor-positive cells compared to ectocervical epithelium, suggesting that the TZ may be at increased risk of developing (pre)neoplastic lesions because of a high sensitivity to sex hormone regulation (Remoue et al., 2003). Although endometrial expression of estrogen and progesterone receptors has been shown to vary during the menstrual cycle (Fujishita et al., 1997; Ravn et al., 1994), no significant difference was demonstrated in hormone receptor-positive cell density between the follicular and luteal phases in both TZ and ectocervical biopsies (Remoue et al., 2003). These data are in agreement with previous studies showing no significant variation, within the menstrual cycle, in hormone receptor concentrations in vaginal tissues (Schwartz, 2000) and in HPV-associated lesions (Monsonogo et al., 1991), suggesting a lower sensitivity to menstrual cycle changes of genital squamous mucosa compared to glandular endometrial tissues.

The expression of sex hormone receptors in human cervical biopsies has also been shown to be higher in the precancerous TZ compared to normal ectocervix and to increase with the grade of SIL, strengthening the hormone-dependent establishment of cervical (pre)neoplastic lesions (Monsonogo et al., 1991).

Estrogen exposure may also contribute to the persistence and malignant progression of cervical cancers. In the HPV transgenic mice model, the continued growth and persistence of tumors that have arisen in the reproductive tract of mice expressing the viral oncogenes remained estrogen-dependent after their initial development, raising the possibility that estrogen dependence

in human cervical cancer makes anti-estrogen therapy valuable (Brake and Lambert, 2005). Accordingly, the anti-estrogen tamoxifen has been reported to induce progesterone receptors and to inhibit cell growth in many human cervical carcinoma cell lines (Vargas Roig et al., 1993). In a more recent study, aromatase expression was found in 35% of cervical tumor samples and was proposed to contribute to tumor growth by increasing cell proliferation and expression of growth or angiogenic factors such as VEGF (Nair et al., 2005), suggesting that *in situ* estrogen production may influence the progression of gynecologic malignancies in the absence of high levels of circulating estrogen (Nair et al., 2005).

### 3. Role of sex hormones in HPV gene expression

The role of sex hormones during the cervical carcinogenesis could be related not only to their ability to induce squamous metaplasia but also to stimulate HPV gene expression through hormone response elements in the viral genome (Yuan et al., 1999). Interestingly, expression of estrogen receptor and HPV oncogenes has been demonstrated in the same basal cell population (Arbeit et al., 1996), suggesting that estrogen can enhance transcription of HPV E6 and E7 oncogenes (Khare et al., 1997).

HPV E6 and E7 proteins of high-risk HPV are necessary for cell transformation whereas the HPV E2 protein is required for viral replication and gene expression (Blachon and Demeret, 2003). Interestingly, it was found that E2 and E7 proteins can induce apoptosis in transformed cells (Cho et al., 2002; Demeret et al., 2003; Desaintes et al., 1997; Webster et al., 2000) and that progesterone and estrogen increase the levels of E2- and E7-induced apoptosis (Webster et al., 2001). Although the mechanism is still not completely understood, the authors suggested that the pro-apoptotic signals from E2 and E7 may be counter-balanced by anti-apoptotic signals from HPV E6 protein. By affecting this balance, steroid hormones can therefore decrease cell growth. However, during the cervical carcinogenesis, HPV DNA is often integrated into the host genome, leading to the loss of E2 gene. Since HPV E2 is a modulator of HPV gene expression and, in the same time, an inducer of apoptosis, this loss might increase cell proliferation (Sanchez-Perez et al., 1997; Webster et al., 2000). In the presence of E2, progesterone and estrogen might therefore protect cells from malignant transformation via upregulation of the cell death whereas, in the absence of E2, these hormones might be a risk factor for the cervical carcinogenesis via their effects on HPV gene expression or other still not identified pathways.

Other synergies may also exist between steroid hormones and HPV in the pathogeny of cervical disease. Estrogen has a well-known mitogenic activity (Liehr, 2000) which may be amplified by viral oncoproteins. It has been shown to stimulate the proliferation of human keratinocytes by promoting the expression of cyclin D2 which induces G1 to S phase progression in the cell cycle (Kanda and Watanabe, 2004). Estrogen also inhibits oxidative stress-induced apoptosis in keratinocytes by promoting expression of the anti-apoptotic protein bcl-2 (Kanda and Watanabe, 2003). Estrogen can also induce direct DNA damage via its catechol metabolites (Newfield et al., 1998; Zhu and

Conney, 1998) and HPV infection has been shown to markedly increase the formation of these potentially carcinogenic estrogen metabolites (Auborn et al., 1991).

### 4. Role of sex hormones on the cervical immune microenvironment

In addition to interactions between sex hormones and HPV gene expression, hormones could also sensitise the TZ to cervical cancer formation by altering the local immune microenvironment. The squamous epithelium of the cervix is composed not only of keratinocytes (the primary target of HPV) but also of a type of immature dendritic cell (DC), the Langerhans cells (LC), which are important for the immunosurveillance of the squamous epithelium (Romani et al., 2003). Interestingly, the density of LC and their function are significantly reduced in the TZ compared to the ectocervix (Al-Saleh et al., 1995; Giannini et al., 2002), suggesting that keratinocyte/DC interactions could play a role in the establishment of SIL in this region. The production of cytokines/chemokines is necessary to maintain a balanced turnover of LC (Griffiths et al., 2005) and is most likely influenced by the complex differentiation state of keratinocytes, which is altered in metaplastic areas of the TZ and during cervical carcinogenesis (Hubert et al., 1999; Smedts et al., 1992).

There is now accumulating evidence that sex hormones have the property to influence the immune system, particularly by acting on cytokine production (Gilmore et al., 1997; McMurray et al., 2001; Rogers and Eastell, 2001; Schuur and Verheul, 1990). For example, progesterone has been shown to increase the production of  $\text{I}\kappa\text{B}\alpha$ , an inhibitor of  $\text{NF}\kappa\text{B}$  (Miller and Hunt, 1998) and to have an inhibitory effect on GM-CSF secretion (Robertson et al., 1996). Estradiol has been also reported to inhibit the expression of GM-CSF in the U2OS cell line via interaction with estrogen receptor alpha ( $\text{E}\text{r}\alpha$ ) and to decrease this production via contact with  $\text{E}\text{R}\beta$  (Brady et al., 2002).

Sex hormones may also directly influence DC/LC by modulating their morphology, density, distribution, maturity and function (Kaushic et al., 1998; Komi et al., 2001; Koyama et al., 1989; Wira et al., 2000; Young and Hosking, 1986). More specifically, sex hormones have been shown to influence the distribution of DC in the epithelium of rat reproductive tract (Kaushic et al., 1998).  $17\beta$ -estradiol inhibits antigen presentation and decreases the number of LC in ovariectomized rodents. In contrast, progesterone given together with  $17\beta$ -estradiol reverses the inhibitory effect of estradiol on antigen presentation and increases the number of LC in ovariectomized rodents (Wira and Rossoll, 1995). Progesterone is also able to increase the number of LC in human vaginal epithelium (Hubert et al., 2001; Wieser et al., 2001). In a context of autoimmune encephalomyelitis, it has been demonstrated that the pretreatment of DC with estradiol suppresses their ability to present antigens to specific T cells (Zhang et al., 2004). Analysis of cytokine production demonstrated that estradiol decreases  $\text{TNF}\alpha$ ,  $\text{IFN}\gamma$  and IL-12 production by immature DC (Liu et al., 2002). In addition, estrogen-exposed DC prevents the expansion of  $\text{CD4}^+$  T cells and increases the proportion of

regulatory T cells producing IL-10 and of CD4+CD28+ suppressor T cells (Pettersson et al., 2004). Moreover, specific T cells cocultured with estradiol-pretreated mature DC in the presence of antigens exhibit a shift towards a production of type II cytokines such as IL4 and IL10 (Liu et al., 2002). Interestingly, a co-expression of IL10 and IL4 has been demonstrated in established (pre)neoplastic lesions of the cervix (Al Saleh et al., 1998; Giannini et al., 1998) and suggests the presence of a type II cytokine pattern instead of a cell-mediated anti-tumor immunity. This inappropriate response against intracellular infectious agents, such as viruses or tumor cells may lead to the persistence of HPV by preventing infected cells from being eliminated by cytotoxic T lymphocytes.

In conclusion, epidemiological, experimental, virological and immunological data suggest that sex hormones play a role, in addition to HPV infection, in the development of cervical HPV infections and associated (pre)neoplastic lesions. These observations may be relevant for studies aiming to develop new anti-neoplastic therapeutic or preventive strategies.

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