Int. J. Cancer: **48**, 533–539 (1991)
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Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

ESTROGEN AND PROGESTERONE RECEPTORS IN CERVICAL HUMAN PAPILLOMAVIRUS RELATED LESIONS

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According to recent studies showing that human papillomavirus (HPV) infections can be influenced by sex steroid hormones, we performed estrogen (ER) and progesterone (PgR) receptor assays in fresh frozen biopsies of genital-HPV-related lesions. Seventy-three women with normal cervix, condyloma, low- and high-grade CIN and squamous carcinoma were evaluated in comparison with 15 persons with vulvar and 9 with penile papillomavirus-associated lesions. HPV genotypes were determined by dot-blot hybridization. Non-cervical lesions did not express HR. Condyloma on squamous metaplasia of the cervix and high-grade CIN expressed high levels of HR, particularly PgR (mean 4,086 and 4,518 fmoles/g tissue, respectively). Cervical squamous carcinoma expressed very low concentrations of PgR in a limited number of cases. High levels of PgR were correlated with high-grade CIN ($p < 0.05$), HPV16-18-associated lesions ($p < 0.01$) and ER were correlated to HPV6-11-related lesions ($p < 0.01$). The levels were independent of age, cycle stage and oral contraception. Morphological localization of PgR, using an immunocytochemical method using a monoclonal antibody (MAb) (PR-ICA), showed intense homogeneous staining in the nuclei of the stromal fibroblasts underlying dysplastic epithelium and condyloma on squamous metaplasia. These results suggest that, under *in vivo* conditions, sex steroid hormones, particularly progesterone, may act indirectly on HPV-infected epithelial cells and be implicated as co-factors in HPV-related cervical neoplasia. They could explain the relative predisposition to malignant transformation of the cervix as compared with vulvar and penile mucosa.

In recent years, a distinct increase in the incidence of viral anogenital condylomatous lesions in men and women has been observed (Oriel, 1971). Indeed, HPV16 DNA has been identified in more than 70% of cervical cancers and cervical intraepithelial neoplasms. Several biological, epidemiological and clinical works suggest that the association of these viruses with condylomatous lesions or with CIN is insufficient by itself to induce malignant transformation of infected cervical lesions (see references in Monsonego, 1990). Therefore it is suggested that infection by HPV, particularly HPV16, is ubiquitous (zur Hausen, 1989). It appears that co-factors are needed for the full expression of oncogenicity (Koss, 1982; Monsonego, 1990). Among these, steroids appear to play a role in carcinogenesis *in vitro*. The recent identification of steroid-responsive elements within the viral promoter region of HPV16 (Gloss *et al.*, 1987) and the effect of steroids on growth-regulating viral genes (Mitrani-Rosenbaum *et al.*, 1989; Pater *et al.*, 1990) suggest that steroids act directly on the virus itself. On the other hand, cervical squamous-cell carcinomas have not been traditionally considered as hormone-dependent cancers. However, an increased risk of cervical neoplasia has been suggested in long-term users of oral contraceptives (Brinton *et al.*, 1986) and in pregnant women (Schneider *et al.*, 1987); therefore, sex steroid hormones could be important co-factors in HPV-associated neoplasia.

The purpose of the present study is to investigate the sex steroid receptors and HPV DNA in normal cervix and in cervical condylomas, cervical intraepithelial neoplasms and squamous carcinomas in comparison with non-cervical lesions.

MATERIAL AND METHODS

1. Clinical specimens

Ninety-seven sexually active patients were investigated in our department between December, 1989 and February, 1990. Eighty-eight female patients were aged from 17 to 51 years. Forty-two of them did not use oral contraceptives and had regular menstrual cycles, 43 were taking combined oral contraceptive preparations and 3 were pregnant. Colposcopy of the genital tract was carried out by one of us (J.M.). Large colposcopic biopsies were collected from 73 cervical and 15 vulvar HPV-associated lesions. The samples of squamous cervical carcinoma were provided by the radiotherapy department (Y.C.). Nine male partners were examined by peniscopy. The 9 samples were taken from condylomata acuminata and from subclinical viral lesions (such as macular and papular acetowhite lesions). Each representative biopsy was divided into 3 parts.

2. Histological samples

One sample was examined histologically and classified according to commonly accepted criteria (Ferenczy, 1977). At the cervical level histological findings were as follows: negative if normal, inflammatory or squamous metaplasia; condylomata acuminata; low-grade CIN (flat condyloma, CIN 1); high-grade CIN (CIN 2–3) and squamous carcinoma. The stage of differentiation was noted. All the analyzed samples were approximately equivalent as regards volume and proportion of stromal tissue. Condylomata acuminata and cervical cancer without a significant amount of stroma were discarded. At the vulvar level, 3 histological groups were considered: negative (normal or inflammatory), condylomata acuminata or flat condyloma and Bowenoid papulosis (VIN3). Finally, in men, we distinguished condylomata (acuminata and plana) and penile intraepithelial neoplasia (PIN2-3).

3. HPV DNA hybridization

The second fragment was analyzed for HPV DNA using dot-blot hybridization with sulfonated probes (Melki *et al.*, 1988). Biopsy samples in TEND buffer were gradually frozen at −80°C and, prior to use, brought back to 37°C. Trypsin was added at a final concentration of 0.25%, and tubes were left at 4°C for 12 hr and homogenized.

Next, 90 µl of the cell suspension were transferred into a microtitration plate, then 10 µl of Proteinase K (Sigma, La Verpillière, France) were added to each well and the plate was incubated at 37°C for 1 hr. Replicate 10-µl samples were spotted onto a nitrocellulose filter, soaked in 0.5 M NaOH, followed by NaCl/Tris 0.6 M and 1.5 M, dried and denatured in ethanol and chloroform. Viral DNA probes were labelled by inserting an antigenic sulfone group into cytidine moieties of denatured DNA. ^{32}P-labelled probes were obtained by nick translation using ^{32}PdCTP (Amersham, Les Ulis, France) and a nick-translation kit (Gibco-BRL, Cergy, France). Prehybridization was done for 6–8 hr at 65°C with 10 µl/ml denatured salmon sperm DNA. Hybridization was performed in the same solution without salmon sperm DNA, replaced by the dena-

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Received: January 21, 1991 and in revised form March 8, 1991.