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-two 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. Condylomata on squamous metaplasia of the cervix and high-grade CIN expressed high levels of HR, particularly PgR (mean 4.966 and 4.518 fmol/mg tissue, respectively). Cervical squamous carcinomas expressed very low concentrations of PgR in a clinical lesions of cases. High levels of PgR were correlated with high-grade CIN (p < 0.05), HPV-16 associated lesions (p < 0.01) and ER were correlated to HPV-16 associated lesions (p < 0.01). The levels were dependent on age, cycle status and oral contraception. Morphological localization of PgR using an immunocytochemical method using a monoclonal antibody (PMA-ICA) showed intense immunostaining in the nuclei of the stromal fibroblasts underlying dysplastic epithelium and condyloma on squamous metaplasia. These results suggest that, under in vitro conditions, sex steroid hormones, particularly progestrone, may act indirectly on HPV-infected cells. Indeed, we hypothesized that they can act 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 neoplasia.

In recent years, a distinct increase in the incidence of viral anogenital condylomatous lesions in men and women has been observed (Oresk 1971). Indeed, HPV 16 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 is strong, and that it can be independently of HPV infected cervical cells considered 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 neoplasia.

2. Histological samples

One sample was examined histologically and classified according to commonly accepted criteria (Fenoglio, 1977). At the cervical level histological findings were as follows: negative (normal), inflammatory or squamous metaplasia; condylomata acuminata; low-grade CIN (flat condylomata, CIN 1); high-grade CIN (CIN 2–3) and squamous carcinoma. The stage of differentiation was noted. All the analyzed samples were approximately equivalent in 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 condylomata and Bowenoid papulosis (VIN 3). Finally, in men we distinguished condylomata acuminata (acuminata and plana) and penile intraepithelial neoplasia (PIN 2–3).

3. HPV DNA hybridization

The second fragment was analyzed for HPV DNA using dot-blot hybridization with sulfonated probes (Meli et al., 1988). Biopsy samples in TEN buffer were gradually frozen at −20°C and, prior to use, brought back to 37°C. Tris-HCl 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 microcentrifuge 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 sulfate group into cytidine triphosphate of denatured DNA. 32P-labelled probes were obtained by nick translation using 32P-CTP (Amersham, Les Ulis, France) and a nick-translation kit (Gibco-BRL, Cergy, France). Prehybridization was done for 6–8 hr at 55°C with 10 µl denatured salmon sperm DNA. Hybridization was performed in the same solution without salmon sperm DNA, replaced by the dena-

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