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Typing of human papillomavirus in genital specimens by DNA hybridization.

More than 50 different types of human papillomaviruses (HPVs) have been identified so far, most of which give rise to benign epithelial lesions. A number of HPV types have also been linked to the development of malignant tumors, including some genital HPVs, which have been associated with cervical carcinoma (COGGIN & ZUR HAUSEN, 1979). Epidemiological studies have demonstrated that certain HPV types, such as HPV6 and HPV11, are found almost exclusively in benign genital papillomas whereas other types, including HPV16, HPV18 and HPV33, have more frequently been detected in cervical cancers (ZUR HAUSEN & SCHNEIDER, 1987). It would seem that these latter types somehow have a greater oncogenic potential than HPV6 and HPV11. However, it is apparent that only a low percentage of women with HPV16 infection develop a cervical carcinoma after a long latent period, and so it is clear that secondary events are important for the progression of the premalignant lesion to an invasive carcinoma (ZUR HAUSEN, 1986).

Because the pathology of the disease and its progress depend upon the type of papilomavirus present in the cervix, virus typing in genital specimes, suspected to be infected, turns out to be very important. The most straightforward technology to type these closely related viruses is DNA hybridization performed in highly stringent conditions. Hybridization between total genomic DNA, extracted from the clinical samples and spotted on nylon filters, and radiolabelled HPV probes have been performed at 65°C in 35% formamide and 1 M NaCl. Stringent washes have been done to get rid of cross-hybridizations.

Nearby 170 clinical samples have been analysed by this dot-blot technique and among them, it appears that 39.5% are HPV's positive. All these samples have been hybridized in conditions where the probes allowed to discriminate between infections associated with HPV's 6b, 11, 16 and 18. The distribution between the two types associated with bengn lesions is the following : 36% are HPV6b-positive and 20.5% are HPV11-positive. In the case of the other two types frequently associated with severe lesions, 34% of the samples contained HPV16 whereas only 9.5% were HPV18-positive. It should be pointed out that multiple infections are rather important : two different HPV types such as 6b and 11, or 6b and 16, or 11 and 16 were detected together in 10.2% of the samples analysed. Two samples (1.2%) contained three different types of HPV (HPV6b, -11 and -16). A detailed table summarizing the results of the typing experiments together with the histological analysis will be presented.

An event which seems to play a role during the progression of the initial, virally-induced lesion towards carcinoma is the integration of the viral genome (mainly the HPV16, -18 and -33) in the host cellular DNA (DURST *et al.*, 1985). The physical states of HPV16 DNA in several biopsic samples have been determined after genomic DNA restriction with *Eco*RI, *Bam*HI and *Pst*I and southern blotting. The analysis of the hybridization patterns demonstrates that the HPV16 DNA, in all samples tested, is not integrated into the host genome but is under an episomal form.

In this work, we have shown that a reliable HPV type assignment in clinical samples can be obtained provided that both the hybridization and the washing conditions are stringent. HPV6b turns out to be the type most frequently associated with bengin lesions, whereas HPV16 has been found associated with severe lesions such as cervical intraepithelial neoplasias and carcinomas.

References

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