Abstract Human papillomaviruses (HPV) are thought to be involved in the malignant evolution of cutaneous lesions from transplant recipients. As E6 proteins from potentially oncogenic HPV types degrade p53 tumour suppressor gene product in vitro, we analysed p53 protein status in benign, premalignant and malignant skin lesions from grafted patients, to determine whether HPV may interfere with p53 function. With immunohistochemistry, p53 protein accumulation was detected in 70% of skin lesions from grafted patients. p53 immunoreactivity was confined to basal keratinocytes in benign lesions (warts, condylomas), while suprabasal keratinocytes were also stained in premalignant and malignant skin lesions (precancerous keratoses, squamous cell carcinomas). Multiple HPV carriage was detected with in situ hybridization in benign and malignant skin lesions from transplant recipients: low risk HPV types 1, 2, 6, 11 and potentially oncogenic HPV types 5, 16, 18 were frequently found. There was no clear correlation between p53 detection and the presence of the HPV types under study. The frequent detection of p53 protein in cutaneous lesions from grafted patients is suggestive of p53 protein accumulation interfering with normal function. Our results may reflect the presence of mutated p53 proteins due to the mutagenic effect of ultra-violet (UV), or wild-type p53 protein accumulation in response to UV-induced DNA damage, or may be produced by the interaction with HPV-encoded E6 proteins.

Key words Transplant recipients · Human papillomaviruses · p53 protein · Ultra-violet

Introduction

The long term immunosuppression required to prevent allograft rejection is associated with a high rate of skin disorders and human papillomavirus (HPV) infections. Organ transplant recipients successively develop numerous benign, premalignant and malignant skin lesions on sun-exposed areas in which histological signs of viral infection and HPV DNA are detected [3, 7, 13]. This suggests that HPV may be involved in the evolution of cutaneous lesions from grafted patients towards malignancy.

Studies on anogenital cancers have pointed out the potential role of HPV in human carcinogenesis. However, clinical and experimental data suggest that HPV infection is not sufficient to induce cancer [47], and additional alterations of host cells may be required for the malignant progression of HPV-infected cells. Alterations of p53 tumour suppressor gene are the most common genetic alterations detected in human tumours [9], and it has been shown that E6 oncoproteins encoded by potentially oncogenic HPV types 16 and 18 bind and promote the degradation of p53 tumour suppressor gene product in vitro [30]. Analysis of cervical carcinoma cell lines showed that p53 gene and protein are mutated in HPV-negative cell lines, whereas HPV-positive cell lines contain wild type p53 sequences and low levels of normal p53 protein [11, 31, 44]. These data strongly suggest that, like p53 gene mutations, interaction of E6 proteins from potentially oncogenic HPV types with p53 protein may interfere with p53 function and lead to cell cycle alterations responsible for tumour progression.

In this study, we analysed p53 protein expression by immunohistochemistry, and the presence of HPV DNA by in situ hybridization in benign, premalignant and malignant cutaneous lesions from transplant recipients. Our aim was to analyse p53 protein status in grafted patient skin lesions, and to determine whether the presence of HPV interfered with p53 expression in these lesions.

Materials and methods

We analysed 40 skin biopsies from renal and cardiac transplant recipients, and 14 lesions from non-immunocompromised control patients. Seven samples of normal unexposed skin and four samples of foreskin from non-grafted patients were also used as controls. Each biopsy was divided into two parts: one part was fixed...
in Bouin's solution, and embedded in paraffin for histological examination after haematoxylin-eosin staining. The other part was snap frozen and stored in liquid nitrogen. Five micron serial cryostat sections were then prepared on aminopropyl-triethoxysilane (Aldrich Chemie, Steinheim, Germany) precoated slides, and fixed for 10 min in cold acetone before immunostaining or in situ hybridization.

For the immunohistochemical detection of p53 protein frozen sections were incubated for 15 min in 0.15% hydrogen peroxide to quench endogenous peroxidase activity, and washed twice in phosphate-buffered saline (PBS; bioMérieux, Marcy l’Etoile, France). The biotin-streptavidin-peroxidase staining was then performed using Dako LSAB kit and amino-ethylcarbazole (Dako, Carpinteria, Calif.). In preliminary assays, three monoclonal antibodies (mAb) purchased from Oncogene Science (Manhasset, NY) were tested: PAb 240 and PAb421 mAb were diluted 1:50 and PAb 1801 was diluted 1:100; they were incubated for 30 min onto sections to detect p53 protein. PAb 240 mAb does not bind to wild type p53 protein, but reacts with a conformational epitope that results from different activating mutations on mutant p53 proteins [14]. PAb 421 and PAb 1801 mAb recognize two different epitopes shared by wild type and mutant p53 proteins, respectively located at the carboxy and the amino end of the proteins [1]. In controls, PBS was applied onto sections instead of primary antibody.

Plasmid DNA probes for HPV types 1a, 2a, 5, 6a, 11a, 16 and 18 were prepared and purified through caesium chloride gradients and labelled using a nick-translation kit (BRL, Gaithersburg, Md.) and biotinylated 11-dUTP (Sigma, St Louis, Mo.). The conditions for HPV DNA detection by in situ hybridization were described previously [16]. Briefly, hybridization was performed under stringent conditions with 50% formamide in the hybridization mixture, at -19°C. The DNA-DNA hybrids were revealed with a three-step reaction and streptavidin-alkaline phosphatase complex. The specificity of HPV typing with in situ hybridization was assessed on typical cutaneous and mucosal lesions previously shown to contain the different HPV types under study with Southern blotting and the polymerase chain reaction (PCR), as describing by Soler et al. [34]. CaSki and HeLa carcinoma cell lines respectively containing 600 copies of HPV type 16 and 10–50 copies of HPV type 18 were also used as positive controls. In negative controls, the specific probe was omitted or replaced by an heterologous probe; cutaneous lesions unrelated to HPV infection were also used.

Results
Typical histological signs of HPV infection were seen in most premalignant and malignant skin lesions from transplant recipients, with vacuolized keratinocytes containing basophilic inclusions in the upper epidermal layers, either in the centre or on the border of the lesions. Squamous cell carcinomas (SCC) often retained architectural features of pre-existing viral warts.

In preliminary experiments, we tested the three mAb to p53 protein on serial frozen sections of two