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Comparative transforming potential of different human papillomaviruses associated with non-melanoma skin cancer

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Abstract

It is well established that high-risk human papillomaviruses (HPVs) that infect mucosal epithelia are the causative agents of cervical cancer. In contrast, the association of cutaneo-tropic HPV types with the development of non-melanoma skin cancer (NMSC) is less well defined. In this study, we have analysed the *in vitro* transforming potential of various cutaneous HPV types. Using oncogene cooperation assays with activated ras, we have shown that diverse cutaneous types, including 12, 14, 15, 24, 36 and 49, have significant transforming potential. Interestingly, most of this activity appears to be encoded by the *E6* gene product. In contrast, the common HPV-10 exhibits no significant transforming potential in these assays. This difference may be a reflection of different patterns of cellular localization, with transforming E6s being nuclear and non-transforming being cytoplasmic. These results provide molecular support for a role of these viruses in the development of certain human malignancies.

Keywords: HPV; Cell transformation; E6; E7

Introduction

A subset of mucoso-tropic HPV types, in particular HPV-16 and HPV-18, are the causative agents of a number of human tumours, the most important of which is cervical cancer (Zur Hausen, 2002). In contrast, the role of certain cutaneous HPV types in the development of human malignancies is more controversial. It has been proposed that the cutaneous types of the genus beta of the HPV phylogenetic tree, such as HPV-5 and HPV-8, are associated with the development of skin tumours in genetically immunosuppressed individuals (Majewski et al., 1997) and transgenic mouse models support a causative role for HPV-8 in the development of these tumours (Schaper et al., 2005). In the case of non-melanoma skin cancer (NMSC), a large number of different HPV types from the B1 subgroup have been detected in these lesions; although the high frequency of detection in normal skin makes conclusions on the role of these viruses in the development of the malignancies contentious (Forslund et al., 2003). This is compounded by the fact that when viral DNA is detected in these tumours it is frequently present at less than 1 genome copy per cell (Weissenborn et al., 2005), implying that the role of HPV in these tumours may be more akin to a 'hit and run' mechanism of transformation (Zur Hausen, 2001).

Although previous studies have shown significant transforming potential for the HPV-8 E6 oncoprotein (Iftner et al., 1988; Kiyono et al., 1992), there is no information on the potential transforming activity of the majority of NMSC-associated beta HPV types. Determining whether these viruses have *in vitro* transforming activity would at least support or refute their potential roles in the development of human malignancies *in vivo*. In this study, we have chosen to analyse the transforming potential of beta HPV types in an oncogene cooperation assay in primary baby rat kidney cells (BRKs).

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Results and discussion

To investigate the transforming potential of the NMSC-associated beta HPV types, we isolated and cloned the *E6* and *E7* oncogenes. These were derived from HPV-12, HPV-14, HPV-15, HPV-24, HPV-36 and HPV-49. For comparison, we also included the common skin cutaneous type HPV-10, which has previously only been found in benign lesions (Kremsdorf et al., 1983). Primary baby rat kidney (BRK) cultures were transfected with *E6* and/or *E7*, together with an activated EJ-ras

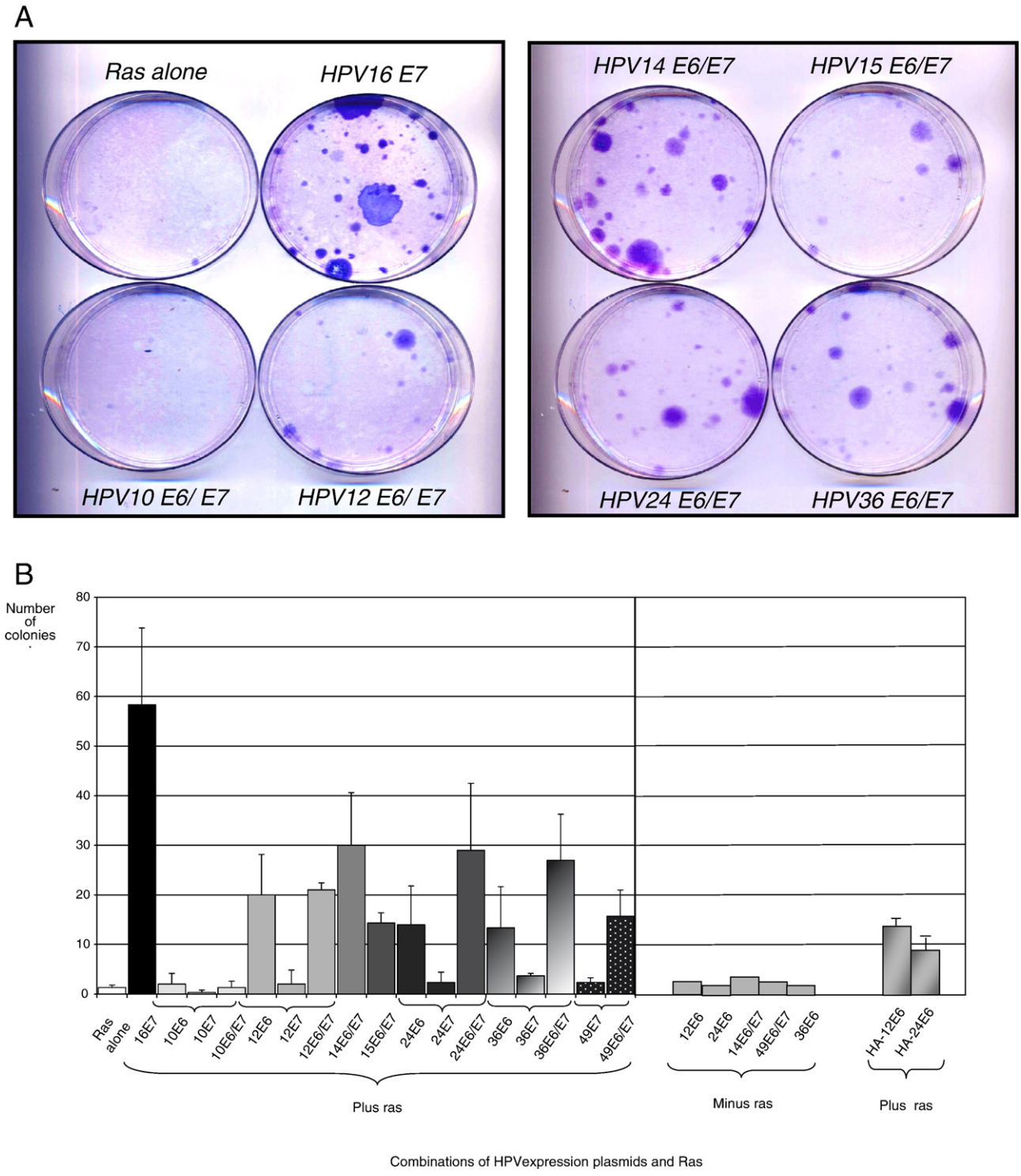

Fig. 1. Transformation of primary BRK cells. The cells were transfected with EJ-ras together with the indicated expression plasmids and placed under selection for 3–4 weeks. After this time, the cells were fixed and stained and the colonies counted. (A) Results from a typical assay. (B) Collated results from multiple assays. Standard deviations are shown.