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UV-B irradiation stimulates the promoter activity of the high-risk, cutaneous human papillomavirus 5 and 8 in primary keratinocytes

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Summary

Human papillomaviruses (HPV) have been implicated in the development of non-melanoma skin cancer (NMSC). HPV types 5 and 8 are strongly associated with NMSC in patients with the inherited disease Epidermodysplasia verruciformis (Ev). In these patients tumours arise predominantly on sun-exposed skin and consistently harbour HPV DNAs. To determine whether UV-B irradiation modulates the noncoding region (NCR) promoter activity of the Ev-HPV types 5, 8, 9, 14, 23, 24, and 25 we performed transient transfection assays with NCR luciferase reporter gene constructs in primary human epithelial keratinocytes (PHEKs) and in p53-null RTS3b cells. Each of the HPVs showed different basal NCR activity in both cell types and reacted differently upon UVB treatment and p53 cotransfection in RTS3b cells. The NCR of HPV5 and 8 were the only ones to be activated by UV-B in PHEKs. The stimulation of the NCR activity of the high-risk cutaneous HPV types 5 and 8 by UV-B irradiation may point to a role of this interaction in the development of NMSC.

Human papillomaviruses (HPVs) are small double-stranded DNA viruses associated with a spectrum of human diseases ranging from benign epithelial proliferations to epithelial cancers [22]. A causal relationship is best characterized in human anogenital malignancies. The first evidence that HPV infection is also associated with non-melanoma skin cancer was found in patients with the rare genetically determined disease Epidermodysplasia verruciformis (Ev) [12]. This syndrome is characterised by the presence of numerous flat warts and macular lesions, which have a high risk of developing into squamous cell carcinomas (SCC) later in life. Warts and SCC of both immunocompromised and immunocompetent patients contain a diverse spectrum of HPV types commonly referred to as Ev-HPV-types including HPV5, 8, 9, 14, 23, 24, and 25 [2, 7, 16]. In contrast to the majority of HPV types in benign lesions, mostly HPV5 or HPV8 are found in SCC of Ev patients and are regarded as high-risk types. The preferential localisation of SCCs and viral skin warts on chronically sun-exposed body sites strongly suggests an important role for sunlight in the development of skin lesions. The UV component of sunlight is the most important physical carcinogen in the environment for the development of skin cancer [3]. UV light affects the skin in different ways depending on its wavelength [20]. UV-B (290–320 nm) is considered the causative agent of many of the effects attributed to UV, giving rise to mutations in DNA and modifying the pattern of cellular gene expression [19]. With

regard to a possible interplay between HPV and UV radiation exposure, it is interesting to note that an UV-inducible p53-responsive element has been found in the noncoding region (NCR) of HPV77 (a HPV type isolated from an SCC of a transplant recipient), mediating activation of viral transcription [17]. Promoter stimulation by UV irradiation was also described for the Ev-related HPV types 5, 20, 23, and 38 [4] in HaCaT skin keratinocytes, containing mutated p53 gene [11]. In this initial study we analysed the effect of UV-B radiation on HPV promoters located within the NCR of the Ev-associated HPV types 5, 8, 9, 14, 23, 24, and 25. Two cell systems were used in this study: primary human epithelial keratinocytes (PHEK), which have wild-type p53 and represent the natural host cells for cutaneous HPVs, and cells of the skin-cancer-derived keratinocyte cell line RTS3b [18], which has no detectable p53 protein [9]. The construct pNCR8-Luc harbouring the NCR of HPV8 (nucleotides [nt] 7077–558), was cloned by inserting the *HindIII*-*BglIII* fragment from pNCR8-CAT [14] into the *HindIII*-*BamHI* sites of pALuc. The cloned fragment comprises the entire HPV8 NCR and parts of the flanking L1 and E6 genes. The NCR of HPV8 is the best characterised one among Ev viruses [8, 13, 15, 21]. Corresponding to the NCR fragment, cloned into pNCR8-luc, the regions of HPV5 (nt 7145–568), HPV9 (nt 6959–550), HPV14 (nt 6837–615), HPV23 (nt 6859–557), HPV24 (nt 6948–508), and HPV25 (nt 7121–559) were generated by PCR amplification and cloned into pALuc upstream of the firefly luciferase reporter gene. Before use, the different pALuc clones were sequenced for confirmation. Although these NCRs have not been fully characterised in terms of promoter activities, functional homology can be assumed in view of the high sequence homology among all EV-HPVs [6, 10]. We cannot rule out however that other DNA elements located outside the regions cloned here may affect promoter activity. These reporter gene constructs were cotransfected with pCMV- β -Gal into PHEKs using TransFast (Promega) and into RTS3b cells using FuGene (Roche) for transient reporter gene assays in accordance with the manufacturer's recommendations. We chose 15 mJ/cm² UV-B (using a UVP CL-1000 ultraviolet cross-linker with F8T5 bulbs giving a spectral peak at 312 nm) as we previously demonstrated that this dose is sufficient to cause p53 activation in PHEKs without inducing a significant level of apoptosis [9]. The number of viable cells 24 h post irradiation was around 90%. Cells were UV-B irradiated 8 h after transfection and harvested 24 h later. The cells were lysed in Cell Lysis Buffer (Promega) and the lysates cleared by centrifugation and analysed for β -galactosidase protein expression and luciferase activities as described before [5]. All results are the average values of three experiments which had been performed in duplicate. The relative promoter activities were determined by calculating the luciferase-to- β -galactosidase activity ratios. The basal NCR activities of pNCR23-Luc and pNCR25-Luc were about the same in nonirradiated PHEKs and RTS3b cells. The relative basal luciferase activity of pNCR25-Luc was set to 1 as reference for the UV experiments in both cell types. In PHEKs the basal level of NCR activities varied among the tested constructs. Compared to pNCR25-Luc, an enhanced transcriptional activity was noted only for HPV5. The luciferase expression after UV-B irradiation increased 1.7-fold in case of HPV5 ($P = 0.05$) and 2-fold for HPV8 ($P = 0.05$) and slightly for HPV25 (1.4-fold) (Fig. 1). UV-B had no effect on the NCRs of HPV9, 14, and 23 in PHEKs. Both the empty luciferase vector pALuc and the NCR of HPV24 showed no activity in PHEKs. In comparison with PHEKs, the NCRs of HPV5, 8, 9, and 14 showed 10-fold (HPV5), 7-fold (HPV8), 8-fold (HPV9), and 13-fold (HPV14) weaker basal activity in RTS3b cells (data not shown), while the NCR of HPV24 was 10 times more active in RTS3b. UV-B irradiation stimulated the NCR activity in these cells by 1.7-fold for HPV8 ($P=0.0143$) and 2-fold for HPV14 ($P=0.043$), whereas only a small effect (1.3-fold) could be observed for HPV5 (Fig. 2). In contrast, UV-B clearly repressed the NCR of HPV25 in RTS3b cells ($P=0.014$). Interestingly, most tested HPV-types showed different relative luciferase activities and reacted differently to UV-B irradiation in PHEKs and RTS3b cells.

It has been demonstrated before that stimulation of the HPV77 promoter activity by UV-B irradiation is mediated through a p53 consensus binding site within the HPV77 NCR [9]. This was demonstrated by cotransfection assays with a p53 expression vector into RTS3b cells. Recently we also described a p53 recognition site within the 3' part of the HPV8 NCR [1]. To investigate how p53 overexpression can modulate the HPV-NCR activities we also cotransfected the luciferase reporter constructs with the p53wt expression vector pCp53wt [14] (Fig. 3). The relative basal luciferase activity of pNCR8-Luc was set to 1 as reference. Overexpressed p53wt activated the NCR of HPV5 (1.3-fold) and HPV8 (2.4-fold) and repressed the NCRs of HPV14, 23, 24, and 25. These results show that the tested EV-HPV types react differently upon p53 cotransfection and that this effect does not correlate with the UV-B response of a particular viral promoter in PHEKs. Only for HPV5 and HPV8 the UV-B response in PHEK may in part be attributed to p53, although there is no p53-responsive element easily recognizable in the NCR of HPV5 at the position described for HPV8 [1]. The differences in these results are suggestive of additional factors rather than p53 alone being involved in the basal NCR activities and UV-B responses of the tested NCRs. It is interesting to note that in particular HPV5 and HPV8 are activated by UV-B irradiation in PHEKs. Whether any stimulation of transcription by UV contributes towards a higher oncogenic potential of a particular viral type remains to be determined.

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Fig. 1.

Relative luciferase activities of pNCR8-Luc, pNCR5-Luc, pNCR9-Luc, pNCR14-Luc, pNCR23-Luc, pNCR24-Luc, and pNCR25-Luc without and after UV treatment. Transient transfections were performed in PHEK and a UV-B dose of 15 mJ/cm² was applied. The pCMV-β-Gal plasmid was cotransfected as an internal control for transfection efficiency. Promoter activities are presented as luciferase-to-β-galactosidase activity ratios. The relative luciferase activity obtained with the pNCR25-Luc is taken as 1. The slash indicates that pNCR24 showed only background levels of luciferase activity

Fig. 2.

Relative luciferase activities of pNCR8-Luc, pNCR5-Luc, pNCR9-Luc, pNCR14-Luc, pNCR23-Luc, pNCR24-Luc, and pNCR25-Luc without and after UV treatment. Transient transfections were performed in RTS3b cells and a UV-B dose of 15 mJ/cm² was applied. The pCMV-β-Gal plasmid was cotransfected as an internal control for transfection efficiency. Promoter activities are presented as luciferase-to-β-galactosidase activity ratios. The relative luciferase activity obtained with the pNCR25-Luc is taken as 1

Fig. 3.

Relative luciferase activities in extracts from RTS3b cells transfected with pNCR8-Luc, pNCR5-Luc, pNCR9-Luc, pNCR14-Luc, pNCR23-Luc, pNCR24-Luc, pNCR25-Luc, and the p53wt expression vector pCp53wt. The total amount of DNA was adjusted with empty pCMV-1 vector. The pCMV-β-Gal plasmid was cotransfected as an internal control for transfection efficiency. Promoter activities are presented as luciferase-to-β-galactosidase activity ratios. The relative luciferase activity obtained with the pNCR8-Luc is taken as 1