

Effect of human papillomavirus 16 E6 and E7 oncoproteins on the expression of involucrin in human keratinocytes

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The productive phase of the human papillomavirus (HPV) life cycle is closely linked to keratinocyte differentiation. Oncogenic HPV infection has been shown to hamper the normal differentiation of keratinocytes; however, the underlying mechanisms responsible for this phenomenon are yet to be clarified. Involucrin is a precursor of the keratinocyte cornified envelope and can be used as a marker for keratinocyte differentiation. The aim of this study was to investigate the effects of HPV16 E6 and E7 oncogenes on the expression of involucrin in human keratinocytes.

Primary human foreskin keratinocytes were maintained in serum free, low calcium medium and transduced by LXS_N (control) retrovirus or virus vectors expressing HPV16 E6, E7 or E6/E7 genes. These cells were induced to differentiate by culture in high calcium and serum containing medium for 24h. The expression level of involucrin in differentiating and non-differentiating infected cells was estimated using real-time RT-PCR. The involucrin protein was detected by western blotting using monoclonal anti-human involucrin antibody. Primary human keratinocytes were co-transfected by luciferase reporter plasmids carrying different fragments of the involucrin promoter along with vectors expressing HPV 16 E6 or E7 genes. After transfection, cells were either left untraeted or induced to differentiate by culture in high calcium and serum containing medium for 24h. Luciferase assay was used to measure the effect of the HPV oncogenes on involucrin promoter activity.

The differentiation of keratinocytes by serum and high calcium significantly increased both the mRNA and the protein levels of IVL. The E6 and E7 oncoproteins of HPV16 together caused down-regulation of involucrin mRNA and protein both in proliferating and in differentiating keratinocytes. In order to verify the effect of HPV oncogenes on the involucrin promoter, we made transient transfection assays and found that the HPV E6 repressed involucrin promoter activity in proliferating keratinocytes. Both HPV oncoproteins caused a down-regulation of involucrin promoter activity in differentiating cells. The effect of HPV oncogenes was localized to the proximal region of the involucrin promoter. These results suggest that the HPV16 oncoproteins decrease endogenous involucrin expression in keratinocyte cells through down-regulating involucrin promoter activity.

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