Biological properties of the oncoproteins E6 and E7 from mucosal and cutaneous HPV types

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Summary

Certain Human papilloma virus (HPV) types infect the mucosa of the genital tract and are the main risk factors for development of cervical cancer. Extensive studies over the last 30 years have led to elucidation of their precise role and mechanisms in the development of cervical cancer. The product of two early genes, E6 and E7, play a key role in the malignant transformation. These proteins are able to interact and inactivate the cellular tumour suppressor proteins 53 and Rb, respectively, and thereby deregulate the cell cycle and cellular response to uncontrolled proliferation. Low activity or inability of the E6 and E7 protein to associate or inactivate p53 and pRb has shown to be linked to an inability of the HPV types to induce malignant lesions. In the last decade several studies suggested that cutaneous HPV types of the genus beta of the HPV phylogenetic tree are associated with non-melanoma skin cancer (NMSC). However, their direct role in this disease remains to be proven. The characterization of the biological properties of the E6 and E7 proteins of the cutaneous HPV types is a very valid approach to clarify the role of these viruses in carcinogenesis.

This thesis describes the characterization of the biological properties of E6 and E7 proteins from mucosal and cutaneous HPV types using novel or previously described assays and models. A particular emphasis was given to the cutaneous HPV type 38, which displays *in vitro* transforming properties and appears to be frequently present in NMSC.

The ability of E7 to associate and degrade the tumour suppressor protein Rb is the first step leading to deregulation of the cell cycle. We have developed an assay, which allows us to analyse the affinity of the E7 protein for pRb. Using the plate-binding assay described in Chapter 2 we could successfully and reproducibly quantify and compare the affinity of different E7 proteins for pRb. Both HPV16 and HPV38 E7 were shown to have high affinity for pRb, which correlates with the ability of these HPV types to extend the lifespan in primary keratinocytes.

We show in Chapter 3 that HPV32 E7, despite its high affinity for pRb, is not able to induce pRb degradation and can not overcome cell cycle arrest induced by ectopic levels of the cyclin-dependent kinase inhibitor $p16^{INK4a}$. However, HPV32 E7 is able to fully transform immortalized rodent cells indicating that there are other properties of E7 important in cellular transformation. In addition, we show that HPV32 E6 is not able to associate and inactivate p53 and these two genes together are not able to immortalize primary keratinocytes. In line with this *in vitro* data, HPV32 infections appear to be exclusively associated with benign lesions.

The E6 and E7 from the cutaneous EV HPV type 38 have been shown to be able to immortalize primary human keratinocytes. In Chapter 4 we evaluate the carcinogenic activities of the viral proteins in an *in vivo* model, we generated transgenic mice expressing HPV38 E6 and E7 in their skin. The expression of the transgenes induced hyperproliferation, hyperplasia, dysplasia and loss of UV-induced cell cycle checkpoints in keratinocytes in the skin of these transgenic mice. In addition, experiments using chemical carcinogens demonstrate that HPV38 E6E7 transgenic mice are more susceptible to the development of skin tumours. These data confirm that HPV38 is able to increase the risk of skin cancer development significantly and is a potential high-risk cutaneous HPV type.

In agreement with this conclusion, we have observed that HPV38 E6 and E7 alter the transcriptional functions of the tumour suppressor p53 *in vitro* in human keratinocytes and *in vivo* in skin keratinocytes of transgenic mice expressing the viral genes. In both experimental models HPV38 E6 and E7 induce an accumulation of specific p53 form, which induces selective transcription of ΔNp73, an isoform of the p53-related protein p73. ΔNp73 inhibits the capacity of p53 to induce the transcription of genes involved in growth suppression and apoptosis. This is a novel mechanism of the alteration of p53 function that is mediated by a cutaneous HPV type and further support the role of HPV38 and ΔNp73 in human carcinogenesis. (Chapter 5)

In summary the synergistic activities of E6 and E7 are required for immortalisation and transformation of primary cells. We demonstrated that HPV38 E6 and E7 are sufficient to immortalize primary keratinocytes and increase the risk of skin cancer development in mice. Our studies strongly support the involvement of cutaneous HPV types in skin cancer development and pave the way for both epidemiological and biological studies on cutaneous HPV types.