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Mastomys natalensis papillomavirus as a model for the analysis of E6 and E7 protein functions during skin tumorigenesis

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The present work describes the initial characterization of two proteins (E6 and E7) of MnPV, a virus involved in skin tumour formation. This work demonstrated that MnPV E7 bound with high affinity to Rb, similar to the E7 protein of high-risk HPV16. This biochemical property of MnPV E7 may be an important factor for skin tumour formation in M. natalensis. Furthermore, the first steps towards the generation of transgenic mouse model using MnPV genes to analyse PV protein functions in skin tumorigenesis were performed.

For the analysis of MnPV E6 and E7 proteins in MnPV skin tumours and transgenic mice, polyclonal antisera against GST.MnPV E6 and GST.MnPV E7 fusion proteins were generated. Affinity-purified anti-MnPV E6 and anti-MnPV E7 antisera specifically recognized the E6 and E7 proteins in transiently transfected COS7 cells. Furthermore, the localization of MnPV E6 and E7 proteins in transfected mammalian cells was determined by immunofluorescence using these affinity-purified antisera. MnPV E6 protein was detected both in the nucleus and cytoplasm. The intracellular localization of MnPV E7 protein was predominantly nuclear and MnPV E7 was excluded from the nucleolus. When MnPV E7 protein was highly produced in transiently transfected cells, MnPV E7 protein was also observed in the cytoplasm.

The affinity purified MnPV E6 or MnPV E7-specific antisera were also used to characterise the translation efficiencies of various MnPV E6 and MnPV E7 expression vectors in mammalian cells. MnPV E6 was produced at higher levels from the bicistronic MnPV E6/E7 region construct than the construct containing monocistronic MnPV E6. In contrast, MnPV E7 could be translated from the bicistronic MnPV E6/E7 region but with a lower efficiency compared to monocistronic MnPV E7 expression constructs. This is very likely due to inefficient translation of the E7 ORF in the bicistronic E6/E7 message. For the generation of MnPV E6 and MnPV E7 expression constructs for the use in transgenic mice, these results revealed that in order to obtain high-level expression of MnPV E7, the MnPV E7 ORF needs to be the first cistron in a MnPV E7 transgenic construct. Furthermore, no splicing of the MnPV E6 transcript was observed in transfected mammalian cells and MnPV-infected skin and skin tumours of *M. natalensis*. Therefore no alterations in the E6 sequence, i.e. mutations in splice donor or acceptors sequences within E6, are necessary in order to assure efficient E6 production in transgenic mice.

In order to analyse the functions of the MnPV E6 protein *in vivo*, transgenic mouse lines that express the MnPV E6 gene and a lacZ reporter under the control of the human keratin 14 promoter were generated. The expression of the lacZ reporter gene was monitored during embryonic

development of transgenic lines. The activity of the β-gal reporter was observed mostly in the epidermis of the face, ears, extremities, tail, and hair follicles. These sites of expression in represent the expected activity of the K14 promoter between 13.5 dpc and 16 dpc.

For a functional analysis of the MnPV E7 protein *in vivo,* the problem of embryonic lethality of transgenic founders constitutively expresssing MnPV E7 needs to be overcome. Therefore, a K14.MnPV E7 construct (pK14βloxZloxMnPV-E7-pA) was generated that can be activated by a tamoxifen-inducible Cre recombinase in transgenic mice. Together the work described here constitutes the basis for the generation and analysis of a transgenic mouse model in which the functions of the E6 and E7 proteins of a cutaneous PV-type (MnPV) and a possible involvement of these viral proteins during tumour formation in the skin can be studied.