

Abstract

The E6 protein of the cervical carcinoma associated human papillomavirus (HPV) 16 (16E6) has a C-terminal PDZ-binding-motif, which is conserved among genital high-risk HPV-types. Via this motif E6 can bind to PDZ-domain proteins and induce their accelerated degradation. This contributes to the oncogenic potential of 16E6. The protein-tyrosine-phosphatase PTPH1 was identified as a new protein bound by the PDZ-binding-motif of 16E6. PTPH1 was suggested to act as a tumor-suppressor-protein. In this work the interaction between 16E6 and PTPH1 was confirmed by GST-pull-down-assays and co-immunoprecipitations. *In vitro* and *in vivo* degradation-assays revealed, that 16E6 induced the proteosomal degradation of PTPH1. This required both the interaction of the PDZ-binding-motif with the PDZ-domain of PTPH1 and the binding of 16E6 to the cellular ubiquitin ligase E6-AP. The targeting of PTPH1 seems to be conserved among high-risk genital E6 proteins, since also HPV18 E6 was able to interact with PTPH1 and to mediate its accelerated degradation. HPV-positive cervical carcinoma cell lines revealed lower levels of endogenous PTPH1 than HPV negative cells. This suggests that the expression of E6 in these cells enhances the degradation of intracellular PTPH1. Correspondingly the introduction of the HPV16 E2 protein, known to repress the expression of E6 resulted in higher amounts of PTPH1 in HPV16 positive SiHa-cells. The interaction with PTPH1 may be essential for the 16E6-mediated morphologic transformation of established rodent cell lines. Here it has been shown, that the reduction of endogenous PTPH1 by specific shRNA induced the morphological transformation of the C127i-mouse fibroblast cell line. Three potential substrates of PTPH1 have been identified by substrate-trapping-assays. Two of them are involved in the development of the cytoskeleton.

E6 proteins of cutaneous high-risk HPV-types also have oncogenic potential. For instance the E6 protein of the cutaneous HPV8 (8E6) has the ability to transform C127i-cells as well. Although 8E6 does not encode a PDZ-binding-motif, an interaction with PTPH1 was observed. The consequence of this interaction on the function of PTPH1 remains unclear. 8E6 neither affected the stability of PTPH1, nor the phosphatase activity. 8E6 binds to PTPH1 via its N-terminal domain. The mutation of the valin at position 68, which is conserved among the cutaneous E6 proteins, to prolin, the conserved amino acid at the corresponding position in the E6 proteins of genital high-risk HPV-types, eliminated the interaction with PTPH1. Moreover the prolin in position 68 enabled 8E6 to induce cell growth in growth factor reduced medium. This activity could not be observed with wildtype 8E6, in contrast to 16E6. Correspondingly, the replacement of the prolin at position 59 in 16E6 by valin did not allow the growth of cells expressing this 16E6 mutant in reduced media. These data implicate, that the amino acid at position 68 of cutaneous E6 proteins mediates the interaction with PTPH1, while genital high-risk E6 proteins bind to PTPH1 via their PDZ-binding-motif. A prolin at the corresponding position in genital E6 proteins confers their ability to reduce growth factor requirements of target cells. This feature is not shared by cutaneous E6 proteins.