

does not appear to be involved in mediating apoptosis required for normal growth and development as the PKR knockout mice have a normal life span and there are no gross abnormalities in peripheral lymphoid compartments. However, while Fas mRNA expression is strongly upregulated in wildtype cells by dsRNA and LPS, this induction, is much reduced in PKR knockout MEFs. The induction of Fas on wildtype MEFs by

dsRNA results in cells becoming sensitive to killing by an antagonist anti-Fas antibody, Jo2, which induces apoptosis specifically by stimulating the Fas receptor. PKR knockout MEFs treated with dsRNA remain insensitive to killing by the Jo2 antibody. Thus, PKR may play a role in other processes where up-regulation of Fas is required for activating apoptotic pathways to control cellular stress responses.

MOLECULAR BASES FOR HUMAN PAPILLOMAVIRUS RESISTANCE TO INTERFERON ACTION

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Introduction

Human Papillomavirus (HPV) is mostly associated with the development of cervical carcinoma (1). The two major transforming proteins, E6 and E7, are generally conserved after viral integration into the cell genome and their expression is necessary for maintaining the malignant cell phenotype. Both viral oncoproteins respectively complex with products from p53 and Rb genes and such events appear to be determinant for cellular transformation produced by these DNA tumor viruses. IFNs are a family of biological response modifiers that exhibit antiviral, antiproliferative and immunomodulating functions. Binding of IFNs to their receptors triggers the assembly of a cytoplasmic protein complex and its translocation to the nucleus, where the activated complex can promote transcription by binding to ISREs (2).

In this work, we examined the effect of E7/E6 oncoproteins on the ISRE activation by IFN and investigated the functional domain of HPV-16 E7 engaged with the interference elicited by such oncoprotein. We found that E7 phosphorylation site by Casein Kinase II (CK II) was essential for inhibiting the ISRE activation by IFN suggesting that this molecular event is involved on the resistance of oncogenic HPVs to IFN action.

Materials and Methods

Cells

Caski (human cervix epidermoid carcinoma, HPV-16 positive), HeLa (human cervix adenocarcinoma, HPV-18 positive), RHEK-1 (human epidermic keratinocytes), Hep-2 (human laryngeal carcinoma).

Plasmids

RSV E6/E7 (E6 or E7 ORFs driven by RSV enhancer/promoter) p24Gly (Rb-binding E7 mutant),

p31/32Arg/Pro (E7 phosphorylation mutant), p566Pro (E7 transformation mutant), 3X ISRE CAT (IRF-1 binding site from fused to CAT gene)

Transient transfection experiments

Cells were transfected by DEAE-dextran method with 3XISRE-CAT vector and constructs expressing either HPV-16 E7 mutants or wild type.

Results and Discussion

We demonstrated that HPV-16 E7 and E6 oncoproteins largely abrogated the activation of a GBP ISRE reporter by IFN suggesting that both viral oncoproteins seem to impair the IRFs/Stats function (Figure 1). These findings are in line with other ones observed in patients where high levels of E7 oncoprotein were correlated with the lack of IFN response in terms of ISGs activation and antiproliferative effect.

We further investigated the HPV-16 E7 functional domain engaged with the impairment of the ISRE activation by IFN. We found that substitution of Ser 31/32 by non-phosphorylatable residues on E7, fails to abrogate the ISRE reporter activation by IFN (Figure 2). In other approach using heparin as CK II inhibitor we observed an increase of the IFN-induced GBP mRNA levels. In CaSki cells, heparin pre-treatment increased the sensitivity of these cells to the antiproliferative effect of IFN.

Taking together these results, we suggest that HPV resistance to IFN action is in part mediated by E7 phosphorylation event. Likewise, such results could lead to the speculation that CK II inhibition would be therapeutically useful in treating HPV-patients resistant to IFN.

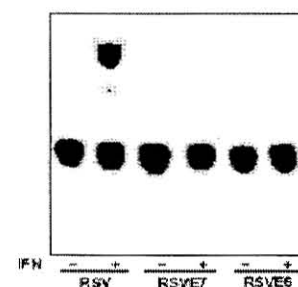


Figure 1. Effect of the E7 and E5 expression of HPV 16 on the activation of the ISRE sequence.

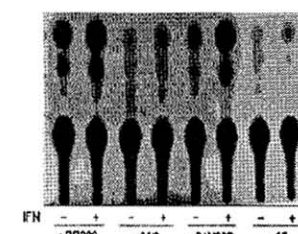


Figure 2. Analysis of the E7 functional domain involved in the inhibitory effect of the activation of the ISRE sequence.

1. Iwasawa et al. Cancer 1996;77:2275-2279.

2. Lerner et al. Biotherapy 1996;8:175-181.