NEW PARADIGMS IN INTERFERON AND CYTOKINE SIGNALING

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Introduction

Interferons (IFNs) are a family of secreted proteins that function as important biological response modifiers. The IFN-genes are transcriptionally induced in response to viral and other microbial infections and IFNs induce rapid activation of gene transcription following binding to specific cell surface receptors. The IFN-stimulated genes (ISGs) modulate the different biological effects of IFNs. The characterization of the molecular mechanisms of IFN-induced gene activation led to the discovery of the Jak-STAT signal transduction pathway. Subsequent studies have shown that many cytokines, growth factors and hormones signal their target cells through this pathway. The Jak-STAT pathway operates through a cascade of specific protein-protein interactions followed by DNA-protein interactions. Protein phosphorylation plays a pivotal role in this process. IFN receptors lacking intrinsic kinase activity are associated with Janus family kinases (Jak) which are activated by receptor engagement. This triggers the activation of the cytoplasmic latent proteins, which are the signal transducers and activators of transcription (STAT), by tyrosine phosphorylation. Activated STATs translocate to the nucleus as oligomeric complexes and interact with the cognate enhancer elements upstream of the ISGs leading to their transcriptional activation and subsequent biological effects.

We have recently identifed roles for other molecules in the Jak-STAT pathway. In particular, type I interferon (alpha/beta) treatment causes the phosphorylation and activation of cytosolic phospholipase A2 (cPLA2) which requires JAK1. Moreover, JAK and cPLA2 can be co-precipitated suggesting a close physical interaction. Inhibitors of cPLA2 inhibit IFN alpha/beta induced expression of interferon-stimulated response element (ISRE)-driven genes, but not gamma activated site (GAS)-driven genes indicating that cPLA2 is required for the formation of ISGF3 which binds the ISRE but not STAT1 homodimers which bind GAS elements. We have also implicated the double-stranded RNA (dsRNA) dependent protein kinase (PKR) in signaling by dsRNA, IFNs, TNF-alpha and lipopolysaccharide. PKR is a serine threonine kinase which is induced at a transcriptional level by IFNs and mediates their antiviral activity. PKR is normally inactive but on binding dsRNA it undergoes a conformational change allowing autophosphorylation and dsRNA-independent phosphorylation of substrates. The antiviral activity of PKR is due to autophosphorylation in response to dsRNA followed by phosphorylation of the alpha subunit of initiation factor eIF2 resulting in rapid inhibition of translation. In human and mouse cells, dsRNA induction of PKR leads to NFkB activation via the PKR-mediated phosphorylation of IkB. The IFNs, dsRNA, TNF and LPS all fail to activate IRF-1 DNA binding activity in PKR knockout mouse embryo fibroblasts (MEFs) resulting in a selective defect in the induction of genes dependent on IRF-1 (or NFkB). Several genes important in mounting different aspects of host resistance to infection can be classified as wholly or partially dependent on PKR including genes involved in antigen presentation (class I MHC), chemotaxis (the chemokines IP-10, Myg, JE, Rantes) antimicrobial activity (iNOS) and apoptosis (FAS). The induction of the cell adhesion molecules VCAM and E-selectin by dsRNA is also mediated via a PKR-dependent pathway. The mechanisms of direct activation of PKR by cytokines remain to be determined. Whether this occurs through Jak-dependent mechanisms or other signals generated by receptor engagement is not known. Direct interactions of PKR with components of the Jak-STAT pathway have been identified. There is deficient STAT3 binding to the GAS element in the c-fos promoter in extracts from PKR knockout MEFs compared to wild type cells in response to PDGF, although the response of STAT3 to other stimuli remains unaffected. PDGF induces a rapid and transient increase in the interaction between PKR and STAT3. While the mitogenic effect of PDGF on PKR wild type and knockout cells is identical, c-fos gene induction by PDGF is deficient. Therefore the mitogenic pathway activated by PDGF is PKR independent and may be distinguished from the PKR dependent pathway required for immediate early gene expression which involves a PKR-STAT3 interaction.

Physiological levels of PKR are required for mediating an apoptotic response to different stimuli including dsRNA. PKR knockout MEFs are resistant to apoptotic cell death in response to dsRNA, TNF-alpha, or LPS. The mechanism for the suppression of apoptosis in PKR knockout cells was linked to a defect in the activation of the DNA- binding activity of the transcription factor, IFN Regulatory Factor-1 (IRF-1) in response to dsRNA, LPS or TNF-alpha. These results reveal an unexpected role for normal cellular basal levels of PKR in mediating several forms of stress-induced apoptosis through regulation of IRF-1 activity. PKR

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, a re 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-phophorylitable 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.

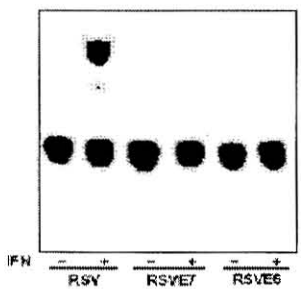

Figure 1. Effect of the E7 and E5 expression of HPV 16 on the activation of the ISRE sequence.

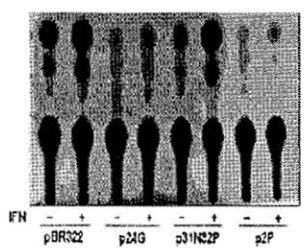

Figure 2. Analysis of the E7 functional domain envolved in the inhibitory effect of the activition of the ISRE sequence.

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

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