The importance of alpha/beta (α/β) interferon receptors and signaling pathways for the treatment of cervical intraepithelial neoplasias

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Summary

Introduction: Immunotherapies have been effective in treating various forms of cancer, including cervical intraepithelial neoplasias (CINs) predominantly caused by human papilloma virus (HPV). *Development:* To establish persistent infections in stratified epithelia, HPV induces proliferative lesions. Viral gene products are able to change gene expression and cellular proteins. Interferons (IFNs) are inducible glycoproteins that have immunomodulatory, antiviral, antiproliferative, and antiangiogenic effects. In particular, interferon-alpha (IFN-α) has been shown to inhibit the development and progression of cervical cancer. In this review, actions of interferons α/beta (α/β), including their receptors and signaling pathways, are described, as well as their clinical importance in the immune response against cervical lesions. *Conclusion:* The interaction of IFN-α/β with its receptor results in a series of phosphorylation events. These mechanisms can be ineffective in IFN response, then it can also compromise the therapeutic effects of immunotherapy.

Key words: Type I Interferons; Interferon receptors; Cervical neoplasia.

Introduction

Human papilloma virus (HPV) has a great affinity for squamous epithelial cells. As a result, HPV has been associated with the formation of cervical lesions, referred to as cervical intraepithelial neoplasias (CINs). These lesions are characterized by the formation of an acetowhite epithelium in the cervix [1], and are classified as slight, moderate, or severe neoplasias - CIN I, II, and III, respectively. As these lesions are considered the precursors of cervical cancer, their early detection, and subsequent intervention, can potentially prevent tumor development [2].

HPV establishes persistent infections in stratified epithelial cells by inducing proliferative lesions and maintaining a low number of episomal copies in infected basal cells. Viral gene products have been shown to interfere with the expression of native genes and cell proteins, such as Rb and p53, thereby altering cell cycle progression. Persistent infections are induced by high risk HPV, which are also associated with high oncological risk due to their ability to escape an immune response. For example, HPV types 16, 18, and 31 are able to block interferon-stimulated gene (ISG) expression and compromise the antiviral function of cytokines involved in an immune response, including the function of interferons (IFNs) [3].

Interferon-alpha/beta (α/β) has been shown to play a key role in mediating both innate and adaptive immune responses. Furthermore, there are the possibility of IFNs in the clinical treatment of cancer, particularly for neoplasias that are a precursor to cervical cancer. However, in order for interferon-a/β to be used effectively as an immunotherapy, the components of its signaling pathways need to be expressed and functionally characterised in target cells.

Therefore, the goal of this review is to present current evidence regarding the actions of IFN-α/β and its associated signaling pathways, and their role in the immune response against cervical intraepithelial lesions. In addition, the clinical importance of treating these lesions is highlighted, to elucidate the systemic response to endogenous IFN-α, for further studies on the efficacy of treatment with IFN-α in these patients are necessary.

The importance of IFNs in CINs

In Table 1, the expression, functions, and receptors associated with the three main types of IFNs are presented. For type I IFNs, their direct antiviral and antitumor properties are complemented by their role in immunovigilance [4]. For example, type 1 IFNs (e.g., IFN-c/β) induce the expression of hundreds of ISGs which regulate antiviral effects, cell growth, and affect immunomodulation. Correspondingly, when epithelial cells are infected with HPV, production of IFN-α/β is induced, and this can lead to an inhibition of viral replication, the induction of cytotoxic

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Table 1. — *The three main types of IFNs and their characteristics.*

IFN type	Main subtypes of IFNs	Expressed by:	Primary Functions	Receptors
I	IFN-α IFN-β	Leukocytes Fibroblasts Cells infected by virus	- Mainly acts on innate immunity - Predominantly mediates antiviral activities	IFNAR1 IFNAR2
II	IFN-γ	Activated T cells	- Acts with IL-12 on acquired immunity to differentiate into Th1 cells - Exhibits antiviral and antitumor activity via activation of macrophages to eliminate pathogens via phagocytosis - Can induce expression of MHC I and IRF-1	IFNGR1 IFNGR2
III	IFN-λ1 IFN-λ2 IFN-λ3	Various cell lineages, except for non-infected cells	- Induction of antiviral protection - Augments expression of MHC I - Its action mechanisms remains to be fully characterized	IL28Rα IL10R2

Sources: Hsieh *et al.* [31]; Müller *et al.* [32]; Kotenko *et al.* [33].

and antiproliferative functions, and negative regulation of angiogenesis [4]. However, the viral proteins, E6 and E7, can block downstream targets of IFN-α/β, thereby providing a mechanism for evasion of the immune system. Moreover, when HPV episomes are lost, proliferation of cells that have the virus integrated into their genome is enhanced [5].

While characterizing the signaling pathways activated by IFN-α, including those involving gene expression, a family of transcription factors was discovered that links cell surface receptors with nuclear events. These proteins were referred to as, signal transducers and activators of transcription (STAT), and they were found to localise outside the nucleus; however, following stimulation with IFN, they are activated by tyrosine phosphorylation, undergo multimerization, and migrate to the nucleus where they recognize regulatory sequences in DNA [6]. Using mouse models, genetic experiments have been performed to define the crucial roles that each STAT has in mammals, thereby characterizing this family of latent cytoplasmic proteins that upon activation, affect gene expression in response to extracellular polypeptides [7].

The interaction of IFN-α/β with its receptor results in a series of phosphorylation events. First, binding of the tyrosine kinases, TYK2 and JAK1, occurs, followed by their binding of the IFN I receptors, IFNAR-1 and IFNAR-2. The receptors are then activated by phosphorylation of tyrosine residues present in the intracellular subunits of each receptor, and these phosphorylated sites become binding sites for phosphorylate STATs. STAT1 binds STAT2 to form a heterodimer that subsequently binds a regulatory factor of IFN (IRF) to form interferon-stimulated gene factor 3 (ISGF3). ISGF3 then localizes to the nucleus and promotes the transcription of specific genes, interferon stimulation response element (ISRE) which participate in IFN stimulation events. This signaling pathway of type I IFN activation is referred to as the JAK-STAT pathway [8,9] (Figure 1).

Type I interferon receptors

IFN-α/β receptors are made up of two subunits, IFNAR1 and IFNAR2, with the latter having three distinct forms: IFNAR-2a (short form), IFNAR-2b (soluble form), and IFNAR-2c (long form). Both IFN-α and IFN-β bind the same receptors, and these receptors are expressed by many different types of cells [10, 11] (Table 1).

For type I IFNs to function, an initial binding event between these cytokines and their specific receptor must occur. Following binding, a chain of intracellular signaling events is induced which results in the responses mediated by IFNs [12]. However, variations in the concentration and the number of receptors expressed by a cell, can affect the intensity of the responses generated by the stimulation to IFN-α/β [13]. In addition, it has been suggested that when IFNAR1 is highly expressed on the cell surface, it undergoes phosphorylation, ubiquitination, and degradation via mechanisms independent of its binding by TYK2 [14]. While this mechanism can be effective in avoiding an excessive IFN response, it can also compromise the therapeutic effects of immunotherapy.

Over the years, the present research group has studied the applicability of IFN-α to the treatment of CINs, with significant clinical results. For example, in approximately 60% of patients who received immunotherapy and have changed a Th1 cytokine profile (e.g., IFN-γ, TNF-a, IL-2) in the stroma, a response to IFN-α treatment was observed [15], with a reduction in HPV load detected. In contrast, patients that did not respond to IFN-α were found to have a Th2 cytokine profile, IL-4, or Treg (e.g., transforming growth factor beta 2 and 3 - TGF-β2, TGF-β3). The latter group also had a history of smoking. In another study which evaluated vaginal secretion in patients with CIN treated with IFN, a reduced viral load and lower levels of the inflammatory cytokines, IL-6 and TNF-α, were detected concomitant with neoplasia regression [16].

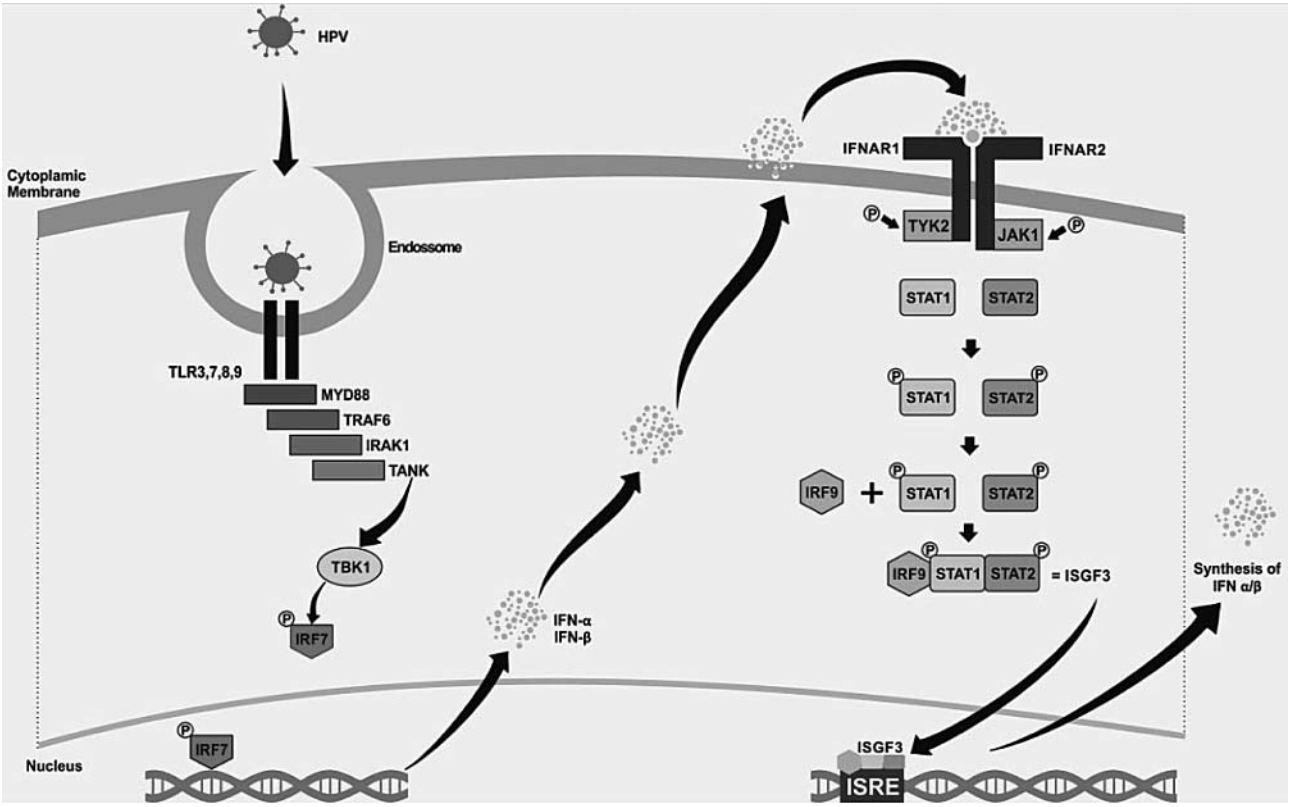

Figure 1. — Mechanisms for the synthesis and main signaling pathways of IFN-α/β. TLRs 3, 7, 8, and 9 are involved in the recognition of HPV, and are expressed in endosomal membranes. Their activity is dependent on MyD88, a protein that mobilizes TRAF6 and IRAK1, signaling proteins that phosphorylate IRF7. Upon phosphorylation, IRF7 translocates to the nucleus and activates genes responsible for IFN production. Following the secretion of IFN-α/β from the cell, this cytokine dimer binds with receptors, IFNAR1 and IFNAR2, in an autocrine or paracrine manner. The bound receptors then undergo phosphorylation by the Janus kinases, JAK and TYK, which is followed by the binding and activation of the signaling transducer proteins, STAT 1 and 2, also by phosphorylation. Subsequently, IRF9 binds to STATs and forms interferon-stimulated gene factor 3 (ISGF3), which dislocates to the nucleus to promote transcription of interferon-sensitive response element (ISRE). This process promotes additional synthesis of IFN-α/β, which then exercises its action. Source: Adapted from Alarcón-Riquelme [22] and Aaronson and Horvath [34].

When expression of receptors in patients with different grades of CIN were analysed versus a healthy control group, both lower local levels of IFN-α mRNA and reduced expression of IFN-α receptors were detected in the patients with CIN. Moreover, simultaneous expression of IFNAR1/IFNAR2 was not detected in the former group, yet was in the latter [17]. Taken together, these findings suggest that IFN-α immunotherapy can be ineffective if there is an insufficient number of receptors present on the cell surface, and may be represents a mechanism by which HPV and neoplastic cells can evade the immune response.

The presence of IFNR has been associated with an improved response to immunotherapies involving IFN-α. For example, patients with hepatocellular carcinoma who were treated with IFN-α were subsequently found to have a greater number of cells expressing the IFNAR-2 receptor, and this expression was proportional to the treatment response observed [18]. In addition, studies of pancreatic cell lines have demonstrated that expression of the IFNR receptors facilitates the apoptopic and antiproliferative effects of IFN-α/β, thereby disrupting the cell cycle of these the cell lines [19].

Activation of the JAK/STAT signaling pathways is an important regulatory mechanism through which host cells are able to inhibit viral infections provoked by a variety of viral RNA and DNAs. Moreover, the STAT proteins, 1, 2, and 3, are the central transcriptional activators of this pathway. HPV has been found to suppress the constitutive expression of STAT-1 at the transcriptional level, while levels of STAT-2, IRF-9, and STAT-3 remain unaffected [3, 20]. In addition, the HPV oncoproteins, E6 and E7, independently suppress the expression of STAT-1, which is necessary for amplification of the HPV genome and maintenance of its episomes. In combination, these results suggest that STAT1 has an important role in viral pathogenesis [3].

After an infection is established, production of IFN-α/β depends on activation of IRF-3 and IRF-7 via phosphorylation by TBK-1 and IKKε kinases, respectively. Upon phosphorylation, IRF-3 and IRF-7 dimerize, then undergo nuclear translocation to activate type I IFN promoter genes [4]. The transcriptional activity of IRF-7 is dependent on phosphorylation of its C-terminus, and expression of this multifunctional protein is restricted to B lymphocytes and dendritic cells. However, in other cell types, IRF-7 production can be induced by virus or IFN [21]. When toll like receptors (TLRs) in endosomes recognize an accumulation of viral DNA, they activate signaling pathways that result in the phosphorylation of transcription factors and the induction of IFN genes as well. Figure 1 schematically represented the modes of activation for type I IFNs.

Final considerations

Based on the antiviral and antitumor potential of IFNs, various studies have explored their effects on the immune system, especially the ability of IFNs to enhance the treatment of many different types of cancer. For example, IFN-α/β has been shown to effectively inhibit tumor cell proliferation, induce cell apoptosis, and increase expression of main histocompatibility complex (MHC) class I molecules [23]. Furthermore, treatment with IFNs has yielded good results in the treatment of patients with neoplasias that are precursors of cervical cancer [15,16], vaginal cancer [24] and pancreatic cancer [19]. In addition, IFN has been shown to interfere with the viral transcription of HPV16 and HPV18 [25], partly by reducing the expression of E6 and E7 proteins in cells infected by HPV [26]. However, a deficiency, or absence of some of the elements involved in the JAK-STAT signaling pathway can result in a loss of responsiveness by IFN-α/β. For example, in recent studies using mouse models, deletion or inactivity of TYK2 did not impede the activation of JAK1, yet, phosphorylation of STATs 1 and 2 were affected both *in vitro* and *in vivo*. With the final step of signaling compromised, effector functions of IFN-α/β were disrupted [27]. Furthermore, STAT needs to be functional in order to achieve adequate gene transcription and signaling [28].

Changes or deformities in IFNAR-1/IFNAR-2, particularly an absence of one of the receptor chains, can also damage the JAK-STAT signaling pathway, and consequently, inhibit its effects [29]. For example, certain types of genetic polymorphisms in one of the subunits can prevent an adequate response from IFN-α/β. This has been demonstrated in chronic hepatitis B carriers where the presence of these changes has been implicated in the elimination of hepatitis B infection in its early stages, and this can also affect the long-term course of the infection [30].

Conclusions

Type I IFNs (IFN-α / β) have important biological functions, from development and activation of cells of the immune system to destroy tumor cells to the inhibition of viral replication. After the viral infection or activation of toll-like receptors (TLR), promotes IFN gene and enhances the production of responsive genes important for the development of an effective antiviral immune response [8]. Therefore, future studies of immunotherapies involving IFN-α/β should monitor the functionality of the elements involved in IFN-α/β signaling pathways in order to better understand the mechanisms involved in the immune response of patients with HPV and CINs. In addition, this would help identify the main obstacles for maintaining a treatment's effectiveness.

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