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IFNA2: The prototypic human alpha interferon

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ABSTRACT

The human interferon α2 (IFNα2) was the first highly active IFN subtype to be cloned in the early eighties. It was also the first IFN and the first cytokine to be produced and commercialized by the pharmaceutical industry. *Ipso facto* it became the favorite IFNα subtype for academic researchers. For this fortunate reason IFNα2 has been at the origin of most discoveries related to the mechanism of action of type I interferons.

Contents

1. Introduction

Type I interferons (IFNs) were discovered in 1957 by Isaacs and Lindenman who reported that cells infected with an inactivated virus release a soluble factor exerting an antiviral action (Isaacs and Lindenmann, 1957). We now know that IFN is a key cytokine of the innate immune response which is produced upon recognition of many pathogens and damage-associated molecular patterns released by infected cells or dying cells (Tomasello et al., 2014).

Type I IFNs are capable to act on virtually all body cells since they recognize a receptor which is ubiquitously expressed. In addition to their function in establishing an antiviral state, they are also capable to decrease the proliferation rate of dividing cells and to exert immunomodulatory activities. Type I IFN action impacts not only innate immunity, but also on almost every aspect of cellular and humoral adaptive immune responses. In particular, the action of IFN on dendritic cells (DC) is crucial. It induces IL-15 trans-presentation for NK cell activation. More importantly, IFN can modulate all 3 types of signals delivered by DC to T cells: MHC-antigenic peptide complex, costimulation and cytokine production (Tomasello et al., 2014; Rizza et al., in press). Type I IFNs are thus critical cytokines for the generation of a protective immune response. When given exogenously as a drug to adult mice, IFN exert a potent antitumor effect (Gresser, 2007). In human, IFNα2 has the longest record of clinical use for the treatment of many types

Abbreviations: AGS, Aicardi-Goutières syndrome; ATF-2, activating transcription factor 2; cDNA, DNA complementary to RNA; CML, chronic myeloid leukemia; CpG, Cytidine-phosphate-Guanosine; DAA, directly acting antiviral agents; DC, dendritic cells; ERK, extracellular signal-regulated kinases; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, Interferon; IFNAR, IFN-α/β receptor; IKK, inducible IκB kinase; IRF, interferon regulatory factors; ISG, interferon-stimulated genes; ISGF3, interferon-stimulated gene factor 3; ISRE, interferon stimulated responsive elements; JAK, Janus kinase; Kd, dissociation constant; MAPK, mitogen-activated protein kinases; MHC, major histocompatibility complex; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NK, Natural Killer; NMR, nuclear magnetic resonance; pDC, plasmacytoïd dendritic cells; PEG, polyethylene glycol; PI3K, phosphatidylinositide 3-kinases; PKC, protein kinase C; PRD-LE, positive regulatory domain-like element; RCC, renal cell carcinoma; ssRNA, single strand RNA; STAT, signal transducers and activators of transcription; SVR, sustained virological response; TBK1, Tank-binding kinase 1; TKI, tyrosine kinase inhibitors; TLR, Toll-like receptor; USP18, Ubiquitin specific peptidase 18; VEGF, Vascular endothelial growth factor; VRE, virus-responsive element.

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of cancer, including some hematological malignancies and solid tumors (Antonelli et al., in press). In the last 3 years, it has been demonstrated that type I IFNs are essential in the processes of immunosurveillance, tumor rejection and regulation of metastasis spread (Diamond et al., 2011; Fuertes et al., 2011; Bidwell et al., 2012). Moreover, IFNs were shown to improve the efficacy of classical antitumor treatments such as radiotherapy, chemotherapy and monoclonal antibody-based therapy (Burnette et al., 2011; Schiavoni et al., 2011; Stagg et al., 2011).

The type I IFNs represent a family of several closely related subtypes. At least 8 subclasses: α, β, δ, ε, κ, τ, ω and limitin have been described in different mammalian species. They all act through the same plasma membrane receptor made of IFNAR1 and IFNAR2. Humans have 17 subtypes: 13α, 1β, 1ω, 1ε and 1κ (Pestka et al., 2004).

Genes and cDNAs encoding human type I IFNs were first cloned in the early eighties (Derynck et al., 1980; Goeddel et al., 1980; Nagata et al., 1980b; Taniguchi et al., 1980). Among them, the IFNα1 and α2 were the first two α subtypes characterized. Given the low specific activity exhibited by IFNα1, IFNα2 was the first highly active human IFN made available to scientists and physicians. IFNα2 thus became the prototypic type I IFN subtype used in fundamental research and most clinical applications. With some rare exceptions, the basic knowledge generated by these studies is essentially valid for the other α, β and ω IFN subtypes in humans and higher mammals. In this review, we will summarize what we know on the structure, mechanism of action and biological activities of IFNα2. When pertinent, we will emphasize its uniqueness with respect to the other family subtypes.

2. IFNα2 gene and expression

Soon after the cloning of the human IFNα2 cDNA (Goeddel et al., 1980; Streuli et al., 1980), the gene was isolated and mapping of the type I IFN gene family began (Nagata et al., 1980a; Lawn et al., 1981). All type I IFN genes are clustered on a region covering 400 kb on the short arm of chromosome 9 (Diaz et al., 1994). All are intronless genes, suggesting that the family has originated from a retroposition event replacing, in higher vertebrates, ancestral intron-containing IFN genes (Qi et al., 2010). If all eutherian mammals have several type I IFN genes, the diversification of the family seems to have arisen independently in each species. Thus, except for very closely related species (eg. human and chimpanzee), there is no orthologues of human IFNα2 in other species (Woelk et al., 2007).

Several IFNα2 alleles have been described. The best known are α2a and α2b, both commercialized for clinical use as RoferonA and IntronA, respectively. They differ by a neutral K/R substitution at position 23 (von Gabain et al., 1990). In human populations, the gene encoding IFNα2 is found to be under constraints which prevent mutations, suggesting an essential role in physiology (Manry et al., 2011).

The expression of type I IFN genes is regulated primarily at the transcriptional level. Upstream of the transcription start site and the TATA box, the IFNα2 promoter contains several virus-responsive elements (VREs), also called positive regulatory domain-like elements (PRD-LEs) (Honda et al., 2005; Genin et al., 2009). These elements are found in the promoter of all IFNα genes. Of relevance, the IFNβ promoter contains two additional regulatory domains engaging NF-κB and ATF-2/c-Jun transcription factors. The VREs are activated by two IFN regulatory factors (IRF) primarily responsible for the initiation of IFNα transcription, IRF-3 and IRF-7. The small variations in the VRE sequences of the different IFNα genes affect the affinities of IRF-3 and IRF-7 binding and may account for some temporal and quantitative differences in gene expression (Genin et al., 2009). While IRF-3 is constitutively expressed in almost all cells, IRF-7 is constitutively expressed at high level mainly in plasmacytoïd dendritic cells (pDCs). IRF-7 is however robustly induced in all cells by type I IFNs. In most cell types, a full IFNα gene expression is thus dependent on a positive feedback loop where the *de novo* synthesis of IRF-7 is critical (Honda et al., 2005).

To act as transcription factors IRF-3 and IRF-7 need to be serine phosphorylated, to homo- or hetero-dimerize and translocate to the nucleus (Fig. 1). Several kinases such as Tank-binding kinase 1 (TBK1) or inducible IκB kinase (IKKε) can mediate phosphorylation. These IRF kinases are themselves activated through signaling cascades initiated by the recognition of many molecular patterns signing virus replication, bacterial infections, viral nucleotide sequences or cellular stresses (Tomasello et al., 2014). If all cells are equipped with the necessary sensors of viral replication, only specific cell types can detect danger patterns upon endocytosis of infected material. Among them pDCs selectively express Toll-like receptor (TLR) 7 and 9 which recognize endosomal ssRNA and DNA with unmethylated CpG motifs, respectively. Since pDCs constitutively express IRF7 and are equipped with a robust protein synthesis and secretion system, they are able to produce very high levels of all IFNα subtypes (Coccia et al., 2004; Ito et al., 2006).

3. IFNα2 structure, receptor binding and signaling

The IFNα2 gene encodes a 188 amino acid precursor that contains a N-terminal 23 amino acid leader sequence that is cleaved during the secretion process. The size of the mature IFNα2 is thus 165 amino acids, which is one amino acid shorter than all other human IFNα subtypes. From sequence alignment, it is clear that the aspartic acid at position 44 in all human IFNα subtypes is missing in IFNα2. No functional property was associated with this deletion. Because IFNα2 is not glycosylated, the biopharmaceutics industry has chosen to produce IFNα2 in *Escherichia coli* fermenter. Up to now, IFNα2 has been classically purified from inclusion bodies and refolded but new methods achieving high soluble protein expression and fast purification are being developed (Bis et al., 2014). The three-dimensional structure of IFNα2 was revealed by nuclear magnetic resonance (NMR) spectroscopy (Klaus et al., 1997) and X-ray crystallography (Radhakrishnan et al., 1996). It contains five α-helices (A to E), the helices A, B, C and E forming a left-handed four helix-bundle characteristic of the helical cytokine family. The long loop between helix A and helix B is perpendicular to the bundle axis; it is linked to helix E by a disulfide bond between cysteine 29 and 138. A second disulfide bond connects the amino-terminal cysteine to position 98 in the helix C.

Type I IFNs exert their biological activity by assembling a ternary IFN-receptor complex with the IFNAR1 and IFNAR2 chains. The structure of the ternary complex IFNAR1–IFNα2–IFNAR2 has been recently solved by X-ray crystallography (Thomas et al., 2011). An excellent recent review analyses in-depth all structural and dynamic aspects of IFNα2 binding (Piehler et al., 2012) and the cartography of the IFNα2 residues interacting with IFNAR1 and IFNAR2 are easily found at Protopedia (http://www.proteopedia.org/wiki/index.php/Journal:Cell:1). Briefly, a surface area of 18 nm^2 formed by part of the helices A and E, and the A–B loop interacts with IFNAR2. The domain of interaction with IFNAR1 is located on the opposite side of the IFN molecule and covers a surface area of 22 nm^2, containing residues located in the helices B, C and D. It is highly probable that all type I IFN subtypes, α, β and ω, form a similar ternary complex as suggested by the perfect superimposition of two ternary complexes assembled by two different IFNs described by Thomas et al. (2011), and by other low and high resolution structural data (Chill et al., 2003; Quadt-Akabayov et al., 2006; Li et al., 2008; Strunk et al., 2008; de Weerd et al., 2013).

All IFN subtypes show a dissymmetry in their affinities for the individual IFNAR chains. Typically, the constant of dissociation (K_d) of the interaction with IFNAR1 is in the μM range and the K_d for IFNAR2 is much lower, being in the nM range (Piehler et al., 2012). Since IFNAR1 and IFNAR2 are not pre-assembled at the cell surface (Wilmes et al., in press), it is likely that IFN binds first to IFNAR2 and then to IFNAR1 in a bi-dimensional reaction to form a ternary complex. Only the ternary complex is capable of signal transduction through the activation of the JAK kinases associated with the receptor chains, the ensuing phosphorylation of STAT1 and STAT2 and the formation of the ISGF3 transcription