JCMM
Available online at
www.jcmm.ro
www.jcmm.org
Phenomenin Review Series

J. Cell. Mol. Med. Vol 9, No 2, 2005 pp. 244-254

IFN-ζ/ limitin: a member of type I IFN with mild lympho-myelosuppression

Kenji Oritani *, Yuzuru Kanakura

Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan

Received: April 28, 2005; Accepted: May 20, 2005

Abstract

Interferon (IFN)-ζ/limitin has been considered as a novel type I IFN by the Nomenclature Committee of the International Society for Interferon and Cytokine Research. IFN-ζ/limitin shows some sequence homology with IFN-α and IFN-β, has a globular structure with five α-helices and four loops, and recognizes IFN-α/β receptor. Although IFN-ζ/limitin displays antiviral, immunomodulatory, and antitumor effects, it has much less lympho-myelosuppressive activities than IFN-α. Treatment of cells with type I IFNs induces and/or activates a number of molecules, which regulate cell cycle and apoptosis. It is noteworthy that IFN-ζ/limitin activates the Tyk2-Daxx and Tyk2-Crk pathways weaker than IFN-α. Because experiments using antisense oligonucleotides have revealed their essential role in type I IFN-related suppression of lympho-hematopoiesis, little ability of IFN-ζ/limitin to activate the Tyk2-dependent signaling pathway may explain its uniquely narrow range of biological activities. Further analysis of structure-function relationship of type I IFNs will establish an engineered cytokine with useful features of IFN-ζ/limitin.

Keywords: interferon - IFN-ζ/limitin - cytokine - structure - signal - Daxx - Crk

Introduction

Limitin was isolated with an expression cloning based on growth inhibitory effects on a WEHI3 myelomonocytic leukemia cell line [1]. It has sequence homology with type I interferons (IFNs) and binds to the IFN-α/β receptor [1]. In addition, it displays antiviral activity and can induce some effectors as IFN-α and IFN-β [2]. Based on these facts, limitin has been considered as a novel type I IFN with the designation of IFN-ζ by the Nomenclature Committee of the International Society for Interferon and Cytokine Research [3]. IFN-ζ/limitin has antitumor, immunomodulatory,

* Correspondence to: Kenji ORITANI, MD,
Department of Hematology and Oncology, Osaka University, Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan.
Tel.: +81-6-6879-3871; Fax: +81-6-6879-3879
E-mail: oritani@bldon.med.osaka-u.ac.jp

Reprinted from:
Journal of Cellular and Molecular Medicine

Published by:
the CMM Foundation

and antiviral activities as strong as IFN-α. However, IFN-ζ/limitin is unique in no or less growth-suppression of normal lympho-hematopoietic progenitors as compared with IFN-α. In this review, we will introduce the structure, biological activities, and signals of this interesting new cytokine in comparison with previously known type I IFNs and discuss issues about growth inhibitory activities of type I IFNs.

IFN-ζ/limitin

Structure

IFN-ζ/limitin cDNA consists of 997 base pair nucleotides whose sequence data is available from EMBL/GenBank/DDBJ under accession number of AB024521 [1]. The deduced protein is composed of 182 amino acid residues, which contain a signal peptide of 21 amino acids at the N-terminal end and a N-linked glycosylation site at the amino acid residue 68. The cleavage site of the signal peptide was confirmed by N-terminal amino acid sequencing, and the N-linked glycosylation was confirmed by the fact that treatment of IFN-ζ/limitin protein with N-glycosydase F changed its mobility in SDS-PAGE [4]. A computer modeling based on the refined crystal structure of IFNs has suggested that IFN-ζ/limitin shows a globular structure with five α-helices (A–E) and four loops and that there are two disulfide bonds between cysteine residues 52 and 157 and between cysteine residues 80 and 130 (Fig. 1A, 1B) [5]. A computer search using FASTA and BLAST programs indicated that IFN-ζ/limitin has some homology with murine IFN-α (31.9% identity in 166 amino acids overlap) and murine IFN-β (25.9% identity in 166 amino acids overlap). Especially, IFN-ζ/limitin has high sequence identity with IFN-α and IFN-β at residues 45-60, 105-115, and 135-165, corresponding to the N-terminal half of the AB loop, the C helix, and the DE loop together with helices D and E, which has been expected to function as receptor-recognition sites [3]. A dendrogram analysis has revealed that IFN-ζ /limitin branches at an early stage and does not fall into any of the IFN-α, IFN-β, IFN-ω, or IFN-τ subclusters while it belongs to one large cluster of type I IFNs (Fig. 1C) [6, 7].

Biological activities

When fibroblasts were treated with IFN-ζ/limitin, the gene and protein expressions of 2',5'-oligoadenylate synthetase (OAS) and RNA dependent protein kinase (PKR) were induced. IFN-ζ/limitin inhibited not only cytopathic effects in encephalo myocarditis virus- or herpes simplex virus-infected L929 cells but also plaque formation in mouse hepatitis virus-infected DBT cells [2]. In addition, treatment of mice with IFN-ζ/limitin suppressed mouse hepatitis virus-induced hepatitis and herpes simplex virus-induced death [2]. The induction of antiviral state by IFN-ζ/limitin was much stronger in BALB/c mice than in athymic nude mice [2]. Thus, in addition to direct antiviral activity, IFN-ζ/limitin seems to have indirect antiviral activities such as the induction of IFNs, the enhancement of apoptosis of virus-infected cells, the activation of natural killer cells, and the induction of nitric oxide *in vivo*.

IFN-ζ/limitin suppressed mitogen- and antigen-induced T-cell proliferation through inhibiting the responsiveness to interleukin (IL)-2 [8]. IFN-ζ/limitin enhanced the killer activity of cytotoxic T-lymphocytes associated with a perforin-granzyme pathway [8]. In addition, IFN-ζ/limitin augmented MHC class I expression on antigen-presenting cells such as spleen cells [8]. In a lethal graft versus host disease assay, IFN-ζ/limitin-treatment of host mice enhanced lethal graft versus host disease and induced early death after lymph node cell-transplantation [8]. Thus, IFN-ζ/limitin modifies immune responses under certain circumstances.

IFN-ζ/limitin reduced clonal proliferation of IL7-responding B-lymphocyte precursors (CFU-IL7) and megakaryocyte progenitors (CFU-Meg) in a dose-dependent manner [1, 9]. In contrast, IFN-ζ/limitin had no influence on colony formation of myeloid precursors responding to colony stimulating factors (CFU-GM) or that of erythroid precursors to erythropoietin (BFU-E) [1]. Similarly, IFN-ζ/limitin had no influence on the production of myeloid cells in Dexter type long-term bone marrow cultures, while it inhibited the production of B-lymphocytes in Whitlock-Witte type long-term bone marrow cultures [1]. When IFN-ζ/limitin was injected to newborn mice, B-lymphocytes, but not myeloid or erythroid cells,

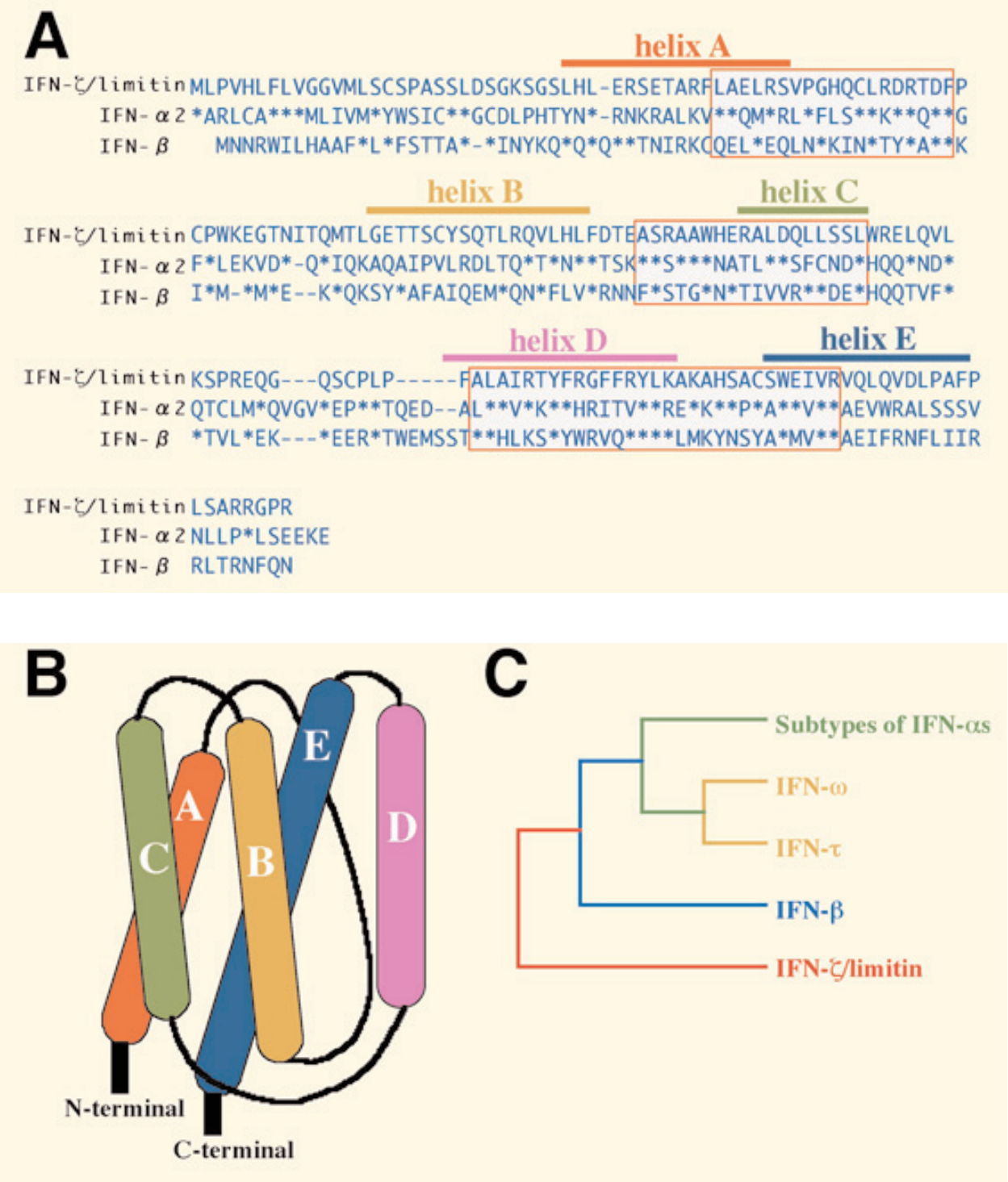

Fig. 1 Similarity of sequence and structure among IFN-ζ/limitin, IFN-α, and IFN-β. (A) Alignment of amino acid sequences. Asterisks indicate amino acid identities with IFN-ζ/limitin, and the dashed lines represent gaps introduced to align sequences. Each possible helix position of IFN-ζ/limitin was indicated. The highly conserved regions are boxed. The accession numbers of EMBL/GenBank/DDBJ for each gene are as follows: murine IFN-ζ/limitin; AB024521, murine IFN-α2; K01238, and murine IFN-β; K00020. (B) Schematic drawing of the side view of IFN-ζ/limitin molecule. Helices A-E and N-, C-termini are labeled. (C) A phylogenetic tree showing the similarities among members of the type I IFN family. Analysis was performed using the NJplot program

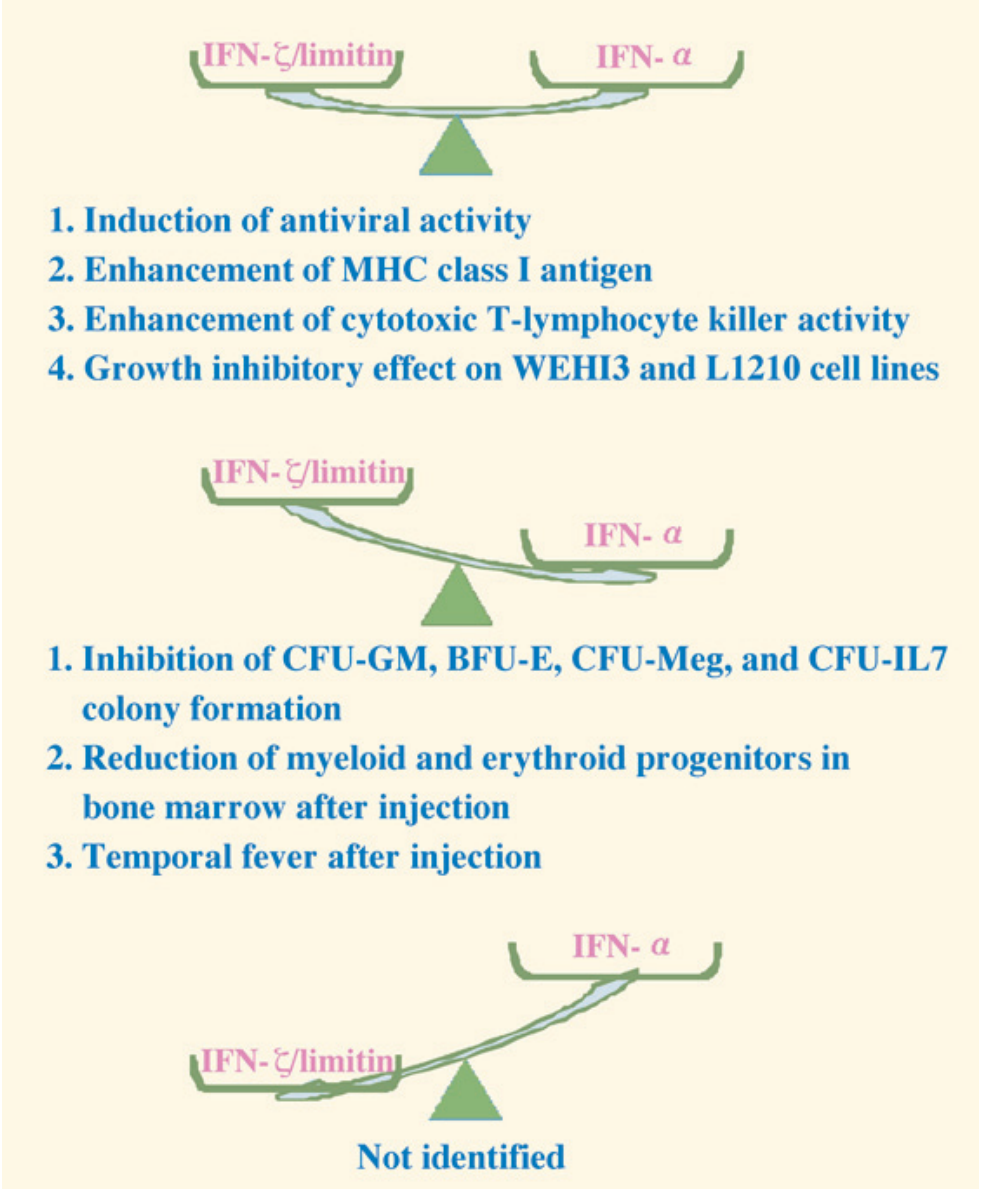

Fig. 2 Direct comparison of biological activities between IFN-ζ/limitin and IFN-α. IFN-ζ/limitin has antiviral activity, antitumor activity, and immunomodulatory activities as strong as IFN-α. However, IFN-ζ/limitin has no or less lympho-myelosuppressive activity than IFN-α. Up to date, we have not identified any IFN-ζ/limitin-specific biological activity

were markedly decreased in bone marrow and spleen of the treated mice [1]. Thus, the sensitivity to IFN-ζ/limitin is different among lineages of lympho-hematopoietic cells.

Signal transduction

When B-lymphocytes were treated with IFN-ζ/limitin, tyrosine phosphorylation of Jak1, Tyk2, and Stat1 was induced [1]. Gene expression of IFN regulatory factor (IRF)-1 was also induced after IFN-ζ/limitin-stimulation. In fibroblasts, IFN-ζ/limitin induced the promoter activity from IFN-stimulated response element (ISRE sequence), which resulted in the induction of OAS and PKR [2]. Treatment of megakaryocytes with IFN-ζ/limitin induced gene expression of suppressor of cytokine signaling (SOCS)-1 and death associated protein (Daxx). IFN-ζ/limitin also induced tyrosine phosphorylation of Tyk2 and Stat1 as well as that of Crk in megakaryocytes [9]. Thus, IFN-ζ/limitin induces similar signals to previously known IFNs. However, their differences will be described in the later part of this review.

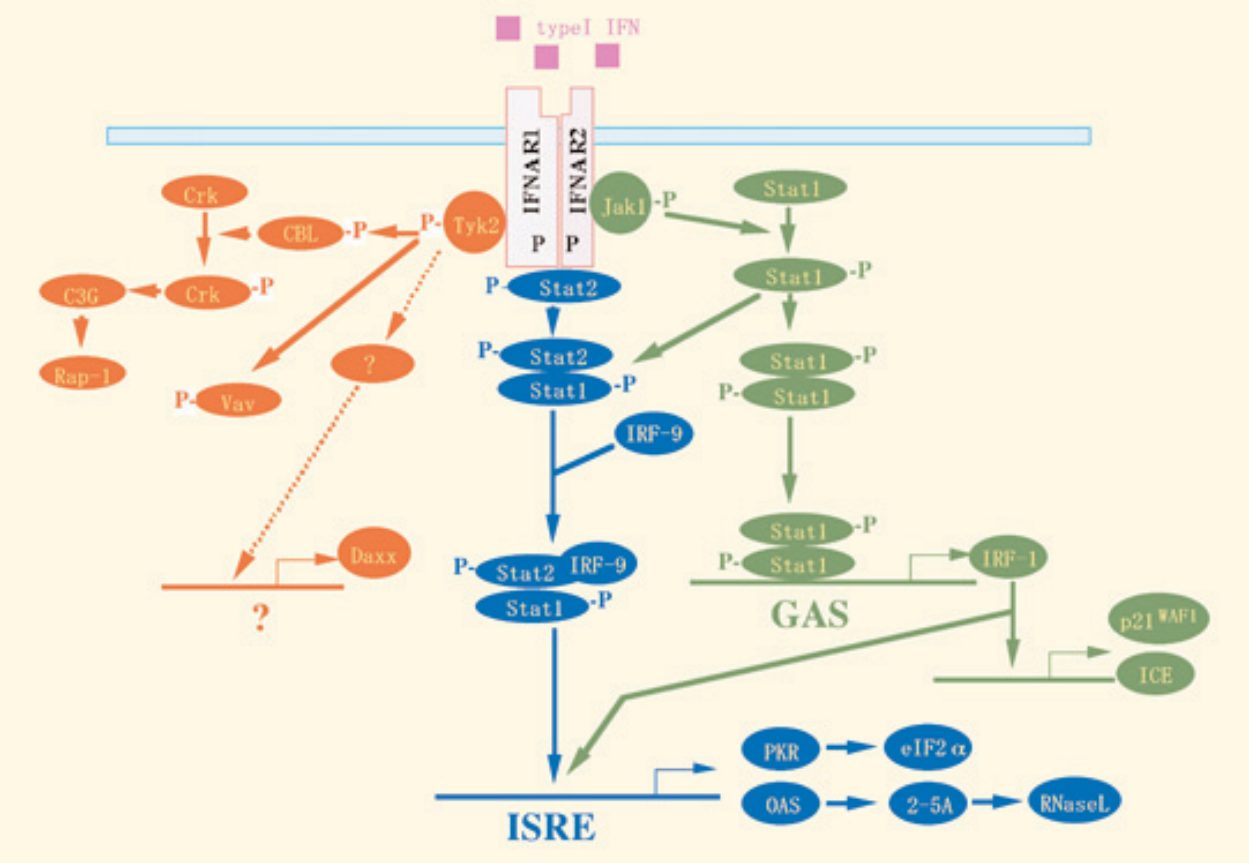

Fig. 3 The type I IFN signal transduction pathway. Type I IFN-binding causes phosphorylation of the receptors as well as two Jaks (Jak1 and Tyk2) and some Stat proteins. The phosphorylated Stat1-Stat2 complexes combine with IRF-9, and migrate to the nucleus where they bind to the ISRE sequence (blue pathway). Another important pathway is mediated by Stat1-Stat1 homodimer, which binds to GAS sequence, resulting in the transcription of IRF-1 (green pathway). IRF-1 recognizes consensus promoter sequences in part overlapping with the ISRE sequence. On the other hand, there are some Stat1-independent signals such as the induction of Daxx and the activation of Crk (red pathway). The phosphorylation of Crk is mediated by $p120^{cbl}$, which is a downstream event of Tyk2. The IFN-α-induced gene expression of Daxx is also dependent on Tyk2.

Comparison between IFN-ζ/limitin and IFN-α

We titrated recombinant IFN-ζ/limitin and IFN-α with a cytopathic effect dye binding assay in encephalo myocarditis virus-infected L929 cells. Half-maximal protection of cytopathic effects was achieved with 30 pg/ml of IFN-ζ/limitin and with 30-300 pg/ml of several subtypes of IFN-αs [2]. We directly compared signals and functions of the titrated IFN-ζ/limitin and IFN-α in same assay systems. The relative activities of IFN-ζ/limitin to IFN-α are summarized in Fig. 2. Similar dose requirement between IFN-ζ/limitin and IFN-α was observed in the enhancement of cytotoxic T-lymphocyte activity and the augmentation of MHC class I expression [10]. The growth inhibition of a myelomoncytic leukemia cell line WEHI3 and a murine lymphoblast cell line L1210 also required similar dose of IFN-ζ/limitin and IFN-α [10]. However, IFN-ζ/limitin did not suppress CFU-GM or BFU-E colony formation while IFN-α did [10]. Much higher concentrations of IFN-ζ/limitin than IFN-α were required for the suppression of CFU-IL7 and CFU-Meg colony formation [9, 10]. Approximately 30% of CFU-GM and 50% of BFU-E in bone marrow were reduced when 4,000 inter-

national unit (IU)/body/day of IFN-α was injected, while there was no influence on these progenitors even when IFN-ζ/limitin was injected at 40,000 IU/body/day [10]. Thus, IFN-ζ/limitin has similar levels of immunoregulatory and antiviral activities to IFN-α. However, IFN-ζ/limitin displays less lympho-myelosuppressive activity *in vitro* and *in vivo* than IFN-α.

We have found that IFN-ζ/limitin fails to display some common functions in type I IFNs. The narrow range of biological activities of IFN-ζ/limitin should be related to the difference of signals between IFN-ζ/limitin and IFN-α. General information about IFN-signaling is illustrated in Fig. 3. Antiviral activity of IFN-ζ/limitin was impaired more severely than that of IFN-α in the absence of IRF-1, while similar antiviral activity was observed in fibroblasts derived from wild type-mice [2]. IFN-ζ/limitin could induce less transcriptional activity from ISRE sequence than IFN-α in IRF-1-deficient fibroblasts [2]. It is interesting that IFN-ζ/limitin induced IRF-1 gene expression more strongly than IFN-α while no differences of the induction of OAS and PKR gene expressions were observed between IFN-ζ/limitin and IFN-α [2]. Thus, IRF-1-dependent pathway is more critical for IFN-ζ/limitin than IFN-α to induce antiviral state and to induce ISRE promoter activity. In megakaryocytes, IFN-ζ/limitin induced weaker Tyk2 phosphorylation than IFN-α, while it induced phosphorylation of Stat1 as strong as IFN-α [9]. The induction of Crk phosphorylation and Daxx gene expression, downstream events of Tyk2, was weaker in IFN-ζ/limitin-treated megakaryocytes than in those treated with IFN-α [9]. Similar level of SOCS-1 gene expression, which was dependent on some Stats, was induced by IFN-ζ/limitin- and IFN-α-treatment [9]. Thus, IFN-ζ/limitin is likely to activate Tyk2-dependent signaling pathway weaker than IFN-α.

Regulation of cell growth and/or cell death by type I IFNs

IFN-α and IFN-β can commonly block cell cycle at G1-phase or sometimes lengthen all phases of cell cycle. They can also induce apoptosis in transformed cell lines and primary tumor cells. While the detailed molecular background remains unclear, a variety of molecules have been known to be induced or to be activated after IFN-α-stimulation. Some are related to the induction of apoptosis, and the others are regulators of cell cycle.

IRFs constitute a family of transcription factors, which regulate a variety of cellular responses. Loss of IRF-1 alleles markedly exacerbates tumor predispositions caused by a transgene Ha-ras or a *p53* nullizygosity [11]. IRF-4-deficient mice progressively develop lymphoadenopathy in their whole body [12]. IRF-8-deficient mice display chromic myelogeneous leukemia-like phenotypes [13]. IRF-9 is a member of IFN-stimulated gene factor-3 (ISGF3), which induces many IFN-inducible genes such as PKR and OAS. Activated PKR can catalyze the phosphorylation of eIF2α, resulting in the inhibition of protein synthesis. PKR also plays a role in NF-κB pathway, which induces transcription of death promoting molecules such as Fas [14]. OAS correlates with the synthesis of 2',5'-oligoadenylic acid (2-5A), which activates latent cellular ribonucleases RNase L. It is known that a dominant-negative RNase L reduces apoptosis in response to IFNs and that RNase L-deficient mice have diminished levels of spontaneous apoptosis in their thymus and spleen [15]. Induction of tumor suppressor *p53* by IFNs is also dependent on the binding of ISGF3 to ISRE in the promoter of the *p53* gene [16]. There are some IRF-1-dependent molecules such as a cell cycle regulator, $p21^{WAF1}$ and a mammalian homologue of the caenorhabditis elegans cell death gene *ced3*, IL-1β-converting enzyme [17, 18].

IFNs activate members of the MAPK family, including Jun N-terminal kinase (JNK) and p38 kinase, which establish antiproliferative enzymatic cascades. Overexpression of JNK augments potential of apoptosis induced by IFN-α [19]. Pharmacological inhibition of p38 activation reverses the type I IFN-dependent suppression of hematopoietic progenitor colony formation [20]. Another signaling molecule, which is involved in IFN-related growth suppression is the Vav proto-oncogene. Vav undergoes rapid tyrosine phosphorylation in response to IFN-stimuli, and the disruption of Vav protein expression with antisense oligonucleotides reverses the growth inhibitory effects of IFN-α on megakaryocytic cell lines [21].

Some IFN-related molecules were recently identified as proapoptotic proteins. X-linked inhibitor of apoptosis (XIAP) associated factor-1, XAF-1, is a novel IFN-inducing gene, which was identified with gene array studies in IFN-sensitive melanoma cells [22]. XAF-1 interacts with XIAP, which has the baculoviral inhibitor of apoptosis repeat domains and inhibits caspase functions. Overexpression of XAF-1 resulted in neutralization of XIAP's ability to inhibit cell death [23]. Regulators of IFN-induced death (RIDs) such as inositol hexakisphosphate kinase 2 were identified with antisense technical knockout approach [24]. Overexpression of RIDs genes sensitized cells to death induced by IFNs and anticancer drugs [24, 25].

In addition to the direct regulators of cell cycle and apoptosis, we have to pay attention to regulators of cytokine signaling. Wang and his colleagues reported that SOCS-1 inhibited the proliferation of megakaryocyte progenitors via blunting TPO-induced signals in IFN-α-treated cells [26].

Daxx and Crk in IFN-systems

Daxx was originally identified as a Fas-binding protein by yeast two-hybrid screening and described as a proapoptotic protein, which can enhance the extrinsic pathway of apoptosis through JNK activation [27]. Daxx in the cytoplasm promotes caspase-independent cell death upon Fas-stimulation [28]. However, Daxx is exclusively present in the nucleus and regulates transcriptional activity. Daxx directly cooperates with promyelocytic leukemia protein (PML) to potentiate FAS-induced apoptosis. In the absence of PML, Daxx is delocalized from the PML-nuclear body, and activation-induced cell death of splenocytes is profoundly impaired [29]. In addition, the interaction of Daxx with PML may control the actylation of p53 and the Rb transcription [30]. Thus, PML and Daxx is likely to cooperate in a novel nuclear body-dependent pathway for apoptosis. Daxx also acts as a transcription corepressor of Pax3 and Ets-1, and as an activator or repressor of Pax5 [31, 32, 33]. We also found that Daxx forms a functional complex composed of DNA methyltransferase 1, DNA methyltransferase 1 associated protein, and tumor susceptibility gene 101 [34, 35]. Although Daxx makes important contributions to apoptosis and has the ability to modulate transcription, many mechanistic aspects of Daxx-function are still unclear. Recently, Daxx was reported to be essential for the inhibition of B-lymphopoiesis by IFN-α [36]. Treatment of B-lymphocytes with IFN-α induces Daxx expression and nuclear body translocation. Antisense oligonucleotides against Daxx rescue IFN-α-treated pro-B cells from growth arrest and apoptosis. Because Stat1 was not required for the IFN-α-induced growth suppression of B-lymphocytes [37], we examined a role of Tyk2 in this IFN-α function by using Tyk2-deficient cells. Both IFN-ζ/limitin and IFN-α inhibited CFU-IL7 colony formation of bone marrow cells derived from wild-type, but not Tyk2-deficient mice [38, 39]. In addition, the IFN-ζ/limitin- and IFN-α-induced up-regulation and nuclear translocation of Daxx were completely abrogated in the absence of Tyk2 [38, 39]. Thus, these events concerning Daxx exist downstream of Tyk2 in the IFN-signaling pathway. Daxx also plays a role in IFN-induced growth suppression of megakaryocyte progenitors. The disruption of Tyk2 as well as the treatment with antisense oligonucleotides against Daxx partially cancelled IFN-ζ/limitin- or IFN-α-induced inhibition of CFU-Meg colony formation [9]. Thus, Daxx might be one of the key molecules, which mediate lympho-myelosuppressive effects of type I IFNs.

The Crk family proteins, cellular homologues of the v-Crk proto-oncogene product, includes three members: CrkL, CrkI, and CrkII. CrkL and CrkII have one SH2-domain, which binds to $p120^{cbl}$, and two SH3-domains, which interact with C3G, a guanine exchange factor for Rap-1 [40, 41]. $p120^{cbl}$ constitutively associates with type I IFN-dependent Tyk2 and undergoes phosphorylation after IFN-stimulation [42]. The activated $p120^{cbl}$ acts as a docking protein for an adapter protein Crk, which provides a link to the C3G-Rap-1 signaling cascade [40, 41]. The activated Rap-1 then antagonizes the Ras pathway, which plays a role in cell proliferation [43]. Most importantly, the inhibition of CrkL and CrkII protein expression by the treatment of cells with antisense oligonucleotides reversed the IFN-α-induced inhibition of CFU-GM and BFU-E colony formation [44]. The Crk family proteins also play a role in IFN-induced growth suppression of megakaryocyte progenitors. The disruption of Tyk2

Table 1 Adverse effects of IFN-therapy

Flu-like symptoms	Hematological disorders	Gastrointestinal symptoms	Neuropsychiatric symptoms
Fatigue	Anemia	Nausea	Depression
Headache	Leukopenia	Anorexia	Anxiety
Chill	Thrombocytopenia	Diarrhea	Irritability
Fever		Constipation	Insomnia
Arthro-myalgia			

as well as the treatment with antisense oligonucleotides against Crk partially cancelled IFN-ζ/limitin- or IFN-α-induced inhibition of CFU-Meg colony formation [9]. Thus, Crk might be another key molecule, which mediates lympho-myelosuppressive effects of type I IFNs.

Studies from IFN-ζ/limitin

The most important and strange issue of IFN-ζ /limitin is why it has the narrow range of biological activities as compared with previously known type I IFNs. Probably, less activation of the Tyk2-Daxx and/or Tyk2-Crk pathways leads to no or less suppression of normal lympho-hematopoiesis. In this connection, there are some explanations for the different functions among type I IFNs. Differences in affinity for IFN-α/β receptor have been described between IFN-α and IFN-β as well as among subtypes of IFN-α [45]. In addition, there are suggestions that different type I IFNs have distinct interactions with IFN-α/β receptor. For example, IFN-β uniquely induces the association of tyrosine phosphorylated IFN-α/β receptor chains in myeloma cell lines [46, 47, 48]. A mutant human 11,1 cells lacking Tyk2 are completely unresponsive to IFN-α, but still retain a partial sensitivity to IFN-β [49]. Those differences could activate IFN-β-specific or IFN-α subtype-specific intracellular signaling pathways, leading to cellular responses specific for each IFN. Thus, unique IFN-ζ/limitin characters such as the restricted biological activities are likely to reflect differences in the receptor interactions and/or the utilization of Jak kinases after ligand-receptor binding. Experiments using monoclonal antibodies against IFN-α or IFN-β and site-directed mutagenesis revealed the functional importance of the N-terminal half of the loop AB and the loop DE together with the nearest segments of the helices D and E [50–52]. Another analysis using hybrid IFNs with combinations of common restriction sites suggested that the helix C interacts directly with IFNAR-1 [53]. The helices A and C present residues to the solvent on one side of the molecule with the AB loop and the helix D positioned on the other side [53]. Recently, Runkel and his colleagues reported that IFN-β mutations in the helix C lost the ability to induce the association of tyrosine-phosphorylated receptor chains, which IFN-β, but not IFN-α, could induce [54]. In addition, Shorts and his colleagues reported that some mutations in the helix A or the first section of AB loop of IFN-τ changed either antiviral or antiproliferative activities [55]. These facts suggest that each IFN-specific effect is related to the small difference of sequences, which provides essential interaction with IFN-α/β receptor, and probably the narrow range of biological activities of IFN-ζ/limitin might be adaptable for this situation.

Type I IFNs are used for the treatment of patients with virus-infection, autoimmune diseases,

and malignant diseases [56]. Remarkable recent advances for IFN-therapy are a combined therapy with ribavirin or amantadine as well as an appearance of a pegylated IFN and a consensus IFN [57–59]. Although these new therapeutic strategies are more efficient than previous IFN monotherapy, adverse effects of IFNs as listed in Table 1 are still severe problems. Indeed, patients receiving the treatment with IFN-α or IFN-β sometimes experience fatigue, anorexia, depression, and myelosuppression, which require the dose reduction or the discontinuation of treatment [60]. An engineered cytokine with useful features of the IFN-ζ/limitin, which displays less lymph-myelosuppressive property, could be superior than previously known IFNs because of less adverse effects. Alternatively, pharmacological inhibition of the Tyk2-Daxx and/or Tyk2-CrkL pathways might reduce lympho-myelosuppression after IFN-treatment. These possible strategies will enable patients with neutropenia or thrombocytopenia to receive IFN-therapy as well as patients with hepatitis C whose genotype is IFN-resistant to receive high-dose and/or long-duration of IFN-therapy.

Concluding remarks

IFN-ζ/limitin belongs to the type I IFN category. IFN-ζ/limitin has similar antiviral, antitumor, and immunomodulatory effects to IFN-α, but no or less lympho-myelosuppressive activities. For the reduction of adverse effects, it is particularly exciting to find a novel IFN with a uniquely narrow range of functions and targets. Further analysis will promise us to understand structure-function relationships of IFNs in greater detail as well as to establish a new strategy for an IFN-based therapy. IFN-ζ/limitin is a truly powerful model for this purpose.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Japan Society for the Promotion of Science.

References

1. **Oritani K, Medina KL, Tomiyama Y, Ishikawa J., Okajima Y, Ogawa M, Yokota T, Aoyama K, Takahashi I, Kincade PW, Matsuzawa Y.** Limitin: An interferon-like cytokine that preferentially influences B-lymphocyte precursors. *Nat Med.* 2000; 6: 659–66.
2. **Kawamoto S, Oritani K, Asada H, Takahashi I, Ishikawa J, Yoshida H, Yamada M, Ishida N, Ujiie H, Masaie H, Tomiyama Y, Matsuzawa Y.** Antiviral activity of limitin against encephalomyocarditis virus, herpes simplex virus, and mouse hepatitis virus: diverse requirements by limitin and alpha interferon for interferon regulatory factor 1. *J Virol.* 2003; 77: 9622–31.
3. **Oritani K, Tomiyama Y.** Interferon-ζ/limitin: novel type I interferon that displays a narrow range of biological activity. *Int J Hematol.* 2004; 80: 325–31.
4. **Oritani K, Hirota S, Nakagawa T, Takahashi I, Kawamoto S, Yamada M, Ishida N, Kadoya T, Tomiyama Y, Kincade PW, Matsuzawa Y.** T lymphocytes constitutively produce an interferonlike cytokine limitin characterized as a heat- and acid-stable and heparin-binding glycoprotein. *Blood* 2003; 101: 178–85.
5. **Oritani K, Kincade PW, Zhang C, Tomiyama Y, Matsuzawa Y.** Type I interferons and limitin: a comparison of structures, receptors, and functions. *Cytokine Growth Factor Rev.* 2001; 12: 337–48.
6. **Oritani K, Kincade PW, Tomiyama Y.** Limitin: an interferon-like cytokine without myeloerythroid suppressive properties. *J Mol Med.* 2001; 79: 168–174.
7. **Pestka S, Krause CD, Walter MR.** Interferons, interferon-like cytokines, and their receptors. *Immunol Rev.* 2004; 202: 8–32.
8. **Takahashi I, Kosaka H, Oritani K, Heath WR, Ishikawa J, Okajima Y, Ogawa M, Kawamoto S, Yamada M, Azukizawa H, Itami S, Yoshikawa K, Tomiyama Y, Matsuzawa Y.** A new IFN-like cytokine, limitin, modulates the immune response without influencing thymocyte development. *J Immunol.* 2001; 167: 3156–63.
9. **Ishida N, Oritani K, Shiraga M, Yoshida H, Kawamoto S, Ujiie H, Masaie H, Ichii M, Tomiyama Y, Kanakura Y.** Differential effects of a novel IFN-ζ/limitin and IFN-α on signals for Daxx induction and Crk phosphorylation that couple with growth control of megakaryocytes. *Exp Hematol.* 2005; 33: 495–503.
10. **Kawamoto S, Oritani K, Asakura E, Ishikawa J, Koyama M, Miyano K, Iwamoto M, Yasuda S, Nakakubo H, Hirayama F, Ishida N, Ujiie H, Masaie H, Tomiyama Y.** A new interferon, limitin, displays equivalent immunomodulatory and antitumor activities without myelosuppressive properties as compared with interferon-alpha. *Exp Hematol.* 2004; 32: 797–805.
11. **Nozawa H, Oda E, Nakao K, Ishihara M, Ueda S, Yokochi T, Ogasawara K, Nakatsuru Y, Shimizu S, Ohira Y, Hioki K, Aizawa S, Ishikawa T, Katsuki M, Muto T, Taniguchi T, Tanaka N.** Loss of transcription factor IRF-1 affects tumor susceptibility in mice carrying

the Ha-ras transgene or nullizygosity for p53. *Genes Dev*. 1999; 13: 1240–5.

12. **Mittrucker HW, Matsuyama T, Grossman A, Kundig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS, Mak TW.** Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science* 1997; 275: 540–3.
13. **Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, Lou J, Knobeloch KP, Gabriele L, Waring JF, Bachmann MF, Zinkernagel RM, Morse HC 3rd, Ozato K, Horak I.** Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell* 1996; 87: 307–17.
14. **Donze O, Dostie J, Sonenberg N.** Regulatable expression of the interferon-induced double-stranded RNA dependent protein kinase PKR induces apoptosis and fas receptor expression. *Virology* 1999; 256: 322–9.
15. **Zhou A, Paranjape J, Brown TL, Nie H, Naik S, Dong B, Chang A, Trapp B, Fairchild R, Colmenares C, Silverman RH.** Interferon action and apoptosis are defective in mice devoid of 2',5'-oligoadenylate-dependent RNase L. *EMBO J.* 1997; 16: 6355–63.
16. **Takaoka A, Hayakawa S, Yanai H, Stoiber D, Negishi H, Kikuchi H, Sasaki S, Imai K, Shibue T, Honda K, Taniguchi T.** Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424: 516–23.
17. **Tanaka N, Ishihara M, Lamphier MS, Nozawa H, Matsuyama T, Mak TW, Aizawa S, Tokino T, Oren M, Taniguchi T.** Cooperation of the tumour suppressors IRF-1 and p53 in response to DNA damage. *Nature* 1996; 382: 816–8.
18. **Tamura T, Ishihara M, Lamphier MS, Tanaka N, Oishi I, Aizawa S, Matsuyama T, Mak TW, Taki S, Taniguchi T.** An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. *Nature* 1995; 376: 596–9.
19. **Caraglia M, Abbruzzese A, Leardi A, Pepe S, Budillon A, Baldassare G, Selleri C, Lorenzo SD, Fabbrocini A, Giuberti G, Vitale G, Lupoli G, Bianco AR, Tagliaferri P.** Interferon-α induces apoptosis in human KB cells through a stress-dependent mitogen activated protein kinase pathway that is antagonized by epidermal growth factor. *Cell Death Differ*. 1999; 6: 773–80.
20. **Verma A, Deb DK, Sassano A, Uddin S, Varga J, Wickrema A, Platanias LC.** Activation of the p38 mitogen-activated protein kinase mediates the suppressive effects of type I interferons and transforming growth factor-β on normal hematopoiesis. *J Biol Chem.* 2002; 277: 7726–35.
21. **Micouin A, Wietzerbin J, Steunou V, Martyre MC.** p95(vav) associates with the type I interferon (IFN) receptor and contributes to the antiproliferative effect of IFN-α in megakaryocytic cell lines. *Oncogene* 2000; 19: 387–94.
22. **Leaman DW, Chawla-Sarkar M, Vyas K, Reheman M, Tamai K, Toji S, Borden EC.** Identification of X-linked inhibitor of apoptosis-associated factor-1 as an interferon-stimulated gene that augments TRAIL Apo2L-induced apoptosis. *J Biol Chem.* 2002; 277: 28504–11.
23. **Liston P, Fong WG, Kelly NL, Toji S, Miyazaki T, Conte D, Tamai K, Craig CG, McBurney MW, Korneluk RG.** Identification of XAF1 as an antagonist of XIAP anti-Caspase activity. *Nat Cell Biol.* 2001; 3: 128–33.
24. **Morrison BH, Bauer JA, Kalvakolanu DV, Lindner DJ.** Inositol hexakisphosphate kinase 2 mediates growth suppressive and apoptotic effects of interferon-β in ovarian carcinoma cells. *J Biol Chem.* 2001; 276: 24965–70.
25. **Morrison BH, Bauer JA, Hu J, Grane RW, Ozdemir AM, Chawla-Sarkar M, Gong B, Almasan A, Kalvakolanu DV, Lindner DJ.** Inositol hexakisphosphate kinase 2 sensitizes ovarian carcinoma cells to multiple cancer therapeutics. *Oncogene* 2002; 21: 1882–9.
26. **Wang Q, Miyakawa Y, Fox N, Kaushansky K.** Interferon-α directly represses megakaryopoiesis by inhibiting thrombopoietin-induced signaling through induction of SOCS-1. *Blood* 2000; 96: 2093–9.
27. **Yang X, Khosravi-Far R, Chang HY, Baltimore D.** Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* 1997; 89: 1067–76.
28. **Muromoto R, Yamamoto T, Yumioka T, Sekine Y, Sugiyama K, Shimoda K, Oritani K, Matsuda T.** Daxx enhances Fas-mediated apoptosis in a murine pro-B cell line, BAF3. *FEBS Lett.* 2003; 540: 223–8.
29. **Zhong S, Salomoni P, Ronchetti S, Guo A, Ruggero D, Pandolfi PP.** Promyelocytic leukemia protein (PML) and Daxx participate in a novel nuclear pathway for apoptosis. *J Exp Med.* 2000; 191: 631–40.
30. **Salomoni P, Pandolfi PP.** The role of PML in tumor suppression. *Cell* 2002; 108: 165–70.
31. **Hollenbach AD, Sublett JE, McPherson CJ, Grosveld G.** The Pax3-FKHR oncoprotein is unresponsive to the Pax3-associated repressor hDaxx. *EMBO J.* 1999; 18: 3702–11.
32. **Li R, Pei H, Watson DK, Papas TS.** EAP1/Daxx interacts with ETS1 and represses transcriptional activation of ETS1 target genes. *Oncogene* 2000; 19: 745–53.
33. **Emelyanov AV, Kovac CR, Sepulveda MA, Birshtein BK.** The interaction of Pax5 (BSAP) with Daxx can result in transcriptional activation in B cells. *J Biol Chem.* 2002; 277: 11156–64.
34. **Muromoto R, Sugiyama K, Takachi A, Imoto S, Sato N, Yamamoto T, Oritani K, Shimoda K, Matsuda T.** Physical and functional interactions between Daxx and DNA methyltransferase 1-associated protein DMAP1. *J Immunol.* 2004; 172: 2985–93.
35. **Muromoto R, Sugiyama K, Yamamoto T, Oritani K, Shimoda K, Matsuda T.** Physical and functional interactions between Daxx and TSG101. *Biochem Biophys Res Commun.* 2004; 316: 827–33.
36. **Gongora R, Stephan RP, Zhang Z, Cooper MD.** An essential role for Daxx in the inhibition of B lymphopoiesis by type I interferons. *Immunity* 2001; 14: 727–37.
37. **Gongora R, Stephan RP, Schreiber RD, Cooper MD.** Stat-1 is not essential for inhibition of B lymphopoiesis by type I IFNs. *J Immunol.* 2000; 165: 2362–6.
38. **Shimoda K, Kamesaki K, Numata A, Aoki K, Matsuda T, Oritani K, Tamiya S, Kato K, Takase K, Imamura R, Yamamoto T, Miyamoto T, Nagafuji K, Gondo H,**

Nagafuchi S, Nakayama K, Harada M. Cutting edge: tyk2 is required for the induction and nuclear translocation of Daxx which regulates IFN-α-induced suppression of B lymphocyte formation. *J Immunol.* 2002; 169: 4707–11.

39. **Aoki K, Shimoda K, Oritani K, Matsuda T, Kamezaki K, Muromoto R, Numata A, Tamiya S, Haro T, Ishikawa F, Takase K, Yamamoto T, Yumioka T, Miyamoto T, Nagafuji K, Gondo H, Nagafuchi S, Nakayama K, Harada M.** Limitin, an interferon-like cytokine, transduces inhibitory signals on B-cell growth through activation of Tyk2, but not Stat1, followed by induction and nuclear translocation of Daxx. *Exp Hematol.* 2003; 31: 1317–22.
40. **Ahmad S, Alsayed YM, Druker BJ, Platanias LC.** The type I interferon receptor mediates tyrosine phosphorylation of the CrkL adaptor protein. *J Biol Chem.* 1997; 272: 29991–4.
41. **Reedquist KA, Fukazawa T, Panchamoorthy G, Langdon WY, Shoelson SE, Druker BJ, Band H.** Stimulation through the T cell receptor induces Cbl association with Crk proteins and the guanine nucleotide exchange protein C3G. *J Biol Chem*. 1996; 271: 8435–42.
42. **Uddin S, Gardziola C, Dangat A, Yi T, Platanias LC.** Interaction of the c-cbl proto-oncogene product with the Tyk-2 protein tyrosine kinase. *Biochem Biophys Res Commun.* 1996; 225: 833–8.
43. **Cook SJ, Rubinfeld B, Albert I, McCormick F.** RapV12 antagonizes Ras-dependent activation of ERK1 and ERK2 by LPA and EGF in Rat-1 fibroblasts. *EMBO J.* 1993; 12: 3475–85.
44. **Platanias LC, Uddin S, Bruno E, Korkmaz M, Ahmad S, Alsayed Y, Van Den Berg D, Druker BJ, Wickrema A, Hoffman R.** CrkL and CrkII participate in the generation of the growth inhibitory effects of interferons on primary hematopoietic progenitors. *Exp Hematol.* 1999; 27: 1315–21.
45. **Foster GR, Finter NB.** Are all type I human interferons equivalent? *J Viral Hepat.* 1998; 5: 143–52.
46. **Abramovich C, Shulman LM, Ratovitski E, Harroch S, Tovey M, Eid P, Revel M.** Differential tyrosine phosphorylation of the IFNAR chain of the type I interferon receptor and of an associated surface protein in response to IFN-α and IFN-β. *EMBO J.* 1994; 13: 5871–7.
47. **Constantinescu SN, Croze E, Murti A, Wang C, Basu L, Hollander D, Russell-Harde D, Betts M, Garcia-Martinez V, Mullersman JE, Pfeffer LM.** Expression and signaling specificity of the IFNAR chain of the type I interferon receptor complex. *Proc Natl Acad Sci USA.* 1995; 92: 10487–91.
48. **Croze E, Russell-Harde D, Wagner TC, Pu H, Pfeffer LM, Perez HD.** The human type I interferon receptor. Identification of the interferon β-specific receptor-associated phosphoprotein. *J Biol Chem.* 1996; 271: 33165–8.
49. **Velazquez L, Fellous M, Stark GR, Pellegrini S.** A protein tyrosine kinase in the interferon α/β signaling pathway. *Cell* 1992; 70: 313–22.
50. **Uze G, Lutfalla G, Mogensen KE.** α and β interferons and their receptor and their friends and relations. *J Interferon Cytokine Res.* 1995; 15: 3–26.
51. **Cebrian M, Yague E, de Landazuri MO, Rodiguez-Moya M, Fresno M, Pezzi N, Llamazares S, Sanchez-Madrid F.** Different functional sites on rIFN-α2 and their relation to the cellular binding site. *J Immunol.* 1987; 138: 484–90.
52. **Kontsek P, Borecky L, Kontsekova E, Macikova I, Kolcunova A, Novak M, Krchnak V.** Mapping of two immunodominant structures on human interferon α2c and their role in binding to cells. *Mol Immunol.* 1991; 28: 1289–97.
53. **Uze G, Di Marco S, Mouchel-Vielh E, Monneron D, Bandu MT, Horisberger MA, Dorques A, Lutfalla G, Mogensen KE.** Domains of interaction between α interferon and its receptor components. *J Mol Biol.* 1994; 243: 245–57.
54. **Runkel L, Pfeffer L, Lewerenz M, Monneron D, Yang CH, Murti A, Pellegrini S, Goelz S, Uze G, Mogensen K.** Differences in activity between α and β type I interferons explored by mutational analysis. *J Biol Chem.* 1998; 273: 8003–8.
55. **Shorts LH, Dancz CE, Shupp JW, Pontzer CH.** Characterization of N-terminal interferon τ mutants: P26L affords enhanced activity and lack of toxicity. *Exp Biol Med.* 2004; 229: 194–202.
56. **Jonasch E, Haluska FG.** Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. *Oncologist* 2001; 6: 34–55.
57. **Bacosi M, Russo F, D'innocenzo S, Santolamazza M, Miglioresi L, Ursitti A, De Angelis A, Patrizi F, Ricci GL.** Amantadine and interferon in the combined treatment of hepatitis C virus in elderly patients. *Hepatol Res.* 2002; 22: 231–9.
58. **Reddy KR, Wright TL, Pockros PJ, Shiffman M, Everson G, Reindollar R, Fried MW, Purdum PP 3rd, Jensen D, Smith C, Lee WM, Boyer TD, Lin A, Pedder S, DePamphilis J.** Efficacy and safety of pegylated (40-kd) interferon α-2a compared with interferon α-2a in noncirrhotic patients with chronic hepatitis C. *Hepatology* 2001; 33: 433–8.
59. **Melian EB, Plosker GL**. Interferon αcon-1: a review of its pharmacology and therapeutic efficacy in the treatment of chronic hepatitis C. *Drugs.* 2001; 61: 1661–91.
60. **Weiss K.** Safety profile of interferon-α therapy. *Semin Oncolo.* 1998; 25: 9–13.