

Translational and post-translational modifications of proteins as a new mechanism of action of Alpha-Interferon: Review article

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Summary. Interferon- α (IFN α) is a recombinant protein widely used in the therapy of several neoplasms such as myeloma, renal cell carcinoma, epidermoid cervical and head and neck tumours and melanoma. IFN α , the first cytokine to be produced by recombinant DNA technology, has emerged as an important regulator of cancer cell growth and differentiation, affecting cellular communication and signal transduction pathways. However, the way by which tumour cell growth is directly suppressed by IFN α is not well known. Wide evidence exists on the possibility that cancer cells undergo apoptosis after the exposure to the cytokine. Here we will discuss data obtained by us and others on the post-translational regulation of the expression of proteins involved in the occurrence of apoptotic process such as tissue transglutaminase (tTG) or in the modulation of cell cycle such as the cyclin-dependent kinase inhibitor p27. This new way of regulation of p27 and tTG occurs through the modulation of their proteasome-dependent degradation induced by the cytokine. We will also review the involvement of protein synthesis machinery in the induction of cell growth inhibition by IFN α . In details, we will describe the effects of IFN α on the expression and activity of the protein kinase dependent from dsRNA (PKR) and on the eukaryotic initiation factor of protein synthesis 5A (eIF-5A) and their correlations with the regulation of cancer cell growth. These data strongly suggest that the antitumour activity of IFN α against human tumours could involve still unexplored mechanisms based on post-translational and translational control of the expression of proteins that regulate cell proliferation and apoptosis.

Keywords: Interferon α – STAT – Ubiquitin – tTG – eIF-5A – Hypusine – PKR

Abbreviations: IFN, interferon; STAT, Signal transducer and activator of transcription; SH2, Src homology 2; PKR, protein kinase dependent from dsDNA; IF, initiation factor; eIF, eukaryotic IF; DRBM, dsRNA binding motifs; TRBP, Tar RNA binding protein; MEFs, mouse embryonic fibroblasts; TNF, tumor necrosis factor; EGF, epidermal

growth factor; LPS, lipopolisaccaride; tTG, tissue transglutaminase; ERK, extracellular signal regulated kinase; MEK, mitogen extracellular signal regulated kinase; GC7, N1-guanyl-1,7-diaminoheptane; IL-1 β , interleukin 1 β ; MAb, monoclonal antibody; JNK-1, NH2 terminal Jun Kinase-1

The interferon superfamily

The interferons (IFNs) represent proteins with antiviral activity that are secreted from cells in response to a variety of stimuli (Pestka et al., 1981a, b, 1986). There are at least 5 classes of IFN alpha, beta, gamma, tau and omega. The interferons are divided into two groups designated type I and type II interferons. IFN γ is the only type II interferon, whereas the type I interferons consist of four major classes: IFN α , IFN β , IFN ω , and IFN τ . There is only one Hu-IFN ω and one Hu-IFN β , but a family of multiple IFN α species exists. It is unlikely that any human IFN τ exists. In general, exposure of cells to viruses and doublestranded RNAs induce the production of IFN α and IFN β species. The classical function of these proteins is the protection against viral infections. However, it emerged soon the ability of IFN of inhibiting tumour cell proliferation and, on the basis of the preclinical data, it entered the clinical practice and conquered a role in the therapy of a defined group of neoplasms. It appeared, therefore, clear that IFN has a limited activity

and several cancers are resistant to anti-proliferative action induced by this cytokine. On the other hand, the mechanisms at the basis of anti-cancer effects of IFN are not still completely clear even if the induction of programmed cell death has been recently involved. The potential role played by apoptosis and the new findings about the signal transduction elicited by this cytokine has given emphasis on the molecular pathways regulated by IFN in the view of potentiate its antitumour activity (Pestka et al., 2000).

Interferon alpha and signal transduction

The IFN receptor family

The IFN- α R1, IFN- α R2, CRFB4, IFN- γ R1, and IFN- γ R2 chains are members of the cytokine type 2 receptor family as described by Bazan and by Thoreau et al. who proposed that the interferon receptors as well as other receptors for cytokines and some growth factors are composed of two folding domains that comprise the ligand binding site that resides in the crevice between the folds (Bazan et al., 1990a, b; Thoreau et al., 1991).

IFN α interacts with the type I IFN receptor class that is composed by two chains that dimerize following the interaction with the ligand. After the dimerization two intracytoplasmic receptor-associated kinases (JAK-1 and Tyk-2) are activated and their activation induces the tyrosine phosphorylation of the receptor itself and of cytoplasmic factors called Signal transducer and activator of transcription (STAT) proteins. The latter are a family of latent cytoplasmic transcription factors involved in cytokine, hormone, and growth factor signal transduction (Schindler et al., 1995; Ihle et al., 1996; Darnell et al., 1997; Imada et al., 2000; Takeda et al., 2000; Williams et al., 2000; Bromberg et al., 2001). STAT proteins mediate broadly diverse biologic processes, including cell growth, differentiation, apoptosis, fetal development, transformation, inflammation, and immune response. Once activated the tyrosine phosphorylated sites of the cytokine receptors become docking elements for Src homology 2 (SH2) and phosphotyrosyl-binding domain-containing proteins present in the membrane or the cytoplasmic compartment. Prominent among these are the STATs. Receptor-recruited STATs are phosphorylated on a single tyrosine residue in the carboxyl terminal portion. The modified STATs are released from the cytoplasmic region of the receptor subunits to form homodimers or heterodimers through reciprocal interaction between the phosphotyrosine of one STAT and the SH2 domain of another. Following dimerization,

STATs rapidly translocate to the nucleus and interact with specific regulatory elements to induce target gene transcription. STAT proteins were originally discovered in interferon (IFN)-regulated gene transcription in the early 1990s (Shuai et al., 1993; Sadowski et al., 1993; Darnell et al., 1994). Since then, a number of cytokines have been recognized to activate various STAT proteins. Seven members of the STAT family of transcription factors have been identified in mammalian cells: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 and convincing evidence from genetic mapping studies indicates a common ancestral origin that gave rise to 3 chromosomal clusters of STAT genes through a series of duplication processes (Copeland et al., 1995). Four different domains can be identified in the three-dimensional structure of STAT: the oligomerization domain (that allow the interaction between different STAT molecules); the DNA binding domain (responsible for DNA binding and gene transcription activation); SH2 domain (that mediates the recognition of p-Tyr signals on receptors and receptor associated kinases and other STATs); carboxyl-terminal domain (a regulatory domain of DNA transcription) (Shuai et al., 1993, 1994; Sadowski et al., 1993; Vinkemeier et al., 1996; Horvath et al., 1995; Leonard et al., 1998; Zhang et al., 1995; Wen et al., 1995; Turkson et al., 1999; Bromberg et al., 1998). STAT has only one tyrosine residue (responsible for the regulation of its activity). Moreover, all STATs except STAT2 and 6 bare a second phosphorylation site located on a serine residue near C-terminus involved in the regulation of STAT1 and 3 activities.

The functional role of the different STATs and their implication in cell proliferation is far to be clear. In fact, some STAT proteins are postulated to play important roles in oncogenesis by 2 distinct mechanisms: constitutive activity of the full-length molecule and expression of a C-terminally mutated one. In details STAT3 and 5 have been implicated in the development of haematological and solid neoplasms. Since type I IFN can activate through cross-talk mechanisms these two STAT factors the latter can be one of the molecular mechanisms of escape of tumour cells to the antiproliferative activity of IFN α .

IFN receptor activation classically leads to the phosphorylation and activation of STAT1 and 2. STAT1 and STAT2 form a heterodimer that associates with a member (designated as 9) of the IFN regulatory factor (IRF) family, p48, resulting in the formation of the mature ISGF3 complex that translocates to the nucleus to initiate gene transcription by binding to interferon-stimulated response elements (ISRE) (Darnell et al., 1994, 1997).

Stat 1:1 homodimers, Stat 3:3 homodimers, Stat 1:3 heterodimers, Stat 5:5 homodimers, and CrkL:Stat5 heterodimers are also formed during engagement of the type I IFN receptor since, as described below, also these STAT molecules can be activated by IFN α . These complexes move to the nucleus where they bind to GAS regulatory elements in the promoters of IFN-activated genes (Darnell et al., 1997; Meinke et al., 1996). Thus, signalling specificity via the IFN α -activated Jak/Stat pathway is established by the formation of multiple different complexes that activate distinct regulatory elements in the promoters of IFN-regulated genes.

The protein synthesis as a target of IFN α action

In the past years, the attention of scientists has focused mainly on the study of the genetic information and alterations that regulate eukaryotic cell proliferation and that lead to neoplastic transformation. All therapeutic strategies against cancer are, to date, directed at DNA either with cytotoxic drugs or gene therapy. Little or no interest has been aroused by protein synthesis mechanisms. However, an increasing body of data is emerging about the involvement of translational processes and factors in control of cell proliferation, indicating that protein synthesis can be an additional target for anticancer strategies (Caraglia et al., 2000).

PKR

IFNs regulate at the transcriptional level more than 200 gene products that determine their responses. The protein kinase dependent from dsRNA, PKR, involved in protein synthesis regulation, is one of the best studied of these genes. Human PKR is a serine-threonine kinase of 551 amino acid residues, with two dsRNA binding motifs (DRBM) at its N terminus and a conserved kinase domain at its C-terminus (Meurs et al., 1990). Upon dsRNA binding or by protein-protein interaction with the newly discovered activator protein PACT/RAX,4;5 PKR dimerizes, later autophosphorylates and thus become activated (Levin et al., 1978). Once activated, PKR phosphorylate exogenous substrates. The best characterized substrate of PKR is the small subunit of the eukaryotic initiation factor 2 (eIF2). PKR inhibits translational initiation by phosphorylating eIF2 on serine (Datta et al., 1999). Additionally, PKR also regulates the action of several transcription factors such as NF- κ B, IRF-1, p53, STAT1, NF-90 and it has been proposed to phosphorylate DRBP76. Modulation of all of these different targets

allows PKR to control diverse cellular processes, such as cell growth (Chong et al., 1992), differentiation (Petryshyn et al., 1984) and to exert antitumour activity (Merus et al., 1993; Koromilas et al., 1992). In addition, PKR is differentially regulated during cell cycle (Zamanian-Daryoush et al., 1999) and also induces apoptosis in a manner strictly dependent of its kinase catalytic activity (Lee et al., 1994). The biological importance of PKR function is further remarked by the existence of a multitude of cellular and viral inhibitors of PKR action. Among cellular inhibitors of PKR action we can mention p58 IPK, a protein induced during influenza virus infection and by heat shock (Barber et al., 1994); the eIF-2-associated glycoprotein p67 (Wu et al., 1996), and the Tar RNA binding protein (TRBP) (Park et al., 1994), a cellular protein that binds to the DRBM of PKR independently of dsRNA.

PKR has been involved in apoptosis induced by many different stimuli. In the case of poxviruses, the stimuli resulting in PKR activation upon its expression from VV is most probably dsRNA produced as a result of symmetrical transcription from late genes during VV infection (Kibler et al., 1997).

Evidences pointing to PKR as playing a role in dsRNA-induced apoptosis are also found in studies carried out in 3T3 cells expressing a noncatalytic mutant PKR or using mouse embryonic fibroblasts (MEFs) derived from PKR 0=0 mice (Srivastava et al., 1998; Der et al., 1997). Evidences pointing to PKR as playing a role in dsRNA-induced apoptosis are also found in studies carried out in 3T3 cells expressing a noncatalytic mutant PKR or using MEFs derived from PKR 0=0 mice (Park et al., 1994; Der et al., 1997). However, it must be noted that dsRNA-induced apoptosis is a complex process involving different pathways (Jacobs et al., 1996). For example, the IFN-inducible 2-5A system, composed of dsRNA dependent 2-5A-oligosynthetases and the RNase L, is involved in apoptosis induction (Diaz-Guerra et al., 1997; Zhou et al., 1997). Clearly, both PKR and the 2-5A system can induce apoptosis by independent mechanisms. RNase L is able to induce apoptosis both in PKR + = + and PKR 0=0 cells and this correlates with RNA breakdown and inhibition of protein synthesis (Diaz-Guerra et al., 1997). Although expression of PKR induces activation of RNaseL through an unknown mechanism (Rivas et al., 1998), expression of PKR is still able to induce apoptosis in RNase L 0=0 cells (Gil et al., unpublished observation). Moreover, studies carried out with cells derived from PKR 0=0 mice have shown that the PKR-defective MEFs are more resistant to apoptosis induced by lipopolisaccaride

(LPS), tumour necrosis factor (TNF), and also dsRNA when compared with wild type MEFs (Der et al., 1997). A role for PKR in TNF- α -induced apoptosis had been previously suggested from experiments performed in U937 cells (Harvat et al., 1997). Other studies using 3T3 cells expressing the noncatalytic trans-dominant negative mutant of PKR (K296P), have also involved PKR in TNF- α -induced apoptosis and in apoptosis induced by serum starvation (Yeung et al., 1996). Interestingly, interleukin 3 (IL-3) withdrawal from the IL-3-dependent NFS/N1.H7 cell line, induces PKR autophosphorylation, eIF-2 phosphorylation and also correlates with increased cell death by apoptosis (Ito et al., 1994), thus suggesting involvement of the PKR pathway in growth factor withdrawal-induced apoptosis. Analysis of

the role of PKR targets on mediating apoptosis induction has revealed a complex scenario. In fact, the role of eIF-2a, NF- κ B and p53 on PKR-induced apoptosis has been implicated by different studies. PKR activation regulates translational and transcriptional pathways (eIF-2a and NF- κ B-dependent) resulting in the specific expression of selected proteins (Fas, p53, Bax and others) that trigger cell death by engaging with the caspase pathway (Fig. 1). Through an unknown mechanism, upon PKR activation, FADD recruits procaspase 8, activating it to its active form, caspase 8 than in turn, activates down-stream caspases such as caspase 3, 6, 7, which cleave multiple targets triggering cell death. The role of the caspase 9 pathway in these events is unknown (Gil et al., 2000).

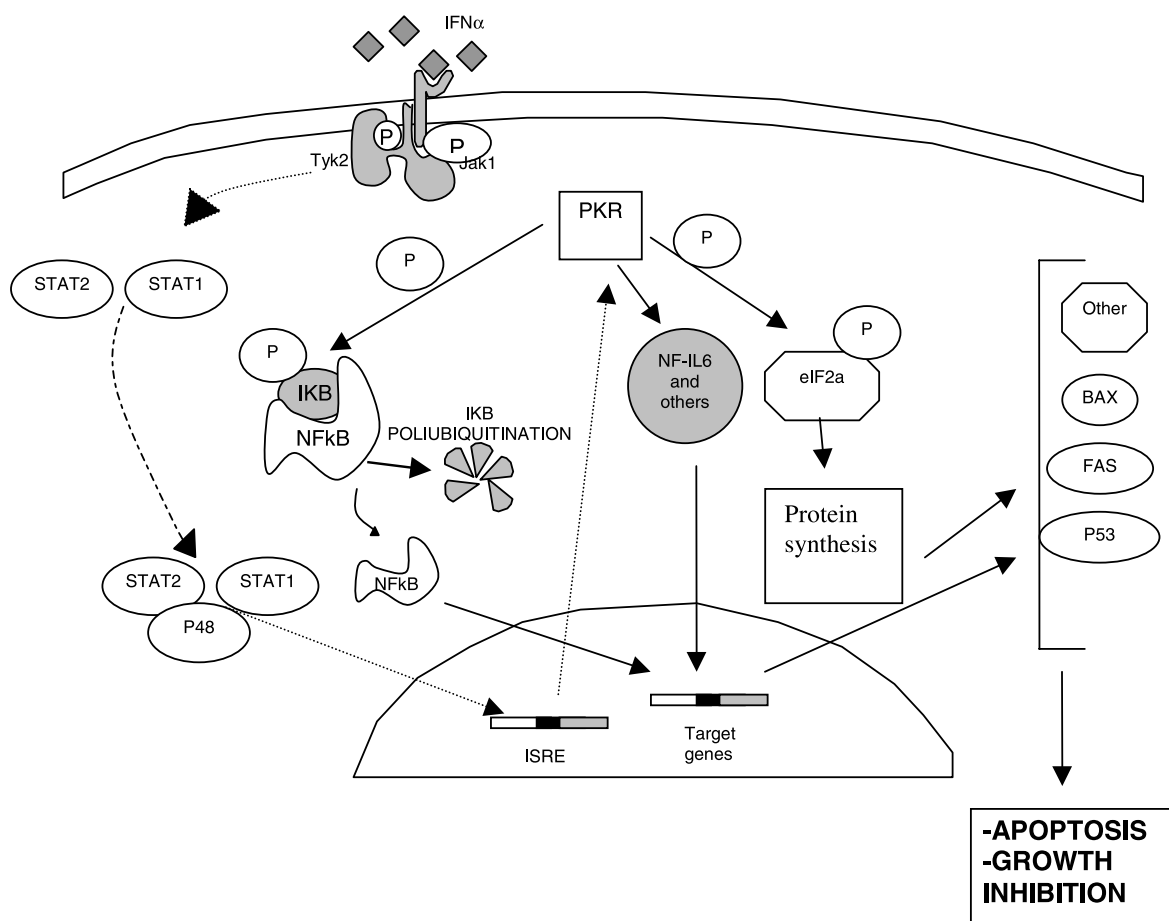


Fig. 1. The regulation of apoptosis and cell cycle by the IFN α -induced PKR. IFN α receptor activation classically leads to the phosphorylation and activation of STAT1 and 2. STAT1 and STAT2 form a heterodimer that associates with a member (designated as 9) of the IFN regulatory factor (IRF) family, p48, resulting in the formation of the mature ISGF3 complex that translocates to the nucleus to initiate gene transcription by binding to interferon-stimulated response elements (ISRE) (dashed arrows, left part of the figure). The interaction of ISGF3 with ISRE induces the transcription of several genes. One of the most relevant among these genes is PKR. PKR, once transcribed and activated, regulates translational and transcriptional pathways (eIF-2a and NF- κ B-dependent) resulting in the specific expression of selected proteins (Fas, p53, Bax and others) that trigger cell death by engaging with the caspase pathway (full arrows, right part of the figure)

The eukaryotic initiation factor-5A of protein synthesis (eIF-5A)

The eukaryotic initiation factor 5A (eIF5A) is peculiar because its activity is modulated by a series of post-translational modifications that culminates in the formation of the unusual amino acid hypusine. Hypusine [N $^{\epsilon}$ -(4-amino-2-hydroxybutyl)lysine] is formed by the transfer of the butylamine portion from spermidine to the ϵ -amino group of a specific lysine residue of eIF-5A precursor (Wolff et al., 1990) and by the subsequent hydroxylation at carbon 2 of the incoming 4-amino-butyl moiety (Park et al., 1993; Abbruzzese et al., 1986). eIF-5A probably acts in the final stage of the initiation phase of protein synthesis by promoting the formation of the first peptide bond (Abbruzzese et al., 1991). Hypusine plays a key role in the regulation of eIF-5A function because its precursors, which do not contain hypusine do not have activity (Park et al., 1991). These biochemical correlates make eIF-5A peculiar. In fact, only the hypusine-containing eIF-5A form is active and, consequently, the dosage of intracellular hypusine content measures also the activity of eIF-5A since hypusine is contained only in this factor. The correlation between hypusine, and thus eIF-5A activity, and cell proliferation (Abbruzzese et al., 1988) suggests that activated eIF-5A might play a role in cell growth and differentiation (Shnier et al., 1991). More recently a correlation has been found between the polyamine-dependent modification of eIF-5A and the triggering of apoptosis in tumour cells (Abbruzzese et al., 1989). In fact, excess putrescine accumulation in hepatoma tissue culture DH23A/b cells induces apoptosis and suppresses the formation of hypusine-containing eIF-5A (Shnier et al., 1991). Furthermore, we have evidenced an *in vitro* post-translational modification of eIF-5A catalyzed by tissue transglutaminase (tTG) (Beninati et al., 1998) that is involved in apoptosis regulation and if stably transfected in Balb-C 3T3 cells strongly reduces hypusine levels. These effects occurred together with a significant reduction of cell proliferation and apoptosis (Beninati et al., 1998). We have reported that IFN α induces growth inhibition and reduction of the activity of eIF-5A in human epidermoid cancer KB cells (Caraglia et al., 1997). The activity of eIF-5A was evaluated through the determination of hypusine levels since this amino acid is essential for the function of this translational factor that is involved in the regulation of cell proliferation and transformation (Caraglia et al., 1997). The cell proliferation regulatory properties of eIF-5A could be correlated by its reported mRNA chaperon

functions since eIF-5A is involved in the transport of mRNAs from the nucleus to the cytoplasm (Lipowsky et al., 2000). It has been also proposed that these mRNAs could encode for proteins involved in the regulation of cell proliferation (Caraglia et al., 2000). We have, moreover, found that IFN α induces cell growth inhibition and apoptosis in human epidermoid cancer cells and these effects are antagonized by EGF. We found also that IFN α is able to induce a strong inhibition of eIF-5A activity since a reduction of hypusine synthesis is recorded with a parallel increase of eIF-5A protein expression. This finding suggests a further reduction of the active fraction of eIF-5A (hypusine-containing eIF-5A:total eIF-5A ratio). On the other hand, when EGF antagonized the apoptosis induced by IFN α a restoration of hypusine synthesis caused by the cytokine and an increase of extracellular signal regulated kinase (ERK) activity are recorded in cancer cells. In the same experimental conditions, we have also found that PD098059, a specific inhibitor of mitogen extracellular signal regulated kinase (MEK-1) and thus of erk, reduces hypusine synthesis and enhanced the decrease of intracellular hypusine content caused by IFN α (Caraglia et al., 2003). Moreover, PD098059 is also able to antagonize the recovery of hypusine synthesis induced by EGF (Caraglia et al., 2003). The reduction of hypusine synthesis could be even higher if tumour cells treated with IFN α did not show an antiapoptotic response based on the hyperactivation of the MEK $\rightarrow$ ERK pathway. Therefore, the addition of PD098059 to IFN α -pretreated cells overcome this survival pathway inducing a potentiation of both hypusine level reduction and apoptosis. On the other hand, the addition of EGF to IFN α -treated cells overstimulated this survival pathway inducing a recovery of both hypusine levels and apoptosis (Caraglia et al., 2003). On the basis of these results we have investigated if eIF-5A could be really critical for the biological effects induced by IFN α . We have used the specific deoxyhypusine synthase inhibitor N1-guanyl-1,7-diaminoheptane (GC7) that avoids hypusine formation and thus blocks eIF-5A activity (Lee et al., 2000). We have found that this agent synergized with IFN α in inducing cell growth inhibition and apoptosis suggesting a critical role for eIF-5A in the modulation of cell proliferation induced by IFN α in human epidermoid cancer cells (Caraglia et al., 2003). All these data support the hypothesis of an involvement of eIF-5A, another protein synthesis regulator, in the apoptosis induced by IFN α in human epithelial cells.

The modulation of protein degradation

The proteasome is a multisubunit enzyme complex that plays a central role in the regulation of proteins that control cell-cycle progression and apoptosis, and has therefore become an important target for anticancer therapy. In fact, the expression of proteins essential for the regulation of cell growth and survival can be also controlled at post-transcriptional and post-translational levels, the latter through the regulation of protein degradation. Before a protein is degraded, it is first flagged for destruction by the ubiquitin conjugation system, which ultimately results in the attachment of a polyubiquitin chain on the target protein. The proteasome's 19S regulatory cap binds the polyubiquitin chain, denatures the protein, and feeds the protein into the proteasome's proteolytic core. The proteolytic core is composed of 2 inner beta rings and 2 outer alpha rings. The 2 beta rings each contain 3 proteolytic sites named for their trypsin-like, post-glutamyl peptide hydrolase-like (i.e., caspase-like), or chymotrypsin-like activity. Inhibition of the proteasome generally results in cell-cycle arrest and apoptosis (Adams et al., 2003). Since 1996 it has been demonstrated that type I IFNs (IFN τ) can regulate the expression of a 16-kDa protein that is produced by the bovine endometrium during early pregnancy and that shares epitopes with hUCRP and ubiquitin (Austin et al., 1996).

These results were confirmed by a study performed with metabolic labeling and two-dimensional gel electrophoresis followed by MS and database searches to identify potentially new IFN α -induced proteins in human T cells. By this analysis, it was shown that IFN α induces the expression of ubiquitin cross-reactive protein (ISG15) and two ubiquitin-conjugating enzymes, UbcH5 and UbcH8. Northern-blot analysis showed that IFN α rapidly enhances mRNA expression of UbcH5, UbcH6 and UbcH8 in T cells. In addition, these genes were induced in macrophages in response to IFN α . Similarly, IFNs enhanced UbcH8 mRNA expression in A549 lung epithelial cells, HepG2 hepatoma cells, and NK-92 cells. Cycloheximide, a protein synthesis inhibitor, did not block IFN-induced upregulation of UbcH8 mRNA expression, suggesting that UbcH8 is the primary target gene for IFN α (Nyman et al., 2000). More recently, it was demonstrated that administration of interleukin 1 β (IL-1 β) *in vivo* attenuates IFN α -induced STAT1 tyrosine phosphorylation in the liver but not in the spleen. The inhibitory action of IL-1 β *in vivo* is not affected by depleting hepatic Kupffer cells, suggesting that IL-1 β may directly target IFN α signalling in hepatocytes. Indeed, pretreatment of human hepatocellular carcinoma HepG2 cells with IL-1 β suppresses IFN α -induced antiviral activity and protein MxA mRNA expression. Furthermore, IL-1 β attenuated IFN α -induced STAT1

Table 1. Data about the correlations between the protein degradative and the IFN α -dependent pathways

Proteasome component	Molecular target	Source	Biological effect	Ref.
16 kD protein similar to hUCRP and ubiquitin	Unknown	Bovine endometrium during early pregnancy	Unknown	(Austin et al., 1996)
● Ubiquitin cross-reactive protein ● Ubiquitinating enzymes (UbcH5 and UbcH8)	Unknown	T cells	Unknown	(Nyman et al., 2000)
Unknown	STAT1	Hepatocellular carcinoma cells	Attenuation of biological effects of IFN α by IL1 β	(Tian et al., 2000)
Unknown	tTG	Epidermoid cancer cells	Mediates apoptosis induced by IFN α	(Esposito et al., 2003)
Unknown	STAT3	Epithelial cells	Attenuation of biological effects of IFN α by Mumps virus	(Ulane et al., 2003)
Unknown	IFN-AR, JAK-1, Tyk-2, STATs	Epithelial cells	Attenuation of biological effects of IFN α by the physiological inactivator SOCS	(Larsen et al., 2002)

binding and tyrosine phosphorylation without affecting the level of STAT1 protein. This inhibitory effect can be reversed by pretreatment with either proteasome inhibitors or transfection of dominant negative NF- κ B inducing kinase mutants. Taken together, these findings suggest that IL-1 β attenuates IFN α -induced STAT1 activation by a proteasome-dependent mechanism (Tian et al., 2000). Moreover, we have recently found that IFN α induces apoptosis through, at least in part, the increase of the expression and activity of tTGase in human epidermoid lung cancer cells (Esposito et al., 2003). The increase of the expression of tTGase was not due to the induction of its transcription, but to a decrease of its degradation via a proteasome-dependent pathway. Therefore, IFN α modulates apoptosis through the regulation of the degradation of intracellular proteins involved in the triggering of apoptotic process. Not only the target of the IFN α -dependent signalling but also the components of the pathway activated by the cytokine itself can be subjected to regulation via proteasome-dependent degradation. In fact, Mumps virus, a common infectious agent of humans, causing parotitis, meningitis, encephalitis, and orchitis, induces degradation of STAT3 mediated by the ubiquitination and subsequent proteasome-dependent degradation (Ulane et al., 2003). The latter could be a mechanism by which viruses protect themselves by the anti-viral action of IFN α . Finally, one of the modes used by SOCS to turn off the IFN α -dependent signalling is the delivery of the transductional components to the degradative proteosomal machinery (Larsen et al., 2002). For a summary see Table 1.

Perspectives and future directions

More germane to clinical practice is the possibility that IFN α treatment could be improved by the concomitant administration of agents known to enhance JAK-STAT responses; the use of retinoids in combination to IFN α in cancer therapy is a salient example (Ransohoff et al., 1998; Harvat et al., 1997). However, on the basis of our previous findings, different therapeutic strategies are under preclinical investigation in order to increase the anti-cancer activity of IFN α . We can hypothesize that the selective interference on eIF-5A activity could be an additional target in order to potentiate the antitumour efficacy of IFN α . In fact, we have found that the hypusine synthesis inhibitors, and thus eIF-5A inactivator, GC7 synergizes with the cytokine in the induction of cell growth inhibition and apoptosis (Caraglia et al., 2003). We have recently performed a computer-based prediction

of the three dimensional structure of eIF-5A in order to define the structure of the hypusine-containing site (Facchiano et al., 2001). We are now planning a pharmacological screening of drugs with potential eIF-5A-inhibiting properties. The inhibition of eIF-5A either through the blocking of hypusine synthesis (mediated by agents similar to GC7) or the selective binding with the hypusine-containing site could represent a new scenario of intervention in anti-cancer therapy based on IFN α administration. Moreover, the involvement of proteasome-dependent degradation of key molecules involved in the regulation of cell cycle and apoptosis (tTG and p27) encourages the investigation on the use of proteasome inhibitors in combination with IFN α in the therapy of human neoplasms. Finally the use of viral vectors encoding tTG or of pharmacological activators of tTG (such as retinoic acid) could be other tools in order to enhance the antitumour activity of the cytokine.

In conclusion, the understanding of the molecular mechanisms regulating apoptosis and cell growth inhibition mediated by IFN α could be useful in the design of new therapeutic strategies based on the use of IFN α and in order to widen the therapeutic window of this cytokine.

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