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Invited Review

Interferons and cell growth control

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Summary. Cytokines modulate cell growth, differentiation, and immune defenses in the vertebrates. Interferons (IFNs) are a unique class of cytokines that stimulate antiviral, antitumor and antigen presentation by inducing the expression of several cellular genes. Recent studies have identified a novel gene regulatory pathway activated by IFNs, which serves as a paradigm for most cytokine signal transduction pathways. A number of genes induced by IFNs participate in cell growth regulation and apoptosis. These include novel tumor suppressor genes. Although discovered as IFN-regulated factors, deletions of these genes cause leukemias in experimental models and in human patients. Genetic approaches have identified several novel regulators of apoptosis. Studies on the mechanism of action of these growth regulatory molecules are not only useful in identifying novel targets for the development of therapeutics but also help understand the molecular basis for loss of cell growth control and resistance to IFNs. This review focuses on the functions and roles of IFN regulated factors in cell growth control and mechanisms of disruption of IFN action in cancer cells.

Key words: Cytokines, Signaling, Gene regulation, Apoptosis, Cancer

Introduction

Interferons (IFNs) area family of proteins that regulate innate immunity, immune surveillance, and homeostasis of peripheral blood cell populations. Binding to their high affinity cell surface receptors, IFNs stimulate a variety of genes employing unique signaling molecules. IFNs inhibit virus replication, and activate antipathogen defenses, and suppress tumor growth. A subclass of IFNs modulates the trophoblastic implantation in domestic ruminants (Imakawa et al., 1987). In this article the mechanisms of IFN action, signal transduction pathways, and the gene products that cause cell growth arrest will be discussed. It is conceivable that genetic or epigenetic perturbations in these pathways can lead to either an over stimulation of certain cellular functions or make cells resistant to a given ligand, manifesting clinical disorders such as cancer.

Distinct genes encode IFN-a, IFN-B, IFN-ω and IFN-γ, the major forms of IFN. There are multiple IFN-a, and single IFN-B and IFN-y genes in humans (Pestka, 1997) IFN-a and IFN-B genes are clustered on human chromosome 9. IFN-y gene is located on human chromosome 12. Most IFNs are 16-18 kDa in size and post-translationally modified prior to their secretion (Pestka, 1997). A number of agents such as viruses, double-stranded (ds) RNA, LPS, TNF and IL-1 induce the expression of IFNs (Fujita et al., 1989a,b). Expression of IFN-y gene is restricted to the activated T-cells and NK cells (Young and Ghosh, 1997). IFN-genes are regulated by specific transcription factors in a stimulus dependent manner (Falvo et al., 1995; Thanos and Maniatis, 1995; Wathelet et al., 1998). Among IFNs the regulation of IFN-B gene is most clearly elucidated. The IFN-B gene promoter consists of multiple positive and negative regulatory elements on which members of Jun-ATF, IFN-gene regulatory factor (IRF) and Rel-NF-kB families of transcription factors and HMG-I(Y) assemble a virus directed transcriptional complex that regulates transcription (Fujita et al., 1989a,b; Taniguchi et al., 1997; Thanos and Maniatis, 1995).

Receptors

IFNs bind to specific cell surface receptors and transmit the signals to upregulate various cellular responses. IFN-α/ß bind to a multimeric cell surface receptor. The IFN-y receptor is a distinct complex consisting of two different polypeptides (Domanski and Colamonici, 1996). These receptors are glycosylated in the extracellular domains and intrinsic tyrosine kinase activities are absent in the cytoplasmic domains. However, tyrosine kinases of the Janus kinase (JAKs)

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family associate and phosphorylate the receptor in ligand dependent manner. JAKs then activate several transcription factors that regulate the expression of IFN-stimulated genes (ISGs). Genes for the two major known subunits of IFN-α/ß receptor, IFNAR1 (Uze et al., 1990), IFNAR2 (Lutfalla et al., 1995) are located on human chromosome 21 (Domanski and Colamonici, 1996). Unlike the IFN-γ receptor, the IFN-α/ß receptor can interact with atleast a dozen IFN-α subtypes, IFN-ß, IFN-ω and IFN-τ with varying affinities and generate various biological responses (Mouchel-Vielh et al., 1992; Ghislain et al., 1995; Domanski and Colamonici, 1996). Genes encoding the IFNGRα, IFN-γ binding poly-peptide, and the IFNGRß, the signal transducing chain are present on human chromosomes 6 and 21 respectively. After the generation of signals, receptor-bound IFN-γ is internalized and the ligand is degraded in the endosomes leaving the receptor chains in the cytoplasm (Marsters et al., 1995). Consistent with their physiologic importance, targeted disruption of IFN-α and IFN-γ genes and their receptors results in an extreme susceptibility of mice to bacterial, viral and parasitic infections (Huang et al., 1993; Muller et al., 1994; Hwang et al., 1995). Mutations in human IFN-γ receptor increase susceptibility to infections (Jouanguy et al., 1996; Newport et al., 1996), probably due to a failure to induce the expression of ISGs. Certain members of the poxvirus family encode analogues of IFN-γ and IFN-α receptors (Alcami and Smith, 1995; Kalvakolanu, 1999). Unlike their vertebrate orthologues, these viroceptors can bind to IFNs from a variety of species and suppress their biological actions.

Regulation of gene transcription by IFNs

IFNs induce disparate biological responses primarily by the transcriptional activation of various ISGs (Sen and Ransohoff, 1997; Stark et al., 1998) using the JAK-STAT signaling pathway (Darnell, 1997; Stark et al., 1998). ISGs can be classified into three major types as: 1) IFNs α/ß inducible, 2) IFN-γ inducible and 3) those inducible by all IFNs. Identification of these genes suggested a clear overlap between the IFN-α/ß and IFN-γ signaling pathways. All IFN-α/ß and some IFN-γ responsive gene promoters contain an IFN-Stimulated Response Element (ISRE). The consensus sequence for ISRE is AGGTTTCNNTTTCCT. The thymine triplets of ISRE are crucial for generating IFN-α/ß responses. IFN-γ stimulated genes contain a variety of response elements (Sen and Ransohoff, 1997). The kinetics and requirements for de novo protein synthesis of these genes are quite variable. Certain IFN-γ stimulated genes are directly induced by STATs (signal transducing activators of transcription) while others are activated by secondary regulatory factors (Sen and Ransohoff, 1997). The γ-IFN activated site (GAS) (Decker et al., 1991) (canonical sequence TTNNNNNAA) was the first described STAT-binding element. Most cytokines activate different STATs that bind to GAS-like elements, which vary in the number of central bases.

Specific IFN-stimulated gene factors (ISGF) bind to the response elements and regulate gene expression. Nuclear extracts from IFN-α treated cells form three major complexes with ISRE, ISGF1, ISGF2 and ISGF3 (Dale et al., 1989; Levy et al., 1989). ISGF1 and ISGF2 are constitutive nuclear proteins, although IFN-α slightly increases the levels of ISGF2. ISGF3 is observed only in IFN-α/ß treated cells. Upon activation in the cytoplasm, it migrates to nucleus and induces gene expression (Dale et al., 1989; Levy et al., 1989). ISGF2 is identical to IRF-1 (IFN-gene Regulatory Factor 1), which regulates the IFN-ß gene transcription (Fujita et al., 1989a,b; Pine et al., 1990). ISGF3 is composed of three proteins: a 48 kDa DNA binding protein (ISGF3γ or IRF9) and 2 tyrosine phosphorylated proteins of 91- and 113-kDa. The latter two are now known as STAT1 and STAT2, respectively. The IFN-γ responsive element, GAS, binds a dimer of STAT1 (Shuai, 1993). P48 belongs to the IRF-myb family of transcription factors (Veals et al., 1992), involved in cell growth and immune cell functions.

Structure and function STAT proteins

STAT (signal transducing activator of transcription) proteins are a unique class of bifunctional molecules. The first two members of this family, STAT1 and STAT2, participate in the IFN-initiated JAK-STAT pathway (Darnell et al., 1994). Four other STATs, which have a similar modular structure, participate in various cytokine signaling pathways (Darnell, 1997). Most STATs: 1) interact with the cytoplasmic tails of cytokine receptors, 2) are tyrosine phosphorylated by JAKs in a ligand dependent manner, 3) possess SH2 and SH3 (SH= src homology) domains (Koch et al., 1991), 4) homo- and hetero-dimerize with other members of the family, 5) translocate to the nucleus after activation and dimerization, 6) bind to DNA in a sequence-specific and activation-dependent manner, and 7) induce gene transcription. STATs consist of five functional domains (Fig. 1A). The first 50-300 amino acids constitute the

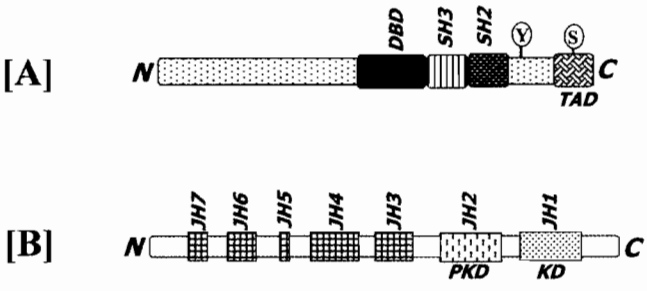

Fig. 1. A. Structure of a STAT molecule. DBD: DNA binding domain; SH: Src homology domain; TAD: Transcription-activating domain; N: amino-terminus; C: carboxyl terminus; Y: tyrosine; S: Serine. **B.** Structure of a Janus tyrosine kinase. JH: JAK homology domain; KD: kinase domain; PKD: pseudo-kinase domain; N: amino-terminus; C: carboxyl terminus. KD has the enzymatic activity.

domains that permit their heteromeric interaction with other STAT or non-STAT proteins (Horvath et al., 1996), ligand-dependent and ligand-independent pre-association with the receptors, and recognition by phosphatases (Darnell, 1997). A module between 300-400 amino acids contains a DNA binding domain (Darnell, 1997). Amino acids 450 to 650 form the SH3, SH2 domains. The SH2 domains play a critical role in homo- or heterodimerization (Shuai et al., 1993, 1994) and maintenance of the signal fidelity (Heim et al., 1995; Leung et al., 1995). The SH2 domains are important not only for the binding of STATs to the ligand-engaged receptor but also to the JAKs (Gupta et al., 1996; Darnell, 1997). 4) A conserved tyrosine residue at 6-8 amino acids downstream of the SH2 domain (Shuai et al., 1993; Darnell, 1997) is crucial for dimerization. 5) The C-terminus contains a trans-activation domain (TAD). Although STATs are primarily regulated by tyrosine phosphorylation, phosphorylation at a specific serine residue in the TAD is essential for optimal gene transcription (Eilers et al., 1995; Wen et al., 1995; Zhang et al., 1995).

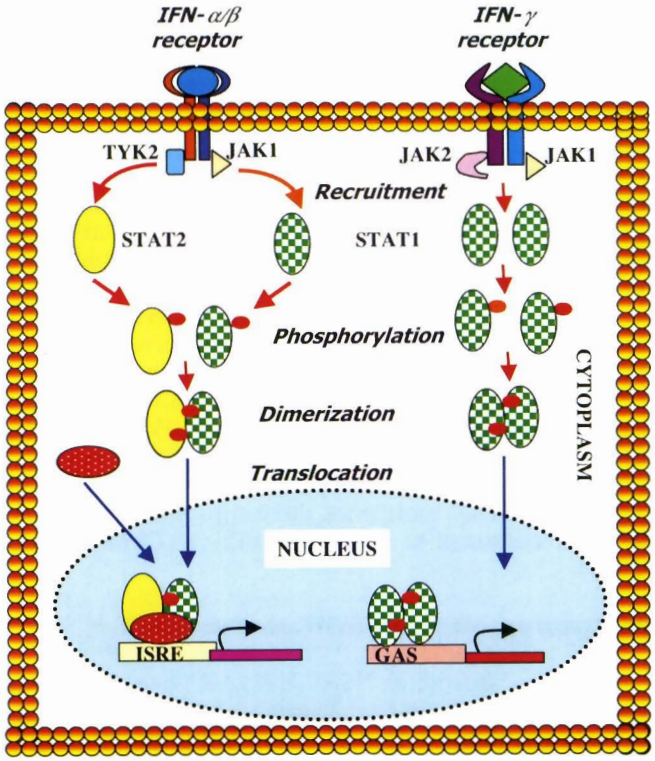

Fig. 2. Signal transduction by Interferons. IFN-α/ß using JAK1 and Tyk2 induce the tyrosine phsophoryaltion of the intracellular domain of receptor, which recruits STAT proteins. Upon phosphorylation a dimer of STAT1, STAT2 is formed. The dimer migrates to the nucleus, associates with the p48 protein (IRF9), binds to the IFN-stimulated Response Element (ISRE) and induces gene expression. Similary, IFN-γ induces the aggregation and tyrosine phosphorylation of a distinct receptor and STAT1. After tyrsoine phosphorylation, a STAT1 dimer is formed which induces gene expression upon translocation to the nucleus.

Janus tyrosine kinases

Most JAKs have been identified by polymerase chain reaction (Firmbach-Kraft et al., 1990; Wilks, 1991). Because their physiologic functions were unclear at the time of identification, they were designated as Just Another Kinases (JAK). These enzymes contain two tyrosine kinase domains only one of which is functional. These two domains are the basis for drawing an analogy with the roman god Janus, who possesses two heads, and hence the name Janus kinases. JAKs are 120-130 kDa in size and contain seven conserved structural motifs unique to their family, known as JAK homology (JH) domains (Wilks, 1991). The tyrosine kinase activity is present only in the JH1 but not in the JH2 domain (Fig. 1B). JAKs are devoid of SH2 or SH3 domains, found in src-family kinases. To date 4 JAKs: JAK1, JAK2, JAK3 and Tyk2 have been identified (Firmbach-Kraft et al., 1990; Wilks, 1991; Kirken et al., 1994; Rane and Reddy, 1994). JAK3 is mostly expressed in hematopoietic cells (Witthuhn et al., 1994; Nosaka et al., 1995). Most JAKs require another JAK to be functional, because intermolecular but not intramolecular tyrosine phosphorylation of JH1 domain activates the signaling (Darnell, 1997). The juxtapositioning of JAKs is accomplished by ligand-induced receptor oligomerization of receptor polypeptide chains, which brings JAKs together. Although an overwhelming number of studies show the interaction of JAKs with cytoplasmic tails of cytokine receptors, other reports have shown the presence of these enzymes in the nucleus (Lobie et al., 1996). Thus, JAKs can participate in undefined nuclear events.

IFN-γ signaling

Binding of dimeric IFN-γ to 2 molecules of IFNGR-α (the ligand binding chain) initiates the ligand-induced assembly of the signaling complex (Bach et al., 1996). IFNGR-α is pre-associated with JAK1 in unstimulated state (Muller et al., 1993; Sakatsume et al., 1995; Kaplan et al., 1996) (Fig. 2). Following this, two molecules of signal transducing IFNGR-ß chain, pre-associated with JAK2 (Watling et al., 1993), are brought into the complex (Greenlund et al., 1995). Such aggregation of the receptor polypeptides triggers the mutual phosphorylation of JAKs and of the IFNGR-α chain by JAK1 (Greenlund et al., 1995). It is not clear which JAK phosphorylates the other first (Muller et al., 1993; Watling et al., 1993). Thus, the JAK-STAT pathway does not involve kinase cascades. Phosphorylated tyrosine residue 440 of IFNGR-α chain serves as a docking site for the SH2 domain of unphosphorylated STAT1 (Greenlund et al., 1994; Heim et al., 1995). Subsequently, JAK2 phosphorylates STAT1 at tyrosine 701 (Muller et al., 1993; Shuai et al., 1993). Phosphorylated STAT1 brings the second STAT1 molecule in to the

complex and promotes its tyrosine phosphorylation. Later on the phosphorylated STAT1 molecules dissociate from the receptor, form a stable head-to-tail dimer, and migrate to the cell nucleus (Shuai et al., 1994). The SH2 domain of STAT1 plays a central role in regulating IFN-γ response (Heim et al., 1995). Mutation of a critical arginine (at 602) in the SH2 domain of STAT1 disrupts its recruitment and phosphorylation following IFN-γ or IFN-α treatment (Heim et al., 1995). Similarly a mutant STAT1 lacking tyrosine 701 fails induce the IFN response (Muller et al., 1993; Shuai et al., 1993). In addition to tyrosine phosphorylation at 701, serine phosphorylation at 727 is essential for strong transcriptional stimulation by STAT1 (Eilers et al., 1995; Wen et al., 1995; Zhang et al., 1995).

IFN-α/ß signaling

The IFN-α/ß receptor consists of atleast two polypeptides, the IFNAR1 and IFNAR2 (also known as IFNAR2.2). The cytoplasmic tail of IFNAR1 (Yan et al., 1996) is pre-associated with Tyk2 (Fig. 2). JAK1, STAT1 and STAT2 weakly interact with the intracellular domain of IFNAR2. Binding of IFN-α/ß to the receptor induces tyrosine phosphorylation of JAK1 and Tyk2 (Velazquez et al., 1992; Barbieri et al., 1994; Gauzzi et al., 1996). Activated Tyk2 then phosphorylates the tyrosine (at 466) of IFNAR1. This phosphotyrosine serves as an anchor for STAT2 (Yan et al., 1996) Tyrosine phosphorylation of STAT2 at position 690 creates a binding site for STAT1 (Leung et al., 1995). STAT1 is then transferred from IFNAR2 to STAT2. JAK1 then phosphorylates STAT1 (Leung et al., 1995). The respective SH2 domains of STATs are important for specific binding to phosphotyrosines.

The STAT2-STAT1 heterodimer dissociates from its receptor; migrates to the nucleus and associates with p48 (ISGF3γ) to form ISGF3 (Horvath et al., 1995), which stimulates transcription. All three proteins, p48, STAT1, and STAT2, make contact with the bases in ISRE (Qureshi et al., 1995, 1996). Unlike STAT1, STAT2 is not phosphorylated at serine. IFN-α also stimulates STAT3, which serves as an adapter for the docking of PI3-kinase to IFN-receptor (Pfeffer et al., 1997). However, the biological significance of such activation is unclear. In summary, ligand interaction with IFN-α/ß receptor activates STAT1, STAT2 and STAT3. In certain hematopoietic cells STAT5 is recruited and phosphorylated by Crk, kinase (Fish et al., 1999). In summary, the biological responses generated by IFN-γ and IFN-α/ß receptors are critically dependent on JAK1 and STAT1 (Muller et al., 1993a,b).

Termination of JAK-STAT signaling

Tyrosine phosphatases inactivate ligand-generated signals, by removing the phosphates from activated substrates (Hunter, 1995). Evidence for the role of tyrosine phosphatases in the IFN induced JAK-STAT pathway is mostly indirect. Inhibitors of phosphatases prolong the STAT activated gene expression (Igarashi et al., 1993). Some studies suggest that proteasomes degrade the activated STAT1α by limiting duration of the signals, because inhibition of proteasomes results in the persistence of STAT1α mediated signals (Kim and Maniatis, 1996). Other studies suggest that STAT activity is switched off primarily by phosphatases and proteasomes play a minimal role in this process (Haspel et al., 1996).

Recent studies identified several cellular inhibitors of JAK-STAT pathway (Hilton et al., 1998). A family of proteins, SOCS (Suppressor of cytokine signaling), JAB (Jak Binding proteins) or SSI (STAT induced STAT inhibitor) inhibit the cytokine inducible activation of STATs (Endo et al., 1997; Naka et al., 1997; Starr et al., 1997). These proteins bind to JAKs and suppress their tyrosine kinase activity (Starr et al., 1997). Interestingly, these inhibitors are induced by the same ligands that activate the STATs (Naka et al., 1997), indicating a feed back inhibition of JAK-STAT pathway. This idea is further strengthened by the observation that certain SOCS proteins are induced only by specific STATs. For example, SSI is specifically induced by STAT3 but not by other STATs (Naka et al., 1997). Over expression of certain SOCS genes blocks the antiproliferative and antiviral actions of IFN-α (Endo et al., 1997) SOCS may play a critical role in the regulation of cytokine induced apoptosis. Deletion of SSI-1 gene causes growth retardation and enhances apoptosis of lymphocytes (Naka et al., 1998). In $SSI^{-/-}$ cells cell death activating protein Bax is strongly upregulated, thus indicating that SOCS may maintain tissue homeostasis by inhibiting excessive cytokine responses in vivo (Naka et al., 1998).

In addition to SOCS, the newly discovered protein inhibitors of activated STATs (PIAS) have also been suggested to terminate JAK-STAT induced signals (Chung et al., 1997). PIAS1 and PIAS3 bind to and inhibit STAT1 and STAT3, respectively. PIAS3 inhibits DNA binding and gene activating functions of STAT3 (Chung et al., 1997). It appears that physical level of PIAS and STAT molecules determine the outcome of cellular response to a given cytokine (Chung et al., 1997).

Biological effects of JAK-STAT gene disruption

Targeted disruption STAT and JAK genes have been reported. $STAT1^{-/-}$ mice develop normally with no gross defects except for a loss of innate immunity (Durbin et al., 1996; Meraz et al., 1996). No other cytokine signaling defects are observed in cells derived from $STAT1^{-/-}$ mice (Durbin et al., 1996; Meraz et al., 1996). These observations indicate that STAT1 is specifically required for IFN signaling. In contrast disruption of STAT2, which is used only by type I IFNs, causes embryonic lethality (Darnell, 1997). This observation suggests that STAT2 is required for the embryonic development. Deletion of STAT3 causes embryonic

Table 1. Cell growth regulators modulated by IFNs.

GENE	FUNCTION	EFFECT
PKR	Protein kinase	Growth arrest and stress-induced apoptosis
IRF1	Transcription factor	Tumor suppression
IRF2	Transcription factor	Oncogenic growth promoter
ICSBP	Transcription factor	Tumor suppressor
STAT1	Transcription factor	Inhibition of cell cycle and promotion of apoptosis
DAP1	Unknown	Apoptosis
Cathepsin-D	Protease	Apoptosis
Thioredoxin	Redox-regulator	Apoptosis
DAPK	Protein kinase	Apoptosis, Antimetastatic
DAP5	Regulator of protein synthesis	Apoptosis
DAP3	GTP binding protein	Apoptosis
ICE	Protease	Apoptosis
Waf/Cip-1	Cell cylce inhibitor	Growth arrest
p202	Cell Cycle regulator	Growth arrest

lethality (Takeda et al., 1997). This observation is consistent with participation of STAT3 in the signaling pathways initiated by several growth promoting ligands (Darnell, 1997). Indeed, STAT3 expression is observed right from the onset of embryogenesis (Duncan et al., 1997). STAT4$^{-/-}$ mice lack T_H1 type of immune responses (Kaplan et al., 1996). STAT5 is essential for the development of mammary gland as evidenced by lack of the breasts in the knock-out mice (Liu et al., 1997). STAT6$^{-/-}$ mice are born with lymphoid defects in their IL4 responses. Consequently, they lack T_H2 immune responses (Shimoda et al., 1996; Takeda et al., 1997).

Similar to STATs deletion of JAKs causes distinct phenotype in mice. Despite their normal birth, postnatal lethality occurs in JAK1$^{-/-}$ mice (Rodig et al., 1998). JAK1$^{-/-}$ pups fail to nurse and die. Neurons from these mice undergo rapid apoptosis compared to those from wild type mice. It is likely that neuronal development is incomplete in JAK1$^{-/-}$ mice. These observations are consistent with a crucial role for JAK-STAT signaling in gliogenesis (Stahl and Yancopoulos, 1994; Bonni et al., 1997). Deletion of JAK2 causes embryonic lethality (Neubauer et al., 1998; Parganas et al., 1998). In these mice definitive hematopoiesis is absent. JAK3$^{-/-}$ mice develop severe combined immune deficiency (Nosaka et al., 1995). This phenotype correlates well with a population of humans who have similar disease owing to a defective JAK3 gene (Lai et al., 1995; Russell et al., 1995; Candotti et al., 1996, 1997). The Tyk2 gene has not yet been knocked out. However, given the role of this kinase in CNTF, IL-6, and LIF signaling pathways, its deletion may be lethal or cause developmental abnormalities (Darnell, 1997).

Functions of IFN-induced genes

A number of IFN-stimulated gene products inhibit viral growth, participate in immune defenses, antigen presentation, and tumor suppression (Sen and Ransohoff, 1997). Using the "gene chip" technology it has discovered that IFNs induce ~250 different genes in human cells. In addition 10 others genes are down regulated. The biological roles of many of these genes are undefined (Der et al., 1998). In this review, ISG products involved in cell growth regulation will be discussed in detail. Table 1 summarizes the functions of various known IFN-stimulated genes and their functions in cell growth control. Several gene products are probably responsible for the antitumor actions of IFNs (Kalvakolanu and Borden, 1996; Sen and Ransohoff, 1997). Among these the most well characterized ones are: i) the 2-5A dependent RNA degradation pathway (Zhou et al., 1993; Silverman, 1997), and ii) the PKR (protein kinase R) dependent protein synthesis inhibitory pathway (Meurs et al., 1990; Williams, 1997). Both these pathways require double stranded RNA for regulating cell growth (Sen and Ransohoff, 1997).

Cell growth inhibition by RNA degradation

IFNs induce cell growth arrest using a ribonucleolysis. Two different IFN stimulated gene products are required for mediating this process. The first step in this pathway includes the synthesis of specific activators of the ribonuclease, which degrades RNAs. A family of 2',5'-oligoadenylate synthetases (2-5AS), that convert ATP into 2'-5' linked oligoadenylates (2-5A) (Hovanessian, 1991). In the second step the 2-5A activates a dormant endoribonculease (RNaseL) leading to the cleavage of RNAs and growth arrest (Zhou et al., 1993; Silverman, 1997). Multiple isoforms of 2-5AS are present in mammalian cells. Based on their size they can be classified in to large (100 kDa), medium (69 kDa) and small (36-45 kDa) isoforms. The intracellular locations are quite different. The mRNAs that encode these enzymes are generated either by different genes or by the same gene via differential splicing (Rutherford et al., 1991). Although the individual functions of these isozymes are unclear, they cause growth arrest upon over-expression in cells (Salzberg et al., 1997).

Under normal circumstances, RnaseL is enzymatically inert. Binding of 2-5A to its N-terminus stimulates its ribonuclease activity. Mutant RnaseL, that

lacks the C-terminus (Hassel et al., 1993), is a dominant inhibitor of IFN-α induced cell growth arrest (Silverman, 1997; Zhou et al., 1997). It has been proposed that a localized activation of RnaseL triggers the degradation of specific RNAs involved in growth promotion (Silverman, 1997). These properties of the enzyme have been exploited for the development of ribozymes that can cleave tumor specific mRNAs. In this approach a chemically synthesized 2-5A is covalently linked to a gene specific antisense oligonucleotide. Following uptake of this oligo by the cells, it binds to the cognate RNA. As a result of RnaseL recruitment to the chimeric oligo only that particular RNA is selectively destroyed. Experimental feasibility of using such oligos for cancer therapy has been recently demonstrated by a targeted degradation of bcr-abl mRNA, an oncogene that promotes the growth of chronic myelogenous leukemia (Maran et al., 1998).

Inhibition of protein synthesis

Certain ISG products inhibit the translation of proteins. This also causes cell growth suppression. For example the IFN-inducible Protein Kinase R (PKR) is activated by double stranded RNA leading to its autophosphorylation, and trans-phosphorylation of eukaryotic protein synthesis factor-2α (eIF-2α). Phosphorylation of the latter casues the cessation of polypeptide chain initiation (Meurs et al., 1990; Williams, 1997). Overexpression of PKR in yeast and mammalian cells inhibits cell growth (Chong et al., 1992). Although a mutant PKR, which does not bind dsRNA, causes cellular transformation in NIH/3T3 cells (Koromilas et al., 1992), no increase in tumor incidence is observed in $PKR^{-/-}$ mice. Suppression of a number of death-activating genes by the dominant negative inhibitor of PKR may explain its transforming properties (Balachandran et al., 1998).

PKR has also been implicated in the regulation of transcription factor NF-κB (Kumar et al., 1994). NF-κB is retained in the cytoplasm by an inhibitory protein IκB in the unstimulated cells. Signal induced phosphorylation and degradation of IκB, causes the nuclear translocation of NF-κB and gene induction. The latter regulates the expression of several genes involved in inflammatory and growth responses (Baldwin, 1997). Double stranded RNA activated NF-κB and the consequent cellular gene induction are defective in $PKR^{-/-}$ cells (Kumar et al., 1994). Immediate early gene induction by PDGF also requires PKR (Mundschau and Faller, 1995). Defects in cytokine responses and induction of apoptosis, due to an impairment of Fas expression, are also reported in $PKR^{-/-}$ cells (Der et al., 1997; Kumar et al., 1997a,b). PKR maps to a locus deleted in myelodysplasia (Hanash et al., 1993). PKR is also implicated in the phosphorylation of transcription factor IRF1, a known growth regulator (Der et al., 1997).

Regulation of PKR activity by dsRNA can be achieved by viral RNAs or replication intermediates of viral nucleic acids. However, the source of dsRNA of PKR activation in cancer cells is unknown. Therefore, it is possible that other factors can potentially regulate PKR. Consistent with this, a recent study has identified a PKR activating protein, PACT. Identification of the physiological signals that activate PACT or molecules like it may further delineate the role of PKR in cell growth regulation.

Alternatively PKR independent pathways can regulate protein synthesis and cell growth in response to IFNs. The product of ISG-561 gene binds to the p48 subunit of eIF3 and inhibits translation in vitro (J. Guo and G.C. Sen submitted). This process is also independent of dsRNA. The physiologic role and impact of ISG-561 encoded protein in cell growth regulation needs to be analyzed further.

The IRF-myb family of transcription factors

The founding member of the IRF family, IRF1 was originally discovered as a transcriptional inducer of IFN-ß gene. Subsequent analyzes have shown that IRFs represent a family of proteins, which have structural similiarities in their DNA binding domain and play diverse roles in cell growth regulation. These molecules regulate both cell proliferation and immune responses. Other members of this family include IRF2, IRF3, IRF4 (Pip), IRF7, IRF8 (ICSBP), IRF9 (also knows as p48 or ISGF3γ, the DNA binding subunit of ISGF3γ, and the V-IRF of Kaposi's sarcoma associated herpes virus (Fujita et al., 1989a,b; Harada et al., 1989; Nelson et al., 1993; Veals et al., 1992; Au et al., 1995; Eisenbeis et al., 1995; Moore et al., 1996; Yamagata et al., 1996; Zhang and Pagano, 1997). IFN-dependent and IFN-independent gene regulation and biological actions of IRFs are known (Taniguchi et al., 1997).

IRF1 induces the expression of IFN-ß and ISGs (Fujita et al., 1989a,b; Reis et al., 1992; Kamijo et al., 1994; Briken et al., 1995). $IRF1^{-/-}$ fibroblasts fail to undergo apoptosis and are rapidly transformed by activated oncogenes, indicating a tumor suppressor role for this protein (Tanaka et al., 1994). Furthermore, drug or radiation induced apoptosis is defective in $IRF1^{-/-}$ cells (Tamura et al., 1995; Tanaka et al., 1996). IRF1 also regulates the expression of ICE (interleukin-1ß converting enzyme), a mediator of apoptosis. Thus, lack of IRF1 may ablate cell death programs. Consequently such cells may develop into tumors. Indeed, IRF1 gene is deleted in myelodysplasia and myelocytic leukemia (Willman et al., 1993; Harada et al., 1994). Accelerated exon skipping, which causes the synthesis of a dysfunctional protein, has been suggested as one mechanism for the loss of IRF-1 activity (Harada et al., 1994). Mice lacking IRF1 gene do not express gene products involved in antigen presentation. T_H1 and NK cell responses are impaired in $IRF1^{-/-}$ mice (Matsuyama et al., 1993; Duncan et al., 1996; White et al., 1996; Hobart et al., 1997; Lohoff et al., 1997; Taki et al., 1997). These animals are therefor immunodeficient.

IRF2 was originally identified as a repressor of IFN and ISG expression (Harada et al., 1989). It acts as an oncogene upon over expression (Harada et al., 1993). IRF2 induces histone gene which is essential for cell proliferation (Vaughan et al., 1995). IRF9 ($p48^{-/-}$) mice fail to survive viral infections (Kimura et al., 1996). Cells lacking p48 gene rapidly die upon exposure to cytotoxic drugs compared to wild type cells (Weihua et al., 1997). These results suggest that IRF9 plays additional roles in cell growth control, in an IFN-independent manner. IRF8 (ICSBP or IFN-consensus sequence binding protein) represses ISG expression (Nelson et al., 1993; Bovolenta et al., 1994). $ICSBP^{-/-}$ mice develop a chronic myelogenous leukemia (CML)-like disease and immunodeficiency (Holtschke et al., 1996). Consistent with these observations, ICSBP gene expression is suppressed in cells derived from patients with myeloid leukemia (Schmidt et al., 1998). IFN-α, a clinically proven therapeutic agent for CML, restores ICSBP expression in these cells. ICSBP thus activated may suppress tumor cell growth (Schmidt et al., 1998).

IRF3 plays a central role in the regulation of IFN genes and ISGs (Lin et al., 1998; Weaver et al., 1998; Yoneyama et al., 1998). This factor seems to be important for the virus-induced expression of cellular genes. IRF4 (Pip, ICSAT, LSIRF) induces immunoglobulin gene expression in association with lymphoid transcription factor PU.1 (Eisenbeis et al., 1995; Yamagata et al., 1996). IRF4 is essential for the maturation and homeostasis of lymphocytes, therefore $IRF4^{-/-}$ mice are immunodeficient (Mittrucker et al., 1997). v-IRF and IRF7 inhibit IFN responses in virus transformed cells (Moore et al., 1996; Zhang and Pagano, 1997; Zimring et al., 1998). Factors that inhibit the function of IRF family of transcriptional activators are present in several human cancer cells (Petricoin et al., 1994). Thus, members of the IRF-myb gene family are involved in regulation of various cell growth and host defense processes (Taniguchi et al., 1997).

Cell cycle regulation

Mouse chromosome 1 has a cluster of IFN inducible genes, which encode a novel class of growth regulatory proteins are encoded by these genes (Choubey et al., 1989). Murine p202, p203, p204, D3 (Choubey et al., 1989; Tannenbaum et al., 1993), and human MNDA and IFI16 genes belong to this family (Dawson et al., 1995; DeYoung et al., 1997; Kao et al., 1997). Since all these proteins share a homologous 200-residue segment at the C-terminus, these genes are named as gene 200 family. p202, the well-characterized member of this family, is a nuclear protein. It inhibits cell proliferation via an interaction with growth regulatory proteins. p202 binds selectively to the hyperphosphorylated form of the retinoblastoma (pRb) tumor suppressor protein, which inhibits cell cycle entry (Choubey and Lengyel, 1995). Furthermore, p202 also inhibits the action of a number of growth promoting transcription factors, such as NF-κB and AP1. p202 also inhibits the transcriptional stimulatory activity of p53 tumor suppressor via an indirect interaction (Datta et al., 1996), but the functional significance of such action is unclear at present. The role of these proteins in cell growth control is further signified by the translocation of MNDA gene in human myeloid leukemia (Xie et al., 1997). It remains to be determined whether all members of this family have similar or different functional specificities.

IFN-γ induces cell growth arrest (Bromberg et al., 1996) by stimulating the expression of p21/WAF/Cip-1 (Chin et al., 1996), a cyclin dependent kinase inhibitor. STAT1 directly induces p21/WAF/Cip-1 (Chin et al., 1996). IFNs also induce lysyl oxidase, a specific regressor of Ha-ras induced transformation (Contente et al., 1990). Differential display analysis has shown that $IRF1^{-/-}$ fibroblasts fail to express lysyl oxidase (Tan et al., 1996). This observation is consistent with the rapid oncogenic transformation of $IRF1^{-/-}$ fibroblasts by activated ras (Tanaka et al., 1994). IFN-α down regulates c-myc gene expression and increases the levels or dephosphorylation of pRb, leading to growth arrest (Kimchi, 1992; Kumar and Atlas, 1992; Resnitzky et al., 1992).

Regulation of apoptosis

During development the number of cells designed to perform a specific function often exceeds the required limit. These superfluous cells are eliminated by apoptosis. Failure of cells to die is an integral mechanism in some cancers (Adams and Cory, 1998; Ashkenazi and Dixit, 1998). Cell surface receptors such as those of TNFR and Fas family activate death signaling by recruiting several proteins to their cytoplasmic death domains upon engagement with killer ligands (Ashkenazi and Dixit, 1998). Among these proteins are certain members of a unique class of proteases called cysteinyl aspartate specific proteases (caspases). These dormant cytoplasmic proteins are activated by trans-catalytic cleavage of their pro-domains upon activation by death signals. These caspases degrade other proteins necessary for cell survival (Thornberry and Lazebnik, 1998).

Recent studies indicate that IFNs regulate the expression of certain caspases. IFN-γ induced apoptosis requires JAK1 and STAT1 and expression of caspase 1 (Chin et al., 1997). Inhibition of caspases abrogates IFN-γ induced cell death. STAT binding elements are present in the promoter of ICE gene (Chin et al., 1997). TNF-α induced apoptosis is absent in $STAT1^{-/-}$ cells (Kumar et al., 1997a,b). Complementation of STAT1 restores apoptotic pathways in these cells. Expression of some caspases such as ICE, Ich-1, Cpp32 is ablated in $STAT1^{-/-}$ cells (Kumar et al., 1997a,b). These results suggest that basal expression of certain caspases is dependent on STAT1. STAT1, either as a monomer or as a heteromer, or in association with another unidentified protein(s) may regulate the expression of caspases

Table 2. Defective interferon system and disease.

GENE	PATHOGENIC CONDITION
IRF-1	Deleted in myeloid leukemias and immunodeficiency
ICSBP	Decreased expression in Chronic myelogenous leukemia and immunodeficiency
JAK2	Constitutive activation in acute lymphoblastic leukemia
JAK	Leukemia-like disease in drosophila
JAK1	Defective expression in certain lung cancers
STAT1	Increased sensitivity to carcinogen induced tumors, loss of innate immunity in mutant mice
DAPK	Loss of expressionin epithelial cancers and lymphoma
MNDA	Gene translocation in myeloid leukemia
PKR	Deleted in leukemia
CIITA	Bare lymphocyte syndrome

(Kumar et al., 1997a.b). Lack of basal caspase expression and apoptotic program probably explains the propensity of $STAT1^{-/-}$ mice to develop tumors in response to methyl colanthrene treatment (Kaplan et al., 1998).

Gene products other than caspases also mediate the cell death induced by IFNs. An antisense technical knockout strategy has identified several novel genes that participate in IFN-γ induced death program. These include thioredoxin, DAP (death associated protein), DAPK (death associate protein kinase), Cathepsin D, and DAP-5 (a homologue of eukaryotic translation initiation factor 4G) (Deiss and Kimchi, 1991; Deiss et al., 1995, 1996; Kissil et al., 1995; Levy-Strumpf et al., 1997). Among these DAPK is a potent tumor suppressor. DAPK is a calmodulin dependent (160kDa) serine-threonine protein kinase (Deiss et al., 1995). Over expression of DAPK gene causes cell death and the enzyme localizes to actin filaments during cell death (Cohen et al., 1997). DAPK expression is absent in several carcinomas and lymphomas (Kissil et al., 1997). Tumor cells lacking DAPK are highly metastatic (Inbal et al., 1997). Cathepsin D is a protease that cleaves other cellular proteins. It may also potentially activate caspases to induce cell death.

Dysregulation of IFN signaling

It is apparent that disruption or subversion of IFN regulated pathways by infectious agents or suppression of signaling molecules in neoplastic may contribute to loss of cellular homeotic balances. Abnormal regulation could be at the level of the IFN receptor gene expression or aberrant activation of intracellular substrates involved in its signaling pathway.

For example certain viruses inhibit the JAK-STAT pathway to evade IFN action (Kalvakolanu and Borden, 1996; Sen and Ransohoff, 1997). The adenovirus E1A oncogene (Ackrill et al., 1991; Gutch and Reich, 1991; Kalvakolanu et al., 1991; Leonard and Sen, 1997) and the terminal protein (TP) of Hepatitis B Virus (Foster et al., 1991) inhibit IFN-induced transcription. E1A represses cellular p48 levels to inhibit IFN-action (Leonard and Sen, 1997). E1A may also interfere with STAT induced gene regulation by squelching the transcriptional co-activator CBP (Bhattacharya et al., 1996). Epstein-Barr viral Nuclear Antigen-2 (EBNA-2) also inhibits IFN-α induced anticellular activities (Aman and von Gabain, 1990). Expression of HBV-open reading frame C also inhibits IFN-ß gene expression (Whitten et al., 1991). These mechanisms are likely to contribute to hepatocellular carcinogenesis. Cytomegalovirus and polyoma virus degrade or inhibit JAK1 to suppress IFN-actions (Miller et al., 1998; Weihua et al., 1998). The Kaposi's Sarcoma associated Herpes virus encodes a homologue of IRF proteins, v-IRF. It downregulates the expression of cell cycle inhibitor, Waf/Cip-1, and causes cellular transformation (Gao et al., 1997). It also inhibits IFN-induced genes (Zimring et al., 1998). Poxviruses encode homologues of IFN receptors, which inhibit IFN action by preventing the binding of IFNs to the cellular receptors (Alcami and Smith, 1995; Kalvakolanu, 1999).

Disruption of signal transducing molecules involved in IFN-induced pathways such as receptors, JAKs and STATs, also causes a deregulation of cell growth (Table 2). For example, tumor cells expressing a dominant negative mutant of IFN-γ receptor are not rejected upon transplantation (Dighe et al., 1994). The carcinogen, 3-methylcolanthrene (3-MC), induces tumors with a higher frequency in mice lacking IFN-γ receptor and STAT1, than in wild type mice (Kaplan et al., 1998). Constitutive activation of STAT1 by mutant FGFR3 results in the abnormal induction of WAF/Cip-1, an inhibitor of cell cycle, and growth stunting (Su et al., 1997). In type II thanatophoric chondroplasia, a form of human dwarfism, hyperactivation of STAT1 causes cell growth suppression (Su et al., 1997). Cells expressing activated src, abl, BCR/ABL and lck oncoproteins, and those transformed by HTLV constitutively activate STAT3 (Danial et al., 1995; Mignoe et al., 1995; Xu et al., 1995; Yu et al., 1995; Carlesso et al., 1996; Lund et al., 1997; Takemoto et al., 1997). Thus, deregulation of STAT activity may contribute to neoplastic cell proliferation.

A hyperactivated form of JAK2 is detected in acute lymphoblastic leukemia (ALL). Chromosomal translocation t(9;12)(p24;p13) in childhood ALL results in the synthesis of a chimeric protein consisting of TEL (a member of ETS family of transcription factors) and JAK2 genes (Lacronique et al., 1997; Peeters et al., 1997). The resultant chimeric protein, TEL-JAK2, is a constitutively active kinase. Transfection of TEL-JAK2 into IL-3 dependent cells causes a factor independent growth of cells. TEL-JAK2 appears to phosphorylate STAT5 constitutively and cause growth promotion. However, stimulation of oncogenic factors other than STATs by TEL-JAK2 can not be ruled out. AG-490, an inhibitor of JAK2, suppresses the growth of ALL cells in vitro and in vivo (Meydan et al., 1996). Similarly,

STAT5 is also constitutively activated in cell lines derived from patients with chronic myelogenous leukemia (CML) (Shuai et al., 1996) and in primary mouse bone marrow cells transduced with BCR-ABL oncogene.

Consistent with these observations, deletion of an orthologue of JAK in drosophila causes a leukemia-like disease. Thus, disruption of JAK-STAT signaling appears to be a mechanism of leukemogenesis.

Conclusions

Investigation of the molecular mechanisms of IFN action has not only uncovered a novel signaling pathway but also identified several potential cell growth regulators. Several intriguing questions arise out of these studies: What specific roles do the multiple isoforms of IFN-α play in host defenses? How does the type I IFN receptor interact with many different IFNs and mediate the specific functions? How do STAT proteins regulate apoptosis, cell growth and development? Are there undiscovered JAKs and STATs? Are JAK/STAT or the molecules downstream of them defective in human diseases? How do the IRF proteins prevent development of myeloid leukemia? Are there more members of IRF family? How do the multiple IRF proteins cross talk to each other in the regulation of host defenses and cell growth? Detailed analysis of these issues should permit a greater understanding of the fundamental mechanisms in tumor cell survival and resistance to cytokine therapy.

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