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Non-Conventional Signal Transduction by Type I Interferons: The NF-κB Pathway

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Abstract Type I interferons (IFNs) regulate diverse cellular functions by modulating the expression of IFN-stimulated genes (ISGs) through the activation of the well established signal transduction pathway of the Janus Kinase (JAK) and signal transducers and activators of transcription (STAT) proteins. Although the JAK–STAT signal transduction pathway is critical in mediating IFN's antiviral and antiproliferative activities, other signaling pathways are activated by IFNs and regulate cellular response to IFN. The NF-κB transcription factor regulates the expression of genes involved in cell survival and immune responses. We have identified a novel IFN mediated signal pathway that leads to NF-κB activation and demonstrate that a subset of ISGs that play key roles in cellular response to IFN is regulated by NF-κB. This review focuses on the IFN-induced NF-κB activation pathway and the role of NF-κB in ISG expression, antiviral activity and apoptosis, and the therapeutic application of IFN in cancer and infectious disease. J. Cell. Biochem. 102: 1087–1094, 2007.

Key words: signal transduction; interferon; NF-κB; JAK; STAT; gene expression

Interferon (IFN), the founding member of the cytokine family, was first described 50 years ago as a secreted factor produced by influenza virus-infected cells that interfered with viral replication [Isaacs and Lindenmann, 1957]. The type I IFN family, consisting of IFN-α, IFN-β, IFN-ω, IFN-ε, IFN-κ, IFN-δ, and IFN-τ, shares homology in amino acid sequences and tertiary structures, as well as binds to a common cell surface receptor complex. IFNs represent the first line of defense against viral and bacterial infections, and have important roles in immunosurveillance for malignant cells [Platanias, 2005]. The essential role of Type I IFNs in the antiviral innate immunity has been elucidated in mouse models that lack genes encoding IFN receptor subunits or JAK–STAT proteins required for IFN signaling. IFNs inhibit multiple steps in viral replication, including virus penetration and/or uncoating, transcription of viral mRNA, RNA stability, initiation of translation and synthesis of viral proteins, replication of viral genome, maturation, and assembly and release of progeny virions. Almost all mammalian viruses have strategies to avoid attack by the IFN system. For example, viruses can block IFN synthesis, interfere with IFN signaling, and inhibit the functions of IFN-induced antiviral proteins. This results in a dynamic equilibrium between the IFN-induced innate immune defense system and virally induced mechanisms to circumvent these pathways.

IFNs also inhibit cell growth as well as regulate apoptosis, which provides a rationale for their use as antitumor agents. IFNs were the first cytokines to be found effective in clinical therapy of several types of cancers, including hairy cell leukemia, Kaposi's sarcoma, laryngeal and genital papillomas, chronic myelogenous leukemia, and metastatic malignant melanoma. In addition, they have proven useful in other diseases, such as condyloma acuminate, labial and genital herpes, chronic viral hepatitis and multiple sclerosis [Pfeffer et al., 1998]. Although

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IFNs have been broadly used, the specific mechanisms that result in the various therapeutic activities, resistance and side effects of IFN treatment are surprisingly elusive in most cases. As is the case for bio-therapeutic agents, the systemic toxicity associated with IFN often requires reducing the dose of IFN or ceasing treatment. On the other hand, a substantial fraction of patients undergoing IFN therapy show no significant response. Therefore, it is important to fully understand the molecular mechanisms that affect IFN's biological activities.

Type I IFNs mediate their action through the induction of hundreds of early response genes, called ISGs. For example, the dsRNA-dependent protein kinase (PKR) is an ISG that is activated by autophosphorylation and consequently phosphorylates the translation initiation factor, eIF2α, resulting in inhibition of protein synthesis, inhibition of viral replication, and the growth arrest and apoptosis of host cells. 2′-5′-Oligoadenylate synthetases are ISGs that polymerize ATP into 2′-5′ linked oligoadenylates to activate endoribonuclease RNase L, resulting in degradation of viral RNA and inhibition of protein synthesis. IFNs also induce the Ifi200 family of proteins that have been implicated in cell cycle regulation and cell differentiation. IFNs induce the expression of GTP-binding proteins or GTPases, which play important roles not only in resistance of host to viruses, but also resistance to intracellular protozoa and bacteria. IFNs induce genes that are involved in MHC class I antigen processing and presentation. IFN enhances the expression of important regulators of cell proliferation and apoptosis, such as cyclin-dependent kinase inhibitor p21, TNF-related apoptosis-inducing ligand and a number of caspases. IFNs also induce chemoattractants, such as CC chemokines to modulate T cell responses. Despite efforts to define specific contributions of ISGs to biological actions of Type I IFNs, the functions of most ISGs remain undefined [Pfeffer et al., 2004].

CLASSICAL IFN ACTIVATED JAK–STAT SIGNALING

There are numerous excellent reviews about the activation of the JAK–STAT pathway by IFN, and the activation of NF-κB pathway by viruses, lipopolysaccharides, and inflammatory cytokines. This review focuses on recent findings on the activation of NF-κB signal pathway by IFN and the role that NF-κB plays in modulating IFN-induced cellular responses. The type I IFN receptor is comprised of two subunits, designated IFNAR1 and IFNAR2 (Fig. 1), which interact with members of the JAK family. The IFNAR1 chain is constitutively associated with tyrosine kinase 2 (TYK2) and acts as a species-specific transducer for the actions of type I IFN, whereas the IFNAR2 chain is associated with JAK1 and acts as the ligand-binding subunit. Upon ligand binding the JAKs become activated through tyrosine phosphorylation and phosphorylate specific tyrosine residues in STAT proteins. Phosphorylated STAT1 and STAT2 dimerize and complex with a 48-kDa DNA-binding protein (IRF9/p48/ISGFγ), which then translocates into the nucleus and binds to a highly conserved IFN-stimulus response element (ISRE) in ISG promoters to directly induce their transcription. In addition, other STAT homodimers and heterodimers are also induced by Type I IFNs. These STAT complexes bind distinct elements, GASs (IFN-gamma activated sites) or sis-inducible elements (SIEs). Some ISGs have ISRE or GAS elements in their promoters, whereas some ISGs have both elements, indicating that combinations of different STAT complexes might be required for the optimal transcriptional regulation of a particular ISG. However, the preference and specificity of STAT complexes for gene activation is not clear. In addition to tyrosine phosphorylation, STATs are also phosphorylated on serine residues, which augments their transcriptional activity.

The JAK–STAT pathway provides an important model for IFN-mediated signaling. However, the activation of JAK–STAT pathway alone is insufficient for generating all IFN-induced responses. Thus, the cooperation of multiple signal transduction cascades with JAK–STAT is necessary for the optimization of transcriptional regulation of ISGs, as well as biological outcomes induced by IFNs. In addition to classical JAK–STAT pathway, IFNs activate the p38 mitogen-activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI3K) signaling cascades that are required for the generation of cellular responses to IFNs [Platanias, 2005].

NF-κB SIGNAL TRANSDUCTION

The nuclear factor kappa B (NF-κB) transcription factor was first discovered as a factor

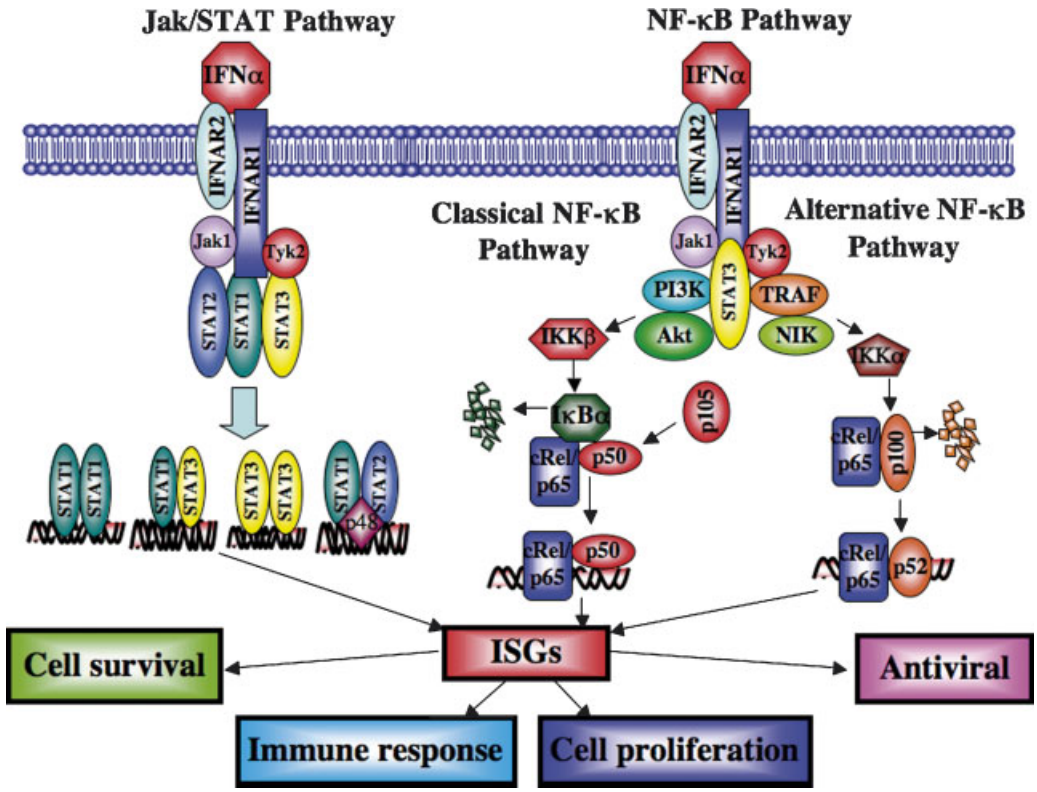

Fig. 1. A schematic of the IFN-activated classical JAK–STAT and novel NF-κB signaling pathways.

in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin gene [Sen and Baltimore, 1986]. The family of NF-κB transcription factors regulates gene expression by binding to *cis*-acting κB sites in their promoters. NF-κB regulated genes play important roles in immunity, inflammation, cell growth and cell survival, which are all processes affected by IFN. In mammals the NF-κB family of related proteins includes NF-κB (p105 processed to p50), NF-κB (p100 processed to p52), RelA (p65), RelB, and cRel. p50 and p52 lack a transcription activation domain, and as homodimers function as repressors. In contrast, p65, cRel and RelB have a transcription activation domain, and thus when complexed with p50 or p52 are capable of activating transcription. Although p50:p65 and p52:RelB heterodimers are the NF-κB complexes most often observed in cells, other Rel heterodimers also form. The NF-κB and NF-κB precursor proteins undergo proteolytic processing into the p50 and p52 proteins, respectively. The processing of p105 is constitutive and largely cotranslational, while the processing of p100 is tightly controlled through phosphorylation induced by various agents and subsequent ubiquitinylation. Under most circumstances, NF-κB homodimers or heterodimers are bound to IκB inhibitory proteins in the cytoplasm of unstimulated cells. Many cytokines promote the dissociation of the cytosolic inactive NF-κB/IκB complexes via the serine phosphorylation and degradation of IκB, leading to NF-κB translocation to the nucleus and DNA binding, which is denoted as the classical NF-κB pathway. The degradation of IκB proteins requires the activation of IκB kinase (IKK), a multiprotein complex consisting of IKKα and IKKβ catalytic subunits and the IKKγ/NEMO regulatory subunit [Baeuerle, 1998]. Targeted gene disruption of individual IKK proteins has determined that IKKβ and IKKγ (but not IKKα) are the major mediators of the classical NF-κB signal transduction [Li et al., 2005]. Recent studies have shown that the LMP1 protein of Epstein-Barr virus, B-cell activating factor, lymphotoxin-β and lipopolysaccharide induce NF-κB activation through an NF-κB signaling pathway that does not involve IκB degradation [Pomerantz and Baltimore, 2002]. This alternative pathway of NF-κB activation involves the linkage of TNF receptor

associated factors (TRAFs) to the activation of the MAP3K related kinase, the NF-κB-inducing kinase (NIK), which results in the ubiquitinylation and proteolytic processing of p100/NFκB2 protein and nuclear translocation of p52:RelB dimers to regulate specific NF-κB target genes [Bonizzi et al., 2004]. The alternative pathway for NF-κB activation is strictly dependent on IKKα [Senftleben et al., 2001], but independent of IKKβ and IKKγ [Dejardin et al., 2002].

In addition, post-translational modifications of NF-κB may play significant roles in modulating the strength and duration of the NF-κB transcriptional responses [Chen and Greene, 2004; Quivy and Van Lint, 2004]. Phosphorylation of p65 optimizes NF-κB activation and facilitates the recruitment of various transcriptional cofactors. Acetylation and deacetylation of p65 by p300/CBP and HDACs are also important for regulating the nuclear function of NF-κB.

The activation of NF-κB is a double-edged sword: while normal functions of NF-κB are needed for proper innate and adaptive immune responses, dysregulation of NF-κB can lead to inflammatory diseases and tumorigenesis. For example, constitutive NF-κB activity results in over-expression of pro-inflammatory genes, which is associated with several acute and chronic inflammatory diseases, including ulcerative colitis, rheumatoid arthritis, and Crohn's disease. Moreover, high constitutive NF-κB activity has been found in many tumor cell lines and in certain types of cancers, including acute myelogenous leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, multiple myeloma, prostate cancer, ovarian cancer, and breast cancer. Inhibitors targeting components of the NF-κB signaling pathway effectively suppress NF-κB activity, protect and relieve inflammatory symptoms, and induce the apoptosis of tumor cells. Thus, NF-κB represents an attractive drug target for therapy of inflammatory and autoimmune diseases, as well as for cancer [Lin and Karin, 2003; Karin et al., 2004; Panwalkar et al., 2004].

IFN ACTIVATES AN NF-κB SIGNALING PATHWAY THAT INVOLVES STAT3, PI3K, AND AKT

Since potential NF-κB binding sites are present in a number of ISG promoters, we tested nuclear extracts of IFN-stimulated cells for NF-κB activation by DNA-binding assays (EMSA) with an oligonucleotide probe based on the κB binding site in the immunoglobulin (Ig) light chain enhancer [Yang et al., 2000]. IFN rapidly promoted NF-κB DNA-binding activity in diverse cell types (lymphoblastoid, fibrosarcoma, renal carcinoma, and normal fibroblasts). NF-κB activity is tightly controlled by inhibitory IκB proteins, which bind to NF-κB complexes and sequester them in the cytoplasm. To determine whether IFN induces classical NF-κB activation, IκBα levels were determined at various times after IFN addition to the IFN-sensitive Daudi lymphoblastoid cell line. IFN induced a progressive decrease in cellular IκBα levels [Yang et al., 2000]. The kinetics of induction of NF-κB activation in Daudi cells paralleled that of IκBα degradation, indicating that IFN promotes the dissociation of IκBα/NF-κB complexes and IκBα degradation. Since serine phosphorylation of IκBα leads to its degradation, IκBα proteins with mutations (IκBαM) or deletions of serine phosphorylation sites (IκBα△N) function as super-repressors of NF-κB activation as determined by EMSA or by κB-dependent reporter assays, and sensitize cells to pro-apoptotic stimuli. Thus, we examined if expression of super-repressor IκBα (SR-IκBα) mutants would sensitize Daudi cells to IFN-induced apoptosis. Expression of SR-IκBα constructs markedly (∼50%) sensitized Daudi cells to IFN-induced death as determined by TUNEL assays. We have observed similar sensitization of other tumor lines (renal cancer and squamous carcinoma cells) to IFN-induced apoptosis by SR-IκBα expression.

We previously established that STAT3 acts as an adapter to couple PI3K to the IFNAR1 subunit of the IFN receptor. A phosphopeptide corresponding to the STAT3-docking site of IFNAR1 was found to block IFN-induced NF-κB activation, indicating that NF-κB activation is directed through the tyrosine phosphorylation-dependent formation of the STAT3/PI3K signaling complex with the IFNAR1 chain. While expression of dominant-negative (DN) PI3K blocked the IFN-induced IκB degradation and NF-κB activation, constitutively active (CA) PI3K promoted NF-κB activity, demonstrating that PI3K is involved in NF-κB activation by IFN. In addition, DN-Akt blocked IFN-promoted NF-κB activation and CA-Akt promoted NF-κB activity in Daudi cells,

demonstrating that Akt is also involved in NF-κB activation by IFN. Furthermore, while IFN stimulated κB-dependent transcription in empty vector-transfected cells, expression of DN-PI3K suppressed IFN-activated κB-dependent transcription. DN-PI3K expression had no effect on ISRE-dependent transcription. These results indicate that NF-κB-activation by IFN via the PI3K pathway is distinct from the ISRE-driven mechanism in regulating gene expression.

A different mechanism of PI3K/AKT activation by IFN that involves the insulin receptor substrate 1 (IRS1) has also been reported [Uddin and Platanias, 2004]. In addition PI3K may directly interact with IFNAR1, and induce NF-κB activity and CXCL11 gene expression [Rani et al., 2002]. The activation of NF-κB was recently found to require the TYK2 but not JAK1 kinase [Yang et al., 2005b]. IFNs induce NF-κB activation in JAK1 deficient cells, an event blocked by the expression of a kinase dead TYK2 construct. The PKR has also has been reported to activate NF-κB by directly activating the IKK complexes and lead to the phosphorylation and degradation of IκB [Deb et al., 2001]. These results suggest that there are multiple IFN-stimulated pathways leading to NF-κB activation, which may be cell context-dependent.

IFN ACTIVATES AN ALTERNATIVE NF-κB PATHWAY THAT INVOLVES NIK AND TRAF PROTEINS

The alternative NF-κB pathway is dependent on NIK and TRAF proteins. To define the role of NIK in IFN-promoted NF-κB activation, a dominant negative kinase-inactive NIK mutant (DN-NIK) was expressed in Daudi cells and the effect on IFN-promoted NF-κB activation was examined [Yang et al., 2005a]. Expression of DN-NIK in Daudi cells blocked IFN-promoted NF-κB DNA binding activity and κB-dependent transcription. However, while expression of dominant negative PI3K blocked IκBα degradation [Yang et al., 2001], expression of DN-NIK had no effect on IFN-promoted IκBα degradation, demonstrating the distinct roles that NIK and PI3K play in IFN-induced NF-κB activation. TRAF2 functions as an adaptor molecule for various members of the TNF receptor superfamily to activate NIK and subsequently mediate NF-κB activation. To assess the role of TRAF2 in IFN-induced NF-κB signal transduction pathway, cells were transfected with an expression plasmid for DN-TRAF2, which lacks the NH2-terminal 86 amino acids of the RING finger domain of TRAF2 and inhibits TRAF2-dependent signaling events [Rothe et al., 1995]. Expression of DN-TRAF2 in Daudi cells blocked IFN-promoted NF-κB DNA binding activity, as well as the stimulation by IFN of κB-dependent transcription. These results indicate that both TRAF2 and its downstream effector NIK are involved in the IFN-induced NF-κB signal transduction pathway. The alternative NF-κB pathway mediates p100 processing into p52 and the formation of p52 heterodimers, which translocate into the nucleus to regulate gene transcription [Pomerantz and Baltimore, 2002]. IFN induced the appearance of p52 in both nuclear and cytoplasmic extracts prepared from Daudi cells, as determined by pull-down assays with glutathione *S*-transferase fused to p65 (RelA), although untreated Daudi cells contained no detectable p52 [Yang et al., 2005a]. We also found that both dominant negative NIK and TRAF2 constructs blocked IFN-induced p52 appearance in nuclear extracts, demonstrating the roles of NIK and TRAF2 in IFN-induced p100 processing. In contrast, dominant negative PI3K expression or treatment with the pharmacological PI3K inhibitor LY294002 had no effect on basal or IFN-promoted appearance of p52, although they inhibited IFN-induced NF-κB activation and NF-κB dependent gene transcription. Thus, these results demonstrate that IFN-induced p100 processing to p52 is dependent on NIK/TRAF signaling but is independent of PI3K/Akt signaling.

To assess the role of NIK and TRAF2 in IFN mediated cell survival, Daudi cells were transfected with dominant-negative expression plasmids, and assessed for apoptosis. Expression of dominant-negative NIK or TRAF2, as well as PI3K, sensitized Daudi cells to IFN-induced cell death as determined by TUNEL or DNA fragmentation assays, while expression of the empty vector alone had no effect. These results indicate that the NIK/TRAF dependent pathway leading to NF-κB activation protects cells against IFN's pro-apoptotic action. Thus, IFN generates cell survival signals through both the alternative NF-κB pathway dependent on NIK/TRAF, and the classical NF-κB pathway dependent on Akt/PI3K [Yang et al., 2001].

These results indicate that there is a dynamic equilibrium between the ability of IFN to promote apoptosis and the ability of IFN to generate cell survival signals through an NF-κB pathway. Inhibiting the NF-κB pathway drives IFN promoted apoptosis. The understanding of the pathways that limit IFN-induced apoptosis may provide new avenues to develop therapeutics to enhance the apoptotic action of IFN. For example, the effectiveness of IFN in cancer therapy is often limited by its inability to induce significant cell death. Better characterization of such pathways could lead to new strategies that improve IFN's efficacy as an antitumor agent.

THE ROLE OF NF-κB IN ANTIVIRAL ACTIVITY AND ISG EXPRESSION

Since IFNs by definition are antiviral proteins, the role of NF-κB activity in the induction of IFN's antiviral action was examined in MEFs that had normal NF-κB function (WT MEFs), or had a germline disruption of both p50 and p65 NF-κB proteins (p50/p65-DKO MEFs). IFN treatment of p50/p65-DKO MEFs did not result in NF-κB activation as determined by EMSA with the "classical" oligonucleotide probe based on the κB binding site in the immunoglobulin light chain enhancer but did in WT MEFs. However, it was very interesting that p50/p65-DKO MEFS were more sensitive to the antiviral action of IFN when compared to WT MEFs [Pfeffer et al., 2004; Wei et al., 2006]. Thus, the NF-κB pathway suppressed the induction of antiviral activity by IFN.

Recently it was reported that the IκB kinase family member, IKKε may also play an important role in IFN-mediated antiviral activity [Tenoever et al., 2007]. IKKε knockout mice are more susceptible to influenza virus infection as compared to WT mice, which appears to reflect a defective IFN response due to the lack of STAT1 phosphorylation directed by IKKε. The role of IKKε in IFN-induced NF-κB activation is unknown.

IFNs produce their biological effects by altering gene expression, most notably through the induction of the ISG family of early response genes. To determine the relationship between gene regulation by IFN and biological activity, gene expression profiling using Affymetrix murine U74Av2 GeneChips was performed on RNA samples collected from IFN-treated WT MEFs and p50/p65-DKO MEFs [Pfeffer et al., 2004]. Comparative analysis of gene expression profiles identified a subset of NF-κB-regulated ISGs, specifically genes encoding GTP-binding and antigen presentation proteins [Wei et al., 2006]. The NF-κB-regulated GTP-binding ISGs play important roles in resistance to viruses as well as intracellular protozoa and bacteria, and include the 65–67 kDa guanylate-binding proteins (Gbp 1 and Gbp2), the Mx proteins (Mx1 and Mx2), and the 47-kDa GTPase Ifi47 (also called Irg47) [Vestal, 2005]. The NF-κB-regulated antigen presentation ISGs (Tap1, Tap2, Psmb9/Lmp2, and Psmb8/Lmp7) are involved in degrading intracellular proteins into antigenic peptides, and contribute to the transport of these peptides to endoplasmic reticulum where they bind to the assembled MHC class I molecules [Ploegh, 2000]. We found that NF-κB suppresses the IFN-induced expression of Gbp1, Ifi47, Mx1, Mx2, Tap1, Psmb9/Lmp2, and Psmb8/Lmp7, while NF-κB enhances the IFN-induced expression of Gbp2 and Tap2 [Pfeffer et al., 2004a]. Thus, the IFN-activated NF-κB pathway not only counterbalances the ability of IFN to induce apoptosis but also differentially regulates the expression of specific ISGs.

THE BINDING OF NF-κB PROTEINS TO THE PROMOTERS OF A SUBSET OF ISGS

To investigate proteins that directly regulated the transcription of Ifi47, Tap1, and Mx1, we performed chromatin immunoprecipitation (ChIP) assays on the promoters of NF-κB-regulated ISGs. IFN treatment induced the binding of STAT1 and STAT2 to Ifi47, Tap1 and Mx1 promoters in WT and p50/p65-DKO MEFs between 15 and 60 min of IFN treatment. Importantly, p50 was basally bound to Mx1, Ifi47, and Tap1 promoters in WT cells and remained bound for up to 2 h after IFN addition. Remarkably, the time course of expression of these ISGs correlated precisely to when p50 detached from these promoters. Interestingly, in p50/p65-DKO MEFs IFN induced the recruitment of IRF1 to the promoters of all three ISGs, and IRF1 recruitment closely correlated with the more rapid and enhanced gene induction upon IFN treatment. Taken together these results demonstrate that for this subset of ISGs that are negatively regulated by NF-κB, p50 is basally bound to the promoters, and thereby

inhibits their induction by IFN, perhaps by inhibiting recruitment of IRF1 to their promoters.

We also examined the role that specific NF-κB proteins play in IFN-induced CXCL11 gene expression in IFN-sensitive Daudi cells and an IFN-resistant Daudi subclone (DRST3) that has a defective STAT3-dependent signaling pathway [Yang et al., 2007]. In IFN-sensitive Daudi cells the p50 NF-κB subunit was found basally bound to the CXCL11 promoter and by 1 h of IFN treatment the p50 subunit of NF-κB was no longer found associated with the CXCL11 promoter. In contrast, p65 was not basally promoter-bound but its binding was induced 1 h after IFN addition. In DRST3 cells, while p50 was not basally bound to the CXCL11 promoter, IFN induced p50 binding within 1 h after addition, and p65 was neither basally promoter-bound nor was its binding IFN-induced. Moreover, IFN induced the binding of the transcriptional activator IRF1 to the CXCL11 promoter in Daudi cells. In contrast, in DRST3 cells IFN induced the binding of another IRF family member, IRF2, which is a transcriptional repressor. WT-STAT3 expression in DRST3 cells rescued the recruitment of NF-κB and IRF proteins to the CXCL11 promoter by IFN. The pattern of transcription factor binding in WT-STAT3 expressing DRST3 cells was indistinguishable from that in IFN-sensitive Daudi cells. These results indicate that the defective STAT3 signaling pathway in DRST3 cells affects the binding of NF-κB and IRF proteins, which cooperate in the transcription regulation of CXCL11.

CONCLUSION AND PERSPECTIVE

As illustrated in Figure 1, IFNs activate not only the JAK/STAT signaling pathway but also the NF-κB signaling pathway. NF-κB regulates ISG expression and the cellular response to IFNs. Although STAT3, TYK2, PI3K, AKT, IKK, TRAF, and NIK have been identified in the IFN-activated NF-κB signaling pathway, the components of this signaling pathway have not been fully elucidated and could be cell context-dependent. ChIP assays on ISG promoters regulated by NF-κB indicate that the outcome is highly dependent on the interplay between different STAT, NF-κB, and IRF proteins. The therapeutic effectiveness of IFN in cancer may be limited by IFN's inability to induce significant cell death because of high constitutive NF-κB activity in tumor cells. Since there is a close relationship of NF-κB between inflammation, tumorigenesis and the cellular response to IFN, we hypothesize that selectively targeting the NF-κB pathways may represent a novel strategy for enhancing therapeutic effectiveness of IFN and/or diminishing undesirable side effects. Inhibiting NF-κB activity in vitro sensitizes cells to IFN's apoptotic and antiviral activities. However, it will be important to determine whether inhibiting NF-κB activity in vivo will enhance IFN's antiviral and antitumor clinical activity.

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