Noncanonical IFN Signaling: Mechanistic Linkage of Genetic and Epigenetic Events

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The canonical model of cytokine signaling via the JAK/STAT pathway dominates our view of signal transduction but provides no insight into the significance of the simultaneous presence of activated JAKs and STATs in the nucleus of cells treated with cytokines. Such a mechanistic shortcoming challenges the usefulness of the model in its present form. Focusing on the interferon (IFN) cytokines, we have developed a noncanonical model of IFN signaling that naturally connects activated JAKs and STATs at or near response elements of genes that are activated by the IFNs. Specifically, cells treated with IFN\(\gamma\) showed association of activated STAT1\(\alpha\) and JAK2 at the GAS element of genes activated by IFN\(\gamma\). For IFN\(\alpha\) treated cells, the association involved activated STAT1\(\alpha\) and TYK2 JAK kinase at the ISRE promoter. The power of the noncanonical model is that it provides mechanistic insight into specific gene activation at the level of the associated epigenetics, akin to that of steroid/steroid receptor signaling.

1. Introduction

The classical or canonical model of signaling by cytokines such as the interferons (IFNs) involves ligand interaction with receptor extracellular domain, followed by “allosteric changes” in the receptor cytoplasmic domain that results in autophosphorylation of the relevant Janus tyrosine kinases (JAKs) and subsequent tyrosine phosphorylation of receptor cytoplasmic domain(s) [1–4]. The climatic event is the association and tyrosine phosphorylation of the appropriate signal transducer and activator of transcription (STATs) factors. The beauty and frailty of the model lies in the simplicity of the STATs being responsible for the specific functional effects attributed to the IFNs. For example, IFN\(\gamma\) signaling via a heterodimeric receptor results in the activation of STAT1\(\alpha\), by receptor-associated JAK1 and JAK2, to form an asymmetric dimer which undergoes nuclear translocation to specific promoters of genes that are activated by IFN\(\gamma\). In the case of the family of the 15 or more type 1 IFN subtypes, all acting through the same heterodimeric receptor, JAK1 and TYK2 kinases activate STAT1\(\alpha\) and STAT2 in conjunction with the receptor cytoplasmic domains. STAT1\(\alpha\) and STAT2 form a trimeric complex with IFN regulatory factor 9 (IRF9), known as ISGF3, followed by nuclear translocation and association with promoters of genes specifically activated by the type I IFNs.

The canonical model of IFN signaling is remarkably lacking in specificity mechanisms, probably attributable to its attractive simplicity and skewed focus on STATs. In the case of IFNs, it has never been shown that activation of the corresponding STATs independent of the IFNs or their receptors has resulted in the induction of an antiviral state. The discovery of a novel member of the type I IFN family, called IFN\(r\), places a particular strain on the canonical model of IFN signaling. Ovine IFN\(r\) was originally identified not as an IFN but as a pregnancy recognition hormone in ruminants [5]. It is produced by the conceptus (placenta) of pregnant sheep. Structurally, the amino acid sequence of IFN\(r\) shows 30 to 70 percent homology to other type I IFNs [6]. IFN\(r\) operates via the same heterodimeric receptor as all the type I IFNs, is as potent antiviral and antiproliferative agent as IFN\(\alpha\), and is equally effective in induction of (2'-5') oligoadenylate synthetase but unlike IFN\(\alpha\) it is relatively nontoxic at high doses [7].
It is noteworthy that IFNr and IFNα had similar specific antiviral activities but IFNα is bound to receptor at a 10-fold higher binding affinity [7]. Antibodies to IFNr C-terminus blocked binding of both IFNr and IFNα to the receptor but antibodies to IFNα N-terminus only blocked IFNr binding, suggesting that they recognized the receptor similarly at their C-terminus but differently at their N-terminus. The findings suggested that maximal IFN antiviral activity required only fractional occupancy of receptor by the IFNs while toxicity was associated with maximal receptor occupancy. Consistent with similar antiviral activity, IFNr and IFNα phosphorylated JAK kinase TYK2 and STAT1 and STAT2 transcription factors similarly, suggesting that phosphorylation of these signal transduction molecules was associated with antiviral activity and not toxicity. The similar and differential effects of these two type I IFNs operating through the same receptor complex are not readily explained by the canonical model of type I IFN signaling. We have discovered, developed, and characterized a noncanonical IFN signal transduction pathway that is remarkably similar to steroid/steroid receptor signaling (reviewed in [8, 9]). It is our contention that the pathway not only provides the mechanism of genetic and epigenetic signaling by IFNs but also provides insight into other cytokine, growth factor, and hormone signaling pathways.

2. The Canonical Model

The central players of IFNγ signaling via the classical or canonical pathway involve the IFNγ, receptor subunits IFNGRI and IFNGR2, tyrosine kinases JAK1 and JAK2, and STAT1α transcription factor [1–4]. Type I IFN signaling involves a type I IFN, receptor subunits IFNAR1 and IFNAR2, tyrosine kinases JAK1 and TYK2, and STAT1 and STAT2 transcription factors. Figure 1 illustrates the sequence of events from IFNγ/receptor binding to the presence of activated STATs at the response elements of genes that are specifically activated by the IFNs. Proponents of these classical pathways point out the interaction of the activated STATs with coactivators. STATs in the nucleus, for example, interacting with epigenetic players such as p300 and CBP (CREB binding protein) where CREB means cAMP response element binding. STATs in the nucleus, for example, interacting with coactivators.

3. IFNγ Receptor Interaction and the Noncanonical Model

There are a number of interesting observations concerning IFN activity that are beyond rational explanation or understanding in the context of the canonical JAK/STAT model of IFN signaling. For example, IFNγ has been shown to be capable of functioning intracellularly. Specifically, human IFNγ delivered by a liposome vector induced an antitumor effect in murine macrophages, expression of nonsecreted human IFNγ in murine fibroblasts induced antiviral activity, and microinjected human IFNγ induced la antigen expression in murine macrophages [14–16]. These findings are at odds with the well-known species preference of exogenous human IFNγ which has no activity on murine cells. These reports suggest that IFNγ can induce function via an intracellular mechanism that is not species restricted. The fact that these observations were essentially ignored by the IFN community would suggest that they were considered to be of no significance in the context of the canonical JAK/STAT pathway. The IFNγ molecule is an asymmetric dimer and the IFNγ receptor consists of a noncovalent linked tetramer consisting of two subunits called IFNGRI and IFNGR2 [12]. According to the canonical model, IFNγ cross-links the IFNGRI subunit, resulting in allosteric changes to the receptor cytoplasmic domain [12]. This allosteric change initiated from the IFN/receptor extracellular interaction is not specified but merely assumed in the context of the model. The intervening hydrophobic transmembrane sequence of the receptor, separating receptor extracellular and cytoplasmic
domain, is similarly ignored as to its role in these allosteric events.

Given the stasis of the canonical model in its lack of predictive appeal, we approached IFNγ signaling by first doing binding studies of IFNγ and IFNGRI. We questioned whether all relevant ligand effects occurred at the receptor extracellular domain. Specifically, we carried out IFNγ bindings to intact soluble receptor subunit IFNGRI consisting of both extracellular and cytoplasmic domains. Using intact IFNγ, overlapping IFNγ peptides, and overlapping IFNGRI extracellular and cytoplasmic peptides along with site specific antibodies, we discovered that the N-terminus of IFNγ is bound to IFNGRI extracellular domain and that the C-terminus of IFNγ is bound to receptor cytoplasmic domain [17]. Murine IFNγ C-terminus peptide, IFNγ(95-132), and the corresponding sequence of human IFNγ are bound to residues 253-287 of IFNGRI cytoplasmic domain. This binding was adjacent to the binding site of JAK2 on IFNGRI and was specifically blocked by anti-(253-287) specific antibodies in fixed/permeabilized cells [17, 18].

It was observed that when cells were treated with IFNγ JAK2 binding shifted from receptor subunit IFNGR2 to IFNGRI, presumably as a result of the allosteric changes referred to above [2, 12]. By comparison, we showed that Sepharose coupled JAK2 (Seph-JAK2) bound our soluble radiolabeled IFNGRI and that such binding was enhanced by both intact IFNγ and its C-terminal peptide, IFNγ(95-132), but not by the receptor extracellular domain-binding peptide IFNγ(1-39) [18]. The enhanced binding of JAK2 was blocked by the IFNGRI peptide, IFNGRI(253-287), that corresponded to the IFNGRI binding site for IFNγ C-terminus, showing specificity of enhanced binding. A receptor peptide corresponding to the JAK2 binding site, IFNGRI(283-309), also blocked JAK2 binding, while a peptide to an adjacent site had no effect on enhanced JAK2 binding, providing further evidence of specificity. IFNγ C-terminus enhancement of
JAK2 binding to IFNGR1 cytoplasmic domain would seem to be consistent with the well-known law of mass action, shifting the equilibrium between IFNGR1 and IFNGR2, rather than by allosteric changes evoked by the canonical JAK/STAT model [18].

Functionally, treatment of murine macrophage cell lines with either murine IFNγ C-terminal peptide, IFNγ(95-132), or its human counterpart resulted in upregulation of MHC class II molecules and induction of an antiviral state similar to IFNγ [19]. The peptides also enhanced binding of JAK2 to IFNGR1 in these cells [18]. The peptides were internalized via pinocytosis by the macrophages and were not effective against nonpinocytic fibroblasts, presumably because they could not access IFNGR1 cytoplasmic domain. This lack of effect on fibroblasts was overcome by attachment of a palmitate to not access IFNGR1 cytoplasmic domain. This lack of effect nonpinocytotic fibroblasts, presumably because they could not access IFNGR1 cytoplasmic domain. The test was to show that IFNγ interaction with receptor on intact cells finds its way to the IFNGR1 cytoplasm binding site, as per IFNGR1(253-287) peptide, during the process of endocytosis [21]. First, specific binding to a murine cell line (P388D1) was established using radiolabeled murine IFNγ, 125I-IFNγ. Binding was carried out at 4°C to prevent endocytosis. Unlabeled IFNγ blocked 125I-IFNγ binding by competing for receptor while IFNGR1(253-287) that corresponds to the IFNGR1 cytoplasmic binding site for IFNγ in soluble receptor binding had no effect on extracellular receptor binding (Figure 2(a)). To assess intracellular binding to the sequence 253-287 of IFNGR1, P388D1 cells were incubated at 37°C to facilitate intracellular loading of the IFNGR1(253-287) peptide. Cells were then incubated at 37°C for a short period with 125I-IFNγ after which they were lowered to 4°C and surface 125I-IFNγ was removed by acid treatment. Cell supernatant was treated with antibodies to IFNGRI and Western blots showed that 125I-IFNγ was associated with the precipitated IFNGRI (Figure 2(b)). Importantly, IFNGR1(253-287) loaded cells blocked binding of 125I-IFNγ to the corresponding site on IFNGR1. An added caveat to this experiment is that blockage of IFNγ binding to IFNGR1 cytoplasmic domain resulted in the absence of activation of STAT1α as assessed by phosphorylation of tyrosine 701 by JAK2 (Figure 2(c)). Taking together, these binding studies show that IFNγ binds extracellular receptor domain and traverses to the cytoplasmic domain of IFNGR1 which is coupled to STAT1α activation.

Historically, the smallpox virus has been responsible for billions of deaths and has been estimated to have wiped out as many as 90% of the South American population as a result of European introduction of the virus [22]. A central reason for the potent virulence is probably due to the remarkable refractiveness of the virus to IFNs as a result of the induction of IFN decoy receptors. The vaccinia virus, for example, codes for secreted, soluble proteins B18R and B8R that are truncated such as to retain only the extracellular, ligand binding domain of the receptor that competes with type I and type II IFN, respectively [23, 24]. The IFN based C-terminus mimetics by comparison are potent inhibitors of vaccinia virus because they are not recognized by virus decoy receptors [25, 26]. Thus, the decoy receptors can neutralize the intact IFNs and not the C-terminal peptides that are devoid of the domain involved in the extracellular binding of the decoy receptor. It should be noted that these mimetics are the result of the noncanonical pathway of IFN signaling.

4. IFN and the Genetics and Epigenetics of Specific Gene Activation

The abovementioned scenario with IFNγ and IFNγ receptor subunit IFNGR1 is applicable with variations to type I IFN signaling system. In IFNγ signaling, the receptor subunit IFNGR2 remains on the plasma membrane during endocytosis, while IFNGR1 is endocytosed with IFNγ [27, 28]. For a type I IFN like IFNα, both receptor subunits IFNARN1 and IFNARN2 are endocytosed with the IFN [29]. In this section, we address issues of complex formation in the cytoplasm and movement to specific genes in the nucleus.

The observation that IFNγ translocates to the nucleus in receptor-expressing cells with kinetics similar to those of activated STAT1α is not considered in the context of the canonical model of JAK/STAT signaling, perhaps because of the central role ascribed to STAT (reviewed in [8, 9]). However, such observations are of potential importance in the context of the noncanonical model of IFNγ signaling. We showed that IFNγ nuclear translocation was driven by a polycationic nuclear localization sequence (NLS), 126RRKKRSR, in its C-terminus that is similar to that of the prototypical SV-40 large tumor antigen (T-ag) NLS(PKKKRKV) [30]. Mutations of the IFNγ NLS resulted in loss of biological activity which was restored by T-ag prototypical NLS [31]. Efficient nuclear transport via polycationic NLSs involves high affinity recognition by members of the importin (IMP) superfamily of nuclear transport molecules [32–34]. IFNγ is actively transported by the heterodimeric IMPαβ complex in the cytoplasm where IMPα binds the IFNγ NLS and IMPβ mediates the interaction with the nuclear pore and Ran, with ATP/GTP as energy source [32]. The IMP association of IFNγ was established by immunoprecipitation with antibody to IMPα (anti-NPI-1) and Western blotting [31]. Related to this, T-ag which binds to IMPα competitively inhibited IFNγ function in cells [30].

We showed above that endocytosed IFNγ interacted with the cytoplasmic domain of receptor subunit IFNGR1 at residues 253-287 which is adjacent to JAK2 binding site. Immunofluorescent confocal microscopy and cell fractionation studies showed that receptor in lipid microdomains on the cell surface played an important role in the endocytosis and that receptor subunit IFNGR2 did not undergo endocytosis but remained on the cell surface [27, 28]. The function of IFNGR2 may be to serve as reservoir for JAK2 that binds to IFNGR1 with higher affinity as a result of IFNγ binding [18].

Given the central emphasis that has been placed on STATs in specific gene activation by cytokines, it seems
Mediators of Inflammation 5

No Compet. IFNGR1 pept. competitive IFNγ

(a)

(b)

(c)

Figure 2: Intracellular presence of peptide IFNGR-1(253-287) inhibits binding to IFNGR of extracellular IFNγ and subsequent activation of STAT1α. (a) Presence of extracellular peptide IFNGR-1(253-287) did not inhibit binding of 125I-IFNγ to P388D1 cells at the concentrations to be used in subsequent experiments. Unlabeled murine IFNγ or peptide IFNGR-1(253-287), as indicated, was added at a final concentration of 1μM to P388D1 cells at 4°C along with 10 nM of 125I-IFNγ, and cells were incubated at 4°C for 30 minutes. Control cells were incubated with 125I-IFNγ in the absence of any competitor. Cells were then washed and bound IFNγ determined. Samples were run in triplicate and values plotted as mean ± s.d. (b) Intracellular accumulation of peptide IFNGR-1(253-287) in P388D1 cells by pinocytosis was accomplished by incubating cells with either 25 μM (lane 2) or 50 μM (lane 3) of peptide at 37°C for 1 hour. Cells used in lanes 1 and 4 did not receive any peptide. Cells were then washed at room temperature to remove extracellular peptide and then incubated with 125I-IFNγ at 4°C without peptide. After 125I-IFNγ incubation, all cells were washed at 4°C and then acid-washed at 4°C to remove surface-bound 125I-IFNγ. Cells were then lysed and immunoprecipitated with antibodies to IFNGR-1. After Western transfer of immunoprecipitates to nitrocellulose membranes, 125I-IFNγ associated with IFNGR-1 was detected by autoradiography. Total IFNGR-1 immunoprecipitated was followed by immunodetection with antibodies to STAT1α (lower panel). (c) Conditions are the same as in (b), except that lysates were immunoprecipitated with STAT1α antibodies and tyrosine phosphorylation of immunoprecipitated STAT1α was followed by immunodetection with antibodies specific for Tyr701-phosphorylated STAT1α. Total immunoprecipitated STAT1α was followed by reprobing blots with antibodies to STAT1α (lower panel).

reasonable in the case of IFNγ to determine what proteins are associated with STAT1α at the GAS element in genes activated by this IFN. Accordingly, we used the combination of immunoprecipitation with Western blotting, nuclear confocal immunofluorescence, chromatin immunoprecipitation (ChIP) followed by PCR, and other focused techniques to show that IFNγ, IFNGR1, JAK1 and JAK2, and STAT1α were all present at the GAS element of genes activated by IFNγ. We initially focused on the role of the IFNγ NLS in translocation of IFNGR1 into the nucleus [8, 9, 31, 35]. We established that cells treated with IFNγ or the internalized IFNγ C-terminus peptide IFNγ(95-132) resulted in IFNGR1 translocation to the nucleus and that the IFNγ NLS was required [8, 9, 31, 35]. We next showed that activated STAT1α (pSTAT1α) and the activated JAKs, pJAK1 and pJAK2, also required the NLS of IFNγ for nuclear translocation, all as a complex of IFNγ/IFNGR1/pSTAT1α/pJAK1/pJAK2 [8, 9, 31, 35]. We showed that the complex played an essential role in the coordinated events of specific gene activation and the associated epigenetics. Thus, STAT1α is but one of the collection of key players in specific gene activation by cytokines such as IFNγ. See Figure 3(a) for an illustration of the IFNγ events.

We similarly examined type I IFN system for noncanonical signaling. Using the same approach with particular use of ChIP followed by PCR, immunoprecipitations, and confocal microscopy, we showed the association of pSTAT1α, IFNAR1, IFNAR2, and TYK2 with the ISRE element of the oligoadenylate synthetase 1 (OAS1) promoter in IFNα2 treated cells [29]. Such association was not shown at the β-actin promoter after IFN treatment as type I IFN does not activate the β-actin gene. See Figure 3(b) for an illustration of the IFNα events.
Figure 3: The noncanonical model of IFN \( \gamma \) (a) and IFN\( \alpha \) (b) signaling. (a) Binding of IFN\( \gamma \) to its receptor extracellular domain is followed by movement to IFNGR1 cytoplasmic domain in conjunction with endocytosis. The cytoplasmic binding increases the affinity of JAK2 for IFNGR1, which is the basis for its movement from IFNGR2 to IFNGR1. This results in autoactivation of the JAKs, phosphorylation of IFNGR1 cytoplasmic domain, and the binding and phosphorylation of STAT1\( \alpha \) at IFNGR1. The complex of IFN\( \gamma \)/IFNGR1/STAT1\( \alpha \)/JAK1/JAK2 undergoes active nuclear transport where the classic polycationic NLS of IFN\( \gamma \) plays a key role for this transport to genes in the nuclei that are specifically activated by IFN\( \gamma \). JAKs are involved in epigenetic events that cause heterochromatin destabilization and promoter activation. Histone H3 phosphorylation at Tyr 41 by JAK1 and JAK2, indicated by arrows, is a key epigenetic event in IFN\( \gamma \) gene activation. (b) IFN\( \alpha \) signaling involves the endocytosis of IFN\( \alpha \) and both of its receptor subunits. This complex binds to p48 to generate interferon stimulated gene factor 3 (ISGF3). IFN\( \alpha \)/IFNAR1/IFNAR2/JAK1/TYK2/p48 are then translocated to the promoter ISRE, where JAK1 and TYK2 are involved in phosphorylation of histone H3. GAS, IFN\( \gamma \) activated sequence; H3, histone H3; ISRE, IFN sensitive response element; NPC, nuclear pore complex.

As indicated earlier, both wild-type and gain-of-function mutated JAK2 were shown to be present in the nucleus [13]. Specifically, constitutively activated JAK2V617F was shown to phosphorylate histone H3 on tyrosine 41 (H3pY41) which caused dissociation of the inhibitor HP1\( \alpha \) from H3. The key result of this was chromatin remodeling to euchromatin which led to gene activation. JAK2V617F mutation is associated with particular hematologic disorders, suggesting that the epigenetic effect involves interaction with the relevant hematological receptor. It is our view that the power of a model is that it both explains and predicts mechanisms. In this regard, it was shown that JAK2V617F association with a homodimeric type I cytokine receptor, the erythropoietin receptor (EpoR), the thrombopoietin receptor, or the granulocyte colony-stimulating factor receptor, was necessary for the induction of the transforming leukemic phenotype [36, 37]. The question of whether receptor/JAK2V617F complexes were present at the promoters of genes that were activated in cancers caused by or associated with JAK2V617F was not addressed. It is our view that hematopoietic receptor activation of JAK2V617F in the cytoplasm is not sufficient to induce the H3pY41 phosphorylation specifically at the genes that are activated by JAKV617F as we are not aware that JAK2V617F possesses such intrinsic properties. Thus, in addition to activation of JAK2V617F, the receptor may also colocalize with the kinase at the specific promoter in the nucleus. These results with mammalian systems were preceded by similar observations in a Drosophila model of hematopoietic tumors, including the suppressive effects of Drosophila HP1 on the mutant JAK in conjunction with inhibition of tumor [38]. Importantly, it provides an explanation for the phenotypes of these JAK2V617F associated cancers.

Perhaps the most intriguing finding of our noncanonical approach to IFN signaling was the association of H3pY41
with nuclear pJAK2 and pSTAT1 at the IRF-1 promoter in cells treated with IFNγ [35], and the association of H3pY41 with nuclear TYK2 and pSTAT1 at the OAS1 promoter in cells treated with IFNα [29]. Genes such as β-actin, which are not activated by IFNs, were negative for the relevant JakS and StatS [29, 35]. The fact that IFNα-associated TYK2 can phosphorylate Y41 on H3 is evidence that the phosphorylation is not restricted to JAK2. In this regard, the mutated JAKV617F appears to be a special case of a more general process. The key is not just the particular JAK but also the particular cytokine, growth factor, or hormone that is the activator of the JAK.

The question arises as to the significance of JAK induction of H3pY41 to nucleosome transient unwrapping so that factors such as the complexes of our noncanonical studies can become involved in DNA transcription. Specifically, it was shown that H3pY41 increased nucleosome unwrapping and access to transcription factor binding by severalfold [39, 40]. H3 at lysine 56, H3K56, is located at the same DNA-histone interface as H3Y41. Acetylated H3K56, H3K56ac, similarly increased nucleosome unwrapping. However, the combination of H3pY41 and H3K56ac had a multiplicative effect and increased unwrapping by 17-fold. It was concluded that the combination of phosphorylation with acetylation significantly increased DNA accessibility to regulatory transcription complexes.

The movement of membrane receptors to the nucleus following endocytosis is not a rare anomaly just limited to some odd IFN result. On the contrary, there are a plethora of plasma membrane receptors that translocate to the nucleus following ligand/receptor interaction. Polycationic NLSs are virtually ubiquitous in cytokine receptors, ligands, or both [41]. Most of the membrane receptors that traffic to the nucleus tend to signal via the JAK/Stat pathway [41]. Receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor (VEGFR), growth hormone receptor (GHR), and insulin receptor are well-known growth factors that have been shown to similarly undergo nuclear translocation with ligand [42, 43]. G protein-coupled receptors (GPCR) involving peptide ligands such as angiotsin also have been found in the nucleus [44]. EGFR was the first RTK to be shown to be a cotranscription factor [45].

Considerable work has also been done on the mechanism of nuclear transport of membrane receptors. We showed that internalized IFNγ is bound to the cytoplasmic domain of IFNγR1 but the challenge is to decipher the mechanism (Figure 2). We have addressed this issue in part where we showed that the presence of IFNγR1 and IFNγR2 in the lipid microdomain was central to the endocytosis that is linked to the noncanonical signaling pathway [28]. The cytoplasmic domain of IFNγR1 in the endocytic vesicle is exposed to the cytoplasm as intracellular injected antibodies specific to IFNγ C-terminus blocked nuclear translocation as well as STAT1α activation while the antibodies had no effect on nuclear accumulation of STAT1α in cells treated with IFNα [31]. Retrograde trafficking of the receptor tyrosine kinase EGFR from the cell surface into the nucleus has been studied extensively [46, 47]. Following EGF induced endocytosis, the endocytic vesicles with the EGFR fuse with early endosomes which traffic to golgi. Retrograde trafficking was blocked by brefeldin A or dominant negative mutants of the small GTPaseARF (ADP-ribosylation factor). Both treatments resulted in disassembly of the COPI (coat protein complex I) which was interpreted as COPI regulation of retrograde vesicular trafficking of EGFR from the Golgi to the ER (endoplasmic endothelium) [46]. The Sec61 translocon was shown to be required for trafficking of EGFR from the ER into the nucleus [47]. Epigenetically, EGFR has been shown to modulate DNA synthesis and repair through phosphorylation of tyrosine on histone H4 at residue H4Y72 which is connected to enhanced methylation at H4 K20 [43] Thus, there are specific mechanistic data on a key epigenetic event associated with activation of EGFR. Mechanisms of nuclear translocation and some epigenetic effects have similarly been reported for other RTKs [48, 49].

5. Conclusions

Receptor-associated tyrosine kinases such as the JAKs and RTKs such as EGFR in the nucleus are probably key players in normal and abnormal cellular activity. Our noncanonical model of IFN signaling where JAKs, receptors, and StatS are physically linked significantly demystifies genetic and epigenetic aspects of cytokine signaling. JAK2V617F associated hematopoietic cancers are much better understood in the context of JAK2V617F linkage with receptors such as EpoR for both activation and specific function in the nucleus. The same pertains to RTKs where the kinase is part of the receptor. The noncanonical signaling model should thus provide insight into regulation of both homeostatic and nonhomeostatic cellular processes.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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