# Interferons and Their Stimulated Genes in the Tumor Microenvironment

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Constitutive expression of interferons (IFNs) and activation of their signaling pathways have pivotal roles in host responses to malignant cells in the tumor microenvironment. IFNs are induced by the innate immune system and in tumors through stimulation of Toll-like receptors (TLRs) and through other signaling pathways in response to specific cytokines. Although in the oncologic context IFNs have been thought of more as exogenous pharmaceuticals, the autocrine and paracrine actions of endogenous IFNs probably have even more critical effects on neoplastic disease outcomes. Through high-affinity cell surface receptors, IFNs modulate transcriptional signaling, leading to regulation of more than 2,000 genes with varying patterns of temporal expression. Induction of the gene products by both unphosphorylated and phosphorylated STAT1 after ligand binding results in alterations in tumor cell survival, inhibition of angiogenesis, and augmentation of actions of T, natural killer (NK), and dendritic cells. The interferon-stimulated gene (ISG) signature can be a favorable biomarker of immune response but, in a seemingly paradoxical finding, a specific subset of the full ISG signature indicates an unfavorable response to DNA-damaging interventions such as radiation. IFNs in the tumor microenvironment thus can alter the emergence, progression, and regression of malignancies.


Although in an oncologic context interferons (IFNs) have been often thought of more as exogenous pharmaceuticals, the autocrine and paracrine actions of endogenous IFNs probably have even more critical effects in contributing to tumor outcomes in patients. Constitutive expression of interferons (IFNs) and activation of their signaling pathways have pivotal roles in host responses to malignant cells in the tumor microenvironment. Induction of IFNs in immune effector cells, together with sustained effects of STAT1, can result in direct alterations in tumor cell survival, inhibition of angiogenesis, and augmentation of actions of T, natural killer (NK), and dendritic cells. These effects derive from immune cell recognition of tumors, endothelial cell proliferation, and response of tumors to exogenous DNA damage. With receptors present on almost every cell type, IFNs through their cellular actions can alter the emergence, progression, and regression of malignancies (Table 1). The interferon-stimulated gene (ISG) signature can be a favorable biomarker of immune response, but, in a seemingly paradoxical finding, a specific subset of the ISG signature indicates an unfavorable response to DNA damaging interventions such as radiation.

IFNs, a family of secreted α-helical cytokines, are induced by the innate immune system through stimulation of Toll-like receptors (TLRs) and other signaling pathways in response to specific extracellular biomolecules (pathogen- or damage-associated molecular patterns [PAMPs or DAMPs]). Through high-affinity cell surface receptors, IFNs activate kinase-driven signaling, leading to the induction of more than 2,000 transcriptionally regulated ISGs with varying patterns of temporal expression after ligand binding. Although most genes ($>$1,500) are stimulated, some are suppressed (~300).[1–7] These ISGs, stimulated by exogenous IFNs at the RNA level up to 100-fold include structural proteins, transcription factors, adaptors, enzymes, and secreted proteins.[5]

Expression arrays and cytogenetic analyses have identified somatic, homozygous deletions of the chromosomal locus for IFN-α and IFN-β and germline mutations of ISGs in colon, lung, prostate, breast,

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**Table 1.** Receptors and Signaling Molecules in IFN Pathways

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| **Receptors responding to pathogenic molecules** |
| - Toll-like receptors (TLRs) |
| TLR3: dsRNA (poly I:C) |
| TLR4: LPS (paclitaxel) |
| TLR7/8: ssRNA (imiquimod) |
| TLR9: CpG DNA |
| - RIG-I, MDA5: dsRNA |
| - cGAS: cytoplasmic ss and dsDNA |
| **Signaling molecules involved in IFN production** |
| - TBK1, IKK |
| - IRF1, IRF2, IRF3, IRF5, IRF7, IRF8 |
| - NF-κB, AP-1 |
| - STING |
| **Receptors responding to IFNs** |
| - IFNAR1/ IFNAR2: type I IFNs |
| - IFNGR1/ IFNGR2: type II IFN |
| - IFNLR1/ IL-10R2: type III IFNs |
| **Signaling molecules responding to IFNs** |
| - JAK1, JAK2, TYK2 |
| - STAT1, STAT2 (PY- and U-STATs) |
| - IRF9 |
| - PI3K, MAPKs |

head and neck, and pancreatic carcinomas, melanoma, and hematologic malignancies.[8–17] Epigenetic and genetic silencing of signaling pathways stimulated by IFNs is also likely to influence tumor development.[18–21] Although we will draw on insights from studies of actions of exogenously added IFNs, our focus is to illustrate how endogenous host IFNs can potently influence early regression or later either stability or progression of the neoplastic process. Since tenets regarding their protein structure, receptors, and intracytoplasmic signaling have been the basis for new insights concerning endogenous IFNs and their activation, we will begin with a short overview of canonical findings and understandings.

## GENES, RECEPTORS, PROTEINS, AND CANONICAL SIGNALING

Classification of the several types and families of IFNs comes from commonality in both primary structures and their influence on three dimeric target receptors. Based on similarities and differences, there are three major classes of IFNs.[22–25] Type I IFNs include the IFN-α family with its many isoforms, IFN-β, and other IFNs of less studied significance in humans, IFN-ω, IFN-τ, IFN-κ, and IFN-ε.[23,26] The sole type II IFN is IFN-γ.[27] A more recently discovered family, type III IFNs or IFNs-λ (interleukin [IL]-28/29) and its isoforms are produced by mucosal epithelial cells.[24] Type III IFNs share structural homology and induction pathways with type I IFNs but with cell lineage distribution of its unique receptor restricted to mucosal epithelial cells and plasmacytoid dendritic cells (pDC).[24,28]

The genes for the human type I IFNs including those encoding 14 subspecies of IFN-α, are clustered at 9p21.[22,26] As proteins, the human IFN-α subspecies have about 50% sequence identity; IFN-β is about 20% identical to IFN-α2. IFNs-α and IFN-β have 186–190 amino acids and have a cleavable signal peptide resulting in secreted proteins of 165 or 166 amino acids. Structure–function analyses have shown that the $NH_2$ terminus of type I IFNs is important for biological activity.[26] The gene encoding IFN-γ, located on human chromosome 12, has three introns, encodes a protein of 146 amino acids, functions as a dimer, and has minimal homology with type I IFNs.[27] NK and T cells are major sources of IFN-γ. Type I IFNs are produced predominantly by dendritic cells but can be induced in all cell types including T cells, monocytes, fibroblasts, and epithelial cells.

Virus or microbial gene products, such as double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), dsDNA, ssDNA, or cell wall constituents (PAMPs) bind to specific membrane proteins (TLRs) to trigger type I IFN synthesis.[29–34] dsRNA can be recognized by TLR3 and also by two cytosolic RNA-helicases, RIG-I and MDA5, both of which are ISGs.[33–35] Viral single-stranded RNAs are recognized by TLR7 and TLR8 and viral and cellular DNAs (DAMPs) by TLR9 and STING, all present in endosomal membranes.[29,30,32,33] Adaptor proteins connect TLRs to specific protein kinases such as TBK1 and IKK to activate transcription factors nuclear factor-κB (NF-κB), interferon regulatory factor (IRF)3, IRF7, and AP-1.[32,33]

For IFN-β induction, NF-κB, the AP-1 complex composed of ATF2/c-jun and IRF3 or IRF7 are required.[35,36] IFN-β induces further IRF7 synthesis, which, in turn, induces transcription of IFN-α1 and other IFN-α genes.[31] Inhibition of IFN signaling blocks robust production of IFNs-α. Synthesis of various IFNs are, therefore, intimately linked and further influenced by IRFs, a family of nine transcription factors that have common DNA binding domains in their N-terminus. IRFs were first identified through the role of IRF1 in inducing IFN-β.[37–39] IRF1 is expressed constitutively and also in response to IFN-γ as is IRF8.[40] Although IRF3 and IRF7 are