



Review

Components of the IFN- γ Signaling Pathway in Tumorigenesis

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Abstract. Many features of the interferon γ (IFN- γ) signaling pathway would suggest that it is anti-tumorigenic. The IFN- γ signaling pathway leads to apoptosis and to the expression of immune function proteins that could cooperate with T cells in the destruction of tumor. Various lines of experimental approach have, in general, supported the hypothesis that the IFN- γ signaling pathway is anti-tumorigenic. However, data also indicate that the idea that the IFN- γ signaling pathway is **exclusively** anti-tumorigenic is too simplistic. Also, to date, very little of the knowledge regarding the anti-tumor effects of the IFN- γ pathway has been useful in the prognosis or therapy of cancer. This review summarizes the current state of knowledge regarding the IFN- γ signaling pathway in tumorigenesis, with an emphasis on MHC class II induction in tumor cells and the induction of apoptosis in tumor cells. The review also indicates some future areas of investigation that offer hope for applying this knowledge in reducing cancer mortality.

Key words: interferon γ ; tumorigenesis; apoptosis; MHC; interferon regulatory factor-1; retinoblastoma protein.

The Main Branches of the Pathway

The best-understood pathway of gene activation by interferon γ (IFN- γ) begins with IFN- γ -mediated receptor dimerization, cross phosphorylation of JAK1 and JAK2, and phosphorylation of STAT1 at the receptor complex (Fig. 1)^{7, 62}. However, it is important to keep in mind that not all IFN- γ -signaling goes through STAT1^{2, 14, 23, 53}. STAT1 phosphorylation leads to STAT1 dimerization, translocation to the nucleus, and activation of promoters with STAT1 binding sites. Many of the end points of distinct branches of the pathway represent the expression of genes that require only STAT1 for the IFN- γ response (Fig. 1). One example is intracellular adhesion molecule-1 (ICAM-1). STAT1

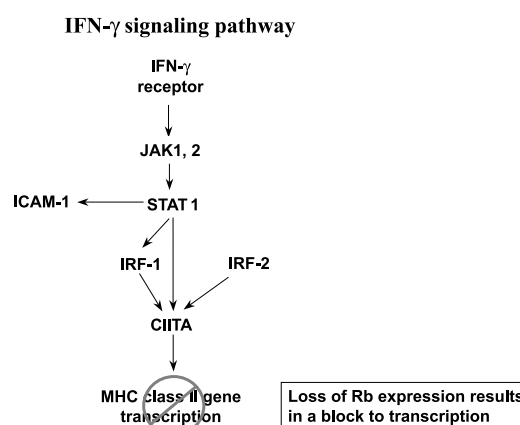


Fig 1. Basic IFN- γ signaling pathway. IFN- γ – interferon γ , IRF-1 and -2 – interferon regulatory factors-1, 2, ICAM-1 – intracellular adhesion molecule-1, CIITA – class II transactivator, Rb – retinoblastoma tumor suppressor protein

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facilitates a substantial upregulation of ICAM-1 transcription, leading to a substantial increase in ICAM-1 function at the cell surface³³. However, STAT1 can lead to the activation of the transcription of genes that encode other transactivators, which can either function in cooperation with STAT1 or independently. The most prominent secondary transactivator is interferon regulatory factor 1 (IRF-1), which activates many IFN- γ responsive genes (Fig. 1)⁶⁹. Among these is yet another transactivator, termed class II transactivator (CIITA)⁶⁵. CIITA is required for the IFN- γ activation of the MHC class II genes and of related immune function genes. CIITA also enhances the expression of the MHC class I genes^{24, 38}. There are no known transactivators that require CIITA for expression and no other transactivators whose expression requires STAT1 and IRF-1. Thus, MHC class II gene activation by CIITA represents the longest branch in the IFN- γ signaling pathway (Fig. 1).

The components of the IFN- γ signaling pathway can also be expressed independently of IFN- γ in various cell-types, especially in immune function cells, such as T cells, where IRF-1, for example, is required for development of CD8⁺ T cells²⁷. Also, CIITA is constitutively expressed in B cells and in melanoma cells^{15, 25, 64}.

Tumorigenesis in Mice Lacking the IFN- γ Receptor

Mice lacking the IFN- γ receptor have a higher incidence of chemically induced tumors and of tumors that arise in p53^{-/-} mice²⁸. Also, transplantation of these tumors indicates that tumors lacking the IFN- γ receptor are more aggressive than control tumors²⁸. The basis for this difference includes a reduced anti-tumor immune response that is apparently dependent on a functioning IFN- γ signaling pathway in the tumor cells. There are several likely connections between the functioning of the IFN- γ signaling pathway in tumor cells and the generation of anti-tumor immunity. In particular, reconstitution of the MHC class I antigen presentation pathway is able to rescue the effect of the IFN- γ signaling pathway in preventing growth of mouse tumors derived from mice lacking the IFN- γ receptor⁶⁰.

Tumor Cell MHC Induction

A tremendous body of data has revealed the importance of tumor cell expression of the MHC class I and class II proteins in immune system-mediated eradica-

tion of the tumor. In general, these proteins have the potential of binding to tumor antigens, thus forming a complex that can be recognized by specific T cell receptors. The MHC class I proteins mediate T cell killing, thus lack of MHC class I represents a generally accepted strategy of tumor cell evasion of the immune system. The first linkage of MHC class I down-regulation and tumorigenesis came from the study of tumor viruses, the infection of which leads to either down-regulation of MHC class I expression or interference in MHC class I function^{8, 51}. Eventually, lack of MHC class I expression in tumors was directly attributed to mutations of the MHC class I genes⁷¹. The MHC class I proteins are constitutively expressed, but can be up-regulated by IFN- γ . Also, accessory proteins, such as the TAP peptide transporter proteins, are up-regulated by IFN- γ . Like the MHC class I proteins, the MHC class I antigen processing proteins have been demonstrated to be unresponsive to IFN- γ in human tumor lines¹⁸, although loss of the antigen processing proteins without loss of IFN- γ inducibility has been more commonly reported^{13, 29, 56, 57, 59}.

The role of MHC class II expression on tumor cells has been more controversial, but the basic data indicating that MHC class II expression on tumor cells has an anti-tumorigenic function is compelling and overwhelming^{4, 6, 22, 41, 49, 50, 52}. The controversy stems from the question of exactly how tumor cell MHC class II expression leads to destruction of the tumor cell. In general, MHC class II molecules function to present antigen to T cell receptors of CD4⁺ T cells, which then leads to stimulation of tumor cell killing by CD8⁺ T cells. However, it is clear that professional antigen-presenting cells, such as dendritic cells, are much more efficient at presenting antigen to CD4⁺ T cells⁶³. Also, the status of potential co-stimulatory molecules on tumor cells has not been resolved. Without the expression of co-stimulatory molecules, it is possible that MHC class II expression could lead to tumor tolerance rather than to an anti-tumor response. However, there are potential co-stimulatory molecules expressed on so-called "non professional" antigen-presenting cells^{5, 40, 61, 70} and the fact remains that tumor cell expression of MHC class II leads to a CD4⁺ dependent anti-tumor immune response⁶. It is worth noting that many human tumor cell lines have lost the ability to express CIITA, the transcriptional activator required for MHC class II promoter activation, in response to IFN- γ , while retaining the main or "early" components of the signaling pathway, such as STAT1 activation^{19, 35}. Unlike the MHC class I genes, there has never been a report of a tumor cell-related mutation directly affecting either the CIITA

gene or the MHC class II genes. The MHC class II gene response to IFN- γ requires expression of the retinoblastoma (Rb) protein (see below), which is mutated in many human tumors. Rb gene expression is not known to be required for any other portion of the IFN- γ signaling pathway.

Apoptosis

Exposure of cells to IFN- γ can result in apoptosis. IFN- γ treatment leads to the upregulation of numerous proteins in apoptotic pathways, for example, IRF-1^{44, 67, 68}, DAPs³², DAP kinase¹², and CD95^{31, 72, 73}. IFN- γ treatment also leads to increased expression of the caspases (and cathepsin D¹⁶) that essentially represent endpoints of apoptotic pathways. The promoter for caspase-1, for example, is activated by IRF-1^{17, 66}. This has led to the question of whether IFN- γ induced apoptosis could eradicate tumor cells. Many human tumor lines have been shown to undergo apoptosis in response to IFN- γ , which might lead to more effective antigen presentation and anti-tumor immunity^{1, 10, 11, 55, 58}. In various mouse model systems, IFN- γ has been shown to reduce tumorigenicity, but in no case has this reduced tumorigenicity

been directly attributable to increased tumor cell apoptosis. In one case IFN- γ inhibited angiogenesis, and in another case IFN- γ led to increased macrophage-dependent tumor eradication^{3, 20}. Thus, it remains to be determined whether IFN- γ -induced apoptosis can be relevant to tumor eradication in an animal model.

Mechanism of Repression of the Pathway in Human Tumor Lines

Repression of MHC class II expression in Rb-defective tumor cells

As noted above, Rb is required for the IFN- γ response of the MHC class II genes in human tumor lines^{34, 36, 47, 48}. Rb is also required for the induction of the mouse MHC class II gene, IA β ⁷⁸. The role of Rb in growth control and as a tumor suppressor protein has been extensively studied, but the role of Rb in facilitating the IFN- γ response of the MHC class II genes is unknown. Recent work has revealed some details about the mechanism of MHC class II silencing in cells lacking Rb⁷⁷ (Fig. 2). First, it is apparent that cells lacking Rb have a high level of Oct-1 DNA binding activity,

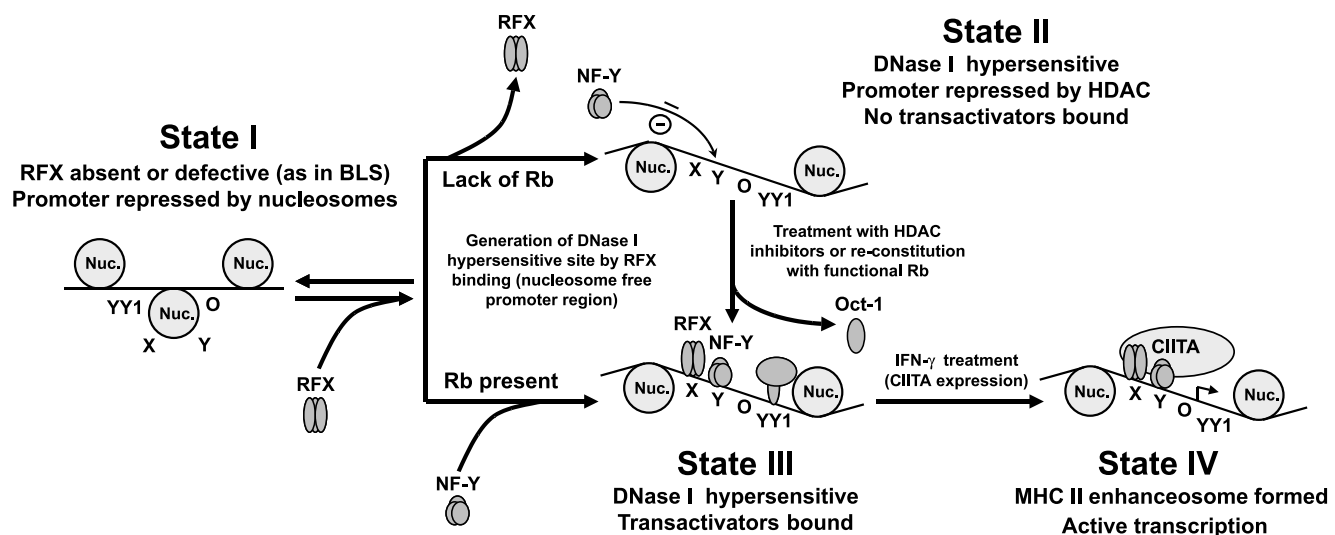


Fig. 2. Proposal for distinct states of HLA-DRA promoter repression. Data from the study of bare lymphocyte syndrome (BLS), where MHC class II is not expressed and where the HLA-DRA promoter DNase I hypersensitive (HS) site is absent (State I), have indicated that RFX can destabilize a nucleosome at the HLA-DRA promoter. This is consistent with data indicating that RFX efficiently interacts with the X1 promoter element when this element is part of a nucleosome⁹⁷. (The X, Y and Oct-1 binding elements of the HLA-DRA promoter are indicated.) NF-Y does not increase the efficiency of RFX interaction with the nucleosome, but NF-Y does substantially increase the efficiency of RFX interaction with otherwise naked DNA⁹⁷, consistent with data indicating that mutation of the NF-Y binding site (Y promoter element) eliminates RFX binding *in vivo*⁷⁴. Interestingly, NF-Y includes a series of histone-like motifs. There is no evidence of RFX defects in Rb-defective cells, consistent with the formation of the DNase I HS site in Rb-defective cells⁴⁸. RFX does not occupy the promoter in these cells, but this is consistent with lack of NF-Y occupancy⁴⁷. In sum, in the Rb-defective cells (State II), RFX apparently functions to establish the DNase I HS site, but then dissociates from the promoter because NF-Y cannot interact with the promoter. This interaction may be prevented by Oct-1 binding to the promoter, once the DNase I HS site has been formed, or by histone-DNA contacts that are not affected by RFX. Rb expression or treatment of the Rb-defective cells with HDAC inhibitors leads to the promoter occupancy required for transcription (States III and IV)⁴⁸

likely to be consistent with the role of Oct-1 in activating the transcription of genes required for rapid cell growth, such as histone genes. Oct-1 has been shown to repress IFN- γ -induced activation of the promoter for the human MHC class II gene, HLA-DRA, which encodes the heavy chain of the MHC class II DR heterodimer. Repression of the HLA-DRA promoter is mediated by an Oct-1 binding site located 5' to the TATA box and 3' to the series of well-studied activator binding sites required for formation of the HLA-DRA enhanceosome. However, other MHC class II promoters that require Rb for activation by IFN- γ do not have an apparent Oct-1 binding site, raising the question of whether Oct-1 plays a role in repressing these promoters in Rb-defective cells.

Recent work has also revealed that histone deacetylase inhibitors can rescue **the IFN- γ activation** of the MHC class II genes in Rb-defective cells⁴⁸ (Fig. 2). Because Rb-defective cells have the main DNase I hypersensitive site present in Rb-positive, normally inducible cells, HDAC function in Rb-defective cells must repress transcription at an intermediate, but stable state in the de-repression of the promoter chromatin. The acquisition of a DNase I hypersensitive site implies either the elimination of a nucleosome from the promoter or a significant alteration in nucleosome structure that reduces the DNA-histone contacts. Thus, HDAC function in Rb-defective cells could prevent further nucleosome destabilization, i.e., a further reduction in the DNA-histone contacts that would be required for loading the promoter binding proteins of the enhanceosome. Or, HDAC function could prevent a second step in de-repression that involves a sequence specific DNA binding protein. Interestingly, Oct-1 DNA binding activity is eliminated by HDAC inhibitors, although there is no evidence for a direct link between Oct-1 and HDAC function.

HDAC inhibitor treatment has also been shown to lead to tumor cell expression of MHC class II on tumor cells where the Rb status or the status of the IFN- γ signaling pathway is unknown³⁷. However, these treatments, unlike that summarized in the paragraph above, were shown to lead to **CIITA-independent** expression of MHC class II, raising the question of whether HDAC inhibitor treatments of different tumor types could lead to MHC class II expression via different mechanisms.

Transient vs. sustained activation of the IFN- γ pathway

Many human tumor lines activate STAT1 and synthesize IRF-1 in response to IFN- γ , but do not express

any detectable CIITA mRNA in response to IFN- γ ^{19, 35}. Although STAT1 activation and IRF-1 synthesis in these human tumor lines can be reduced, compared with normal cells, it was apparent that the reduction was minor in comparison with the complete lack of CIITA mRNA induction. Subsequently, it was determined that CIITA induction requires a saturating and sustained level of STAT1 activation and a saturating and sustained level of IRF-1 synthesis¹⁹. This is likely due to the requirement that STAT1 and IRF-1 simultaneously occupy the type IV promoter of CIITA, the promoter responsible for most, if not all, of the response of CIITA to IFN- γ ⁴². Presumably, because the above-mentioned human tumor lines achieve neither saturating nor sustained levels of activated STAT1 or IRF-1, simultaneous occupancy of the CIITA promoter by STAT1 and IRF-1 does not occur. The molecular basis for the transient activation of the IFN- γ pathway is unknown. In sum, transient activation of the IFN- γ pathway is an apparent tumor-associated mechanism for minimizing the adverse effects of IFN- γ on the growth of the tumor.

Repression of IFN- γ -induced apoptosis in tumorigenicity

In several cases, it is apparent that tumor cells have developed a mechanism to inhibit IFN- γ -induced apoptosis, for example hypermethylation of the DAP kinase gene promoter³⁰, which inhibits transcription of the DAP kinase gene in response to IFN- γ . Tumor-related mutations in IRF-1 have also been reported^{26, 46}. Malignant T cell lines and hepatoma cells have been shown to have low levels of the IFN- γ receptor (IFN- γ R)^{43, 45}. Interestingly, low levels of IFN- γ R are associated with IFN- γ -induced proliferation rather than apoptosis in T cells, although there has yet to be a determination of whether a specific reduction in the level of surface IFN- γ R could alter an IFN- γ signal for apoptosis to a signal for tumor cell proliferation. The relationship between the amount of IFN- γ R and the signal for apoptosis has not been fully elucidated, but it is apparent that a durable, i.e. sustained, activation of the IFN- γ pathway is required for IFN- γ -induced apoptosis, and that a durable activation of the pathway is often lacking in tumor lines¹⁹. The lack of a sustained activation of the pathway has been shown to have specific effects on gene regulation, such as the specific lack of a CIITA response discussed above, but as yet none of these gene-specific effects have been directly connected with preventing apoptosis. This type of direct connection awaits a more detailed molecular understanding of how

sustained vs. transient activation of the pathway affects formation of promoter-protein complexes required for promoter activation.

Emerging Issues

The most pressing goal in the field of tumor-associated, IFN- γ pathway defects is a more complete understanding of the molecular mechanisms underlying the defects. As noted above, there is no explanation for Rb's role in facilitating the MHC class II response, although there have been recent advances in understanding the molecular basis of the repression of the MHC class II response in Rb-defective cells, as described above (Fig. 2). There is no molecular basis to explain (tumor-associated) transient activation of the pathway in some cells vs. sustained activation of the pathway in others, when the amount of IFN- γ exposure is the same for the two different cell types¹⁹. There is a complete basic understanding of how IRF-2 mutations can reduce the induction of CIITA (Fig. 1), but these mutations are apparently very, very rare, in contrast with Rb mutations or with the frequency of tumor-associated, transient activation of the pathway^{75, 76}.

Keeping in mind that MHC class II expression is associated with reduced tumorigenicity, the fact that HDAC inhibitors can restore MHC class II expression leads to the question of whether these inhibitors can be used for MHC class II-dependent anti-tumor therapy. This may amount to a situation where the technology is preceding the science, as HDAC inhibitors are already in use in clinical trials. However, it will be very important to know whether any patient responses involve stimulation of patient T cells dependent on MHC class II expression. Further advances will likely come from a determination of whether HDAC-mediated rescue of MHC class II leads to the expression of MHC class II epitopes that represent peptides of tumor antigens, as have been detected in cases where tumor cells retain the expression of MHC class II. If so, this would lend considerable support to the idea that loss of MHC class II expression on tumor cells is a means of tumor escape from the anti-tumor immune response.

Another important issue is which parts of the pathway are anti-tumorigenic? It is possible that some parts of the pathway are pro-tumorigenic. For example, ICAM-1 expression is associated with metastasis^{21, 39}. Also, human tumor lines that are completely unresponsive to IFN- γ are very rare, although these lines do exist³⁵. IFN- γ affects the expression of a very large number of genes. Addressing the issue of which parts

of the pathway are pro- or anti-tumorigenic will likely require isolating specific effects of the pathway, such as high-level ICAM-1 expression, in tumor-prone mice and assaying for the effects of the isolated portions of the pathway on tumor growth and metastasis. In particular, it will be of great interest to learn whether the connection between a low-level of IFN- γ receptor and IFN- γ -induced proliferation applies to tumor cells.

As noted above, components of the IFN- γ signaling pathway can be expressed in the absence of IFN- γ in certain cell types. This raises the question of whether proteins such as IRF-1 are anti-tumorigenic in tumors related to those cell types, such as lymphoma. Melanoma represents a particularly perplexing case, where a very large percentage of tumors, derived from what is apparently a non-professional antigen-presenting cell, is constitutive for MHC class II and CIITA. Because melanoma represents a clear example of a tumor that can lead to an anti-tumor response, including an MHC class II-dependent anti-tumor response, the common CIITA constitutive phenotype in these tumors is worthy of further investigation.

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Received in December 2001

Accepted in January 2002