Interferon-gamma in Brain Tumor Immunotherapy

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KEYWORDS

• Interferon gamma • Gliomas • Immunotherapy
• Cytokines • Brain tumors • Gene transcription
• Tumor angiogenesis

Each year, approximately 17,000 new patients in the United States are diagnosed with uniformly fatal, malignant glioblastoma.[1] Over the past several decades there have been only marginal gains in treatment. The median survival and five-year survival for the most common type of primary brain tumor, malignant glioma, remains less than 1 year and 2% respectively.[2–4] Current treatment modalities are largely unsuccessful in altering patients' mortality and are also associated with adverse side effects. During tumor genesis, neoplasms acquire several characteristics including the ability to evade the host immune response, proliferate, and recruit a vascular supply. Novel approaches using immunotherapy offer the potential to specifically target neoplasms, their unique characteristics, and decrease adverse effects.[5,6]

Interferon-gamma (IFNγ) is a cytokine that acts on cell-surface receptors, activating transcription of genes that offer treatment potential by increasing tumor immunogenicity, disrupting proliferative mechanisms, and inhibiting tumor angiogenesis. However, abnormally low levels of IFNγ are produced by tumor cells and local T cells in the glioma microenvironment.[7,8] Current investigations into the immunomodulating effects of IFNγ suggest that IFNγ has the potential to be used clinically in the treatment of brain tumors and as a promising adjunct to other immunotherapeutic modalities. Here the authors review the published literature that highlights the potential role of IFNγ in the treatment and immunotherapy of malignant gliomas.

INTERFERON-GAMMA AND THE CELL CYCLE

IFNγ has numerous effects on transcriptional gene regulation involving the cell cycle. Several lines of evidence demonstrate the utility of IFNγ to inhibit actively dividing cells and induce apoptosis. IFNγ has significant cytotoxic effects on actively dividing neural cells, but much less on immature cells, and no apparent effect on mature cells.[9] IFNγ preferentially disrupts the cell cycle of proliferating cells by causing a delay in the G1/S-phase transition.[9–12] Discrete mechanisms by which IFNγ can cause cell cycle arrest have recently been further characterized (**Fig. 1**). Horiuchi and colleagues[9] demonstrated a reversal of IFNγ-induced cell growth inhibition by partially inhibiting the MEK-mitogen-activated protein kinase and extracellular signal regulated kinase (ERK) pathway. This finding supported the postulation that IFNγ induces a transient increase in ERK activity which has downstream effects of inhibiting G1/S transition. Kominksy and colleagues[11,12] have presented evidence that IFNγ has antiproliferative effects on glioblastoma cells by way of the p21 pathway, although having little effect on normal human astrocyte cell proliferation. They have reported that the percent inhibition across glioma cell lines is directly proportional to the level of p21 expression post-IFNγ exposure.

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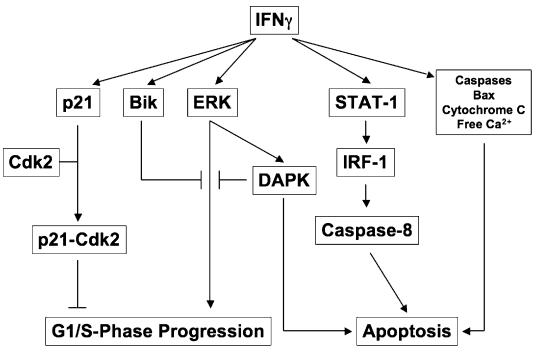

Fig. 1. IFNγ disrupts proliferative mechanisms in gliomas by several mechanisms. p21 is upregulated by IFNγ which binds cdk2 inactivating cdk2 and arresting cell cycle progression. IFNγ upregulates ERK and Bik. Bik blocks ERK's proliferative effects, while ERK increases death associated protein kinase activity leading to apoptosis. IFNγ induces apoptosis through further activation of capsases and downstream mediators of STAT-1.

Additionally, the level of p21 expression directly correlates with the level of cyclin dependent kinase 2 (cdk2) bound to p21 and subsequently the inhibition of cdk2 activity. Janardhanan and colleagues[10] have also reported their findings that IFNγ inhibits cdk2. These experiments collectively suggest the potential ability of IFNγ to arrest cell cycle progression at the G/S1-phase transition by way of ERK and p21 signaling and act as an antiproliferative agent in the potential treatment of malignant glioma.

IFNγ has also been shown to promote apoptosis by way of the mechanism of signal transducer and activator of transcription 1 (STAT-1) and caspase activity. IFNγ has been demonstrated by several studies to be a potent inducer of STAT-1 activity,[9,13–15] which subsequently induces transcription of interferon regulatory factor 1, promoting caspase-8 activity.[14] This mechanism has been further validated in that inhibition of STAT-1 blocks the proapoptotic effects of IFNγ and the increase in caspase-8 activity.[14] In addition, other laboratory investigations have found that IFNγ also causes the upregulation of caspase-1, -3, -8, and -9[10,13,16] and is associated with increased Bax/Bcl-2 ratio, cytochrome C, and free intracellular calcium.[13]

INTERFERON-GAMMA SIGNALING

IFNγ is encoded by a gene on chromosome 12.[17] It is a homodimer in its functionally active state. The IFNγ membrane receptor is called the type II interferon receptor and is composed of the distinct subunits IFNGR1 and IFNGR2, which are constitutively associated with Janus Kinases (JAK) 1 and 2 respectively.[17–21] The IFNγ homodimer binds to two IFNGR1 subunits causing dimerization and recruitment of two IFNGR2 subunits.[18] This interaction results in the cytoplasmic domain autophosphorylation of the associated JAK creating a docking site for STAT-1.[18,19,21] Two free STAT-1 molecules localize to the cytoplasmic binding site, are phosphorylated by the kinases, and associate with each other to form a homodimer. The homodimer then translocates to the cell nucleus and can interact with other coactivator proteins. These oligomers bind IFNγ-activated sites (GAS) in the promoter region of IFNγ-inducible genes and stimulate the transcription of various types of proteins including transcription factors, adaptor proteins, enzymes, and numerous other classes of molecules.[17–19]

The genetic products include proapoptotic elements, such as death-associated proteins.[18,19] Immunogencity is increased by production of proteasomes and major histocompatibility complex (MHC) subunits that increase antigen processing, loading, and presentation.[20] Antiproliferative transcripts result in p21, p27, p38, repressor activator protein 1 (RAS1) and RAS, which are involved in controlling the cell cycle.[17–21] More complex cascades are initiated by inducing transcription factors, such as interferon regulatory factors and class II transactivator (CIITA).[20,21]

More recently it has become clear that IFNγ signaling involves more than the well-described JAK-STAT pathway. Studies suggest that interferon receptors can form higher order complexes and that other molecules have influential control on the interferon signaling pathway. Candidate molecules include PI3-K, protein kinase C, MyD88, and c-Cbl.[17,21] Upon activation of interferon receptors these interacting molecules can initiate independent signaling pathways, such as the MEK-ERK, mammalian target of rapamycin (MTOR), and peroxisome proliferator-activated receptor (PPAR) pathways, or augment the STAT1 pathways.[17,21]

MAJOR HISTOCOMPATIBILITY COMPLEX REGULATION BY INTERFERON-GAMMA

MHC molecules are proteins that display peptide antigens on the cell surface and are crucial to the cellular immune response.[22–24] MHC class I molecules are found on all nucleated cells and function to present to cytotoxic T lymphocytes (CD8), whereas MHC class II molecules are more commonly found on antigen presenting cells (APC) and present to T helper cells (CD4).[24] MHC class I is expressed, but at low levels in malignant gliomas[24–28] and other forms of neoplasms.[29–31]

MHC class II molecules are expressed at varying levels on malignant gliomas[25,27,32–35] and infiltrating APC.[35–37] IFNγ has the ability to upregulate surface expression of class I and II MHC molecules (**Fig. 2**).[24,38–40]

Increasing MHC class I expression on glioma cells could potentially increase the immunogenicity of the glioma and elicit a tumor-specific CD8 cytotoxic response.[24] Findings in animal and human studies indicate that IFNγ upregulates MHC class I expression in gliomas[24,25,27,28,41–44] and other neoplasms.[40,45] In addition, IFNγ may alter antigen processing allowing for better antigen quality control,[39] presentation, and increased expression of tumor-specific peptide antigens.[24] The upregulation of MHC class I molecules also promotes tumor-cell apoptosis by CD8 T cell interactions and has been shown to decrease mortality in animal models.[25,42,44,46] These effects can be abrogated by MHC class I antibody, which inhibit the MHC molecule.[25,42] Additional evidence also suggests that tumors that survive in the presence of IFNγ administration may have preferential mutations causing resistance to IFNγ-induction.[29]

Increasing MHC class II expression on glioma cells and local infiltrating APC may serve to increase immunogenicity by way of a tumor-specific CD4 helper T-cell response. Findings in animal and human studies indicate that IFNγ upregulates MHC class II expression in gliomas[47–50] and infiltrating APC.[35,36,41,47,51] IFNγ also induces STAT-1α expression concurrently with MHC class II upregulation.[48] STAT-1α in turn induces MHC CIITA, a regulator of MHC class II expression, by way of two CIITA promoters.[48–50] STAT-1α inhibitors have been demonstrated to block IFNγ-induced upregulation of MHC class II molecules. These potential mechanisms suggest that IFNγ may elicit malignant glioma cells to process and present MHC II associated native antigen to CD4 helper T cells.[50] IFNγ treatment has also been shown to be associated with an increase in APC MHC Class II expression and an increase in infiltrating tumor-specific APC.[35,36,41,51] One recent study reported a concomitant increase in survival with an associated increase in infiltrating APC with MHC class II molecules on glioma cells.[51]

GENE THERAPY USING INTERFERON-GAMMA TRANSFECTION

Gene therapy offers another potential therapeutic approach to use IFNγ and to induce its possible antitumor effects in the micro environment surrounding gliomas. Several studies have demonstrated the efficacy and feasibility of using IFNγ gene treatment as either monotherapy or as an adjuvant therapy against gliomas and other neoplasms.[52–64] Various methods have been devised to transfect IFNγ genes into cells; however, only several general systems-based approaches have been investigated and explored in gliomas. IFNγ expression has been accomplished in vitro and in vivo by transfecting APC, T cells, and glioma tumor cells. These reports demonstrate the feasibility and potential for durable IFNγ expression[52] along with inhibition of tumor growth,[52,60,65–67] increased T-cell infiltration and T cell-mediated killing,[25,56,57,60,61,66–68] decreased tumor size,[52,66] prolonged survival,[52,61–63,65,66,68,69] and in some cases tumor eradication.[65,68,69] Nishihara and colleagues[57] reported that the level of IFNγ expression in their study correlated with the level of CD8-mediated tumoricidal activity, and that these tumoricidal

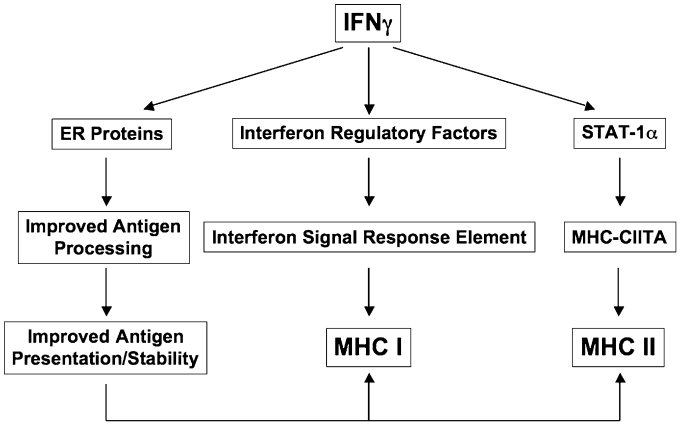

Fig. 2. IFNγ alters endoplasmic reticulum regulatory proteins to improve antigen processing and stability of MHC complexes. Further through Interferon regulatory factors and STAT-1α, IFNγ upregulates expression of both MHC class I and class II molecules increasing the immunogenicity of gliomas to CD4 and CD8 T cells.

processes could be inhibited with the administration of IFNγ-antibody, further suggesting a mechanism that specifically implicates the involvement of the IFNγ pathway in an antitumor effect.

Regardless of the cell type, transfection of the IFNγ gene causes specific changes in the phenotypic status of the tumor and its interaction with immune cells. First, the expression of cell-surface proteins is altered to induce antitumor effects and immunogenic pathways. Paul and colleagues[67] and Ehtesham and colleagues[66] have recently demonstrated modification of cell-surface proteins with the upregulation of MHC class I and II molecules on glioma cells modified to express IFNγ. Mizuno and colleagues[70] have reported that the insertion of an IFNγ gene induces expression of intercellular adhesion molecule 1 (ICAM-1) and FAS antigen, and they also report that enhanced CD8-mediated killing was blocked by ICAM-1 antibody. In another recent animal study, Saleh and colleagues[68] have reported their investigation where all animals who have IFNγ-modified glioma survived to an arbitrary endpoint of 3 months compared with 14 days for their control counterpoints. Pathologic examination of these animals who have IFNγ-modified tumor reportedly revealed eradication of the tumor with normal-appearing brain tissue remaining.

INTERFERON-GAMMA INHIBITS TUMOR ANGIOGENESIS

Inhibiting tumor angiogenesis is another potential mechanism for limiting tumor growth and metastasis.[71–75] IFNγ is one of several cytokines that has been reported to effectively inhibit angiogenesis in tumors.[76–79] Several potential mechanisms have been shown to be associated with this vascular inhibition (**Fig. 3**). Friesel and colleagues[80] have demonstrated that IFNγ causes a decrease in vascular endothelial proliferation. Furthermore, IFNγ enhances the release of antiangiogenic chemokines, such as CXC chemokine, γ-IFN, monokine induced by gamma interferon (MIG), and IFN-inducible protein 10.[81–84] IFNγ has also been shown to down-regulate platelet endothelial cell-adhesion molecule 1 (PECAM-1),[85,86] a molecule constitutively expressed at vascular endothelial cell junctions. Ruegg and colleagues[87] has also recently reported that IFNγ down-regulates integrin alphaVbeta3, an adhesion receptor that plays a key role in tumor angiogenesis. This alteration may lead to decreased endothelial cell adhesion, survival, detachment, and apoptosis of angiogenic endothelial cells in tumors.

Elegant experiments also support the antiangiogenic potential of IFNγ in gliomas. Saleh and

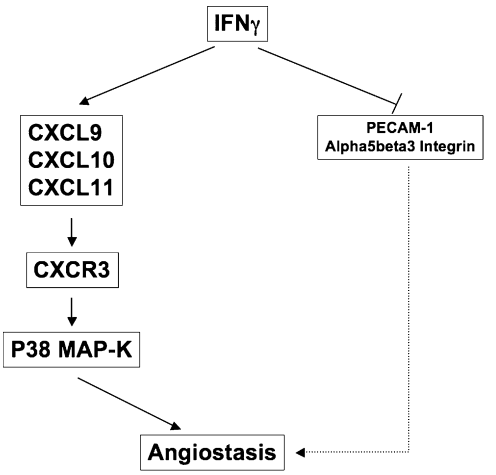

Fig. 3. IFNγ inhibits tumor angiogenesis through chemokines and cell surface proteins involved in neovascularization. IFNγ induces CXCL9, CXCL10, and CXCL11 which act through their receptor, CXCR3, via MAP-K pathway to promote angiostasis. Additionally, IFNγ promotes angiostasis by downregulating pro-angiogenic PECAM-1 and alpha5beta3 integrin.

colleagues[68] treated established rodent gliomas with in situ retroviral IFNγ cDNA. This treatment resulted in dramatically increased survival and eradication of glioma tissue in this animal glioma model. Anti-PECAM antibody stains revealed significantly reduced numbers of tumor vessels, decreased vessel caliber, and thinner vascular walls. In another animal study, Fathallah-Shaykh and colleagues[69] transfected established glioma cells in vivo with IFNγ or beta-galactosidase and compared the effects on animal survival and tumor pathology. They found that the animals that received IFNγ had significantly prolonged survival, and on pathologic examination 38% rejected the tumor with resultant cavity formation. They also showed that the tumors exhibited decreased hemoglobin content and spheroid growth (as opposed to linear in the control) in a gelatinous protein mixture assay in vivo. Furthermore, they report that the transfected cells inhibited neovascularization of tumor cells and induced apoptosis of endothelial cells.

INTERFERON-GAMMA IN COMBINED IMMUNOTHERAPY

Combined therapy offers the advantage of disrupting multiple oncogenic pathways thereby simultaneously promoting discreet yet synergistic therapeutic mechanisms. As the effects of IFNγ are further elucidated, other agents could be used in combination to specifically augment parts

of the IFNγ pathway to increase its antitumor efficacy. IFNγ has been used in combination in gene transfer models and recombinant form in addition to other forms of immuno- and chemotherapy including granulocyte-macrophage colony-stimulating factor (GM-CSF),[88] retinoid compounds, and inducible nitric oxide synthase (iNOS) inhibitors.

The importance of GM-CSF and IFNγ in regulating antitumor surveillance has been demonstrated as double-knockout mice spontaneously develop tumors.[89] Additionally, in other tumor lines combination therapy has demonstrated antitumor effects.[90,91] Relevant to antitumor strategies, GM-CSF encourages development of APC and T cells, which may subsequently have their antitumor effects enhanced by IFNγ.[92–94] As described, IFNγ has the capability to augment T cell-mediated tumor killing, thus combining a leukocyte growth and differentiation stimulating agent could serve to augment the local effects of IFNγ on tumor cytolysis and phagocytosis. Indeed, Smith and colleagues[88,92] have demonstrated this in an established glioma rodent model. They administered GM-CSF gene-modified glioma cells and recombinant IFNγ into established intracranial gliomas and found not only tumor volume reduction and increased lymphocytic infiltration but an 88% tumor eradication rate. Furthermore, they demonstrated that combination therapy was associated with increases in CD4 and CD8 counts, an increase in IFNγ-producing T cells, and rejection of tumors upon rechallenge post-initial eradication.

IFNγ is a potent inducer of iNOS that results in dramatic increases in Nitric oxide (NO).[95,96] NO itself has immunosuppressive effects including suppression of lymphocyte proliferation and lymphocyte-derived chemokines.[97–99] Thus, although IFNγ has many potent antitumor and immunogenic effects, it may also be paradoxically engaging immunosuppressive mechanisms. Medot-Pirenne and colleagues[100] established that inhibiting NO could augment antitumor cytotoxic T lymphocyte activity. Demonstrating the feasibility of this therapy in an animal glioma model, Badn and colleagues[101] showed that iNOS inhibitors administered concurrently with IFNγ transduced glioma cells in rat intracerebral tumors lead to prolonged survival over IFNγ-modified glioma cells alone.

The theory of combining retinoid compounds with IFNγ is based on the concept that chemoimmunotherapy could simultaneously reduce tumor burden and overcome immune resistance. All-trans retinoic acid (ATRA) has been shown in vitro and in vivo to induce differentiation and suppress proliferation by arresting the G1 phase of the cell cycle and down regulating telomerase activity.[13,102,103] Combination therapy in human glioma demonstrates that ATRA causes differentiation, decreased proliferation, and down regulation of telomerase, thus sensitizing tumor cells to IFNγ activity leading to increased apoptosis compared with IFNγ alone.[13,102,103] Another retinoid compound, N-(4-Hydroxphenyl)retinamide, used in combination with IFNγ has shown similar results.[10]

CLINICAL TRIALS

There has been few clinical trials using IFNγ as therapy for gliomas, and all have thus far used recombinant IFNγ (rIFNγ) as the form of delivery. Although rIFNγ has been shown to decrease cell viability, proliferation, and migration while increasing cell death in human glioma cell lines, clinical trials have not yet been proven successful.[104] One recent trial[105] followed 14 subjects given an intravenous dose between IFNγ twice weekly for 8 weeks. Evidence of CT response was only observed in one subject with stabilization between 12 to 86 weeks. Additionally, side effects potentially attributable to rIFNγ administration were observed. In another study,[106] 31 subjects were randomized to receive either intravenous or subcutaneous rIFNγ in escalating triweekly doses over 4 weeks plus adjuvant radiotherapy. Although the treatment was tolerated well, there was no difference between the groups in tumor progression or survival. The most recent study[107] treated 40 subjects who had pediatric glioma with induction radiation and chemotherapy followed by increasing daily doses of rIFNγ for 7 weeks. Although a historical control was used as a comparison group, no difference in the median survival was observed with this treatment in these subjects who had pediatric glioma.

SUMMARY

Treatment gains have been significant for a variety of neoplastic processes. However, malignant gliomas remain seemingly impervious to the range of current clinical therapeutic modalities. Evidence is accumulating that IFNγ has the potential to overcome this barrier by simultaneously targeting multiple mechanisms that typically render gliomas treatment resistant. Research demonstrates that IFNγ interrupts glioma proliferation, induces apoptosis, enhances tumor immunogenicity, and inhibits glioma neovascularization. The diverse array of intra- and extracellular signaling cascades

that IFNγ influences continues to be discovered, particularly in relevance to its antitumor properties.

Although clinical trials have been disappointing, scientific insight into IFNγ antitumor effects continues to progress. There remains a huge opportunity to devise improved methods of treatment and expand clinical trials to larger populations. As with any therapeutic intervention, immunotherapy with IFNγ requires establishing durable treatment, refining delivery techniques, optimizing the pharmacokinetic and pharmacodynamic parameters, reducing systemic toxicity, and devising appropriate clinical selection criteria. Initial clinical trials involved small numbers of patients and used only recombinant systemic delivery. Newer technologies allowing for effective gene transfer, local delivery systems, and multiagent therapies all hold promise to facilitate the efficacious use of IFNγ as immunotherapy for malignant gliomas.

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