

Challenges of Using IFN γ in Clinical Settings

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ABSTRACT

Cytokines in the tumor microenvironment can affect tumor growth, progression, and response to therapy, making them compelling therapeutic agents and targets. IFN γ is a pleiotropic cytokine predominantly secreted by immune cells that binds to its receptors IFNGR1 and IFNGR2 on target cells. Multiple clinical

trials have investigated the efficacy of IFN γ in combination with other therapies for treating patients with cancer and have shown varying results. Here, we summarize the known effects of IFN γ signaling on tumor cells and explore the possibility of its use in clinical settings.

Introduction

According to the World Health Organization, approximately 685,000 women died of breast cancer globally in the year 2020 (www.bcrf.org). Because of early diagnosis, survival is better in developed nations as compared with developing or underdeveloped countries where diagnosis itself remains a challenge. Researchers and medical experts around the globe have been continuously working to save the lives of women diagnosed with breast cancer. Increasing survival rates and decreasing recurrence rates for patients with breast cancer show that there has been progress in the last few decades; however, much still needs to be done. Immune checkpoint inhibitor therapy initially generated a lot of hope, but the recent clinical findings suggest limited responses of patients with cancer to checkpoint inhibitors. Therefore, it is imperative to explore the possibility of combination therapies including cytokines, like IFN γ , along with immunotherapy and chemotherapy.

IFN γ , first discovered in 1965 by Wheelock and recognized for its function to inhibit viral replication, is a 17 kDa pleiotropic molecule that elicits its effects on target cells by binding to IFNGR1 and IFNGR2 and activating the downstream JAK/STAT pathway. It is predominantly secreted by immune cells such as cytotoxic CD8⁺ T cells, CD4⁺ Th cells, natural killer cells, and $\gamma\delta$ T cells. The presence of high numbers of immune cells in the tumor microenvironment (TME) provides a pool of cytokines that affects the growth, metastasis, and survival of tumor cells in response to therapy. These cytokines could potentially be harnessed to improve cancer treatment. The FDA has approved ACTIMMUNE (interferon gamma-1b) for the treatment of chronic granulomatous disease and severe malignant osteopetrosis (accessdata.fda.gov); however, the effect of IFN γ on tumor cells remains far from obvious. Multiple clinical trials have shown varying results with IFN γ when used with combination therapy for patients with cancer. We overview here how IFN γ signaling affects tumor cells and explore its possible clinical utility.

Protumorigenic Function of IFN γ Signaling

Several studies have demonstrated that IFN γ signaling is protumorigenic. Zaidi and colleagues showed that exposure to UVB radiation causes migration of IFN γ -producing macrophages into neonatal skin and that IFN γ induces expression of genes involved in immunoevasion in mouse melanocytes (1). The immunosuppressive effect of IFN γ was further supported by a study from Benci and colleagues that showed that exposure to persistent IFN γ is sufficient to render melanoma cells resistant to radiation and immune checkpoint therapy (2). Persistent IFN γ signaling initiated epigenetic changes in cancer cells characterized by enhanced STAT1-associated open chromatin, which the authors suggested constituted a PD-L1-independent resistance mechanism (2). In addition, Song and colleagues demonstrated that exposure of non-small cell lung cancer cells to a low dose of IFN γ induces expression of intercellular adhesion molecule 1 (ICAM1) and cancer stem cell (CSC) properties in tumor cells, thus connecting IFN γ signaling to the CSCs, which are a small population of tumor cells that has been connected to drug resistance, metastasis, and recurrence (3).

Recent work by Singh and colleagues showed that in triple-negative breast cancer (TNBC), IFNGR1 stabilization leads to activated IFN γ signaling and increased tumor growth, elevated metastasis, and decreased survival (4). Using multiple mice models, including a novel TNBC model, they found that loss of a single allele of transcription factor *Elf5* leads to stabilization of IFNGR1 protein by reducing the ubiquitination and subsequent degradation of IFNGR1 by FBXW7. Tumors from *Elf5*^{+/-} mouse with IFNGR1 stabilization expressed elevated epithelial-to-mesenchymal transition signatures as compared with tumors from *Elf5*^{+/+} mouse. IFN γ signaling has also been reported to increase resistance to immunotherapy. Jacquilot and colleagues demonstrated that chronic PD-L1 blockade induces IFN γ signaling in the TME, upregulating PD-L1 and NOS2 expression on tumor and immune cells, leading to resistance (5). Together, these studies indicate that IFN γ has protumorigenic and prometastatic functions (Fig. 1).

Antitumorigenic Function of IFN γ Signaling

Despite the established tumor supportive effects of IFN γ signaling, multiple other reports over the years have demonstrated the antitumorigenic potential of IFN γ . IFN γ has been shown to induce tumor cell death by enhancing the expression of apoptosis promoting molecules and promoting necroptosis. IFN γ signaling also exerts antitumorigenic effects by targeting the TME. IFN γ inhibits

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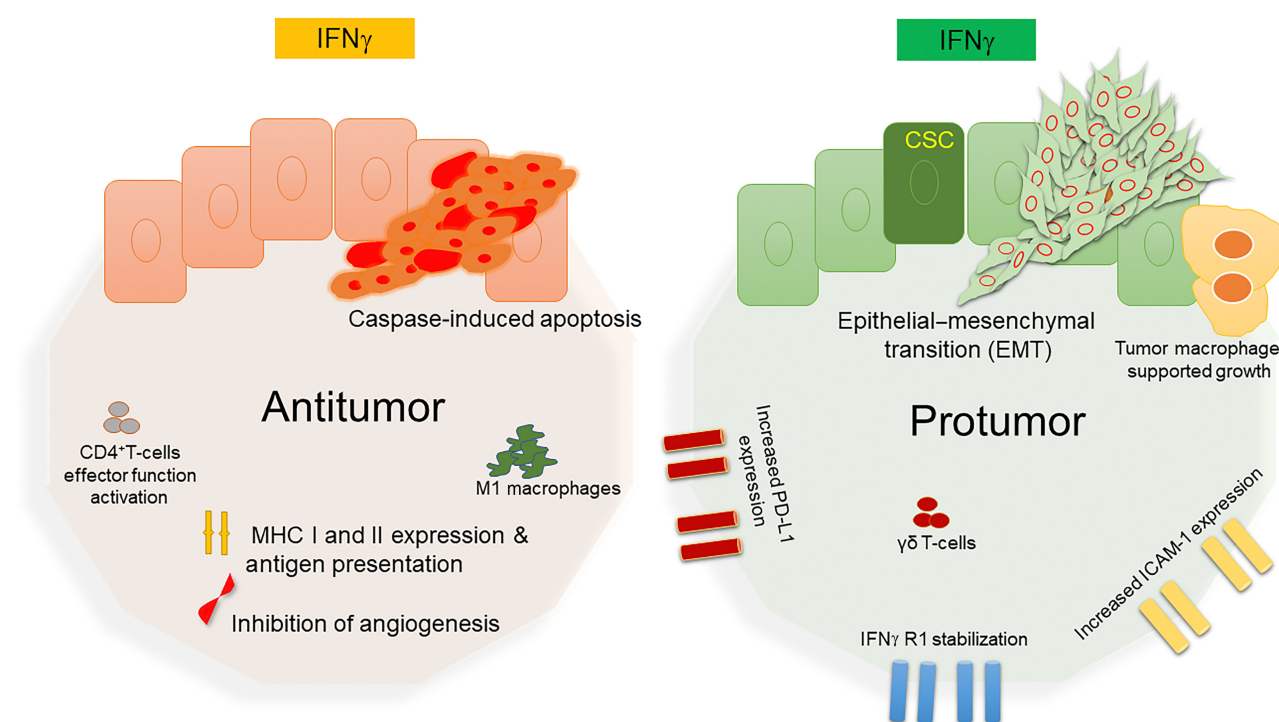


Figure 1.

Major mechanisms through which IFN γ exerts its antitumor or protumor effects on tumor cells and the TME.

angiogenesis by inducing endothelial cell destruction and promotes immune surveillance by increasing cancer cell antigen presentation, enhancing T-cell effector function, and activating antitumor macrophages (Fig. 1; ref. 6).

An interesting study by Benci and colleagues (2) showed that IFN γ signaling may have opposing functions in cancer based on the tumor cell or immune cell context. The authors demonstrated that inhibiting tumor IFN γ signaling using IFNGR knockout cancer cells stimulates both adaptive and innate immune functions in response to immune checkpoint blockade by increasing IFN γ signaling in immune cells. However, the degree to which each of these signaling axes contributes to response is context dependent. Thus, the cell context and timing of IFN γ signaling need to be carefully evaluated in cancer.

IFN γ in the Clinic

The evidence supporting that IFN γ has antitumorigenic functions in tumors has led to clinical investigations of the impact of IFN γ on tumor growth. In a phase III trial, Windbichler and colleagues studied the efficacy of IFN γ in patients with ovarian cancer by including it in the first-line therapy (7). Patients receiving IFN γ showed better progression-free survival than control patients. It has also been shown that recombinant IFN γ treatment is helpful for preventing bladder cancer recurrence (8). On the other hand, multiple reports have shown that inclusion of IFN γ in treatment regimens does not lead to any improvement in clinical parameters in melanoma (9), soft-tissue sarcoma (shows adverse effects, trial terminated, clinicaltrials.gov. NCT01957709), and breast cancer (no improved response, huge toxicity; ref. 6). In addition, in 2008, a clinical trial was prematurely terminated because of toxicity in patients receiving IFN γ treatment

(peritoneal and ovarian cancer; ref. 10). These studies indicate that IFN γ response varies among different types of tumors and might be dependent on tumor responsiveness to IFN γ , along with the presence of different types on immune cells and the cytokine concentration in the TME.

As the presence of immune cells in the TME affects tumor response to IFN γ and tumors are highly heterogeneous, the effects of exposure to proinflammatory IFN γ on both tumor and immune cells should be investigated in detail to understand when the protumorigenic effects of IFN γ will be more prominent and when the antitumorigenic effect due to the immunostimulatory activity of IFN γ will be more detrimental to tumor growth. It is possible that between the time of exposure to IFN γ and subsequent T cell-mediated killing, a few cellular and immunologic pathways might be playing a role in shifting the overall phenotype to growth or death. Also, a wide scale study comparing expression of different genes involved in IFN γ pathway in clinical samples from different tumor-bearing patients and their correlation with prognosis is needed. Variations in immune cells in the TME of different tumors could be a driving factor determining the protumorigenic or antitumorigenic effect of IFN γ . A better understanding of the prognostic correlation with the extent of immune infiltration and local IFN γ concentration in tumors would also be helpful in dissecting the cause and effect. As of now, due to lack of clarity, clinicians need to be cautious of providing IFN γ treatment to patients with cancer.

Future of IFN γ in the Clinics

Considering that IFN γ elicited varied responses in different types of tumors, detailed studies are required to fully categorize tumors. Tumors could be categorized as IFN γ responsive and unresponsive,

by the degree and type of immune cell infiltration, and based on the local concentration of IFN γ in tumors. Preclinical mouse studies using intratumoral injection of IFN γ that comprehensively assess the effects on tumor cell populations and immune cells, conduct pharmacokinetic studies on the rate of clearance of the drug from the system, and evaluate toxicity for different types of tumors would be beneficial in determining its possible course in human population. Additional insights into the mechanisms and biomarkers of sensitivity and

resistance are necessary to fully realize the clinical potential of IFN γ for treating cancer.

Authors' Disclosures

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