Dual Faces of IFNγ in Cancer Progression: A Role of PD-L1 Induction in the Determination of Pro- and Antitumor Immunity

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Abstract

IFNγ is a cytokine that plays a pivotal role in antitumor host immunity. IFNγ elicits potent antitumor immunity by inducing Th1 polarization, CTL activation, and dendritic cell tumoricidal activity. However, there are significant discrepancies in our understanding of the role of IFNγ as an antitumor cytokine. In certain circumstances, IFNγ obviously acts to induce tumor progression. IFNγ treatment has negatively affected patient outcomes in some clinical trials, while it has favorably affected outcomes in other trials. Several mechanisms, including IFNγ insensitivity and the downregulation of the MHC complex, have been regarded as the reasons for this discrepancy, but they do not fully explain it. We propose IFNγ-induced programmed cell death 1 ligand 1 (PD-L1) expression as a novel mechanism by which IFNγ impairs tumor immunity. When tumor cells encounter CTLs in the local environment, they detect them via the high concentration of IFNγ secreted from CTLs, which induces PD-L1 expression in preparation for an immune attack. Thus, tumor cells acquire the capability to counterattack immune cells. These findings indicate that although IFNγ is thought to be a representative antitumor cytokine, it actually has dual roles: one as a hallmark of antitumor immunity and the other as an inducer of the immune escape phenomenon through various mechanisms, such as PD-L1 expression. In this context, the optimization of immunotherapy according to the local immune environment is important. Anti–PD-1/PD-L1 treatment may be particularly promising when efficient tumor immunity is present, but it is disturbed by PD-L1 expression.

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the basic mechanism of tumor immunity, especially of the role of IFNγ in the expression of PD-L1 in the local tumor environment.

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IFNγ as an Antitumor Cytokine in Cancer Biology and Tumor Immunity

Role of IFNγ in physiologic and tumor immunity

IFNγ is a multifunctional cytokine that is primarily secreted by activated T, natural killer cells (NK), and NK T cells. IFNγ plays a pivotal role in systemic and local immunity and is involved in almost all inflammatory responses. IFNγ is a key cytokine in the polarization of Th1 cells. The ability to secrete IFNγ is a hallmark
Recently, cancer immunotherapies, especially those using immune checkpoint inhibitors, such as anti-programmed cell death 1 ligand 1 (anti-PD-L1) or anti-PD-1 antibodies, are being focused upon because of the efficacy they have shown in clinical trials. Nevertheless, they are effective in only a portion of patients with cancer, and it is necessary to personalize these treatments by selecting patients who will benefit from these immunotherapies. IFNγ is one of the representative immune-activating cytokines that has been tested in cancer immunotherapy, but its efficacy is still controversial. We investigated the role of PD-L1 expression in the local tumor environment and found that IFNγ plays a pivotal role in PD-L1 expression in cancer cells and the consequent immune escape by the tumor cells. Here, we focus on the dual aspects of IFNγ in tumor immunity and propose personalized immunotherapies according to the local immune status.

Immune activation mechanisms of IFNγ

Although the biologic mechanism by which IFNγ exerts its antitumor effect is not fully understood, it is likely that the effect depends on multiple processes. IFNγ primarily activates the JAK-STAT pathways that lead to the induction of the expression of multiple genes. In cancer cells, the alterations in gene expression that are caused by IFNγ are presumably associated with increased immunogenicity, which thereby induces immune stimulation. The most typical example of this is the upregulation of HLA class I molecules by IFNγ (7). IFNγ-induced MHC class I expression has been shown to activate a tumor-specific immune response in a mouse model of prostate cancer (8). Sarcoma cells engineered to secrete IFNγ acquire sensitization to being killed by immune cells (9). The retrovirally mediated gene transfer of human IFNγ upregulates MHC antigen expression in human breast cancer and leukemia cell lines (7). The treatment of cervical carcinoma cells expressing low levels of class I and class II MHCs along with IFNγ results in the increased expression of these molecules and significantly enhances the lysis of the tumor cells by specific CTLs (10). It has also been reported that IFNγ upregulates survivin and Bfl-20 expression and induces the survival and proliferation of tumor-specific T cells (11).

Conflicting Data from Basic and Clinical Research on IFNγ Treatment

Negative effects of IFNγ on tumor inhibition

Although a large amount of data indicate that IFNγ acts as a key factor in anticancer immunity, there is also significant evidence demonstrating the opposite effect of this molecule. IFNγ-mediated hepatocarcinogenesis has been observed in mice treated with diethylaminoetosmine (12). Suppressor of cytokine signaling-1 (SOCS1)-deficient mice spontaneously developed colorectal carcinomas in an IFNγ-dependent manner (13). IFNγ has been demonstrated to promote papilloma development (14). Mouse mammary adenocarcinomas transfected with the murine IFNγ gene give rise to progressive tumors (15). IFNγ induces lung colonization following intravenous inoculation with B16 melanoma cells, although this process also enhances MHC class I expression (16). These data clearly contrast with the aforementioned tumor-inhibiting effects of IFNγ.

Inconsistent clinical results regarding the effects of IFNγ

Reflecting the controversial results from basic research findings, the clinical data obtained in several trials are also inconsistent. In the relatively early studies on this topic, several reports suggested the efficacy of IFNγ for use in cancer treatment. The treatment of patients who had melanomas on their extremities using hyperthermic-isolated limb perfusion with melphalan, TNF, and IFNγ resulted in a 76% complete response rate (17). The inclusion of IFNγ in the first-line treatment of ovarian cancer resulted in an improvement in progression-free survival (18). In a prospective randomized study of patients with superficial transitional cell carcinomas who underwent transurethral tumor resection, prophylactic treatment with intravesicular IFNγ administration resulted in a better tumor-free rate compared with that of the nontreated group. Importantly, significant increases in T cells, Th cells, cytotoxic T cells, natural killer cells, and total leukocytes, as well as the numbers of B cells expressing intercellular adhesion molecule-1 and the total leukocytes expressing HLA-DR were observed following IFNγ treatment (19). In contrast, IFNγ treatment did not result in any difference in the outcomes of patients with metastatic renal cell carcinomas (20). No clinically meaningful benefit was observed in a controlled trial testing the use of IFNγ as a postoperative surgical adjuvant therapy for colon cancer (21). Furthermore, a phase III trial of IFNγ plus carboptatin/paclitaxel versus carboptatin/paclitaxel alone for treating advanced ovarian carcinomas was stopped early due to the significantly shorter overall survival (OS) time of the patients receiving IFNγ (22). Similarly, the time to progression and survival were inferior (although nonsignificantly) in patients treated with IFNγ compared with the outcomes of randomized control subjects in a trial including patients with small cell lung cancer with complete response following chemotherapy (23). These results indicate that the effects of IFNγ on tumor suppression are inconsistent and that IFNγ can even be detrimental depending upon the type of tumor and treatment protocol.
A Possible Mechanism Underlying the Controversial Effects of IFNγ in Tumor Immunity

IFNγ insensitivity and tumor development/progression

In sensitivity to IFNγ may contribute to tumor development and progression. Mutations in the IFNγ receptor lead to impaired IFNγ signal transduction. In an animal model, Meth A fibrosarcoma cells overexpressing a dominant-negative IFNγ receptor display enhanced tumorigenicity (24). Mice lacking sensitivity to IFNγ, such as IFNγ receptor-deficient mice, developed tumors more rapidly and with greater frequency than IFNγ-sensitive mice (25). Tumor escape variants that survive CTL adoptive immunotherapy exhibit decreased expression levels of the IFNγ receptor (26). In humans, IFNγ receptor α expression is lower in cases of infiltrating breast cancer than in cases of in situ tumors (27). Rare malignant cutaneous squamous cell carcinomas have been reported in a patient with an IFNγ receptor 2 deficiency (28). Functionally, the expression of the IFNγ receptor is downregulated by the overexpression of the activating protein (AP)-2 (29). The loss of the IFNγ receptor is an independent prognostic factor in ovarian cancer (30). These data all suggest that the lack of responsiveness of tumor cells to IFNγ signaling due to impairment of the IFNγ receptor results in cancer development and/or progression.

Even if the IFNγ receptor is normally expressed, the signal mediated by the receptor can be disrupted by various mechanisms. SOCS1 contributes to the attenuation of IFNγ signaling in vivo by binding to tyrosine-441 of the IFNγ receptor subunit 1 (31). The inhibitory effect of αGalCer on B16F10 lung metastases, of which IFNγ is known to be a critical mediator, is significantly more prominent in mice with mutations in tyrosine-441 of the IFNγ receptor subunit 1 (31). The IFNγ pathway has been demonstrated to be negatively regulated by IFN regulatory factor 2 in esophageal cancer (32).

MHC downregulation and the loss of immunogenicity

MHC molecule expression induced by IFNγ is a major mechanism involved in the immunostimulatory effect of IFNγ, as mentioned above. Therefore, an MHC deficiency and decreased immunogenicity are believed to be important consequences of IFNγ insensitivity. The downregulation of HLA class I molecules has been reported in various malignancies, including breast, cervical, colorectal, esophageal, gastric, ovarian, and renal cell carcinomas (33). However, the frequency of this downregulation varies significantly between tumor types. It can be as high as 48% in esophageal cancer but only 29% in ovarian cancer (34, 35). These findings suggest that MHC downregulation is not the only cause of immune escape by tumors. It has been shown in a uveal melanoma model that treatment with IFNγ boosted MHC class I presentation, but MHC class I–restricted CTL lysis was suppressed (36). Similarly, in human malignant melanomas, low-dose IFNγ treatment induced MHC expression, but this expression was not associated with a tumor response (37). A test using a sporadic tumor mouse model demonstrated that the tumors that develop in immunocompetent mice did not necessarily lose immunogenicity or escape from immunorecognition by T cells; instead, they induced tolerance accompanied by the expansion of anergic CD8+ T cells (38).

Induction of an immune-inhibitory microenvironment

If MHC downregulation is not the only cause of immune escape, what else could be a possible mechanism by which cancer fights against host immunity? One possibility is that IFNγ alters the immune microenvironment and consequently attenuates local tumor immunity. IFNγ is known to induce indoleamine 2,3-dioxygenase (IDO), which results in the induction of regulatory T cells (39). IFNγ has been reported to be essential for myeloid-derived suppressor cell (MDSC) development and its immunosuppressive function (40). Mundy-Bosse and colleagues demonstrated that the nitric oxide produced by MDSCs can reduce IFNγ responsiveness in immune cells such as CD4+, CD8+, and NK cells (41). Finally, we reported that IFNγ induces programmed cell death 1 (PD-1) ligand 1 (PD-L1) in cancer cells, as described below.

IFNγ Induces PD-L1 in Cancer Cells and Impairs Local Tumor Immunity

PD-L1 expression affects patient outcomes in various cancers

It has been reported that PD-L1 expression is associated with the prognosis of various types of malignant tumors. Meta-analyses of studies of non–small cell lung cancer, renal cell cancer, and gastrointestinal tract cancer have revealed that PD-L1 expression is associated with poor OS (42, 43). Wu and colleagues conducted a meta-analysis of 28 studies involving a total of 3,107 patients with solid tumors and concluded that the expression of PD-L1 is associated with lower survival rates in solid tumor patients (44). We also reported that PD-L1 expression is associated with a poor prognosis in patients with ovarian cancer (45). Although there are some variations in the clinical significance of PD-L1 expression in relation to tumor type, its expression is generally associated with poor outcomes for patients with cancer.

Anti–PD-L1/PD-1 therapy has been shown to be effective in clinical trials

Anti–PD-L1/PD-1 therapy is currently the focus of much attention in clinical oncology, and this therapy may change the conventional medical treatment strategy. Nivolumab and pembrolizumab are anti–PD-1 antibodies and have been approved by the FDA for the treatment of metastatic melanomas, and other chemicals, including anti–PD-L1 antibodies, have also been demonstrated to be effective in the treatment of various cancers, including malignant melanoma, non–small cell lung cancer, renal cell cancer, and hematologic malignancies (46). We have reported on the possible usefulness of nivolumab in treating ovarian cancer (47). These results suggest that PD-L1/PD-1 signaling plays not only an important biologic role but also an important clinical role in the treatment malignant tumors in terms of tumor immunity. However, how PD-L1 expression is induced and regulated in human cancers has not been clarified.

PD-L1 expression is induced by IFNγ secreted from T cells in vitro

Using an ovarian cancer model, we investigated the mechanism underlying PD-L1 expression (48). The expression of PD-L1 in vitro varied from high expression to no expression in human and mouse ovarian cancer cells as detected by flow cytometric analysis. However, in most of the human and mouse ovarian cancer cells, PD-L1 expression was strongly induced by IFNγ. Other cytokines, including IL2, IL6, and TGFβ, did not induce PD-L1 expression in vitro. Next, we cocultured mouse ovarian cancer cells with
mouse CD8⁺ T cells recovered from the ascites of cancer-inoculated mice or with the supernatants of the ascites fluid. Notably, PD-L1 expression by the cancer cells was strongly induced by coculture with CD8⁺ T cells but not with the ascetic supernatant, which suggests that direct contact with T cells is necessary for the induction of PD-L1 (48). It is possible that paracrine exposure to the IFNγ secreted by T cells induces PD-L1.

PD-L1 expression is induced by IFNγ in vivo and attenuates local tumor immunity

We have demonstrated the correlation between PD-L1 expression and positive ascetic cytology in human ovarian cancer. Notably, when mouse ovarian cancer cells were inoculated in the mouse abdominal cavity and ascetic cancer cells were subsequently recovered, the expression of PD-L1 in the cancer cells was apparently elevated compared with expression in the cells cultured in vitro (48). On the basis of the in vitro findings, we speculated that direct contact with CD8⁺ T cells in the mouse abdominal cavity induced PD-L1 expression in the cancer cells via paracrine exposure to IFNγ. To test this hypothesis, the IFNγ receptor was knocked down in ovarian cancer cells using shRNA, and mice were intra-abdominally inoculated with these cells (49). The expression of PD-L1 by the IFNγ receptor–depleted cancer cells was reduced, which indicates that IFNγ also mediated PD-L1 expression in vivo. Consequently, CD8⁺ T-cell infiltration into the tumor site was significantly increased, and the survival of the mice was significantly improved compared with the mice inoculated with control mouse ovarian cancer cells, which suggests the recovery of antitumor immunity.

These findings indicate one of the mechanisms by which tumor cells escape immunity and survive despite an immunocompetent environment (Fig. 1). When tumor cells encounter T cells, they detect them via the high concentration of IFNγ secreted from T cells, which induces PD-L1 expression on their surface in preparation for an immune attack. Consequently, local immune cells, especially tumor-specific CTLs, are paralyzed and become unable to attack the tumor cells. Thus, the IFNγ-dependent induction of PD-L1 could serve as a potent immune escape mechanism for cancer cells. This hypothesis is consistent with and partly explains the results of controversial clinical trials examining the efficacy of IFNγ treatment.

Future Directions for Cancer Immunotherapy Based on the Expression of PD-L1

IFNγ is thought to be a representative antitumor cytokine. However, IFNγ actually has dual roles: one as a hallmark of antitumor immunity and the other as an inducer of the immune escape phenomenon via PD-L1 expression. On the basis of these findings, we should consider the use of personalized immunotherapy according to the immune status of each case. For example, in cases with low IFNγ activity, active immunization either via IFNγ treatment or other methods, such as cancer vaccination, may be generally needed, and its further combination with anti-PD-L1/PD-1 therapy should be considered. In cases with high IFNγ activity and high PD-L1 expression, anti–PD-L1/PD-1 therapy alone is expected to be useful. We have shown that some chemotherapy reagents may induce PD-L1 expression in tumor cells (50). Therefore, during chemotherapy, using these drugs, the inclusion of anti–PD-L1/PD-1 therapy may augment the efficacy of the treatment. Although the actual immune condition of an individual patient might be complicated, a better understanding of tumor immunity, especially the effect of IFNγ in each case, should lead to the effective individualization of immunotherapy.
Development of methodology:


Collectively, an overview of the role of IFNgamma in tumor immunity indicates that the local immune microenvironments of malignant tumors are complicated and variable. For effective future immunotherapy, a comprehensive understanding of local tumor immunity and the establishment of personalized treatments according to the evaluation of the immune status of each case appears to be necessary.

Authors’ Contributions

Conception and design: M. Mandai, N. Matsumura, T. Baba
Methodology of development: M. Mandai, K. Abiko, T. Baba

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