

RESEARCH ARTICLE

Induction of Indoleamine 2,3-dioxygenase (IDO) Enzymatic Activity Contributes to Interferon-Gamma Induced Apoptosis and Death Receptor 5 Expression in Human Non-small Cell Lung Cancer Cells

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Abstract

Interferon-gamma (IFN- γ) has been used to treat various malignant tumors. However, the molecular mechanisms underlying the direct anti-proliferative activity of IFN- γ are poorly understood. In the present study, we examined the *in vitro* antitumor activity of IFN- γ on two human non-small-cell lung carcinoma (NSCLC) cell lines, H322M and H226. Our findings indicated that IFN- γ treatment caused a time-dependent reduction in cell viability and induced apoptosis through a FADD-mediated caspase-8/tBid/mitochondria-dependent pathway in both cell lines. Notably, we also postulated that IFN- γ increased indoleamine 2,3-dioxygenase (IDO) expression and enzymatic activity in H322M and H226 cells. In addition, inhibition of IDO activity by the IDO inhibitor 1-MT or tryptophan significantly reduced IFN- γ -induced apoptosis and death receptor 5 (DR5) expression, which suggests that IDO enzymatic activity plays an important role in the anti-NSCLC cancer effect of IFN- γ . These results provide new mechanistic insights into interferon- γ antitumor activity and further support IFN- γ as a potential therapeutic adjuvant for the treatment of NSCLC

Keywords: Non-small-cell lung carcinoma - nterferon- γ - apoptosis - indoleamine 2,3-dioxygenase - death receptor 5

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Introduction

Lung cancer is the most common cancer in terms of both incidence and mortality worldwide. Among lung cancers, non-small cell lung carcinomas (NSCLC) account for approximately 80%–85% of lung cancer cases (Mitsudomi, 2010; Cancer, 2014). Despite improvements in survival through early detection and treatment of NSCLC, many patients suffer from rapid disease recurrence and progression (Xu et al., 2012; Zheng et al., 2013; Sun et al., 2014). Therefore, the search for new or alternative therapeutic approaches remains important and urgently needed in clinical cancer therapeutics.

Interferon gamma (IFN- γ) is a pleiotropic cytokine with diverse physiological functions. IFN- γ is a major activator of various types of immune cells (Wakita et al., 2009; Hayakawa et al., 2011) and also can induce the expression of the major histocompatibility complex in both normal and malignant cells and provokes inflammatory

infiltrate infiltration (Chen et al., 2012; Hong et al., 2014). In addition, IFN- γ can directly inhibit the growth of cells, in some cases, through the induction of cell cycle arrest with or without the concomitant induction of apoptosis (Tate et al., 2012; Liu et al., 2012; Jin et al., 2013; Zhao et al., 2013 Wang, 2014). Recognition of the direct anti-proliferative properties of IFN- γ and its ability to stimulate the immune system has led to the investigation of its therapeutic activity in a number of malignant conditions.

IFN- γ has been used in treating patients with advanced NSCLC, and the results from these initial small clinical studies suggest that IFN- γ can mediate a measurable antitumor effect *in vivo* (Mattson et al., 1991). In addition, for patients with advanced NSCLC, an adjunctive dose of 100 μ g IFN- γ , given three times weekly in the induction and maintenance phase, is feasible. Survival data seem favorable; thus, this regimen may warrant further investigation in a phase III study (Prior et al., 1999). However, the mechanisms of action of IFN- γ in NSCLC

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are merely speculative. Thus, the present study aimed to elucidate the direct effect of IFN- γ on cell growth in H322M and H226 human NSCLC cells and to investigate the detailed mechanism of its anti-cancer activity.

Materials and Methods

Cell Culture and Reagents

The human non-small cell lung carcinoma cells, A549 (adenocarcinoma), H226 (squamous cell carcinoma) and H322M (bronchi alveolar carcinoma) were obtained from Dr. Jeremy J.W. Chen at Chung Hsing University. The cells were grown in RPMI 1640 medium (GIBCO/Life Technologies) containing 10% fetal bovine serum (FBS, GIBCO/Life Technologies), 100 U/ml penicillin and streptomycin. Recombinant human IFN- γ (R&D) was prepared a 100 mg/ml stock solution in PBS, stored at -80°C until use.

Cell viability assay

Cells were seeded into 24-well tissue culture plates and treated with IFN- γ at various concentrations (0-100 ng/ml) for the indicated time, and the cell viability was determined by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide (MTT) assay (Sigma-Aldrich Co.).

Cell cycle analysis

Cells were treated with IFN- γ for the indicated time point. Harvested cells were then washed with PBS and fixed in 70% ethanol at -20°C for overnight. The fixed cells were stained in solution containing RNase A (50 μ g/ml Sigma-Aldrich Co.) and propidium iodide (PI, 50 mg/ml, Sigma-Aldrich Co.) in the dark for 30 min at room temperature. Flow cytometry was performed using a FACSCalibur flow cytometry (FACSCalibur; BD BioSciences).

Measurement of mitochondrial membrane potential (MMP)

Changes in mitochondrial membrane potential were examined by flow cytometry using JC-1 (Molecular Probes). For this assay, cells were treated with IFN- γ for the indicated time point. Ten minutes prior to flow cytometry analysis, JC-1 was added to a final concentration 10 μ g/ml at 37°C, 5% CO₂ atmosphere. At the design time, cell were analyzed for each treatment group on a FL-1 (530 nm) versus FL-2 (580 nm) dot plot on a flow cytometer (FACSCalibur, BD BioSciences).

Western blot analysis

Cells were solubilized in cell lysis buffer (Cell signaling). Lysates were clarified by centrifugation, and protein concentrations were measured using the BCA Protein Assay Reagent (PIERCE). Cell lysates were resolved by electrophoresis using a SDS-polyacrylamide gel (12 or 15%) and electrophoretically transferred to a PVDF membrane. The membranes were blocked at room temperature for 1 h in 5% non-fat milk and blotted with primary antibodies against phospho-STAT1, STAT1, caspase-3, caspase-8, caspase-9, Bax, Bcl-2,

Bcl-Xl, Bak, truncated Bid, and cleaved PARP (all from cell signaling) and IDO (Santa Cruz) at 4°C overnight, followed by secondary antibodies linked to HRP (The Jackson Laboratory) for 2 hours. The immunoactive bands were detected with the enhanced chemiluminescence (ECL) system and developed using the LAS3000 system (Fujifilm, Japan).

In vitro assay for cytochrome c release

Cells were treated with 100 ng/ml IFN- γ for 96 hr. Harvested cells were collected by centrifugation. Cytosolic fractions were isolated using the Mitochondria/Cytosol Fraction Kit (BioVision). The quality of the cytosolic fraction was then determined by western blotting using an anti-cytochrome c antibody (BD Pharmingen).

Transient transfection and RNA interference

Cells were transiently transfected with 400 pmol of FADD siRNA (Santa Cruz) using the Lipofectamine 2000 reagent. Twenty-four hours after transfection, cells were treated 10 ng/ml concentration of IFN- γ for the indicated time. The cell viability was examined by MTT assay.

IDO enzymatic activity assay

Cells were treated with different doses of IFN- γ . At the indicated time point, the cells were disrupted by three freeze/thaw cycles in 200 μ L of PBS buffer, and the lysates was cleared by centrifugation at 3000 \times g. An equal amount of 2 x IDO assay buffer (potassium phosphate buffer (100 mmol/L, pH 6.5), ascorbic acid (40 mmol/L, neutralized with NaOH), catalase (400 μ g/mL), methylene blue (20 μ mol/L), and L-tryptophan (800 μ mol/L; all reagents were purchased from sigma-Aldrich) was added. After 45 min incubation at 37 °C, the reaction was stopped with the addition of 20 μ l of 30% w/v trichloroacetic acid at 65°C for an additional 15 min, and the reaction mixture was centrifuged at 6000 \times g for 5 min. After centrifugation, 20 μ l of supernatant was analyzed by a high performance liquid chromatography system with a reverse-phase column (Mightysil RP-18 GP 4.6 \times 250 mm; Kanto Chemical Co. Inc.). L-Kynurenine (5-100 μ mol/L, sigma) was used as the standard.

Analysis of death receptors and ligands by flow cytometry

After IFN- γ treatment at indicated times, the cells were suspended in FACS buffer (PBS supplemented with 5% FBS and 0.1% NaN₃) and then incubated with anti-DR4-PE, anti-DR5-PE, and anti-Fas-PE, (eBioscience) at 4°C for 30-45 minutes. Subsequently, the stained cells were fixed with 1% paraformaldehyde (Sigma), and the expression level of each molecule was analyzed by flow cytometry.

IDO inhibitor (1-MT) and tryptophan treatment assay

The cells were pre-treated with IDO inhibitors of 1-methyl-tryptophan 1-MT (sigma) or L-tryptophan for 1 hr and then treated with 10 ng/ml IFN- γ for 96 h. The treated cells were subjected to MTT assay, flow cytometry analysis following staining with Annexin V-FITC and propidium iodide (PI) or western blotting assay.

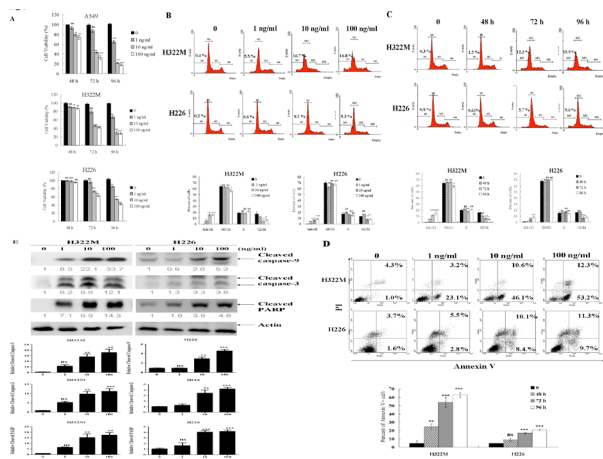


Figure 1. IFN- γ reduced cell viability and apoptosis in NSCLC cells. (A) Cells were treated with different concentrations of IFN- γ (0-100 ng/ml) for 48-96 h and subjected to MTT assay. The viability of PBS-treated cells was considered 100%. (B) Cells were treated with different concentration of IFN- γ (0-100 ng/ml) for 96 h or (C) 10 ng/ml IFN- γ for 48-96 h and subject to cell cycle analysis. (D) Cells were treated with different concentrations of IFN- γ (0-100 ng/ml) for 96 h and subject to Annexin V/PI staining. (E) Cells were treated with different concentrations of IFN- γ (0-100 ng/ml) for 96 h. Total cell lysates were prepared to detect the cleaved forms of caspase-9, caspase-3 and PARP. The bands were analyzed by ImageJ and normalized to actin. The means \pm SD of the experimental triplicates are presented in the bar graph. All data presented are representatives of three independent experiments with similar results. nsp>0.05, * p <0.05, ** p <0.01, *** p <0.001, compared with the PBS-treated group

Statistical Analysis

The results are expressed as the mean \pm SD. Statistical analyses were performed by one-way ANOVA followed by Tukey's post-hoc test. A p <0.05 was considered statistically significant.

Results

IFN- γ inhibits the growth of NSCLC cell lines

To study the anti-proliferative effect of IFN- γ on human Non-small-cell lung carcinoma (NSCLC) cell, three NSCLC cell lines, A549, H322M and H226, were chosen. The cells were treated with various concentrations of IFN- γ to investigate the cytotoxicity of IFN- γ against these cells. Cell viability was then examined using the MTT assay. IFN- γ inhibited cell growth in a dose- and time-dependent manner in all three cell lines (Figure 1A). However, the growth of A549 and H322M cell lines were inhibited to a greater extent than the growth of H226 cells.

IFN- γ induces apoptosis in H322M and H226 cells

Furthermore, to determine whether IFN- γ -induced growth inhibition occurs via cell cycle blockade or apoptosis, H322M and H226 cells were assayed using PI staining and subjected to flow cytometric analysis. Figure 1B and 1C show that IFN- γ treatment did not cause cycle

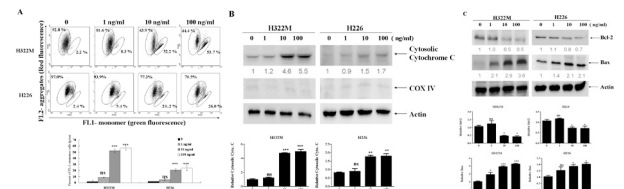


Figure 2. IFN- γ triggered mitochondrial apoptotic events in H322M and H226 cells. Cells were treated with different concentration of IFN- γ (0-100ng/ml) for 96 h and (A) subject to JC-1 fluorescence staining and (B) Immunoblotting for cytochrome c using cytosolic fractions. Blots were stripped and reprobred with cytochrome c oxidase subunit IV (COX IV) as a mitochondrial marker. (C) Total cell lysates were prepared and subjected to western blot with antibodies to detect the expression of Bcl-2 and Bax, The bands were analyzed by ImageJ and normalized to actin. The means \pm SD of the three independent experiments are presented in the bar graph. All data shown are representatives of three independent experiments with similar results. nsp>0.05, * p <0.05, ** p <0.01, *** p <0.001, compared with the PBS-treated group.

arrest during the 48 to 96 hr period. However, the sub-G1 population, which is typically considered an apoptosis, significantly increased in a dose- and time-dependent manner after IFN- γ treatment. To further examine the observed IFN- γ -induced apoptosis, we utilized flow cytometry following staining with annexin V-FITC. As presented in Figure 1D, the percentages of early apoptotic death (Annexin V+/PI-) increased in a dose-dependent manner, suggesting that IFN- γ predominantly induced apoptosis in H322M and H226 cells. This result was confirmed by analyzing the level of cleaved caspase-9, caspase-3 and PARP after IFN- γ treatment (Figure 1E).

IFN- γ activates the mitochondria-mediated apoptotic pathway in H322M and H226 cells

Mitochondria play an important role in the intrinsic and extrinsic apoptotic death pathways (Sankari et al., 2012; Wang et al., 2014). Loss of mitochondrial membrane potential ($\Delta\psi_m$) is an indicator of mitochondrial damage during apoptosis. The $\Delta\psi_m$ was estimated using JC-1 fluorescent dye by flow cytometry. In healthy cells with high $\Delta\psi_m$, JC-1 incorporates into mitochondria and forms aggregates (fluorescence in red, 590 nm). However, in apoptotic or unhealthy cells with low $\Delta\psi_m$, JC-1 incorporates into mitochondria and forms monomers (fluorescence in green, 527 nm) (Smiley et al., 1991). As shown in Figure 2A, treatment of H322M and H226 cells with IFN- γ for 96 h led to a dose-dependent increase in red fluorescence relative to the PBS-control group, suggesting that IFN- γ treatment led to a reduction in $\Delta\psi_m$. In addition, the release of cytochrome c from the mitochondria into the cytosol is a key initiative step in the mitochondrial apoptotic pathway. The Bcl-2 family is known for its ability to target mitochondria and induce cytochrome c release (Kluck et al., 1997; Wang et al., 2014). As shown in Figure 2B, IFN- γ induced an increase in the level of cytosolic cytochrome c in H322M and H226 cells. In addition, western blot analysis revealed

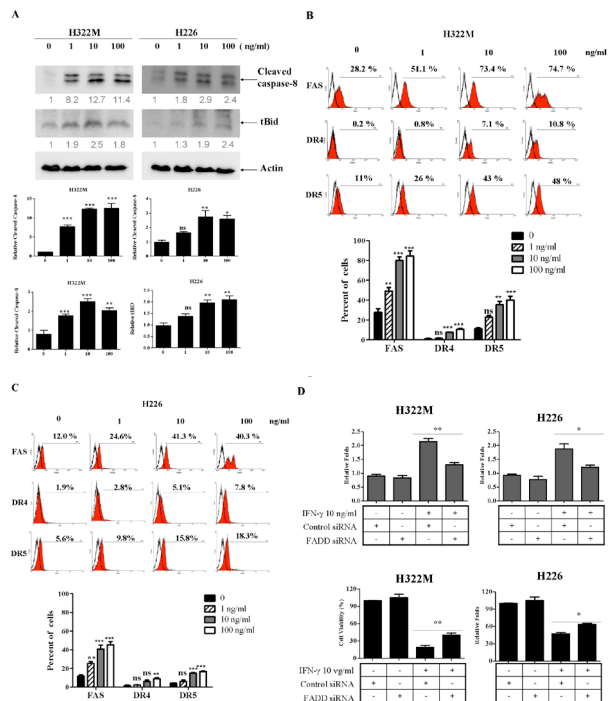


Figure 3. IFN- γ induced the activation of the FADD/caspase-8-tBid cascade and up-regulated death receptor expression in H322M and H226 cells. Cells were treated with different concentrations of IFN- γ for 96 h. (A) Total cell lysates were prepared to detect the cleaved caspase-8 and tBid. The bands were analyzed by ImageJ and normalized to actin. (B) The expression of FAS, DR, and DR5 receptor surface expression in H322M and (C) H226 were examined by flow cytometry after staining with respective PE-conjugated antibodies. (D) After transfection of negative control siRNA or FADD-siRNA for 24 h, FADD protein expression was determined with real time PCR. The effect of 10 ng/ml IFN- γ treatment on cell viability was detected by MTT assay for 72 h. Data are the mean \pm SD of triplicate wells. All data shown are representatives of three independent experiments with similar results. nsp>0.05, * p <0.05, ** p <0.01, *** p <0.001 compared with the PBS-treated group. # p <0.001, compared with the 10 ng/ml IFN- γ treated group.

that the anti-apoptotic protein Bcl-2 was down-regulated and that the pro-apoptotic protein Bax was up-regulated (Figure 2C). Taken together, these findings suggest that treatment with IFN- γ induces apoptosis primarily through the mitochondrial apoptosis pathway.

IFN- γ increases caspase-8 activity and death receptor expression in H322M and H226 cells

Active caspase-8 can induce the mitochondrial apoptotic pathway by cleaving Bid, freeing the truncated form to translocate to the mitochondria, which leads to mitochondria damage and activates mitochondrial apoptotic downstream signals, including cytochrome c release into the cytosol (Sankari et al., 2012; Tao et al., 2012). As shown in Figure 3A, IFN- γ treatment could dose independently increases the level of cleaved caspase-8 protein and truncated Bid. In addition, the death receptor

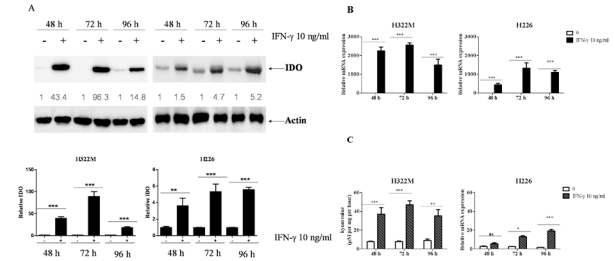


Figure 4. IFN- γ induced IDO expression and activity in H322M and H226 cells. Cells were treated with 10 ng/ml IFN- γ for the indicated time. (A) Total cellular protein was extracted, and the IDO expression was determined by western blot analysis. The bands were analyzed by ImageJ and normalized to actin. (B) Total RNA was extracted, and the IDO mRNA expression levels were measured by quantitative real-time RT-PCR. (C) IDO enzymatic activity was measured as the concentrations of L-kynurenine as detected through HPLC. Data are presented as the mean \pm SD of triplicate wells. All data shown are representatives of three independent experiments with similar results. nsp>0.05, * p <0.05, ** p <0.01, *** p <0.001 compared with the PBS-treated group

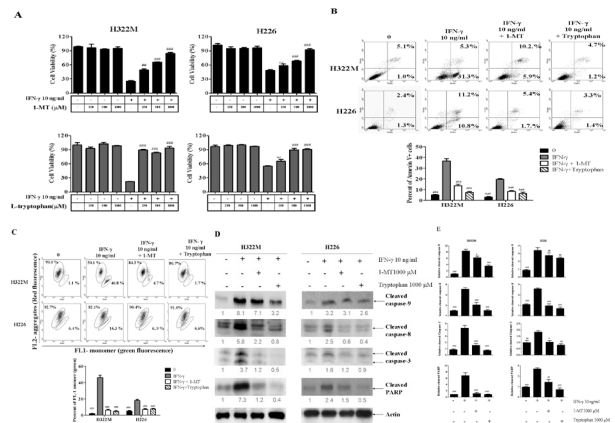


Figure 5. Inhibition of IDO activity reduces IFN- γ -induced apoptosis in H322M and H226 cells. (A) Cells were treated with 10 ng/ml IFN- γ alone or in combination with the IDO inhibitor 1-MT (250-100 μ M) or L- tryptophan (250-100 μ M) for 96 h, and viability was assessed using the MTT assay. The cells were stained with (B) annexin V-FITC/propidium iodide (PI) or (C) JC-1, and flow cytometry analysis was used to detect cell apoptosis and mitochondrial membrane potential. (D) Cleaved Caspase-8, Caspase-9, caspase-3 and PARP levels were assessed by western blot analysis. The bands were analyzed by ImageJ and normalized to actin. All data are presented as the mean \pm SD of triplicate wells. The figure was representatives of three independent experiments with similar results. nsp>0.05, # p <0.05, ## p <0.01, ### p <0.001, compared with the IFN- γ -treated group.

signaling complex (DISC) formation results in the activation of caspase-8, which plays the important role in cell apoptosis. In particular, the DR4/5 and Fas receptors and their ligands have been extensively investigated (Guo et al., 2012; Hong et al., 2013). As shown in Figure 3B and 3C, the expression of Fas and DR5 significantly increased

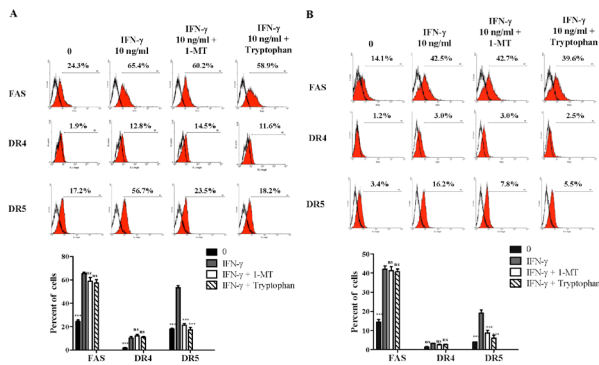


Figure 6. Inhibition of IDO activity reduces IFN- γ -induced death receptor 5 expression in H322M and H226 cells. Cells were treated with 10 ng/ml IFN- γ alone or in combination with the IDO inhibitor 1-MT (1000 μ M) or L-tryptophan (1000 μ M) for 96 h. The expression of FAS, DR, and DR5 receptor surface expression in A) H322M and B) H226 was examined by flow cytometry after staining with the respective PE-conjugated antibodies. The mean \pm SD of the experimental triplicates are presented in the bar graph. nsp>0.05, ###p<0.001, compared with the IFN- γ -treated group.

in a time-dependent pattern after IFN- γ treatment in H322M and H226 cells. Moreover, given that activated death receptors recruit an adaptor protein called Fas-Associated Death Domain (FADD) and that activated caspase-8 induces apoptosis, we further used specific siRNA to silence FADD expression and assessed the cell viability. We found that FADD silencing rescues IFN- γ -induced reduction of cell viability (Figure 3D). Taken together, these data suggest that IFN- γ -induces apoptosis in H322M and H226 lung cancer cells via a caspase-8- and death receptor-dependent mechanism.

The induced expression and activation of IDO by IFN- γ is associated with cell death in H322M and H226 cells

Indoleamine 2,3-dioxygenase (IDO) is the first and rate-limiting enzyme of tryptophan catabolism (Fujigaki et al., 2012; Sagan et al., 2012). Recent studies have reported that IFN- γ can induce the expression of IDO in many types of human tumors, and IDO activation is responsible for the antiproliferative action of IFN- γ in some cancer cell lines (de la Maza and Peterson, 1988; Ozaki et al., 1988). In this study, our data also indicated a marked elevation of IDO protein (Figure 4A) and RNA expression (Figure 4B) and an increase in IDO enzymatic activity (Figure 4C) in H322M and H226 lung cancer cells following 10 ng/ml IFN- γ treatment. However, the IFN- γ -treated H322M cells exhibited higher IDO expression and activity than did IFN- γ -treated H226 cells. These data were consistent with the Figure 1 and Figure 2 cell viability and apoptosis results. To confirm the role of IDO in IFN- γ -induced apoptosis, H322M and H226 cells were pretreated with the IDO-inhibitor 1-methyl-tryptophan (1-MT) or L-tryptophan, followed by IFN- γ treatment for 96 h. As shown in Figure 5, the addition of 1-MT or L-tryptophan significantly decreased the IFN- γ -induced reduction in cell viability (Figure 5A), apoptosis (Annexin V+/PI-) (Figure 5B), loss of mitochondrial membrane potential

($\Delta\psi_m$) (Figure 5C) and cleaved forms of caspase -8, -9, -3 and PARP expression (Figure 5D and 5E) in these two cell lines. Thus, these results suggest that IDO enzymatic activity plays an important role in IFN- γ -induced H322M and H226 cell apoptosis.

IDO enzymatic activity participates in IFN- γ -triggered DR5 expression in H322M and H226 cells

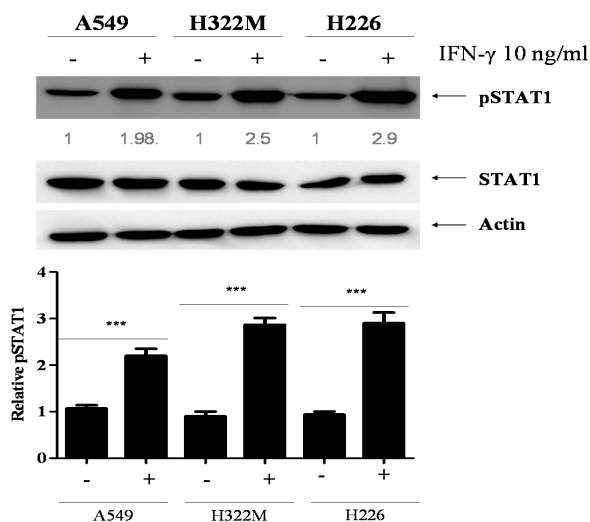
Our results in Figure 3B demonstrated that IFN- γ can induce Fas, DR4 and DR5 expression, in H322M and H226 cells. Therefore, we further examined whether IDO is involved in IFN- γ -induced death receptor expression in H322M and H226 cells. As illustrated in Figure 6A and 6B, 1-MT and L-tryptophan could significantly repress IFN- γ -induced DR5 expression without significantly affecting Fas and DR4 expression, therefore suggesting that IDO enzymatic activity plays a key role in modulating IFN- γ -induced death receptor DR5 expression.

Discussion

IFN- γ is confirmed to possess immune-activating properties and hence is proven to have anti-cancer effects *in vivo* (Wakita et al., 2009; Hayakawa et al., 2011, Yuan et al., 2014). In addition, recent studies also have indicated that IFN- γ has the ability to directly inhibit cancer cell growth (Tate et al., 2012; Liu et al., 2012; Jin et al., 2013; Zhao et al., 2013; Wang, 2014); however, the mechanism remains unclear. Our present study demonstrated that the FADD/caspase 8/tBid/mitochondria pathway plays an important role in IFN- γ -induced apoptosis in the NSCLC cell lines H322M and H226. Notably, our results also demonstrated for the first time that IDO enzymatic activity plays a significant role in the IFN- γ -induced apoptosis and death receptor 5 (DR5) expression in NSCLC cells.

Recently, IDO enzymatic activity has been reported to be associated with apoptosis in many types of cells. Two possible mechanisms of apoptosis are proposed: 1) depletion of tryptophan (de la Maza and Peterson, 1988; Ozaki et al., 1988; Lee et al., 2002; Platten et al., 2012) and 2) production of toxic tryptophan metabolites in the kynurenine pathway, such as kynurenines and 3-hydroxyanthranilic (3-HAA) (Morita et al., 2001; Fallarino et al., 2003; Mailankot et al., 2009; Lee et al., 2010; Mailankot and Nagaraj, 2010; Song et al., 2011; Hou et al., 2014). In this study, we demonstrated that the addition of either the IDO inhibitor 1-MT or L-tryptophan could decrease IFN- γ -induced reduction of cell viability (Figure 5A) and apoptosis (Figure 5B-E). Therefore, we suggested that IDO-mediated tryptophan depletion rather than the production of toxic tryptophan metabolites plays a more important role in the anti-NSCLC cancer effect of IFN- γ , as many studies have indicated that degradation of excess L-Trp via IDO leads to the production of large amount of various types of tryptophan metabolites.

However, these different susceptible effects of tryptophan metabolite-induced apoptosis between H322M and H226 cancer cells and other sensitive cells such as T cell or lens epithelial cells may be explained by previous study demonstrated that significant apoptosis of thymocytes and T lymphocytes cells was evident at 10



Supplemental Figure 1. IFN- γ induced STAT1 phosphorylation in A549, H322M and H226 cells. Cells were treated with 10 ng/ml IFN- γ for 15 min. Total cellular protein was extracted, and the phospho- and non-phospho-STAT1 expression was determined by western blot analysis. The bands were analyzed by ImageJ and normalized to actin. The means \pm SD of the three independent experiments are presented in the bar graph. *** p <0.001 compared with the PBS-treated group.

μ M 3-HAA and QUIN, whereas apoptosis of macrophages or dendritic cells required >10-fold higher concentrations (Fallarino et al., 2002).

In this study, we observed that inhibition of IDO activity by 1-MT or L-tryptophan suppressed DR5 expression but did not affect the expression of DR4 and FAS in IFN- γ -treated H322M or H226 cells (Figure 6A). To our knowledge, no previous study has reported the relationship between IDO activation and death receptor expression. Some studies have reported that certain intracellular molecules can selectively affect the expression of different death receptors. For example, inhibition of p53 expression affects the expression of DR5 but not that of DR4 in myeloma cells (Surget et al., 2012). Thus, further clarification on the mechanism underlying IDO activity-regulated DR5 expression and further research is still needed.

Our results in Figure 4 result demonstrated that IFN- γ can increase IDO expression and activity in A549 and H322M to a greater extent than in H226 cells. The interaction of IFN- γ with its cell surface receptor leads to the activation of JAK1 and JAK2 (phosphorylation), which in turn phosphorylates and activates the STAT1 molecules (Chen et al., 2011). In addition, the JAK-STAT1 pathway was also demonstrated to play a critical role in IFN- γ induced IDO expression in several cell types (Chon et al., 1996). However, our result demonstrated that IFN- γ induced comparable STAT1 activation among three cells cultured under optimal growth conditions (Supplemental Figure 1). Thus, this research remains unable to explain the diverse reactions to IFN- γ -induced IDO expression and apoptosis among different NSCLC cells and requires further study.

IDO activation appears to play a role in the suppression of adaptive T-cell-mediated immunity by inducing the apoptosis of T cells (Lee et al., 2002; Fallarino et al., 2002), Treg induction (Sun et al., 2011), and the down-regulation of MHC class I (Li et al., 2004). Therefore, determining the differences in the mechanisms of IDO-induced direct growth inhibition of tumor cells and IDO-induced suppression of adaptive immunity is also an important area of study in the future for the application of IFN- γ in clinical practices against cancer.

In summary, our findings demonstrated that the proliferation of H322M and H226 NSCLC cells was reduced by IFN- γ treatment through the induction of IDO enzymatic activity and the activation of the caspase-8/tBid- and mitochondria-dependent apoptotic pathways. These data provide new insights into the molecular mechanisms underlying IFN- γ -mediated NSCLC cell death and may provide an efficacious therapy for patients harboring NSCLC.

Acknowledgements

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