Type III interferon induces apoptosis in human lung cancer cells

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Abstract. The apoptotic effects of interferon lambdas (IFNλs) have been described in several types of cancers. However, their effects on human lung cancer cells and the mechanisms are elusive. In addition, the interaction between IFNλs and other interferons remains unclear. The interplay between IFNα and IFNλ has been reported. However, although IFNγ is a well-known regulatory interferon, the mechanisms through which it regulates IFNλs in lung cancer cells are unknown. These issues are critical for the application of IFNλs in lung cancer therapy. In this study, we used A549, a cell line derived from a human lung carcinoma, to characterize the antiproliferative and apoptotic effects of IFNλs on lung cancer, and the interplay between IFNγ and IFNλ. Because overexpression of full-length ectopic IFNλR1 led to cell death, we generated A549 cells stably expressing a chimeric receptor (10R1/λR1), which is composed of the extracellular domain of IL-10 receptor (IL10R1) fused in tandem to the transmembrane and intracellular domains of the IFNλ receptor (IFNλR1). By comparing with A549 cells stably expressing its cognate vector, we demonstrated that IL-10 stimulation triggered the intracellular IFNλ signaling via 10R1/λR1 receptor. By using A549 cells expressing 10R1/λR1, we report that the IFNλR1 chain of IFNλ receptor possesses an intrinsic ability to trigger apoptosis in human lung cancer cells. Although it did not suppress cell proliferation, IFNλ signaling via 10R1/λR1 receptor induced cell cycle arrest, externalization of phosphatidylserine, DNA fragmentation, activation of caspase-3, caspase-8 and caspase-9. However, the caspase inhibitor Z-VAD-FMK did not prevent apoptosis. In addition, the extent of induced apoptosis correlate with the expression levels of the IFNλ receptor and the levels of STAT1 activation. Lastly, we demonstrated that IFNγ sensitized A549 cells to IFNλ-induced apoptosis, via upregulation of IFNλR1. These data indicate the potential of IFNλ, alone or in combination with IFNγ, in the treatment of human lung carcinoma.

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Introduction

Lung cancer is one of the most deadly cancers in the world (1,2). It was estimated that more than 326,000 deaths of lung cancer in China in 2005 (3). The death rate of lung cancer in China soared to 306.1 per 1,000,000 persons in 2008, in a sharp contrast to 5.47 and 17.27 per 1,000,000 persons in the mid 1970s and in the early 1990s, respectively (4). Treatment of lung cancer remains a challenge.

Interferons (IFNs) have been used to treat various types of cancer in clinical practice (5). Interferons, including type I, type II and type III IFNs, are a group of secreted cytokines that possess antiviral and antitumor capacities (5-7). The receptors of IFNs are composed of a ligand-binding subunit and an accessory subunit. The ligand-binding subunit is usually type-specific, while the accessory subunit is commonly shared by receptor complexes of various cytokines. Upon the ligation of IFNs to their respective receptors, the receptor-associated Janus activated kinases (JAKs) are phosphorylated and activated, which in turn phosphorylate and activate various signal transducer and activator of transcription (STAT) family members. STATs undergo homo- or hetero-dimerization, translocate to nuclei and bind to the promoter region of IFN-stimulated genes leading to downstream gene transcription (8). Crosstalk between the JAK-STAT pathway and other signaling pathways, such as phosphotidylinositol 3-kinase and mitogen-activated protein kinase pathways, modulates cellular responses to IFNs and IL-10-related cytokines (9,10).

Type I IFNs, including IFNα and IFNβ, have intense antiviral and antitumor activities, as well as severe and common side effects, such as bone marrow suppression, psoriasis,

thyroid disorders, diabetes, retinal changes and psychiatric disorders, which significantly limit their clinical applications (11,12). Type II IFN, including only IFNγ, generally has weak antiviral and antitumor activities (12). Type III IFNs, including IFNλ1, IFNλ2 and IFNλ3, possess comparable antiviral and antitumor capacities to type I IFNs, however, with much less side effects, which makes them new hotspots in the development of antiviral and antitumor agents (13).

Evasion of apoptosis is an important step in the development of cancer (14). Therapeutic activation of apoptosis in cancer cells is a potential anticancer strategy (14). More than 300 genes regulated by IFNs are implicated in apoptosis (15-18). Previous studies have demonstrated the antitumor potential of type III IFNs in mouse melanoma (19) and fibrosarcoma (20,21), human colorectal adenocarcinoma (22), glioblastoma (23), pancreatic neuroendocrine tumor (24) and colorectal carcinoma (25). There is also a report indicating that IFNλ induces apoptosis in non-small cell lung cancer (NSCLC) cells (26). But, its molecular mechanisms and effects when combines with type II IFN still remain elusive.

In this study, we selected IFNλ1 as a model to study the antitumor effects of type III IFNs on human lung cancer. By using an established chimeric receptor (22), we observed the apoptotic effects of IFNλ1 on human lung cancer with a concomitant activation of STAT1. In addition, we observed that IFNγ renders human lung cancer cells sensitivity to IFNλ-induced apoptosis.

Materials and methods

Plasmid, cells and transfection. The plasmid FL-10R1/λR1 (10R1/λR1) was previously described (22). Human lung cancer cells A549 (from PriCell Research Institute, Wuhan, P.R. China) were maintained in RPMI-1640 medium (Sigma-Aldrich) with 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich). A549 cells were transfected with expression plasmid 10R1/λR1 or its cognate vector using TransIT transfection kit (Mirus) following the manufacturer's instruction. G418-resistant transfectants were selected in medium containing antibiotic G418 (400 μg/ml).

Cell cycle analysis and TUNEL assay. To determine distribution of cells through the cell cycle, 10^5-10^6 cells (attached and floating) were collected, rinsed with phosphate-buffered saline (PBS) containing 5% FBS, permeabilized by incubation with 0.1% Triton X-100 at 22˚C for 5 min, washed with PBS containing 5% FBS, precipitated by centrifugation at 2000 g, 4˚C and re-suspended in PBS containing 5% FBS, 8 mg/l PI and 40 mg/l RNase A at 22˚C for 10 min in the dark. Cell distribution through the cell cycle was analyzed by flow cytometry.

TUNEL assay to determine DNA fragmentation in apoptotic cells was performed according to the manufacturer's suggested protocols (Promega). Briefly, 3-5x10^6 cells were trypsinized, washed twice with cold PBS, fixed in 4% paraformaldehyde at 4˚C for 20 min, washed again with PBS and permeabilized with 0.5 ml 0.5% saponin at 22˚C for 5 min. The cells were washed with PBS, incubated with 80 μl equilibration buffer at 22˚C for 5 min, washed with PBS, re-suspended in 50 μl Nucleotide mix and incubated in the dark at 37˚C for 1 h. Cells were washed again with PBS then analyzed by fluorescence microscopy.

To induce apoptosis, A549 cells were treated with a combination of IFNγ (10 ng/ml) and TNFα (1 ng/ml). A549 cells expressing 10R1/λR1 (A549/10R1/λR1) were treated with IL-10 (10 ng/ml) at 37˚C for 48 h. The concentrations and durations of cytokine treatments were indicated in other apoptosis-related assays. Where indicated, caspase inhibitor Z-VAD-FMK (Calbiochem) was added to the medium.

Flow cytometry and antibodies. Flow cytometry was performed as previously described (27). FITC-conjugated mouse anti-human activated STAT1 antibody was purchased from Cell Signaling, USA. FITC-conjugated mouse anti-human major histocompatibility complex class II (MHC II) antibody, FITC-conjugated mouse anti-human caspase-3 antibody, FITC-conjugated mouse anti-human caspase-8 antibody, APC-conjugated mouse anti-human caspase-9 antibody and FITC-conjugated Annexin V antibody were purchased from BD Biosciences. FITC-conjugated rabbit anti-FLAG antibody was purchased from Sigma-Aldrich. Phycoerythrin-conjugated mouse anti-human IFNλR1 antibody was purchased from R&D Systems.

Proliferation and cell viability assays. Proliferation and cell viability assays were performed as previously described (22). Briefly, to determine cell proliferation, equal amount of cells (10^5/well) were plated in 6-well plates and treated with various concentrations of cytokines, as indicated in the text. Floating cells were collected and combined with adherent cells released from the wells by trypsinization before cell counting.

To determine cell viability, an equal amount of cells (3x10^4/well) were plated in all wells of 96-well microtitre plate and treated with various concentrations of cytokines as described in the text. Dead cells lost their attachment. At indicated time points live adherent cells were visualized by staining with crystal violet at 22˚C for 5 min.

Statistical analysis. Statistical analyses were performed using Prism software (GraphPad Prism). Untreated and treated groups were compared using the Student's t test when the data were normally distributed. When the data were not normally distributed, the two groups were compared using the non-parametric Mann-Whitney U test. All tests were two-tailed. The P-values <0.05 were considered statistically significant.

Results

IFNλ signaling does not inhibit the proliferation of human lung cancer cells. Treatment of A549 cells with either type I, type II or type III IFNs increased the expression levels of MHC II to equivalent levels, indicating that A549 cells responded to all three types of IFNs (Fig. 1A). In contrast to type II IFNs, type I and type III IFN did not inhibit the proliferation of A549 cells, even when the concentration was up to 100 ng/ml (Fig. 1B). In summary, IFNλ1 did not inhibit A549 cell proliferation.

Signal induction through chimeric receptor 10R1/λR1. The expression level of IFN receptors can determine the

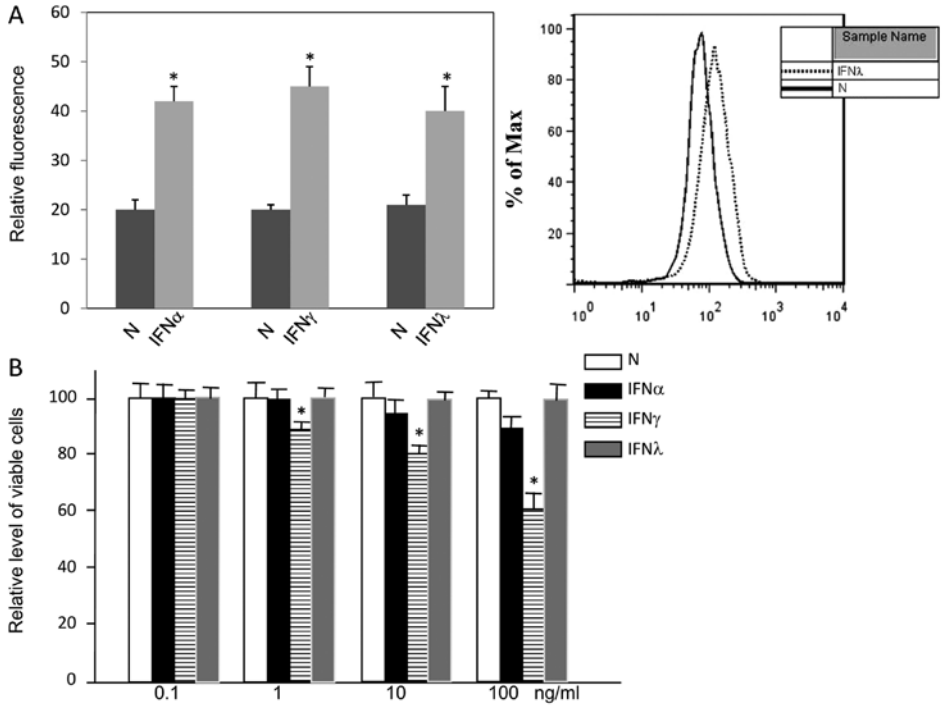

Figure 1. IFNλ inhibits the proliferation of human lung cancer cells. (A) A549 cells were treated respectively by mock (N), IFNα, IFNγ or IFNλ1 at the concentration of 10 ng/ml for 72 h. MHC II expression levels in these cells were determined by flow cytometry. The ordinate represents cell number. The abscissa represents relative fluorescent intensity. (B) A549 cells were treated respectively by mock (N), IFNα, IFNγ or IFNλ1 at indicated concentrations for 72 h. Cell proliferation was evaluated by cell counting and presented as a percentage of the mock treated cells. *P<0.05 if compared with mock treated cells. Data represent at least three independent experiments.

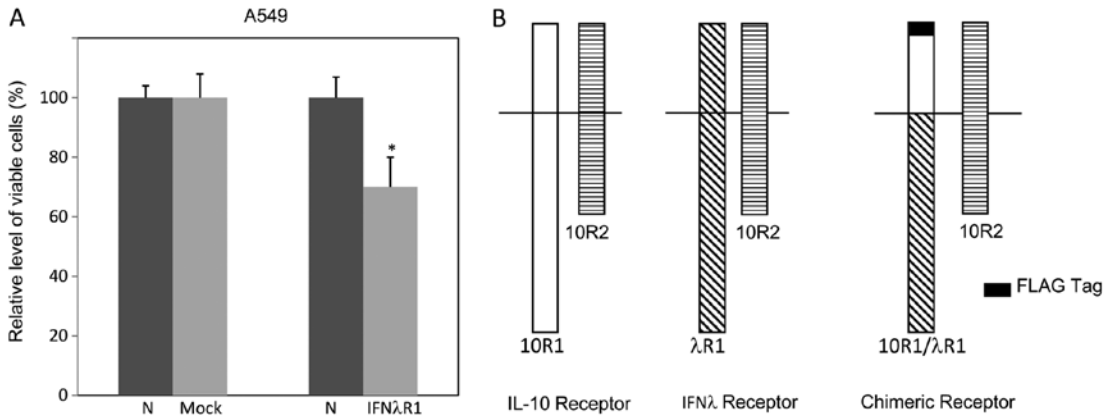

Figure 2. The receptors used in this study are schematically shown. (A) A549 was transfected with plasmid expressing IFNλR1 receptor (IFNλR1) or its cognate vector (Mock). Viable cells were counted 48 h post-transfection. The viability was presented as a percentage of non-transfected parental cells (N). (B) IL-10 receptor complex and IFNλ receptor complex are composed of a common accessory receptor chain (10R2), which has a short intracellular domain, and a unique signaling receptor chain (10R1 and λR1, respectively), which has a long intracellular domain. Chimeric receptor complex is composed of an intact accessory receptor chain (10R2) and an N-terminal FLAG-tagged extracellular domain of 10R1 fused in tandem to the transmembrane and intracellular domains of λR1 (10R1/λR1).

responsiveness of cells to IFNs. It has been reported that the expression level of IFNλR1, the signal competent of IFNλ receptor complex, correlates to the ability of IFNλ to block cell proliferation (23,28). We wondered if overexpression of IFNλR1 could render A549 cell responsiveness to IFNλ-induced antiproliferative or apoptotic effects. However, ectopic expression of full-length IFNλ receptor R1 (IFNλR1) in A549 cells resulted in cell death even without the presence of IFNs. Clones expressing detectable levels of ectopic IFNλR1 could not be obtained (Fig. 2A).

We previously managed this obstacle in other cell lines with a FLAG-tagged chimeric receptor 10R1/λR1 (Fig. 2B), via which treatment of IL-10 could induce intracellular IFNλ signaling (6,22). Therefore, we generated A549 cells expressing 10R1/λR1 (A549/10R1/λR1) and its cognate vector. To characterize the signaling mediated by 10R1/λR1 in A549 cells, Cl.1, a clonal population expressing 10R1/λR1 at high level, and Cl.2, a clonal population expressing 10R1/λR1 at low level, were selected. Expression levels of the FLAG-tagged proteins were examined by flow cytometry (Fig. 3A). In response to

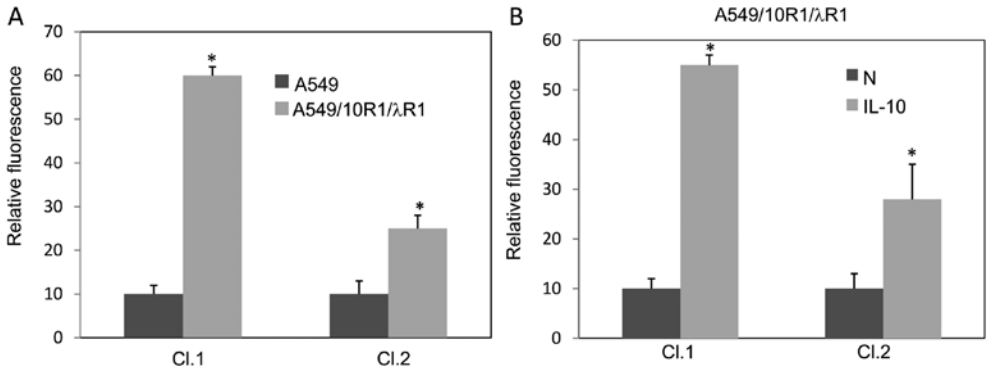

Figure 3. Chimeric receptor-mediated IFNλ signaling upon IL-10 stimulation. (A) Two clones (Cl.1 and Cl.2) with high and low expression levels of chimeric receptor were selected. (B) Both clones were treated with mock solution (N) or 0.3 ng/ml of IL-10 for 72 h. Expression levels of MHC II were determined by flow cytometry. *P<0.05 if compared with cells treated by mock solution. Data represent at least three independent experiments.

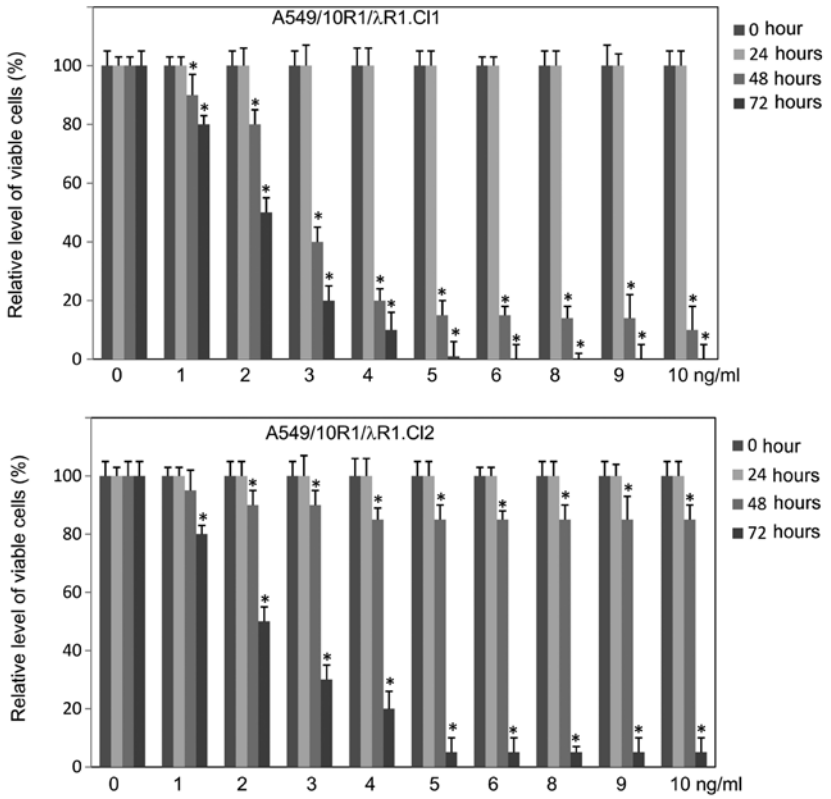

Figure 4. Cell viability reciprocally correlates to the intensity of chimeric receptor-mediated IFNλ signaling. Cell line Cl.1 and Cl.2 were treated by IL-10 at various concentrations (0-10 ng/ml). Cell viability was determined at time points of 0, 24, 48 and 72 h. The abscissa represents concentration of IL-10 (ng/ml). The ordinate represents percentage of live cells relative to the mock treated cells. *P<0.05 if compared with mock treated cells. Data represent at least three independent experiments.

a low concentration of IL-10 when the possible apoptotic response was minimal, i.e., at the concentration of 0.3 ng/ml for 72 h, the cell line Cl.1 and Cl.2 showed increased MHC II expression (about 5.5- and 2.8-fold, respectively) when compared with cells treated by mock solution (Fig. 3B).

Characteristics of apoptosis induced by IFNλ signaling. To determine the antiproliferative or apoptotic effects of IFNλ signaling in A549 cells, we treated the cell line Cl.1 and Cl.2 with IL-10 at various concentrations and determined cell viability at various time points. IL-10 induced a strong antiproliferative response and cell death in a dosage- and time-dependent manner. The cell death was more intense in Cl.1 cells than in Cl.2 cells (Fig. 4).

IFNλ signaling may affect cell progression through the cell cycle (22). We treated A549 cells expressing 10R1/λR1 with IL-10 (10 ng/ml). Cell cycle analyses by PI staining were performed at 0 and 48 h. In response to IL-10 stimulation (10 ng/ml), a majority of A549 cells expressing 10R1/λR1 was in G0/G1 phase or dead at 48 h. Cells in G2 phase disappeared completely (Fig. 5A).

Upon apoptosis, cells may undergo numerous physiological changes, including the redistribution of phosphatidylserine (PS) to the external cell surface, activation of caspases and

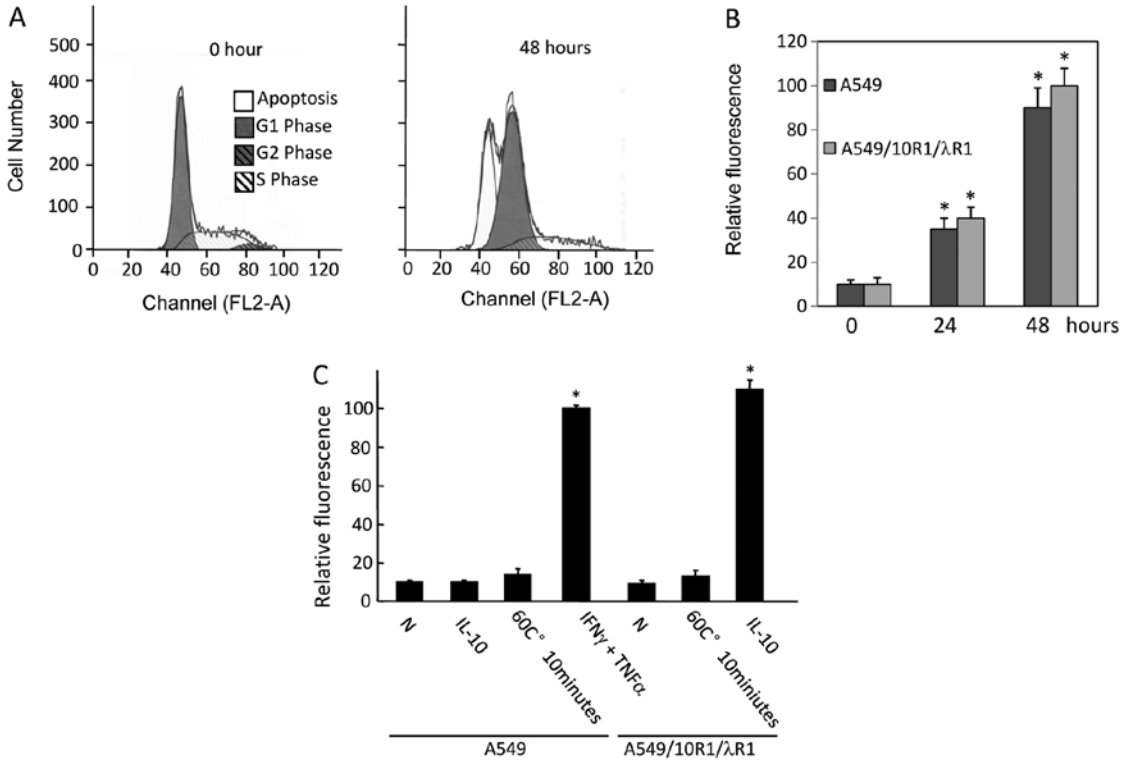

Figure 5. IFNλ signaling induces apoptosis on A549/10R1/λR1 cells. (A) A549 cells expressing 10R1/λR1 were treated by IL-10 (10 ng/ml). Cell cycle analyses by PI staining were performed at 0 and 48 h and analyzed by flow cytometry. (B) A549 cells expressing 10R1/λR1 were treated by IL-10 (10 ng/ml, grey bars). A549 cells expressing vector were treated by a combination of IFNγ (10 ng/ml) and TNFα (1 ng/ml) as a positive control (black bars). Annexin V staining analyses were performed at 0, 24 and 48 h and analyzed by flow cytometry. (C) A549 cells expressing 10R1/λR1 or vector were treated by mock (N) or IL-10 (10 ng/ml) for 72 h. In a parallel experiment, these cells were heated at 60˚C for 10 min. In another parallel experiment, A549 cells expressing vector were treated by IFNγ (10 ng/ml) and TNFα (1 ng/ml) for 72 h. DNA fragmentation was detected by TUNEL assay. *P<0.05 if compared with mock treated condition. Data represent at least three independent experiments.

DNA fragmentation (5). Externalization of PS and DNA fragmentation assays are often performed to indicate induced apoptosis in cells. Externalization of PS can be detected by Annexin V. DNA fragmentation can be detected by TUNEL assay, in which the ends of DNA fragments can be fluorescently labeled by terminal deoxynucleotidyl transferase.

PS externalization was observed in A549/10R1/λR1 cells in response to IL-10 treatment (10 ng/ml) as early as 24 h and further increased at 48 h (grey bar in Fig. 5B). A similar response was observed in the A549 cells expressing vector treated by a combination of IFNγ (10 ng/ml) and TNFα (1 ng/ml), an established treatment to induce apoptosis in A549 (black bar in Fig. 5B) (29).

DNA fragmentation was examined in A549/10R1/λR1 cells in response to IL-10 treatment (10 ng/ml) at 72 h (Fig. 5C). In contrast to A549 cells expressing vector treated by IL-10 (second column in Fig. 5C), we observed substantial DNA fragmentation in A549/10R1/λR1 cells treated by IL-10, indicating the DNA fragmentation resulted from chimeric receptor-mediated IFNλ signaling. The A549 cells expressing 10R1/λR1 or vector were heated at 60˚C for 10 min as a non-apoptotic cell death control and no DNA fragmentation was observed (Fig. 5C).

Activation of caspase-3, caspase-8 and caspase-9 were not detectable in A549 cells expressing vector in response to IFNλ treatment up to 100 ng/ml (Fig. 6A). In contrast, activation of caspase-3, caspase-8 and caspase-9 (about 12.2-, 6.3- and 8.1-fold, respectively) were observed in A549/10R1/λR1 cells in response to IL-10 stimulation (10 ng/ml), suggesting that the caspases were activated by IFNλ signaling (Fig. 6B). In addition, pancaspase inhibitor Z-VAD-FMK inhibited the activation of caspase-3 and caspase-8, but surprisingly promoted caspase-9 activation. This might be a result of compensatory activation, since caspase-3, a substrate of caspase-9, was inhibited by Z-VAD-FMK (Fig. 6B). Because caspase-3 activation is indispensable for caspase-8- and caspase-9-mediated apoptosis, Z-VAD-FMK was capable of inhibiting caspase cascade. However, TUNEL assay indicated that Z-VAD-FMK did not prevent these cells from apoptosis induced by IFNλ signaling, indicating that caspase-3 may not be critical for the apoptosis induced by IFNλ signaling (Fig. 6C). As in Fig. 6B, Z-VAD-FMK did not completely inhibit caspase-3 activation. We increased Z-VAD-FMK concentration to 100 μM, but still failed to see a full inhibition (data not shown). Results from a previous study have shown by ELISA that Z-VAD-FMK (100 μM) inhibited caspase-3 completely in A549 cells (30). So, Z-VAD-FMK should be able to block caspase-3 activation. Therefore, apoptosis induced by IFNλ signaling should be caspase-independent.

STAT1 is activated by IFNλ signaling. STATs, especially STAT1, are important components in IFN signaling. To investigate the molecular mechanism of apoptosis induced by IFNλ signaling, we examined the STAT1 activation in parental A549

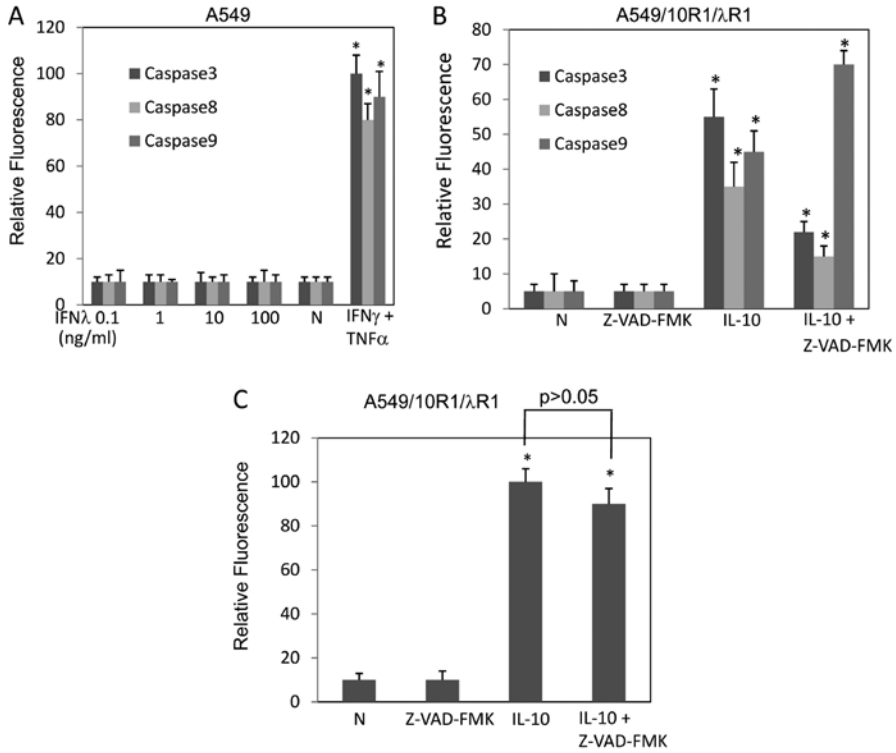

Figure 6. Caspase activation was induced by IFNλ signaling. (A) A549 cells expressing vector were stimulated by mock, IFNλ1 at various concentrations (0.1, 1, 10 and 100 ng/ml) or a combination of IFNγ (10 ng/ml) and TNFα (1 ng/ml). Activation of caspase-3, caspase-8 and caspase-9 was examined at 72 h by flow cytometry. (B) A549 cells expressing 10R1/λR1 were treated by mock (N), Z-VAD-FMK (30 μM), IL-10 (10 ng/ml) with or without Z-VAD-FMK (30 μM). Activation of caspase-3, caspase-8 and caspase-9 was examined at 72 h by flow cytometry. (C) A549 cells expressing 10R1/λR1 were treated by mock (N), Z-VAD-FMK (30 μM), IL-10 (10 ng/ml) with or without Z-VAD-FMK (30 μM). TUNEL assay were performed at 72 h to detect apoptosis. *P<0.05 when compared with mock treated condition. Data represent at least three independent experiments.

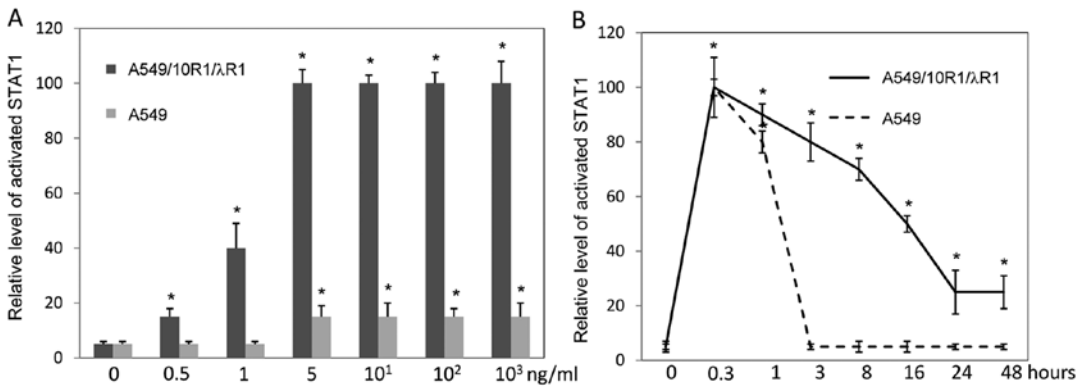

Figure 7. The effects of IFNλ signaling intensity and duration on STAT1 activation. (A) A549 cells expressing 10R1/λR1 (black bars) and A549 cells (grey bars) were treated by IL-10 and IFNλ1, respectively, at various concentrations (0, 0.5, 1, 5, 10^1, 10^2 and 10^3 ng/ml). Levels of activated STAT1 were examined by flow cytometry at 15 min. (B) A549 cells expressing 10R1/λR1 and A549 cells were stimulated by IL-10 (0.5 ng/ml) and IFNλ (100 ng/ml), respectively. Levels of activated STAT1 were determined at time points of 0, 0.3, 1, 3, 8, 16, 24 and 48 h by flow cytometry. *P<0.05 if compared with time point 0. Data represent at least three independent experiments.

cells treated by IFNλ1 and A549/10R1/λR1 cells treated by IL-10. STAT1 activation was much stronger in A549/10R1/λR1 cells treated by IL-10 than that in parental A549 cells treated by IFNλ1 (Fig. 7A). Next we examined the duration of STAT1 activation in A549 cells treated by IFNλ1 and A549/10R1/λR1 cells treated by IL-10. A549 cells were treated by 100 ng/ml of IFNλ1, while A549/10R1/λR1 cells were induced with 0.5 ng/ml of IL-10, the concentration at which IL-10 activated STAT1 in A549/10R1/λR1 cells to a similar level of that induced by 100 ng/ml of IFNλ in A549 cells (Fig. 7A) and did not kill the cells. Apparently, IFNλ signaling induced by IL-10 in A549/10R1/λR1 cells was maintained much longer than that by IFNλ1 in A549 cells (Fig. 7B). These results implied a key role of STAT1 in apoptosis induced by IFNλ. The prolonged and intensified signaling may determine whether IFNλ can induce apoptosis.

IFNγ sensitizes A549 cells to IFNλ-induced apoptosis. It is known that IFNγ potentiates the activity of IFNα by inducing important signaling components, such as STAT1, STAT2

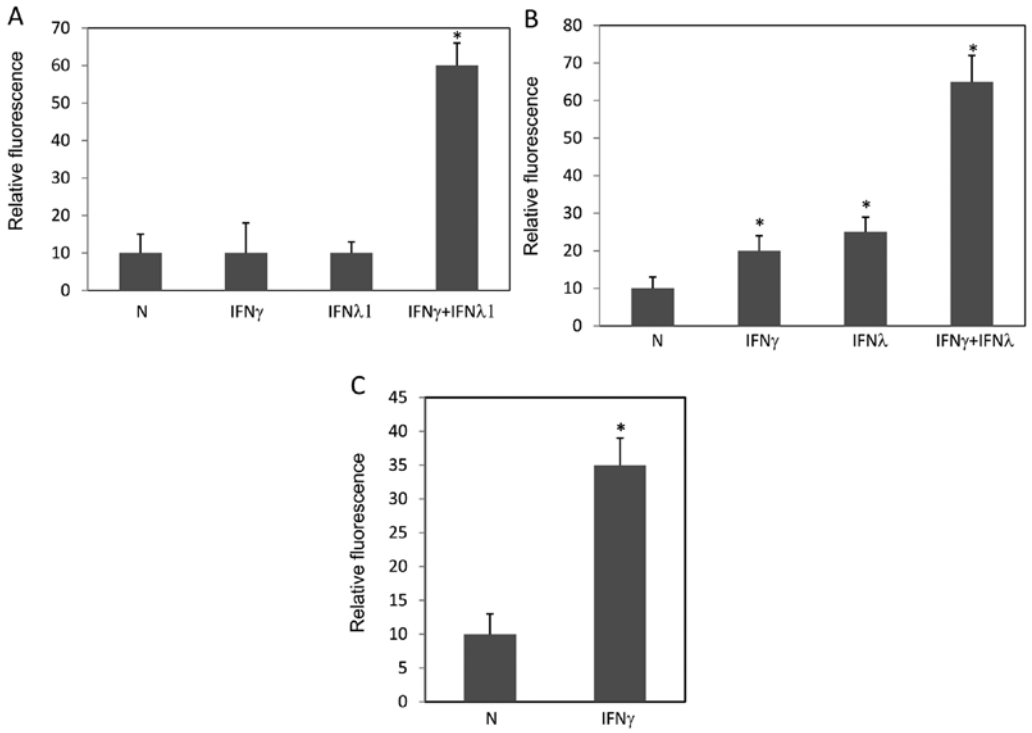

Figure 8. IFNγ sensitizes A549 cells to IFNλ-induced apoptosis. (A) Parental A549 cells were treated with mock solution (N), IFNγ (0.1 ng/ml), IFNλ1 (100 ng/ml) or a combination of IFNγ (0.1 ng/ml) and IFNλ1 (100 ng/ml). Apoptosis was measured by TUNEL assay at 72 h. (B) Parental A549 cells were treated with mock solution (N) or IFNλ1 (100 ng/ml) for 24 h. In parallel, parental A549 cells were treated by IFNγ (0.1 ng/ml) for 24 h then treated by mock or IFNλ1 (100 ng/ml) for 15 min. Levels of STAT1 activation were measured by flow cytometry. (C) A549 cells were treated by mock (N) or IFNγ (0.1 ng/ml) for 48 h. The expression levels of IFNλR1 were determined by flow cytometry. *P<0.05 when compared with mock treated condition. Data represent at least three independent experiments.

and IRF9 (31-33). These proteins are also involved in IFNλ signaling (6). Thus, we examined whether IFNγ could make A549 cells more sensitive to IFNλ-induced apoptosis. First, we observed pronounced apoptosis induced by a combination of IFNγ and IFNλ1 instead of sole treatment of IFNγ or IFNλ1 in TUNEL assay (Fig. 8A). Next we examined the levels of STAT1 activation by these treatments. IFNγ at low concentration (0.1 ng/ml) sensitized STAT1 activation in response to IFNλ (Fig. 8B). Next, since the expression level of IFNλR1 could be a limiting factor for IFNλ signaling to induce apoptosis, we examined expression levels of IFNλR1 in A549 cells treated by IFNγ. IFNγ significantly increased the expression level of IFNλR1 (Fig. 8C). In summary, these results demonstrated that IFNλ was able to induce apoptosis in A549 cells when the cells were sensitized by other stimuli, such as IFNγ.

Discussion

IFNs are essential members in the family of antiviral and anticancer drugs, which are widely used in modern clinical practice. Although type I interferons, including IFNα, IFNβ and other members, are well studied and widely used, their side effects are obvious. It is urgent to find alternatives with similar functions but fewer side effects to satisfy current clinical needs. IFNλs were discovered less than 10 years ago. Their antiviral function is similar to that of type I interferons. In addition, the distribution of receptors of IFNλs is much more restricted than that of type I interferons, implying a less severe side effect profile (23). Therefore, IFNλs become one of the current hot points in research.

Apoptosis is an important approach in tumor therapy and antiviral therapy. Tumors have a variety of mechanisms to evade apoptosis. IFNs inhibit tumor growth and clear viruses by inducing apoptosis (34-36). Our study explored the mechanisms of type III interferons to induce apoptosis in lung cancer cells.

The expression levels of cytokine receptors are critical for cytokines to activate intracellular signals. In many cases they decide the amplitude of downstream biological effects (23). Therefore, when we realized that cells overexpressing IFNλ receptor were not viable, we utilized an established chimeric receptor 10R1/λR1 to study the apoptotic effect of IFNλs in human lung cancer cells.

We did not observe any inhibitory effect of IFNλ on the growth of A549 cells (Fig. 1). This was consistent with previous observations from mouse BW5147 T cells and other cell lines (23-25,28). Expression of the chimeric receptor 10R1/λR1 rendered cells responsive to apoptosis induced by IFNλ signaling, as indicated by cell cycle analysis by PI staining, TUNEL assay and Annexin V staining (Fig. 5). This is consistent with a previous report in colorectal carcinoma cell line (22). Caspases are key effectors in apoptosis, activation of which is critical for canonical apoptosis (34). We observed that, in contrast to parental A549 cells treated by IFNλ, A549 cells expressing 10R1/λR1 treated by IL-10 showed pronounced activation of caspase-3, caspase-8 and caspase-9. Although Z-VAD-FMK inhibited the activation

of caspase-8 and caspase-3, it did not prevent cell apoptosis (Fig. 6), suggesting that IFNλ-induced apoptosis may be caspase-independent. Interestingly, caspase-9 activation was promoted by Z-VAD-FMK. This probably resulted from a compensation of the suppression of its downstream caspase-3. This is consistent with our previous report in colorectal carcinoma cells (22). We also demonstrated that both the intensity and duration of STAT1 activation were increased, when IFNλ signal was intensified (Fig. 7). These results demonstrated that IFNλ signal was able to trigger apoptosis in human lung cancer cells, which probably was mediated via STAT1. More detailed mechanism is under investigation.

Many experiments in this study utilized the chimeric receptor 10R1/λR1, rather than the full length IFNλ receptor, IFNλR1. This was because clonal populations expressing the ectopic IFNλ receptor were not available. A549 cells expressing high levels of IFNλ receptor may not be viable in physiological conditions. By using the artificial chimeric receptor 10R1/λR1, we found the intrinsic capability of IFNλ signaling on inducing apoptosis. But there should be physiological or pathological conditions that this apoptosis-mediating capability could be activated. Therefore, at the end of this study, we tested the treatment combination of IFNλ and IFNγ on A549 cells, and discovered that IFNγ upregulated expression of IFNλR1, which facilitated IFNλ-mediated apoptosis (Fig. 8). IFNγ-induced upregulation of IFNλR1 did not kill the cells, possibly because the level of IFNλR1 is not as high as in the clonal populations expressing the ectopic IFNλ receptor. Further experiments are required to prove this hypothesis. These results indicated the potential of IFNλR1 to induce apoptosis in special conditions, such as a pathological condition where IFNγ is upregulated. We know that the regulatory effects of IFNγ are very broad. So, in our next step, we will try to find other physiological or pathological stimulations that upregulate IFNλ receptor specifically to further confirm our findings.

In summary, we report that the apoptotic potential of IFNλs on human lung cancer cells in concomitant with STAT1 activation and increased expression of IFNλ receptor R1. This apoptosis could be enhanced by IFNγ. Our results provided a theoretical basis for IFNλs to treat lung cancers.

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