Int J Clin Exp Med 2015;8(9):14999-15004
www.ijcem.com /ISSN:1940-5901/IJCEM0012457

Original Article

Interferon-λ1 suppresses invasion and enhances autophagy in human osteosarcoma cell

Dongmei Gao[1*], Jun Zhao[2*], Xiuyi Li[1], Yuanyuan Xia[1], Yu Cai[1], Jiwen Pan[1], Hong Zhou[1], Yong Fang[1], Shouzhu Zhang[1], He Wen[1]

[1]Department of Clinical Laboratory, Third Affiliated Hospital of Anhui Medical University, Hefei 230032, China; [2]Department of Microbiology, Anhui Medical University, 69 Meishan Road, Hefei 230032, China. [*]Equal contributors.

Received July 6, 2015; Accepted September 6, 2015; Epub September 15, 2015; Published September 30, 2015

Abstract: Objective: The purpose of the present study was to determine whether type III IFN can modulate the autophagic response in human osteosarcoma cell. Methods: Human osteosarcoma cell were treated with Interferon-λ1. We investigated that Interferon-λ1 could inhibit the invasive ability of osteosarcoma cells by Matrigel invasion assay. Autophagy were assessed by acridine orange staining, MDC staining and Transmission electron microscopy. Results: In this study, we found that Interferon-λ1 could inhibit the invasive ability of osteosarcoma cells. Acridine orange staining and MDC staining showed that Interferon-λ1 triggered the accumulation of acidic vesicular and autolysosomes in osteosarcoma cell. The acridine orange osteosarcoma cell ratios were 3.6 ± 0.5%, 4.5 ± 0.8%, and 12.4 ± 1.7% after treatment with 1, 10, and 100 ng/mL Interferon-λ1 for 48 h. Osteosarcoma cell cells treated with 100 ng/mL Interferon-λ1 for 48 h developed autophagy some-like characteristics, including single or double-membrane vacuoles containing intact and degraded cellular debris. Conclusions: Interferon-λ1 could inhibit the invasive ability of osteosarcoma cells. Autophagy can be induced in a dose-dependent manner by treatment with Interferon-λ1 in osteosarcoma cell.

Keywords: Interferon-λ1, autophagy, acridine orange, transmission electron microscopy

Introduction

IFN lambdas were just identified during the recent years and classified as a new group, type III IFN. In human, 3 distinct proteins called IFN-λ1, IFN-λ2, and IFN-λ3 have been identified, they are also named interleukin-29 (IL-29), IL-28A, and IL-28B, respectively [1-3]. Study on type III IFN has revealed that these cytokines exhibit a variety of biological effects which are different from those on viral replication, such as antitumor activity [4, 5]. In the present study we report for the first time that Interferon-λ1 induce autophagy in osteosarcoma cells using several different methods that measure autophagy levels. We further show that the Beclin1 signaling pathway was stimulated by Interferon-λ1.

Materials and methods

Cell culture

Human osteosarcoma MG-63 cell line was procured from ATCC (American type culture collection). We propagated MG-63 cells in Dulbecco's modified Eagle's medium (GBICO) supplemented with 10% fetal bovine serum (GBICO) in a humidified incubator containing 5% CO_2 at 37°C. Recombinant Human IL-29/ IFN-λ1 (R&D Systems) was diluted in serum-free medium.

Matrigel invasion assay

Matrigel invasion assay was performed to assess the effects of IFN-λ1 on the invasive properties of MG-63 cells. Transwell inserts (12-well, 12 mm with 12.0 μm pore size) from Corning (Corning, NY) were coated with 200 μl of matrigel (final concentration, 1.0 mg/ml in ice-cold serum-free medium) (BD Biosciences, San Jose, CA) and allowed to dry at 37°C for 3-5 h. Cells were treated for 3 days with IFN-λ1 at the following concentrations: 1×10^2, 1×10^3, 1×10^4 IU/ml. The control and treated cells were washed twice with serum-free medium and were trypsinized. 200 μl of cell suspension (2×10^5 cells) from each sample was added to each well in triplicate.

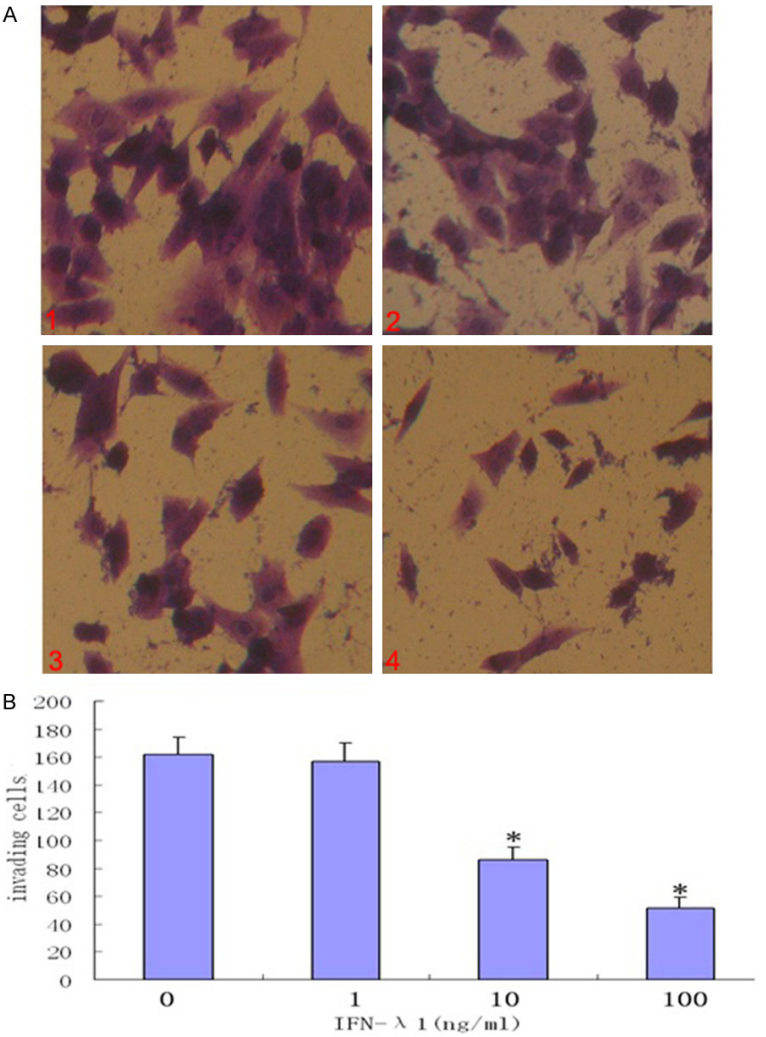

Figure 1. Cell invasion assay after treatment with IFN-λ1 for 48 h. A. Representative cell invasion assay for MG-63 cells. Invasion assays were carried out in 12-well transwell inserts of polycarbonate filters with 12.0 μm pores coated with 200 μl of 0.1% matrigel. After 48 h incubation period, the membranes were collected and stained. A significant reduction in the number of invaded cells indicated the decrease in invasive capacity. B. Quantitative evaluation of matrigel invasion assay. The data represented are mean ± SD of 10 randomly selected microscopic fields from 3 independent wells (*P < 0.01 compared with the control mean values). 1: Control group. 2: Treated with 1 ng/ml IFN-λ1 group. 3: Treated with 10 ng/ml IFN-λ1 group. 4: Treated with 100 ng/ml IFN-λ1 group.

Filters were incubated for 48 h at 37°C in a humidified incubator containing 5% CO_2, fixed and stained with 0.5% crystal violet in methanol. The non-migratory cells were removed with a cotton tip and the migratory cells were counted at the light microscope, with 400 × magnification. Each experiment was run in duplicate. Results were expressed as the mean number of cells counted in each field, ± standard deviations.

Cell proliferation assay

For the assessment of cell growth, MG-63 Cells (5000 cells/well) were treated with various concentrations of IFN-λ1 in 96-well plates. After 48 h posttreatment, cell growth was evaluated using methyl thiazolyl tetrazolium assay (MTT assay). MTT (Sigma) was added to the culture medium in each well at the concentration of 500 μg/ml. After 4 h at 37°C, 100 μl DMSO was added to each well and the 550 nm absorption was measured. Each experiment was reproduced in six different wells and repeated at least three times.

Acridine orange staining for autophagy

Autophagy is characterized by the formation and promotion of acidic vesicular organelles (AVOs). MG-63 Cells were treated with various concentrations of IFN-λ1 in 6-well plates. After 48 h post treatment, then incubated with 1 mg/mL acridine orange (Sigma) for 15 min. Pictures were obtained with a fluorescence microscope.

MDC assay for autophagy

For MDC assay, cells cultured in 6-well plate were treated with 0.05 mM MDC and incubated at 37°C for 20 minutes. After staining, cells were fixed in 4% paraformaldehyde for 10 minutes and intracellular autophagy was detected using a fluorescence microscope with 380 nm excitation wavelength.

Transmission electron microscopy

Cells were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer for 1 h. After fixation, the samples were post-fixed in 1% OsO_4 in the same buffer for 30 min. Ultrathin sections were

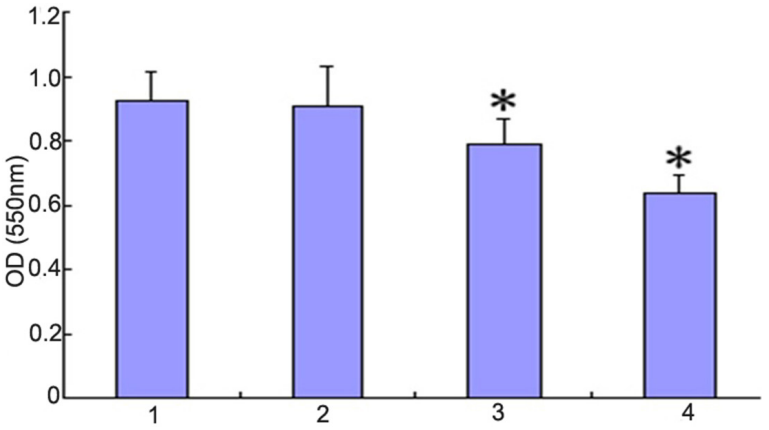

Figure 2. Antiproliferative effect of IFN-λ1. MG-63 cells were treated with IFN-λ1. Cell viability was determined by MTT assay. 1: Control group. 2: Treated with 1 ng/ml IFN-λ1 group. 3: Treated with 10 ng/ml IFN-λ1 group. 4: Treated with 100 ng/ml IFN-λ1 group. (*P < 0.01 compared with the control mean values).

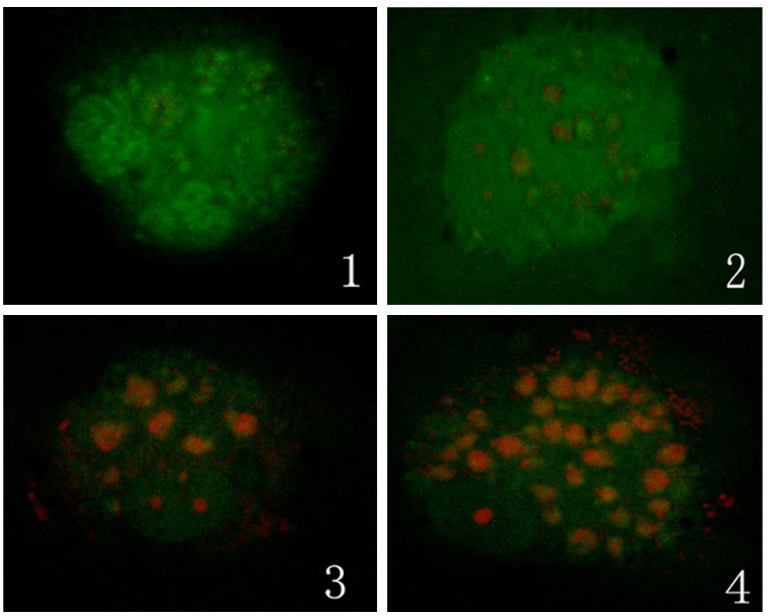

Figure 3. Modulation of autophagy by IFN-λ1 in MG-63 cells. Cells were treated with IFN-λ1 for 48 h at concentrations of 1 ng/ml, 10 ng/ml, and 100 ng/ml. Cells were then stained with acridine orange 1: Control group. 2: Cells treated with 1 ng/ml IFN-λ1. 3: Cells treated with 10 ng/ml IFN-λ1. 4: Cells treated with 100 ng/ml IFN-λ1.

then observed under a transmission electron microscope.

Statistical analysis

The experimental data were expressed as mean ± SD. Group means were compared by T-test using the statistical software program SPSS 13.0. *P* values < 0.05 were considered to be statistically significant.

Results

Marked reduction in tumor cell invasion

We evaluated cell invasion after the treatments (**Figure 1**). Remarkable reductions in the invasive properties of MG-63 cells after the treatment with IFN-λ1 were demonstrated through matrigel invasion assays. The staining of invaded cells through the membrane demonstrated that the number of cell invasion was significantly reduced in cells treated with IFN-λ1, compared with that of untreated control cells.

Anti-proliferative effects of IFN-λ1 in MG-63 cells

We investigated whether the anti-proliferative effect of IFN-λ1. As shown in **Figure 2**, the cell proliferation was inhibited by exposing cells to IFN-λ1 10 ng/ml and 100 ng/ml for 48 h.

Effect of IFN-λ1 derivatives on autophagy in MG-63 cells

MG-63 cells were treated with IFN-λ1. IFN-λ1 was found to trigger the accumulation of acidic vesicular and autolysosomes in MG-63 cells (**Figures 3**, **4**). The acridine orange MG-63 cell ratios were 3.6 ± 0.5%, 4.5 ± 0.8%, and 12.4 ± 1.7% after treatment with 1, 10, and 100 ng/mL MG-63, respectively (**Figure 5**). Additionally, treatment of the IFN-λ1 for 48 h developed autophagosome-like characteristics, including single and double membrane vacuoles containing intact and degraded cellular debris (**Figure 6**).

Discussion

The discovery and initial description of the interferon-λ(IFN-λ) family in early 2003 opened an exciting new chapter in the field of IFN

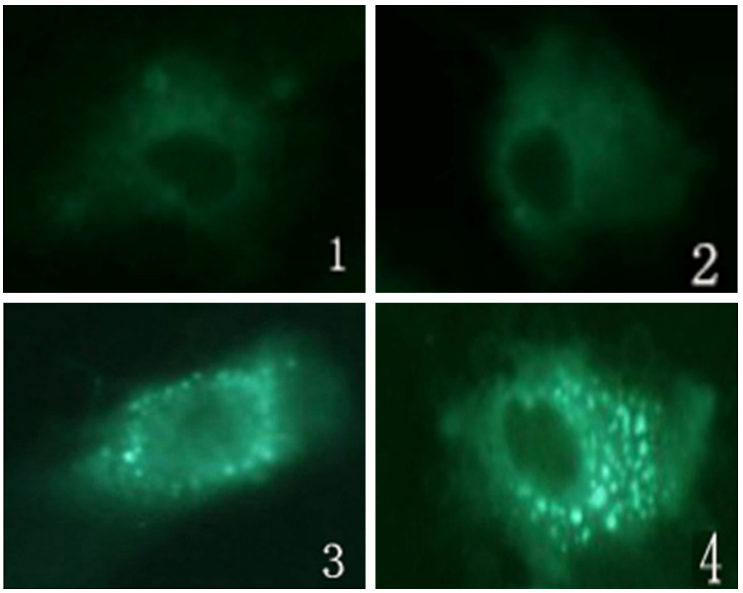

Figure 4. Modulation of autophagy by IFN-λ1 in MG-63 cells. Cells were treated with IFN-λ1 for 48 h at concentrations of 1 ng/ml, 10 ng/ml, and 100 ng/ml. Cells were then stained with MDC. 1: Control group. 2: Cells treated with 1 ng/ml IFN-λ1. 3: Cells treated with 10 ng/ml IFN-λ1. 4: Cells treated with 100 ng/ml IFN-λ1.

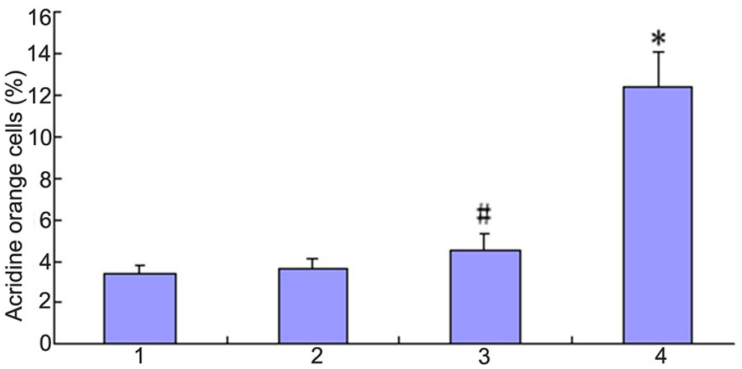

Figure 5. Autophagic vacuoles quantified by flow cytometry. 1: Control group. 2: Cells treated with 1 ng/ml IFN-λ1. 3: Cells treated with 10 ng/ml IFN-λ1. 4: Cells treated with 100 ng/ml IFN-λ1. ($^{\#}P < 0.05$ relative to control values and $^{*}P < 0.01$ relative to control values).

research. There are 3 IFN-λ genes that encode 3 distinct but highly related proteins denoted IFN-λ1, IFN-λ2, and IFN-λ3. These proteins are also known as interleukin-29 (IL-29), IL-28A, and IL-28B, respectively. Collectively, these 3 cytokines comprise the type III subset of IFNs. They are distinct from both type I and type II IFNs for a number of reasons, including the fact that they signal through a heterodimeric receptor complex that is different from the receptors used by type I or type II IFNs [6-8].

Several of the biological functions of type III IFN, including its regulation of innate and adaptive immunity and its antiangiogenic and proapoptotic effects, make it an obvious candidate for anticancer therapy. Indeed, type III IFN has been used with some success for the treatment of several types of cancer [9, 10]. The importance of the different mechanisms of action underlying the response in patients is still a matter of debate. Our study on Human osteosarcoma MG-63 cells showed that IFN-λ1 treatment suppressed human osteosarcoma cell invasion. Matrigel invasion assays demonstrated remarkable reductions in the invasive properties of MG-63 cells after treatment with IFN-λ1. MTT assays demonstrated the cell proliferation was inhibited by exposing cells to IFN-λ1.

Autophagy is a self-degradation process whereby cytosolic components and organelles are sequestered in double membrane-bound vesicles and delivered to lysosomes for degradation and recycling. In normal tissue, autophagy maintains cellular homeostasis by clearing damaged organelles or misfolded proteins. However, the role of autophagy in cancer is complex and paradoxical as it is an adaptive process that is responsive to changes in the cellular microenvironment. Thus, autophagy can either suppress or support the growth of tumor cells depending on the cellular context [11, 12].

Given the critical roles of autophagy in tumor progression and maintenance, various preclinical and clinical studies have been undertaken to develop therapeutic agents targeting autophagy. As most anticancer agents inevitably cause cellular stress, autophagy is often activated in cancer cells after drug treatment. Indeed, many therapies targeting growth factor

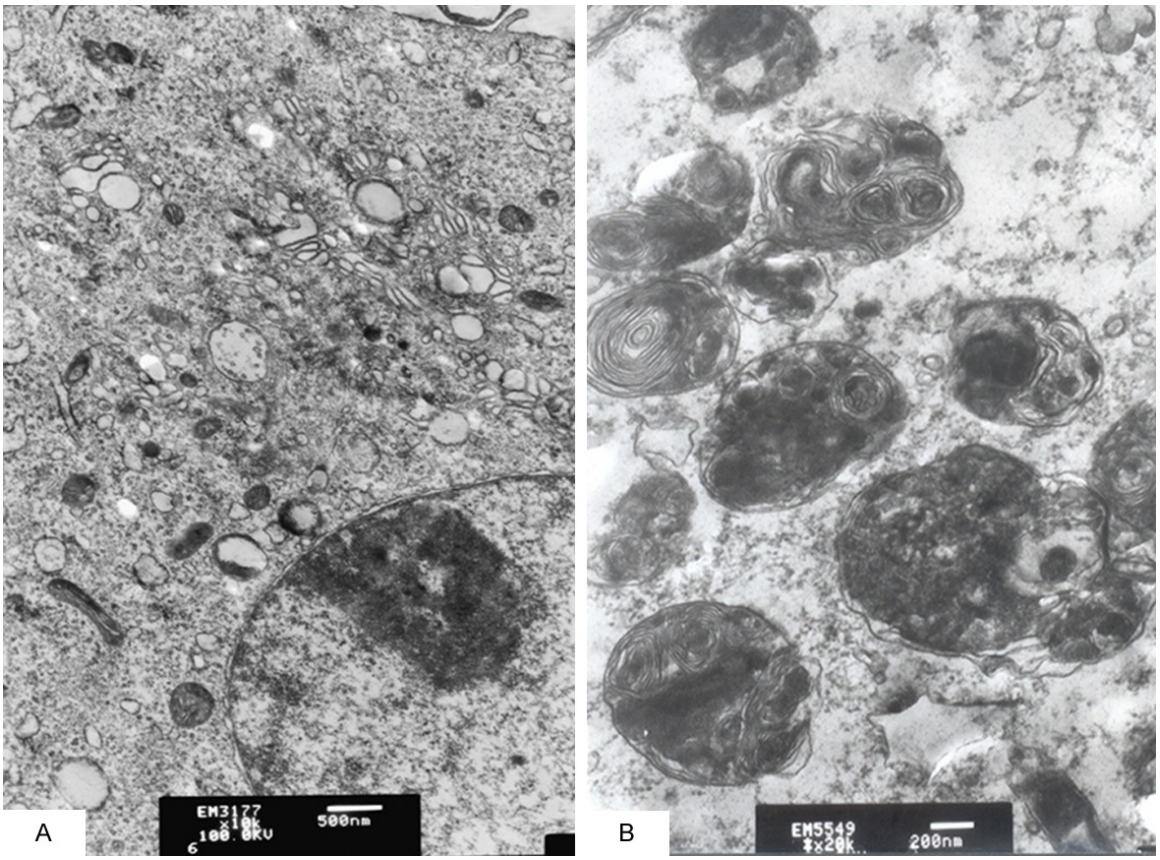

Figure 6. Transmission electron images of MG-63 cells treated with IFN-λ1. A: Control group. B: Cells treated with 100 ng/ml IFN-λ1 group.

signaling-either singly or in combinations targeting two different pathways-lead to autophagy induction. For example, several allosteric and catalytic inhibitors of mTOR, PI3K-AKT, and the tyrosine kinase signaling and activators of energy sensing pathway induce autophagy in cells. It was originally proposed that autophagic cell death is part of the mechanism of action of anticancer drugs [13].

In this study, Human osteosarcoma MG-63 cells were treated with IFN-λ1. Autophagy were assessed by acridine orange staining, MDC staining and Transmission electron microscopy. We found that autophagy can be induced in a dose-dependent manner by treatment with IFN-λ1 in MG-63 cells.

Acknoledgements

This work was supported by the National Natural Science Foundation of China (No. 81101273), and the Scientific Support Project of Anhui Province Education Department of China (No. KJ2012Z162).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. He Wen, Department of Clinical Laboratory, Third Affiliated Hospital of Anhui Medical University, Hefei 230032, China. Tel: 86-551-62183273; E-mail: wenhewh@163.com

References

[1] Ichihashi T, Asano A, Usui T, Takeuchi T, Watanabe Y and Yamano Y. Antiviral and antiproliferative effects of canine interferon-lambda1. Vet Immunol Immunopathol 2013; 156: 141-146.

[2] Li Q, Kawamura K, Tada Y, Shimada H, Hiroshima K and Tagawa M. Novel type III interferons produce anti-tumor effects through multiple functions. Front Biosci (Landmark Ed) 2013; 18: 909-918.

[3] Donnelly RP and Kotenko SV. Interferon-lambda: a new addition to an old family. J Interferon Cytokine Res 2010; 30: 555-564.

[4] Zheng YW, Li H, Yu JP, Zhao H, Wang SE and Ren XB. Interferon-lambdas: special immuno-

modulatory agents and potential therapeutic targets. J Innate Immun 2013; 5: 209-218.
[5] Tagawa M, Kawamura K, Li Q, Tada Y, Hiroshima K and Shimada H. A possible anticancer agent, type III interferon, activates cell death pathways and produces antitumor effects. Clin Dev Immunol 2011; 2011: 479013.
[6] Bolen CR, Ding S, Robek MD and Kleinstein SH. Dynamic expression profiling of type I and type III interferon-stimulated hepatocytes reveals a stable hierarchy of gene expression. Hepatology 2014; 59: 1262-1272.
[7] Liu D, Chang CH, Rossi EA, Cardillo TM and Goldenberg DM. Interferon-lambda1 linked to a stabilized dimer of Fab potently enhances both antitumor and antiviral activities in targeted cells. PLoS One 2013; 8: e63940.
[8] Zhou P, Cowled C, Todd S, Crameri G, Virtue ER, Marsh GA, Klein R, Shi Z, Wang LF and Baker ML. Type III IFNs in pteropid bats: differential expression patterns provide evidence for distinct roles in antiviral immunity. J Immunol 2011; 186: 3138-3147.
[9] Li M, Liu X, Zhou Y and Su SB. Interferon-lambdas: the modulators of antivirus, antitumor, and immune responses. J Leukoc Biol 2009; 86: 23-32.
[10] Zitzmann K, Brand S, Baehs S, Goke B, Meinecke J, Spottl G, Meyer H and Auernhammer CJ. Novel interferon-lambdas induce antiproliferative effects in neuroendocrine tumor cells. Biochem Biophys Res Commun 2006; 344: 1334-1341.
[11] Nagelkerke A, Bussink J, Geurts-Moespot A, Sweep FC and Span PN. Therapeutic targeting of autophagy in cancer. Part II: pharmacological modulation of treatment-induced autophagy. Semin Cancer Biol 2015; 31: 99-105.
[12] Thorburn A, Thamm DH and Gustafson DL. Autophagy and cancer therapy. Mol Pharmacol 2014; 85: 830-838.
[13] Gewirtz DA. The four faces of autophagy: implications for cancer therapy. Cancer Res 2014; 74: 647-651.