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IL-35 over-expression increases apoptosis sensitivity and suppresses cell growth in human cancer cells

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ABSTRACT

Interleukin (IL)-35 is a novel heterodimeric cytokine in the IL-12 family and is composed of two subunits: Epstein-Barr virus-induced gene 3 (EBI3) and IL-12p35. IL-35 is expressed in T regulatory (Treg) cells and contributes to the immune suppression function of these cells. In contrast, we found that both IL-35 subunits were expressed concurrently in most human cancer cell lines compared to normal cell lines. In addition, we found that TNF-α and IFN-γ stimulation led to increased IL-35 expression in human cancer cells. Furthermore, over-expression of IL-35 in human cancer cells suppressed cell growth *in vitro*, induced cell cycle arrest at the G1 phase, and mediated robust apoptosis induced by serum starvation, TNF-α, and IFN-γ stimulation through the up-regulation of *Fas* and concurrent down-regulation of *cyclinD1*, *survivin*, and *Bcl-2* expression. In conclusion, our results reveal a novel functional role for IL-35 in suppressing cancer activity, inhibiting cancer cell growth, and increasing the apoptosis sensitivity of human cancer cells through the regulation of genes related to the cell cycle and apoptosis. Thus, this research provides new insights into IL-35 function and presents a possible target for the development of novel cancer therapies.

1. Introduction

Interleukin (IL)-35 is a novel immune-suppressing cytokine that consists of the EBI3 (Epstein-Barr virus-induced gene 3) and IL-12p35 subunits [1]. IL-35 is expressed in non-stimulated mouse Tregs [1] and in stimulated human Tregs [2–4] but has not previously been detected in non-stimulated human Tregs [5]. Previous researches by our group and others have shown IL-35 to be an immune-suppressing cytokine that suppresses the activity of Th1 and Th17 and leads to Treg expansion [1,2,6]. Although elevated numbers of Tregs in various tumorigenic tissues have been consistently shown, the role of Tregs in tumorigenesis remains controversial [7].

IL-35 shares the IL-12p35 and EBI3 subunits with IL-12 and IL-27, respectively [1,6], and both IL-12 and IL-27 can induce distinct immune responses against tumors [8]. The anti-tumor activities of IL-12 have been extensively studied in a variety of murine and human tumor models. Studies have shown that the anti-tumor activity of IL-27 is mediated not only through its direct effects on tumors but also through angiogenesis and through lymphocytes such as $CD8^+$ T cells and NK cells [9,10]. However, the role of IL-35 in tumor immunity has not yet been elucidated.

To investigate the possible role of IL-35 in tumor immunity, we first examined IL-35 expression levels with EBI3- and IL-12p35-specific antibodies in human lung cancer, colon cancer, esophageal carcinoma, hepatocellular carcinoma and cervical carcinoma biopsies using immunohistochemistry analysis. To our surprise, we observed positive staining of both IL-35 subunits in these cancer cells, leading us to examine IL-35 expression levels in human tumorigenic cell lines.

Recently, it was reported that IL-35 signals through a unique heterodimer receptor of IL-12Rb2 and gp130 or through homodimers of each chain [11]. Therefore, in the present study, we examined the expression levels of the IL-35 subunits (EBI3 and IL-12p35) and its receptor (IL-35R) subunits (IL-12Rb2 and gp130) in a variety of human tumorigenic cell lines. In addition, we determined the mRNA expression levels of the IL-35 subunits in these cell lines and investigated a possible role for IL-35 expression in the growth and apoptosis of human cancer cells.

2. Materials and methods

2.1. Cell culture, reagents and treatments

Human cell lines were isolated from lung adenocarcinoma (A549), colon cancer (SW480), hepatocellular carcinoma (BEL-7402 and HepG2), cervical carcinoma (SiHa), pancreatic cancer (HPAC), breast cancer (MDA-MB-231 and MCF-7), normal bronchial epithelial cells (Beas-2B), neuroblastoma (SH-SY5Y), B cell lymphoma (Raji) and human embryonic kidney cells (HEK293).

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The cells were cultured *in vitro* in RPMI 1640 (Hyclone, USA) containing 10% fetal bovine serum (Hyclone, USA) at 37 °C in a humidified incubator containing 5% CO_2. In some experiments, the cells were stimulated with the indicated concentration of recombinant human TNF-α and IFN-γ (PeproTech, USA). After a set period of stimulation, the cells were harvested using trypsin for FACS analysis or were lysed for total RNA extraction.

2.2. RT-PCR and quantitative real-time PCR

RNA was isolated from cells using TRIzol Reagent (Invitrogen, USA). Two micrograms of RNA was used for cDNA synthesis using the High-Capacity RNA-to-cDNA Kit (Invitrogen, USA). The primer sequences used in this study are shown in Table 1. The PCR products were amplified using the following thermal cycles parameters: 95 °C for 30 s, 58 °C for 60 s and 72 °C for 60 s. The PCR amplicons were visualized using a 1.5% agarose gel containing ethidium bromide and verified through direct DNA sequencing. The relative gene expression levels were determined using quantitative real-time PCR and the SYBR Green labeling method in a CHROMO4 continuous fluorescence detector (Bio-Rad, USA). cDNA was mixed with primers and SYBR Green Supermix following the manufacturer's recommended protocols (Bio-Rad, USA). All samples were run in triplicate. The C_t value of each sample was acquired, and the relative gene expression levels were calculated using the ΔC_t method, which was normalized to the endogenous *β-actin* as a control. The data are expressed as n-fold relative to the control.

2.3. Immunocytochemical analysis

The cells were cultured in a 24-well plate with a glass slide in each culture well. The cells were washed with PBS before being fixed with 4% (v/v) paraformaldehyde. To block peroxidase activity in the cells, the slides were treated with 3% H_2O_2 before permeabilizing the cell membranes with 0.2% Triton X-100 at room temperature (RT). Non-specific antibody binding was blocked with 10% (v/v) goat serum at RT. Anti-human EBI3 (Abcam, UK) and anti-human IL-12p35 antibodies (Santa Cruz Biotechnology, USA) were diluted with PBS and the cells were stained. HRP conjugated anti-rabbit IgG was used as secondary antibody to detect anti-human EBI3 and IL-12p35 antibody binding. Staining was visualized with 3,3′-diaminobenzidine (DAB) substrate before counterstaining with hematoxylin.

2.4. FACS analysis

Single cell suspensions were obtained by trypsinizing cultured cells in 6-well plates. The cell density was diluted to 5×10^5 cell/tube for each antibody staining. The cells were fixed in 4% (v/v) paraformaldehyde and permeabilized with a permeabilization buffer (eBiosciences, USA). Anti-human EBI3-allophycocyanin (APC) and IL-12p35-phycoerythrin (PE) antibodies (R&D Systems, USA) were used for intracellular protein staining; isotype control antibodies (R&D Systems, USA) were used as negative controls. The expression of IL-35 was detected using a flow cytometer (BD FACSAria, USA). The data were analyzed using FACSDiva4.1 software (BD Biosciences, USA).

2.5. Establishing stable cells with IL-35 over-expression

Human IL-35-Fc and Fc expression vectors were constructed as described previously [6]. The cells were cultured to 80% confluence in a 24-well plate before transfection with IL-35-Fc and Fc expression vectors using Lipofectamine 2000 (Invitrogen, USA). The stably transfected cell clones were selected using a culture medium containing Zeocin (Invitrogen, USA) for 10 days with a change of medium every 3 days. IL-35 expression was confirmed through RT-PCR, quantitative real-time PCR, and FACS analyses.

2.6. Cell counting kit-8 (CCK-8) assay for cell growth

Cell proliferation and viability were determined using the CCK-8 assay (Dojindo, Japan). Briefly, 2.5×10^3 cells were seeded into the wells of a 96-well plate in quadruplicate. Cell proliferation at days 0, 1, 2, 3, 4, 5 and 6 was evaluated by the addition of 10 μl of CCK-8 reagent followed by 2 h of incubation. The optical density (OD) of the cultures was measured at 450 nm using a spectrophotometer.

2.7. Cell cycle distribution analysis

The cells were incubated in 6-well plates. At the indicated time points of 0, 24 and 48 h in serum-free culture, the cells were harvested, fixed with 70% ethanol, treated with 0.002% RNase solution, and stained with a 0.05% propidium iodide solution. The DNA content in the cells was detected using a flow cytometer (COULTER EPICS@XL, USA). The data were analyzed using EXPO32 ADC v1.1C and MultiCycle software.

2.8. Induction of apoptosis

The cellular levels of apoptosis were measured using the TACS Annexin V kit (Trevigen, USA). The cells were incubated with 0.1 ng/ml TNF-α or 10 ng/ml IFN-γ for 24 h, washed twice with cold PBS, and stained with Annexin V-FITC and PI for 15 min. Apoptosis was detected using a flow cytometer (BD FACSAria, USA). The data were analyzed using FACSDiva4.1 software. The cells that were negative for PI and positive for Annexin V were scored and counted as apoptotic cells.

2.9. Statistical analyses

The results are presented as the mean ± standard deviation (SD). All statistical analyses were performed using the SPSS 11.5 software package for Windows. Significance was determined using

Table 1
The primer sequences of the target genes.

Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Product size (bp)
EBI3	TTCGTGCTTTCATAACAGAGCACATCA	CTCCAGTCACTCAGTTCCCCGTAGTCTG	302
IL-12P35	ACATGCTGGCAGTTATTGATGA	TGAAGAAGTATGCAGAGCT	127
IL-12RB2	GCCCGCTGGCAGGCGACACGTGGAAGAA	CCCAGGTGCAGGCCACAGTCCCCTGTTC	494
Gp130	ACAGATGAAGGTGGGAAGGAT	AGATGACATGCATGAAGACCC	423
Cyclin D1	TACACCGACAACTCCATC	ACATCTGTGGCACAGAGG	206
Survivin	GCTTCAAGGAGCTGGAAG	CGCAGTTTCCTCAAATTC	206
Fas	AATGCCCAAGTGACTGAC	TGTGTACTCCTTCCCTTC	207
Bcl-2	TGTGGCCTTCTTTGAGTTC	GAGACAGCCAGGAGAAATC	209
c-myc	CAGCAAACCTCCTCACAG	ACATTCTCCTCGGTGTCC	201
β-Actin	GTGGACATCCGCAAAGA	CTCGTCATACTCCTGCTTG	234

Student's *t* test. A value of $p < 0.05$ was deemed significant, and values of $p < 0.01$ and $p < 0.001$ were considered highly significant.

3. Results

3.1. Both IL-35 subunits were expressed concurrently in most human cancer cell lines compared to normal cell lines

To determine whether IL-35 plays a biological role in tumor cells, mRNA expression levels of the IL-35 and IL-35R subunits were detected in both human tumor and normal cell lines using RT-PCR analysis. The results showed that both IL-35 subunits *EBI3* and *IL-12p35* were presented in nine cell lines (9 out of 12): A549, SW480, BEL-7402, HepG2, SiHa, HPAC, MDA-MB-231, MCF and Raji cells. *EBI3* was not detected in neuroblastomas (SH-SY5Y) and the normal cell lines (Beas-2B and HEK293). For the IL-35R subunits, all cell lines tested expressed *gp130* (12 out of 12), which is ubiquitously expressed and is a receptor component for many cytokines. However, only SH-SY5Y and HEK293 cells expressed *IL-12Rb2* (2 out of 12; Fig. 1A). All PCR products were verified by direct DNA sequencing (data not shown).

Furthermore, quantitative real-time PCR analysis showed that the expression levels of *EBI3* and *IL-12p35* varied greatly among the cell lines compared to the controls (SiHa for *EBI3* and Beas-2B for *IL-12p35* expression). Raji cells are derived from a human hematopoietic lymphoma. Interestingly, we found that Raji cells expressed extremely high levels of *EBI3* but very low levels of *IL-12p35* compared to the other cell lines (Fig. 1B and C).

Subsequently, immunocytochemical analysis was performed to examine the localization of endogenous IL-35 subunits in the three cancer cells and control cells. The results showed that EBI3 and IL-12p35 were visualized in the cytoplasm of the cancer cells, while the control cells exhibited negative staining for EBI3 and positive staining for IL-12p35, agreeing with the PCR results (Fig. 1D).

These results indicated that both IL-35 subunits were expressed concurrently in most human cancer cell lines compared to normal cell lines, suggesting that IL-35 may play a functional role in regulating cancer progression.

3.2. The expression of IL-35 was up-regulated by TNF-α and IFN-γ stimulation in human cancer cells

EBI3 and IL-12p35 are cytokine subunits whose expression can be induced in immune cells. To understand the effect of pro-inflammatory cytokines on IL-35 expression in human cancer cells, HepG2 cells were stimulated with TNF-α (10 ng/ml) or IFN-γ (10 ng/ml) for 24 h. Elevated expression levels of EBI3 and IL-12p35 were observed in cells using FACS analysis (Table 2). This result indicated that the expression of IL-35 in human cancer cells was inducible.

3.3. Over-expression of IL-35 reduced human cancer cell growth

To understand the role of IL-35 expression in human cancer development, we established stable cell lines over-expressing IL-35 and confirmed IL-35 expression with RT-PCR, quantitative real-time PCR, and FACS analysis (Fig. 2A–C). The HepG2 cells with stable IL-35 over-expression showed a significant growth reduction compared to the controls (Fig. 2D). This result demonstrated that IL-35 may inhibit cancer activity by suppressing cancer cell growth.

3.4. Over-expression of IL-35 in human cancer cells caused cell cycle arrest at the G1 phase and sensitized cells to serum starvation-induced apoptosis

To determine the mechanism of IL-35-dependent cancer cell growth inhibition *in vitro*, the cell cycle distribution was examined

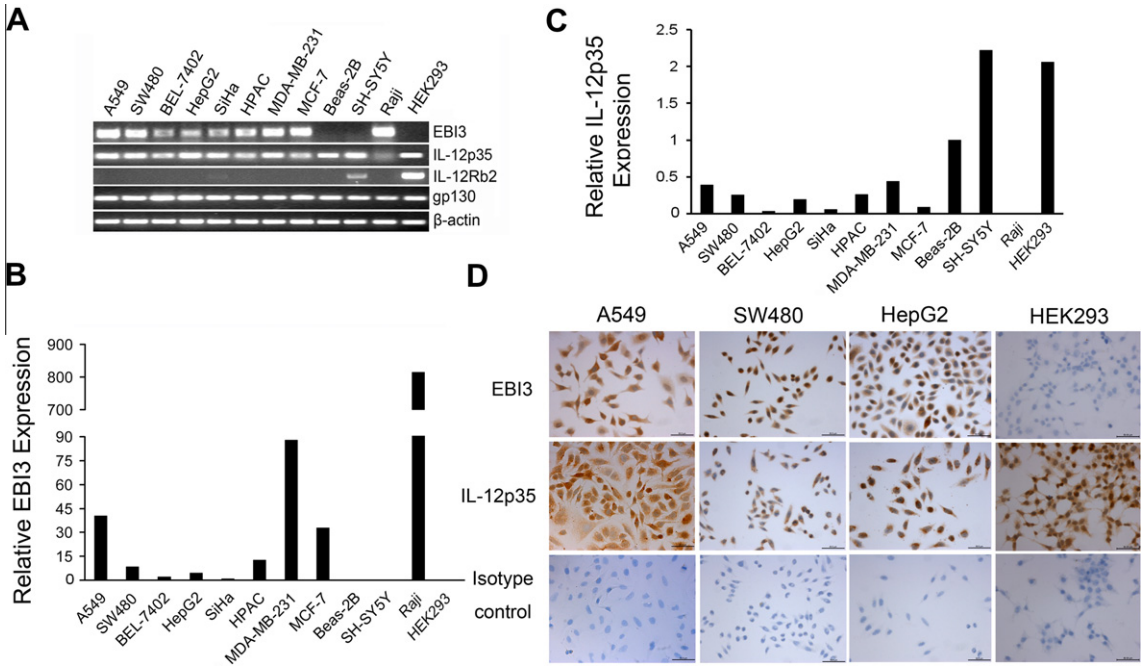

Fig. 1. The expression and localization of IL-35 or IL-35R in a variety of human cell lines. Expression of the IL-35 subunits *EBI3* and *IL-12p35* and the IL-35R subunits *IL-12Rb2* and *gp130* in a variety of human cell lines were detected using RT-PCR. All PCR products were verified through direct DNA sequencing. (B and C) Quantification of *EBI3* and *IL-12p35* expression levels was performed using quantitative real-time PCR. The data shown are the relative expression levels of each cell line compared to the controls (SiHa for *EBI3* and Beas-2B for *IL-12p35* expression). The data in A, B, and C are representative of three independent experiments. (D) The localization of endogenous EBI3 and IL-12p35 in human cancer and control cell lines was determined through immunocytochemical analyses. EBI3 staining was observed in the cytoplasm of A549, SW480, and HepG2 cells, whereas no staining was observed in HEK293 cells. IL-12p35-positive staining was observed in the cytoplasm of all cell lines. Isotype control antibodies did not result in positive staining for any cell type. The scale bar is 50 μm.

Table 2
TNF-α and IFN-γ stimulation increased the expression of IL-35 in human cancer cells ($n = 3$).

Group	EBI3$^+$IL-12p35$^+$ (%)	EBI3$^+$ (%)	IL-12p35$^+$ (%)
Control[a]	10.10 ± 1.73	13.27 ± 2.05	49.33 ± 3.50
TNF-α[b]	18.23 ± 1.90**	23.30 ± 3.48*	65.07 ± 2.11*
IFN-γ[c]	24.07 ± 4.20*	25.07 ± 3.97*	87.13 ± 4.27***

[a] HepG2 cells stimulated without TNF-α or IFN-γ.
[b] HepG2 cells stimulated with TNF-α for 24 h.
[c] HepG2 cells stimulated with IFN-γ for 24 h.
* $p < 0.05$; versus control, Student's t test.
** $p < 0.01$; versus control, Student's t test.
*** $p < 0.001$; versus control, Student's t test.

using flow cytometry to examine DNA content in human cancer cells after being maintained in serum-free culture for 0, 24, and 48 h (Fig. 2E).

At the starting time point, we found an increased number of cells in the G1 phase and a decreased number of cells in the S phase in HepG2 cells with stable IL-35 over-expression compared to the controls ($p < 0.01$). These results indicated that IL-35 over-expression in human cancer cells caused a cell cycle arrest at the G1 phase, which correlated with the reduced cell growth (Fig. 2D).

After 24 h of serum-free culture, both IL-35 over-expression and control cells were synchronized in the cell cycle. No significant differences were detected in number of cells in either the G1 or S

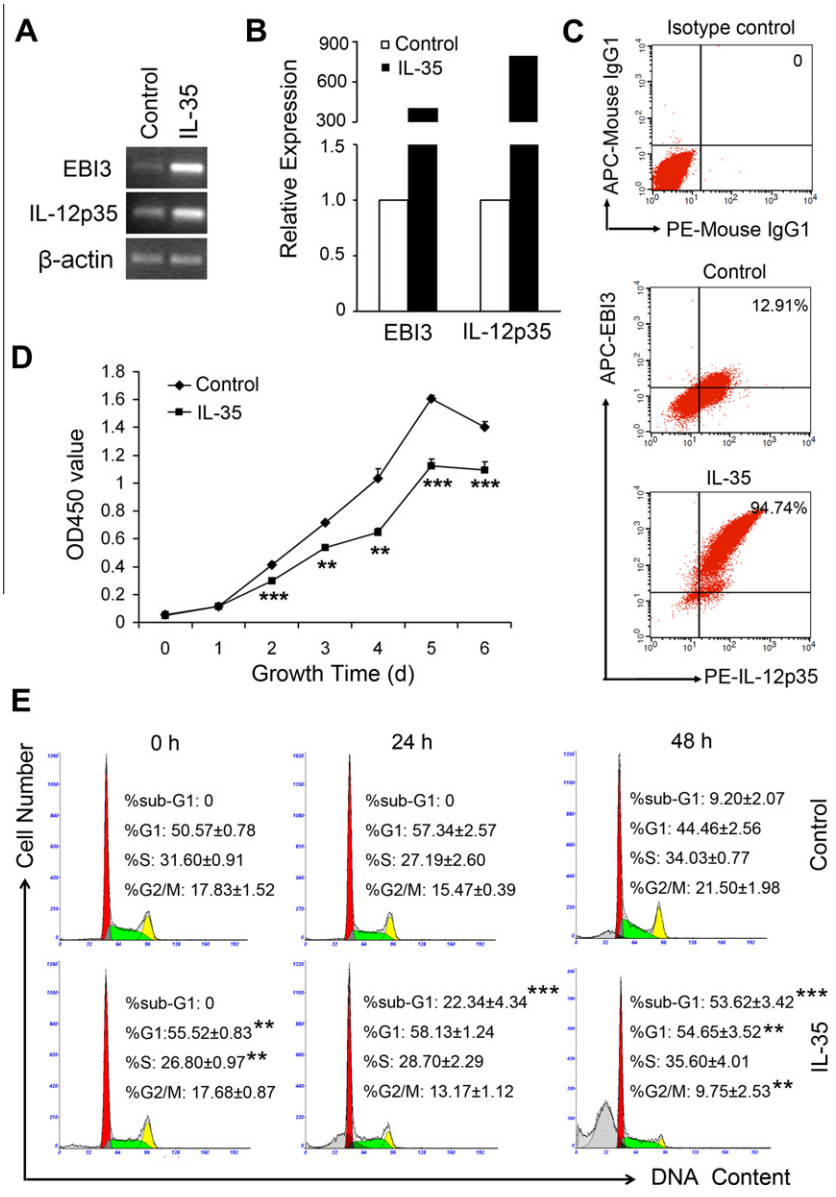

Fig. 2. IL-35 over-expression in human cancer cells reduced growth, resulted in cell cycle arrest at the G1 phase, and increased apoptosis induced by serum starvation. (A–C) Detection of IL-35 expression was performed in established HepG2 cells stably transfected with IL-35-Fc or Fc expression vector by RT-PCR (A), quantitative real-time PCR (B), and FACS analysis (C). All PCR products were verified by direct DNA sequencing. The relative *EBI3* and *IL-12p35* expression levels were determined using quantitative real-time PCR. The data shown are the relative *EBI3* and *IL-12p35* expression levels between the IL-35 group and the controls. The established stably transfected cells were stained with an APC-anti-human EBI3 and PE-IL-12 p35 antibody or with isotype control antibodies before FACS analysis. (D) Comparative analysis of cell growth using the CCK-8 assay was performed between the IL-35 group and the controls at the indicated time points. (E) The histograms of cell cycle distribution using HepG2 stable cells in 0, 24 and 48 h of serum-free culture using FACS analysis showed an increased number of G1 phase cells, a decreased number of S or G2/M phase cells and a substantially increased number of sub-G1 phase cells in the IL-35 group compared to the controls. The data in A, B, C, D, and E are representative of three independent experiments. Significant values are indicated with asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, versus control, Student's t test). Control, HepG2 cells stably transfected with the Fc expression vector; IL-35, HepG2 cells stably transfected with the IL-35-Fc expression vector.

Table 3
The over-expression of IL-35 sensitized human cancer cells to apoptosis induced by TNF-α and IFN-γ stimulation ($n = 3$).

Group	TNF-α apoptotic cells (%)	IFN-γ apoptotic cells (%)
Control[a]	4.40 ± 0.26	3.84 ± 1.00
IL-35[b]	12.48 ± 1.87**	10.28 ± 2.89*

[a] HepG2 cells stably transfected with the Fc expression vector. The cells were stimulated with TNF-α or IFN-γ for 24 h.
[b] HepG2 cells stably transfected with the IL-35-Fc expression vector. The cells were stimulated with TNF-α or IFN-γ for 24 h.
* $p < 0.05$; versus control, Student's t test.
** $p < 0.01$; versus control, Student's t test.

phases. However, a higher number of apoptotic cells in the sub-G1 phase were detected in IL-35-transfected cells ($p < 0.001$). After 48 h of serum-free culture, IL-35-transfected cells also showed a higher number of cells in the G1 phase, a decreased number of cells in the G2/M phase, and, most important, a substantially higher number of apoptotic cells in the sub-G1 phase compared to the controls ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively). These results indicated that IL-35 over-expression sensitized human cancer cells to serum starvation-induced apoptosis.

3.5. Stimulation with TNF-α and IFN-γ induced a higher level of apoptosis in IL-35-over-expressing human cancer cells

To evaluate whether the increased expression of IL-35 sensitized cancer cells to apoptosis induced by anti-tumor cytokines, we induced apoptosis using TNF-α and IFN-γ stimulation in human cancer cells. At 24 h post-stimulation, both TNF-α and IFN-γ stimulation significantly increased apoptosis in HepG2 cells stably transfected with IL-35 compared to the controls using FACS analysis (Table 3). These results verified that IL-35 over-expression sensitized human cancer cells to apoptosis induced by anti-tumor cytokines.

3.6. Regulation the expression of genes related to cell cycle and apoptosis in IL-35- over-expressing human cancer cells

To elucidate the mechanism underlying how IL-35 expression inhibits cell growth and induces apoptosis, we examined the expression levels of genes related to cell cycle and apoptosis using RT-PCR and quantitative real-time PCR. HepG2 cells stably transfected with IL-35 induced an increase in *Fas* and a decrease in *cyclin D1*, *survivin*, and *Bcl-2* expression levels compared to the controls ($p < 0.05$, $p < 0.001$, $p < 0.01$, and $p < 0.001$, respectively) (Fig. 3).

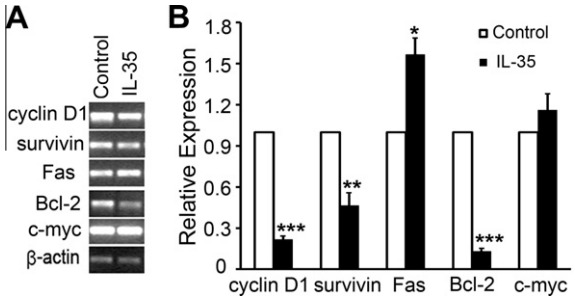

Fig. 3. The expression of genes related to cell cycle and apoptosis in IL-35 over-expressing human cancer cells. The expression levels of *cyclin D1*, *survivin*, *Fas*, *Bcl-2*, and *c-myc* in the HepG2 stable cells were analyzed using RT-PCR (A) and quantitative real-time PCR (B). The results showed that *Fas* expression was up-regulated, and *cyclin D1*, *survivin*, and *Bcl-2* expression levels were down-regulated compared to the controls. The data in A and B are representative of three independent experiments. Significant values are indicated with asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, versus control, Student's t test). Control, HepG2 cells stably transfected with the Fc expression vector; IL-35, HepG2 cells stably transfected with the IL-35-Fc expression vector.

These results suggested that IL-35 exhibited an inhibitory effect on growth and promoted apoptosis of human cancer cells through selective effect on genes related to cell cycle and apoptosis.

4. Discussion

It is well known that IL-35 is predominantly expressed in Tregs [1–4,6]. Recently, a study found that EBI3 expression in human lung cancer but not in normal tissues (liver, kidney, heart and lung) is a potent biomarker with clinical prognostic value for lung cancer [12]. Several studies have indicated that EBI3 expression is present in lymphomas [13,14]. Our previous studies showed that both IL-35 subunits were expressed in lung, colon, esophageal, hepatocellular and cervical carcinomas (data not shown). In this study, our data revealed that both IL-35 subunits were expressed concurrently in most human cancer cell lines compared to normal cells.

The function of IL-35 in Tregs has been demonstrated in immune regulation [1,2,6]; however, the function of IL-35 in tumor cells has not been extensively studied. In contrast to previous studies in Tregs, our findings assign a new role to IL-35 by showing that over-expression of IL-35 in human cancer cells inhibits cancer cell growth *in vitro*. A recent study has shown that transfection of the IL-27 gene into human pancreatic carcinoma cells produces anti-tumor effects *in vivo* through the induction of a cell cycle arrest and apoptosis by up-regulating p21 expression and down-regulating survivin expression [15]. Currently, no link between IL-35 expression in cancer cells and cell cycle arrest has been reported in the literature. Our data, using human cancer cells stably transfected with IL-35, showed that IL-35 suppressed cancer cell growth via cell cycle arrest at the G1 phase and substantially sensitized cells to serum starvation-induced apoptosis through down-regulation of *cyclin D1* and *survivin* expression. Cyclin D1 is associated with the G1/S transition in cell cycle and is over-expressed in a variety of cancers [16]; survivin regulates the cell cycle, inhibits apoptosis proteins, and is involved in cell division and apoptosis suppression [17].

The link between IL-35 over-expression and the increased susceptibility to serum starvation-induced apoptosis suggests that IL-35 expression in human cancer cells might play a role in the induction of apoptosis. Cytokine production has convincingly been demonstrated in many types of tumor cells [18,19]. Certain cytokines, such as TNF-α and IFN-γ, can play a role in the induction of tumor cell apoptosis [20,21]. Others studies have reported that IL-12 up-regulated Fas expression in human osteosarcoma and Ewing's sarcoma, leading to an increase in their sensitivity to Fas-induced cell apoptosis by enhancing Fas promoter activity [22]. It is now well known that apoptosis occurs through two main pathways, namely, the extrinsic and intrinsic apoptosis signalling pathways. The extrinsic pathway is triggered through the Fas death receptor, and the intrinsic pathway is regulated by the Bcl-2 family of proteins, which are involved in the positive and negative regulation of apoptosis. The anti-apoptotic Bcl-2 and the pro-apoptotic Bax are two central members of the Bcl-2 family. Our present study shows that TNF-α and IFN-γ stimulation not only increases the expression of IL-35 in human cancer cells but also increases apoptosis in human cancer cells over-expressing IL-35 through the up-regulation of *Fas* and the concurrent down-regulation of *Bcl-2* expression. Although the detailed function of IL-35 in cancer immunity and the precise mechanism by which IL-35 affects cancer progression are not clear, our results indicate that cell cycle arrest and apoptosis may be central to the inhibitory cancer activity of IL-35.

Cancer develops when the balance between cell proliferation and cell death is disturbed, and aberrant cell proliferation leads to cancer growth. Apoptosis is a form of physiological cell death

essential to normal tissue development and homeostasis. However, apoptosis is down-regulated in cancer as well as autoimmune diseases and up-regulated in degenerative diseases [23]. A successful clinical therapy against cancer is one that suppresses proliferation and promotes apoptosis. Our findings may have clinical implications for cancer therapy and for understanding how intracellular cytokine expression limits immune cell expansion resulting in the suppression of inflammation and autoimmunity.

In summary, this study demonstrates a novel function of IL-35 in human cancer cells by suppressing cancer cell growth via cell cycle arrest at G1 phase and, most important, sensitizing these cells to apoptosis through selective effects on cell cycle and apoptotic gene expression levels.

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