Fluoxetine regulates cell growth inhibition of interferon-α

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Pharmacy, Chi-Mei Medical Center, Tainan; [8]Department of Pharmacy, Chia Nan University of Pharmacy and Science,
Tainan; [9]Department of Public Health, College of Medicine, Taipei Medical University, Taipei, Taiwan, R.O.C.

Abstract. Fluoxetine, a well-known anti-depression agent, may act as a chemosensitizer to assist and promote cancer therapy. However, how fluoxetine regulates cellular signaling to enhance cellular responses against tumor cell growth remains unclear. In the present study, addition of fluoxetine promoted growth inhibition of interferon-alpha (IFN-α) in human bladder carcinoma cells but not in normal uroepithelial cells through lessening the IFN-α-induced apoptosis but switching to cause G1 arrest, and maintaining the IFN-α-mediated reduction in G2/M phase. Activations and signal transducer and transactivator (STAT)-1 and peroxisome proliferator-activated receptor alpha (PPAR-α) were involved in this process. Chemical inhibitions of STAT-1 or PPAR-α partially rescued bladder carcinoma cells from IFN-α-mediated growth inhibition via blockades of G1 arrest, cyclin D1 reduction, p53 downregulation and p27 upregulation in the presence of fluoxetine. However, the functions of both proteins were not involved in the control of fluoxetine over apoptosis and maintained the declined G2/M phase of IFN-α. These results indicated that activation of PPAR-α and STAT-1 participated, at least in part, in growth inhibition of IFN-α in the presence of fluoxetine.

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Introduction

Interferon-alpha (IFN-α) has a variety of biological properties, including antiviral effects, antiproliferation and immune response modulation. Besides, IFN-α also exerts antitumor activities in a range of haematological and non-haematological malignancies (1,2). Following IFN-α bond to its receptor, it affects Janus kinases JAK1 and TYK2 on the phosphotyrosine residue and sites at the intracellular domain of each receptor chain. The signal transducer and transactivator (STAT) proteins are phosphorylated by JAK1 and TYK2. Moreover, they dissociate from the receptor, dimerize via SH2 domain, and form the mature ISGF3 complex associated with the IFN regulatory factor family. This complex further translocates to the nucleus and binds to interferon-stimulated response elements (ISRE) that initiate gene transcription contributing to the activation of the cytoplasmic targets of IFN-α. Of note, the interaction of ISGF3 with ISRE induces several transcriptional genes such as protein kinase dependent on dsRNA (PKR) that modulates cancer cell growth. Through the translational and transcriptional pathways, PKR activates protein expressions of Fas, p53 and Bax to trigger apoptosis. IFN-α-induced apoptosis possibly activates the caspase cascade mediated by mitogen-activated protein kinases (1,3,4). Alternatively, IFN-α activates its receptors and induces antiproliferative signaling via the STAT by cross-talking with the extracellular signal-regulated kinase (ERK) pathway; it further leads to the slowing down of G1/S transition without apoptosis in human hepatocellular carcinoma cells (HCC) (5). Conversely, IFN-α reduces activation of ERK in haematological malignancies (6). IFN-α also exerts growth inhibition of human T-cell leukaemia line Jurkat through p38a and p38b (7). These discrepancies depend on the cell types, time of treatment and dosage used (1). Although the cell growth inhibition and apoptosis of IFN-α are thought to be a possible explanation for its antitumor action, the precise mechanisms of this issue are restricted.

Besides antitumor activity with IFN-α monotherapy, several studies suggest a combinatorial strategy with IFN-α in cancer therapies. For instance, the synergic cell growth inhibition and apoptosis of IFN-α are observed in human T-cell lymphotropic virus type I-transformed cells combined with arsenic trioxide (8), in transformed T- and monocytoid cell lines combined with IL-21 (9), in HCC combined with 5-fluorouracil (10), or in bladder cancer cells combined with proanthocyanidin (11). These results indicate that a combinatorial strategy is more effective to antitumor action. Nevertheless, serious adverse effects still exist, and they are limited due to their tolerability and efficacy.

Fluoxetine is widely used in treatment of depression in patients with cancer or infection of hepatitis C virus (12,13). The potential of antitumor action of fluoxetine is still inconclusive due to the dependence on the dosage used and the cell types (14,15). Besides, fluoxetine is kept to the range between 5-20 μM as a multidrug resistance reversal agent and it has been proposed to be considered a fourth-generation chemosensitizer in clinic (15). However, how fluoxetine regulates cellular signaling to enhance cellular responses in chemotherapy is still unclear. In the present study, we used human bladder superficial carcinoma cells, T24, to investigate the possible mechanisms through which fluoxetine promotes the antitumor activity of IFN-α. Recent evidence suggests that peroxisome proliferator-activated receptor alpha (PPAR-α), a member of the ligand-activated nuclear receptor superfamily, may regulate cell survival and apoptosis (16). Interaction between PPAR-α and STAT transcription factors contributes to PPAR-α-mediated transcriptional repression (17); however, whether PPAR-α regulates the growth inhibition of IFN-α associated with the regulation of STAT-1 remains unclear. Thus, after pretreatments with PPAR-α and STAT-1 inhibitors, we have examined the IFN-α-mediated anti-proliferation and apoptosis in the presence of fluoxetine, including cell growth, cell cycle, cyclins, and signal molecules as well as the levels and co-localization of activations of STAT-1 and PPAR-α.

Materials and methods

Chemicals. IFN-α-2b (Intron A) was purchased from Schering-Plough Brinny Co. (Cork, Ireland). Fluoxetine, fludarabine and GW6471 were purchased from Tocris Bioscience (Ellisville, MO, USA).

Antibodies. Antibodies against β-actin, phospho-STAT1 (Tyr701 and Ser727), STAT1, cyclin A, cyclin B1, cyclin D1, p27 and p53 were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). PPAR-α was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lamin A was purchased from BD Biosciences (San Jose, CA, USA).

Cell cultures. The human bladder carcinoma cell line T24 and normal uroepithelial cell line SV-HUC-1 (Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan) were grown in Dulbecco's modified Eagle's medium and Ham's F12 medium, respectively, with 10% fetal calf serum (FCS) and 100 μg/ml gentamicin.

Cell proliferation assay. Cell growth of cultured cells was studied by colorimetry with a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS] assay kit (Promega Corp., Madison, WI, USA). Briefly, cells (8x10^3 cells/well) were seeded on 96-well plates for 24 h and the culture medium was later changed to a new medium containing IFN-α, fluoxetine, or in combination for 3 days. The number of viable cells was measured with SpectraMax 340PC (Molecular Devices, Inc., Sunnyvale, CA, USA) at a wavelength of 490 nm after reacting with the tetrazolium reagent for 1.5 h.

Flow cytometry. Cells (2x10^6) were harvested from 10-cm culture dishes, washed with phosphate-buffered saline (PBS), suspended in 200 μl of ice-cold 70% ethanol and incubated on ice for a least 1 h. After cells were washed and exposed to RNase A at 37°C for 30 min, these cells were then suspended in propidium iodide (PI) (Sigma-Aldrich, St. Louis, MO, USA) in PBS. DNA was analyzed via flow cytometry (FACSCalibur; BD Biosciences) to evaluate the cell cycle by measuring the percentage of subG1, G0/G1-, S- and G2/M-phase after treatment with IFN-α and/or fluoxetine after 24 h.

Caspase-3 activity. Cells (7x10^5) were treated with GW6471 or fludarabine for 1 h before IFN-α and fluoxetine, and then harvested at day 1 for performing ICE-family proteases/caspases activation to initiate apoptosis. The caspase-3/CPP32 colorimetric assay kit (BioVision, Inc., Milpitas, CA, USA) was used according to the manufacturer's protocols and measured with a spectrophotometer at 405 nm.

Western blot analysis. Cells (7x10^5) were harvested at indicated times and lysed with lysis buffer containing 1% Triton X-100, 50 mM Tris (pH 7.5), 10 mM EDTA, 0.02% NaN$_3$, and protease inhibitor cocktail (Sigma-Aldrich). The membrane (Millipore, Billerica, MA, USA) was blocked with 5% skim milk in TBS-T [50 mM Tris, 150 mM NaCl and 0.05% Tween-20, (pH 7.6)] at room temperature for 1 h and probed with primary antibodies at 4°C overnight. After being washed with TBS-T, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (1:2,500) at room temperature for 1 h. The protein expression was visualized via enhanced chemiluminescence reagent (Perkin-Elmer, Boston, MA, USA) and analyzed using VisionWorks LS software (Upland, CA, USA) for the optical densities of phospho-protein/total protein when using β-actin as the internal control.

Nuclear extraction. The commercially available CHEMICON® nuclear extraction kit (Millipore) was used according to the manufacturer's protocols.

Indirect immunofluorescence. The cells (2x10^4) were fixed with 4% paraformaldehyde in PBS for 10 min and permeabilized with 0.1% Triton X-100 for another 10 min at room temperature. These cells were later stained with primary and secondary antibodies after being washed with PBS twice. Primary antibodies used were anti-phospho-STAT1 (Tyr701) or anti-PPAR-α, and Alexa Fluor 568-conjugated goat anti-rabbit IgG and 488-conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA) were used as secondary

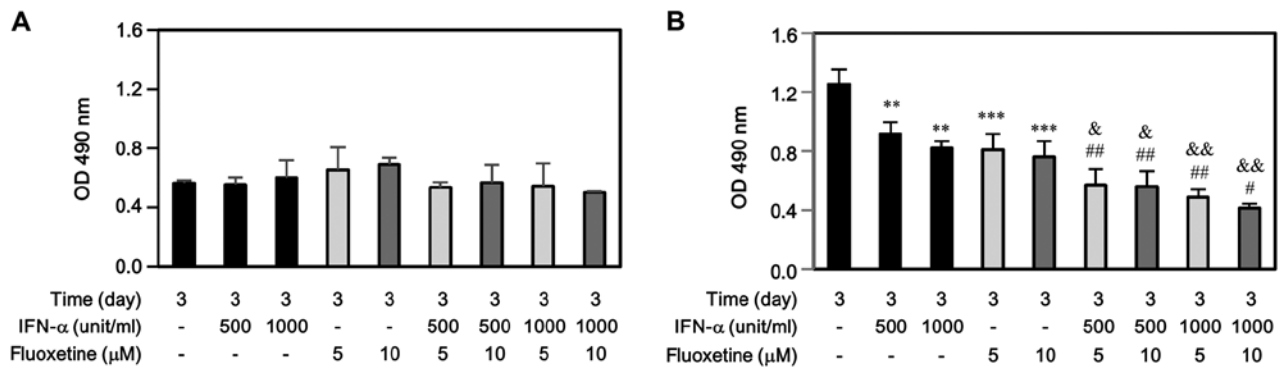Figure 1. Effects of IFN-α and fluoxetine on cell growth inhibition. SV-HUC-1 and T24 cells were treated with 500 and 1000 U/ml of IFN-α and/or 5 and 10 μM of fluoxetine, and then harvested at day 3 to perform cell proliferation by MTS assay. Data are expressed as mean ± SEM obtained from three individual cultures. **P<0.01 and ***P<0.001 as compared with medium control group; #P<0.05 and ##P<0.01 as compared with IFN-α-treated group; &P<0.05 and &&P<0.01 as compared with fluoxetine-treated group.

antibodies. Stained cells were later washed with PBS and counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) at room temperature for 1 h. After staining, these cells were mounted on glass slides and observed under confocal laser scanning microscope (Olympus FluoView™ FV1000; Olympus).

Statistical analysis. One-way ANOVA test was used to examine various experiments among the IFN-α-, fluoxetine-, IFN-α plus fluoxetine-treated, and the medium control groups. Statistical significance was set at P<0.05.

Results

Fluoxetine sensitizes cell growth inhibition to IFN-a. Normal human uroepithelial SV-HUC-1 and bladder carcinoma T24 cells were treated with IFN-α (500 and 1000 U/ml), fluoxetine (5 and 10 μM), or in combination, and then harvested for performing cell proliferation by MTS assay. As shown in Fig. 1A, IFN-α, fluoxetine, or in combination did not affect SV-HUC-1 cell growth at day 3. However, either IFN-α or fluoxetine only impeded significantly T24 cell growth at day 3 (Fig. 1B). Moreover, the decreased levels of T24 proliferation induced by IFN-α combined with fluoxetine-treated group were much more than those induced by IFN-α-treated or fluoxetine-treated group.

Fluoxetine sensitized cell growth inhibition to IFN-α via the STAT-1- and PPAR-α-dependent pathways. The levels of phospho-STAT-1 at Tyr701 and Ser727 residue were markedly increased by IFN-α after 6 h to 2-day post-treatment, whereas fluoxetine alone had no effect on the phosphorylation of STAT-1 (Fig. 2A). Addition of fluoxetine facilitated the IFN-α-mediated phosphorylation of STAT1 at Tyr701 after 6 h, but declined after 2 days. Moreover, an elevated level of phospho-STAT1 at Ser727 was also detected after 6 h and maintained continuously to day 3 (Fig. 2A).

The phospho-STAT1 localization was also determined by indirect immunofluorescence. In the medium of control cells, phospho-STAT1 was found around the cell surface with slight fluorescence intensity. Following treatment with IFN-α and fluoxetine for 6 h, a marked increase in phospho-STAT1

fluorescence intensity and nuclear translocation was observed, which was repressed by 0.2 μM of fludarabine, a specific STAT-1 blocker (18-20) (Fig. 2B). Similarly, an increase in PPAR-α protein expression was observed during 6-12 h (data not shown). An addition of fluoxetine to IFN-α caused a translocation of PPAR-α to the nucleus, which was inhibited by 5 μM of GW6471, a PPAR-α antagonist (Fig. 3).

Notably, co-localization of STAT-1 and PPAR-α was observed to the some extent in the cytoplasm by IFN-α or fluoxetine alone after a 6 h post-treatment (Fig. 4A). We also found that IFN-α-treated cells partially activated and translocated both proteins to the nucleus, whereas fluoxetine-treated cells predominately triggered PPAR-α translocation to the nucleus (Fig. 4). As compared with IFN-α-treated cells, co-localization of STAT-1 and PPAR-α was clearly seen in the nucleus while cells were exposed to IFN-α in combination with of fluoxetine. These results indicated that fluoxetine promoted the IFN-α-induced activation and translocation of STAT1 and PPAR-α.

PPAR-a and STAT-1 were partially involved in the growth inhibition of IFN-α in the presence of fluoxetine via the alterations of cell cycle subpopulations and cell cycle regulatory proteins. First, in order to investigate the roles of STAT-1 and PPAR-α proteins in cell growth inhibition of IFN-α or fluoxetine, fludarabine and GW6471 were used before treatment with IFN-α, fluoxetine, or in combination. Fludarabine or GW6471 partially prevented the cell growth inhibition induced by IFN-α or in combination with fluoxetine, whereas pretreatment of fludarabine could not reverse this inhibition induced by fluoxetine. Otherwise, either fludarabine or GW6471 alone did not have this result (Fig. 5).

Second, we stained the cells with PI to carry out flow cytometry and detect changes of cell cycle subpopulations to examine the cellular mechanisms of IFN-α growth inhibition in the presence of fluoxetine (Fig. 6). IFN-α at 1000 U/ml caused apoptosis, slightly increased S phase, and decreased G2/M phase, but it did not affect G1 phase markedly. However, fludarabine and GW6471 could reverse the IFN-α-mediated apoptosis, whereas fludarabine but not GW6471 could reverse the decreased G2/M phase. On the contrary, fluoxetine at 10 μM caused G1 arrest without apoptosis, but it did not affect
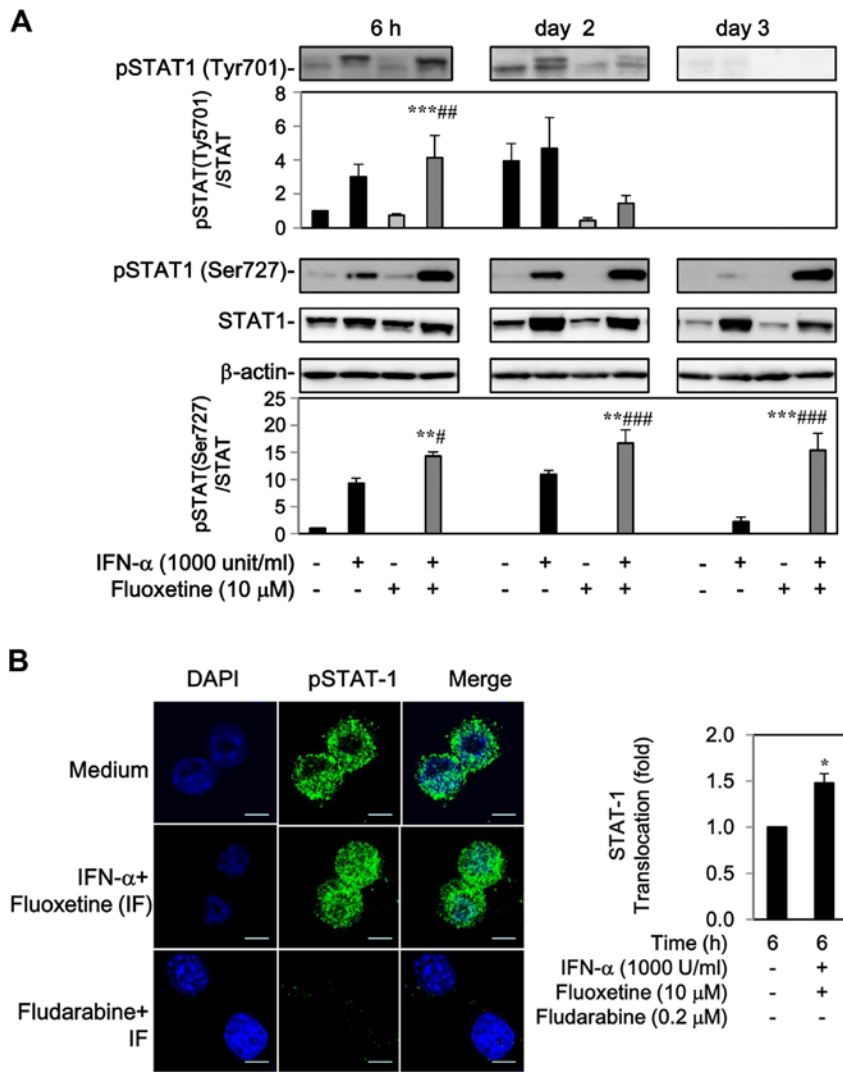
Figure 2. Fluoxetine boosts IFN-α-induced STAT1 activation and translocation. (A) T24 cells were treated with 1000 U/ml of IFN-α and 10 μM of fluoxetine in a 6-well plate. Cell lysates were collected for 3 days and the levels of phospho-STAT1 Tyr701 and Ser727 were examined with western blot analysis. (B) Cells were treated with 0.2 μM of fludarabine for 1 h before treatment with IFN-α and fluoxetine for 6 h in a 24-well plate, fixed with 1% paraformaldehyde, and stained with phospho-STAT1 Tyr701 antibody for the detection of activation and translocation of STAT1 by immunocytometry staining (magnification, x600). The arbitrary units of immunofluorescene intensity were assessed using Image-Pro Plus 6.0 software. Data are expressed as mean ± SEM obtained from three individual experiments. *P<0.05, **P<0.01 and ***P<0.001 as compared with medium control group; #P< 0.05, ##P<0.01 and ###P<0.001 as compared with IFN-α-treated group.
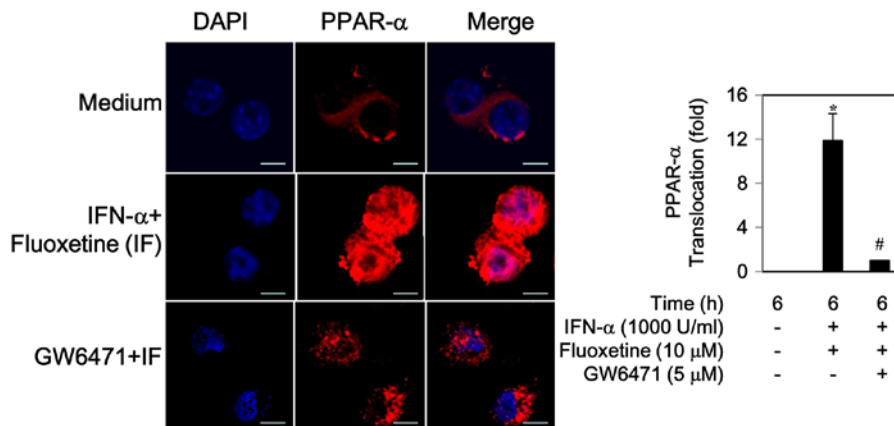

Figure 3. Fluoxetine boosts the IFN-α-induced activation and translocation of PPAR-α. T24 cells were treated with 5 μM of GW6471 before treatment with 1000 U/ml of IFN-α and 10 μM of fluoxetine for 6 h in a 24-well plate, fixed with 1% paraformaldehyde, and stained with PPAR-α antibody for detection of activation and translocation of PPAR-α by immunocytometry staining (magnification, x600). The arbitrary units of immunofluorescene intensity were assessed using Image-Pro Plus 6.0 software. Data are expressed as mean ± SEM obtained from three individual experiments. *P<0.05 as compared with medium control group; #P<0.05 as compared with IFN-α-treated group.

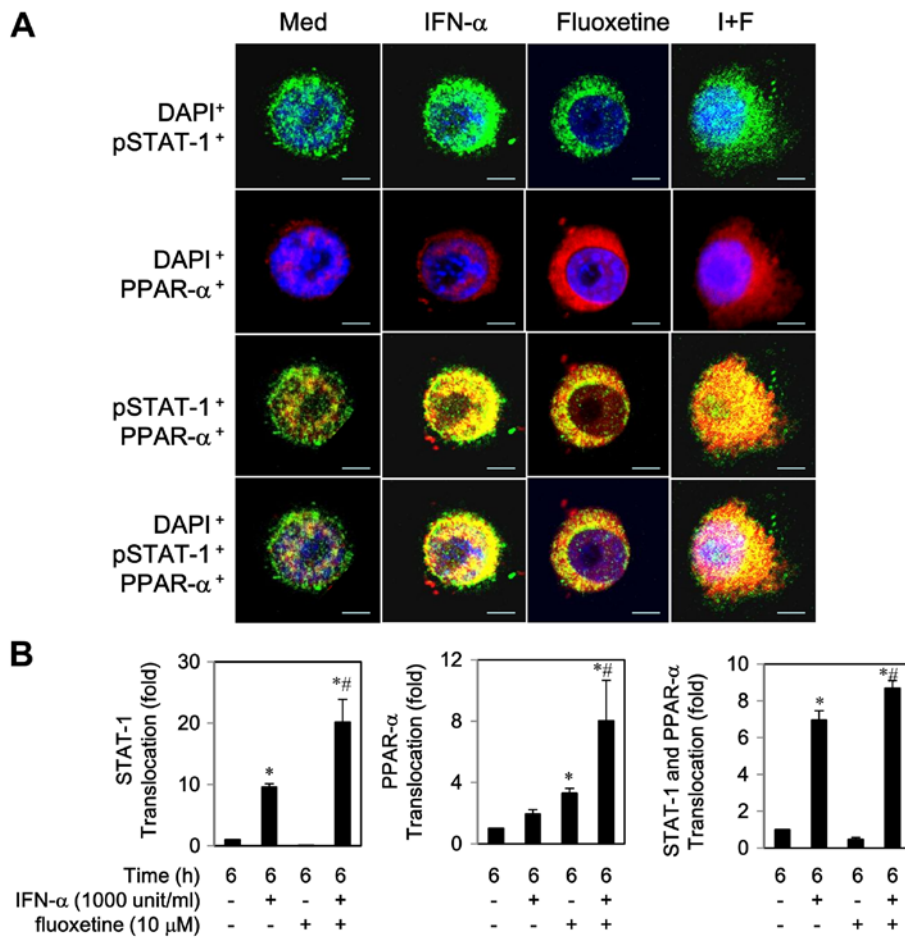Figure 4. IFN-α and fluoxetine caused co-localization of STAT-1 and PPAR-α proteins. (A) T24 cells were treated with 1000 U/ml of IFN-α, 10 μM of fluoxetine, or in combination (I+F) in a 24-well plate, fixed with 1% paraformaldehyde, and stained with phospho-STAT-1 Tyr 701 and PPAR-α antibodies for the detection of activation and translocation of STAT-1 and PPAR-α by immunocytometry staining (magnification, x600). (B) The percentages of translocation to nucleus were determined using the Image-Pro Plus 6.0 software. Data are expressed as mean ± SEM obtained from three individual experiments. *P<0.05 as compared with medium control group; #P<0.05 as compared with IFN-α-treated group.
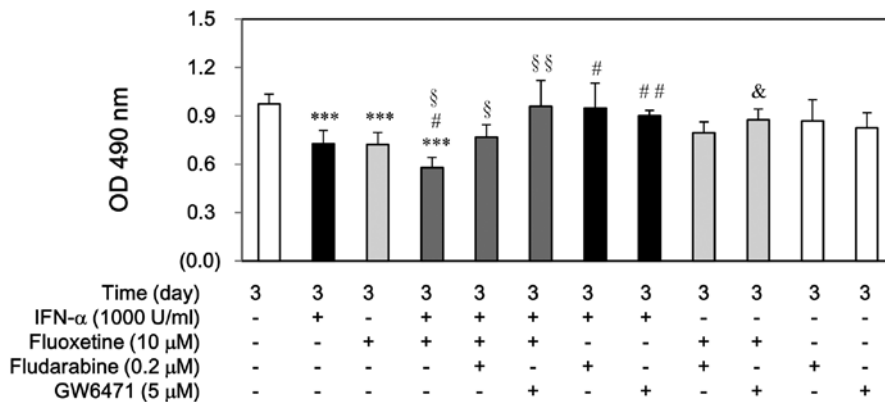

Figure 5. Fludarabine or GW6471 rescue T24 cells from IFN-α-induced growth inhibition in the presence of fluoxetine. Cells were treated with 0.2 μM of fludarabine or 5 μM of GW6471 before treatment with 1000 U/ml of IFN-α, 10 μM of fluoxetine, or in combination, and then harvested at day 3 for performing cell proliferation by MTS assay. Data are expressed as mean ± SEM obtained from three individual cultures. ***P<0.001 compared with medium control; #P<0.05 and ##P<0.01 compared with IFN-α-treated group; &P<0.05 compared with fluoxetine-treated group; §P<0.05 and §§P<0.01 compared with IFN-α plus fluoxetine-treated group.

S and G2/M phase. GW6471 but not fludarabine attenuated the fluoxetine-mediated G1 arrest.

Compared with the IFN-α-treated group, addition of fluoxetine attenuated the IFN-α-induced apoptosis signifi-

cantly, which was not significantly reversed by fludarabine and GW6471 (Fig. 6). Similarly, IFN-α increased the caspase-3 activity at day 1, whereas fluoxetine alone or in combination with IFN-α did not show this effect. Moreover, fludarabine
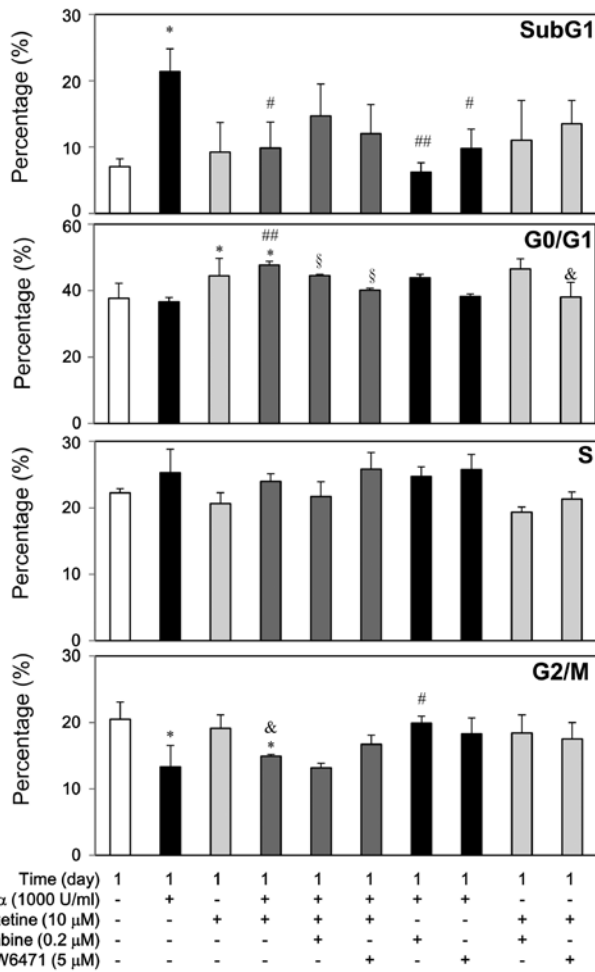
Figure 6. Determinations of IFN-α, fluoxetine, or the combinations on phases of cell cycle. T24 cells were treated with 0.2 μM of fludarabine or 5 μM of GW6471 before treatment with 1000 U/ml of IFN-α and/or 10 μM of fluoxetine, and then harvested at day 1 for performing cell cycle using flow cytometry with PI. Data are expressed as mean ± SEM obtained from three individual cultures. *P<0.05 as compared with medium control group; #P<0.05 and ##P<0.01 as compared with IFN-α-treated group; &P<0.05 as compared with fluoxetine-treated group; §P<0.05 as compared with IFN-α plus fluoxetine-treated group.

Figure 8. Determinations of IFN-α, fluoxetine, or the combinations on cell cycle regulatory proteins. T24 cells were treated with 0.2 μM of fludarabine or 5 μM of GW6471 before treatment with 1000 U/ml of IFN-α and/or 10 μM of fluoxetine and then harvested at day 1 for determining cell cycle regulatory proteins. Cell lysates were collected and the cyclin A, cyclin B1, cyclin D, p53 and p27 levels were detected by western blot analysis. Data shown are representative of three individual experiments. Data are expressed as mean ± SEM obtained from three individual cultures. *P<0.05, **P<0.01 and ***P<0.001 as compared with medium control group; #P<0.05, ##P<0.01 and ###P<0.001 as compared with IFN-α-treated group; &P<0.05, &&P<0.01 and &&&P<0.001 as compared with fluoxetine-treated group; §P<0.05 and §§P<0.01 as compared with IFN-α plus fluoxetine-treated group.
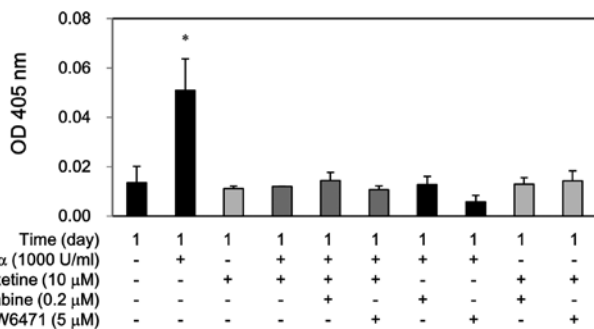

Figure 7. Effects of IFN-α, fluoxetine or the combinations on the caspase-3 activity. T24 cells were treated with 5 μM of GW6471 or 0.2 μM of fludarabine for 1 h before 1000 U/ml of IFN-α and 10 μM of fluoxetine, and then harvested at day 1 for performing caspase-3 activity with a spectrophotometer at 405 nm. *P<0.05 as compared with medium control group.

and GW6471 hampered the caspase-3 activity by IFN-α but not by fluoxetine alone or in combination (Fig. 7). Conversely, this c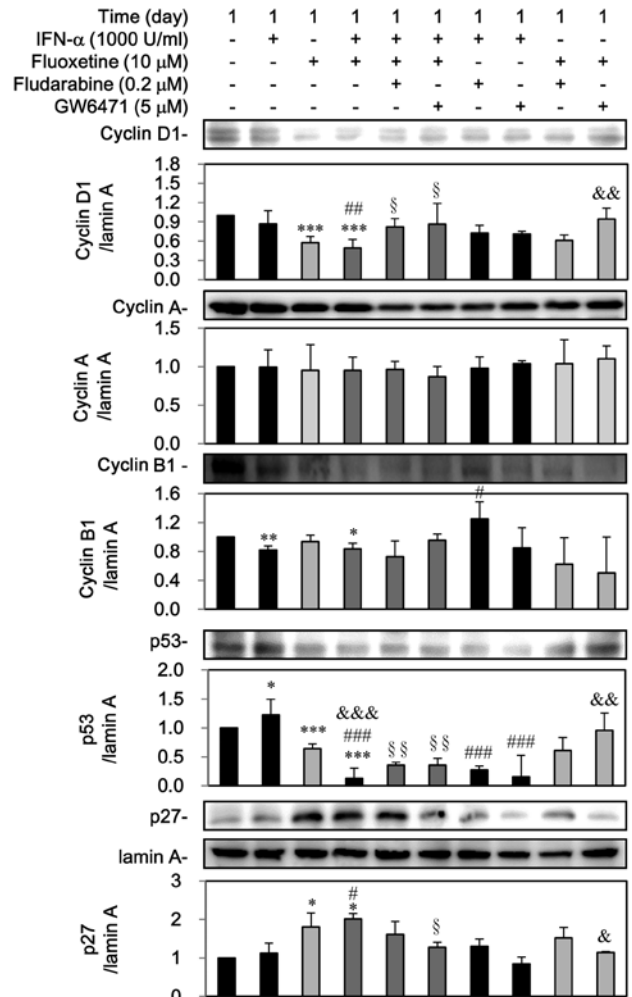o-treatment also significantly caused G1 arrest, which was partially reversed by fludarabine and GW6471. Otherwise, fluoxetine did not affect the S phase and the G2/M reduction of IFN-α (Fig. 6).

Third, as shown in Fig. 8, IFN-α can decline cyclin B1 but induced p53, which was reversed by fludarabine. Otherwise, GW6471 can block the IFN-α-mediated upregulation of p53, however, IFN-α did not notably affect the expressions of cyclin D1, cyclin A and p27. On the contrary, fluoxetine itself decreased the expression of cyclin D1 and p53 as well as induced the nuclear p27 expression, which was reversed by GW6471 but not by fludarabine. However, fluoxetine did not affect cyclin A and cyclin B1.

A reduction in the expression of cyclin D1, cyclin B1, and p53 as well as a marked increase of p27, but not cyclin A, was observed in the group of IFN-α in combination of fluoxetine.
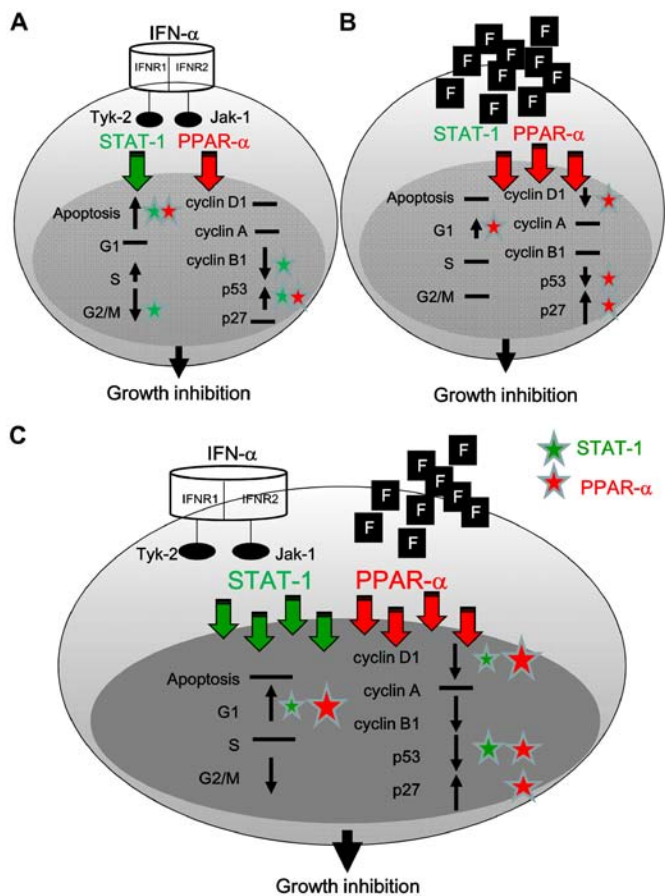
Figure 9. A schematic model of the involvement of STAT-1 and PPAR-α in cell growth inhibition of IFN-α, fluoxetine, or the combinations. (A) IFN-α activated STAT-1 and PPAR-α, translocated to the nucleus, and induced apoptosis via the induction of p53. (B) Fluoxetine activated predominately PPAR-α to further cause cell G1 arrest via reduction of cyclin D1 and p53 and induction of p27. (C) Addition of fluoxetine facilitated the growth inhibition of IFN-α and caused cell arrest via a boosted activation of STAT-1 and PPAR-α accompanied with downregulation of cyclin D1 and p53 and upregulation of p27. Alterations of phases and cyclins of cell cycle by IFN-α, fluoxetine, or the combinations were blocked by STAT-1 inhibitor (green asterisk) or PPAR-α inhibitor (red asterisk).

Pretreatment with fludarabine and GW6471 could reverse downregulations of cyclin D1 and p53 but not cyclin B1, whereas GW6471 could block the upregulation of p27 by IFN-α combined with fluoxetine (Fig. 8).

Discussion

The present study demonstrate that IFN-α can activate STAT-1 and PPAR-α, translocate to the nucleus, and induce apoptosis via the induction of p53 (Fig. 9A). Fluoxetine predominately activated PPAR-α to further cause G1 arrest via the reduction of cyclin D1 and p53 and induction of p27 (Fig. 9B). The addition of fluoxetine facilitated the cell growth inhibition of IFN-α and caused cell arrest via a boosted activation of STAT-1 and PPAR-α accompanied with the downregulation of cyclin D1 and p53 and the upregulation of p27 (Fig. 9C).

In the present study, IFN-α caused apoptosis and blocked the G2/M phase, but did not affect markedly G1 and S phases of cell cycle accompanied by the induced p53 as well as declined cyclin B1 and showed no effect on the expression of cyclin D1, cyclin A and p27. Our results were similar to previous studies with IFN-α treatment in various cell lines, including hematopoietic (H9) (21), Ba/F3 pro-B cells (22), and HCC (23); yet, discorded with some reports in chronic myelogenous leukemia (K562) (24), carcinoid tumor Bon1 (25), and human glioblastoma, U-373MG and T98G (26). Therefore, the influence of IFN-α on the cell cycle progression appears to be variable. These discrepancies depend on the cell types, the length of time in treatment and the dosage used (1).

In synergistic and human xenograft mouse tumor models, fluoxetine impedes multidrug resistance extrusion pumps and enhances the responses to chemotherapy. Thus, fluoxetine may help a drug to be sensitive to tumor cells and accumulate to a sufficient level that culminates and undergoes cell growth inhibition (15,27). In addition to our observation in T24 cells, similar results of IFN-α in combination with fluoxetine were observed in human Jurkat-T, Huh7.5 and U2OS cells (data not shown). Since fluoxetine belongs to the pump-protein of ATP-binding cassette sub-family B member 1 (also known as P-glycoprotein 1 or multidrug resistance protein 1), our preliminary results indicated that IFN-α and fluoxetine alone could inhibit the P-glycoprotein expression in early stage cancer. Unfortunately, fluoxetine failed to facilitate the downregulation of this protein in the presence of IFN-α (data not shown). Instead, fluoxetine itself caused a slight G1 arrest along with a marked induction of p27 as well as slight reduction of cyclin D1 and p53. Our results were similar to previous studies related to the effect of fluoxetine on G1 arrest and upregulations of p27 in human cervical cancer cells (SiHa), breast cancer cells (MDA-MB-231) and colon cancer cells HT29 (28-30). In addition, the dosage of fluoxetine as a chemosensitizer is kept under the range of 5-20 μM, where this agent itself does not affect cell viability (15). In the present study, fluoxetine at 10 μM did not induce apoptosis, whereas higher concentrations of fluoxetine (>25 μM) could suppress glioblastoma cells by calcium-dependent apoptosis (31). Alternatively, the addition of fluoxetine lessened the IFN-α-induced apoptosis but switched to cause G1 arrest, and maintained the IFN-α-mediated reduction in G2/M phase (Fig. 6). Accordingly, fluoxetine sensitized the growth inhibition of IFN-α via the alteration of cell cycle progression, especially G1 arrest.

Activation of STAT-1, especially on Ser727, might modulate pro- and anti-apoptotic genes upon stress-induced responses via the caspase-3 dependent pathway. Moreover, STAT-1, a novel modulator of p53 function, can interact with p53 and induce apoptosis (32), whereas the STAT-1-deficient cells are more resistant to apoptosis-inducing agents (33). In the present study, the IFN-α-mediated STAT-1 activation caused apoptosis related to the induction of p53. Alternatively, through the enhancement of STAT-1 activation, the addition of fluoxetine lessened the IFN-α-mediated apoptosis and lowered the p53 expression but enhanced G1 arrest related to subsiding expression of cyclin D1 and upregulation of p27. These results might possibly be explained by the fact that STAT-1 serine 727 phosphorylation site can interact directly with cyclin D1, accelerate cyclin D1-dependent proteosomal degradation, and downregulate cyclin D1, which further mediates cell cycle arrest (34). Conversely, inhibiting STAT-1 could reverse the cell growth delay of IFN-α (Fig. 5), but this blockade did not affect the increased amounts of cell cycle inhibitors p27 of

IFN-α in the presence of fluoxetine (Fig. 8). These findings were partially consistent with previous a study that STAT-1 deficient cells proliferated but reduced p27 in human fibrosarcoma cell lines, 2fTGH and U3A (34).

Besides controlling lipid metabolism, recent evidence suggests that PPAR-α suppresses apoptosis and induces proliferation in hepatocytes, in response to peroxisome proliferators (16,35). Conversely, loss of PPAR-α inhibits radiation-induced apoptosis in the mouse kidney through the activation of NF-κB and the upregulation of anti-apoptosis factors (36). PPAR-α activation also causes the release of STAT-1 from gene promoters concomitant with the downregulation of gene expression in human hepatocellular carcinoma HepG2 cells (17). IFN-α2b affects the expression of various drug-metabolizing enzymes and transporters in human primary hepatocytes related to the upregulation of STAT-1 and PPAR-α-regulated genes (37). However, cross talk between PPAR-α-mediated survival signaling and cell cycle associated with STAT-1 activation remains unclear. In the present study, co-localization of STAT-1 and PPAR-α protein to some extent were observed in the cytoplasm by IFN-α or fluoxetine alone. We also found that IFN-α-treated cells partially activated and translocated both proteins to the nucleus, whereas fluoxetine-treated cells predominantly triggered PPAR-α translocation to the nucleus. Notably, the addition of fluoxetine to IFN-α-treated cells caused significant co-localization of both proteins in the nucleus (Fig. 4), which mediated cell growth inhibition.

In conclusion, STAT-1 and PPAR-α might contribute distinct functions in cell cycle progression to achieve the growth inhibition of IFN-α, fluoxetine, or in combination. Moreover, fluoxetine regulates cell growth inhibition of IFN-α via a boosted activation of STAT-1 and PPAR-α.

Acknowledgements

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