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Interferons Induce the Expression of IFITM1 and IFITM3 and Suppress the Proliferation of Rat Neonatal Cardiomyocytes

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ABSTRACT

Cardiovascular diseases have been one of the leading killers among the human population worldwide. During the heart development, cardiomyocytes undergo a transition from hyperplastic to hypertrophic growth with an unclear underlying mechanism. In this study, we aim to investigate how interferons differentially stimulate the interferon-inducible transmembrane (IFITM) family proteins and further be involved in the process of heart development. The expression levels of three IFITM family members, IFITM1, IFITM2, and IFITM3 were investigated during Sprague–Dawley rat myocardial development and differentiation of H9C2 cardiomyocytes. The effects of interferon-α, -β, and -γ on DNA synthesis in H9C2 cells were also characterized. Up-regulation of IFITM1 and IFITM3 were observed during the heart development of Sprague–Dawley rat and the differentiation of H9C2 cells. Moreover, interferon-α and -β induce the expression of IFITM3 while interferon-γ up-regulates IFITM1. Finally, interferon-α and -β were demonstrated to inhibit DNA synthesis during H9C2 cell differentiation. Our results indicated interferons are potentially involved in the differentiation and cell proliferation during heart development. J. Cell. Biochem. 113: 841–847, 2012.

KEY WORDS: INTERFERON-INDUCIBLE TRANSMEMBRANE PROTEIN; CARDIOMYOCYTES; CELL PROLIFERATION

Cardiovascular diseases have been one of the leading killers among the human population worldwide. During neonatal development, cardiomyocytes in men, mice, and rats undergo a transition from hyperplastic to hypertrophic growth, so any further increases in myocardial mass are not accompanied by cardiomyocyte proliferation and the loss of the cardiac cells cannot be replenished. For decades, scientists have focused on elucidating the molecular events underlying the rapid switch from hyperplasia to hypertrophy in heart development.

Interferons are multifunctional cytokines that play key roles in body defense against viral and parasite infection, in exhibiting antiproliferative and differentiating activities, and immune-modulating responses. There are two basic types of interferons: type I interferons (α and β predominately) are encoded by a large family of genes and secreted principally by leukocytes (α) and fibroblasts (β) and are induced directly in response to a viral infection; type II interferons (γ), which are structurally unrelated and encoded by a single gene, are synthesized by T lymphocytes or natural killer cells following detection of infected cells by antigen presentation. Type I and type II interferons signal through distinct but related pathways [Stark et al., 1998]. They bind to their cognate receptors, which bind to the Janus kinases (JAKs) and the signal transducers and activators of transcriptions (STATs) [McGillicuddy et al., 2009; Dedoni et al., 2010]. These in turn initiate a signaling cascade that eventually leads to the transcriptional induction of genes whose products mediate interferon actions such as inhibition of proliferation.

Interferons have been demonstrated to inhibit cell growth, even though exhibiting various degrees of sensitivity to the antiproliferative activity in different cells in vitro. In some cases, growth arrest may be due to differentiation, particularly when interferons are used in combination with other agents such as retinoids [Higuchi et al., 1991; Nason-Burchenal et al., 1996]. Although specific interferon-induced genes have not been linked directly to the

Abbreviations: IFITM, interferon-inducible transmembrane protein; MLC-2V, myosin light chain ventricular isoform 2; PCNA, proliferating cell nuclear antigen; ISRE, interferon-stimulated response element; ATCC, American Type Culture Collection; HS, horse serum.

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antiproliferative activity, interferons have been shown to target specific cell-cycle regulatory components, including c-myc [Yasuoka et al., 2001], retinoblastoma protein [Xin et al., 2003], cyclin D3, and cdc25A [Tiefenbrun et al., 1996].

The roles of interferons in the cardiovascular system have not been extensively explored. Most of the current studies of interferons in cardiomyocytes focus on cardiac protection against viruses [Wang et al., 2007; Eid et al., 2009; Yuen et al., 2011]. Nonetheless, there is a growing body of evidence that interferon-γ plays a critical role in cardiomyocyte apoptosis in vitro. Recent studies also found that interferon-β stimulates vascular smooth muscle cell proliferation [Schirmer et al., 2010] while interferon-γ is required for efficient skeletal muscle regeneration [Cheng et al., 2008]. Moreover, upregulation of interferon-α is associated with vascular diseases in systemic lupus erythematosus patients [Lood et al., 2010].

Previously, our group focused on the isolation of differential expressed genes during rat neonatal heart development [Chim et al., 2000ab]. Later, Anisimov and his group isolated a number of expressed sequence tags clones that were differentially expressed during differentiation of P19 embryonic stem cells to cardiomyocytes by serial analysis of gene expression [Anisimov et al., 2002]. One of the up-regulated genes identified by this study was the interferon-inducible transmembrane protein (IFITM) 1, which was first identified to encode a T-cell surface antigen Leu-13 and predominately expressed in heart tissues [Anisimov et al., 2002]. Together with the other two family members, IFITM2 and IFITM3, they correlate to inhibition of cell growth in cancer cells and immune cells and play a role in development in mice [Bradbury et al., 1993; Deblandre et al., 1995]. We hypothesize that the up-regulation of IFITM1, or even IFITM2 and IFITM3, is also involved in the growth suppression in the differentiation of rat neonatal cardiomyocytes.

Recently, IFITM family members have been found being capable of inhibiting human immunodeficiency virus type 1 (HIV-1) infection and mediating cellular resistance to influenza A H1N1 virus [Lu et al., 2011], SARS coronavirus [Huang et al., 2011], West Nile virus, and dengue virus [Brass et al., 2009]. In addition, a polymorphism (T-204G) in the promoter region of IFITM3 gene has been associated with ulcerative colitis in a Korean population [Seo et al., 2010].

In this study, we aim at studying the role of interferons on the expression of IFITM family proteins and the cell proliferation as well as differentiation of rat cardiomyocytes. We found that interferons stimulate the IFITM1 and IFITM3 differentially and are potentially involved in the process of heart development.

RESULTS

DIFFERENTIAL EXPRESSION OF IFITM1, IFITM2, AND IFITM3 DURING RAT MYOCARDIAL DEVELOPMENT

The expression levels of IFITM1, IFITM2, and IFITM3 during myocardial development were determined by quantitative real-time PCR at postnatal 1-, 7-, 14-day-old, and 3-month-old Sprague–Dawley rat ventricular myocardium. We found that the mRNA level of IFITM1 increased steadily from 1- to 14-day-old, and reach 11-fold in 3-month-old rat ventricular myocardium. For IFITM2 and IFITM3, the mRNA levels increased drastically by sevenfolds in 14-day-old rat ventricles, then slightly dropped in the adult myocardium (Fig. 1).

ALTERED mRNA LEVELS OF IFITM1 AND IFITM3 DURING DIFFERENTIATION OF H9C2 CELLS

To see whether IFITM1, IFITM2, and IFITM3 were involved in the process of heart formation, H9C2, a rat myoblast cell line derived from embryonic heart tissue, was used to mimic the differentiation status during heart development by culturing in differential medium. The successful differentiation was proven by both the morphological evidence including elongated shape, multiple nuclei, extensive networks of myotubes, and the significantly increased level (more than twofolds) of myosin light chain ventricular isoform 2 (MLC-2V), a commonly used cardiac differentiation marker. Consistent with the observation in vivo, IFITM1 and IFITM3 were up-regulated during differentiation of H9C2 cells (Fig. 2). Compared with the undifferentiated H9C2 cells, the mRNA levels of IFITM1 and IFITM3 continuously increased and reached for more than threefolds and fivefolds at day 8, respectively (Fig. 2).

INDUCTION OF IFITM PROTEINS BY INTERFERON-α, -β, AND -γ IN H9C2 CELLS

Real-time PCR result revealed that both IFITM1 and IFITM3 were upregulated upon treatment by either interferon-α or interferon-β in H9C2 cells (Fig. 3A,B). Their expression levels increased for around eightfolds and 40-folds at the concentration of 500 U/ml compared with untreated controls, respectively. However, IFITM2 showed no response to either interferon-α or interferon-β treatment. When treated with interferon-γ, the mRNA level of IFITM1 increased in a dose-dependent manner while the expression of IFITM2 and IFITM3 was only slightly affected (Fig. 3C).

DNA SYNTHESIS AFTER TREATMENT OF INTERFERON-α, -β, AND -γ IN H9C2 CELLS

As shown in Figure 4, the relative BrdU uptakes in H9C2 cells decreased upon the treatment of either interferon-α or interferon-β

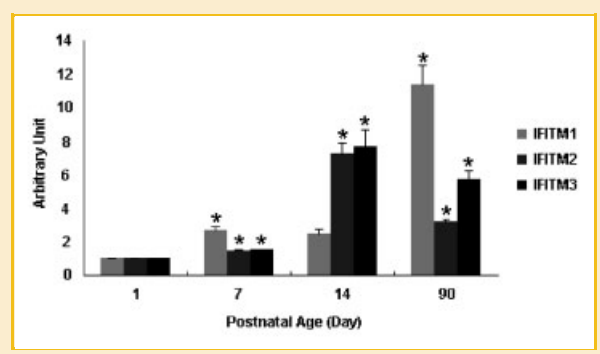

Fig. 1. Expression profiles of IFITM1, IFITM2, and IFITM3 in 1-, 7-, 14-day, and 3-month-old SD rat ventricular myocardium by real-time PCR. β-actin acted as the internal control for the normalization of cDNA templates. Filled bars indicate the means and T bars indicate the standard errors of the means for three separate experiments with triplicates. The expression levels were presented in relative arbitrary units. $^{*}P \leq 0.05$ versus control.

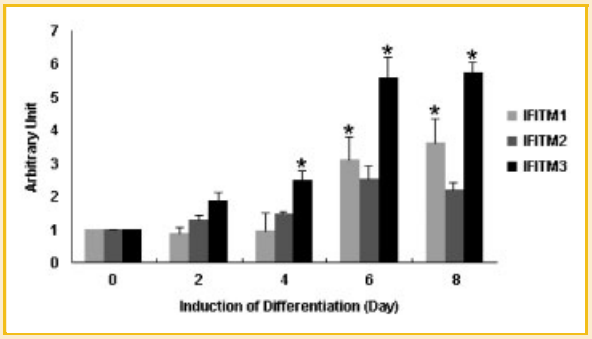

Fig. 2. Expression profiles of IFITM1, IFITM2, and IFITM3 during differentiation of H9C2 cells by real-time PCR. The H9C2 cells were induced to differentiate by 1.5% HS for 8 days. GAPDH acted as the internal control for normalization of cDNA templates. Filled bars indicate the means and T bars indicate the standard errors of the means for three separate experiments with triplicates. The expression levels were presented in relative arbitrary units. $^{*}P \leqq 0.05$ versus control.

when compared with that of the untreated control. These results indicated that both interferon-α and -β inhibit cellular DNA synthesis in H9C2 cells. On the contrary, interferon-γ displays stimulating effect on cellular DNA synthesis. The relative BrdU uptakes increased for 38% and 42% when the concentration of interferon-γ is 20 and 40 ng/ml, respectively.

PROLIFERATING CELL NUCLEAR ANTIGEN EXPRESSION AFTER THE TREATMENT OF INTERFERON-α, -β, AND -γ IN H9C2 CELLS

Upon the interferon-α treatment, the mRNA level of proliferating cell nuclear antigen (PCNA) decreased by 2.5-folds, threefolds and nearly fivefolds at the concentration of 100, 200, and 500 U/ml, respectively (Fig. 5A). Similar result was observed in the presence of interferon-β (Fig. 5B). Taken together, interferon-α and -β elicit anti-proliferation effect and consequently inhibit the DNA synthesis in H9C2 cells. Interferon-γ only triggered a mild induction of the mRNA level of PCNA at the concentration of 40 ng/ml (Fig. 5C). This result is consistent with the increase of BrdU uptake when H9C2 cells were treated by interferon-γ.

DISCUSSION

The proliferative capability of rat cardiomyocytes still retains from postnatal 1 to 3 days [Li et al., 1996], during which low-mRNA expression of IFITM1, IFITM2, and IFITM3 were detected. Thereafter, from 6 to 14 days of age, a transitional phase from hyperplasia to hypertrophy occurs and this rapid switch is critical for the molecular events behind terminal differentiation and the loss of proliferative ability in cardiomyocytes to take place [Clubb and Bishop, 1984; Clubb et al., 1987]. In agreement with the timing of the rapid switch, our results demonstrated a continuous increase in the expression of IFITM1 from postnatal 7 days and IFITM2 and IFITM3 from postnatal 14 days in rat ventricles. This result is consistent with the differential expression of IFITM1 in fetal and adult heart tissues [Anisimov et al., 2002]. Taken together, interferons are potentially involved in the cell proliferation of rat neonatal cardiomyocytes.

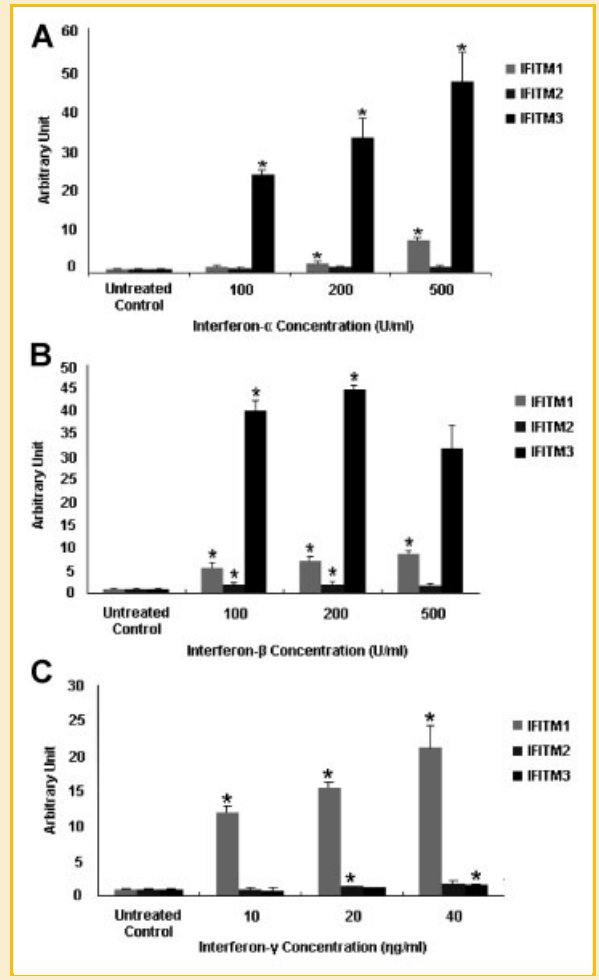

Fig. 3. Expression levels of IFITM1, IFITM2, and IFITM3 determined by real-time PCR upon the treatments of interferon-α (A), interferon-β (B), and interferon-γ (C) under different concentrations for 24 h. H9C2 cells were treated with interferon-α or -β under concentrations of 100, 200, and 500 U/ml. For interferon-γ, H9C2 cells were treated under concentrations of 10, 20, and 40 ng/ml. GAPDH acted as the internal control for normalization of cDNA templates. Filled bars indicate the means and T bars indicate the standard errors of the means for three separate experiments with triplicates. The expression levels were presented in relative arbitrary units. $^{*}P \leqq 0.05$ versus control.

Our real-time PCR results demonstrated different responsiveness of IFITM1, IFITM2, and IFITM3 in the presence of type I (α and β) and type II (γ) interferons in H9C2 cells. IFITM1 was moderately induced in response to type I interferons but significantly up-regulated by type II interferon. Expression of IFITM3 was notably increased by type I interferons, but did not change significantly when exposed to type II interferon. IFITM2 was non-responsive to both type I and type II interferons. The similar responsiveness of the IFITM proteins towards interferon-α and -β supports the notion that interferon-α and -β share the common signaling pathways [Mitani et al., 2001].

Notably, IFITM2 was neither responsive to type I nor type II interferons in cardiomyocytes, but it is interferon-inducible in other cell types [Lewin et al., 1991]. IFITM3 was not induced by interferon-γ in H9C2 cells. However, the transcript level of IFITM3 is increased

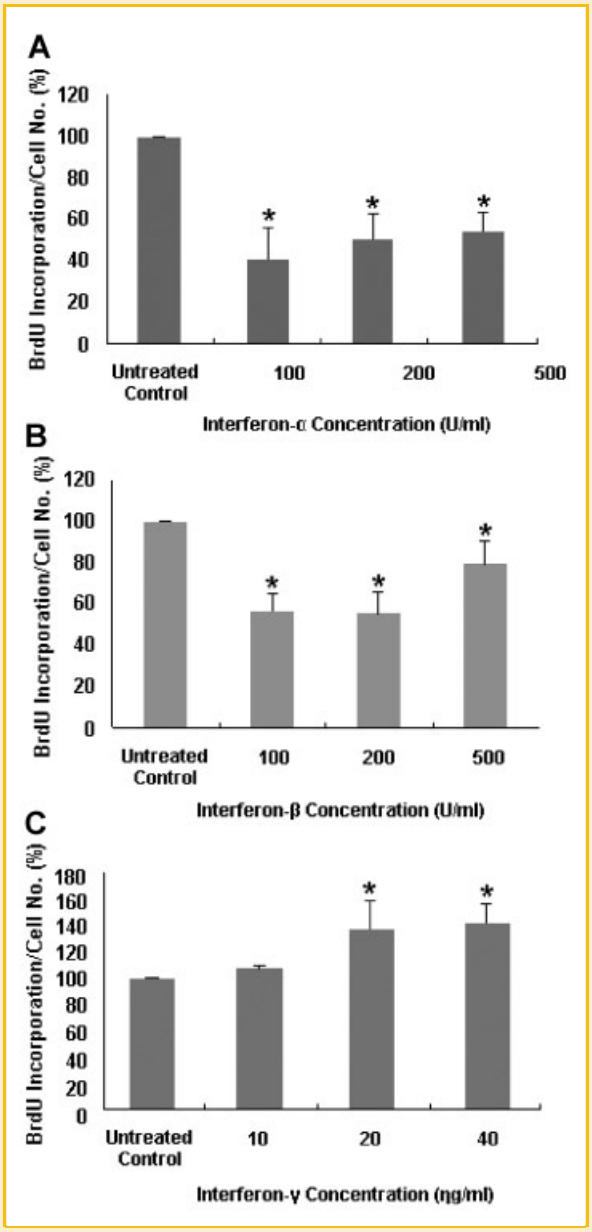

Fig. 4. DNA synthesis measured by BrdU incorporation upon the treatment of interferon-α (A), interferon-β (B), and interferon-γ (C) for 24 h. H9C2 cells were treated with interferon-α or interferon-β under concentrations of 100, 200, and 500 U/ml. For interferon-γ, H9C2 cells were treated under concentrations of 10, 20, and 40 ng/ml. The relative BrdU uptakes of the samples were divided by the respective cell number and presented in percentage, relative to the BrdU uptake of the untreated cells. Filled bars indicate the means and T bars indicate the standard errors of the means for three separate experiments with triplicates. $^{*}P \leqq 0.05$ versus control.

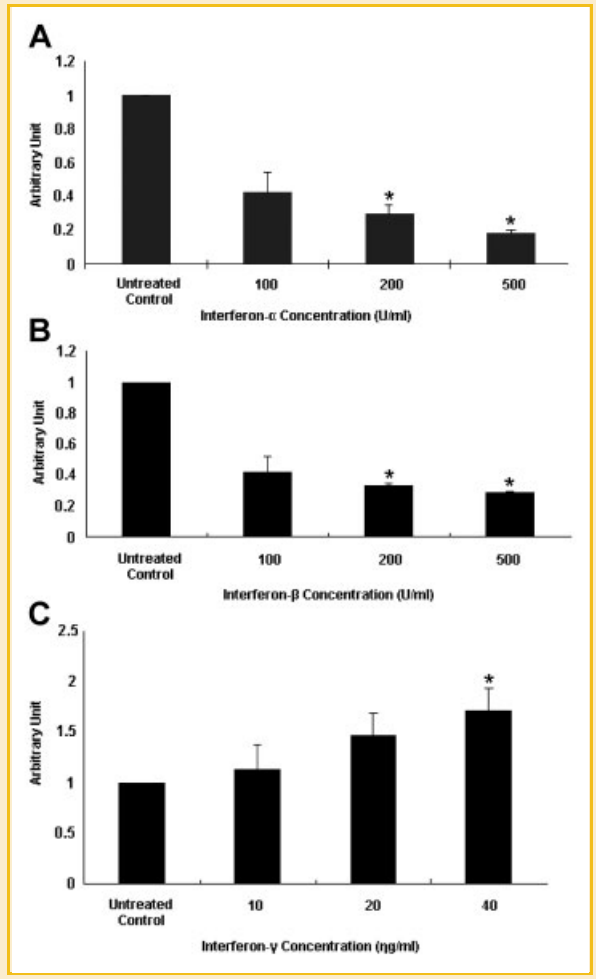

Fig. 5. Panels A–C: Expression levels of PCNA upon the treatment of interferon-α (A), interferon-β (B), and interferon-γ (C) for 24 h in H9C2 cells. H9C2 cells were treated with interferon-α or -β under concentrations of 100, 200, and 500 U/ml. For interferon-γ, H9C2 cells were treated under concentrations of 10, 20, and 40 ng/ml. mRNA expression analyzed by real-time PCR. GAPDH acted as the internal control for normalization of cDNA templates. Filled bars indicate the means and T bars indicate the standard errors of the means for three separate experiments with triplicates. $^{*}P \leqq 0.05$ versus control.

in the presence of the cytokine in other cell types [Brem et al., 2003]. Such different expression patterns of IFITM2 and IFITM3 in response to interferons in various cell types may be a consequence of cell type specificity to perform specialized physiological functions. However, another possibility of the variations of interferon-stimulated response element (ISRE) and gamma-activating sequences in the promoter regions in different species cannot be excluded. Further investigation on the ISRE sequences of IFITM2 and IFITM3 in rats by deletion or mutagenesis would be helpful to determine their importance in the expression of these two genes.

Interferons are cytokines that regulate a variety of biological effects and one of the profound properties of interferons is inhibition of proliferation. The interferon-induced growth inhibition affects different phases of the mitotic cycle, including a block in G1, lengthening of the S phase or a general prolongation of the cell cycle [Sangfelt et al., 1999; Murphy et al., 2001; Vannucchi et al., 2005]. It is not clear which genes are involved in cell cycle control induced by interferons although several studies link the IFITM family to antiproliferative activity. However, the findings are quite controversial. Higher IFITM1 expression has been correlated with improved survival in chronic myeloid leukemia patients [Akyerli et al., 2005]. Downregulation of IFITM1 and IFITM3 also correlates

with brain tumor progression [Huang et al., 2000]. Notably, overexpression of IFITM1 negatively regulated cell growth, whereas suppression of IFITM1 blocked the antiproliferative effect of interferon-γ [Yang et al., 2007]. On the contrary, a recent study showed that IFITM gene expression was significantly up-regulated specifically in colorectal tumors and IFITM family proteins may serve as a molecular marker in those tumors [Andreu et al., 2006]. Consistently, the knockdown of IFITM1 inhibits proliferation, migration, and invasion of glioma cells [Yu et al., 2010]. Moreover, IFITM1 is able to promote invasion at early stage of head and neck cancer progression [Hatano et al., 2008]. We speculate that the proliferative and antiproliferative effects of IFITM proteins are quite cell type-specific, depending on the differences in interacting partners in different scenarios.

According to our results, interferon-α and -β induce IFITM1 and IFITM3, and interferon-γ promotes IFITM1 expression in H9C2 cells. We further investigate whether interferons elicit inhibitory effects on proliferation in cardiomyocytes. In general, both interferon-α and -β remarkably decreased relative BrdU uptakes in the proliferation assay, maximally for 66% and 47%, respectively, while interferon-γ moderately increased the BrdU uptakes for 20% on average under relatively higher concentrations of the cytokine (20 ng/ml and 40 ng/ml), but not under a lower concentration (10 ng/ml). We also examined the expression level of PCNA after the interferon treatments. In consistent with the observation in the BrdU proliferation assay, interferon-α and -β reduced the expression of PCNA. Therefore, these findings further support that the treatment of interferon-α and -β decreased cellular DNA synthesis. On the other hand, interferon-γ slightly promoted PCNA mRNA expression.

In conclusion, our study found that IFITM1 and IFITM3 were upregulated during Sprague–Dawley rat myocardial development and differentiation of H9C2 cells. Interferon-α and -β were demonstrated to inhibit DNA synthesis during H9C2 cell differentiation. These observations indicated that interferons are potentially involved in differentiation and cell proliferation during heart development.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND SAMPLING

Sprague–Dawley rats of different ages were obtained from the animal facilities of the Chinese University of Hong Kong. Hearts were carefully dissected from decapitated animals and rinsed in diethyl pyrocarbonate-treated phosphate-buffered saline. Only the lower two-thirds of the myocardium were isolated. The ventricles were frozen in liquid nitrogen to avoid RNA degradation.

CELL CULTURE

The cell line H9C2 was obtained from the American Type Culture Collection (ATCC) (ATCC No: CRL-1446) and grown in a monolayer culture in Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 10% (v/v) heat-inactivated, certified fetal bovine serum (FBS; HyClone), and 1% (v/v) antibiotics (penicillin, 100 U/ml; streptomycin sulfate, 100 μg/ml) at 37°C in 5% CO_2 in a humidified atmosphere. Myoblasts were passed and harvested before reaching the confluent state. All experiments were done using cells between 5 and 20 passages.

INDUCTION OF DIFFERENTIATION OF H9C2 CELLS

In brief, $5 \times 10^6 - 6 \times 10^6$ H9C2 cells were seeded on 100-mm dish and grown for 2–3 days. When the cell confluence reached 70–80%, the complete medium was replaced by low-serum differentiation medium [DMEM containing 1.5% (v/v) horse serum (HS) and 0.5% (v/v) antibiotics (penicillin, 100 U/ml; streptomycin sulfate, 100 μg/ml)] and grown for 2, 4, 6, and 8 days.

IN VITRO INDUCTION OF IFITMS BY INTERFERON TREATMENTS

H9C2 cells were incubated in DMEM medium with interferon-α (100, 200, 500 U/ml) (Sigma), interferon-β (100, 200, 500 U/ml) (Sigma), and interferon-γ (10, 20, 40, 100 ng/ml) (Sigma) for 24 h under standard conditions. After each treatment, the cells were harvested for total RNA extraction, protein isolation or subjected to proliferation assays.

RNA ISOLATION

Total RNA was extracted using the TRIZOL Reagent (Invitrogen) following the manufacturer's instruction. To remove the residual genomic DNA from the RNA samples, 4 μg of each sample was further purified by RNeasy columns (Qiagen) and then digested by 4 U DNase I (Invitrogen) under 1× DNase I Reaction Buffer [200 mM Tris–HCl (pH 8.4), 20 mM $MgCl_2$, 500 mM KCl] (Invitrogen) at room temperature for 15 min. To inactivate the DNase I, 4 μl of 25 mM EDTA was added and the samples were heated at 65°C for 10 min.

FIRST-STRAND cDNA SYNTHESIS

The first-strand cDNA was synthesized from total RNA by oligo (dT) primer and reverse transcriptase (RT) with the first-strand synthesis kit from Thermoscript RT-PCR System III (Invitrogen), according to the manufacturer's instructions. Briefly, 4 μg purified RNA was first mixed with 4 μl of 50 mM oligo (dT) primer and 4 μl of 10 mM dNTPs and added to a total volume of 52 μl. The reaction mixture was denatured at 65°C for 5 min and then on ice for 1 min. Then cDNA synthesis reaction mix, containing 5× synthesis buffer, 0.1 M DTT, 4 U Reverse transcriptase (RT), 4 U RNaseOUT was added to RNA and cDNA synthesis reaction was proceeded for 1 h at 55°C and an heat-inactivation step at 70°C for 15 min. Then the first-strand cDNA was stored at −20°C until use.

QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION (REAL-TIME PCR)

Real-time PCR analyses were carried out with the Prism 7500 Fast System Sequence Detection System (Applied Biosystems). PCR primers were designed with the software GeneTool and the sequences were listed in Table I. Cycling reaction conditions consisted of the following: 50°C for 2 min, 95°C for 10 min, followed by 95°C for 15 s and 60°C for 1 min cycled 40 times. Reactions were performed in 96-well MicroAmp Optical plates (Applied Biosystems) in a final volume of 20 μl. Each reaction mix included 1 or 2 μl cDNA templates, 0.625 μM forward and reverse primers and 1X Power SYBR Green Reaction Mix (Applied Biosystems). Glyceraldehyde-3-phosphate-dehydrogenase or β-actin was used to normalize the

TABLE I. Sequence of Primers

Primers name	Forward (F) and reverse (R) primers
β-Actin for real-time PCR	F: 5′-GGCAATGAGCGGTTCCGAT-3′ R: 5′-TGCCTGGGTACATGGTGGT-3′
IFITM1 for real-time PCR	F: 5′-CAACCACCACAATCAACATG-3′ R: 5′-TGGGGTAAACAGGCAGGACT-3′
IFITM2 for real-time PCR	F: 5′-CGGTAGTCTTTCAGTCGCTTTC-3′ R: 5′-TGTGGACAGATATACGAAGGT-3′
IFITM3 for real-time PCR	F: 5′-CTGTCGTCATCATTGCTCTTA-3′ R: 5′-ATGGCTATCAAGTGCGTTTTAT-3′
GAPDH for real-time PCR	F: 5′-TGCTGAGTATGTCGTGGAGT-3′ R: 5′-GGCTAAGCAGTTGGTGGTG-3′
PCNA for real-time PCR	F: 5′-GCGAGTGGGGAGCTTGGCAAT-3′ R: 5′-AAGACCTCAGAACACGCTGGC-3′
IFITM1 for cloning	F: 5′-CCCAAGGAACAGCAAGAGGT-3′ R: 5′-CTATGTTCTTTTAGTGGCGTAA-3′
IFITM2 for cloning	F: 5′-AGCCACAATTCCCAAGCCTTC TTGCCC-3′ R: 5′-CTAGAATCCAGAATGTGGCGTTCTTT-3′
IFITM3 for cloning	F: 5′-AACCACACTTCTCAAGCCTTCGTGAAC-3′ R: 5′-TCAAGTCTGGAGACGAGGAGCATTAA-3′

cDNA templates in each panel. Non-template controls were included in each plate. All data were analyzed by the SDS 1.3.1 software (Applied Biosystems).

BROMODEOXYURIDINE PROLIFERATION ASSAY

Transiently transfected or interferon-treated H9C2 cells in 12-well plates (5×10^4) were incubated with 10 μM bromodeoxyridine (BrdU) in DMEM for 14–18 h, with the use of BrdU detection kit III (Roche Ltd.), according to the manufacturer's instructions. The cells were first washed with PBS containing 10% FBS for three times and fixed by chilled fixative solution at −20°C for 30 min. After that, the cells were washed with PBS containing 10% FBS for three times, then digested by nuclease solution at 37°C for 30 min and subjected to washing for three times again. After that, the cells were incubated in anti-BrdU-POD at 37°C for 30 min and washed three times with 1× washing solution to remove excess antibody, followed by incubation in peroxidase substrates in a dark area at room temperature for 5–30 min. The samples were then transferred from the 12-well plate to a 96-well plate and triplicate aliquots from each sample were applied. Extinction of the samples was measured in a microtiter plate reader at 405 nm with a reference wavelength at 490 nm. Background BrdU incorporation was subtracted from the samples. Cellular DNA synthesis was measured by the BrdU incorporation divided by the respective cell number.

STATISTICAL ANALYSIS

The statistical analyses were performed using SPSS software. Statistical differences were assessed by one-way ANOVA followed by Levene test for homogeneity of variances and post hoc tests for comparison of individual means. A P-value $\leqq 0.05$ was considered statistically significant.

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REFERENCES

Akyerli CB, Beksac M, Holko M, Frevel M, Dalva K, Ozbek U, Soydan E, Ozcan M, Ozet G, Ilhan O, Gürman G, Akan H, Williams BR, Ozçelik T. 2005. Expression of IFITM1 in chronic myeloid leukemia patients. Leuk Res 29: 283–286.

Andreu P, Colnot S, Godard C, Laurent-Puig P, Lamarque D, Kahn A, Perret C, Romagnolo B. 2006. Identification of the IFITM family as a new molecular marker in human colorectal tumors. Cancer Res 66:1949–1955.

Anisimov SV, Tarasov KV, Riordon D, Wobus AM, Boheler KR. 2002. SAGE identification of differentiation responsive genes in P19 embryonic cells induced to form cardiomyocytes in vitro. Mech Dev 117:25–74.

Bradbury LE, Goldmacher VS, Tedder TF. 1993. The CD19 signal transduction complex of B lymphocytes. Deletion of the CD19 cytoplasmic domain alters signal transduction but not complex formation with TAPA-1 and Leu 13. J Immunol 151:2915–2927.

Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, Ryan BJ, Weyer JL, van der Weyden L, Fikrig E, Adams DJ, Xavier RJ, Farzan M, Elledge SJ. 2009. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 139:1243–1254.

Brem R, Oraszlan-Szovik K, Foser S, Bohrmann B, Certa U. 2003. Inhibition of proliferation by 1-8U in interferon-alpha-responsive and non-responsive cell lines. Cell Mol Life Sci 60:1235–1248.

Cheng M, Nguyen MH, Fantuzzi G, Koh TJ. 2008. Endogenous interferon-gamma is required for efficient skeletal muscle regeneration. Am J Physiol Cell Physiol 294:C1183–C1191.

Chim SS, Cheung SS, Tsui SKW. 2000a. Differential gene expression of rat neonatal heart analyzed by suppression subtractive hybridization and expressed sequence tag sequencing. J Cell Biochem 80:24–36.

Chim SS, Fung KP, Waye MMY, Lee CY, Tsui SKW. 2000b. Expression of replication factor C 40-kDa subunit is down-regulated during neonatal development in rat ventricular myocardium. J Cell Biochem 78:533–540.

Clubb FJ, Jr, Bishop SP. 1984. Formation of binucleated myocardial cells in the neonatal rat. An index for growth hypertrophy. Lab Invest 50:571–577.

Clubb FJ, Jr, Bell PD, Kriseman JD, Bishop SP. 1987. Myocardial cell growth and blood pressure development in neonatal spontaneously hypertensive rats. Lab Invest 56:189–197.

Deblandre GA, Marinx OP, Evans SS, Majjaj S, Leo O, Caput D, Huez GA, Wathelet MG. 1995. Expression cloning of an interferon-inducible 17-kDa membrane protein implicated in the control of cell growth. J Biol Chem 270: 23860–23866.

Dedoni S, Olianas MC, Onali P. 2010. Interferon-β induces apoptosis in human SH-SY5Y neuroblastoma cells through activation of JAK-STAT signaling and down-regulation of PI3K/Akt pathway. J Neurochem 115: 1421–1433.

Eid RE, Rao DA, Zhou J, Lo SF, Ranjbaran H, Gallo A, Sokol SI, Pfau S, Pober JS, Tellides G. 2009. Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation 119:1424–1432.

Hatano H, Kudo Y, Ogawa I, Tsunematsu T, Kikuchi A, Abiko Y, Takata T. 2008. IFN-induced transmembrane protein 1 promotes invasion at early stage of head and neck cancer progression. Clin Cancer Res 14:6097–6105.

Higuchi T, Hannigan GE, Malkin D, Yeger H, Williams BR. 1991. Enhancement by retinoic acid and dibutyryl cyclic adenosine 3':5'-monophosphate of the differentiation and gene expression of human neuroblastoma cells induced by interferon. Cancer Res 51:3958–3964.

Huang H, Colella S, Kurrer M, Yonekawa Y, Kleihues P, Ohgaki H. 2000. Gene expression profiling of low-grade diffuse astrocytomas by cDNA arrays. Cancer Res 60:6868–6874.

Huang IC, Bailey CC, Weyer JL, Radoshitzky SR, Becker MM, Chiang JJ, Brass AL, Ahmed AA, Chi X, Dong L, Longobardi LE, Boltz D, Kuhn JH, Elledge SJ,

Bavari S, Denison MR, Choe H, Farzan M. 2011. Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog 7:e1001258.

Lewin AR, Reid LE, McMahon M, Stark GR, Kerr IM. 1991. Molecular analysis of a human interferon-inducible gene family. Eur J Biochem 199:417–423.

Li F, Wang X, Capasso JM, Gerdes AM. 1996. Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. J Mol Cell Cardiol 28:1737–1746.

Lood C, Amisten S, Gullstrand B, Jönsen A, Allhorn M, Truedsson L, Sturfelt G, Erlinge D, Bengtsson AA. 2010. Platelet transcriptional profile and protein expression in patients with systemiclupus erythematosus: up-regulation of the type I interferon system is strongly associated with vascular disease. Blood 116:1951–1957.

Lu J, Pan Q, Rong L, Liu SL, Liang C. 2011. The IFITM proteins inhibit HIV-1 infection. J Virol 85:2126–2137.

McGillicuddy FC, Chiquoine EH, Hinkle CC, Kim RJ, Shah R, Roche HM, Smyth EM, Reilly MP. 2009. Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. J Biol Chem 284:31936–31944.

Mitani Y, Takaoka A, Kim SH, Kato Y, Yokochi T, Tanaka N, Taniguchi T. 2001. Cross talk of the interferon-alpha/beta signalling complex with gp130 for effective interleukin-6 signalling. Genes Cells 6:631–640.

Murphy D, Detjen KM, Welzel M, Wiedenmann B, Rosewicz S. 2001. Interferon-alpha delays S-phase progression in human hepatocellular carcinoma cells via inhibition of specific cyclin-dependent kinases. Hepatology 33:346–356.

Nason-Burchenal K, Gandini D, Botto M, Allopenna J, Seale JR, Cross NC, Goldman JM, Dmitrovsky E, Pandolfi PP. 1996. Interferon augments PML and PML/RAR alpha expression in normal myeloid and acute promyelocytic cells and cooperates with all-trans retinoic acid to induce maturation of a retinoid-resistant promyelocytic cell line. Blood 88:3926–3936.

Sangfelt O, Erickson S, Castro J, Heiden T, Gustafsson A, Einhorn S, Grandér D. 1999. Molecular mechanisms underlying interferon-alpha-induced G0/G1 arrest: CKI-mediated regulation of G1 Cdk-complexes and activation of pocket proteins. Oncogene 18:2798–2810.

Schirmer SH, Bot PT, Fledderus JO, van der Laan AM, Volger OL, Laufs U, Böhm M, de Vries CJ, Horrevoets AJ, Piek JJ, Hoefer IE, van Royen N. 2010. Blocking interferon β stimulates vascular smooth muscle cell proliferation and arteriogenesis. J Biol Chem 285:34677–34685.

Seo GS, Lee JK, Yu JI, Yun KJ, Chae SC, Choi SC. 2010. Identification of the polymorphisms in IFITM3 gene and their association in a Korean population with ulcerative colitis. Exp Mol Med 42:99–104.

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. 1998. How cells respond to interferons. Annu Rev Biochem 67:227–264.

Tiefenbrun N, Melamed D, Levy N, Resnitzky D, Hoffman I, Reed SI, Kimchi A. 1996. Alpha interferon suppresses the cyclin D3 and cdc25A genes, leading to a reversible G0-like arrest. Mol Cell Biol 16:3934–3944.

Vannucchi S, Chiantore MV, Fiorucci G, Percario ZA, Leone S, Affabris E, Romeo G. 2005. TRAIL is a key target in S-phase slowing-dependent apoptosis induced by interferon-beta in cervical carcinoma cells. Oncogene 24:2536–2546.

Wang YX, da Cunha V, Vincelette J, White K, Velichko S, Xu Y, Gross C, Fitch RM, Halks-Miller M, Larsen BR, Yajima T, Knowlton KU, Vergona R, Sullivan ME, Croze E. 2007. Antiviral and myocyte protective effects of murine interferon-β and -α2 in coxsackievirus B3-induced myocarditis and epicarditis in Balb/c mice. Am J Physiol Heart Circ Physiol 293:H69–H76.

Xin H, Pramanik R, Choubey D. 2003. Retinoblastoma (Rb) protein upregulates expression of the Ifi202 gene encoding an interferon-inducible negative regulator of cell growth. Oncogene 22:4775–4785.

Yang G, Xu Y, Chen X, Hu G. 2007. IFITM1 plays an essential role in the antiproliferative action of interferon-gamma. Oncogene 26:594–603.

Yasuoka Y, Naomoto Y, Yamatsuji T, Takaoka M, Kimura M, Uetsuka H, Matsubara N, Fujiwara T, Gunduz M, Tanaka N, Haisa M. 2001. Combination of tumor necrosis factor alpha and interferon alpha induces apoptotic cell death through a c-myc-dependent pathway in p53 mutant H226br non-small-cell lung cancer cell line. Exp Cell Res 271:214–222.

Yu F, Ng SS, Chow BK, Sze J, Lu G, Poon WS, Kung HF, Lin MC. 2010. Knockdown of interferon-induced transmembrane protein 1 (IFITM1) inhibits proliferation, migration, and invasion of glioma cells. J Neurooncol 103:187–195.

Yuen S, Smith J, Caruso L, Balan M, Opavsky MA. 2011. The coxsackie-adenovirus receptor induces an inflammatory cardiomyopathy independent of viral infection. J Mol Cell Cardiol 50:826–840.