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MDA7/IL-24 is an anti-viral factor that inhibits influenza virus replication

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Melanoma differentiation associated gene-7 (mda-7)/interleukin-24 (IL-24) is a secreted cytokine, which plays an essential role in tumor suppression. Although its role as a multifunctional protein affecting broad types of cancers is well described, functions of IL-24 in host defense against virus infection are yet to be determined. In this study, we explored the anti-viral effect of recombinant IL-24 treatment during influenza infection. Infection of human lung adenocarcinoma cells (A549) with the influenza A virus up-regulated IL-24 mRNA and protein expression in a time-dependent manner. Pre-treatment of A549 cells with recombinant IL-24 protein effectively suppressed viral plaque formation. Furthermore, IL-24 treatment of A549 cells reduced viral non-structural protein 1 (NS1) synthesis, whereas IL-24 knockdown resulted in increased viral replication. Interestingly, IL-24 treatment following influenza A virus infection led to up-regulation of interferon (IFN)-induced antiviral signaling. Taken together, our results suggest that IL-24 exerts a potent suppressive effect on influenza viral replication and can be used in the treatment of influenza infection.

Keywords: IL-24, influenza, antiviral activities

Introduction

Interleukin-24 (IL-24) is a secreted protein of the IL-10 family, which is expressed in hematopoietic and skin cells, and functions as a cytokine at normal physiological levels (Huang *et al.*, 2001; Mhashilkar *et al.*, 2001; Wang and Liang, 2005). IL-24 was initially described as a melanoma differentiation-associated (MDA)-7 antigen, derived from cultured human melanoma cells after treatment with interferon-β (IFN-β) and the protein kinase C activator mezerein (Jiang *et al.*, 1995). IL-24 is known to be highly conserved in amino acid sequences across species. Upon binding its receptors, IL-20R1/IL-20R2 and IL-22R1/IL-20R2, IL-24 can trigger the Janus kinase and Signal Transducer and Activator of Transcription (JAK/STAT) pathway, which is essential for cell survival and proliferation. The tumor suppressive function of IL-24 has been well characterized by many studies. Exogenous treatment of a broad spectrum of cancer cell models with IL-24 led to pro-apoptotic, anti-tumor, and anti-metastatic activities *in vitro* (Sarkar *et al.*, 2002; Lebedeva *et al.*, 2003; Su *et al.*, 2003; Yacoub *et al.*, 2003a, 2003b; Fisher, 2005). Moreover, its clinical potential as an anti-cancer drug has been demonstrated in a Phase I clinical trial using adenovirus-mda7-based cancer gene therapy (Cunningham *et al.*, 2005; Tong *et al.*, 2005). In this study, the authors found that IL-24 is not toxic, and a single injection of IL-24-containing recombinant virus therapy could induce approximately 70% apoptosis in tumors. Although IL-24 is gaining recognition as an effective cancer therapy, its immunomodulatory and antiviral roles during inflammation and host defense are not completely understood.

Influenza is the most common infectious disease, which poses a great threat to public health because of its rapid transmissibility and highly contagious nature (Collin and Briand, 2009). Influenza virus can be highly pathogenic, causing annual acute respiratory diseases in human and several animal species, resulting in high mortality and morbidity rate, and significant economic loss. The influenza virus belongs to the *Orthomyxoviridae* family and has vast genetic diversity, comprising 18 hemagglutinin subtypes and 9 neuraminidase (NA) subtypes (Yoon *et al.*, 2014). Anti-influenza drugs targeting the NA active sites, such as oseltamivir and zanamivir, have been widely used for more than 10 years (Englund, 2002). However, accumulating evidence suggests that resistance to NA inhibitors has emerged rapidly in recent years, specifically affecting reduced sensitivity to influenza B virus infection (Hatakeyama *et al.*, 2007). Therefore, it is important to develop novel target molecules that can be exploited to inhibit viral replication and prevent the spread of influenza infections.

In response to influenza infection, host cells or tissues produce and secrete a wide range of cytokines and chemokines to recruit immune cells to the infected sites and to defend against the spread of influenza infection. Based on RNA-sequencing results, we previously found that IL-24 was one of the most upregulated differentially expressed genes (DEGs) in the lungs of H5 avian influenza virus-infected mice (Park *et al.*, 2015). Interestingly, recent studies demonstrated that IL-24 has anti-viral effects potentially induced by toll-like receptor 3 (TLR3)-mediated apoptotic pathways (Weiss *et al.*, 2015). Additionally, IL-24 expression by an influenza A virus vector with deleted NS1 (delNS1) in mice induced mi-

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nimal weight change even after influenza A virus infection *in vivo*, indicating that IL-24 may selectively induce apoptosis in virus-infected cells (Weiss *et al.*, 2015). Given the importance of IL-24 during influenza infection, it may be worthwhile to further investigate its role in controlling replication of other viruses.

In the present study, we investigated the effect of treatment with recombinant IL-24 protein in response to influenza A virus infection. Our results demonstrate that IL-24-treated cells have enhanced ability to control viral replication, reduce viral plaque formation and viral antigen expression. Furthermore, IL-24 increased phosphorylation in JAK/STAT pathways to induce expression of anti-viral molecules such as myxovirus resistance protein A (MxA) and 2-5′ oligoadenylate synthetase (OAS) in response to infection with the human influenza virus strain PR8. Collectively, our data provide evidence for the anti-viral role of IL-24, and further studies on the *in vivo* application of IL-24 therapy could be beneficial to developing therapeutic approaches to combat influenza.

Materials and Methods

Cells and viruses

Human lung adenocarcinoma cells (A549) and Madin-Darby canine kidney (MDCK) cells were obtained from the American Type Culture Collection (ATCC). A549 cells were cultured in RPMI 1640 medium (HyClone) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin and maintained at 37°C in a humidified atmosphere with 5% CO_2. MDCK cells were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Recombinant IL-24 protein was purchased from R&D, dissolved in sterile PBS and stored at -80°C. Interferon-α was purchased from R&D and interferon-β protein was obtained from PBL Assay Science.

Human influenza virus A/Puerto-Rico/8/34 (H1N1) PR8, and recombinant PR8 virus expressing green fluorescent protein (GFP) (rPR8-GFP) were obtained as previously described (Kim *et al.*, 2012). Influenza virus lacking the NS1 open reading frame (delNS1) was provided by Dr. Adolfo Garcia-Sastre (Icahn School of Medicine at Mount Sinai) and generated using reverse genetics from PR8 as previously described (Garcia-Sastre *et al.*, 1998). Seasonal A/H1N1 and A/H3N2 strains were obtained from Korea Bank for Pathogenic Viruses (KBPV). For viral infection, cells were washed with PBS and incubated with virus diluted in infection media (RPMI 1640 containing 7.5% bovine albumin fraction V, 1 M HEPES, 2 μg/ml tolylsulfonyl phenylalanyl chloromethyl ketone [TPCK]-trypsin, and antibiotics at the indicated multiplicity of infections (MOIs) for 1 h at 37°C. The inoculum was aspirated, and cells were incubated with RPMI 1640 containing 2% FBS without antibiotics.

Plaque assay

The virus infectivity titers of supernatants were determined by a plaque assay. Briefly, viral supernatants diluted in DMEM were added to MDCK cells in 6-well plates. After 2 h of attachment, viral supernatants were removed, and cells were overlaid with Eagle's minimum essential medium (EMEM) (without phenol red, with L-glutamine) (Lonza), 1.5% LE agarose (Lonza), and 2 μg/ml (TPCK)-trypsin, after which the cells were incubated for 3 days. After the incubation, the infected cells were fixed with 4% formaldehyde (Duksan Pure Chemicals Co., Ltd.) in PBS and stained with 0.5% crystal violet (JUNSEI) solution. Plaque-forming units (PFU) were calculated.

MTT assay

A549 cells were seeded in 96-well plates. After 24 h, the medium was changed, recombinant protein IL-24 (1, 10, and 100 ng/ml) was added, and the incubation was continued for 24 h at 37°C. After the incubation, 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) solution was added for 4 h. The optical density was then measured at 570 nm using a spectrophotometer.

Confocal microscopy

A549 cells were seeded onto coverslips in 24-well plates and treated with recombinant protein IL-24 followed by rPR8-GFP infection for 24 h. For knockdown of IL-24, transient transfections with either control scrambled or IL-24-specific siRNA (Bioneer) were performed with Lipofectamine RNAiMAX reagent® (Invitrogen), according to the manufacturer's protocol as previously described. After 24 h of siRNA transfection, cells were infected with influenza in infection media for 1 h at 37°C, after which the cells were washed with PBS and cultured in DMEM with 2% FBS for 24 h. After the cells were washed with PBS, they were fixed with methanol and mounted onto glass slides using mounting media containing 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories), which were examined by confocal microscopy (LSM700; Carl Zeiss).

Quantitative real time RT-PCR

Total RNA (1 μg, isolated with Trizol reagent [Invitrogen]) was reverse transcribed to generate cDNA using the RT system (Promega) for 1 h at 42°C. The resulting cDNA was used as the template for real-time PCR quantification. Using a Power SYBR Green PCR Master Mix (Thermo Fisher Scientific), mRNA expression levels of human and influenza genes were measured. Primer sequences are available upon request. The cycling parameters were as follows: 95°C for 15 min, followed by 40 cycles of 30 sec at 95°C and 1 min at 60°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as a normalizing control.

Western blotting

Cells were lysed at the specified times post-infection with RIPA buffer (Sigma-Aldrich). Lysates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10–12% acrylamide gels. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes, and blocked with 5% (w/v) skim milk in Tris-buffered saline (0.2 M Tris, 1.36 M NaCl) supplemented with 0.1% (v/v)

Tween 20 (TBS-Tw) for 1 h at room temperature. This was followed by overnight incubation with primary antibodies (Cell Signaling Technologies) against pIRF-3, pSTAT1/tSTAT1, pSTAT3/tSTAT3, pERK/tERK, pp38, IL-24 (Abcam) at 4°C. For influenza A virus protein expression, anti-NS1 antibodies (Santa Cruz Biotechnology), anti-matrix protein 1 (M1) antibodies (Santa Cruz Biotechnology), and anti-nucleoprotein (NP) antibodies (Sino biological) were used. After 3 washes in TBS/Tw, the membranes were incubated with HRP-conjugated anti-rabbit or anti-mouse IgG secondary antibodies for 1 h at 25°C. Membranes were washed with TBS-Tw, incubated with Western Lumi Pico solution (ECL solution kit) (DoGen), and exposed to film.

Statistical analysis

Results were expressed as the mean ± standard deviation (SD) of the mean. Data were analyzed using Student's *t*-test to determine the significance of differences between the means of 2 groups. Statistics were performed using Prism software. *P*-values < 0.05 were considered significant.

Results and Discussion

Influenza virus infection results in the up-regulation of IL-24 expression

To investigate the induction of IL-24 expression in response to influenza A virus infection, A549 cells were infected with either mock control, PR8 (A/Puerto-Rico/8/34 [H1N1]), or seasonal A/H1N1 and A/H3N2 strains of influenza A virus for 4, 8, and 24 h (MOI = 0.1). Transcript level of IL-24 was determined by quantitative RT-PCR. In response to PR8

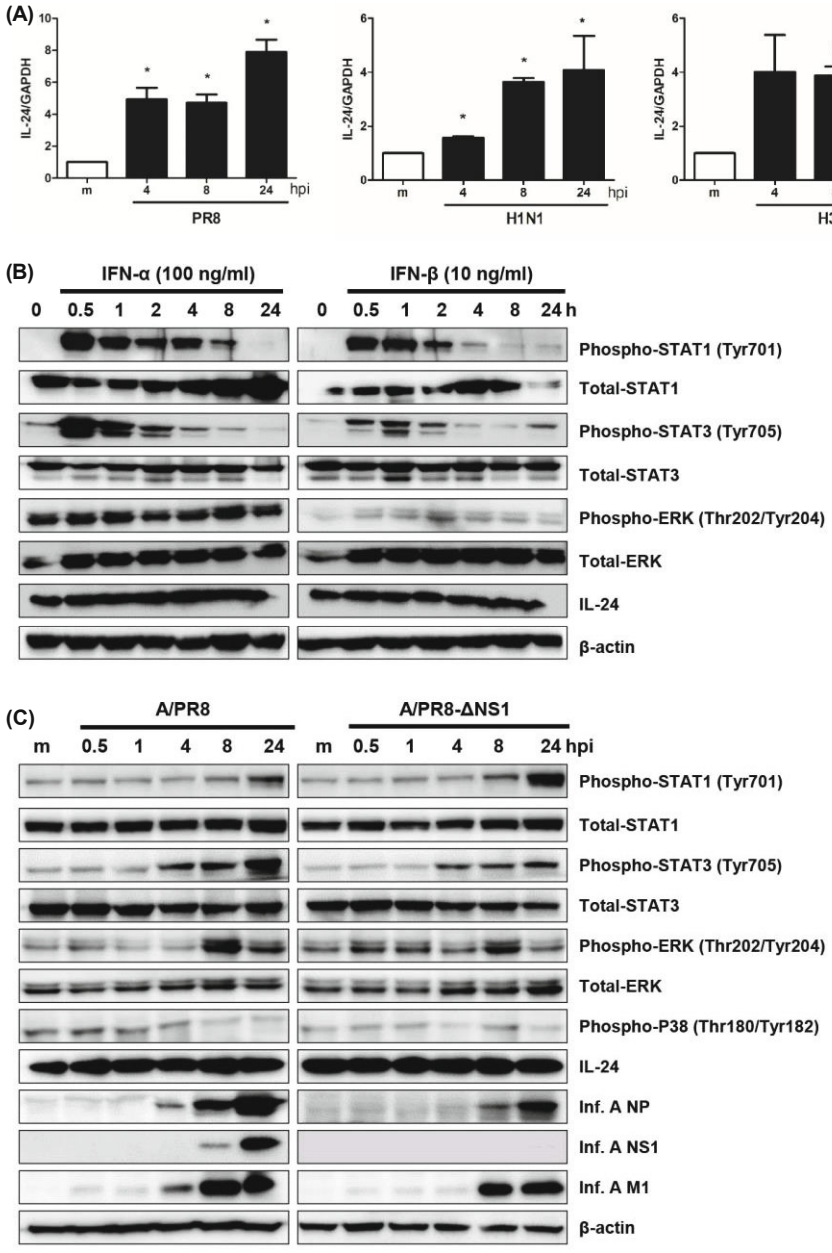

Fig. 1. IL-24 expression patterns during influenza A virus infection. (A) A549 cells were infected with influenza virus strains PR8, seasonal A/H1N1, and A/H3N2, at a multiplicity of infection (MOI) of 0.1 for the different time points indicated. Total cellular RNA was isolated, and IL-24 mRNA expression was measured using qRT-PCR. The results are shown as fold induction compared to mock control (m) and are representative of three independent experiments. (B) IFN-α (100 ng/ml) and IFN-β (10 ng/ml) were added to cells and incubated for different time periods (0, 0.5, 1, 2, 4, 8, and 24 h). Protein levels of IL-24, phospho-ERK, phospho-STAT1, and phospho-STAT3 were determined using Western blotting. The levels of cellular actin are shown as loading controls. Results are representative of three independent experiments. (C) A549 cells were mock-infected or infected with PR8 or PR8/delNS1 (MOI = 1) for 0.5, 1, 4, 8, and 24 h post-infection (hpi) and analyzed using western blotting with antibodies specific for IL-24, phospho-MAPK (ERK, p38), phospho-STAT1, and phospho-STAT3, using total cell lysates. Levels of cellular actin are shown as loading controls. Results are representative of three independent experiments.

and H1N1 infection, IL-24 transcriptional level was significantly increased in a time-dependent manner (Fig. 1A). Upregulation of H3N2-mediated IL-24 mRNA expression was highest at 4 and 8 h post infection time points, but had declined by 24 h post infection.

IL-24 is known to induce rapid activation of Stat-1 and Stat-3 transcription factors, and this can lead to regulation of cellular proliferation (Wang and Liang, 2005). To examine whether IL-24's downstream JAK/STAT pathway is activated upon treatment with recombinant type I IFN, A549 cells were pre-treated with IFN-α (100 ng/ml) or IFN-β (10 ng/ml) at different time points. Because all IFNs signal through JAK-STAT signaling pathways, rapid phosphorylation of STAT1/3 pathways was confirmed by IFN-α and -β pre-treatment, whereas phosphorylation of extracellular signal regulated kinase (ERK) remained similar following IFN-α treatment (Fig. 1B).

Influenza virus is known to have strategies to evade and overcome the antiviral response induced by IFN, and influenza NS1 is considered an essential antagonist for the induction of retinoic acid-inducible gene-1 (RIG-I)-mediated IFN-β production and interferon-stimulated genes (ISGs) (Schneider *et al.*, 2014). Given that NS1 can modulate the activation of host signaling pathways, we examined whether IL-24 expression is influenced by influenza viruses with or without NS1. A549 cells were infected with wildtype PR8 or PR8/delNS1 virus for the indicated time periods (MOI = 1). As expected, the protein synthesis of major influenza antigens such as NP and M1 began to significantly increase at 4 h post infection. For both wildtype PR8 and PR8/delNS1 virus infection, the phosphorylation of STAT1 and STAT3 began to increase at 4 h post infection and continued to increase

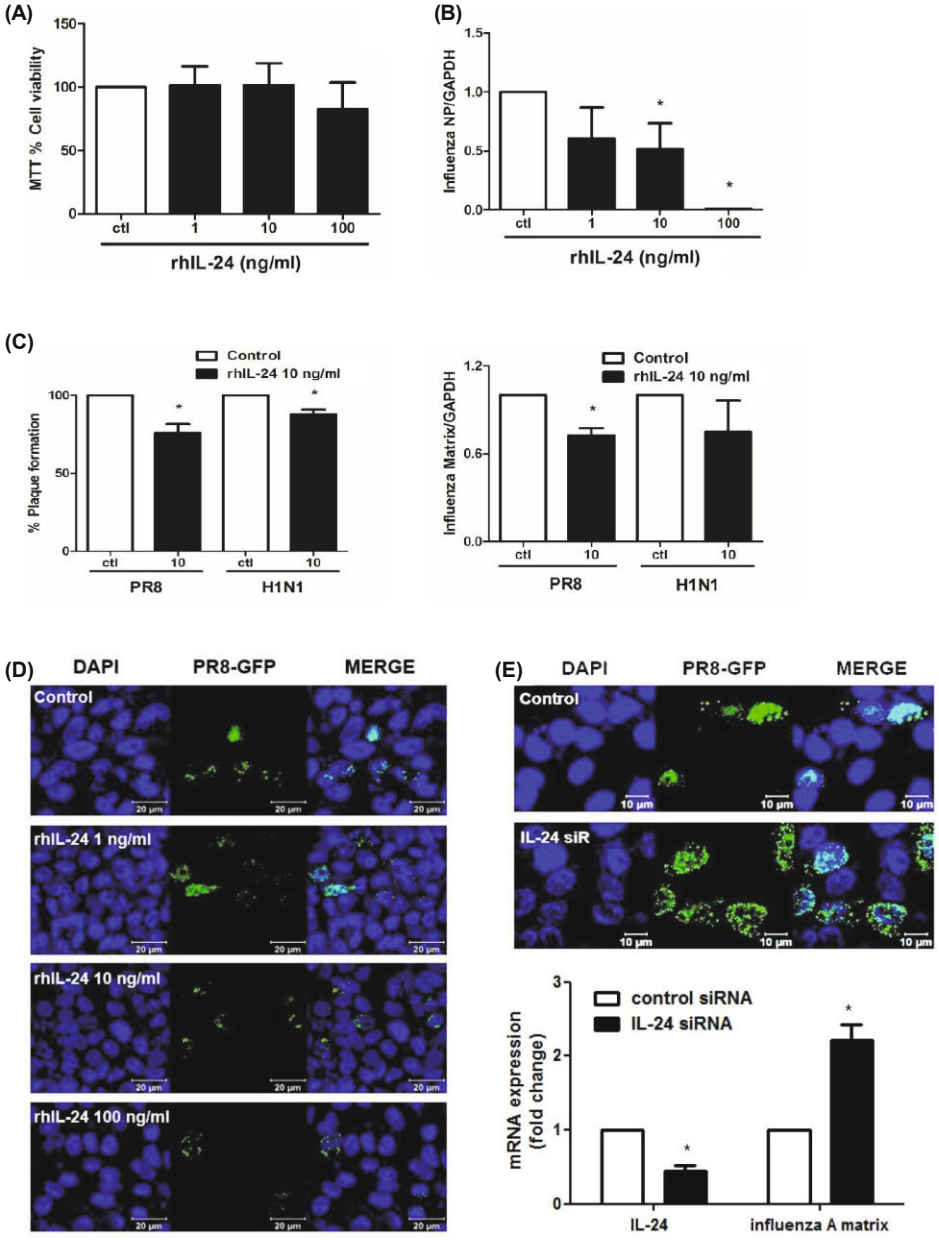

Fig. 2. Anti-viral effect of recombinant IL-24 protein. (A) A549 cells were treated with various concentrations (1, 10, 25, and 100 ng/ml) of recombinant IL-24 protein for 24 h, after which cell viability was measured using an MTT assay. Data are presented as a percentage (%) of cell viability of the control PBS-treated cells (100%). The average of two independent experiments is shown. (B) Transcriptional expression of the viral NP gene was quantified by real-time RT-PCR in A549 cells infected with the H1N1-PR8 virus at a multiplicity of infection (MOI) of 1. The expression levels of viral genes were normalized to GAPDH. The expression level of the mock-infected control (m) group was arbitrarily set to 1, and relative expression is shown in the graph. Data are shown as the mean ± standard deviation (SD) of the means from two independent experiments. Statistical analysis: * $P < 0.05$ vs. the control-treated group. (C) Plaque assays were performed. Data are presented as the percentage (%) decrease in the number of plaque forming units with respect to that of the control-treated cells, which was normalized to 100%. Three independent experiments were performed. The average of all experiments is shown. * $P < 0.05$ vs. mock (PBS control)-treated group. (D) A549 cells were infected with rPR8-GFP virus at MOI = 3. After 24 h, GFP expression was measured by confocal microscopy. (E) Knockdown of IL-24 was performed with IL-24-specific siRNA transfection, and knockdown efficiency of IL-24 was confirmed by qRT-PCR. Viral matrix gene expression was also measured. IL-24 knockdown cells were infected with rPR8-GFP, and GFP expression was measured by confocal microscopy. * $P < 0.05$ vs. control siRNA-transfected group.

until 24 h post infection, whereas there was a slight increase in IL-24 expression after wildtype PR8 infection, compared with the mock control (Fig. 1C).

IL-24's antiviral function

To evaluate the effect of recombinant IL-24 on cell viability, MTT assays were performed. As shown in Fig. 2A, various concentrations (1, 10, and 100 ng/ml) of IL-24 did not significantly affect cell viability. To assess the role of IL-24 during influenza virus infection, we first measured the transcript levels of NP in the presence of IL-24 using quantitative PCR. At 24 h post infection (hpi) with PR8 (MOI = 1), there was a significant reduction in viral NP transcript levels in the presence of IL-24 (Fig. 2B), suggesting that IL-24 inhibited viral gene expression. Consistent with these data, IL-24 treatment also significantly suppressed the transcript level of the viral matrix (M) gene and reduced the number of plaques regardless of influenza strain (PR8 and seasonal A/H1N1 strains), although the antiviral effect of IL-24 was lower in seasonal H1N1 compared with PR8 (Fig. 2C). Next, we used a recombinant influenza virus expressing green fluorescent protein (rPR8-GFP) virus to measure the antiviral effect of IL-24 on viral replication. Recombinant PR8-induced GFP expression patterns were measured by confocal microscopy. A549 cells were treated with either a mock control or IL-24 overnight and infected with rPR8-GFP for 24 h (MOI = 3). IL-24-treated A549 cells exhibited weaker GFP intensity than the mock control, implying IL-24-mediated reduction of viral replication efficiency (Fig. 2D). We also examined whether the knockdown of IL-24 changes viral replication efficiency. qRT-PCR data from IL-24 siRNA-transfected cells revealed effective knockdown of IL-24 but enhanced viral matrix gene expression. Moreover, there was an increased number of cells expressing GFP, indicating anti-viral role of IL-24 against influenza A virus infection (Fig. 2E).

IL-24 induces the type I IFN response during influenza infection

To assess whether the antiviral effect of IL-24 involves the activation of IFN-induced signaling, cell lysates from A549 cells infected with the PR8 virus (MOI = 1) in the presence or absence of IL-24 were analyzed using western blotting. Protein levels of viral NS1 were measured. As shown in Fig. 3A, influenza NS1 protein expression was attenuated in the presence of IL-24 compared with control PBS-treated influenza-infected cells. Additionally, we observed that IL-24 treatment led to enhanced phosphorylation of interferon regulatory factor 3 (IRF-3), which is important for the initiation of the innate antiviral response, and leads to interferon cascade, and induction of the transcription of specific genes such as IFN- β. Moreover, total cellular RNA was isolated from IL-24-treated, PR8-infected cells, and the transcript levels of interferon-inducible genes (ISGs), such as *MxA*, *OAS*, and *IFN-β* were measured to determine whether IL-24 treatment can induced antiviral gene expression. Both IFN-β and IFN downstream molecules, MxA and OAS, were significantly up-regulated following treatment with 10 ng/ml of IL-24 (Fig. 3B).

In this study, we characterized the anti-influenza activity of recombinant IL-24 *in vitro* by measuring transcript levels of influenza essential genes, viral protein expression, and the extent of plaque formation. Our previous study involving RNA-sequencing from H5 avian influenza virus-infected mice demonstrated that *IL-24*, along with inflammatory chemokines such as *IL-8* and *IP-10*, was the most highly up-regulated gene from H5 virus-infected lungs of mice (Park *et al.*, 2015). Given that IL-24 is a pro-apoptotic factor, an increase in expression may have contributed to a significant up-regulation of death receptor signaling and severe pathogenicity, which we observed in the H5N1-infected mice. Consistent with this, another group's study indicated that the anti-viral

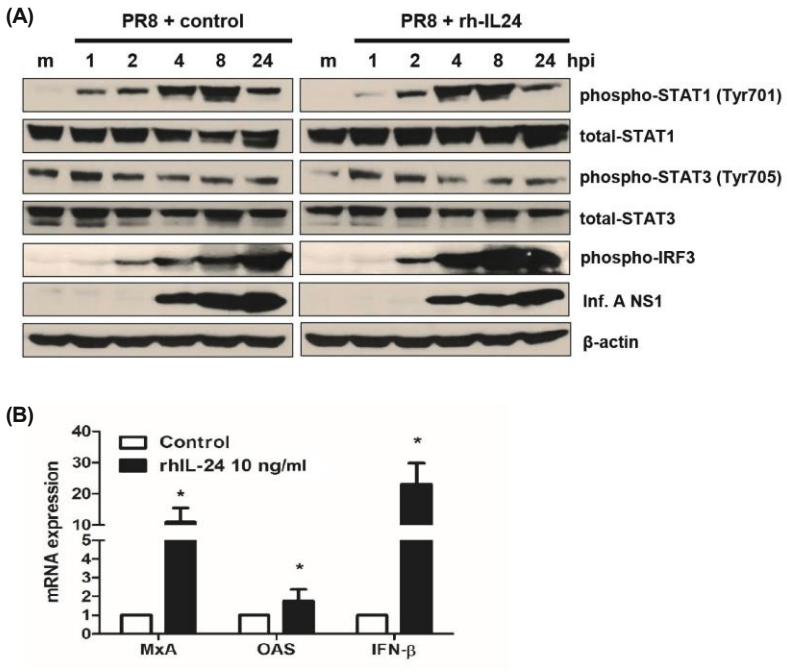

Fig. 3. Effect of IL-24 on host interferon-induced signaling and gene expression in influenza-infected A549 cells. (A) A549 cells were treated with 10 ng/ml of recombinant IL-24 protein overnight and infected with mock control (m) or the PR8 virus at a multiplicity of infection (MOI) of 1. Cells were harvested at 1, 2, 4, 8, and 24 h post-infection and analyzed using western blotting with antibodies specific for influenza NS1, phospho-IRF3, and phospho-STAT1, and phospho-STAT3. Levels of cellular actin are shown as loading controls. Results are representative of three independent experiments. (B) Changes in the transcriptional expression of myxovirus resistance gene (*MxA*), 2-5′ oligoadenylate synthetase (*OAS*), and interferon-β (*IFN-β*) in A549 cells infected with PR8 in the presence of phosphate buffered saline (PBS control) or IL-24 were measured using real-time qRT-PCR. Transcript expression levels were calculated in relation to the expression level of GAPDH and expressed as a fold-change in comparison with the expression level in mock-infected control cells. * $P < 0.05$ vs. mock-infected control cells.

activities of IL-24 can be attributed to TLR3-mediated apoptosis (Weiss *et al.*, 2015). Moreover, a recombinant virus expressing IL-24 is incapable of producing toxicity *in vivo* in comparison to an empty vector control, suggesting that IL-24 is highly likely to target virus-infected cells specifically. Thus, the results of this study and others provide further evidence that IL-24 is a promising target for the development of new anti-viral drugs.

Conflict of Interest

The authors declare no conflict of interest.

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References

Collin, N. and Briand, S. 2009. Influenza vaccine: globalization of public health stakes. *Med. Trop. (Mars)* **69**, 322.

Cunningham, C.C., Chada, S., Merritt, J.A., Tong, A., Senzer, N., Zhang, Y., Mhashilkar, A., Parker, K., Vukelja, S., Richards, D., *et al.* 2005. Clinical and local biological effects of an intratumoral injection of *mda*-7 (IL24; INGN 241) in patients with advanced carcinoma: a phase I study. *Mol. Ther.* **11**, 149–159.

Englund, J.A. 2002. Antiviral therapy of influenza. *Semin. Pediatr. Infect. Dis.* **13**, 120–128.

Fisher, P.B. 2005. Is *mda*-7/IL-24 a "magic bullet" for cancer? *Cancer Res.* **65**, 10128–10138.

Garcia-Sastre, A., Egorov, A., Matassov, D., Brandt, S., Levy, D.E., Durbin, J.E., Palese, P., and Muster, T. 1998. Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems. *Virology* **252**, 324–330.

Hatakeyama, S., Sugaya, N., Ito, M., Yamazaki, M., Ichikawa, M., Kimura, K., Kiso, M., Shimizu, H., Kawakami, C., Koike, K., *et al.* 2007. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. *JAMA* **297**, 1435–1442.

Huang, E.Y., Madireddi, M.T., Gopalkrishnan, R.V., Leszczyniecka, M., Su, Z., Lebedeva, I.V., Kang, D., Jiang, H., Lin, J.J., Alexandre, D., *et al.* 2001. Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (*mda*-7) gene with cancer specific growth suppressing and apoptosis inducing properties. *Oncogene* **20**, 7051–7063.

Jiang, H., Lin, J.J., Su, Z.Z., Goldstein, N.I., and Fisher, P.B. 1995. Subtraction hybridization identifies a novel melanoma differentiation associated gene, *mda*-7, modulated during human melanoma differentiation, growth and progression. *Oncogene* **11**, 2477–2486.

Kim, J.I., Park, S., Lee, I., Lee, S., Shin, S., Won, Y., Hwang, M.W., Bae, J.Y., Heo, J., Hyun, H.E., *et al.* 2012. GFP-expressing influenza A virus for evaluation of the efficacy of antiviral agents. *J. Microbiol.* **50**, 359–362.

Lebedeva, I.V., Su, Z.Z., Sarkar, D., Kitada, S., Dent, P., Waxman, S., Reed, J.C., and Fisher, P.B. 2003. Melanoma differentiation associated gene-7, *mda*-7/interleukin-24, induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species. *Cancer Res.* **63**, 8138–8144.

Mhashilkar, A.M., Schrock, R.D., Hindi, M., Liao, J., Sieger, K., Kourouma, F., Zou-Yang, X.H., Onishi, E., Takh, O., Vedvick, T.S., *et al.* 2001. Melanoma differentiation associated gene-7 (*mda*-7): a novel anti-tumor gene for cancer gene therapy. *Mol. Med.* **7**, 271–282.

Park, S.J., Kumar, M., Kwon, H.I., Seong, R.K., Han, K., Song, J.M., Kim, C.J., Choi, Y.K., and Shin, O.S. 2015. Dynamic changes in host gene expression associated with H5N8 avian influenza virus infection in mice. *Sci. Rep.* **5**, 16512.

Sarkar, D., Su, Z.Z., Lebedeva, I.V., Sauane, M., Gopalkrishnan, R.V., Dent, P., and Fisher, P.B. 2002. *mda*-7 (IL-24): signaling and functional roles. *Biotechniques* **Suppl 4**, 30–39.

Schneider, W.M., Chevillotte, M.D., and Rice, C.M. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu. Rev. Immunol.* **32**, 513–545.

Su, Z.Z., Lebedeva, I.V., Sarkar, D., Gopalkrishnan, R.V., Sauane, M., Sigmon, C., Yacoub, A., Valerie, K., Dent, P., and Fisher, P.B. 2003. Melanoma differentiation associated gene-7, *mda*-7/IL-24, selectively induces growth suppression, apoptosis and radiosensitization in malignant gliomas in a *p*53-independent manner. *Oncogene* **22**, 1164–1180.

Tong, A.W., Nemunaitis, J., Su, D., Zhang, Y., Cunningham, C., Senzer, N., Netto, G., Rich, D., Mhashilkar, A., Parker, K., *et al.* 2005. Intratumoral injection of INGN 241, a nonreplicating adenovector expressing the melanoma-differentiation associated gene-7 (*mda*-7/IL24): biologic outcome in advanced cancer patients. *Mol. Ther.* **11**, 160–172.

Wang, M. and Liang, P. 2005. Interleukin-24 and its receptors. *Immunology* **114**, 166–170.

Weiss, R., Laengle, J., Sachet, M., Shurygina, A.P., Kiselev, O., Egorov, A., and Bergmann, M. 2015. Interleukin-24 inhibits influenza A virus replication *in vitro* through induction of toll-like receptor 3 dependent apoptosis. *Antiviral Res.* **123**, 93–104.

Yacoub, A., Mitchell, C., Brannon, J., Rosenberg, E., Qiao, L., McKinstry, R., Linehan, W.M., Su, Z.S., Sarkar, D., Lebedeva, I.V., *et al.* 2003a. MDA-7 (interleukin-24) inhibits the proliferation of renal carcinoma cells and interacts with free radicals to promote cell death and loss of reproductive capacity. *Mol. Cancer Ther.* **2**, 623–632.

Yacoub, A., Mitchell, C., Lebedeva, I.V., Sarkar, D., Su, Z.Z., McKinstry, R., Gopalkrishnan, R.V., Grant, S., Fisher, P.B., and Dent, P. 2003b. *mda*-7 (IL-24) inhibits growth and enhances radiosensitivity of glioma cells *in vitro* via JNK signaling. *Cancer Biol. Ther.* **2**, 347–353.

Yoon, S.W., Webby, R.J., and Webster, R.G. 2014. Evolution and ecology of influenza A viruses. *Curr. Top. Microbiol. Immunol.* **385**, 359–375.