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Galectin-3 is involved in HIV-1 expression through NF-κB activation and associated with Tat in latently infected cells

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ABSTRACT

Galectin-3 (Gal-3) is involved in many biological processes and pathogenesis of diseases in part through nuclear factor (NF)-κB activation. We demonstrated that Gal-3 expression was significantly induced by tumor necrosis factor (TNF)-α or phorbol 12-myristate 13-acetate in OM-10.1 and ACH-2 cells, which are considered as a model of HIV-1 latently infected cells. The expression of Gal-3 was also associated with their viral production. However, the induction of Gal-3 by TNF-α was not observed in their uninfected parental cells. Knockdown of Gal-3 resulted in the suppression of NF-κB activation and HIV-1 replication in the latently infected cells. The expression level of Gal-3 was highly correlated with that of HIV-1 Tat in the latently infected cells stimulated with TNF-α. Furthermore, colocalization and possible interaction of Gal-3 and Tat were observed in the stimulated cells. These results suggent that Gal-3 expression is closely correlated with HIV-1 expression in latently infected cells through NF-κB activation and the interaction with Tat.

1. Introduction

Current antiretroviral therapy (ART) has achieved successful reduction of plasma viral load in human immunodeficiency virus type 1 (HIV-1)-infected patients below the undetectable level and improved the prognosis of infected individuals (Pomerantz and Horn, 2003; Weiss, 2008). However, ART cannot eradicate the virus from patients (Persaud et al., 2004; Cotton et al., 2013) due to the existence of HIV-1 latently infected cells (Finzi et al., 1997; Wong et al., 1997). Most of the latently infected cells are resting $CD4^+$ T cells with a memory phenotype (Chun et al., 1997; Pierson et al., 2000) and do exist for a long period of time in patients as viral reservoirs, which leads to viral rebound in case of treatment interruption (Finzi et al., 1999). They can escape from immune surveillance, since viral antigens are not expressed during ART, except for some proteins like Nef (Chagnon-Choquet et al., 2015; Ferdin et al., 2018; Kulpa et al., 2013). “Shock and Kill” approach has recently been proposed. This concept is based on reactivating HIV-1 latently infected cells to produce viral antigens in anticipation of their clearance by immune functions (Archin et al., 2012). Therefore, it is quite important to elucidate the mechanism of HIV-1 reactivation in latently infected cells for establishing curable treatment for HIV-1 infection.

Since HIV-1 transcription from proviral DNA is initiated in the absence of the viral transactivator Tat, cellular transcriptional factors, such as nuclear factor (NF)-κB, nuclear factor of activated T cells (NFAT), and activator protein 1 (AP-1), play important roles in HIV-1 gene expression in latently infected cells (Nabel and Baltimore, 1987; Kinoshita et al., 1998; Yang et al., 1999; Mbonye and Karn, 2014). Among the factors, NF-κB is required for efficient HIV-1 gene expression. Upon IκB (inhibitory molecule of NF-κB) degradation induced by various stimuli, such as T-cell mitogens and inflammatory cytokines, NF-κB translocates into the nucleus and binds to its binding sites in the HIV-1 long terminal repeat (LTR), followed by the activation of HIV-1 transcription (Nabel and Baltimore, 1987; Tong-Starksen et al., 1987; Duh et al., 1989; Baeuerle and Baltimore, 1988; Jiang and Dandekar, 2015). Although a number of cellular factors seem to be involved in HIV-1 gene expression in latently infected cells, its detailed mechanism has not been fully understood yet.

Galectin-3 (Gal-3) is a member of the lectin family characterized by specific binding to β-galactosides through the highly conserved carbohydrate recognition domain (CRD) (Barondes et al., 1994). It is a unique “chimera-type” galectin, containing a single CRD fused to the non-lectin amino-terminal region with a proline-glycine-alanine-tyrosine-rich repeat between the CRD and the N-terminal region. Gal-3 is widely expressed in numerous types of cells, including immune cells, and localized in the nucleus, cytoplasm, and cell surface (Dumic et al., 2006).

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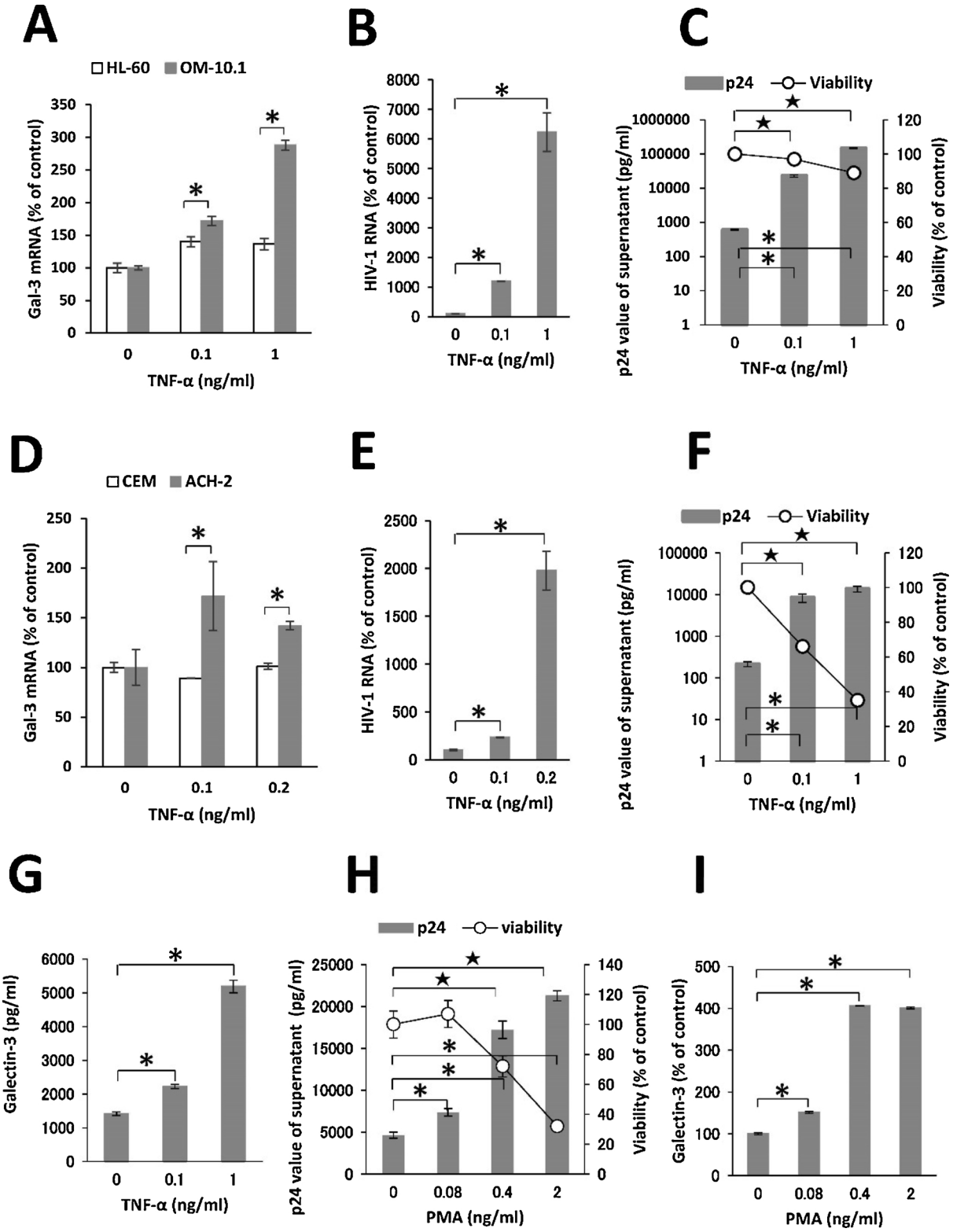

Fig. 1. Gal-3 expression and HIV-1 replication in latently infected cells. Gal-3 mRNA expression in (A) HL-60 and OM-10.1 cells and (D) CEM and ACH-2 cells and HIV-1 RNA expression in (B) OM-10.1 cells and (E) ACH-2 cells were determined by real-time RT-PCR after stimulation with the indicated concentrations of TNF-α for 24 h. HIV-1 p24 antigen levels of culture supernatants and viability of TNF-α-treated (C) OM-10.1 and (F) ACH-2 cells, and (H) PMA-treated OM-10.1 cells were determined by ELISA and colorimetric assay, respectively, after incubation for 3 days. Gal-3 protein levels in (G) TNF-α-treated and (I) PMA-treated OM-10.1 cells were determined by ELISA after incubation for 3 days. For panels A and D, each Gal-3 mRNA level was adjusted by a corresponding GAPDH mRNA level (internal control). All experiments were conducted three times and means ± sem are shown. Statistical significance among samples was evaluated by Student's *t*-test. *, ★ $p < 0.05$.

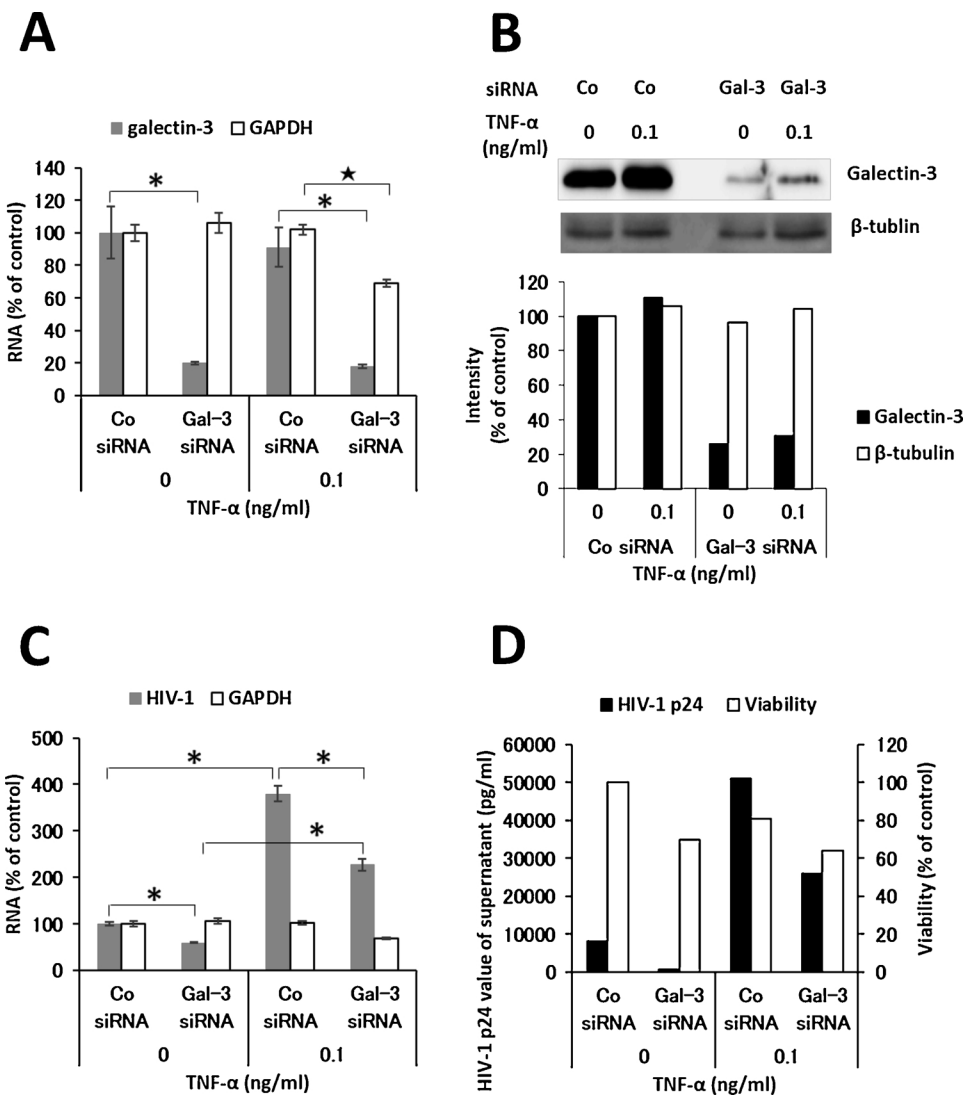

Fig. 2. Effects of Gal-3 knockdown. OM-10.1 cells were untreated or treated with the indicated concentrations of TNF-α at 24 h after transfection with a control siRNA or Gal-3-specific siRNA. The levels of (A) Gal-3 mRNA and GAPDH mRNA and (C) HIV-1 RNA of the cells were determined by real-time RT-PCR at 24 h after TNF-α treatment. (B) The expression of Gal-3 and β-tubulin proteins were analyzed by Western blot at 72 h after TNF-α treatment. (D) HIV-1 p24 antigen levels of culture supernatants were determined by ELISA at 72 h after TNF-α treatment. All experiments were conducted three times (A and C) or twice (B and D) and means ± sem (A and C) or representative results (B and D) are shown. Statistical significance among the samples were evaluated by Student's *t*-test. *, ★ $p < 0.05$.

It is also secreted to the outside of cells. Gal-3 has a variety of functions, such as RNA splicing, cell growth, apoptosis, cell adhesion, and immune responses (Dumic et al., 2006; Radosavljevic et al., 2012), and is involved in the pathogenesis of many diseases, including cancers (Fukumori et al., 2007), heart failure (Filipe et al., 2015), autoimmune diseases (Jensen-Jarolim et al., 2001; Lim et al., 2002; Ohshima et al., 2003), diabetes (Menini et al., 2016), and brain diseases (Shin, 2013). In addition, Gal-3 is also involved in acute HIV-1 infection. Upon acute HIV-1 infection, Gal-3 expression was upregulated in several T-cell lines and human primary $CD4^+$ T cells (Schroder et al., 1995; Wang et al., 2014). However, until now, there is no report that describes the involvement of Gal-3 in latent or chronic HIV-1 infection.

NF-κB has been reported to regulate Gal-3 expression in tumors (Dumic et al., 2000), while Gal-3 is known to play a certain role in the prolongation of inflammation through NF-κB activation (Hsu et al., 2000). Since NF-κB activation is known to trigger off HIV-1 gene expression in latently infected cells, we postulate that Gal-3 induces HIV-1 gene expression and following viral replication in latently infected cells. In this study, we investigated Gal-3 for its expression and functions in HIV-1 latently infected cells and found that Gal-3 expression and HIV-1 gene expression were closely correlated each other through NF-κB activation and the interaction with Tat.

2. Materials and methods

2.1. Cells

The promyelocytic cell line HL-60 (Gallagher et al., 1979) and T-lymphocytic cell line CEM (Foley et al., 1965) were purchased from the ATCC. OM-10.1 (Butera et al., 1991) and ACH-2 cells (Clouse et al., 1989) were obtained from the AIDS Research and Reference Reagent Program (National Institute of Health, Bethesda, MD). OM-10.1 and ACH-2 cells are clones of HL-60 and CEM cells chronically infected with HIV-1, respectively. Previous studies have demonstrated that OM-10.1 and ACH-2 cells produce little amount of HIV-1 particles without stimulation, yet they start producing a large amount of viral particles after TNF-α treatment (Butera et al., 1991; Folks et al., 1989). Therefore, these cell lines were considered as a model of HIV-1 latently infected cells. The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics.

2.2. Measurement of cell proliferation, viral production and Gal-3 expression

Cell viability of each sample was determined by a colorimetric assay. HIV-1 p24 antigen levels of culture supernatants were determined by an enzyme-linked immunosorbent assay (ELISA) kit (Zeptometrix, Buffalo, NY). The expression of Gal-3 was measured by a

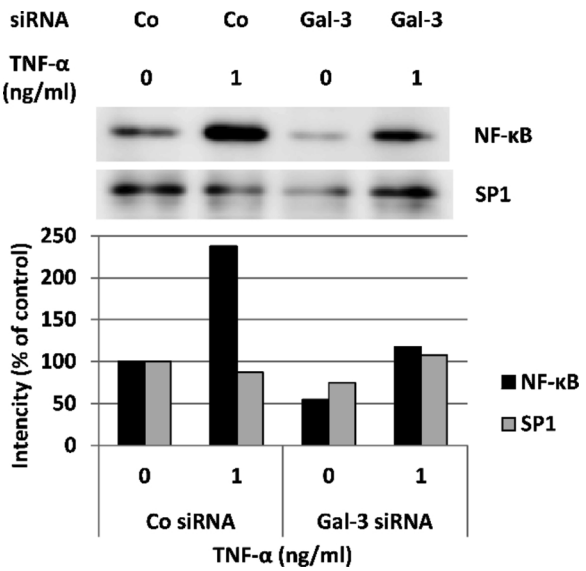

Fig. 3. Effect of Gal-3 expression on NF-κB and SP1 levels. OM-10.1 cells were transfected with a control siRNA or Gal-3 specific siRNA. At 24 h after transfection, the cells were untreated or treated with the indicated concentrations of TNF-α for 1 h. The nuclear proteins were isolated and examined for their NF-κB and SP1 levels by Western blot. All experiments were conducted twice, and representative results are shown.

human Gal-3 assay kit (Immuno-Biological Laboratories, Gunma, Japan). All assays were conducted, according to the manufacturers' instructions.

2.3. Real-time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from 1×10^6 cells in each sample using a RNeasy® Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Each cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The levels of Gal-3 mRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were measured by real-time RT-PCR using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) with TaqMan® Gene Expression Assay Reagents (Applied Biosystems; ID: HS00173587_ml), and a human GAPDH endogenous control (Applied Biosystems), respectively. HIV-1 RNA levels were also determined by real-time RT-PCR, as previously described (Wang et al., 2007).

2.4. Western blot analysis

One million cells were collected from each sample and washed twice with ice-cold phosphate-buffered saline (PBS). The cell pellets were lysed in RIPA buffer (Nacalai Tesque, Kyoto, Japan). Nuclear protein was isolated by the method, as previously described (Schreiber et al., 1989). Each sample was analyzed for its protein amount using a Quick Start™ Bradfold Protein Assay Kit (BIO-RAD Laboratories, Hercules, CA). The lysates containing an equal protein amount were separated by denaturating sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) with 10% polyacrylamide gel and transferred to an Immobilon-P polyvinylidene difluoride membrane (EMD Millipore, Billenca, MA). The membrane was incubated with the first antibody, such as an anti-human Gal-3 mouse monoclonal antibody (Clone: B2C10, 1:100) (Thermo Fisher Scientific, Waltham, MA), an anti-human β-tublin mouse monoclonal antibody (Clone: AA2, 1:1000) (EMD Millipore, Temecula, CA), an anti-human NF-κB p65 rabbit monoclonal antibody (Clone: D14E12, 1:1000) (Cell Signaling Technology, Danvers, MA), and an anti-human SP1 rabbit monoclonal antibody (Clone: D4C3, 1:1000) (Cell Signaling Technology). As the second antibody, a sheep anti-mouse IgG antibody (1:1000) (GE Healthcare Bio-Science, Piscataway, NJ) and a goat anti-rabbit IgG antibody (1:1000) (Cell Signaling Technology) were used, both of which were conjugated with horseradish peroxidase. The protein signals were detected by an ECL Plus Western Blotting Detection System (GE Healthcare) and analyzed by a LAS-1000 mini luminescent image analyzer (Fujifilm, Tokyo, Japan).

2.5. Galectin-3 knockdown

Three pmol of Stealth™ RNAi siRNA for Gal-3 gene knockdown or Stealth RNAi™ Negative Control Duplexes (Invitrogen Co, Carlsbad, CA) was introduced *via* an Amaxa Nucleofector™ (Amaxa Biosystems, Gaithersburg, MD) into 1.2×10^7 OM-10.1 cells using a Human T Cell Nucleofector™ Kit (Lonza, Rochester, NY), according to the manufacture's protocol. The efficiency of gene knockdown was assessed by Gal-3 mRNA and protein levels in siRNA-transfected cells by real-time RT-PCR and Western blot, respectively.

2.6. Immunocytochemistry

OM-10.1 cells were seeded on a culture slide (BD Biosciences, San Jose, CA) and cultured in the presence or absence of 10 ng/ml TNF-α. After incubation for 3 days, the cells were fixed and washed with PBS twice. After blocking the samples, they were incubated overnight with an anti-human Gal-3 mouse monoclonal antibody and an anti-Tat rabbit polyclonal antibody (Abacam, Cambridge UK) or their isotype controls, followed by incubation with each secondary antibody conjugated with fluorescein isothiocyanate or phycoerythrin for 1 h. After washing with PBS containing 0.5% bovine serum albumin (BSA) for three times, they were fixed with 1% paraformaldehyde and observed with a Zeiss LSM 5 Pascal Confocal Laser Scanning Microscope (Carl Zeiss Microscopy, Jena, Germany).

2.7. Flow cytometric analysis

OM-10.1 cells were untreated or treated with 10 ng/ml TNF-α for 3 days, washed twice with PBS containing 0.5% BSA, and incubated with the antibodies to Gal-3 and Tat on ice for 30 min, followed by incubation with the secondary antibody. After washing twice with PBS containing 0.5% BSA, the cells were fixed with 1% paraformaldehyde and analyzed by a flow cytometer (FACS Calibur™, BD Biosciences, San Jose, CA) and its software.

2.8. Co-immunoprecipitation

Co-immunoprecipitation was performed using Pierce Co-Immunoprecipitation (Co-IP) Kit (Thermo Fisher Scientific), according to the manufacturer's protocol. Briefly, 2×10^8 OM-10.1 cells were treated with 10 ng/ml TNF-α for 72 h and lysed with RIPA buffer. The lysate was incubated overnight with 25 μg of an anti-Tat antibody or its isotype control coupled covalently onto an amino-reactive resin at 4 °C. Then, the unbound fraction was collected (flow-through sample), and the co-immunoprecipitation complex was eluted from the antibody-coupled resin (elute sample). Both flow-through and elute samples were analyzed by immunoblotting.

3. Results

3.1. Gal-3 expression in HIV-1 latently infected cells

The levels of Gal-3 mRNA were examined in the HIV-1 latently infected cell lines OM-10.1 and ACH-2 and their uninfected parental counterparts HL-60 and CEM, respectively. Gal-3 mRNA expression was significantly upregulated by tumor necrosis factor (TNF)-α in a dose-

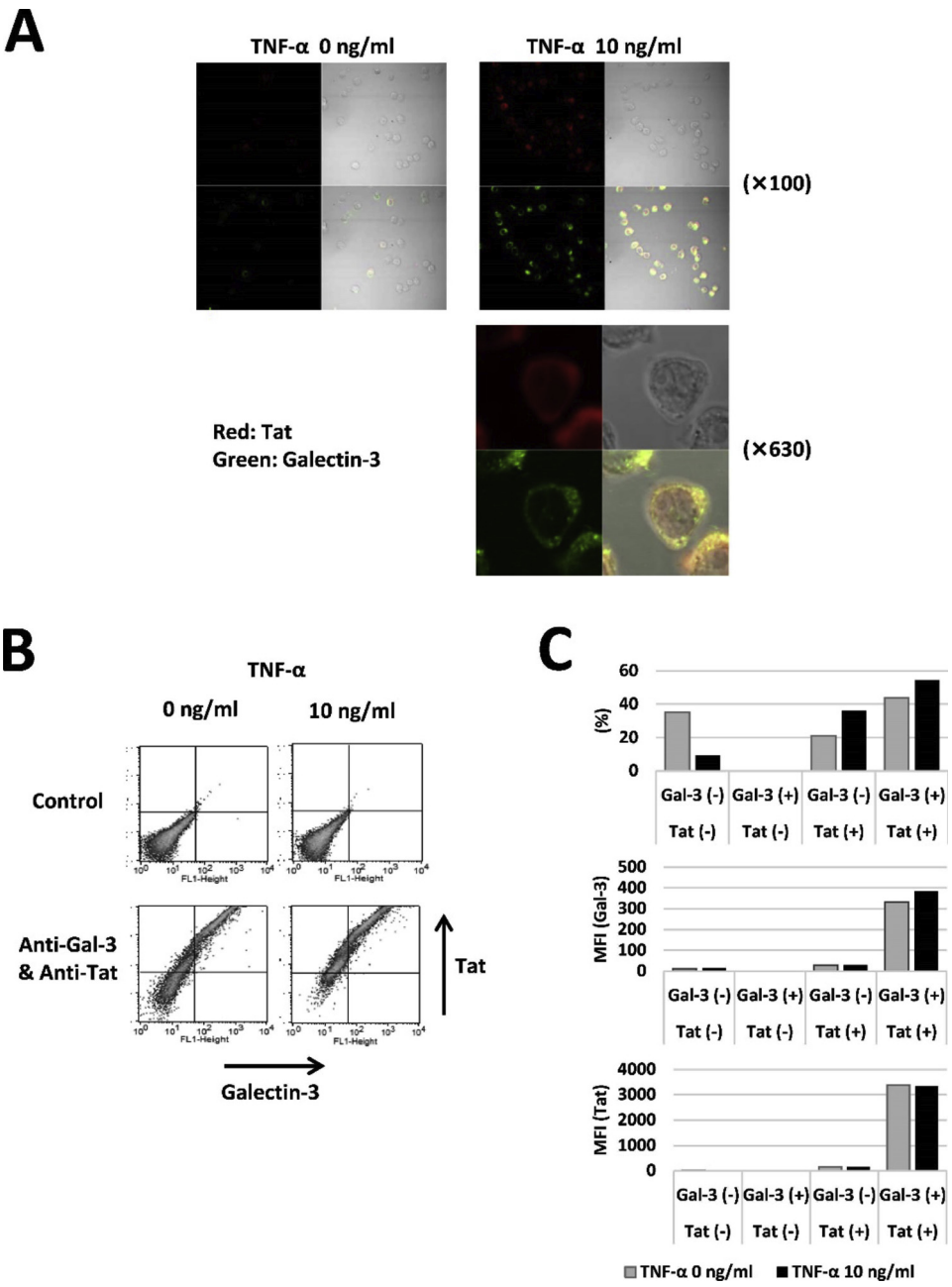

Fig. 4. Co-expression of Gal-3 and Tat. OM-10.1 cells were untreated or treated with the indicated concentrations of TNF-α for 72 h. (A) The cells were subjected to double immunostaining with specific antibodies to Gal-3 and Tat and observed by a confocal laser scanning microscope. (B) OM-10.1 cells were also analyzed by flow cytometry using anti-Gal-3 and anti-Tat antibodies. (C) Percent of the cell number and mean fluorescent intensity (MFI) in each quadrant is shown in a bar graph. All experiments were conducted twice and representative results are shown.

dependent manner in OM-10.1 and ACH-2 cells, whereas such upregulation was not observed in the uninfected cells (HL-60 and CEM) even in the presence of TNF-α (Fig. 1A and D). TNF-α also significantly upregulated HIV-1 RNA expression (Fig. 1B and E) and viral production (Fig. 1C and F) in OM-10.1 and ACH-2 cells. The number of viable ACH-2 cells decreased with increasing concentrations of TNF-α. This may be due to the fact that ACH-2 cells are sensitive to stimulation-induced apoptosis (Vandergeeten et al., 2007). In accordance with HIV-1 RNA expression and viral production, the protein level of Gal-3 was also upregulated by TNF-α in OM-10.1 cells (Fig. 1G). The protein kinase C-activator phorbol 12-myristate 13-acetate (PMA) is also known to induce HIV-1 expression in latently infected cells (Griffin et al., 1989; Doppler et al., 1992). In fact, PMA increased HIV-1 production (Fig. 1H) and Gal-3 expression (Fig. 1I) in OM-10.1 cells in a dose-dependent manner. The number of viable OM-10.1 cells appeared to be reduced by PMA (Fig. 1H), which was due to the induction of differentiation to macrophage-like cells. These results indicate that Gal-3 expression is upregulated in latently infected cells upon their activation by various stimuli.

3.2. Involvement of Gal-3 in HIV-1 gene expression and replication

To determine whether Gal-3 is involved in HIV-1 gene expression in latently infected cells, Gal-3 expression was downregulated by Gal-3-specific siRNA in OM-10.1 cells. Irrespective of TNF-α treatment, the transfection with the specific siRNA could reduce Gal-3 expression at both mRNA and protein levels to approximately 20–30% of their control levels in OM-10.1 cells, whereas the transfection with a control

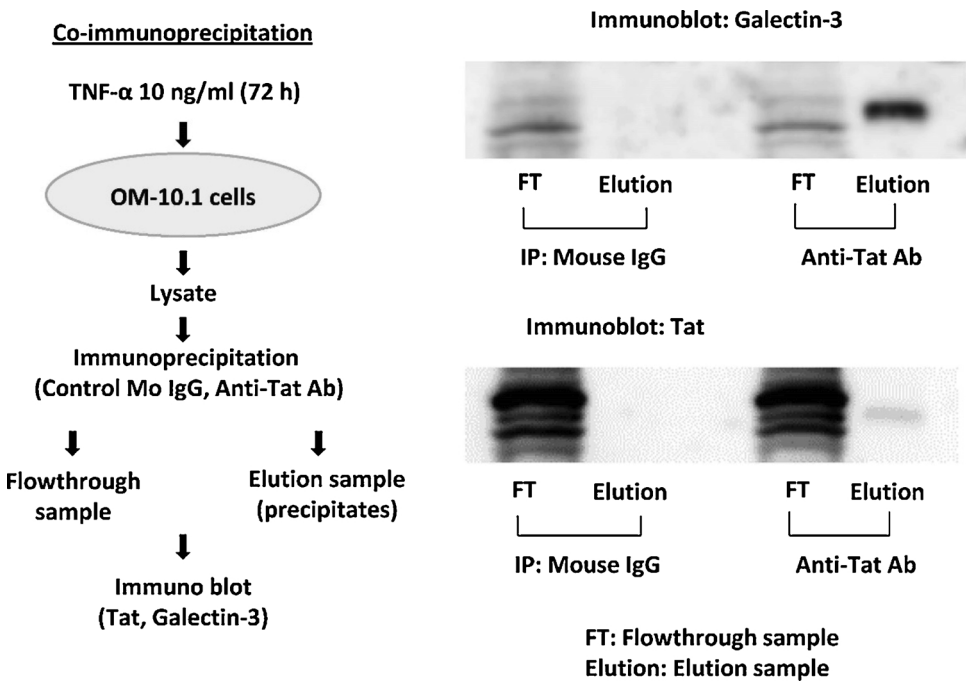

Fig. 5. Association of Gal-3 with Tat. OM-10.1 cells were treated with the indicated concentration of TNF-α for 72 h. The cell lysate was immunoprecipitated with a control mouse IgG or an anti-Tat antibody. The precipitates (elution) and flowthrough samples were analyzed by immunoblot using antibodies specific to Gal-3 and Tat. All experiments were conducted twice or three times, and representative results are shown.

siRNA had no effect on Gal-3 expression (Fig. 2A and B). These results indicate the effective suppression of Gal-3 expression by the specific siRNA. Therefore, the effect of Gal-3 on HIV-1 gene expression and viral replication was investigated in OM-10.1 cells transfected with a control or Gal-3-specific siRNA. As shown in Fig. 2C, the downregulation of Gal-3 expression resulted in significant reduction of HIV-1 gene expression in OM-10.1 cells in the absence or presence of TNF-α. HIV-1 replication was also suppressed by Gal-3 downregulation in OM-10.1 cells (Fig. 2D). These results suggest that Gal-3 is involved in HIV-1 gene expression and viral replication in latently infected cells.

3.3. Involvement of Gal-3 in NF-κB activation

Several inflammatory cytokines including TNF-α and chemical compounds such as PMA are capable of inducing HIV-1 gene expression in latently infected cells through transactivating HIV-1 promoter with NF-κB (Nabel and Baltimore, 1987; Duh et al., 1989; Butera et al., 1993; Griffin et al., 1989; Doppler et al., 1992). Based on the results on the involvement of Gal-3 in HIV-1 expression in latently infected cells (Fig. 2C and D), we postulated that Gal-3 induced HIV-1 gene expression in part through NF-κB activation in the cells. When nuclear NF-κB and SP1 levels were analyzed, both basal and TNF-α-induced NF-κB levels in the nucleus were markedly reduced in OM-10.1 cells transfected with Gal-3-specific siRNA (Fig. 3). In contrast, nuclear SP1 levels were less affected by the specific siRNA. These results suggest that Gal-3 is involved in activating NF-κB in latently infected cells.

3.4. Correlation between Gal-3 and Tat expression

HIV-1 Tat is a potent transactivator of HIV-1 gene expression in latently infected cells (Tong-Starksen et al., 1987). In addition, NF-κB is involved in *de novo* synthesis of Tat, which is required for efficient HIV-1 transcription (Williams et al., 2007). On the other hand, it was reported that the transfection of a Tat-expressing vector increased Gal-3 expression in the transfected cells (Fogel et al., 1999). These reports together with our results prompted us to investigate the correlation between Gal-3 and Tat in latently infected cells. OM-10.1 cells were untreated or treated with TNF-α and analyzed for their Gal-3 and Tat expression by immunostaining and flow cytometry. Treatment of the cells with TNF-α resulted in upregulating Tat expression (Fig. 4A and B), which correlated well with the results in Fig. 1A and C. Interestingly, Gal-3 expression increased proportionally with increasing Tat expression (Fig. 4B), and the cells expressing Gal-3 without Tat expression were not identified in our experiments (Fig. 4C). These results suggest that there is a strong correlation between Gal-3 and Tat upon their expression in latently infected cells.

3.5. Association of Gal-3 with Tat

Since the immunostaining microscopy showed that Gal-3 closely colocalized with Tat in TNF-α-treated OM-10.1 cells (Fig. 4A), the association of Gal-3 with Tat was further investigated. When the lysate of TNF-α-treated OM-10.1 cells was immunoprecipitated with a control mouse IgG or an anti-Tat antibody, both Gal-3 and Tat were detected for the sample immunoprecipitated with the anti-Tat antibody. However, they could not be detected for the sample immunoprecipitated with the control mouse IgG (Fig. 5), suggesting that Gal-3 and Tat are closely associated each other in latently infected cells.

4. Discussion

In approaches to the cure of HIV-1 infection, the reactivation of latently infected cells may trigger off the clearance of those cells from HIV-1-infected patients by their immune systems (Cary et al., 2016). Although many cellular factors are involved in this process (Sato et al., 2012), it is still mandatory to elucidate the key factors that control HIV-1 gene expression in latently infected cells.

In this study, we demonstrated that, along with viral replication, Gal-3 expression was upregulated in HIV-1 latently infected cells stimulated with either TNF-α or PMA (Fig. 1). There are several controversial reports on the relation between Gal-3 and HIV-1 infection. It was reported that Gal-3 mRNA markedly increased in Molt-3 cells on day 2 after HIV-1 infection (Schroder et al., 1995). However, it occurred transiently, and Gal-3 mRNA decreased rapidly after reaching the maximum in an early period of infection, nevertheless Tat expression and HIV-1 production continued to increase during the whole culture period. It was also reported that the elevation of Gal-3 mRNA expression was still observed on day 5 in acutely infected T-cell lines and $CD4^+$ T cells (Wang et al., 2014). Thus, the effect of acute HIV-1 infection on Gal-3 expression was not conclusive from those studies,

which may be due to the difference in cell types.

Another report demonstrated that, unlike galectin-1 (a member of the galectin family), Gal-3 did not increase the susceptibility of host cells to HIV-1 infection by associating with HIV-1 gp120 (West et al., 2001). In contrast, Wang et al. showed that the release of viral particles was suppressed by treating with Gal-3-specific siRNA in acutely infected cells, suggesting that Gal-3 promoted HIV-1 infection by facilitating viral budding (Wang et al., 2014). We also revealed that the knockdown of Gal-3 expression by Gal-3-specific siRNA reduced HIV-1 expression in OM-10.1 cells (Fig. 2), indicating that Gal-3 is involved in HIV-1 expression in the cells. Based on these results, Gal-3 seems to promote HIV-1 infection in both acutely and latently infected cells.

We found in this study that Gal-3 expression was involved in HIV-1 gene expression through NF-κB activation (Figs. 1–3). NF-κB is a transcriptional factor essential for the transcriptional initiation and elongation of HIV-1 mRNA transcripts (Nabel and Baltimore, 1987; Wang and Guo, 2016), especially in latently infected cells (Kimata et al., 2016). Furthermore, it was reported that NF-κB itself mediated Gal-3 expression in some tumors and inflammatory lesions (Wang and Guo, 2016). Taken together, it is assumed that Gal-3 enhances HIV-1 gene expression in latently infected cells *via* NF-κB activation, leading to further upregulation of Gal-3 expression. This positive feedback mechanism between Gal-3 and NF-κB is functioning in latently infected cells, which may strongly activate the cells and significantly produce HIV-1.

We also showed a close correlation between Gal-3 and Tat expressions in OM-10.1 cells (Fig. 4 and 5), which corresponds to the previous reports that the transfection of a Tat expression vector upregulated Gal-3 expression in several human cell lines (Fogel et al., 1999). Furthermore, NF-κB activation was involved in *de novo* Tat synthesis in latently infected cells (Williams et al., 2007). Therefore, it is possible that NF-κB activation may play an important role in the correlation between Gal-3 and Tat expressions in latently infected cells. It was also reported in the previous study that Gal-3 induction by Tat was dependent on SP1 (Fogel et al., 1999), although such direct evidence was not obtained in our study. In addition, the colocalization and possible interaction of galectin-3 and Tat were observed in OM-10.1 cells (Fig. 4 and 5). These results suggest that Gal-3 is required for the function of Tat in the cells. In the meantime, Gal-3 was found to be incorporated into HIV-1 particles released from infected cells (Wang et al., 2014). Tat can shuttle between the nucleus and cytoplasm and is released from infected cells. Tat influences on various cellular events, such as gene expression, cell growth, and the blood-brain barrier function (Musinova et al., 2016; Ensoli et al., 1993; Banks et al., 2005). Thus, from these reports and our findings, it is assumed that Gal-3 and Tat are incorporated into viral particles together and/or released from HIV-1 infected cells as a complex form and that Gal-3 participates in the function of Tat and *vice versa*.

It was recently reported that human recombinant Gal-3 induced cell death in HIV-1-infected THP-1 cells and human monocyte-derived macrophages *in vitro* (Xue et al., 2017). Although we observed that Gal-3 expression was significantly upregulated in acutely infected peripheral blood mononuclear cells (PBMCs) compared with uninfected cells, the viability of infected PBMCs was similar to that of uninfected cells (data not shown). Such difference may be due to the source of Gal-3. In general, intracellular Gal-3 acts as an anti-anpoptotic factor, whereas extracellular Gal-3 mainly acts as a pro-apoptotic factor (Dumic et al., 2006). Futhermore, as described above, Gal-3 is invoved in the activation of NF-κB that is known as a potent anti-apoptotic factor (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996). Thus, Gal-3 expression may also contribute to the protection of latently infected cells from their activation-induced cell death.

Finally, considering the fact that the expression of Gal-3 mRNA increases in the lymph nodes of HIV-1-infected patients during the acute to asymptomatic stages (Li et al., 2009), Gal-3 may become a potential target for killing the latently infected cells by immune systems after their activation with certain stimuli. Therefore, our findings may provide a new insight into a strategy for the eradication of latently infected cells and cure of HIV-1-infected patients.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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