STP-A11, an oncoprotein of *Herpesvirus saimiri* augments both NF- κ B and AP-1 transcription activity through TRAF6

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Abbreviations: AP-1, Activator Protein-1; HVS, *Herpesvirus saimiri*; STP, Saimiri transforming protein; TRAF, TNF- α receptor associated factor; TRANCE, TNF related activation-induced cytokine

Abstract

Herpesvirus saimiri (HVS), a member of the γ -herpesvirus family, encodes an oncoprotein called Saimiri Transforming Protein (STP) which is required for lymphoma induction in non-human primates. However, a detailed mechanism of STP-A11-induced oncogenesis has not been revealed yet. We first report that STP-A11 oncoprotein interacts with TNF- α receptor-associated factor (TRAF) 6 *in vivo* and *in vitro*. Mutagenesis analysis of the TRAF6-binding motif ₁₀PQENDE₁₅ in STP-A11 reveals that Glu (E)₁₂ residue is critical for binding to TRAF6 and NF- κ B activation. Interestingly, co-expression of E12A mutant, lack of TRAF6 binding, with cellular Src (Src) results in decreased transcriptional activity of Stat3 and AP-1, a novel target of STP-A11 compared to that of wild type. Furthermore, the presence of STP-A11 enhances the association of TRAF6 with Src and induces the translocation of both TRAF6 and Src to a nonionic detergent-insoluble fraction. Taken together, these studies suggest that STP-A11 oncoprotein up-regulates both NF- κ B and AP-1 transcription activity through TRAF6, which would

ultimately contribute cellular transformation.

Keywords: herpesvirus 2, saimirine; NF- κ B; oncogene protein pp60 (v-src); Src; TNF receptor-associated factor 6; transcription factor AP-1

Introduction

Despite a structural homology between TNF receptor-associated factor (TRAF) molecules, each TRAF harbors a distinct biological function involved in cell death, survival, and immune responses (Bradley and Pober, 2001; Wajant and Scheurich, 2001; Chung *et al.*, 2002). TRAF family members 2, 5, and 6 share functions to activate Jun-N terminal kinase (JNK) and p38 MAP kinase (Baud *et al.*, 1999; Dempsey *et al.*, 2003), whereas neither TRAF3 nor TRAF4 activates these pathways (Chung *et al.*, 2002; Ely and Li, 2002). Recent studies have shown that the structural determinant of the TRAF6-binding motif; PxExxE/ Φ (acidic/hydrophobic residue) is distinct from TRAF2, 3 and 5-binding motif; PxQxT/S (Ye *et al.*, 2002). Gene knockout studies have shown that TRAF2 is responsible for JNK activity and TRAF6 is critical for NF- κ B signaling (Lomaga *et al.*, 1999; Nguyen *et al.*, 1999). Thus, TRAF6 functions as an adaptor protein for various receptors such as IL-1/Toll-like-, TNF-related activation-induced cytokine (TRANCE)-, and CD40-receptor, leading to NF- κ B activation (Lomaga *et al.*, 1999; Nguyen *et al.*, 1999; Jabara *et al.*, 2002).

Src kinase, a member of the SH2 family tyrosine kinase, is ubiquitously expressed and activated by many pathways including tyrosine kinase growth factor receptors, G-protein-coupled receptors and integrin cell surface adhesion molecules (Frame, 2002). Src activity is regulated by tyrosine phosphorylation at two sites with opposing effects. Phosphorylation of Tyr₄₁₆ in the activation loop of SH1 kinase domain elevates the enzyme activity whereas phosphorylation of Tyr₅₂₇ in the C-terminal tail by Csk reduces the enzyme activity (Fujimoto *et al.*, 2000). Activated Src family kinases have been preferentially found in raft domain (Kramer *et al.*, 1999; Petrie *et al.*, 2000; Dykstra *et al.*, 2001), which is enriched in sphingolipids and cholesterol and displays a resistance to a nonionic detergent such as TritonX-100 (Brown and London, 2000). Interestingly, it has been reported that TRAF6 enhances Src

activity upon TRANCE signaling in dendritic cells and osteoclast cells (Wong *et al.*, 1999). In addition, IL-1 has been shown to regulate cytoskeletal organization in osteoclasts via the association of TRAF6 and Src (Nakamura *et al.*, 2002).

AP-1, composed of a heterodimer of Jun and Fos family protein is activated by various stimuli including growth factors, cytokines, cell-matrix interactions and physical stresses (Shaulian and Karin, 2001; Lee *et al.*, 2004; Milde-Langosch, 2005). AP components can be activated through a direct phosphorylation of c-Jun at its N-terminus by JNK (Derijard *et al.*, 1994) or an enhanced transcription of c-Jun by ERK and p38 MAP kinase (Han *et al.*, 1997). Additionally, c-Fos protein expression is induced by ERK or JNK in response to growth factors or CD28 signaling (Li *et al.*, 2001). AP-1-regulated genes include important regulators of invasion, metastasis, proliferation, and survival. Many oncogenic signaling pathways converge at the AP-1 transcription factor complex (Shaulian and Karin, 2001; Milde-Langosch, 2005).

Due to reproducible induction of lymphoma in New World primates (Jung *et al.*, 1999; Damania *et al.*, 2000), studies on *Herpesvirus saimiri* (HVS), a member of γ -herpesvirus family have been extensively performed as an alternative animal model for studies on Epstein Barr Virus- or Kaposi's sarcoma-associated herpesvirus-mediated pathogenic disease of human (Jung and Desrosiers, 1991; 1995; Jung *et al.*, 1999). HVS can be further sub-classified into three subgroups (A, B, and C) on the basis of the extent of DNA sequence divergence at the left end of L-DNA. Mutational analysis on the gene products in this region, designated with saimiri transforming protein (STP-A, or C) has revealed that STP is not required for viral replication but for immortalization (Desrosiers *et al.*, 1984; 1985). Previous studies have reported that STP-A11 interacts with Src at the residue of ¹¹⁵YAEV¹¹⁸ (Lee *et al.*, 1997). Furthermore, STP-A11 binds to and activates Stat3 in concert with Src, leading to enhancement of cell proliferation and cell survival (Chung *et al.*, 2004). Another study has shown that STP-A11 interacts with TRAF1, 2 and 5 at the residue of ⁶⁰PVQES⁶⁴ (Lee *et al.*, 1999).

On the basis of the previous studies (Lee *et al.*, 1997; 1999; Chung *et al.*, 2004), we wondered how STP-A11-binding proteins such as Src, Stat3 and TRAF proteins would talk to each other through STP-A11. In this study, we reported that TRAF6, a new binding protein of STP-A11, exerts as a mediator of Src activation for AP-1 transcriptional enhancement and NF- κ B activation.

Materials and Methods

Cell culture and transfection

Human embryonic kidney (HEK) 293 T cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin. HEK 293 T cells were plated at 1×10^6 per 100-mm-diameter plate 24 h before transfection and the cells were transfected with 8 to 15 μ g of DNA using calcium phosphate precipitation method (Clontech, Palo Alto, CA). Cells were harvested 48 h after transfection.

Antibodies

Anti-HA, anti- β -tubulin antibody, bead-conjugated anti-Src antibody and HRP-conjugated secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA). Anti-Flag antibody (M2) was obtained at Sigma (St Louis, MO) and anti-Src and anti-phospho Src antibody (Tyr416), which represents an active Src protein, was purchased at Upstate Biotech (Charlottesville, VA).

Plasmid construction

P10A and E12A mutants of STP-A11 were generated by PCR and subcloned to the pEF6 vector (Invitrogen, Carlsbad, CA). Q62D and Y115A mutants were subcloned to the pEF6 vector. To construct glutathione S-transferase (GST) fusion recombinant DNA, STP-A11 fragment was amplified by PCR and inserted into pGEX4T-1 vector (Amersham, Piscataway, NJ). In addition, pCDNA3-Flag-TRAF6 vector (provided by Dr. Yongwon Choi, University of Pennsylvania) and pUSE-cSrc (Upstate Biotech.) vector were used.

Immunoprecipitation and immunoblotting

Cells were harvested and lysed with lysis buffer [0.15 M NaCl, 1% Nonidet P-40, 50 mM Tris(pH7.5)] containing 0.1 mM Na₂VO₃, 1 mM NaF, and protease inhibitors (Sigma). For immunoblotting, proteins from whole cell lysate were resolved by 10% or 12% SDS-PAGE and transferred to nitrocellulose membranes. Primary antibodies were used at 1:1,000 or 1:2,000 dilutions, and secondary antibodies were used at 1:2,000 dilution in 6% nonfat dry milk. After final washing, nitrocellulose membranes were exposed for an enhanced chemiluminescence assay (Amersham).

In vitro GST pull-down assay

The GST and GST-A11 recombinant protein depleted of C-terminal transmembrane region (GST- Δ TM-STPA11) were purified from *Escherichia coli*

BL21 (Invitrogen) with glutathione Sepharose 4B beads as recommended by the manufacturer (Amersham). At 48 h after transfection of HEK 293T cells with TRAF6 expression vector (pCMV-Flag-TRAF6), HEK 293T cells were lysed with binding buffer [20 mM Tris (pH7.5), 150 mM NaCl, 1% Triton X-1000, 1% Protease inhibitor cocktail solution (Sigma)] and mixed with 10 μ g of the GST fusion protein for 2 h at 4°C. Then glutathione Sepharose 4B beads were extensively washed and subjected to 10% SDS-PAGE, followed by an immunoblot assay.

NF- κ B, Stat3 and AP-1 reporter assay

HEK 293T cells were transfected with wild-type (WT) or its mutant STP-A11 vector together with NF- κ B-, Stat3 (pLucTKS3)- or AP-1-luciferase reporter vector by the calcium phosphate precipitation method. To normalize transfection efficiency, the pGK- β gal vector, which expresses β -galactosidase from a phosphoglucokinase promoter, was included in the trans-

fection mixture. At 48 h post-transfection, cells were washed with cold PBS and lysed in lysis solution [25 mM Tris(pH7.8), 2 mM EDTA, 2 mM DTT, 10% glycerol, and 1% Triton X-100]. Luciferase activity was measured with a luminometer by using a luciferase assay kit (Promega, Madison, WI).

Cellular fractionation

As described elsewhere (Chang *et al.*, 2004), cells cultured in 100 mm plate were washed and harvested with ice-cold PBS and cell pellets were lysed with 800 μ l of TTN buffer [20 mM Tris-HCl(pH7.4), 0.05% Triton-X100, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.5 mM PMSF, and 1 $\times$ cocktail protease inhibitor] on ice for 20 min followed by centrifugation at 10,000 *g* for 15 min. The supernatant was taken as soluble protein and the pellets were washed with 500 μ l of TTN buffer and the remaining pellets were subsequently solubilized in 800 μ l of RIPA buffer [50 mM Tris-HCl(pH7.4), 150

Figure 1. Interaction of STP-A11 with TRAF6 *in vitro* and *in vivo*. (A) Bacterially purified GST or GST-A11 protein depleted of C-terminal trans-membrane region (GST- Δ TM-STPA11) was mixed with cell lysates containing flag-tagged TRAF6 protein. GST bead complexes were separated on 10% SDS-PAGE and TRAF6 was detected with an anti-flag mAb. (B) After transfection with STP-A11 together with or without pCDNA3-Flag-TRAF6 vector, cell lysates were used for immunoprecipitation (IP) with anti-flag mAb, followed by immunoblotting (IB) with an anti-HA mAb for detection of STP-A11. (C) Designation of STP-A11 mutants; P10A and E12A of STP-A11. (D) After transfection with Wild type (WT) of STP-A11, its mutants P10A, or E12A together with or without TRAF6 vector, the cell lysates were used for IP with a bead-conjugated anti-flag mAb for precipitation of TRAF6, followed by IB with anti-HA mAb. (E) After transfection with Wild type (WT) of STP-A11, its mutants P10A, E12A, or Y115A together with or without pUse-c-Src vector, the cell lysates were used for IP with a bead-conjugated anti-Src mAb for precipitation of Src, followed by IB with anti-HA mAb (STP-A). (F) After transfection with NF- κ B luciferase reporter vector and expression vectors encoding WT or its mutants E12A, Y115A or Q62D of STP-A11, luciferase activity was measured at 48 h. Transfection efficiency was normalized with β -galactosidase activity. Results are averages from three independent experiments. Error bar indicates standard deviation.

mM NaCl, 1 mM EDTA, 1 mM DTT, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 10% glycerol, 0.5 mM PMSF, and 1 $\times$ cocktail protease inhibitor] on ice for 30 min. Extracts were centrifuged at 12,000 *g* for 15 min and the supernatant was taken as the insoluble fraction.

Results

STP-A11 interacts with TRAF6 *in vitro* and *in vivo*, leading to NF- κ B activation

Since it has been reported that a TRAF6-binding motif is structurally distinct from a TRAF2-binding

Figure 2. Involvement of TRAF6 in transcriptional activity of Stat3 and AP-1 with Src during STP-A expression. Stat3 and AP-1 luciferase activity were measured at 48 h, (A) After transfection with Stat3 luciferase reporter vector and expression vectors encoding WT or its mutants E12A, Y115A, or Q62D of STP-A11 with or without Src expression vector. (B) After transfection with AP-1 luciferase reporter vector and expression vectors encoding Src, TRAF6 or both. (C) After transfection with AP-1 luciferase reporter vector and WT or its mutants E12A, Y115A or Q62D of STP-A11 with or without Src expression vector. Transfection efficiency was normalized with β -galactosidase activity. Results are averages from three independent experiments. Error bar indicates standard deviation.

motif (Ye *et al.*, 2002), we wondered whether STP-A11 carries a potential binding motif for TRAF6; PxExxE/ Φ . We found that STP-A11 indeed contains a potential binding motif for TRAF6 $_{10}\text{PQENDE}_{15}$, which prompted us to investigate the interaction of STP-A11 with TRAF6. To this end, we constructed a GST fusion protein GST- $\Delta\text{TM-A11}$ (C-terminal transmembrane deletion), and purified GST and the GST- $\Delta\text{TM-A11}$ protein from *E. coli*, and mixed them with cell lysate containing TRAF6 protein. TRAF6 was pulled down by GST- $\Delta\text{TM-A11}$ but not GST, suggesting that STP-A11 interacts with TRAF6 (Figure 1A) *in vitro*. Next, we confirmed this interaction of STP-A11 with TRAF6 in HEK 293T cells by co-precipitation of TRAF6 with STP-A11 *in vivo* (Figure 1B). To further dissect which residue in the TRAF6-binding motif ($_{10}\text{PQENDE}_{15}$) of STP-A11 is necessary for binding, we constructed two mutants, designated as P10A and E12A (Figure 1C). STP-A11 wild type (WT) or its mutants were co-expressed with TRAF6, and then lysates were immunoprecipitated with anti-flag mAb for TRAF6. WT strongly bound to TRAF6, whereas P10A mutant weakly interacted with TRAF6. Furthermore, E12A mutant lost the binding to TRAF6 (Figure 1D), suggesting that Glu $_{12}$ of STP-A11 is necessary for TRAF6 binding. In addition, E12A mutant displayed the interaction with Src, whereas Y115A lost the interaction with Src (Figure 1E) as reported previously (Lee *et al.*, 1997). We also observed that E12A mutant still keep the capacity of binding to TRAF2 and Q62D mutant [lack of binding to TRAF2; (Lee *et al.*, 1999)] interacts with TRAF6 (data not shown).

As TRAF6 has been shown to be a critical mediator of NF- κB activation for various receptors (Lomaga *et al.*, 1999), we next examined whether E12A mutant of STP-A11 activates NF- κB . When NF- κB luciferase activity was measured after transfection with WT, E12A, Y115A (lack of binding to Src) (Lee *et al.*, 1997), or Q62D of STP-A11 into HEK 293T cells, E12A mutant exhibited a drastic reduced NF- κB activity, compared to that of WT. The result suggests that the interaction of STP-A11 with TRAF6 is crucial for STP-A11-mediated NF- κB activation. The level of NF- κB activity in the P10A mutant of STP-A11 decreased to approximately one third the level of NF- κB activity in WT (data not shown). Interestingly, Y115A mutant displayed a similar activity of NF- κB , compared to that of WT (Figure 1F), indicating that Src activity is dispensable for the activation of STP-A11-mediated NF- κB . Q62D mutant showed a slight diminished NF- κB activity compared to that of WT, suggesting that TRAF6 is more important than TRAF2 in STP-A11-mediated NF- κB activation.

Both TRAF6 and Src are necessary for STP-A11-mediated transcriptional activation of Stat3 and AP-1

Because STP-A11 has been shown to activate Stat3 (Chung *et al.*, 2004), we questioned whether these mutants would have a similar effect on Stat3 activation as NF- κB . To test this, STP-A11 WT, E12A, Y115A or Q62D was co-expressed with Stat3 luciferase. As expected, Y115A mutant exhibited significantly lower Stat3 activity, compared to that of WT; E12A mutant also exhibited reduced Stat3 activity (Figure 2A). However, Q62D mutant displayed similarity to WT in Stat3 activation (Figure 2A). This result indicates that unlike NF- κB activation, both TRAF6 and Src are required for Stat3 activation.

Since AP-1 promoter has been recently reported to be regulated by TRAF6 and Src during IL-1 β signaling (Funakoshi-Tago *et al.*, 2003), we wondered whether TRAF6 and Src alone can activate AP-1 and if this activation is further enhanced by STP-A11. To test this, Src and TRAF6 were transfected alone with AP-1 luciferase vector, resulting in 2 and 6 fold activation of AP-1 reporter, respectively (Figure 2B). However, co-transfection of Src and TRAF6 synergistically activated AP-1 transcriptional activity 20 fold (Figure 2B). This result suggests that there may be cross-talk between TRAF6 and Src. To investigate this possibility, WT STP-A11 vector was transfected with or without Src vector. We found low activation of AP-1 without Src, but an enhancement of AP-1 activity with Src (Figure 2C). Moreover, when E12A and Y115A of STP-A11 mutants were introduced, both E12A and Y115A mutant exhibited a drastic reduction of AP-1 transcriptional activity compared to that of WT (Figure 2C). The result indicates that STP-A11 activates AP-1 in a TRAF6 and Src dependent manner. However, Q62D mutant showed a similar level of AP-1 activity to that of WT, suggesting that TRAF6 rather than TRAF2 is more closely related to STP-A11-mediated AP-1 activation. Taken together, the results suggest that both TRAF6 and Src are necessary for STP-A11-mediated transcriptional activation of Stat3 and AP-1.

STP-A11 recruits TRAF6 and Src to nonionic detergent insoluble fractions

Due to the fact that TRAF6 binds to Src during TRANCE and IL-1 receptor signaling (Wong *et al.*, 1999; Nakamura *et al.*, 2002), we questioned whether TRAF6 and Src also interact upon STP-A11 expression. To prove this possibility, Src and TRAF6 were co-expressed with or without STP-A11, followed by Src protein immunoprecipitation with a bead-conjugated anti-src mAb from cell lysates. TRAF6

Figure 3. Co-localization of TRAF6 with Src during STP-A11 expression. (A) HEK 293T cells were transfected with Src (pUse-c-Src vector) and TRAF6 (pCDNA3-Flag-TRAF6) with or without STP-A11 expression vectors and the cell lysates were used for IP with an anti-src mAb for precipitation of Src, followed by IB with an anti-flag mAb for the detection of TRAF6. (B) After transfection with expression vectors of Src and TRAF6 together with WT or E12A mutant of STP-A11, soluble fraction was extracted with 0.5% TritonX-100 from the cell lysates and thereafter, insoluble fraction was extracted with RIPA buffer. Localization of Src, TRAF6 and STP-A11 was detected with anti-src, -flag, and -HA mAb, respectively.

was weakly immunoprecipitated with Src in the absence of STP-A11 (Figure 3A). In contrast, TRAF6 was strongly immunoprecipitated with Src in the presence of STP-A11 (Figure 3A), implying that STP-A11 enhances the interaction between Src and TRAF6.

TRAF6 has been shown to localize in a nonionic detergent-insoluble fraction important for its function during TRANCE signaling (Ha *et al.*, 2003). We thus explored whether the expression of STP-A11 induces a similar translocation of TRAF6 and Src. To do this, Src, TRAF6, and WT or E12A were co-expressed and then the cell lysates were divided into a nonionic detergent (0.5% Triton X100)-soluble and insoluble fraction, which represents cytoplasmic and membrane/cytoskeletal (including lipid rafts) complexes, respectively. Co-expression of WT with TRAF6 and Src induced translocation of TRAF6 and Src to some extent from soluble to insoluble fraction where STP-A11 was also localized (Figure 3B); whereas co-expression of E12A mutant with Src and TRAF6 did not (Figure 3B). Interestingly, unlike STP-A11 WT which is found in the insoluble fraction, E12A mutant was found mostly in the soluble fraction (Figure 3B). Accordingly, our results suggest that similar to TRANCE signaling, STP-A11, Src and TRAF6 form a complex which found in an insoluble fraction; furthermore, this translocation is dependent on the integrity of TRAF6 binding motif in STP-A11.

Discussion

Herein, we first reported that STP-A11, an onco-protein interacts with TRAF6 *in vitro* and *in vivo*. According to previous result, STP-A11 interacted with TRAF2, but failed to activate NF- κ B (Lee *et al.*, 1999). Although we do not know the reason for discrepancy between the previous study and ours, we speculate that Lee *et al.* (1999) overlooked STP-A11-mediated NF- κ B activation due to their interest on STP-C-mediated NF- κ B activation. Nevertheless, we observed that introduction of IKK- β -dominant negative vector abolishes STP-A11-mediated NF- κ B activity (data not shown) and STP-A11 furthermore activates ICAM expression through NF- κ B (personal communication to Dr. Jae Jung; Harvard Medical School) and an alternative NF- κ B signal pathway (Cho *et al.*, 2006). It is evident that STP-A11 harbors separate binding motifs for TRAF2 and TRAF6, which are crucial adaptors for NF- κ B activation. Interestingly, E12A mutant, lack of binding to TRAF6 exhibited a drastic reduction of NF- κ B activity whereas Q62D mutant, lack of binding to TRAF2 showed a partially diminished NF- κ B activity, suggesting that TRAF6 plays more important role in STP-A11-mediated NF- κ B activation. Therefore, we believe that the interaction of STP-A11 with TRAF6 delivers a signal to a complex of TAK1, TAB1 and TAB2, subsequently IKK complex with ligand independent manner. Activated IKK- β induces phos-

phorylation of I κ -B α , leading to degradation of the protein *via* ubiquitination. Then, released RelA and p50 complex translocate to the nucleus, resulting in turning on genes related to cell survival and proliferation. Furthermore, E12A mutant affects not only NF- κ B activation but also Stat3 activation. Stat3 activation by STP-A11 was previously found to be dependent on the interaction with Src and activation of Src (Chung *et al.*, 2004). This interesting result prompted us to propose a hypothesis that there may be cross-talk between TRAF6 and Src during STP-A11 expression. This is supported by previous studies which show the enhanced interaction between TRAF6 and Src in dendritic cells and osteoclast during TRANCE signaling (Wong *et al.*, 1999) and co-localization of TRAF6 and Src in osteoclast cells during IL-1 signaling (Nakamura *et al.*, 2002). Here, we observed a weak interaction between TRAF6 and Src which was strengthened with STP-A11 expression. We therefore propose that STP-A11 acts as a molecular scaffold to strengthen TRAF6 and Src interaction (Figure 4). This is further supported by the presence of triple complex composed of TRAF6, Src and STP-A11 in a detergent-insoluble fraction, which includes raft domains. In contrast, TRAF6 and Src preferentially localized in nonionic detergent-soluble fraction during co-expression of E12A mutant, suggesting that TRAF6 binding to STP-A11 is also playing a role in this location. The insoluble fraction may function as a domain within the cell where signaling factors such as TRAF6 and Src are active or activated. This is supported by the fact that Lyn and Lck also display altered localization into raft domain during BCR- or TCR-CD28-mediated

signaling, respectively (Janes *et al.*, 1999; Petrie *et al.*, 2000). Additionally, as previously mentioned, TRAF6 translocation to nonionic detergent-insoluble fraction is necessary for exerting its biological role during TRANCE signaling (Ha *et al.*, 2003). This leads to an interesting question which will be the subject of our future studies; how TRAF6 and Src cross-talk during STP-A11 expression?

We also showed that STP-A11 oncoprotein induces AP-1 activation through TRAF6 and Src. AP-1 protein regulates genes including invasion (MMPs, uPA)-, anti-apoptosis (Bcl-2, Bcl-XL)-, metastasis (CD44, osteopontin)-, and proliferation (CyclinD1, p16)-related genes (Shaulian and Karin, 2001; Milde-Langosch, 2005). IL-1 has also been shown to induce AP-1 activity through Src kinase and further augmented by co-expression of TRAF6 (Funakoshi-Tago *et al.*, 2003) similar to results reported in our study. Q62D mutant displayed AP-1 transcriptional activity to a level comparable with WT. Neither STP-A11 mutant Y115A (Src binding) nor E12A mutant (TRAF6) could activate AP-1 transcription, indicating that both Src and TRAF6 are required for STP-A11-mediated AP-1 activation. Furthermore, a previous study (Funakoshi-Tago *et al.*, 2003) has reported that cross-talk between TRAF6 and Src delivers signals to PI3-kinase-Akt and JNK pathways. Particularly, JNK, an effector molecule of TRAF6-Src signaling induces the activation of c-Jun, a component of AP-1 complex at the N-terminus. Thus, we speculate that STP-A utilizes JNK pathway which targets the AP-1 complex through TRAF6 and Src. However, we do not know the detailed mechanism, which should be assigned to the next study.

Figure 4. Model of STP-A11-mediated transcriptional activation of Stat3 and AP-1 through TRAF6.

Taken together, we propose that STP-A11 elevates transcriptional activity of both NF- κ B and AP-1 through TRAF6. Taken together, in HVS infection, STP-A11 may activate these transcription factors in a ligand-independent manner, which consequently contributes STP-A11-mediated pathogenesis.

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