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HTLV-1 Tax-associated hTid-1, a Human DnaJ Protein, Is a Repressor of IκB Kinase β Subunit*

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hTid-1, a human DnaJ protein, is a novel cellular target for HTLV-1 Tax. Here, we show that hTid-1 represses NF-κB activity induced by Tax as well as other activators such as tumor necrosis factor α (TNFα) and Bcl10. hTid-1 specifically suppresses serine phosphorylation of IκBα by activated IκB kinase β (IKKβ), but the activities of other serine kinases including p38, ERK2, and JNK1 are not affected. The suppressive activity of hTid-1 on IKKβ requires a functional J domain that mediates association with heat shock proteins and results in prolonging the half-life of the NF-κB inhibitors IκBα and IκBβ. Collectively, our data suggest that hTid-1, in association with heat shock proteins, exerts a negative regulatory effect on the NF-κB activity induced by various extracellular and intracellular activators including HTLV-1 Tax.

Nuclear factor-κB (NF-κB)[1] is an inducible eucaryotic transcription factor that belongs to the Rel/NF-κB family of transcription factors and consists of several subunits that are conserved in *Drosophila* and humans (1, 2). In quiescent cells, the predominant form, a p50/p65 of the NF-κB heterodimer, is retained in the cytoplasm by interaction with its major cellular inhibitors IκBs (3, 4). These inhibitors, IκBα and IκBβ bind to and mask the nuclear transport signal peptide sequence in NF-κB, forming an inactive NF-κB-IκB complex (3, 4). Activation of NF-κB, as induced by numerous extracellular stimuli, is initiated by phosphorylation of IκBs by IκB kinases and degradation of the phosphorylated inhibitors in proteasomes (5). NF-κB heterodimer freed from the NF-κB-IκB complex then enters the nucleus for binding to the κB *cis*-element to induce expression of the target genes. In addition to extracellular stimulation by proinflammatory cytokines such as TNFα and interlukin-1 (6), infection of some viruses, such as human T cell leukemia viruses type 1 (HTLV-1), herpes simplex virus, and hepatitis B virus, also induces NF-κB activation (7–9). Furthermore, NF-κB can regulate HIV-1 replication by enhancing transcription of viral genes (10), and HIV-1 replication can be attenuated by expression of a constitutively active IκBα (11–13), suggesting the importance of an NF-κB activity in promoting viral replication and contributing to the pathological events of AIDS.

It is well recognized that infection of T lymphocytes by HTLV-1, a human retrovirus and an etiological agent of adult T cell leukemia (ATL) (14), induces persistent NF-κB activation. NF-κB activation is essential for the induction and maintenance of T cell proliferation and transformation by HTLV-1 and is mediated by Tax, a 40-kDa viral transactivator (15–18). Recent discoveries indicate that the downstream events of multiple stimuli of the NF-κB signaling pathway converge at a 700-kDa IκB kinase complex that is composed of at least three subunits: IKKα, IKKβ, and IKKγ (5, 19–22). IKKβ exhibits a high intrinsic kinase activity and is the major kinase that mediates specific serine phosphorylation of IκBs at their N termini. IKKγ, a regulatory subunit of the IκB kinase complex, serves as an indispensable mediator to bridge IKKβ to its substrate, the IκBs (23, 24). Using a complementation cloning approach (22), IKKγ was identified to be one of the cellular targets for Tax (25–27), binding to Tax with much higher affinity than other potential targets including MEKK1, IKKα and IKKβ (28–31). Through modulation of IKKγ, the kinase activity of IκB kinases, particularly IKKβ, is significantly enhanced, leading to subsequent phosphorylation and degradation of IκBs and release of NF-κB for translocation into the nucleus.

We previously reported on the identification of a novel Tax-interacting cellular partner hTid-1, a human DnaJ chaperone protein (32). The 52-kDa protein shares strong homology with the *Drosophila* tumor suppressor protein Tid56 (33, 34) and displays an *in vitro* transformation suppressive activity in human cancer cells (32). In HEK cells, Tax associates with a molecular chaperone complex containing hTid-1 and Hsp70 and sequesters the complex in a cytoplasmic "hot spot" structure (32). As a first step toward understanding the functional significance of Tax/hTid-1 interaction, the effect of hTid-1 on the NF-κB signaling pathway was examined. Here, we report that hTid-1 antagonizes the activities of various NF-κB activators including Tax, TNFα, and Bcl10 by repressing IKKβ activity and enhancing the stability of the IκB molecules.

EXPERIMENTAL PROCEDURES

Cell Cultures—HEK and COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. Jurkat cells were cultured in RPMI medium with 10% fetal calf serum and antibiotics.

DNA Plasmid Constructs and Site-directed Mutagenesis—Full-length DNA fragments coding for IKKα, IKKβ, Bcl10, p65 subunit of

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[1] The abbreviations used are: NF-κB, nuclear factor-κB; TNFα, tumor necrosis factor α; HIV, human immunodeficiency virus; HTLV, human T cell lymphotrophic virus; ATL, adult T cell leukemia; IKK, IκB kinase; HEK, human embryonic kidney; HA, hemagglutinin; SEAP, secreted alkaline phosphatase; aa, amino acids; GST, glutathione *S*-transferase.

NF-κB, JNK1, p38, and ERK2 (GenBank™ accession numbers AF009225, AF029684, AF082283, M62399, L26318, L35253 and M84489, respectively) were obtained from a cDNA library derived from human lymph node (Edge BioSystems) using PCR with high fidelity *pfu* DNA polymerase (Stratagene) and subsequently cloned into the pCEF vector with an N-terminal FLAG tag or a C-terminal HA tag. Site-directed mutagenesis was performed to generate the dominant-negative mutants $IKK\alpha_{KM}$ and $IKK\beta_{KM}$ (Lys was replaced by Met at amino acid 44) using the PCR method. Full-length IκBα and IκBβ genes (GenBank™ accession numbers U36277 and U19799, respectively) were amplified from a murine spleen mRNA by reverse transcription-PCR and were cloned in the pBEFneo vector with an HA tag at their C termini. The pCEF/hTid-1-FLAG, pCEF/hTid-$1_{\Delta HPD}$-FLAG, pCEF/hTid-$1_{\Delta Cys}$-FLAG, and pBEF/Tax-HA constructs had been described previously (32), and the hTid-1 isoform used in this study is hTid-1L. The FLAG epitope tag from hTid-1 and its mutant constructs were also replaced with an AG tag that provided an alternative detection of the expressed protein. The AG tag, which can be recognized by the monoclonal antibody AG11 (kindly provided by James Hoxie), was generated to correspond to the nucleotide sequence encoding a C-terminal 10 amino acids (ELHPEYFKNC) of HIV-1 Nef. pNF-κB/SEAP was purchased from CLONTECH, and pNF-κBβ-gal was generated by replacing the SEAP fragment with a β-galactosidase fragment derived from pCIβ-gal. An N-terminal fragment of IκBα consisting of 54 amino acids was amplified by PCR and inserted into pGEX-2T to generate a pGST-IκBα (aa 1–54) construct for expression of the recombinant protein in *Escherichia coli*. Purification of GST-IκBα was performed according to the manufacturer's recommended protocol (Pharmacia).

Transfection, Immunoprecipitation, and in Vitro Kinase Assay—DNA transfection for HEK and COS-7 cells was performed with SuperFect reagent (Qiagen) and for Jurkat T cells with DMRIE-C reagent (Invitrogen) following the manufacturer's recommended protocols. The transfected cells were harvested and lysed in buffer containing 50 mM Tris, pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.5% Nonidet P-40 plus phosphatase inhibitors (10 mM β-glycerol-phosphate, 1 mM Na_3VO_4, and 1 mM NaF), and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 5 μg/ml leupeptin). Equal amounts of the cellular protein extracts were incubated with anti-IKKα/β (Santa Cruz, sc-7607) or with anti-FLAG for FLAG-tagged IKKβ for 4 h at 4 °C followed by the addition of 30 μl of protein A-agarose beads (Invitrogen) and incubation at 4 °C for an additional 2 h. The immunoprecipitates were washed extensively (two times with the lysis buffer and two times with the kinase buffer (25 mM Tris-Cl, pH 8.0, 5 mM $MgCl_2$ and 1 mM EDTA)) and resuspended in 15 μl of kinase buffer. 0.5 μl of [γ-^{32}P]ATP (Amersham Biosciences, no. PB-10218, 6000 Ci/mmol) and 5 μg of GST-IκBα (aa 1–54) were added to the beads and incubated for 30 min at 30 °C. The reaction mixture was then analyzed by SDS-PAGE and autoradiography.

Analyses of the Activities of p38, ERK2, and JNK1—HEK cells were transiently transfected with p38-HA, ERK2-HA, or JNK1-HA with or without hTid-1-FLAG. 20 h posttransfection the cells were stimulated with anisomycin (5 μg/ml for activating p38), phorbol 12-myristate 13-acetate (50 ng/ml, for activating ERK2), and TNFα (20 ng/ml for JNK1) for 30 min. The cells were then washed with phosphate-buffered saline and rapidly lysed in buffer (20 mM Tris-Cl, pH 8.0, 1% SDS). Equal amounts of total cell lysates were analyzed by immunoblotting using phospho-specific antibodies for pATF2, pERK, and pc-JUN (Santa Cruz, numbers sc-8398, sc-7383, and sc-822, respectively).

NF-κB Reporter Assay—β-galactosidase activity was measured using a standard color reaction with chlorophenol red-β-D-galactopyranoside as substrate. SEAP activity was analyzed using a chemiluminescence substrate (Tropix) following the manufacturer's recommended protocol.

RESULTS AND DISCUSSION

hTid-1 Suppresses NF-κB Activity Induced by Various Activators—To determine whether hTid-1 has an effect on Tax activation of NF-κB, transient co-transfection of Tax and hTid-1 together with the NF-κB β-galactosidase reporter construct was performed in both HEK and Jurkat T cells. Expression of Tax-HA promoted NF-κB β-galactosidase activity by at least 10-fold (data not shown). Consistent with a previous report that Tax-induced activation was mediated predominantly through IKKβ (28), activation of NF-κB-dependent β-galactosidase activity by Tax was inhibited potently by a dominant-negative mutant of IKKβ ($IKK\beta_{KM}$) (Fig. 1*A*). In contrast, neither $IKK\alpha_{KM}$, a dominant-negative mutant of IKKα, nor $JNK1_{APF}$, a dominant-negative mutant of JNK1, had any suppressive effect on NF-κB activation by Tax.

Co-expression of hTid-1 suppressed the NF-κB activation induced by Tax in both HEK and Jurkat T cells in a dose-dependent manner (Fig. 1*B*). The level of hTid-1 suppression paralleled that exhibited by IκBα, a cellular inhibitor of NF-κB (Fig. 1*B*). As this suppressive activity was comparably seen in HEK and Jurkat T cells, the inhibitory effect of hTId-1 does not appear to be cell type-dependent. Furthermore, hTid-1 did not repress β-galactosidase or SEAP activities driven by housekeeping gene promoters such as the human elongation factor promoter (data not shown), suggesting that hTid-1 is not a general inhibitor of cellular gene transcription.

NF-κB is also activated in response to pro-inflammatory cytokines such as TNFα. In HEK cells, hTid-1 repressed NF-κB activation induced by TNFα by 4-fold (Fig. 1*C*). As controls, FLAG-$IKK\beta_{KM}$ and IκBα-HA potently suppressed the NF-κB-driven β-galactosidase activity, whereas $JNK1_{APF}$ had no effect (Fig. 1*C*). Bcl10, a caspase recruitment domain-containing protein associated with TRAF2 (35, 36), is an apoptosis-inducing protein that can also promote NF-κB activation (35). The mechanism of Bcl10 activation of NF-κB remains unknown. However, because Bc10 is associated with the cytoplasmic membrane, it is likely to act relatively upstream in the NF-κB signaling pathway. We found that hTid-1-FLAG also suppressed Bcl10-induced NF-κB-dependent β-galactosidase activity by at least 5-fold (Fig. 1*D*). The activation of NF-κB by Bcl10 was repressed by IκBα and FLAG-$IKK\beta_{KM}$ but not by FLAG-$IKK\alpha_{KM}$ (Fig. 1*D*), indicating an involvement of IKKβ activity. Taken together, the observation that hTid-1 suppressed NF-κB activation by both extracellular and intracellular activators suggests that hTid-1, a human DnaJ protein and a novel Tax-binding protein, is a general cellular inhibitor of the NF-κB signaling cascade.

hTid-1 Down-modulates NF-κB Signaling through IKKβ—Although upstream stimuli of the NF-κB signaling cascade can differ, the transduction pathways all converge at the 700-kDa protein complex of IκB kinases (5). Because Tax was reported to activate NF-κB by stimulating the IκB kinase activity (29–31), an *in vitro* kinase assay was performed to determine whether hTid-1 has an inhibitory activity on the activation of IκB kinases by Tax. HEK cells were transiently transfected with Tax-HA alone or with various amounts of the hTid-1-FLAG construct. *In vitro* kinase assay was performed on IKKβ immunoprecipitates obtained from transfected cells using GST-IκBα (aa 1–54) as substrate. In the absence of hTid-1, specific phosphorylation of GST-IκBα (aa 1–54) was observed, indicative of an activation of IκB kinase activity by Tax (Fig. 2*A*). Significantly, in the presence of hTid-1-FLAG, phosphorylation of GST-IκBα was suppressed in a dose-dependent manner (Fig. 2*A*).

Because Tax was previously shown to activate the IκB kinase complex activity predominantly through IKKβ (28), a potential inhibitory effect of hTid-1 on the IKKβ subunit was further evaluated. Transient transfection of FLAG-IKKβ stimulated NF-κB-dependent β-galactosidase activity at least 10-fold. In the presence of hTid-1-FLAG, an inhibitory effect on the NF-κB-driven β-galactosidase activity induced by FLAG-IKKβ was observed (Fig. 2*B*). Accordingly, *in vitro* kinase assay showed that hTid-1 suppressed the phosphorylation of GST-IκBα induced by the kinase-active FLAG-IKKβ in a dose-dependent fashion (Fig. 2*C*).

Potential effects of hTid-1 on other serine kinases and signaling cascades were also examined. HEK cells were transiently transfected with p38-HA, ERK2-HA or JNK1-HA in the absence or presence of hTid-1-FLAG. Following transfection,

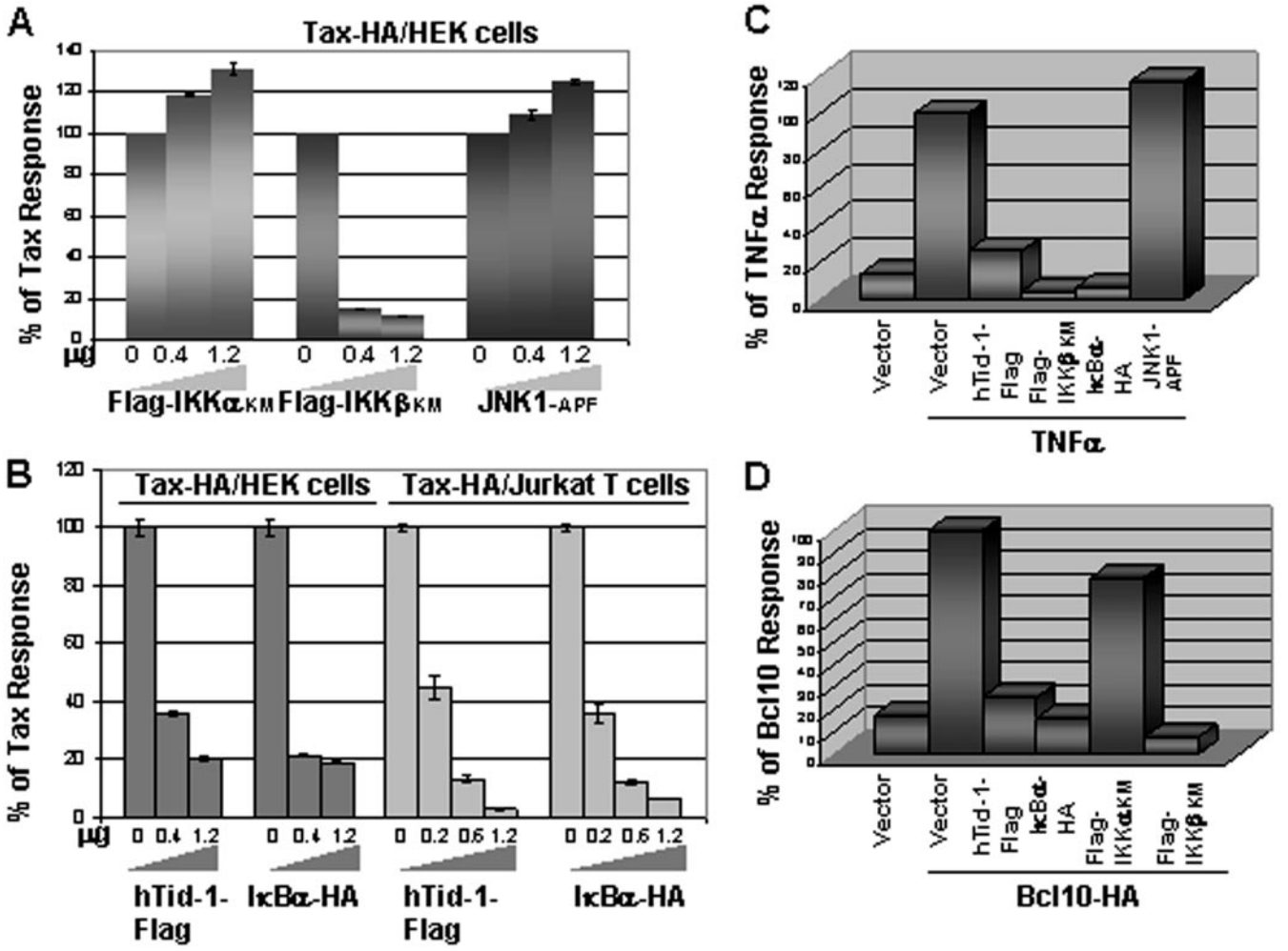

FIG. 1. **hTid-1 suppresses NF-κB activity induced by various activators including Tax, TNFα, and Bcl10.** *A*, inhibition of Tax mediated NF-κB activation by a dominant-negative IKKβ. HEK cells were transfected with a fixed amount of pBEF/Tax-HA (0.8 μg) and pNF-κBβ-gal (0.8 μg) with or without dominant mutants of IKKα (FLAG-IKKα$_{KM}$), IKKβ (FLAG-IKKβ$_{KM}$), or a dominant-negative mutant of JNK1 (JNK1$_{APF}$) at the indicated DNA amounts. Cellular protein extracts were prepared and β-galactosidase activity was determined according to procedures outlined under "Experimental Procedures." The data are presented as a percentage of the β-galactosidase activity in cells transfected with Tax-HA alone (expressed as 100%). *B*, repression of NF-κB-driven reporter activity by hTid-1 in both HEK and Jurkat T cells. Fixed amounts of Tax-HA (0.8 μg) and pNF-κBβ-gal (0.8 μg) were co-transfected with hTid-1-FLAG or IκBα-HA at the indicated doses in HEK (*left panel*) and Jurkat (*right panel*) cells. NF-κB reporter assay was performed similarly in Jurkat T cells, except that the pNF-κB/SEAP reporter plasmid replaced the pNF-κBβ-gal reporter construct. β-galactosidase and SEAP activities were determined as described under "Experimental Procedures." *C*, suppression of TNFα-induced NF-κB activation by hTid-1. pNF-κBβ-gal reporter plasmid was co-transfected with hTid-1-FLAG, IκBα-HA, FLAG-IKKβ$_{KM}$, or JNK1$_{APF}$ in HEK cells. 20 h post-transfection, portions of the transfected cells were stimulated with TNFα (20 ng/ml) for 5 h. β-galactosidase activity was determined as described under "Experimental Procedures." The data shown are representative of four independent experiments. *D*, the effect of hTid-1 on the NF-κB activation induced by Bcl10. HEK cells were transfected with pNF-κBβ-gal construct (0.8 μg) together with hTid-1-FLAG, IκBα-HA, FLAG-IKKβ$_{KM}$, or JNK1$_{APF}$ (1.2 μg/each) in the absence or presence of Bcl10-HA (0.8 μg). β-galactosidase activity was measured 24 h following transfection. The results shown are representative of four independent experiments.

the cells were stimulated with anisomycin (for p38), 12-*O*-tetradecanoylphorbol-13-acetate (for ERK2), and TNFα (for JNK1) as described under "Experimental Procedures." Activation of the kinases or their downstream signaling events was assessed using phospho-specific antibodies that detect the activated kinases and their phosphorylated substrates. As shown in Fig. 2*D*, phosphorylation of ATF2, a substrate for activated p38, was seen following stimulation by anisomycin and was not altered in the presence of hTid-1-FLAG (*top panel*). Similarly, phosphorylation of the ERK2 kinase or c-JUN, the substrate for the activated JNK1 kinase, was detected following stimulation by 12-*O*-tetradecanoylphorbol-13-acetate and TNFα, respectively, and the extent of phosphorylation was not changed by co-expression of hTid-1-FLAG (*middle* and *bottom panels*). These results indicate that hTid-1 has no significant effects on the activities of p38, ERK2, and JNK1 kinases. Although hTid-1 also exhibited some degree of repression of IKKα kinase activity (data not shown), given the principal role of IKKβ in NF-κB signaling and its significant suppression by hTid-1, we conclude that hTid-1 is a novel cellular inhibitor of the NF-κB signaling cascade by targeting predominantly the IKKβ subunit.

The NF-κB Suppressive Activity of hTid-1 Requires a Functional J Domain—We previous showed that Tax associates with a molecular chaperone protein complex containing both hTid-1 and Hsp70, with Tax binding to a Cys-rich region of hTid-1 and the J domain of hTid-1 interacting with Hsp70 (32). To determine whether formation of the molecular chaperone complex is necessary for the inhibitory effect of hTid-1 on the IκB kinases, the activity of two hTid-1 mutants, hTid-1$_{\Delta HPD}$ and hTid-1$_{\Delta Cys}$, were assessed. We found that a low level expression of hTid-1$_{\Delta HPD}$ marginally inhibited NF-κB activation mediated by either Tax or IKKβ, whereas at a higher dose (1.2 μg of DNA) it regained some inhibitory activity but still at a

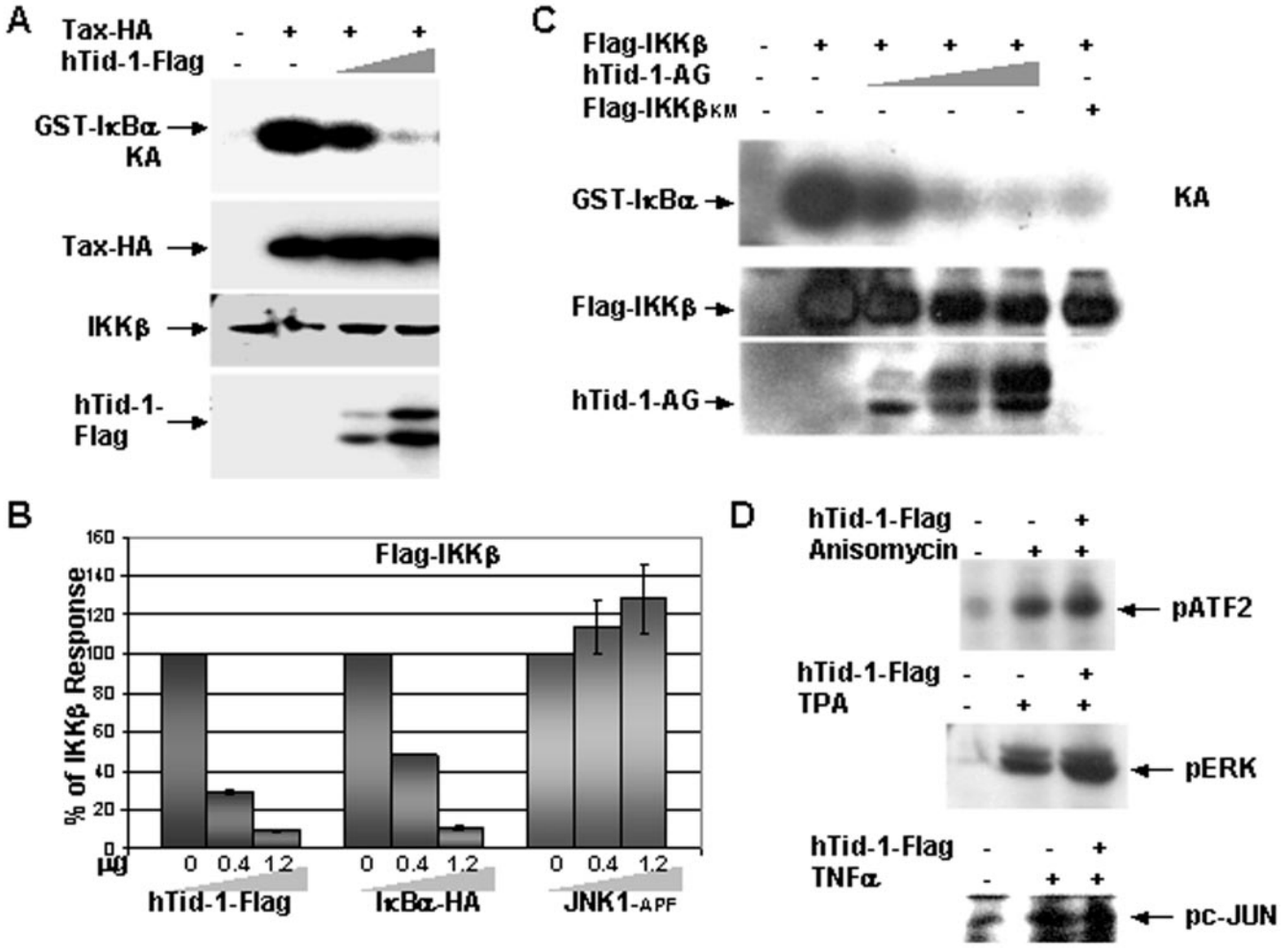

FIG. 2. **hTid-1 represses the kinase activity of IKKβ**. *A*, repression of the Tax-induced IκB kinase activity by hTid-1. HEK cells were transfected with vector or with Tax-HA (0.8 μg) together with hTid-1-FLAG at two DNA doses: 0.2 μg and 0.6 μg. Total cellular protein extracts were prepared and immunoprecipitated with rabbit anti-IKKα/β. *In vitro* kinase assay was performed on the immune complex, and GST-IκBα (aa 1–54) phosphorylation was detected as described under "Experimental Procedures."(*upper first panel*). Expression levels of Tax, endogenous IKKβ, and hTid-1-FLAG in total cellular extracts were detected using immunoblot analysis with antibodies for the HA epitope, IKKα/β, and the FLAG epitope, respectively. *KA*, kinase assay. *B*, inhibition of the kinase-active IKKβ-induced NF-κB activity by hTid-1. Transient co-transfection of pNF-κBβ-gal reporter plasmid and FLAG-IKKβ (0.8 μg each) with hTid-1-FLAG (0.4 μg, 1.2 μg), IκBα-HA (0.4 μg, 1.2 μg), or $JNK1_{APF}$ (0.4 μg, 1.2 μg) was performed in HEK cells. β-galactosidase activity was determined as described previously. *C*, suppression of the kinase activity of IKKβ. Various DNA amounts of hTid-1-AG (0.2 μg, 0.6 μg, and 1.2 μg) were co-transfected with a fixed amount of FLAG-IKKβ (0.8 μg each) in HEK cells. FLAG-$IKK\beta_{KM}$ was used as control. GST-IκBα phosphorylation was detected by the *in vitro* kinase assay (*upper panel*), FLAG-IKKβ, and hTid-1-AG expression levels were detected with anti-FLAG and AG11 immunoblottings, respectively (*middle* and *bottom panels*). *D*, the effect of hTid-1 on the activities of p38, ERK2, and JNK1. p38-HA, ERK2-HA, or JNK1-HA was transiently transfected into HEK cells in the presence or absence of hTid-1-FLAG. 20 h posttransfection, the cells were stimulated with anisomycin (5 μg/ml for activating p38), 12-*O*-tetradecanoylphorbol-13-acetate (50 ng/ml for ERK2) or TNFα (20 ng/ml for JNK1) for 30 min. Equal amounts of whole cell protein extracts were analyzed using immunoblot with phospho-specific antibodies for pATF2 (*top panel*), pERK (*middle panel*), or pc-JUN (*bottom panel*).

significantly reduced level compared with the inhibition mediated by wild type hTid-1-FLAG (Fig. 3*A*). Although the $hTid\text{-}1_{\Delta Cys}$ mutant displayed an inhibitory activity on NF-κB activation induced by FLAG-IKKβ, it was less effective than the activity of wild type hTid-1. Consistent with findings in the NF-κB-dependent reporter assay system, *in vitro* kinase assay showed that compared with wild type hTid-1, $hTid\text{-}1_{\Delta Cys}$ inhibited less potently the IKKβ kinase activity and that $hTid\text{-}1_{\Delta HPD}$ was the least efficient of the three (Fig. 3*B*). While $hTid\text{-}1_{\Delta Cys}$ maintained a full capacity for binding to Hsp70 (32), $hTid\text{-}1_{\Delta HPD}$ exhibited a reduced but not complete absence of binding activity. It is likely that overexpression of $hTid\text{-}1_{\Delta HPD}$ could recruit a small amount of Hsp70 for formation of the molecular chaperone complex, which may explain the partial recovery of the suppressive activity of $hTid\text{-}1_{\Delta HPD}$ at high doses. Indeed, we find that the hTid-1 mutant with complete deletion of the J domain disabled the repression of hTid-1 on NF-κB activity even at high doses (data not shown). Thus, it appears that the molecular chaperone complex formation is necessary for the inhibitory effect of hTid-1 on IKKβ.

hTid-1 Enhances the Stability of IκBα and IκBβ—The observation that hTid-1 suppresses IκB phosphorylation by IKKβ implies that hTid-1 may have an indirect role in protecting the IκB molecules from degradation in proteasomes. We therefore determined whether hTid-1 has any effect on the stability of IκBα and IκBβ. IκBα-HA and IκBβ-HA were transfected into HEK cells. The transfected cells were treated with cycloheximide for 30 min followed by samplings at the indicated time points. As shown in Fig. 4, both IκBα and IκBβ decayed over time; the half-life of IκBα was about 1 h (*top first panel*), while that of IκBβ was less than 30 min (*middle first panel*). In the presence of hTid-1-FLAG however, the half-life of both IκBα and IκBβ appeared to be prolonged (*top second* and *middle second panels*). hTid-1-FLAG itself was stable over the 5-h

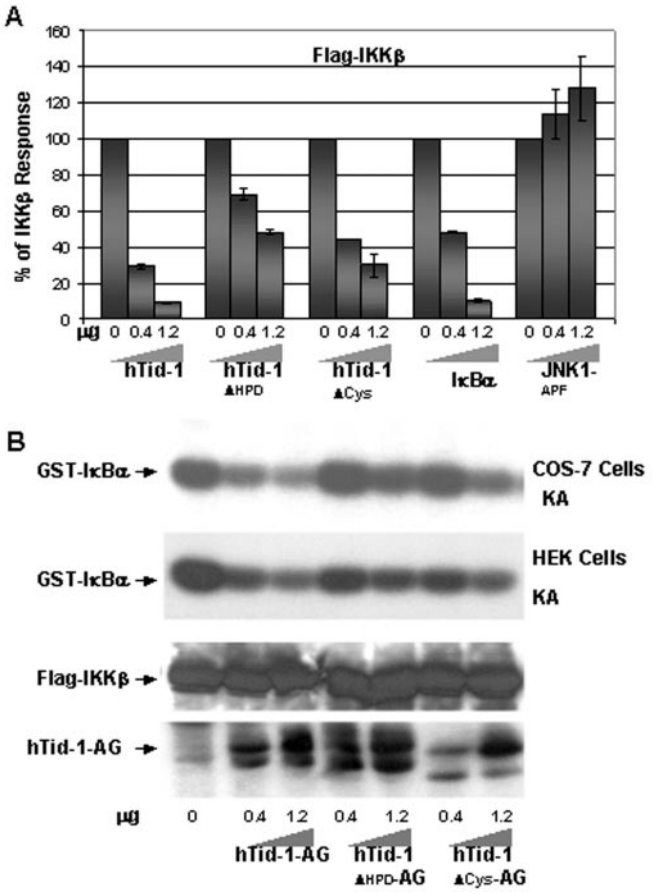

FIG. 3. **Suppression of IKKβ by hTid-1 requires a functional J domain.** *A*, comparison of the suppressive activity of the wild type hTid-1, hTid-$1_{\Delta HPD}$, and hTid-$1_{\Delta Cys}$-FLAG in the reporter assay. NF-κB-dependent β-galactosidase activity was determined in HEK cells co-transfected with fixed amounts of both pNF-κBβ-gal (0.8 μg) and FLAG-IKKβ (0.8 μg) along with various amounts of hTid-1-FLAG (0.4 μg, 1.2 μg), hTid-$1_{\Delta HPD}$ (0.4 μg, 1.2 μg), hTid-$1_{\Delta Cys}$-FLAG (0.4 μg, 1.2 μg), IκBα-HA (0.4 μg, 1.2 μg), or $JNK1_{APF}$ (0.4 μg, 1.2 μg). *B*, *in vitro* suppression of IKKβ by hTid-1 and its mutants. Transfection of wild type hTid-1 (hTid-1-AG) and two hTid-1 mutants (hTid-$1_{\Delta HPD}$-AG and hTid-$1_{\Delta Cys}$-AG) at various DNA amounts indicated with FLAG-IKKβ was performed using both COS-7 and HEK cells. *In vitro* kinase assay was performed, and the kinase activity of IKKβ was shown in the *top two panels*. The *lower two panels* are controls for FLAG-IKKβ, hTid-1-AG, and its mutant protein expression from the whole cellular extracts in transfected HEK cells as detected using anti-FLAGM2 and anti-AG11 antibodies, respectively.

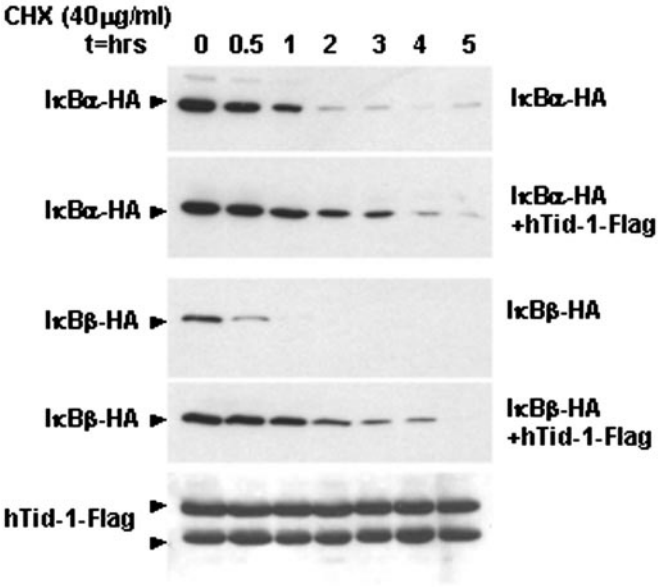

FIG. 4. **hTid-1 enhances the stability of IκBα and IκBβ.** IκBα-HA or IκBβ-HA (1 μg each) was transfected into HEK cells either alone (*top* and *third panels*, respectively) or with hTid-1-FLAG (*second* and *fourth panels*). 24 h following transfection, the cells were treated with cycloheximide (*CHX*, 40 μg/ml) for 30 min, and subsequently cells were collected at indicated time points and lysed immediately in 1% SDS/Tris-Cl, pH 8.0, buffer. Equal amounts of whole cell protein extracts were analyzed by immunoblotting with anti-HA for detection of expression of IκBα-HA and IκBβ-HA or anti-FLAGM2 for analysis of the hTid-1-FLAG protein (*bottom panel*).

period of observation. Thus, it appears that hTid-1, by inhibiting the kinase activity of IKKβ, provides a protective effect on IκBs degradation, enhancing the stability of both IκBα and IκBβ.

hTid-1 is a novel human DnaJ protein whose functions in mammalian cells have not been fully characterized. Several reports indicate that hTid-1 regulates apoptotic and anti-apoptotic processes in response to TNFα stimulation (37) and inhibits IFNγ-induced signaling by complexing with Jak2 kinase and repressing its activity (38). An interaction of a murine homolog mTid-1 with RasGAP protein has also been observed, suggesting that mTid-1 may regulate the Ras signaling pathway (39). We show here that hTid-1 antagonizes NF-κB activity induced by various activators including HTLV-1 Tax, TNFα, and Bcl10 by repressing IKKβ kinase activity.

The molecular mechanism(s) underlying the suppressive activity of hTid-1 on IKKβ remain undefined. Direct binding of hTid-1 to the NF-κB heterodimer is unlikely because hTid-1 does not contain ankyrin repeats that are found in IκBs. It is conceivable that hTid-1 forms a protein complex with the IκBs to prevent their specific phosphorylation by activated IκB kinases and subsequent degradation. Alternatively, the suppressive activity could be mediated through its association with Hsp70 and Hsc70 (32, 38). The finding that the functional J domain of hTid-1 is required for the suppressive activity on IKKβ supports this view. Hsp70 is an inducible protein whose expression is low under physiological conditions but can be induced under stress conditions such as heat shock, oxidation, and heavy metals. In contrast, Hsc70 is expressed constitutively even under non-stressful situation. Induction or activation of heat shock proteins has been reported to be associated with an inhibitory effect on NF-κB (40–43). Indeed, we find that overexpression of an inducible Hsp70 inhibited NF-κB-dependent reporter activity and suppressed *in vitro* IKKβ kinase activity (data not shown). Activation of Hsp70 and Hsc70 as a result of complexing with hTid-1 under stressful and non-stressful conditions, respectively, therefore could lead to repression of the IκB kinase complex and inhibition of NF-κB activity. Tax, by forming a supercomplex with hTid-1 and Hsp70, may abrogate the inhibitory activity of hTid-1 as a part of its multimechanisms in induction of NF-κB activation. Regardless, the discovery of hTid-1 as a novel negative modulator of the IκB kinase complex provides additional insight into the regulation of the NF-κB signaling pathway. Further investigation of the suppressive mechanism of NF-κB by hTid-1 is warranted.

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