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α7 Helix Region of αI Domain Is Crucial for Integrin Binding to Endoplasmic Reticulum Chaperone gp96

*A POTENTIAL THERAPEUTIC TARGET FOR CANCER METASTASIS**

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Background: Integrins are chaperoned by gp96, but the binding region to gp96 remains unknown.
Results: Deletion of α7 helix from αI domain of integrin abolished the interaction between integrin and gp96. Targeting this region by corresponding peptide blocked cell invasion.
Conclusion: We successfully mapped the binding region of integrin to gp96.
Significance: This study will facilitate the development of new cancer therapeutics.

Integrins play important roles in regulating a diverse array of cellular functions crucial to the initiation, progression, and metastasis of tumors. Previous studies have shown that a majority of integrins are folded by the endoplasmic reticulum chaperone gp96. Here, we demonstrate that the dimerization of integrin αL and β2 is highly dependent on gp96. The αI domain (AID), a ligand binding domain shared by seven integrin α-subunits, is a critical region for integrin binding to gp96. Deletion of AID significantly reduced the interaction between integrin αL and gp96. Overexpression of AID intracellularly decreased surface expression of gp96 clients (integrins and Toll-like receptors) and cancer cell invasion. The α7 helix region is crucial for AID binding to gp96. A cell-permeable α7 helix peptide competitively inhibited the interaction between gp96 and integrins and blocked cell invasion. Thus, targeting the binding site of α7 helix of AID on gp96 is potentially a new strategy for treatment of cancer metastasis.

Integrins are a large family of cell surface type I transmembrane receptors that mediate adhesion to the extracellular matrix and immunoglobulin superfamily molecules. At least 24 integrin heterodimers are formed by the combination of 18 α-subunits and 8 β-subunits (1). A wide variety of integrins have been shown to promote cancer cell proliferation, invasion, and survival. For example, in melanoma, the αV subunit has been found to be strongly expressed in both benign and malignant lesions, whereas the β3 subunit is exclusively expressed in vertical growth stage and metastatic disease (2, 3). In addition, increased expression of the integrin α6β4 stimulates the survival of breast cancer cells (4, 5), and elevated expression of integrin α5β1 correlates with decreased survival in patients with lymph node-negative non-small-cell lung carcinoma (6). Moreover, integrin αL is up-regulated in CD44 stimulation-induced adhesion of colon cancer cells (7), and integrin αL, αX, β1, β2, and ICAM are highly expressed in marginal zone B-cell lymphoma (8, 9). Furthermore, integrins on cancer stem cells have also been reported to play essential roles for cancer initiation and progression (10). In recent years, novel insights into the mechanisms that regulate tumor progression have led to the development of integrin-based therapeutics for cancer treatment. Integrin inhibitors, including antibodies, peptides, and nonpeptidic molecules, are considered to have direct and indirect antitumor effects by restricting tumor growth and blocking angiogenesis. Several inhibitors have shown promise in preclinical studies and phase I and phase II trials, but phase III trials have reached no clinically significant results (11–13). Vitaxin, a specific monoclonal antibody that targets the αvβ3 integrin, has shown significant antiangiogenetic effects in preclinical studies and phase I/II trials (14–16). However, phase III trials have thus far shown no significant clinical benefits. Cilengitide is an L-arginine-glycine-L-aspartic acid-based peptide which antagonizes αVβ3 integrins and has been administered to patients with cancers of the breast, lung, and head and neck, but the results of those trials were not sufficiently encouraging to indicate further use in clinical practice (17, 18). Thus, novel integrin inhibitors for cancer therapy need to be discovered.

gp96 (also known as grp94, endoplasmin, and HSP90b1) is the ER-resident member of the Hsp90 family. Its expression is up-regulated by metabolic stress or the unfolded protein response, which results from the accumulation of misfolded proteins in the ER[2] (19–21). gp96 has been implicated in cancer biology. Clinically, gp96 expression correlates with advanced stage and poor survival in a variety of cancers and is closely linked to cancer growth and metastasis in melanoma, breast, prostate, multiple myeloma, lung cancer, and colon cancer (22–

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[2] The abbreviations used are: ER, endoplasmic reticulum; AID, αI domain; TLR, Toll-like receptor; KD, knockdown; EV, empty vector; TAT, trans-activating transduction protein.

29). gp96 has also been found to confer decreased sensitivity to x-ray irradiation (30), and it is required for the canonical Wnt pathway (31).

Recently, our group showed that the maturation of a majority of integrins is dependent on gp96, which folds integrins in the ER and controls their surface expression (32–34). In addition, we have identified a C-terminal loop structure formed by residues 652–678 of gp96 that constitutes the critical client-binding domain for chaperoning both integrins and the Toll-like receptor (35). Interestingly, we previously showed that all of the integrin α subunits that contain the αI domain (AID) are gp96-dependent, suggesting that this domain may play an important role in the gp96-mediated cell surface expression of integrins (33). In this study, we have confirmed this hypothesis and, furthermore, demonstrated that AID is a critical region for integrin binding to gp96. Finally, we show that the AID-based TAT-tagged peptide inhibitor disrupts the interaction between integrins and gp96 and blocks cancer cell invasion.

EXPERIMENTAL PROCEDURES

Cell Lines—All gp96 mutant-transduced PreB leukemia cell lines were generated from parental gp96-null E4.126 PreB cell line, which was a kind gift from Brian Seed (Harvard Univeristy). RAW 264.7 leukemia cell and HCT116 colon cancer cell lines were purchased from ATCC. Phoenix Eco packaging cell line from ATCC was used for retrovirus production. All culture conditions have been previously described in Ref. 36.

Antibodies, Reagents, and Peptides— gp96 N terminus antibody 9G10 and gp96 C terminus antibody SPA851 were purchased from Enzo Life Sciences and detected both endogenous and overexpressed proteins. β-Actin antibody, Myc (9E10), and FLAG antibody were from Sigma Aldrich. HA antibody (clone 16B12) was purchased from Covance, Inc. Biotin-conjugated anti-mouse CD11a (clone M174), CD49d (clone R1–2), CD18 (clone M18/2), TLR2 (clone 6C2), and TLR4 (clone MTS510) antibodies used for flow cytometry were purchased from eBioscience and detected endogenous proteins. TAT-α7 peptide, containing TAT sequence (YGRKKRRQRRR) and amino acids 316–327 of integrin αL, was synthesized by NEO Biolab to >98% purity as verified by HPLC and mass spectrometry. Other reagents were obtained from Sigma-Aldrich unless otherwise specified. H39, a gp96-specific Hsp90 inhibitor of the purine scaffold class, was synthesized using the protocol described in Ref. 37.

Constructs and Site-directed Mutagenesis—Wild type murine integrin αL and β2 cDNA were used as templates for all PCR. Primers for integrin αL are 5′- ATTAGCGGCCGCGCCACCATGAGTTTCCGGATTGCGGG-3′ and 5′-TAATGCGGCCGCTTAAGCATAATCTGGAACATCATATGGATAGTCCTTGTCACTCTCCCGGAGG-3′. Primers for integrin β2 are 5′-ATTAGCGGCCGCGCCACCATGCTGGGCCCACACTCACTG-3′ and 5′-TAATGCGGCCGCCTACAGATCCTCTTCTGAGATGAGTTTTTGTTCGCTTTCAGCAAACTTGGGGTTCATG-3′. Integrin αL ΔAID were constructed by fusion PCR utilizing respective primers with Pfu (Invitrogen). All constructs were subcloned into MigR1 retroviral vector for retrovirus production as described previously (38).

Retrovirus Production and Transduction—MigR1-integrin αL, β2, or AID plasmids were transfected into Phoenix Eco cell line using Lipofectamine 2000 (Invitrogen). Six hours after transfection, medium was replaced by prewarmed fresh culture medium. Virus-containing medium was collected at 48 h after transfection. To facilitate the virus adhesion, spin transduction was performed at 1800 $\times$ *g* for 1.5 h at 32 °C in the presence of 8 μg/ml hexadimethrine bromide (Sigma).

Blasticidin Selection—A blasticidin-resistant gene was bicistronically expressed downstream of the target gene in the MigR1 vector. All transduced PreB or RAW 264.7 cells were selected for a week in RPMI or DMEM culture medium containing 10 μg/ml blasticidin to ensure a relatively homogenous population and comparable expression levels between all mutants.

Pulse-Chase Experiment—HA-tagged integrin αL-overexpressing RAW 264.7 (WT and gp96 KD) cells were incubated with methionine- and cysteine-free medium for 2 h, followed by pulsing with 110 μCi [^{35}S]methionine at 37 °C for 1 h, and chased at 0, 1, 2, and 4 h. Cells were washed with PBS and lysed in PBS containing 5% SDS. Cells were freeze thawed three times to enhance lysis. 200 μg of lysate were immunoprecipitated by using anti-HA antibody, followed by SDS-PAGE and autoradiography.

Flow Cytometry—All staining protocol, flow cytometry instrumentation, as well as data analysis were performed as described previously without significant modifications (34, 36, 39). For cell surface staining, single cell suspension of living cells was obtained and washed with FACS buffer twice. Fc receptor blocking with or without serum blocking was performed depending on individual primary antibody used for staining. Primary and secondary antibodies staining were performed stepwise, with FACS buffer washing in between steps. Propidium iodide was used to gate out dead cells. Stained cells were acquired on a FACS Calibur or FACS verse (BD Biosciences) and analyzed using the FlowJo software (Tree Star).

GST Pulldown Assay—AID of mouse integrin and deletion mutants of α7 helix region of AID were subcloned into pGEX-pMagEmcs vector. GST fusion proteins were isolated on glutathione-Sepharose 4B beads (Amersham Biosciences). Cell lysate was incubated with GST alone or with GST-AID in the presence of 20 mM HEPES, pH 7.2, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MO_4, 0.5% Nonidet P-40, and 1 mM ATP, followed by incubation with glutathione-Sepharose 4B beads at 4 °C overnight, and then washed three times, boiled in Laemmli buffer, and resolved by SDS-PAGE.

Invasion Assay—Cells (1×10^5) were seeded in the upper chamber of a 1% gelatin-coated Transwell membrane (Corning). At 15 h, cells were fixed in 90% ethanol for 10 min and stained with 1% crystal violet for 10 min. Cells in the lower chamber were eluted with 10% acetic acid for 10 min, and the cell number was determined by OD at 595 nm.

Statistical Analysis—The Student's *t* test was used for statistical analysis. $p < 0.05$ was considered significant.

RESULTS

Formation of the Integrin Heterodimer Is gp96-dependent—To test whether gp96 is required for formation of the integrin heterodimer, we used shRNA to knock down gp96 in RAW

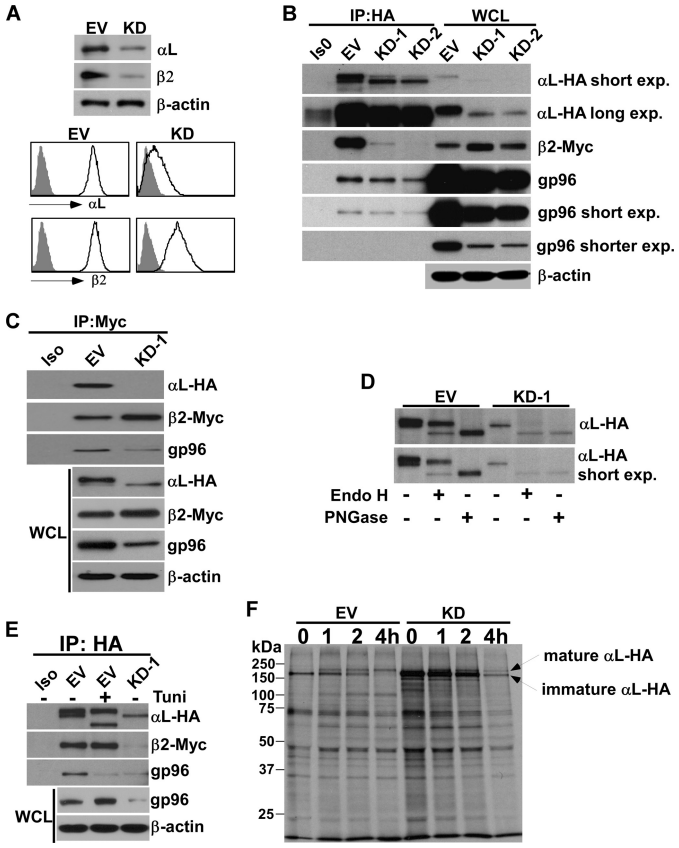

FIGURE 1. **Integrin αL-β2 interaction is gp96-dependent.** *A*, RAW264.7 cells were transduced with either EV or gp96 shRNA (KD), and then levels of endogenous αL and β2 were immunoblotted. Surface expression of αL and β2 was analyzed by flow cytometry. *B*, HA-tagged integrin αL and Myc-tagged β2 were overexpressed in EV-transduced wild type (*EV*) and gp96 knockdown (KD-1, KD-2) RAW264.7 cells. Immunoprecipitation of HA-tagged integrin αL from EV and gp96 KD cells was done, followed by immunoblot (*IB*) for indicated proteins. Whole cell lysates (*WCL*) were used as control. *Iso* indicated immunoprecipitation with isotype control antibody. *Exp.*, exposure. *C*, immunoprecipitation of Myc-tagged integrin β2 from gp96 EV and KD (KD-1) cells, followed by immunoblot for indicated proteins. Whole cell lysates (*WCL*) were used as control. *D*, total lysates of HA-tagged αL-overexpressed EV-transduced and KD-1 RAW 264.7 cells were untreated or treated with endoglycosidase H (*Endo H*) or peptide-*N*-glycosidase F (*PNGase F*), followed by immunoblot for integrin αL using anti-HA antibody. *E*, EV and KD-1 cells were untreated or treated with 5 μg/ml tunicamycin (*Tuni*) for 12 h, followed by immunoprecipitation for indicated proteins. WCLs were used as control. *F*, HA-tagged αL-overexpressed EV-transduced WT and KD-1 RAW 264.7 cells were pulse labeled with [^{35}S]Met, followed by chasing with cold Met for the indicated time point and immunoprecipitation (*IP*) for αL-HA. The precipitated proteins were analyzed by SDS-PAGE and autoradiography.

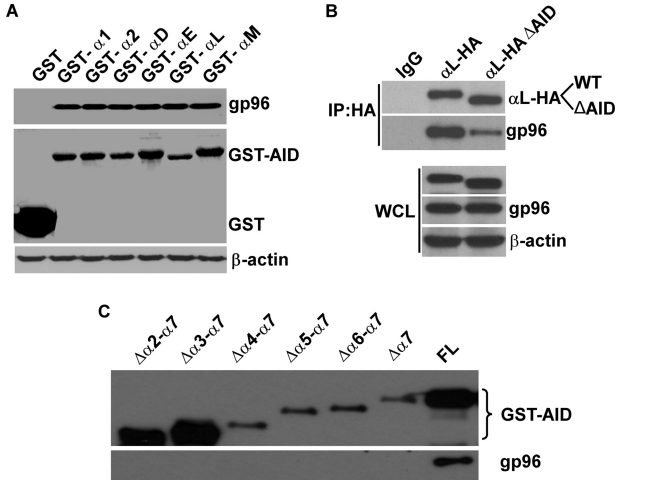

FIGURE 2. **αI domain is critical for αL integrin to interact with gp96.** *A*, AID binds to gp96 *in vitro*. Murine B cell lysates were incubated with GST or GST-AID, recovered by glutathione-Sepharose 4B, and then resolved by SDS-PAGE. The associated gp96 and GST-AID were detected by immunoblot. Equal amount of lysate were used as indicated by β-actin immunoblot. *B*, WT αL-HA or AID deletion mutant (ΔAID) were transiently transfected into HEK293T cells. αL precipitates (IP:HA) were resolved by SDS-PAGE and immunoblotted for indicated proteins. The expression level of αL-HA and ΔAID mutant in the whole cell lysates (*WCL*) were shown. *C*, α7 helix is the critical region of AID to bind to gp96. Sequential deletion mutants of AID were fused with GST. GST pulldown assay was carried out. GST-AID deletion mutants and gp96 were detected by immunoblot. *FL*, full-length integrin αL.

264.7 macrophages. We found that both total and surface expression of αL and β2 were reduced in gp96 knockdown RAW 264.7 cells (KD), comparing with that in wild type cells transduced with empty vector (EV) (Fig. 1*A*). We further overexpressed HA-tagged integrin αL and Myc-tagged integrin β2 in EV-transduced WT or two KD RAW 264.7 leukemia cell lines (KD1 and KD2). We found that the level of αL-HA in KD cells was much less than that in EV-transduced WT cells (Fig. 1*B*). The dimerization of αL-HA and β2-Myc was also reduced dramatically in gp96 KD RAW 264.7 cells, compared with that in EV-transduced WT cells (Fig. 1*B*). Immunoprecipitation of β2-Myc failed to pull down αL-HA in gp96 KD cells, indicating inefficient dimerization between integrin αL and β2 in gp96 KD cells (Fig. 1*C*). This suggests that gp96 is required for integrin αL binding to β2. However, αL-HA presented as a doublet in both EV-transduced WT and KD RAW 264.7 cells (Fig. 1, *B* and *D*). The top band was the major form in EV-transduced WT cells, whereas the lower band was dominant in KD RAW 264.7 cells. The top band was shown to be resistant to endoglycosidase H treatment, suggesting that this is the matured cell surface form of αL-HA, whereas the lower band was sensitive to endoglycosidase H, indicating it as the immature ER form of αL-HA (Fig. 1*D*). Additionally, both bands were sensitive to peptide-*N*-glycosidase F, which cleaves the entire *N*-linked glycan. The immature ER αL-HA was also sensitive to tunicamycin, an *N*-linked glycosylation inhibitor, causing reduction in binding to gp96 even though tunicamycin induced gp96 up-regulation via unfolded protein response. However, the matured cell surface αL-HA was resistant to this blockade and had no change in forming the dimerization with β2-Myc (Fig. 1*E*). Our previous study showed that <5% of gp96 was superglycosylated and preferentially binds to its clientele such as TLR9. Massively increased gp96 upon tunicamycin treatment was deglycosylated and failed to interact with TLR9 (34). All of these observations suggest that *N*-linked glycosylation on both gp96 and its clients are required for their optimal interaction. We also performed the pulse-chase experiment to follow the newly synthesized αL-HA in gp96 KD cells. In EV-transduced WT cells, the mature αL-HA started to show up 1 h after chasing and had completely changed to the mature form 4 h later. However, in gp96 KD cells (KD), the level of αL-HA was dramatically reduced after 4-hour chasing, and a majority of αL-HA remained immature (Fig. 1*F*).

AID Is Crucial for the Interaction between Integrins and gp96—To determine whether AID is required for AID-containing integrin binding to gp96, we generated GST-tagged AID

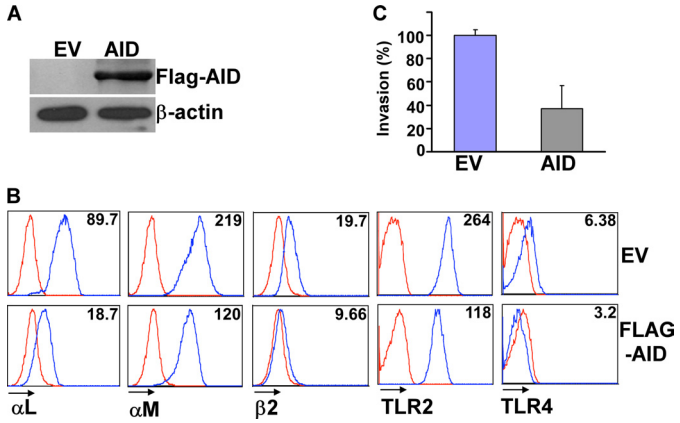

FIGURE 3. **Overexpression of AID results in reduced surface expression of multiple integrins and cell invasion.** *A*, confirmation of expression of FLAG-AID in RAW 264.7 macrophages by immunoblot. *B*, reduced surface expression of multiple gp96 clients (*blue* histogram) by flow cytometry. *Red* histograms represent isotype controls. *Numbers* represent mean fluorescence intensity of integrin or TLR stain as indicated. *C*, invasion potential of EV-transduced or AID-overexpressing RAW 264.7 leukemia cells through an 8-μm diameter Transwell membrane after 15 h of incubation. *, $p < 0.03$.

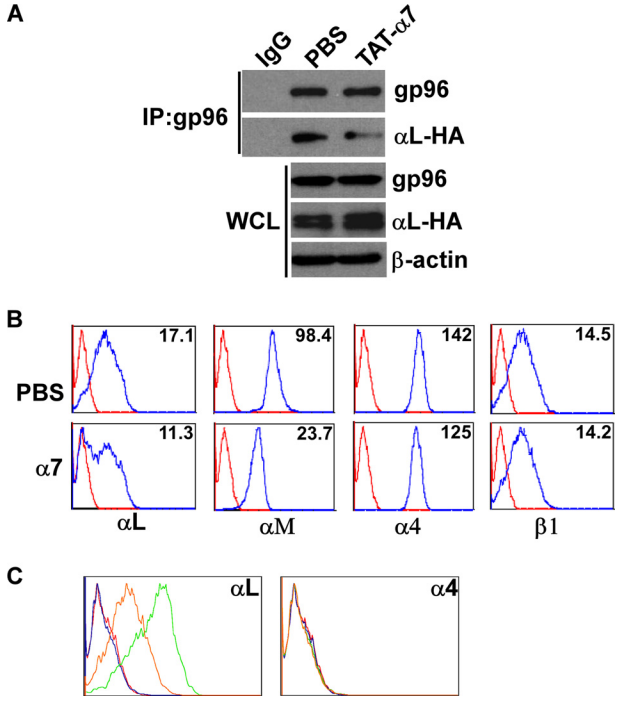

FIGURE 4. **α7 helix peptide blocked interaction between gp96 and αL and surface expression of multiple integrins.** *A*, immunoprecipitation (*IP*) of gp96 was carried out after 10 μM TAT-α7 helix peptide treatment for 12 h, followed by immunoblot for gp96 and αL-HA. Expression level of indicated proteins in whole cell lysates (*WCL*) were verified. β-Actin is shown as a loading control. *B*, PreB cells were treated with PBS or 10 μM TAT-α7 helix peptide for 12 h, and then surface expression of integrin αL, αM, α4, and β1 was measured by flow cytometry. *Numbers* represent mean fluorescence intensity of integrin stain. *C*, CD44-stimulated αL expression was inhibited by cell permeable α7 helix peptide. HCT116 cells were pretreated with 10 μM TAT-α7 peptide for 12 h and then incubated with control 2nd antibody or CD44 cross-link antibody for additional 12 h. Cells were harvested, and flow cytometry was carried out for cell surface integrins. Colors are as follows: *blue*, IgG control; *red*, non-cross-linked; *green*, CD44 cross-link; *orange*, CD44 cross-link + TAT-α7 peptide.

proteins from six AID-contained integrins including α1, α2, αD, αE, αL, and αM subunits. We found that all six GST-tagged AID proteins bound to gp96 (Fig. 2*A*). Moreover, when AID was deleted from integrin αL, the deletion resulted in significantly reduced interaction between integrin αL and gp96 (Fig. 2*B*). These results suggested that AID is a major binding region for integrin association with gp96. To further define which region of AID is critical for binding gp96, sequential deletion mutants of AID were generated. α7 helix is composed of 12 amino acids. Deletion of this region (Δα7) resulted in failure of AID to bind to gp96, indicating that α7 is integral to the binding of AID to gp96 (Fig. 2*C*).

AID Overexpression Decreased Cell Invasion in Vitro—If AID is needed for integrin binding to gp96, then intracellular expression of isolated AID mini-protein in the ER should competitively bind to gp96, thereby reducing gp96 binding and surface expression of multiple endogenous clienteles. To test this hypothesis, we overexpressed FLAG-tagged AID in RAW 264.7 cells by retroviral-mediated transduction (Fig. 3*A*) and found that surface expression of integrin αL, along with αM, β2, TLR2, and TLR4, was indeed decreased (Fig. 3*B*). In addition, AID-overexpressing cells also showed decreased cell invasion in a Transwell system (Fig. 3*C*).

Cell-permeable TAT-α7 Peptide Blocked Interaction between gp96 and Integrin αL—Because the α7 helix region is critical for AID binding to gp96, we synthesized a cell-permeable TAT-tagged α7 helix peptide to test whether or not it competes with the endogenous integrin αL. TAT is an HIV protein that plays a pivotal role in both the HIV-1 replication cycle and in the pathogenesis of HIV-1 infection. An HIV TAT-derived peptide enables the intracellular delivery of cargos of various sizes and physicochemical properties, including small particles, proteins, peptides, and nucleic acids (40). We performed a competition experiment by incubating cells with this TAT-α7 peptide for 24 h prior to cell lysis. We then performed IP analysis to examine the interaction between gp96 and HA-tagged αL integrin. We found that TAT-α7 peptide inhibited the ability of gp96 to interact with αL-HA (Fig. 4*A*). This further supports the suggestion that there is a direct interaction between gp96 and the AID of αL integrin through the α7 helix region. In further support of this hypothesis, we also found that TAT-α7 peptide partially blocked surface expression of integrin αL, αM, and α4, but not β1 (Fig. 4*B*).

CD44 cross-linking on cancer cells has been shown to increase the cell surface expression of integrin αL, resulting in increased cancer invasion (7). To determine whether the α7 helix peptide reduces CD44 cross-linking induced surface expression of integrin αL, we treated the human colon cancer cell line, HCT116, with 10 μM TAT-tagged α7 helix peptide. Such treatment resulted in complete abrogation of CD44-stimulated surface up-regulation of αL (Fig. 4*C*).

TAT-α7 Helix Peptide Prevented Cell Invasion in Vitro—Next, we tested whether TAT-α7 helix peptide can inhibit cell survival and invasion. As shown in Fig. 5*A*, a PreB leukemia cell line was treated with the indicated doses of TAT-α7 helix peptide, which had little effect on cell survival. However, when PreB and RAW 264.7 cells were pretreated with 10 μM of TAT-α7 helix peptide and then incubated in a Transwell system, cell invasion showed significant compromise, compared with PBS-treated cells (Fig. 5*B*). This reduced invasion was also

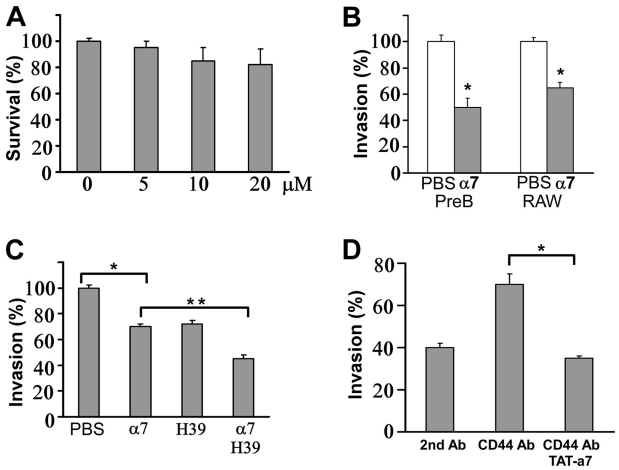

FIGURE 5. **α7 helix peptide blocked cell invasion.** *A*, PreB leukemia cells were treated with the indicated concentrations of TAT-α7 helix peptide. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was carried out. *B*, PreB and RAW264.7 cells were pretreated with PBS or 10 μM TAT-α7 helix peptide for 12 h and then were incubated in a Transwell chamber for an additional 15 h to measure cell invasion. *, $p < 0.05$. *C*, RPMI 8226 myeloma cells were treated with PBS, 10 μM TAT-α7 helix peptide, 5 μM H39 or TAT-α7 plus H39 for 12 h, and then the Transwell assay was performed. *, $p < 0.05$. *D*, HCT116 cells were pretreated with 10 μM TAT-α7 peptide for 12 h and then seeded into a Transwell chamber and incubated with control 2nd antibody (*Ab*) or CD44 antibody with/without a 12-h pretreatment of TAT-α7 peptide for 12 h. The numbers of invaded cells were counted. *, $p < 0.05$.

observed in CD44 antibody-treated HCT116 cells with a pretreatment of the TAT-α7 helix peptide (Fig. 5*D*). We also tested whether this novel peptide inhibitor can potentiate the antitumor effect of H39, a gp96-specific Hsp90 inhibitor of the purine scaffold class (41). H39 inhibits gp96 by directly binding to the ATP-binding pocket but not the client-binding domain of gp96. We found that the TAT-α7 helix peptide and gp96-specific inhibitor, H39, had at least an additive effect on preventing invasion of RPMI 8226 human myeloma cells (Fig. 5*C*).

DISCUSSION

Many integrin-based inhibitors have thus far been introduced to the field for cancer therapy. However, these inhibitors only showed promising results in some preclinical studies, phase I/II clinical trials but largely failed during clinical phase III trials (11–17). The failure of these phase III trials can be ascribed to three causes. 1) It is difficult to deliver the antibodies or peptides to tumors in humans even though preclinical studies show that the drugs have benefits in animal models. 2) Integrin blockade is incomplete due to dose, affinity, or accessibility problems. 3) Most of the inhibitors block the function of a single integrin, and it is possible that blocking multiple integrins could have better therapeutic effects. However, this approach has proven to be difficult because most of the current integrin inhibitors are designed to compete with the ligands that bind to specific integrins. Such a strategy still allows for some ligand binding to other integrins that could trigger the outside-in signaling cascade in tumor cells. Our study is the first to show that AID is required for the interaction between integrin and gp96 (Fig. 2, *A* and *B* and that the α7 helix of AID is critical for binding to gp96 (Fig. 2*C*). Of particular interest, gp96 plays a key role in the folding and cell surface expression of multiple integrin subunits, including α1, α2, α4, αD, αL, αM, αX, αV, αE, β2, β5, β6, β7, and β8 (32, 34, 35, 42), many of which are critically required for tumor growth and metastasis (2–9). In this study, we found that competitive blocking of the gp96-integrin interaction by TAT-α7 helix peptide decreased surface expression and maturation of not only integrin αL but also of other integrins (*i.e.* αM and α4) (Fig. 4, *B* and *C*). Our unique strategy thus allows us to target multiple integrins simultaneously, which is based on integrin substrate-derived peptide to occupy the client-binding site of gp96 to impair maturation of other gp96 clients. We have previously demonstrated that the residues 652–678 of client-binding domain of gp96 are critical for its binding to both integrins and TLRs (35). Thus, it is tempting to speculate that TAT-α7 helix peptide binds and blocks the 652–678 region of the client-binding domain. Further structural studies should not only define the structural basis of gp96-integrin interaction but also facilitate the rational design of inhibitors against this pathway for cancer therapy.

As a proof-of-principle experiment, we found that TAT-α7 helix peptide caused reduction of cell surface expression of multiple integrins (Fig. 4, *B* and *C*), as well as blocked cancer cell invasion *in vitro* (Fig. 5). Further studies are necessary to improve the druggability of this compound, including enhancing its intracellular delivery, its binding affinity to gp96, and its *in vivo* bioavailability and anti-cancer activity. Notwithstanding, chaperone-based and client-specific inhibitors could potentially hold a promise as a new class of therapeutics against cancer in the future.

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