Integrin α3β1, a Novel Receptor for α3(IV) Noncollagenous Domain and a Trans-dominant Inhibitor for Integrin αvβ3*

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Corina M. Borza¹, Ambra Pozzi², Dorin-Bogdan Borza¹, Vadim Pedchenko¹, Thomas Hellmark¹, Billy G. Hudson², and Roy Zent³,‡,§

From the ¹Division of Nephrology, Department of Medicine, Vanderbilt University School of Medicine, and the ²Department of Research Medicine, Veterans Affairs Hospital, Nashville, Tennessee 37232-2372 and the ³Kidney Research Laboratory, Lund University, S-22185 Lund, Sweden

Exogenous soluble human α3 noncollagenous (NC1) domain of collagen IV inhibits angiogenesis and tumor growth. These biological functions are attributed to the binding of α3NC1 to integrin αvβ3. However, in some tumor cells that express integrin αvβ3, the α3NC1 domain does not inhibit proliferation, suggesting that integrin αvβ3 expression is not sufficient to mediate the anti-tumorigenic activity of this domain. Therefore, in the present study, we searched for novel binding receptors for the soluble α3NC1 domain in cells lacking αvβ3 integrin. In these cells, soluble α3NC1 bound integrin α3β1; however, unlike αvβ3, α3β1 integrin did not mediate cell adhesion to immobilized α3NC1 domain. Interestingly, in cells lacking integrin α3β1, adhesion to the α3NC1 domain was enhanced due to activation of integrin αvβ3. These findings indicate that integrin α3β1 is a receptor for the α3NC1 domain and trans-dominantly inhibits integrin αvβ3 activation. Thus integrin α3β1, in conjunction with integrin αvβ3, modulates cellular responses to the α3NC1 domain, which may be pivotal in the mechanism underpinning its anti-angiogenic and anti-tumorigenic activities.

The NC1⁴ domain of certain α-chains of type IV collagen display activity as inhibitors of angiogenesis and tumor growth. The capacity of the exogenous α1NC1 and α2NC1 domains to disrupt basement membrane assembly, blocking tissue development in vivo, was first described in Hydra vulgaris (1). Subsequent to these observations, we were the first to demonstrate that the recombinant α2NC1 and α3NC1 domains of human collagen IV potently inhibited tumor growth and angiogenesis by binding to endothelial cells in an integrin αvβ3-dependent manner (2). Since these initial observations, NC1 domains of different collagen IV chains have emerged as a new class of anti-angiogenic and anti-tumorigenic molecules (3, 4). These domains exert their effects by direct binding to tumor and endothelial cells where they induce apoptosis and/or inhibit cell proliferation. The mechanism of action of the NC1 domains is attributed to their interactions with integrins, transmembrane receptors for extracellular matrix components (5). NC1 domains bind to distinct integrins, for example α1NC1 to integrin α1β1 (3), α2NC1 to integrins α1β1, α1β3, and αvβ5 (6, 7), and α3NC1 to integrins αvβ3 and αvβ5 (2, 4, 8).

The α3NC1 domain is the best characterized of these domains and its anti-tumorigenic effects are predominantly ascribed to its potent anti-angiogenic properties. Endothelial cells adhere to this domain in an integrin αvβ3-dependent manner (2, 8). Furthermore, integrin αvβ3 is thought to mediate α3NC1-dependent inhibition of endothelial cell proliferation (9). In addition to its anti-angiogenic effects, the α3NC1 domain or peptides derived from its C-terminal third also inhibit melanoma cell growth both in vivo and in vitro in an integrin αvβ3-dependent manner (10–13). Interestingly, the tumor cell inhibition is cell type specific, as the α3NC1 domain does not inhibit the proliferation of PC-3 prostate carcinoma cells or 786-O renal carcinoma, although these cells express functionally active integrin αvβ3. This dichotomy raises the issue of how integrin αvβ3 mediates the anti-tumorigenic activity of the α3NC1 domain. One possible mechanism is by transdominant inhibition and activation of αvβ3 by other integrins. In this context, αvβ3 affinity is higher in cells deficient in integrin α5β1 and increased expression of α5β1 reduces αvβ3-mediated adhesion and migration (14). Similarly, integrin α3β1 has been demonstrated to alter the function of other integrins and the formation of stress fibers in mouse keratinocytes (15).

We therefore undertook an unbiased approach to determine whether other integrin receptors might bind the α3NC1 domain and modulate integrin αvβ3 functions. Utilizing flow cytometry, we found that integrin α3β1, a non-classical collagen binding integrin, is a novel receptor for soluble α3NC1 domain. Furthermore, we provide evidence that integrin αvβ3 affinity is negatively modulated by integrin α3β1. Thus integrin

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* The abbreviations used are: NC1, noncollagenous domain of type IV collagen; mAb, monoclonal antibody; HUVEC, human umbilical vein endothelial cells; FITC, fluorescein isothiocyanate; TBS, Tris-buffered saline; BSA, bovine serum albumin.

¹ To whom correspondence may be addressed: Rm. C3210, Dept. of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232-2372. Tel.: 615-322-2089; Fax: 615-322-7156; E-mail: Corina.Borza@vanderbilt.edu.

² To whom correspondence may be addressed: Rm. C3210, Dept. of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232-2372. Tel.: 615-322-7298; Fax: 615-322-7381; E-mail: Billy.Hudson@vanderbilt.edu.

³ To whom correspondence may be addressed: Rm. C3210, Dept. of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232-2372. Tel.: 615-322-4632; Fax: 615-322-4689; E-mail: Roy.Zent@vanderbilt.edu.

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α3β1 may play a key role in mediating the anti-tumorigenic activity of the α3NC1 domain.

**EXPERIMENTAL PROCEDURES**

**Materials**—Monoclonal antibodies (mAb) to FLAG peptide (M2) and fibronectin were from Sigma. Cyclo-RGD peptide was purchased from Peptides International (Osaka, Japan). mAb EB3 (an antibody to the minor Goodpasture epitope of the α3NC1 domain of human collagen IV) (16) was purified on protein G-agarose from hybridoma supernatants. Anti-integrin mAbs Ha2/5 (anti-mouse β1), H9.2B8 (anti-mouse αβ), 2C9G2 (anti-mouse β3), HMα2 (anti-mouse α2), Ha31/8 (anti-mouse α2), HMα5-1 (anti-mouse α5), and GoH3 (anti-human α6, which cross-reacts with mouse) were purchased from Chemicon (Temecula, CA), WOW-1 Fab, recognizing the active αv integrin subunit was provided by Dr. Sanford Shattil (University of California San Diego) (17). FITC-conjugated anti-mouse IgG1 antibodies, FITC-conjugated anti-rat and phycoerythrin-conjugated anti-hamster antibodies were purchased from Pharmingen and His6 mAb FITC-conjugated was purchased from Covance Research Products (Berkley, CA).

**Cell Culture**—Human umbilical vein endothelial cells (HUVECs), obtained from BioWhittaker, were grown in EGM-2 MV medium (BioWhittaker) and used between passages 4 and 8. Human melanoma HT-144 and lung carcinoma A549 were from ATCC and maintained in McCoy modified medium or F12K medium supplemented with 10% fetal bovine serum.

Renal papilla cells from kidney of E18 integrin α3-deficient mice (B10) and integrin α3-deficient cells reconstituted with the human α3 integrin subunit (R10) (kindly provided by Dr. Jordan Kreidberg, Childrens Hospital, Boston, MA) were cultured as described previously (18). Mouse colon carcinoma cells CT26 were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics, as described (19). Temperature-sensitive, conditionally immortalized mouse pulmonary micro vascular endothelial cells were isolated and cultured as described previously (20).

**Recombinant Proteins**—In all studies where soluble human α3NC1 domain has shown anti-tumorigenic activity in vivo, the domain contains a 12-residue collagenous sequence at the N terminus containing an RGD motif in addition to the 232-amino acid noncollagenous region. This domain was originally produced by collagenase digestion of native basement membranes, to ensure the preservation of epitopes for Goodpasture lent to tumstatin (NCBI accession number AAF72632 (GenBankTM)) in other reports (4, 23). All the assays reported in this manuscript, except where indicated, were performed with this recombinant protein. Recombinant human NC1 domains that cross-react with mouse) were purchased from Pharmingen (Temecula, CA), WOW-1 Fab, recognizing the active αv integrin subunit was provided by Dr. Sanford Shattil (University of California San Diego) (17). FITC-conjugated anti-mouse IgG1 antibodies, FITC-conjugated anti-rat and phycoerythrin-conjugated anti-hamster antibodies were purchased from Pharmingen and His6 mAb FITC-conjugated was purchased from Covance Research Products (Berkley, CA).

To evaluate integrin expression levels cells were incubated with specific integrin antibodies for 45 min, washed twice, and then incubated with phycoerythrin-conjugated anti-mouse or anti-hamster antibodies and analyzed with a FACScan.

**Proliferation Assays**—CT26 cells (5 x 10⁶ cells/well) were seeded into 96-well plates and treated with α3NC1 at various concentrations in Dulbecco’s modified Eagle’s medium supplemented with 2% fetal bovine serum and proliferation was measured using [³H]thymidine incorporation as described (26).

**Solid Phase Ligand Binding Assays**—Purified integrin αβ1 (Chemicon) was coated on 96-well plates at 1 μg/ml in TBS overnight at 4 °C. The plates were blocked with TBS with 0.1% BSA and 0.3% Tween 20. The NC1 domains preincubated with mAb EB3 were added to integrin coated wells in binding buffer (TBS, 0.1% BSA, 1 mm MgCl₂, 5 mm octyl glucoside) and incubated for 90 min at room temperature. After extensive washes (TBS, 1 mm MgCl₂, 0.05% Tween), the bound proteins were detected with alkaline phosphatase-conjugated anti-mouse IgG antibodies. p-Nitrophenyl phosphate substrate (Sigma) was added to wells, and the absorbance was measured at 405 nm.

**Statistical Analysis**—The Student’s t test was used for comparisons between two groups, and analysis of variance using Sigma-Stat software for statistical differences between multiple groups. p < 0.05 was considered statistically significant.
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RESULTS

The Binding of Soluble α3NC1 Domain to HUVECs Is Integrin-dependent—The α3NC1 domain is thought to mediate its effects primarily by binding to αv integrins. These receptors were identified using cell adhesion assays or affinity chromatography where the NC1 domain was immobilized on a fixed substratum. However, soluble, and not the immobilized NC1 domain, is the exogenous physical form used in cell culture and animal experiments. Hence, we developed a flow cytometry-based cell binding assay to determine whether soluble α3NC1 domain interacts with other membrane receptors.

As integrins αvβ3 and αvβ5, the major α3NC1 domain-binding receptors (8), are highly expressed on endothelial cells, we initially performed our flow cytometry assay on HUVECs. As shown in Fig. 1A, the α3NC1 domain bound to HUVECs, and this binding was significantly inhibited by EDTA, suggesting that the principal receptors for this ligand were integrins. As HUVECs express αvβ3, the principal integrin to which the α3NC1 domain binds, we investigated whether this binding was dependent on the RGD sequence located at the N terminus of the α3NC1 domain. Thus, the α3NC1 domain, with or without the RGD motif, was utilized for flow cytometric assays. Both forms of the domain bound to HUVECs with equal efficiency, indicating that the binding is independent of the RGD sequence (Fig. 1B). The binding was specific for the α3NC1 domain as C6, a recombinant protein derived from the α1NC1 domain, which contains the epitope for mAb EB3 (22), did not bind to HUVEC (Fig. 1C). To determine whether binding of the α3NC1 domain was primarily dependent on αv integrins, flow cytometry was performed in the presence of cyclo-RGD peptides, which block the ligand binding site of αv integrins. Surprisingly, these peptides did not affect α3NC1 domain binding (Fig. 1D), suggesting that non-RGD binding receptors for the α3NC1 domain are present on HUVEC. To determine whether α3NC1 domain binding was dependent on a β1 integrin, similar flow cytometry assays to those described above were performed on mouse endothelial cells. Human cells cannot be used for antibody-dependent inhibition experiments because functional blocking antibodies, like EB3, are of the IgG1 isotype and interfere with detection by flow cytometry. A small decrease in α3NC1 binding to mouse endothelial cells was seen in the presence of an antibody to β1 integrin (Fig. 1E), suggesting that αβ1 integrins could potentially be receptors for the α3NC1 domain in the absence of integrin αvβ3.

α3NC1 Domain Binds to Integrin α3β1—To identify these putative αβ1 receptors, we used mouse colon carcinoma CT26 cells, as these cells do not express integrin β3 and only very low levels of integrin αv (Ref. 27 and data not shown). As seen in Fig. 2A, despite the lack of integrin αvβ3 expression, the α3NC1 domain bound to CT26 cells. To confirm whether a β1 integrin was the receptor, we preincubated the cells with anti-β1 integrin antibody before addition of the α3NC1 domain. This anti-
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Integrin α3β1 binding to α3NC1 requires the C-terminal third of α3NC1. A, schematic representation of α3NC1 and α1NC1 domains indicating the amino acid positions where fragments of α3NC1 were replaced with homologous fragments from α1NC1 in the α3/α1NC1 chimeras. The black boxes indicate the binding sites for integrin αβ3, 56–75 and 185–203, and the gray box indicates the mAb EB3 epitope. B, analysis of α3/α1NC1 chimeras binding to immobilized integrin α3β1. Immobilized integrin α3β1 (1 μg/ml) was incubated with α3/α1NC1 chimeras (10 μg/ml) and binding measured as described in the legend to Fig. 3. The data represent the mean ± S.D. of triplicate wells. The experiment was repeated twice with similar results. *, difference between 133 and C6 was significant with p < 0.05 and between 333 and C6 with p < 0.01 (**). C, flow cytometry of CT26 cells incubated with 20 μg/ml α3/α1 chimeras indicated. Shown is a representative experiment of two independent experiments performed.

B10 cells. This difference is exclusively attributed to integrin α3β1 since the levels of expression for other αβ1 and β4 integrins is similar in both cell populations (26). Furthermore the expression of integrins β3 and αv was similar in the two cell lines (2.19 ± 0.58-fold versus 1.966 ± 0.91-fold increase in fluorescence β3 integrin and 7.003 ± 4.14 versus 4.89 ± 2.42 for αv integrin in B10 and R10 cells, respectively).

To verify that integrin α3β1 does indeed bind α3NC1, a solid phase ligand binding assay was performed. The α3NC1 domain bound to immobilized integrin α3β1, while C6, used as a negative control, showed no binding (Fig. 3B). Thus, integrin α3β1 is a receptor for the α3NC1 domain.

Integrin α3β1 Binding to α3NC1 Domain Requires Residues 177–232—Three distinct binding sites for integrin αβ3 have been mapped in the α3NC1 domain: a RGD site at the N terminus, a second site between amino acids 56–75 and a third site between amino acids 185–203 (8) (Fig. 4A). To identify the binding sites for integrin α3β1, we used α3/α1NC1 chimeric proteins in which fragments of α3NC1 were replaced with homologous fragments of α1NC1 (1-3-1, 3-3-1, 1-3-3) (Fig.

FIGURE 3. Integrin α3β1 is a receptor for α3NC1 domain. A, flow cytometry of mouse integrin α3-null (B10) or reconstituted with the human integrin α3 subunit (R10) cells incubated with increasing amounts of soluble α3NC1. α3NC1 binding was calculated as the difference between the absorbance of sample incubating with increasing concentrations and bound proteins were detected with mAb EB3. Specific binding was calculated as the difference between the absorbance of sample with and without integrin. The data represent the mean ± S.D. of triplicate wells. The experiment was repeated twice with similar results. *, difference between α3NC1 and C6 was significant with p < 0.01.

To identify the α subunit, we analyzed integrin expression of CT26 cells by flow cytometry and Western blot analysis and found high levels of integrin α2, α3, α5, and α6 (data not shown). Blocking antibodies to mouse α2, α5, α6, and αv integrins had no significant effect on α3NC1 binding (data not shown), suggesting that these integrins were not involved. Thus, integrin α3β1 was the best candidate receptor for the α3NC1 domain. Since neutralizing antibodies to mouse integrin α3β1 are not available, integrin α3-null cells were used for subsequent experiments.

Integrin α3-null cells from kidney papillae (B10) and integrin α3-null cells reconstituted with human α3 integrin (R10) have been characterized (18, 26). As shown in Fig. 3A, R10 cells bound soluble α3NC1 domain at much higher levels than
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A. 

B. 

Figure 5. Soluble αNC1 domain inhibits CT26 cell proliferation, while immobilized αNC1 domain does not support CT26 cell adhesion. A, CT26 cells (5 × 10⁵ cells/well) were plated onto 96-well plates and subsequently treated with αNC1 at the concentrations indicated. Two days later cells were labeled with [³H]thymidine (1 mCi/well) and cell proliferation evaluated as described under “Experimental Procedures.” The data represent the mean ± S.D. of quadruplicate wells. Three independent experiments were performed with similar results. Differences between untreated and treated cells (*) were significant with p < 0.05. B, adhesion of CT26 cells to immobilized αNC1 domain at concentrations indicated or to fibronectin (10 μg/ml). Values represent the mean ± S.D. of triplicate wells. Three experiments were performed with similar results.

Figure 6. Integrin α3β1 expression inhibits integrin αvβ3 activation. A, binding of WOW-1 to R10 and B10 cells was determined by flow cytometry in the presence or absence of αNC1 domain. WOW-1 binding is expressed as a relative percentage of WOW-1 positive cells compared with R10 cells. This is a representative of two independent experiments. B, adhesion of B10 and R10 cells on increasing concentrations of immobilized αNC1 domain in the presence or absence of Mn²⁺. The data represent the mean absorbance and standard deviation of triplicate wells. This is a representative of four separate experiments. Differences between adhesion of B10 and R10 cells (*) were significant with p < 0.01.

to immobilized αNC1 domain (Fig. 5B) at concentrations where endothelial cells adhered well (data not shown). CT26 cells, however, adhered well to their natural ligand fibronectin (Fig. 5B). Thus, the binding of soluble αNC1 domain to CT26 cells expressing integrin α3β1 correlates with the inhibition of cell proliferation. However, in the absence of integrin αvβ3, CT26 cell adhesion to immobilized αNC1 domain is minimal.

Integrin α3β1 Is a Trans-dominant Inhibitor of Integrin αv—Full-length and fragments of the αNC1 domain do not decrease cell proliferation in all tumor cells that express integrin αvβ3 (4, 9, 23, 28). However, as shown above, integrin α3β1 binds to the αNC1 domain in solution. Furthermore, it is well known that the affinity of αv integrins can be altered by the transdominant effect of other integrins (14, 15). To test whether integrin α3β1 can indeed alter the affinity of αv integrins, we measured the activation status of αv integrins in B10 and R10 cells. WOW-1, an antibody that recognizes only the αv integrin (18), and only 1.63% of R10 cells expressed activated αv integrin (17), was used to measure αv integrin activation. R10 and B10 cells express similar but low levels of αv integrin (18), and only 1.63% of R10 cells expressed activated αv integrins. In contrast, the percentage of B10 cells (4.25%) that bound WOW-1 in the absence of the αNC1 domain was more than double that of R10 cells (Fig. 6A). This difference was enhanced in B10 cells (8.15%) by incubation with the αNC1

4A). The binding site for mAb EB3, required for detection, is located in the middle of all the chimeric proteins. In a solid phase binding assay, no binding to immobilized integrin α3β1 was detected with 1-3-1 or 3-3-1 chimeras, while both the 1-3-3 chimera and αNC1 bound to integrin α3β1 (Fig. 4B). Similar results were obtained by flow cytometry on CT26 cells with the 1-3-3 and αNC1 domain showing significant binding, while the 1-3-1 and 1-3-3 chimera bound poorly (Fig. 4C). Thus, the C-terminal third of the αNC1 domain encompassing residues 177–232 is required for binding to α3β1 integrin.

CT26 Cells Proliferation Is Decreased by αNC1—As HUVEC proliferation is decreased by the αNC1 domain via interactions with αv integrins and cell adhesion to the αNC1 domain is primarily mediated by the same integrins (8, 28), we determined whether either of these cell functions could be induced by αNC1 in the absence of integrin αvβ3. As shown in Fig. 5A, CT26 cell proliferation was inhibited in a dose-dependent manner with increasing concentrations of soluble αNC1 domain. In contrast, there was minimal adhesion of CT26 cells
domain suggesting that α3NC1 domain per se can activate integrin αvβ3. Together these data suggest that αv integrins are transdominantly inhibited by the expression of integrin α3β1.

Cell adhesion assays were then performed to determine whether this transdominant effect changed cell adhesion on immobilized α3NC1 domains. As shown in Fig. 6B, R10 cells adhered significantly less than B10 cells. When Mn2+ was added, R10 and B10 cell adhesion to α3NC1 increased, but the enhancement was greater with the R10 cells (5.5-fold versus 1.8-fold for 1.6 μg/ml α3NC1). Thus α3β1-dependent transdominant inhibition of αv integrin affinity decreases cell adhesion to the α3NC1 domain.

Since most cells expressing αv integrins also express α3β1 integrin, we examined whether the expression levels of α3β1 influences integrin αv affinity. We screened for human cell lines that expressed varying levels of integrins α3β1 and αvβ3, as functional blocking antibodies to human α3 integrin are available. We found that melanoma HT-144 cells express high levels of αvβ3 and α3β1, HUVECs express high levels of integrin αvβ3 but slightly lower levels of integrin α3β1 than HT-144 cells, and the lung carcinoma cells A549 express similar levels of integrin α3β1 but lower levels of integrin αvβ3 compared with HT-144 cells (Fig. 7A). As expected, the adhesion of these cells to the α3NC1 domain correlated with the levels of αvβ3 integrin with HT-144 and HUVEC cells adhering significantly more than A549 cells (Fig. 7B). In the presence of α3 integrin blocking antibody, adhesion of A549 cells increased significantly. Interestingly, the adhesion of HT-144 cells to the α3NC1 domain also increased when integrin α3β1 was blocked, although not to the same extent as for A549 cells. In contrast, this antibody had a minimal effect on HUVECs (which express the lowest levels of integrin α3β1). Adhesion of all three cell lines to the α3NC1 domain is integrin αvβ3-dependent as antibodies to this integrin completely inhibited their adhesion (Fig. 7B). These data suggest that modulating both the expression and occupancy of integrin α3β1 alters the affinity and adhesive functions of integrin αvβ3.

DISCUSSION

The efficacy of soluble α3NC1 domain as an anti-tumorigenic agent has been ascribed to its binding to αvβ3 integrin (2, 9, 11, 23, 28–32). This binding was defined by either integrin αv-dependent cell adhesion or affinity chromatography with immobilized NC1 domain. To identify whether the physical immobilization influenced integrin binding, a flow cytometry assay was devised to explore the cellular receptors for soluble α3NC1 domain. This assay is similar to a novel flow cytometry method recently described to quantify integrin affinity and avidity changes at the single cell level (25). Unexpectedly, the nonclassical collagen receptor, integrin α3β1, was shown to bind α3NC1 domain. CT26 cell proliferation was inhibited by soluble α3NC1 domain; however, these cells only minimally adhere to immobilized α3NC1 domain. These results reveal the limitation of assays that rely on immobilized ligands, when screening for receptors for soluble NC1 domain. In addition, we demonstrate that functional blocking of integrin α3β1 transdominantly increases αv-integrin affinity for the α3NC1 domain. Taken together, these results raise the possibility that, in addition to αv integrins, integrin α3β1 might play a role in the anti-tumorigenic effects of the α3NC1 domain by directly affecting cell proliferation or altering the affinity of αv integrins on either tumor or endothelial cells.
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The identification of receptors that bind to the soluble form of NC1 domain is important as this is the physical state in which they are administered and exert their biological effects. Our result that integrin α3β1 binds soluble α3NC1 domain, but cell adhesion is not mediated upon mobilization of the same ligand, highlights this point. Thus only performing assays with NC1 domain attached to fixed substrata might not identify critical receptors that are expressed by tumor cells and may mediate anti-tumoral effects.

We showed that the integrin α3β1 binding site encompasses residues 177–232 of the α3NC1 domain. This region overlaps with residues 185–203 that constitutes a peptide that promotes adhesion of human melanoma cells and inhibits their proliferation in vitro (12). This peptide is proposed to mediate its effects by interacting with the integrin αvβ3 CD47-integrin-associated protein complex (31). However, our data suggest the mechanism of action of this peptide on tumor cell proliferation might be mediated through integrin α3β1 as CT26 cells do not express integrin αvβ3, and residues 185–203 are encompassed in the integrin α3β1 binding site on the α3NC1 domain.

Overexpression of the C-terminal fragment of α3NC1 (residues 183–232) inhibits tumor growth of B16F1 melanoma cells in vivo (12). Furthermore mice treated with a plasmid DNA encoding the α3NC1 domain develop smaller CT26 cell-derived tumors than control animals (27). Our results suggest that these in vivo anti-tumorigenic effects of the α3NC1 domain might be mediated by its binding to integrin α3β1, rather than via proposed αvβ3-mediated effects on endothelial cells.

The mechanisms whereby the α3NC1 domain induces its anti-tumorigenic effects are becoming increasingly complex. Some groups proposed that its action is mediated by anti-angiogenic activity residing in residues 54–132, while the C-terminal derivatives do not inhibit angiogenesis (4, 9, 23, 28, 30). Furthermore the same group proposed that these effects are mediated by both endogenous and exogenously administered α3NC1 domain. In contrast, others found that polypeptides encompassing amino acids 179–208 within the C terminus have both anti-angiogenic (33) and anti-tumorigenic effects (11, 13, 29, 31–33). These effects have only been observed with exogenously administered α3NC1 domain or when the α3NC1 domain is produced by tumor cells. We now demonstrate that the C-terminal third of the α3NC1 domain interacts with integrin α3β1 where it decreases tumor cell proliferation in vitro. Taken together the data suggest that the anti-angiogenic effects of the α3NC1 domain in vivo might be mediated by both the C and N terminus of the domain. In contrast, the anti-tumorigenic effects are mediated only by the C terminus of the domain, via interactions with either integrin αvβ3 or α3β1. The relative expression levels of these integrins on tumor cells in vivo might determine their response to exogenous α3NC1 domain exposure.

We demonstrated that both expression levels and occupancy of integrin α3β1 by ligand alters integrin αvβ3 affinity. Endothelial cells and most carcinomas express both of these integrins in varying amounts. Thus, soluble NC1 domain interactions with integrin α3β1 might alter αvβ3-dependent cell functions by transdominant activation or inhibition. This mechanism of modulating integrin function is well described in αv integrins. Expression of integrin α5β1 reduces αvβ3-mediated adhesion (14), and αvβ3-mediated endothelial cell migration is altered by the ligation state of integrin α5β1 (14). Furthermore, antibodies against αvβ3 can inhibit both integrin α5β1-mediated phagocytosis (34) as well as α3β1/αvβ3-mediated cell adhesion to α4-laminin (35). Moreover, the observation that cells lacking integrin α3β1 adhere to fibronectin and collagen better than wild type cells (15) shows that integrin α3β1 is also a trans-dominant inhibitor of integrin activation. Thus, our data that the ligation state and expression levels of integrin α3β1 can exert significant alterations on integrin αvβ3 function might explain why certain tumor cells are not responsive to the anti-proliferative effects of the α3NC1 domain despite their expression of integrin αvβ3.

In conclusion, we demonstrate that the α3NC1 domain binds integrin α3β1, and cell proliferation is decreased in cells that lack integrin αvβ3 but express integrin α3β1. In addition, the ligation state of integrin α3β1 can modulate the affinity of αvβ3 integrin, a key integrin required for tumor angiogenesis. Thus, integrin α3β1 might be a direct mediator of the anti-tumorigenic actions of the α3NC1 domain. In addition, interactions between the α3NC1 domain and α3β1 integrin might play a key role in the inhibition of tumor angiogenesis by altering αvβ3 integrin affinity on endothelial cells.

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