ITGAV targeting as a therapeutic approach for treatment of metastatic breast cancer

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Abstract: During tumorigenesis and metastasis, integrins regulate localization and activity of proteolytic enzymes that remodel the extracellular matrix. Previous studies have demonstrated blocking of αVβ3 to effectively inhibit proliferation, angiogenesis, and the survival of various cancer cell types. However, little is known about the functional role of the integrin subunit alpha-V gene (ITGAV) in metastatic breast cancer. In this study, ITGAV knockdown was used to identify the molecular mechanism by which ITGAV promotes tumorigenesis, metastasis, proliferation, invasion, and cellular self-renewal. The effectiveness of an ITGAV antagonist, cilengitide, for breast cancer treatment was investigated in vivo. Analysis of publicly available data demonstrated that overexpression of ITGAV was associated with poor relapse free survival of breast cancer patients. Silencing of ITGAV inhibited cell proliferation, invasion, and self-renewal of breast cancer cell lines by altering expression of BCL2 and PXN. The use of cilengitide significantly reduced lung metastasis in a metastatic breast cancer animal model. In conclusion, overexpression of ITGAV contributes to breast cancer metastasis through upregulation of PXN. Targeting ITGAV is a potential treatment for metastatic breast cancer as well as primary breast tumors with high ITGAV expression. ITGAV expression levels may be useful predictors of patient treatment and outcome responses.

Keywords: ITGAV, cilengitide, breast cancer, metastasis

Introduction

Worldwide, breast cancer is the most common cancer in women, affecting 2.09 million women with an age-standardized rate of 46.3 per 100,000 in 2018 [1]. There is a 25% increase in the incidence of breast cancer since 2012 [2]. Of note, loco-regional relapse and distant metastasis remain the major causes of breast cancer-related mortality. Up to 30% of breast cancer patients relapse with distant metastasis even when diagnosed at an early stage [3]. Therefore, there is an urgent need to identify genes associated with breast cancer progression and distant metastasis in order to develop targeted therapies that improve clinical outcomes.

During cancer development, epithelial cancer cells acquire a mesenchymal phenotype by epithelial to mesenchymal transition (EMT), a key process involved in carcinogenesis and metastasis. Cancer cells that have undergone EMT show increased cell motility, reduced intracellular adhesion, stem-cell like characteristics, and are capable of cell renewal and differentiation, conferring tumor heterogeneity [4, 5]. Heterogenous cancer cells, with acquired phenotypes, have increased invasiveness that results in cancer progression and metastasis. Fundamental components of the extracellular matrix (ECM) are fibrous proteins, such as fibronectin and collagen, that regulate cell adhesion and cell attachment [6]. When cancer cells metastasize and invade, EMT-induced degradation of the cytoskeleton and ECM disrupts tight junctions and adherence between cells, which allow cancer cells to disseminate to distant organs [4, 5]. Degradation of the ECM can be achieved by dissociation or blocking of cellular receptors, such as integrins [7].
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Integrins are heterodimeric cell surface receptors that regulate the localization and activity of proteolytic enzymes that promote cancer cell invasion and migration during ECM remodeling [7]. Formed by transmembrane α and β subunits, there are 24α and 9β human integrin subunits [8]. Expression of integrins varies in different cell types and tissues. For instance, αIIbβ3 is only expressed by platelets and α5β4 expression is limited to keratinocytes, while α4β1 is widely distributed in different cell types and tissues [9]. Further, several integrins are ligand-specific. Integrin α1β1 is a fibronectin-specific receptor, while α5β3 binds a wide range of fibrous proteins such as fibronectin, fibrinogen, and vitronectin [10]. During tumor progression and metastasis, integrin expression profiles are altered in a manner that enhances cell invasion and migration [11]. Moreover, integrins promote cancer invasion and metastasis by interacting with other cell types, such as endothelial cells and fibroblasts in the tumor microenvironment [10]. During tumor progression, growth factors such as vascular endothelial cell growth factor (VEGF) and basic fibroblast growth factor (bFGF) released by tumor cells induce expression of integrins in angiogenic endothelial cells, thereby promoting the formation of new blood vessels [12].

Associations between integrin expression and cancer progression have been reported. The α2β1, α3β1, and α5β3 integrins all contain the integrin subunit alpha-V (ITGAV). Each regulates angiogenesis through a distinct pathway [11]. Overexpression of α2β1 has been associated with breast cancer metastasis and tumor progressiveness [10, 13]. Overexpression of ITGAV has been reported in several solid tumors including bladder, colorectal, prostate, and breast [14-17]. Changes in integrin expression activate downstream proteins through phosphorylation of the mitogen-activated protein kinase (MAPK) cascade. The paxillin (PXN) gene is a 68-kDa tyrosine-containing protein that acts as an adaptor protein that integrates integrin, adhesion molecule, and growth factor signals [18]. PXN interacts with integrins during matrix organization and tissue remodeling by destabilizing focal adhesions, such as the linkage between PXN and actin filaments [19]. PXN is overexpressed in many cancers with that overexpression resulting in activation of ERK and Src signaling pathways [18]. Overexpression of PXN has also been reported to increase chemoresistance in lung cancer [20].

Cilengitide is an ITGAV antagonist, which targets α5β1 and α5β3 and reduces angiogenesis. Cilengitide was first developed as a novel therapy for glioblastoma [21]. Several registered U.S. National Institutes of Health clinical trials have used cilengitide as a single agent or in combination with other agents for treatment of metastatic or unresectable cancers including breast, lung, and prostate [22]. In vivo preliminary results have shown cilengitide to reduce breast cancer bone metastasis [23]. However, the mechanistic basis for this result is unresolved. An understanding of the molecular mechanism(s) of ITGAV-mediated breast cancer tumorigenesis and metastasis would facilitate development of potential drug targets.

Hence, the aims of this study were to delineate ITGAV-mediated regulatory pathway in breast cancer and to explore the potential use of cilengitide for the treatment of metastatic breast cancer. Integrin-mediated signaling cascades are important contributors to cancer development. Treatment decisions that include individual gene expression profiles would allow for stratification of patient subsets with similar gene expression characteristics. Inclusion of ITGAV in those expression profiles may further improve treatment efficacy.

Methods

Ethics approval and consent to participate

Ethics approval of animal experiments was obtained from the Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong. The collection and storage of tissue samples from breast cancer patients were approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority West Cluster. Written informed consent was obtained from all participants.

Cell lines and transfection

Five human breast cancer cell lines, MDA-MB-231 (MB231), MDA-MB-468 (MB468), SKBR3, MCF7, and T-47D, were used in this study. MB231, MB468, and T-47D were cultured in RPMI-1640 medium. MCF7 was cultured in DMEM/F12 medium and SKBR3 was cultured in DMEM medium. Cells were cultured in the respective medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Life tech-
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Technologies, Carlsbad, CA, USA) and 1% antibiotic-antimycotic (anti-anti) (Gibco) and kept at 37°C in a humidified atmosphere containing 5% carbon dioxide. All transfection experiments were performed with Lipofectamine 3000 for 3 days (Invitrogen, Life technologies, Carlsbad, CA, USA) according to the manufacturer’s instructions.

RNA extraction and real-time polymerase chain reaction (PCR)

Total RNA from cells and tumor tissues was extracted using a Qiagen RNeasy mini kit (Qiagen, Hilden, Germany). Extracted RNAs were reverse-transcribed into cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions. Target gene expression was determined with a LightCycler 480 Real-time PCR system (Roche, Basel, Switzerland) by the comparative threshold cycle (Ct) method. Expression levels were calculated with the formula $2^{-\Delta\Delta\text{Ct}}$. Beta-actin (β-actin) was used as an internal control to calculate relative fold change.

Cell viability assay

Cell viability of transfected cells or cells treated with cilengitide was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium reduction assay. Briefly, 5,000 cells in 100 µl of culture medium were seeded into 96-well flat-bottom culture plates in triplicate and were incubated for 1 day before transfection or drug treatment. After 3 days, culture medium was removed and replaced with 100 µl of culture medium containing 3% MTT. Medium containing 3% MTT was removed after 2 hours incubation. Dimethylsulfoxide (DMSO) was added to each well, followed by a 30 minute incubation at room temperature. Absorbance at 570 nm was detected with a microplate photometer (Thermo Scientific, USA).

Tumorsphere assay

Cells were seeded into 24-well ultra-low attachment plates in serum free medium supplemented with 20 ng/ml human recombinant EGF (Gibco), 20 ng/ml FGFb (Merck Millipore), 1X N2 supplement (Gibco), and 1X anti-anti. After 7 days, the number of tumorspheres was determined with a microscope.

Soft agar colony formation assay

Culture medium containing 10% FBS, 1X anti-anti, and 0.5% noble agar were loaded into each well of a 6-well plate as the bottom layer and allowed to solidify for 30 minutes. Untreated cells and transfected cells resuspended in culture medium containing 10% FBS, 1X anti-anti and 0.3% noble agar at a density of 5,000 cells/well were loaded over the bottom layer and allowed to solidify for 30 minutes. Cells were cultured for 17 days to allow formation of colonies. Colonies were stained with crystal violet for quantification.

Cell invasion assay

Invasion potential of transfected cells was assessed in 24-well BioCoat Matrigel invasion chambers (BD Biosciences) according to the manufacturer’s protocol. Briefly, trypsinized cells were resuspended in serum free medium at a density of $2\times10^4$ cells/ml. Cells were incubated for 24 hours and then washed with PBS to remove non-invasive cells. Invaded cells were stained with crystal violet for 1 hour and chambers were then washed with PBS before quantification by microscope.

Immunofluorescence staining

Cells were plated into 12-well plates containing coverslips in each well. After overnight incubation, cells were transfected with siRNA for 3 days. Cells were fixed with 4% paraformaldehyde for 15 minutes and then permeabilized with 0.1% Triton X-100 in PBS for 1 hour. Permeabilized cells were incubated with primary antibodies diluted in blocking solution overnight at 4°C. After washing, cells were incubated with Alexa fluor-conjugated secondary antibodies and DAPI (4',6-diamidino-2-phenyldole), used as nuclear counterstain. Cells were visualized with a Nikon Eclipse E600 microscope.

Western immunoblot

Protein expression of integrin αv was examined by western blot analysis. Cell pellets were lysed with ice-cold buffer containing proteinase and phosphatase inhibitors. Protein concentrations in lysed cells were quantified using the Bio-Rad DC$^\text{TM}$ protein assay. Forty µg of protein was mixed with loading buffer and resolved by SDS-polyacrylamide gel electrophoresis. Proteins
were transferred to a Polyvinylidene difluoride (PVDF) membrane followed by 1 hour of membrane blocking. Membranes were immunoblotted with anti-ITGAV (Abcam, 1:1000) and anti-β-actin (CST, 1:5000) primary antibodies overnight at 4°C, followed by incubation with peroxidase-conjugated secondary antibodies for 1 hour. Membranes were developed with enhanced chemiluminescence (ECL) and exposed to X-ray film (FUJI photo Film, Tokyo, Japan) for visualization. Protein intensity was quantified using ImageJ.

**Immunohistochemistry (IHC)**

Anti-Ki67 (CST) and anti-ITGAV (Abcam) were used to examine protein levels in formalin-fixed, paraffin-embedded xenograft tumors. Protein expression of ITGAV (Abcam) was examined in formalin-fixed, paraffin-embedded paired tumor and adjacent normal tissues from breast cancer patients. In brief, tissue sections (6 μm) were dewaxed and rehydrated with ethanol. Tissue sections were incubated with 10 mM citrate buffer solution and 3% hydrogen peroxide, followed by 1 hour incubation with blocking solution at room temperature. After blocking, sections were incubated with primary antibodies overnight at 4°C. Tissue sections were incubated with secondary antibodies for 30 minutes and visualized using Nikon Eclipse E600 microscope using ultraView Universal DAB detection kit (Ventana).

**Flow cytometry**

Apoptotic rate and stem-like phenotypes of breast cancer cells were investigated using flow cytometry. All experiments were performed using FACSCalibur flow cytometry (Becton Dickinson) and were analyzed by FlowJo. For apoptosis assessment, cells were washed and resuspended in 50 μl of binding buffer containing 2.5 μl of annexin V-FITC and 2.5 μl of propidium iodide (PI). Resuspended cells were incubated for 15 minutes and 300 μl of buffer added to the resuspended cells before quantification.

The stem-like phenotype of breast cancer cells was characterized by cell surface markers CD44<sup>high</sup>/CD24<sup>low</sup> and aldehyde dehydrogenase (ALDH) enzymatic activity. For cell surface marker expression, cells were incubated with fluorescently-conjugated anti-CD24 and anti-CD44 in the dark for 20 minutes at room temperature before quantification. For ALDH enzymatic activity, cells were incubated at 37°C with ALDEFLUOR assay buffer containing ALDH substrate for 40 minutes. Cell aliquots stained with diethylaminobenzaldehyde (DEAB) were used as negative controls.

**Tumorigenicity**

The expression of ITGAV was investigated in a human xenograft breast cancer model and a metastatic breast cancer model in NOD/SCID mice. For the human xenograft breast cancer model, 1×10<sup>6</sup> cells/ml (100 μl) were injected into the mammary fat pad of the mice. For the metastatic breast cancer model, breast cancer cells were trypsinized and resuspended in PBS at a concentration of 5×10<sup>6</sup> cells/ml (200 μl) for intravenous injection. Mice were sacrificed after 6 weeks and tumors extracted for histological examination.

**Clinical data analysis**

Relapse-free survival (RFS) analysis of overall breast cancer, luminal A, luminal B, HER2, and TNBC was conducted with the Kaplan Meier (KM) plotter for breast cancer based on the 2017 version of the database with probe ID ITGAV (202351_at) [24].

**Statistical analysis**

Statistical analysis was performed using ANOVA or Student’s t test with SPSS, release 20 or PRISM 5. All p-values are two-sided with a P<0.05 considered statistically significant.

**Results**

**ITGAV is highly expressed in the tumor tissue of breast cancer patients**

To investigate the expression levels of ITGAV in breast cancer, IHC was performed in paired tumor and adjacent normal tissue of breast cancer patients. Results demonstrated ITGAV to be highly expressed in tumor tissue when compared to adjacent normal tissue (Figure 1).

**ITGAV expression is selectively linked to RFS of breast cancer patients**

To assess the prognostic value of ITGAV in different intrinsic subtypes of breast cancer, the association between ITGAV expression and RFS was analyzed using publicly available mi-
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Croarray data. Overexpression of ITGAV in primary tumors was strongly associated with poor RFS in luminal B, HER2, and triple-negative breast cancer patients (Figure 2A-E).

Silencing of ITGAV inhibits cell proliferation, invasion and migration of breast cancer cells

To investigate whether there is differential ITGAV expression in different breast cancer molecular subtypes, baseline gene expression levels of five breast cancer cell lines were evaluated. There was no significant difference in ITGAV baseline expression level for any of the cell lines (Figure 3A). Cell proliferation, invasion, and migration potential for different breast cancer subtypes were assessed in vitro by knockdown of ITGAV in MB231 and T47D cells. ITGAV-silencing was confirmed by qPCR, western blot (Figure 3B), and by immunofluorescence staining of MB231 cells (Figure 3C). Cell proliferation of MB231 and T47D was assessed after silencing of ITGAV with siRNA or the antagonist, cilengitide. Silencing of ITGAV with siRNA significantly inhibited cell proliferation of MB231 and T47D (Figure 3D). As well, treatment with cilengitide significantly inhibited cell proliferation of MB231 and T47D (Figure 3D). Further, silencing ITGAV with siRNA and cilengitide also significantly inhibited cell proliferation of MCF7, MB468, and SKBR3 with varying degrees of effect (Supplementary Figure 1). Colony formation by ITGAV-siRNA transfected cells was significantly inhibited in soft agar, anchorage-free cultures (Figure 3F). These results demonstrate breast cancer invasiveness to be compromised by ITGAV silencing (Figure 3G).

Effect of ITGAV upregulation on breast cancer cell tumorigenesis and metastasis

To investigate the expression levels of integrins during cancer progression, gene expression of several integrins was examined in MB231-induced tumorspheres. Stem cell markers COX2, NOTCH2, SNAI2, as well as ITGAV, were significantly upregulated in tumorspheres (Figure 4A). Furthermore, the CD44\textsuperscript{high}/CD24\textsuperscript{low} subpopulation was increased in subsequent passages of the tumorspheres, suggesting an increase in stem-cell like features (Figure 4B). Expression of ITGAV and Ki67 was higher in tumorsphere-induced tumors than in MB-231-induced tumors (Figure 4C). Further, silencing ITGAV with siRNA significantly reduced tumorsphere formation (Figure 4D). High levels of ALDH activity have been associated with stem-cell like properties. Analysis of ALDH by flow cytometry demonstrated silencing of ITGAV to significantly reduce the number of ALDH-positive cells (Figure 4E). These data suggest that ITGAV confers stem cell-like properties on breast cancer cells.

Expression of PXN is correlated with ITGAV expression

To delineate genes involved in ITGAV-mediated regulatory pathways, gene expression in MB231 cells after silencing of ITGAV was com-
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Figure 2. Kaplan-Meier plot of relapse-free survival (RFS) of breast cancer patients, stratified by expression of \textit{ITGAV} in their primary tumors. A. Kaplan-Meier plot of breast cancer patients. B. Kaplan-Meier plot of breast cancer patients with luminal A subtype. C. Kaplan-Meier plot of breast cancer patients with luminal B subtype. D. Kaplan-Meier plot of breast cancer patients with HER2 subtype. E. Kaplan-Meier plot of breast cancer patients with triple-negative subtype. \(P\) values were calculated using log-rank test.
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Figure 3. Silencing of *ITGAV* inhibits cell proliferation, invasion and migration of breast cancer cells. A. Expression of *ITGAV* in five breast cancer cell lines. B. Relative expression of *ITGAV* after siRNA silencing in MB231 and T47D cells. C. Immunofluorescence images of MB231 and *ITGAV*-siRNA transfected cells. D. Effect of *ITGAV*-siRNA on cell proliferation of MB231 and T47D cells. E. Effect of Cilengitide on cell proliferation of MB231 and T47D cells. F. Effect of *ITGAV*-siRNA on colony formation abilities of MB231 cells. Representative images of colonies induced from MB231 (left) and *ITGAV*-siRNA transfected cells (right). G. Effect of *ITGAV*-siRNA on cell migration of MB231 cells. Representative images (10× magnification) of invaded cells of MB231 (top) and *ITGAV*-siRNA transfected cells (bottom). All experiments were performed in triplicate. *P<0.05, **P<0.01 and ***P<0.005 are considered statistically significant.

pared to parental cells by Partek pathway analysis software. Silencing of *ITGAV* resulted in downregulation of *BCL2* in both MB231 and T47D (Figure 5A). Notably, *PXN*, which encodes for the focal adhesion protein paxillin, was also downregulated after silencing of *ITGAV* in both MB231 and T47D (Figure 5B).

Overexpression of *ITGAV* promotes lung metastasis in vivo

Expression of *ITGAV* was compared between primary tumors extracted from the mammary fat pad and metastatic tumors isolated from the lung. Higher expression of *ITGAV* and *PXN* was observed in metastatic tissues when compared to primary tumors (Figure 6A, 6B). These results suggest that upregulation of *ITGAV* promotes breast cancer tumorigenesis and metastasis through regulation of *PXN*. Furthermore, treatment with the *ITGAV* antagonist, cilengitide, significantly decreased lung metastatic area percentage and proliferative index in comparison to the control group (Figure 6C, 6D). Taken together, these data suggest that *ITGAV* upregulation plays a key role in the promotion of lung metastasis.
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A

ITGAV

COX2

Relative fold change

MB231   G1   G2

G1

FL2-H: CD34

G2

FL2-H: CD44

G3

FL2-H: CD44

Relative fold change

MB231   G1   G2

NOTCH2

SNAI2

% G5A+ in CD44+ cells

G1

FL1-H: CD44

G2

FL1-H: CD44

G3

FL1-H: CD44

Relative fold change

MB231   G1   G2

FL2-H: CD44

FL2-H: CD44
Figure 4. Upregulation of ITGAV in breast cancer tumorigenesis and metastasis. A. Expression of COX2, ITGAV, NOTCH2 and SNAI2 in tumorspheres. B. Representative images of CD44+/CD24- subpopulation in primary (G1) (top left), secondary (G2) (top right), and tertiary (G3) (bottom left) tumorspheres. C. Representative images of ITGAV and Ki67 expressions in tumor tissues induced by MB231 and MB231-induced tumorspheres. D. Effect of ITGAV-siRNA on tumorsphere formation. Representative images (40×) of tumorspheres induced from MB231 (left) and ITGAV siRNA transfected cells (middle). E. Representative images of ALDH flow cytometry of MB231 (left) and ITGAV siRNA transfected cells (right). All experiments were performed in triplicate. *P<0.05 and **P<0.01 are considered statistically significant.
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**Figure 5.** Expressions of PXN and BCL2 are correlated with ITGAV expression. Relative expression of BCL2, PXN, CDH1, ZEB1 and UCP2 after siRNA silencing in MB231 and T47D cells. All experiments were performed in triplicate. *P<0.05 and **P<0.01 are considered statistically significance.

**Figure 6.** Overexpression of ITGAV mediates lung metastasis in vivo. (A, B) Relative expression of (A) ITGAV and (B) PXN in metastatic tissues (MT) when compared with primary tumors (PT). (C) Percent metastatic area in the lung was quantified in control mice (n=4) and mice treated with cilengitide (n=4) after tail vein injection of MB231 cells (5 visual fields per mice). (D) Representative photographs of H&E-stained and expression of ki67 of lung sections (20×). *P<0.05 is considered statistically significance.
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Discussion

Metastasis is one of the major causes of death of breast cancer patients even for those diagnosed at an early stage. Finding effective molecular targets to prevent/treat metastasis will help to improve the prognosis for these patients. Integrins are adhesion molecules that play a pivotal role in cancer progression by modulating cell-cell and cell-ECM interactions. Cancer cells that have undergone EMT show increased motility and reduced cell adhesion, suggesting a critical role for integrins in cancer progression. As such, integrins are an appealing target for cancer therapy.

High expression of the αβ3 integrin has been demonstrated in tumors and angiogenic cells [11]. Overexpression of the αβ3 integrin has been reported in various types of cancers including glioblastoma, melanoma, ovarian, pancreatic, prostate, and breast cancer [10, 11, 23, 25-27]. With regard to ITGAV, only limited reports have evaluated expression levels and the functional role of ITGAV in cancer progression. The integrin αv subunit pairs with multiple beta-subunits to form a number of integrin receptors. A previous in vivo study demonstrated that αβ3 promoted organ-specific breast cancer metastasis to bone and lung [28]. Herein, breast cancer ITGAV overexpression conferred stem-cell like properties in vitro and lung tissue metastasis in vivo, indicating a potential role for ITGAV in breast cancer progression. Knockdown of ITGAV in breast cancer cells inhibited proliferation, invasion, and colony formation, consistent with previous results for colorectal cancer, laryngeal, and hypopharyngeal carcinoma [15, 29]. Waisberg et al. reported overexpression of ITGAV to be associated with higher progression and spread of colorectal cancer. A direct correlation between ITGAV and Ki67 was also reported [15]. Further, ITGAV overexpression is an unfavorable prognostic marker for several other cancers including stomach, liver, and pancreatic cancer [30, 31]. Herein, ITGAV silencing downregulated BCL2 and PXN in vitro and treatment with cilengitide reduced lung metastasis and expression of Ki67 in vivo. KM analysis using publicly available data demonstrated a strong correlation of ITGAV overexpression and poor prognosis for breast cancer patients. The relationship between ITGAV and PXN expression and the association of that relationship with breast cancer metastasis requires further investigation.

Integrin inhibitors have been investigated for therapeutic application in both preclinical and clinical studies of various types of solid cancers. For example, LM609 is a mouse monoclonal antibody that targets the human αβ3 integrin, inhibiting angiogenesis [32]. It was the first integrin antagonist assessed in a preclinical study as a therapeutic cancer intervention [10]. LM609 inhibits angiogenesis by suppressing FGF-2 and TNF-a signaling [33]. Further, inhibited tumor invasion, tumor growth, and organ-specific metastasis to bone have been demonstrated for LM609 with breast cancer and melanoma [32, 34]. Due to the success of LM609 in preclinical studies, humanized versions of LM609, such as Vitaxin and etaracizumab have been developed to investigate potential clinical applications for cancer treatment [35]. Vitaxin was the first humanized form of LM609 tested in a clinical setting [36] and was later reformulated as etaracizumab (Abegrin) [11]. Etaracizumab is an αβ3 antagonist that blocks the binding of vitronectin and osteopontin to integrins, inhibiting angiogenesis and metastasis [33]. Combinations of etaracizumab and antiangiogenetic agents have produced promising outcomes in ovarian cancer [27]. The therapeutic potential of etaracizumab is under investigation in several clinical trials for treatment of solid tumors as well as treatment of psoriasis and rheumatoid arthritis [11]. Other integrin αv antagonists such as CNT0 95 and GLPG0187 also have antitumor activity in breast [17], prostate [16], and bladder cancer [37].

Cilengitide is a cyclic arginylglycylaspartic acid (RGD) pentapeptide that specifically targets αβ3 and α6β3 integrin receptors [38]. Cilengitide is the most advanced integrin inhibitor, initially used in preclinical studies of melanoma and glioblastoma [32, 38]. Subsequent preclinical studies of various types of cancer have investigated cilengitide as a therapeutic cancer treatment modality. Cilengitide has been shown to inhibit metastasis of breast cancer [23, 25, 26] and osteosarcoma [39]. Consistent with these previous findings, our results demonstrate cilengitide to inhibit breast cancer proliferation in a dose-dependent manner, inhibit lung cancer metastasis, and reduce Ki67 expression.

Conclusion

In conclusion, high levels of ITGAV expression were demonstrated in cancer metastatic tissue and in cancer cells with stem-cell like proper-
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ties. As such, ITGAV should be considered as a potential therapeutic target for treatment of metastatic breast cancer as well as primary breast tumors with high ITGAV expression. Such treatment may improve therapeutic efficacy and treatment outcomes.

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Disclosure of conflict of interest

None.

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[31] Human Protein Atlas [www.proteinatlas.org].


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Supplementary Figure 1. A. Effect of ITGAV-siRNA on cell proliferation of MCF7 cells. B. Effect of ITGAV-siRNA on cell proliferation of MB468 cells. C. Effect of ITGAV-siRNA on cell proliferation of SKBR3 cells. D. Effect of Cilengitide on cell proliferation of MCF7 cells. E. Effect of Cilengitide on cell proliferation of MB468 cells. F. Effect of Cilengitide on cell proliferation of SKBR3 cells. All experiments were performed in triplicate. *p<0.05, **p<0.01 and ***p<0.005 are considered statistically significance.