Mutational and Functional Analysis Reveals ADAMTS18 Metalloproteinase as a Novel Driver in Melanoma

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Abstract
The disintegrin-metalloproteinases with thrombospondin domains (ADAMTS) genes have been suggested to function as tumor suppressors as several have been found to be epigenetically silenced in various cancers. We performed a mutational analysis of the ADAMTS gene family in human melanoma and identified a large fraction of melanomas to harbor somatic mutations. To evaluate the functional consequences of the most commonly mutated gene, ADAMTS18, six of its mutations were biologically examined. ADAMTS18 mutations had little effect on melanoma cell growth under standard conditions, but reduced cell dependence on growth factors. ADAMTS18 mutations also reduced adhesion to laminin and increased migration in vitro and metastasis in vivo. Melanoma cells expressing mutant ADAMTS18 had reduced cell migration after short hairpin RNA–mediated knockdown of ADAMTS18, suggesting that ADAMTS18 mutations promote growth, migration, and metastasis in melanoma. Mol Cancer Res; 8(11): OF1–13. ©2010 AACR.

Introduction
It is widely accepted that genetics plays a major role in cancer development (1). The progression of melanoma, which is one of the most aggressive forms of skin cancer (2), is accompanied by a series of genetic changes that affect at least several oncogenes and tumor-suppressor genes (3). Further identification of such genes in melanoma is crucial to promote our understanding of the disease and to develop successful molecular targeted therapies. In this study, we systematically evaluated the disintegrin metalloproteinases with thrombospondin domains (ADAMTS) genes through their comprehensive mutational analysis in melanoma.

The ADAMTS gene family is part of a superfamily of zinc-based proteases, the metzincins (4). The matrix metalloproteinase enzymes, which also belong to the metzincins superfamily, have recently been shown to be highly mutated in melanoma (5). All ADAMTS proteins have proteolytic potential, but have not yet been studied systematically with respect to their ability to systematically delivered drugs makes them excellent therapeutic targets.

In the current study, we examined melanoma samples for somatic mutations in 19 human genes that encode ADAMTS proteins. Remarkably, we found that one ADAMTS gene, ADAMTS18, which was highly mutated in melanoma, was previously found to be a candidate cancer gene (CAN gene) in large-scale whole exome sequencing of colorectal cancer (9, 10). In addition to the ability of the mutated versions of this gene to cause increased proliferation of melanoma cells, we found that they increased cell migration and metastasis. These results suggest that genetic alteration of ADAMTS18 plays a major role in melanoma tumorigenesis.

Materials and Methods

Tumor tissues
Tissue and melanoma cell lines used for the discovery and first validation in this study were described previously (5).

For the melanoma second validation set, optimum cutting temperature–embedded frozen clinical specimens were
obtained from Melanoma Informatics, Tissue Resource, and Pathology Core, and the Central Nervous System Tissue Bank at The University of Texas M.D. Anderson Cancer Center under institutional review board–approved protocols. Hematoxylin and eosin (H&E)–guided dissection and isolation of DNA from the tumor-enriched isolates has been described previously (11).

PCR, sequencing, and mutational analysis of melanoma samples

PCR and sequencing was done as previously described (5, 7, 12). The primary-phase mutation screen was analyzed using Consed (13). Variants were called using Polyphred 6.11 (14) and DIPDetector, an indel detector for improved sensitivity in finding insertions and deletions. Sequence traces of the secondary screen were analyzed using the Mutation Surveyor software package (SoftGenetics).

To increase our confidence that the mutations in the M.D. Anderson set, for which no matched normal DNA sample was available, did not represent germline polymorphisms, we searched the corresponding exons of ADAMTS18 in a total of 145 DNA samples and detected no abnormalities.

Construction of wild-type and mutant ADAMTS18 expression vector

Human ADAMTS18 (NM_199355.2) was cloned by PCR as previously described (5) using a clone purchased from Open Biosystems with primers in Supplementary Table S5. The PCR product was cloned into the mammalian expression vector pCDF-MCS2-EF1-Puro (Systems Biosciences, Inc.) or pCDNA3.1 (−) (Invitrogen) via the Xba I and Not I restriction sites. The G312E, P452S, C638S, Q904X, Q1002X, and P1035S point mutants were made using Phusion PCR for site-directed mutagenesis using the primers listed in Supplementary Table S5.

Cell culture and transient expression

Metastatic melanoma tumor lines were maintained as previously described (15). HEK 293T cells were purchased from American Type Culture Collection and maintained in complete DMEM supplemented with 10% fetal bovine serum, 1× nonessential amino acids, 2 mmol/L l-glutamine, and 0.75% sodium bicarbonate. A375 cells were purchased from Open Biosystems, and two were confirmed previously described (5). HEK 293T cells were transfected with Lipofectamine 2000 reagent (Invitrogen) at a 6:1 ratio with DNA (μL:μg) using 3 to 5 μg of plasmid DNA per T75 flask.

Immunoprecipitation and Western blotting

Transfected cells were gently washed three times in PBS and then lysed using 0.5 to 1.0 mL of 1% NP40 lysis buffer [1% NP40, 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, Complete Protease Inhibitor tablet, EDTA-free Roche], 1 μmol/L sodium orthovanadate, 1 mmol/L sodium fluoride, and 0.1% β-mercaptoethanol per T-75 flask for 20 minutes on ice. Lysed cells were scraped and transferred into a 1.5-mL microcentrifuge tube. Extracts were centrifuged for 10 minutes at 14,000 rpm at 4°C. The supernatant (500 μL) was immunoprecipitated overnight by using 20 μL of anti-FLAG (M2) beads (Sigma-Aldrich). Conditioned medium was immunoprecipitated as previously described (5). The immunoprecipitates were washed and subjected to SDS-PAGE and Western blotting as previously described (16). The primary antibodies used in our analysis were antiFLAG horseradish peroxidase conjugated (Sigma-Aldrich) and anti-α-tubulin (Calbiochem-EMD Biosciences).

Pooled stable expression

To make lentivirus, pCDF-MCS2-EF1-Puro ADAMTS18 constructs were cotransfected into HEK 293T cells seeded at 1.5 × 10^6 per T75 flask with pVSV-G and pFIV-34N (kind gifts from Todd Waldman, Georgetown University) helper plasmids using Lipofectamine 2000 as described by the manufacturer. Virus-containing medium was harvested 48 to 60 hours after transfection, filtered, aliquoted, and stored at −80°C.

A375 cells were seeded at 1.5 × 10^6 per T75 flask 24 hours before infection. Lentivirus for ADAMTS18 (wild-type WT), G312E, P452S, C638S, Q904X, Q1002X, and P1035S or empty vector control were used to infect A375 cells as previously described (17). Stable expression of ADAMTS18 proteins (WT and mutants) was determined by SDS-PAGE analysis followed by immunoprecipitation and immunoblotting with anti-FLAG and anti-α-tubulin to show equivalent expression among pooled clones.

Mel-STR cells were seeded at 1.5 × 10^6 per T75 flask 24 hours before transfection with ADAMTS18 (WT, G312E, P452S, C638S, Q904X, Q1002X, and P1035S) or empty vector control in pcDNA3.1(−) using Fugene6 (Roche 11814443001) as per the manufacturer’s protocol. Transfected cells were selected using normal complete growth medium supplemented with 300 μg/mL G418 and pooled for future experiments. Stable expression of ADAMTS18 proteins (WT and mutants) was determined by SDS-PAGE analysis followed by immunoprecipitation and immunoblotting with anti-FLAG and anti-α-tubulin to show equivalent expression among pools.

Lentiviral short hairpin RNA

Constructs for stable depletion of ADAMTS18 were obtained from Open Biosystems, and two were confirmed to efficiently knock down ADAMTS18 at the message and protein levels. Lentiviral stocks were prepared as previously described (5). Melanoma cell lines (5T, 12T, 85T, and A375) were infected with short hairpin RNA (shRNA) lentiviruses for each condition (vector and scrambled controls) and three independent ADAMTS18–specific shRNAs whose sequences are presented in Supplementary Table S6). Selection and growth were done as described above. Stably infected pooled clones were tested in functional assays.

Reverse transcription-PCR

Total RNA was extracted from pooled clones of melanoma cells A375 stably knocked down for endogenous
ADAMTS18 following the manufacturer’s protocol for RNase Mini Kit (Qiagen). Total RNA was eluted in 30 μL DEPC-treated dH2O. A total of 1 μg of total RNA was used for single-strand cDNA synthesis using a SuperScript III First Strand kit (Invitrogen). CDNA was amplified using the oligo dt20 primer supplied in the kit. To test for loss of ADAMTS18 message, we used 1 μL of cDNA in the PCR with either ADAMTS18 primers (forward primer 5′-acccctgattcagttcaca-3′ and reverse primer 5′-tggacgccatctcgtgca-3′), (forward primer 5′-cgagttggcagtagtgg-3′ and reverse primer 5′-ggcata-gaactggacaga-3′), or GAPDH primers (forward primer 5′-tggaaggacctacgtaccaca-3′ and reverse primer: 5′- tgcctagcataacctctc-3′). The product was then analyzed on a 1% agarose gel.

Proliferation assays
To examine growth potential, pooled A375 and Mel-STR ADAMTS18 clones were seeded into 96-well plates at 250 cells per well in either 1%, 2.5%, or 10% serum-containing medium and incubated for 13 to 17 days. Cells were counted every 48 hours by lysing cells in 50 μL of 0.2% SDS per well and incubating for 2 hours at 37°C before addition of 150 μL of SYBR Green I solution (1:750 SYBR Green I (Invitrogen-Molecular Probes) diluted in dH2O). Plates were analyzed using a BMG Labtech FLOUstar Optima.

Foci formation assays
A375 and Mel-STR pooled clones were seeded at 500 cells per T25 flask in normal complete serum–containing medium and incubated for 8 to 10 days before staining with Hema 3 Stat Pack (Protocol) to visualize foci for counting.

Migration assays
A375 or melanoma cells with stable knockdown of ADAMTS18 were seeded into preconditioned migration wells (8.0 μm; BD Biocoat, BD Biosciences) at 10,000 to 30,000 cells per well in serum-free medium in the top chamber and incubated for 16 to 18 hours with complete serum-containing medium in the bottom chamber before harvesting. Inserts were fixed and stained using Hema 3 Stat Pack (Protocol). Inserts were analyzed and counted for cells that migrated per field view and quantitated using ImageJ (NIH software).

Adhesion assay
Ninety-six-well plates were coated with 5 μg/mL laminin-I or 5 μg/mL fibronectin in 200 μL of 1× PBS and incubated overnight at 4°C. Before plating cells, the coated wells were washed once in PBS and then blocked with 0.1 mg/mL heat-inactivated bovine serum albumin (BSA; dissolved in PBS) for 1 hour at room temperature. Cells were seeded into the plates at 30,000 per well and incubated at 37°C for 2 hours with the lid off. Cells were shaken off plate vigorously then washed three times with PBS. The remaining cells were fixed using 4% paraformaldehyde in PBS overnight at 4°C. Plates were then washed three times using ddH2O. Attached cells were stained with 0.1% crystal violet (w/v; 20% methanol in PBS) for 30 minutes at room temperature followed by three washes with ddH2O. Dye was solubilized with 0.1 N HCl for 10 minutes at room temperature. Absorbance was measured at 610 nm on Molecular Devices SpectraMax and quantitated using Microsoft Excel.

Immunoflourescence analysis of pooled clones
A375 pooled ADAMTS18 clones expressing either WT, G312E, C638S, and P1035S or empty vector were seeded on eight-well chamber slides at a cell density of 50,000 per well. Cells were grown for 24 hours before fixing and staining. Chamber slides were washed once with 1× PBS followed by fixation with 4% paraformaldehyde (PBS) for 15 minutes at room temperature. Cells were subsequently washed three times with ice-cold 1× PBS for 5 minutes per wash. Slides were blocked with 1% BSA (PBS-T) for 30 to 60 minutes at room temperature followed by washing with 1× PBS twice. Chamber slides were immunostained with anti-FLAG (rabbit; Sigma) in 1% BSA (PBS-T) at a dilution of 1:50 for 18 hours at 4°C. Chamber slides were washed three times with PBS at 5 minutes per wash followed by incubation with anti-rabbit (Alexa Fluor 568; Invitrogen) diluted at 1:200 in 1% BSA (PBS-T) for 2 hours at room temperature. Slides were washed three times in PBS followed by fixation/ mounting with 4′,6-diamidino-2-phenylindole and analyzed on a Zeiss AX10 (Scope.A1) at 40× using spot imaging software for image acquisition. Further analysis was done using Adobe Photoshop and ImageJ/NIH software.

Xenograft studies in mice
Nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice were purchased from The Jackson Laboratory. All mice were housed in a pathogen-free facility and were given autoclaved food and water. Mel-STR pooled clones expressing empty vector or with WT ADAMTS18 or mutant ADAMTS18 were grown in T-75 flasks to 70% to 80% confluency. Cells (1 × 10⁶) were resuspended in 100 μL of sterile 1× PBS and injected s.c. into 11-week-old male NOD/SCID mice. Mice were monitored biweekly, and final tumor weights were measured after the tumor was excised from euthanized mice at day 22 postinjection. Lungs from each mouse were harvested in 4% paraformaldehyde and embedded in paraffin for H&E staining followed by histologic evaluation.

Statistical analysis
Statistical analyses were done using the R statistical environment and Microsoft Excel (two-tailed t test, binomial test).

Results and Discussion
Comprehensive mutational analysis of the ADAMTS gene family in human melanoma
The human ADAMTS family consists of 19 genes (Supplementary Table S1). To evaluate whether these are genetically altered in melanoma, we analyzed the coding exons of this gene superfamily in 31 melanoma patients. A total of 408 exons from the ADAMTS genes were extracted from genomic
### Table 1. Mutations identified in ADAMTSs

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**NOTE:** “X” refers to stop codon. “LOH” refers to cases wherein the WT allele was lost and only the mutant allele remained. “None” refers to mutations outside any identifiable domain.

Abbreviation: CCDS, Consensus Coding Sequence project.

*Accession numbers for mutated ADAMTSs in Santa Cruz and Genbank.

†Number of nonsynonymous mutations observed and percentage of tumors affected for each of the 11 genes in the panel of 79 melanoma cancers.

‡Nucleotide and amino acid change resulting from mutation.
ADAMTS18 is highly mutated in melanoma

To evaluate the most highly mutated gene, ADAMTS18 (affecting ~18% of cases analyzed), we further extended our sequencing analysis to an additional human melanoma tumor panel consisting of 65 melanoma specimens (11). In this screen, we detected 10 nonsilent somatic mutations and 1 silent somatic mutation, affecting ~14% of the cases analyzed (Supplementary Table S3 and examples in Fig. 1A). The number of C>T mutations in the melanoma tumors was significantly greater than other nucleotide substitutions, resulting in a high prevalence of C>G>T:A transitions ($P < 0.0001$; Supplementary Fig. S1), confirming previously reported melanoma signatures (18).

ADAMTS18 mutations promote growth factor–

independent cell proliferation

To evaluate the effect of some of these mutations on ADAMTS function, we decided to focus on ADAMTS18, which was the most highly mutated gene, harboring 14 somatic mutations in our initial screen (Table 1). The large number of mutations identified in ADAMTS18 and the fact that the affected residues in ADAMTS18 are highly conserved evolutionarily, retaining identity in rat and mouse, suggest that these mutations may be functionally important in melanoma.

To assess the effects of ADAMTS18 mutations on tumorigenic phenotypes, we created stable clones expressing WT or six tumor-derived ADAMTS18 mutants (G312E, P452S, C638S, Q904X, Q1002X, and P1035S) in two different human melanoma cell lines (A375 and Mel-STR; ref. 19), both of which were confirmed to harbor WT ADAMTS18. We chose to focus on these six particular mutations as these were found in important functional domains and showed high species conservation. Western blot analysis showed a similar expression level of ADAMT18 in A375 cells in all clones except mutations Q904X and P1035S, which had lower expression levels (Supplementary Fig. S3). These clones were used for succeeding studies.

We first assessed the transformation abilities of the ADAMTS18 mutants. As seen in Fig. 2Ai-ii, expression of all the ADAMTS18 mutants in either A375 or Mel-STR cells (except mutant Q1002X in Mel-STR cells) elicited a significantly higher cell transformation ability compared with clones expressing vector or WT ADAMTS18 ($P < 0.05$, t test). When the same set of clones was evaluated for growth, it was apparent that expression of WT or mutant ADAMTS18 genes did not affect the growth rate of A375 and Mel-STR cells in tissue culture in the presence of medium with 10% serum (Fig. 2Bi-ii). However, if the serum concentration was reduced to 2.5% (for A375 cells) or 1% (for Mel-STR cells), WT clones grew at a slower rate than mutant clones on plastic (Fig. 2Biii-iv).

Mutant ADAMTS18 increases cell migration through

modulation of cell adhesion

ADAMTS18 is a secretory protein and similarly to virtually all ADAMTS family members, it is strongly associated with the extracellular matrix at the pericellular space. This location likely facilitates the interaction of ADAMTS18 with integrins and other extracellular matrix components as well as with growth factors such as vascular endothelial growth factor and hepatocyte growth factor (20). To test whether mutant forms of ADAMTS18 have alterations in these interactions, we performed an adhesion assay by using either fibronectin or laminin-I as substrates. Analysis of cell adhesion to these extracellular matrix components revealed that WT ADAMTS18–expressing cells had an increased adhesion to laminin-I when compared with cells expressing ADAMTS18 mutations (Fig. 3Ai). In contrast, adhesion to fibronectin was similar between WT and mutant ADAMTS18–expressing cells (Fig. 3Aii).
Alterations in adhesion have previously been shown to lead to changes in signaling properties. Based on the above results, mutant forms of ADAMTS18 will likely have profound effects on signaling proteins that have the ability to induce changes in gene expression programs. These effects on gene expression will ultimately result in further differences in cell adhesion and migration between ADAMTS18-mutant cells and WT cells. This situation is not unprecedented and it has been previously described in detail for other extracellular metalloproteinases, including members of the ADAMTS family such as ADAMTS12 (21-23).

It has previously been shown that mutations in ADAMTS genes can affect their cell localization (7). To test whether the identified mutations affect their localization, we immunoprecipitated concentrated conditioned medium or cell lysates derived from the A375 pooled clones using FLAG-M2 beads followed by Western blot analysis. Equal levels of FLAG-tagged ADAMTS18 bands were seen in immunoprecipitated cell lysates expressing WT and mutant ADAMTS18 immunoblotted for FLAG (Fig. 3Bi). However, only WT ADAMTS18 was observed in immunoprecipitates from
conditioned medium using the same clones (Fig. 3Bi). Similar results were observed when the same clones were analyzed by immunofluorescence staining. As seen in Fig. 3Bii, more total protein is retained and attached to the cell surface for mutant ADAMTS18-expressing cells, explaining its absence in the conditioned medium. In contrast, immunostaining of WT ADAMTS18-expressing cells revealed diminished cell surface localization of FLAG-tagged WT protein (Fig. 3Bii). The effect of ADAMTS18 mutations on its localization could be the

FIGURE 2. Mutant ADAMTS18 causes reduced cell dependence on growth factors. A, focus formation assay of A375 (i) or Mel-STR (ii) pooled clones expressing the indicated constructs was done. The graph indicates the number of colonies observed after 2 weeks of growth. B, cellular proliferation of A375 (i and iii) or Mel-STR (ii and iv) pooled clones transduced with an empty vector, WT ADAMTS18, or the indicated ADAMTS18 mutants was assessed in plastic culture plates in the presence of 10% serum (i and ii) or reduced serum (iii and iv) for 12 days. The average cell number at each time point was measured by determining DNA content in eight replicate wells using SYBR Green I.
underlying mechanism for the differential adhesion described above.

As previous studies reported that reduced adhesion facilitates cell migration (24, 25), our finding that cells expressing mutant ADAMTS18 have reduced laminin-I adhesion prompted us to investigate whether these cells also have increased migration ability. Boyden chamber assays showed that A375 mutant ADAMTS18-expressing clones had an increased ability to migrate through porous membrane (Fig. 3Ci-ii; \( P < 0.05, t \text{ test} \)). Based on these results, we can postulate that ADAMTS18 modulates cell adhesion, and this might be a candidate mechanism to explain how mutated forms of this protease stimulate cell migration.

**Mutant ADAMTS18 is required for migration in melanoma cells**

To assess whether melanoma cells harboring endogenous ADAMTS18 mutations are dependent on ADAMTS18 for migration, we used shRNA to stably knock down ADAMTS18 in melanoma lines harboring either WT (5T, A375) or mutant ADAMTS18 (85T, Q1002X; 12T, P452S). We confirmed specific targeting of ADAMTS18 by shRNAs in transfected HEK 293T cells and in one of the melanoma cell lines by reverse transcription-PCR (Supplementary Fig. S4A and B). Two unique shRNA constructs targeting ADAMTS18 had minimal effect on the migration of cells expressing WT protease but substantially reduced the migration of melanoma lines carrying mutant ADAMTS18 (Fig. 3Di-vi). Thus, mutant ADAMTS18 is essential for the migration of melanoma cells harboring these mutations.

**Mutant ADAMTS18 causes increased metastases in vivo**

To determine whether ADAMTS18 mutations affect growth in vivo, Mel-STR clones expressing empty vector, WT, or mutant ADAMTS18 were s.c. injected into NOD/SCID mice. Twenty-two days after injection, the mice were evaluated for skin ulceration and metastasis formation by examining H&E-stained sections of paraffin-embedded lungs. As seen in Fig. 4A, most of the tumors expressing mutant ADAMTS18 presented with ulcerations, whereas few of the mice with cells expressing WT ADAMTS18 had ulcerating lesions. In addition, most mice injected with mutant ADAMTS18-expressing cells had micrometastases. In contrast, no lung metastases were found in the mice injected with WT clones (Fig. 4B and C). This suggests that, in some cases, ADAMTS18 may have an assay-specific suppressive effect. This scenario has precedent and has been described for Mdm2 (26). Although the underlying mechanism for the lack of a metastatic phenotype seen in the WT cells is unclear, it is consistent with ADAMTS proteins being inhibited by proteins such as tissue inhibitor of metalloproteinases in vivo (27). It must be noted, however, that the number of identified endogenous protease inhibitors is significantly lower than that of proteases (28). It is therefore conceivable that another, as yet unknown, ADAMTS18 inhibitor is being expressed in vivo, specifically inhibiting the metastasis of cells expressing WT ADAMTS18.

Clearly, ADAMTS18 mutations are dispersed throughout its domains. This is reminiscent of the driver mutations reported in ERBB4 (12), CARD11 (29), and FLT3 (30). In addition to the catalytic domain, ADAMTS proteins have noncatalytic ancillary domains that regulate interaction of the protein with substrates or inhibitors such as the tissue inhibitor of metalloproteinases, and these domains have been shown to mediate recognition and cleavage of numerous substrates (31-33). Furthermore, the ancillary domain of several ADAMTS proteins is modified by COOH-terminal proteolysis (34-36), which might alter substrate recognition and enzyme localization. As several of the mutations identified in ADAMTS18 lie within the COOH terminus and some cause its truncation, they might affect this recognition. Thus, elucidation of the specific interactions of ADAMTS18 with particular substrate(s) will provide an important understanding of the biochemical effects of the discovered mutations. Recently, a novel method of detecting protease substrates has been developed by Kleifeld et al. (37). Upon radioisotopic labeling of the NH\(_2\)-termini amine groups of cellular proteins and enzymatic reaction with known proteases, fragmented peptides are purified and run on a tandem mass spectrometer, thus identifying new cleavage sites. Utilization of such a detection method in determining the physiologic substrates for ADAMTS18 will prove beneficial.

As mentioned above, several ADAMTSs have been suggested to harbor antitumorigenic properties (38, 39). These reports focused on the antiangiogenic effects of ADAMTS1 and ADAMTS8 (40, 41) as well as the modulation of the extracellular signal-regulated kinase pathway by ADAMTS12 through extracellular matrix interactions (21). El Hour et al. (42) showed that lack of ADAMTS12 in mice resulted in increased angiogenesis and tumor progression. In addition, ADAMTS15 was shown to have a protective role in breast cancer as increased expression along with decreased expression of ADAMTS8 resulted in prolonged relapse-free progression in these patients (43). Genetic inactivation of ADAMTS15 through somatic mutation lead to decreased ability to suppress colony formation or invasion of colorectal cancer cells compared with expression of the WT gene (7). Furthermore, epigenetic silencing of ADAMTS genes such as ADAMTS9 or ADAMTS18 has been observed in different types of carcinomas implying them to be tumor suppressors (44, 45).

Conversely, genetic silencing of ADAMTS20 in mice resulted in increased melanoblast apoptosis, decreased soluble Kit ligand stimulation of the prosurvival pathway, and decreased processing of the extracellular matrix protein versican (46). These results suggest that ADAMTS20 is a prosurvival molecule that might act as an oncogene in melanoma. This is supported by the observation that ADAMTS20 is overexpressed in brain, colon, and breast cancers (47). Importantly, ADAMTS20 was the second most highly mutated gene in our study, harboring 12 somatic mutations (11.4%). Future functional evaluation of the identified mutations in...
FIGURE 3. Mutant ADAMTS18 is essential for melanoma cell migration possibly through modulating cell adhesion. A, adhesion assay of A375 clones expressing the indicated constructs was done. Laminin-I (i)– or fibronectin (ii)–coated plates were assessed for adhesion after 1-hour incubation by crystal violet staining. Plates were analyzed by reading the absorbance at 610 nm. B, localization of ADAMTS18 proteins was assessed using immunoprecipitation and immunofluorescence staining. (i) Cellular conditioned medium and lysates were immunoprecipitated using anti-FLAG (M2) beads and analyzed by Western blot analysis. Cell lysates were blotted with anti-α-tubulin as a loading control. (ii) A375 pooled ADAMTS-18 clones (WT, G312E, C638S, P1035S, or empty vector) were plated and fixed on slides for immunofluorescence staining with anti-FLAG and 4′,6-diamidino–2-phenylindole (DAPI) for nuclear localization. C, A375 clones expressing the indicated constructs were grown in Boyden chambers and assessed for their ability to migrate. (i) The graph indicates the number of cells that migrated 18 hours after seeding. (ii) Representative pictures of migrated cells. D, melanoma cell lines expressing WT ADAMTS18 (i and ii) or mutant ADAMTS18 (iii and iv) were infected with either control shRNAs or two different shRNA constructs targeted against ADAMTS18 (#4 and #5). The migration ability of the cells was assessed and plotted. Representative images of migrated cells are shown in v and vi.
ADAMTS20 will further verify whether ADAMTS20 participates in melanoma progression.

Taken together, our results provide genetic, cellular, and in vivo evidence that ADAMTS18 has a role in promoting melanoma tumorigenesis. We postulate that the genetic alteration of ADAMTS18 contributes to the aggressive biological behavior of melanoma through modulation of proliferative, migratory, and metastatic mechanisms.
Importantly, this is the first genetic identification of an ADAMTS gene that is functionally proven to have an oncogenic role in human disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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