The MIG-2/Integrin Interaction Strengthens Cell-Matrix Adhesion and Modulates Cell Motility*

Xiaohua Shi1, Yan-Qing Ma2,1, Yizeng Tu1, Ka Chen1, Shan Wu1, Koichi Fukuda3, Jun Qin3, Edward F. Plow1, and Chuanyue Wu1,2

From the 1Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261 and the 2Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Integrin-mediated cell-matrix adhesion plays an important role in control of cell behavior. We report here that MIG-2, a widely expressed focal adhesion protein, interacts with β1 and β3 integrin cytoplasmic domains. Integrin binding is mediated by a single site within the MIG-2 FERM domain. Functionally, the MIG-2/integrin interaction recruits MIG-2 to focal adhesions. Furthermore, using e1ββ3 integrin-expressing Chinese hamster ovary cells, a well described model system for integrin activation, we show that MIG-2 promotes integrin activation and enhances extra-cellular matrix adhesion. Although MIG-2 is expressed in many cell types, it is deficient in certain colon cancer cells. Expression of MIG-2, but not of an integrin binding-defective MIG-2 mutant, in MIG-2-null colon cancer cells strengthened cell-matrix adhesion, promoted focal adhesion formation, and reduced cell motility. These results suggest that the MIG-2/integrin interaction is an important element in the cellular control of integrin-mediated cell-matrix adhesion and that loss of this interaction likely contributes to high motility of colon cancer cells.

Cell-extracellular matrix (ECM)3 adhesion is a fundamental process that is mediated by transmembrane receptors such as integrins (1–6). The interactions of integrins with ECM ligands can be controlled by integrin activation via “inside-out” signaling. Talin, a FERM (Band 4.1 (ezrin/radixin/moesin) domain-containing focal adhesion (FA) protein, can play a key role in this process (for recent reviews, see Refs. 7–10). Binding of the talin FERM domain to the β integrin cytoplasmic domains results in separation of the α and β integrin cytoplasmic tails and consequently in an increase in integrin extracellular ligand-binding affinity (i.e. integrin activation) (11–13). Integrin extracellular ligand-binding affinity plays an important role in control of initial cell-ECM adhesion. Additionally, integrin-mediated cell-ECM adhesion can be enhanced through interactions with cytoskeletal proteins, a process that has been termed cytoskeletal strengthening (14–16). The physical basis underlying the cytoskeletal strengthening of cell-ECM adhesion has been well described (16). However, the molecular interactions that mediate this process remain to be defined.

MIG-2 (mitogen-inducible gene-2, also known as kindlin-2) is a widely expressed and evolutionarily conserved cytoplasmic protein (17–21). Genetic studies have shown that Caenorhabditis elegans UNC-112, a homolog of MIG-2, is required for attachment of body-wall muscle cells to the hypodermis (17, 19). Loss of UNC-112 in C. elegans results in an embryonic lethal Pat (paralyzed, arrested elongation at two-fold) phenotype resembling that of α or β integrin loss (17, 19). In mammalian organisms, MIG-2 has been detected in many cell types, including fibroblasts, muscle cells, endothelial cells, and epithelial cells (20, 22). In these cells, it concentrates at FAs. MIG-2 interacts with migfilin (20), a filamin- and VASP (vasodilator-stimulated phosphoprotein)-binding protein (20, 21, 23). Through this interaction, it recruits migfilin to FAs and provides a link from FAs to the actin cytoskeleton (20). Although MIG-2 is crucial for recruiting migfilin to FAs, how MIG-2 is recruited to FAs was not known. Structurally, MIG-2 contains an N-terminal region that exhibits no obvious structural motif and a C-terminal FERM domain. Notably, the MIG-2 FERM domain contains a region that shares considerable sequence similarity with the integrin-binding site of the talin FERM domain. Furthermore, kindlin, a protein that shares significant (62%) sequence identity with MIG-2, interacts with β1 and β3 integrin cytoplasmic domains (24). However, the functions of the kindlin/integrin interaction remain unknown.

Cell-ECM adhesion is intimately involved in the regulation of cell behavior such as cell motility. Theoretical consideration and experimental studies have shown that the relationship between cell-ECM adhesion and motility is biphasic (25, 26). Thus, the regulation of cell-ECM adhesion strength is important in the control of cell motility. Consistent with this, alterations of proteins that are pertinent to the control of cell-ECM adhesion are frequently associated with human diseases such as cancer. Determining the molecular basis underlying the regulation of cell-ECM adhesion and migration is therefore not only general biological importance but also considerable clinical significance. In this work, we show that MIG-2 interacts...

*This work was supported in part by National Institutes of Health Grants GM65188 and DK54639 (to C. W.), Grant HL58758 (to J. Q.), and Grant HL073311 (to E. F. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by a fellowship from the American Heart Association, Ohio Valley Affiliate.

2 To whom correspondence should be addressed: Dept. of Pathology, University of Pittsburgh, 7078 Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15261. Tel.: 412-648-2350; Fax: 509-561-4062; E-mail: carnywu@pitt.edu.

3 The abbreviations used are: ECM, extracellular matrix; FA, focal adhesion; mAb, monoclonal antibody; Ab, antibody; GST, glutathione S-transferase; GFP, green fluorescent protein; CHO, Chinese hamster ovary; talin-H, talin head domain; BSA, bovine serum albumin; MFI, mean fluorescence intensity(ies).
with β1 and β3 integrin cytoplasmic domains and functions as an important regulator of integrin activation, cell-ECM adhesion, and migration.

EXPERIMENTAL PROCEDURES

Cells, Antibodies, and Other Reagents—Human SK-LMS-1 cells and HT-1080 cells were from American Type Culture Collection. RKO, HT-29, DLD-1, LoVo, and HCT-116 colon cancer cells were from Dr. Lin Zhang (University of Pittsburgh Cancer Institute). Caco-2 colon cancer cells were from Dr. Craig C. Garner (Stanford University). The SK-LMS-1, HT-1080, and RKO cells were cultured in minimum Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate. HT-29 and DLD-1 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and 2 mM L-glutamine. Caco-2 cells were cultured in Dulbecco’s modified Eagle’s medium with 20% fetal bovine serum and 2 mM L-glutamine. LoVo and Caco-2 cells were cultured in Ham’s F-12 and McCoy’s 5A medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Mouse anti-MIG-2 monoclonal antibody (mAb) 3A3 has been described (20). Mouse anti-kindlin mAb (clone 4A5) was generated using glutathione S-transferase (GST) fusion protein containing human kindlin residues 216–677 as an antigen followed by the previously described protocols (20). Function-blocking mouse anti-α2 integrin (clone P1E6) and anti-β1 integrin (clone 6S6) monoclonal antibodies were purchased from Chemicon (Temecula, CA). Mouse anti-vinculin mAb was from Sigma. Rhodamine Redfluor™-conjugated goat anti-mouse IgG antibody (Ab) and horseradish peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). Cell culture media were from Sigma or Invitrogen. All other chemicals were from Fisher or Sigma.

Mutagenesis, DNA Constructs, and Transfection—The vector encoding green fluorescent protein (GFP)-tagged MIG-2 has been described (20). Point mutations were introduced into MIG-2 coding sequence as specified in each experiment using a QuikChangeTM site-directed mutagenesis system (Stratagene). DNA fragments encoding MIG-2 deletion mutants were generated by PCR. DNA fragments encoding MIG-2 mutants were inserted into the pEGFP-C2 vector (Clontech). All mutations were confirmed by DNA sequencing. Cells were transfected with vectors encoding GFP-tagged wild-type or mutant MIG-2 or GFP alone as a control using Lipofectamine 2000 (Invitrogen).

GST Fusion Protein Pulldown Assays—The vectors encoding GST fusion proteins containing the β1A integrin cytoplasmic domain (residues 775–786), the β3 integrin cytoplasmic domain (residues 716–762), or a mutant form of the β3 integrin cytoplasmic domain in which Tyr747 was substituted with Ala were generated as described previously (27, 28). The vectors were used to transform *Escherichia coli* DH5α cells. Expression of GST and GST fusion proteins was induced with isopropyl β-D-thiogalactopyranoside. The bacterial cells were lysed with 150 mM NaCl, 10 mM Tris (pH 8.0), and 1 mM EDTA (STE buffer) containing 100 µg/ml lysozyme for 15 min (on ice), followed by sonication in STE buffer containing 5 mM dithiothreitol, 1.5% Sarkosyl, and protease inhibitors. After the cell debris was removed by centrifugation, the lysates were incubated with glutathione-Sepharose beads (Pierce) at 4 °C for 1 h. The glutathione-Sepharose beads were precipitated by centrifugation, washed three times with STE buffer containing 0.1% Triton X-100, and used for GST pulldown assays as we described previously (20). Brief, Chinese hamster ovary (CHO) cells, SK-LMS-1 cells, or SK-LMS-1 cells transfected with vectors encoding GFP or GFP-tagged wild-type or mutant MIG-2 were lysed with 1% Triton X-100 in 20 mM Tris (pH 7.1) containing 150 mM NaCl, 10 mM Na2P2O7, 2 mM Na3VO4, 100 mM NaF, 10 mM EDTA, and protease inhibitors (lysis buffer). The lysates were incubated with glutathione-Sepharose beads containing GST or GST-integrin cytoplasmic domain fusion proteins for 4 h or longer at 4 °C. The glutathione-Sepharose beads were precipitated by centrifugation, washed four times with lysis buffer, and then analyzed by Western blotting and Coomassie Blue staining as specified in each experiment.

Integrin Activation—The effect of MIG-2 on integrin activation was analyzed using CHO cells stably expressing αIIbβ3 integrin and activation-specific anti-αIIbβ3 integrin mAb PAC1 as described (28). Briefly, CHO cells expressing αIIbβ3 integrin were transfected with GFP-tagged MIG-2, the GFP-tagged talin head domain (talin-H; residues 1–429), or GFP. Twenty-four hours after transfection, the cells were harvested; suspended in Hanks’ balanced salt solution/bovine serum albumin (BSA); and stained with mAb PAC1 (20 µg/ml) for 30 min at 22 °C, followed by incubation with Alexa Fluor® 633-conjugated goat anti-mouse IgM Ab for 30 min on ice. After washing, the cells were fixed and analyzed using a FACScalibur flow cytometer. mAb PAC1 binding was analyzed only on a gated subset of cells positive for GFP expression (i.e. GFP- or GFP fusion protein-expressing cells). The mean fluorescence intensity (MFI; generated with CellQuest software) of mAb PAC1 bound to the GFP-MIG-2- or GFP-talin-H-expressing cells was divided by the MFI of mAb PAC1 bound to the control GFP-expressing cells in the same experiment to obtain a relative MFI value. Seven independent experiments were performed, and the relative MFI in each experimental set, GFP-MIG-2 or GFP-talin-H, were compared with the MFI of the GFP control by a paired *t* test to determine statistical significance. *p* values <0.05 were considered to be statistically significant.

Adenoviral Vectors and Infection—Adenoviral vectors encoding wild-type or mutant MIG-2 were generated using the AdEasy system following a previously described protocol (29, 30). Briefly, MIG-2 coding sequences were cloned into the NotI/XbaI sites of the pAdTrack-CMV shuttle vector. The resultant plasmids were linearized with Pmel, purified, and mixed with supercoiled pAdEasy-1. The vectors were transferred into *E. coli* BJ5183 by electroporation using a Bio-Rad Gene Pulser electroporator. Recombinants that were resistant to kanamycin were selected, and recombination was confirmed by Pael digestion. The positive plasmids were then transformed into DH5α by heat shock for large-scale amplification. The plasmid DNAs were digested with Pael, ethanol-precipitated, and used to transfect 293 cells with Lipofectamine PLUS (Invitrogen). The transfectants were harvested 10 days after transfection. The cells were lysed by three cycles of freezing in a
methanol/dry ice bath and rapid thawing at 37 °C, and the lysates containing the recombinant adenovirus were collected. The control adenoviral expression vector encoding β-galactosidase was kindly provided by Drs. Tong-Chuan He and Bert Vogelstein (Howard Hughes Medical Institute, Johns Hopkins Oncology Center, Baltimore, MD). Adenoviral vectors were purified by CsCl gradient centrifugation as described (31) and then used to infect cells. The infection efficiency was monitored by the expression of GFP encoded by the viral vectors. The percentage of GFP-positive cells typically reached 80–90% within 1 day after infection. Overexpression of wild-type or mutant MIG-2 in the infected cells was confirmed by Western blotting.

FA Localization of GFP-tagged MIG-2—SK-LMS-1 cells were transfected with GFP-tagged wild-type or mutant MIG-2. One day after transfection, the cells were trypsinized, replated on fibronectin-coated coverslips, and cultured for 24 h. The cells were fixed with 4% paraformaldehyde, stained with anti-vinculin mAb and Rhodamine Red™-conjugated anti-mouse IgG Ab, and observed under a Leica DMR fluorescence microscope.

Cell-ECM Adhesion—Cell-ECM adhesion was assessed by centrifugation assays either at 4 °C (to measure initial cell-ECM adhesion, which is controlled primarily by integrin ligand-binding activity) or after incubation of the cells at 37 °C (to allow cytoskeletal strengthening) as described previously (16). CHO cells, the cells were transfected with expression vectors encoding GFP-MIG-2, GFP-talin-H (as a positive control), or GFP (as a negative control) using Lipofectamine PLUS. Thirty-six hours after DNA transfection, the transfectants (1 × 10⁵ cells/well) were seeded in fibrinogen-coated 96-well plates (Greiner Bio-One). GFP-positive cells were quantified by measuring fluorescence at excitation and emission wavelengths of 485 and 535 nm, respectively, with anti-vinculin mAb and Rhodamine Red™-conjugated anti-mouse IgG Ab, and observed under a Leica DMR fluorescence microscope equipped with GFP and rhodamine filters.

In some experiments, SK-LMS-1 and RKO cells were preincubated with function-blocking mouse anti-β1 integrin mAb (clone 6S6; 6.7 μg/ml IgG), function-blocking mouse anti-α2 integrin mAb (clone P1E6; 6.7 μg/ml IgG), or control mouse IgG Ab (6.7 μg/ml) at 37 °C for 15 min. Integrin-mediated initial cell adhesion was then analyzed as described above.

To assess FA formation, SK-LMS-1 or RKO cells infected with the control β-galactosidase adenovirus or adenoviruses encoding wild-type or mutant MIG-2 were plated on coverslips and cultured as specified in each experiment. The cells were fixed with 4% paraformaldehyde, stained with mouse anti-vinculin mAb and Rhodamine Red™-conjugated anti-mouse IgG Ab, and observed under the Leica DMR fluorescence microscope.
RESULTS

MIG-2 Interacts with β1 and β3 Integrin Cytoplasmic Domains—To test whether MIG-2 interacts with the β1 integrin cytoplasmic domain, we incubated lysates of MIG-2-expressing mammalian cells with GST or GST fusion protein containing the β1A integrin cytoplasmic domain (residues 775–786; referred to as GST-β1). GST, GST-β1 fusion protein, and associated proteins were precipitated with glutathione-Sepharose beads. Western blot analyses showed that MIG-2 was readily coprecipitated with GST-β1 (Fig. 1A, lane 3) but not with GST (lane 2). The presence of GST and GST-β1 in the precipitates was confirmed by staining the membrane with Coomassie Blue (Fig. 1B). In additional control experiments, no protein bands in the GST-β1 fusion protein preparation were recognized by anti-MIG-2 Ab in the absence of cell lysates (Fig. 1A, lane 4), confirming the specificity of the Western blotting.

The β3 integrin tail shares considerable sequence similarity with the β1 integrin tail. To test whether MIG-2 recognizes the β3 integrin tail, we incubated the cell lysates with GST fusion protein containing the β3 integrin tail (residues 716–762; referred to as GST-β3) and analyzed MIG-2 binding by the GST pulldown assay. The results show that MIG-2 was readily coprecipitated with GST-β3 (Fig. 1, C and D, lanes 3) but not with GST (lanes 2). In control experiments, no protein bands in the GST-β3 fusion protein preparation were recognized by anti-MIG-2 Ab in the absence of cell lysates (Fig. 1, C and D, lanes 4). These results suggest that MIG-2 interacts with both β1 and β3 integrin cytoplasmic domains.

Structure-based Mutagenesis Identifies a Single Integrin-binding Site within the MIG-2 FERM Domain—MIG-2 contains multiple protein-binding motifs, including a C-terminal FERM domain. To facilitate studies aimed at determining the functions of the MIG-2/integrin interaction, we sought to identify the MIG-2 site(s) that are involved in integrin binding. The F3 subdomain within the MIG-2 FERM domain shares considerable sequence similarity with the F3 subdomain of the talin FERM domain, including Trp615 and Asp616 in MIG-2 and several other residues that are at the integrin-binding interface (Fig. 2A) (38). To better understand the structural basis of the MIG-2/integrin interaction, we constructed a homology model of the MIG-2 F3 subdomain bound to the β1 or β3 integrin cytoplasmic tail using the atomic coordinates of the talin F3 subdomainβ3 integrin complex as a template (Fig. 2B). Consistent with the β integrin tail pulldown experiments, structural modeling suggested that the MIG-2 C-terminal region folds into a canonical FERM phosphotyrosine-binding domain capable of recognizing β integrin tails. The integrin-binding surface mostly lies on its β strand through polar and hydrophobic interactions as well as intermolecular backbone hydrogen interaction. In particular, Gln614 and Trp615 of the MIG-2 F3 subdomain appear to play a significant role (Fig. 2B) by interacting with Trp775 and Asp776 in the β1 integrin tail or Trp359 and Asp360 in the β3 integrin tail via hydrophobic and hydrophilic interactions, resembling those in the talin F3 subdomainβ3 integrin tail complex (38).

To test experimentally whether the MIG-2 FERM domain indeed mediates integrin binding, we introduced deletion and substitution mutations into this domain. A GFP-tagged MIG-2 deletion mutant with the FERM domain truncated (GFP-ΔFERM), a substitution mutant with Gln614 and Trp615 (colored blue in Fig. 2, A and B) substituted with Ala (GFP-Q614A/W615A), and GFP-MIG-2 as a control were expressed in mammalian cells by DNA transfection. Expression of GFP-Q614A/W615A (Fig. 2D, lane 1), GFP-ΔFERM (lane 2), and GFP-MIG-2 (lane 3) was confirmed by Western blotting. To test the integrin-binding activity, we incubated the lysates with GST-β1 or GST. GST and GST-β1 were precipitated with glutathione-Sepharose beads (Fig. 2E). As expected, both GFP-MIG-2 and endogenous MIG-2 were readily coprecipitated with GST-β1 (Fig. 2D, lane 7) but not with GST (lane 8).
MIG-2/Integrin Interaction in Cell Adhesion and Migration

FIGURE 2. A single site within the MIG-2 F3 subdomain mediates integrin binding. A, sequence alignment of the MIG-2 and talin F3 subdomains by ClustalW analysis. The residues in the MIG-2 F3 subdomain that are conserved in the talin F3 integrin-binding interface are highlighted in red. The loss-of-function mutation sites in the MIG-2 F3 subdomain, demonstrated by the GST pulldown assay (D), are highlighted in blue. B, computer-aided three-dimensional model of the MIG-2 F3 subdomain bound to the β3 integrin cytoplasmic domain. The key residues of MIG-2 at the integrin-binding interface are highlighted in red and blue as described for A. The residues colored in blue (Gln614 and Trp615) were used for the mutational studies (see “Results”). C, overlay of the MIG-2 F3 subdomain model structure (blue) and the talin F3 subdomain structure (yellow) (39). The dotted circle highlights the difference in the S1–S2 loop between the MIG-2 and talin F3 subdomains. D, overlay of the MIG-2 F3 subdomain model structure (blue) and the talin F3 integrin binding interface are highlighted in red and blue as described for A. E, Coomassie blue-stained gel of GST-fused MIG-2 and talin F3 subdomains.

Collectively, these results suggest that, although the MIG-2/integrin and talin/integrin interactions may share certain structural similarities, they are not identical.

contrast, neither GFP-Q614A/W615A (Fig. 2D, lane 9) nor GFP-ΔFERM (lane 5) was coprecipitated with GST-β1, although endogenous MIG-2 was readily detected in the same samples (lanes 5 and 9). In control experiments, no MIG-2 was detected in the GST precipitates (Fig. 2D, lanes 6 and 10). Collectively, these results suggest that, as predicted by the structural modeling, Gln614-Trp615 within the MIG-2 F3 subdomain is essential for integrin binding.

The MIG-2- and Talin-binding Sites in the β3 Integrin Cytoplasmic Domain Are Not Identical—Although the F3 subdomain of MIG-2 shares considerable sequence homology with that of talin (Fig. 2, A and B), overlaying the MIG-2 F3 model structure with the talin F3 structure revealed that the S1–S2 loop, which is crucial for talin interaction with the membrane proximal region of the β integrin cytoplasmic domains (39), is absent in the MIG-2 F3 subdomain (Fig. 2C). There are also other differences between MIG-2 and talin binding. The interaction of the talin FERM domain with the β integrin tails has been extensively characterized (reviewed in Ref. 7–10). Structurally, the talin-binding site encompasses both the membrane proximal and central regions of the β integrin cytoplasmic tails (11, 38, 39). The first NPY3 (Tyr747 in the β3 integrin tail) site located in the central region of the β integrin tails is crucial for the formation of the talin-β integrin complexes. It has been shown, for example, that substitution of Tyr747 with Ala abrogates the talin-binding activity (40, 41). To test whether this site is required for the formation of the MIG-2-β3 integrin complex, we incubated cell lysates with a GST-β3 protein bearing the Y747A substitution mutation and precipitated it with glutathione-Sepharose beads. Western blotting of the GST-Y747A precipitates showed that MIG-2 was readily pulled down by GST-Y747A (Fig. 1, C and D, lanes 5), suggesting that the first NPY3 site is not essential for MIG-2 binding.
β3 integrin activation, we overexpressed GFP-MIG-2, GFP-talin-H as a positive control, and GFP as a negative control in αIIbβ3 integrin-expressing CHO cells. In seven transfection experiments that we performed, the expression level of GFP-MIG-2, as indicated by the MFI, was only 15–20% of that of GFP-talin-H. Despite the relatively low expression level, flow cytometry analyses of the cells showed that, in all seven experiments, overexpression of MIG-2 increased mAb PAC1 binding (Fig. 4C). Although modest, this increase induced by MIG-2 was significant ($p < 0.05$ compared with the GFP control by paired $t$ test analysis) but was substantially less than that induced by talin-H (Fig. 4D), which itself gives only partial activation (28). Thus, MIG-2 can promote integrin activation, albeit weakly. This weak activation may be due to the low MIG-2 expression level and/or to additional regulatory requirements for optimal effects.

To confirm that MIG-2 promotes integrin activation, we analyzed the adhesion of αIIbβ3 integrin-expressing CHO cells to fibrinogen at 4 °C. Under this experimental condition, the strength of cell-ECM adhesion is controlled primarily by integrin ligand-binding activity (16). As expected, overexpression of talin-H markedly increased CHO cell adhesion to fibrinogen (Fig. 4E). Notably, overexpression of MIG-2 also significantly increased CHO cell adhesion to fibrinogen (Fig. 4E). These results are highly consistent with the results of the integrin activation assay (Fig. 4, C and D). Collectively, these results reveal a role of MIG-2 in promoting integrin activation and cell-ECM adhesion.

Many Colon Cancer Cell Lines Express No or Very Low Levels of MIG-2 Proteins—Because alteration of integrin-mediated cell-ECM adhesion is intimately associated with human diseases such as cancer, we next investigated the roles of MIG-2 in cancer cells. As an initial step, we analyzed the levels of MIG-2 in various cancer cells. Previous RNA profiling studies have shown that the MIG-2 mRNA level is reduced in colon carcinoma cells (see harvester.embly.de/harvester/Q96A/Q96AC1.htm and genome-www.stanford.edu/nci60) (43, 44). Thus, we focused our analyses on these cells. SK-LMS-1 leiomyosarcoma cells, which express MIG-2, were used as a positive control. Fig. 5A shows that, although MIG-2 was readily detected in SK-LMS-1 cells (lane 7), no or very low levels of MIG-2 were detected in many colon carcinoma cell lines, including RKO, HT-29, DLD-1, LoVo, and HCT-116 (lanes 2–6). These results are consistent with the RNA profiling studies, suggesting that the level of MIG-2 protein, like that of MIG-2 mRNA, is diminished or lost in many colon cancer cells. One notable exception is Caco-2 cells (Fig. 5A, lane 8), a relatively well differentiated colon carcinoma cell line derived from primary colon tumor. Although Caco-2 cells expressed the highest level of MIG-2 among the colon carcinoma cell lines that were tested, their MIG-2 protein level was lower than that of SK-LMS-1 cells (Fig. 5A, compare lanes 7 and 8). HT-1080 cells, a highly metastatic fibrosarcoma cell line, also expressed a relatively low level of MIG-2 (Fig. 5A, lane 1). Interestingly, the order of abundance was reversed when the same samples were probed with anti-kindlin mAb (Fig. 5B). In control experiments, similar levels of actin were detected in all cell lines (Fig. 5C).

Integrin Binding Is Essential for MIG-2 Localization to FAs—We showed previously that MIG-2 is a component of FAs (20). However, the interaction that mediates MIG-2 localization to FAs was not known. To test whether integrin binding plays a role in this process, we transfected cells with vectors encoding GFP-tagged wild-type MIG-2 or integrin binding-defective mutant ΔFERM or Q614A/W615A. The transfectants were stained with antibodies that specifically recognize components of FAs (e.g. vinculin, paxillin, and α-parvin). GFP-MIG-2 (Fig. 3A) was readily recruited to FAs where abundant vinculin (Fig. 3B) and other FA proteins such as paxillin and α-parvin (data not shown) were detected. By contrast, the integrin binding-defective ΔFERM mutant (Fig. 3C) or the Q614A/W615A substitution mutant (Fig. 3E) failed to localize to FAs where clusters of vinculin were detected (Fig. 3, D and F). These results suggest that integrin binding is essential for MIG-2 localization to FAs.

MIG-2 Can Promote Integrin Activation and Cell-ECM Adhesion—Binding of the talin FERM domain to the β integrin cytoplasmic domains is a key step in integrin activation. Overexpression of FERM domain-containing talin-H in αIIbβ3 integrin-expressing CHO cells, a well described model system for integrin activation, promotes αIIbβ3 integrin activation (as measured by activation-specific anti-αIIbβ3 integrin mAb PAC1) (28, 40, 42). We used the same cell system to assess whether MIG-2 can play a role in integrin activation. Like human MIG-2, hamster MIG-2 bound readily to the β3 integrin cytoplasmic domain (Fig. 4, A and B, lanes 3). MIG-2 binding was not abolished by the Y747A mutation in the β3 integrin tail (Fig. 4, A and B, lanes 5). To test whether MIG-2 can promote
plated cells on a collagen I-coated surface and analyzed cell adhesion either at 4 °C, a condition that permits integrin-ECM ligand interaction but not cytoskeletal strengthening (15, 16), or at 37 °C, a condition that permits cytoskeletal strengthening (15, 16). When cell adhesion was analyzed under the condition that does not permit cytoskeletal strengthening (i.e. at 4 °C), no significant differences between the control cells and cells overexpressing MIG-2 or the Q614A/W615A mutant were observed (Fig. 6B). In control experiments, we treated MIG-2-overexpressing cells with function-blocking antibodies to α2β1 integrin, a major collagen-binding receptor. Treatment of the cells with either function-blocking anti-α2 or anti-β1 integrin Ab nearly completely eliminated the cell-collagen adhesion (Fig. 6C), confirming that the adhesion of these cells to collagen was mediated by α2β1 integrin.

When the integrin-mediated cell adhesion was analyzed under the condition that permits cytoskeletal strengthening (i.e. at 37 °C), a significant increase in cell adhesion was observed in response to overexpression of MIG-2 (Fig. 6D). Notably, the increase in the cytoskeletal strengthened cell-ECM adhesion was eliminated by the Q614A/W615A mutation, which disrupted integrin binding (Fig. 6D), suggesting that integrin binding is essential for MIG-2-mediated cytoskeletal strengthening of cell-ECM adhesion.

To further study the influence of MIG-2 on cell-ECM adhesion, we analyzed the effect of the MIG-2/integrin interaction on FA formation. To do this, we plated SK-LMS-1 cells on collagen I and immunofluorescently stained them with mAb to vinculin, a marker of FAs. Under the conditions used, the control cells formed relatively weak vinculin-rich clusters (Fig. 6E). Overexpression of MIG-2 (Fig. 6F), but not of the integrin binding-defective Q614A/W615A mutant (Fig. 6G), induced the formation of much more evident vinculin clusters. These results are highly consistent with the results of the centrifugation cell-ECM adhesion experiments. Collectively, these results suggest that the MIG-2/integrin interaction contributes to cytoskeletal strengthening of SK-LMS-1 cell-ECM adhesion.

Next, we analyzed cell-ECM adhesion and FA formation in MIG-2-null RKO colon carcinoma cells. MIG-2-null RKO colon carcinoma cells were able to adhere to ECM (Fig. 7, C

**FIGURE 4.** Overexpression of MIG-2 modestly promotes integrin activation. A and B, a GST pulldown experiment was performed as described under "Experimental Procedures." CHO cell lysates (1.8 μg of protein/lane; lanes 1); precipitates derived from CHO cells and GST (lanes 2), GST-β3 (lanes 3), or GST-Y747A (lanes 4); and GST-β3 (lanes 4) and GST-Y747A (lanes 6) in the absence of cell lysates were analyzed by Western blotting with anti-MIG-2 mAb 3A3 (A) or by Coomassie Blue staining (B). C and D, integrin activation was analyzed using CHO cells stably expressing wild-type β3 integrin and activation-specific anti-β3 integrin mAb PAC1 as described under "Experimental Procedures." The MFI of mAb PAC1 bound to GFP-talin-H- or GFP-MIG-2-expressing cells was calculated using CellQuest software. The values were compared with the MFI of mAb PAC1 bound to control GFP-expressing cells (normalized to 1) in each of the seven experiments performed (C). The bars in D represent the means ± S.D. from the seven experiments. **, p < 0.05 versus the control. E, CHO cell adhesion was analyzed as described under "Experimental Procedures." The adhesion of GFP-MIG-2- or GFP-talin-H-expressing cells was compared with that of control GFP-expressing cells (normalized to 1). Bars represent the means ± S.D. from two independent experiments. **, p < 0.05 versus the control.

The MIG-2/Integrin Interaction Strengthens Cancer Cell-ECM Adhesion—Next, we investigated the functions of MIG-2 in the regulation of cancer cell behavior. Two different model systems were used: the MIG-2-expressing SK-LMS-1 leiomyosarcoma cells and the MIG-2-null RKO colon carcinoma cells. To facilitate the functional studies, we generated adenoviral systems: the MIG-2-expressing SK-LMS-1 leiomyosarcoma cells and the MIG-2-null RKO colon carcinoma cells. MIG-2-null RKO colon carcinoma cells were able to adhere to ECM (Fig. 7, C

**FIGURE 4.** Overexpression of MIG-2 modestly promotes integrin activation. A and B, a GST pulldown experiment was performed as described under "Experimental Procedures." CHO cell lysates (1.8 μg of protein/lane; lanes 1); precipitates derived from CHO cells and GST (lanes 2), GST-β3 (lanes 3), or GST-Y747A (lanes 4); and GST-β3 (lanes 4) and GST-Y747A (lanes 6) in the absence of cell lysates were analyzed by Western blotting with anti-MIG-2 mAb 3A3 (A) or by Coomassie Blue staining (B). C and D, integrin activation was analyzed using CHO cells stably expressing wild-type β3 integrin and activation-specific anti-β3 integrin mAb PAC1 as described under "Experimental Procedures." The MFI of mAb PAC1 bound to GFP-talin-H- or GFP-MIG-2-expressing cells was calculated using CellQuest software. The values were compared with the MFI of mAb PAC1 bound to control GFP-expressing cells (normalized to 1) in each of the seven experiments performed (C). The bars in D represent the means ± S.D. from the seven experiments. **, p < 0.05 versus the control. E, CHO cell adhesion was analyzed as described under "Experimental Procedures." The adhesion of GFP-MIG-2- or GFP-talin-H-expressing cells was compared with that of control GFP-expressing cells (normalized to 1). Bars represent the means ± S.D. from two independent experiments. **, p < 0.05 versus the control.

The MIG-2/Integrin Interaction Strengthens Cancer Cell-ECM Adhesion—Next, we investigated the functions of MIG-2 in the regulation of cancer cell behavior. Two different model systems were used: the MIG-2-expressing SK-LMS-1 leiomyosarcoma cells and the MIG-2-null RKO colon carcinoma cells. To facilitate the functional studies, we generated adenoviral systems: the MIG-2-expressing SK-LMS-1 leiomyosarcoma cells and the MIG-2-null RKO colon carcinoma cells. MIG-2-null RKO colon carcinoma cells were able to adhere to ECM (Fig. 7, C

**FIGURE 4.** Overexpression of MIG-2 modestly promotes integrin activation. A and B, a GST pulldown experiment was performed as described under "Experimental Procedures." CHO cell lysates (1.8 μg of protein/lane; lanes 1); precipitates derived from CHO cells and GST (lanes 2), GST-β3 (lanes 3), or GST-Y747A (lanes 4); and GST-β3 (lanes 4) and GST-Y747A (lanes 6) in the absence of cell lysates were analyzed by Western blotting with anti-MIG-2 mAb 3A3 (A) or by Coomassie Blue staining (B). C and D, integrin activation was analyzed using CHO cells stably expressing wild-type β3 integrin and activation-specific anti-β3 integrin mAb PAC1 as described under "Experimental Procedures." The MFI of mAb PAC1 bound to GFP-talin-H- or GFP-MIG-2-expressing cells was calculated using CellQuest software. The values were compared with the MFI of mAb PAC1 bound to control GFP-expressing cells (normalized to 1) in each of the seven experiments performed (C). The bars in D represent the means ± S.D. from the seven experiments. **, p < 0.05 versus the control. E, CHO cell adhesion was analyzed as described under "Experimental Procedures." The adhesion of GFP-MIG-2- or GFP-talin-H-expressing cells was compared with that of control GFP-expressing cells (normalized to 1). Bars represent the means ± S.D. from two independent experiments. **, p < 0.05 versus the control.
and F–H), suggesting that MIG-2 is not absolutely required for integrin/ECM interactions. Immunofluorescence staining with anti-vinculin mAb showed, however, that the MIG-2-null RKO colon carcinoma cells were devoid of large FAs (Fig. 7C). To test whether this is caused by the lack of MIG-2, we expressed MIG-2 in the MIG-2-null cells by infecting them with adenoviral vectors encoding MIG-2 (10 μg of protein/lane; A) and kindlin (20 μg of protein/lane; B). The membrane used in A was reprobed in C with anti-actin Ab.

FIGURE 5. MIG-2 protein expression in colon carcinoma cells. HT-1080 fibrosarcoma cells (lane 1); RKO (lane 2), HT-29 (lane 3), DLD-1 (lane 4), LoVo (lane 3), HCT-116 (lane 6), and Caco-2 (lane 8) colon carcinoma cells; and SK-LMS-1 leiomyosarcoma cells (LMS; lane 7) were analyzed by Western blotting with antibodies specific for MIG-2 (10 μg of protein/lane; A) and kindlin (20 μg of protein/lane; B). The membrane used in A was reprobed in C with anti-actin Ab.

MIG-2 prompted the formation of FAs that could be readily detected by anti-vinculin mAb (Fig. 7D). Thus, the deficiency of large FAs in colon cancer cells is indeed caused by the lack of MIG-2 protein.

To test whether the MIG-2-induced FA formation depends on integrin binding, we infected RKO cells with adenovirus encoding the integrin binding-defective Q614A/W615A mutant (ωQ). Expression of the Q614A/W615A mutant was confirmed by Western blotting (Fig. 7A, lane 3). Expression of the integrin binding-defective mutant, unlike that of wild-type MIG-2, failed to induce the formation of large FAs (Fig. 7, compare D and E). These results confirm the specificity of the MIG-2-induced FA formation. Furthermore, they suggest that MIG-2 promotes FA formation in colon cancer cells through its interaction with the integrins.

Next, we employed the centrifugation assay to quantify the effect of the MIG-2/integrin interaction on colon cancer cell-ECM adhesion. The results show that MIG-2, but not the inte-
grin binding-defective Q614A/W615A mutant, significantly enhanced the cytoskeletal strengthening phase of RKO cell adhesion to collagen (Fig. 7F). No significant increase in the initial cell-collagen adhesion was observed in response to the expression of MIG-2 or the Q614A/W615A mutant (Fig. 7G). In control experiments, treatment of the cells with function-blocking anti-α2 or anti-β1 integrin Ab nearly completely eliminated the cell-collagen adhesion, confirming that α2β1 integrin mediates the cell-collagen adhesion. Collectively, these results suggest that the MIG-2/integrin interaction functions primarily in the cytoskeletal strengthening of cell-ECM adhesion in these colon cancer cells.

The MIG-2/Integrin Interaction Regulates Cancer Cell Motility—Cell-ECM adhesion is intimately involved in the regulation of cell behavior such as cell motility. The findings that the MIG-2/integrin interaction promotes cell-ECM adhesion raised an interesting possibility that it may play a role in the regulation of cell motility. The MIG-2-null RKO colon carcinoma cells were highly motile (Fig. 8A). Consistent with the strengthening of colon cancer cell-ECM adhesion, the expression of MIG-2 significantly reduced colon cancer cell migration (Fig. 8, A, B, and D). No significant inhibition of cell migration was observed in RKO cells expressing the integrin binding-defective Q614A/W615A mutant (Fig. 8, C and D), suggesting that, through integrin binding, MIG-2 suppresses colon cancer cell migration.

To test whether MIG-2 plays a role in the regulation of SK-LMS-1 cell motility, we compared the motility of SK-LMS-1 cells overexpressing MIG-2 or the integrin binding-defective Q614A/W615A mutant with that of the control cells. The results show that the MIG-2-overexpressing SK-LMS-1 cells migrated substantially slower than the control cells (Fig. 9, A, B, and D). No alteration of cell migration was observed in cells overexpressing the integrin binding-defective Q614A/W615A mutant (Fig. 9, A, C, and D). Thus, consistent with the results...
activation. Consistent with a positive role in promoting interaction suppresses leiomyosarcoma cell motility.

DISCUSSION

MIG-2 is a widely expressed and evolutionarily conserved FA protein (17–21). The experiments presented in this study have demonstrated a specific interaction between MIG-2 and the β1 and β3 integrin cytoplasmic domains. Using a structure-based mutagenesis approach, we have characterized the sites that mediate the MIG-2/integrin interaction. Functionally, we have shown that the MIG-2/integrin interaction is essential for recruiting MIG-2 to FAs. In addition, using CHO cells expressing αIIβ3 integrin, a well described model system for integrin activation, we found that MIG-2 can weakly promote integrin activation. Consistent with a positive role in promoting αIIβ3 integrin activation, MIG-2 enhances the adhesion of αIIβ3 integrin-expressing CHO cells to fibrinogen. Finally, we have investigated the functions of MIG-2 in cancer cells. Using two different types of cancer cells (MIG-2-expressing leiomyosarcoma cells and MIG-2-null colon carcinoma cells), we have demonstrated that the MIG-2/integrin interaction promotes cell-ECM adhesion and FA formation and reduces cell motility. These results provide new insights into the molecular basis underlying the cellular control of cell-ECM adhesion. Furthermore, our findings suggest that alterations of certain components (e.g. MIG-2) that participate in the cellular control of cell-ECM adhesion may contribute to high motility of certain types of cancer cells (e.g. colon carcinoma cells).

Structurally, the MIG-2/integrin interaction shares certain but not all features with the talin/integrin interaction. One of the most obvious common features of MIG-2 and talin is that both contain FERM domains. In particular, the F3 subdomains within the MIG-2 and talin FERM domains share considerable sequence homology. On the basis of the sequence homology, we constructed a model of the MIG-2 F3 subdomain bound to the β1 integrin tail, which suggests a canonical FERM phosphotyrosine-binding domain fold capable of recognizing the β1 integrin tail. On the basis of the structural modeling, we predicted that several residues, including Glu614-Trp615, within the MIG-2 F3 subdomain constitute the integrin-binding interface. We predicted, for example, that Glu614 and Trp615 in the MIG-2 F3 subdomain directly interact with Trp775 and Asp776 in the β1 integrin tail via hydrophobic and hydrophilic interactions. Consistent with this model, substitution of Glu614-Trp615 with alanine residues abrogated integrin binding. The fact that the Glu614-Trp615 substitution mutation eliminated integrin binding also suggests that other regions of MIG-2 are incapable of interacting with the β integrin tails with high affinity.

Although it is clear that the MIG-2 FERM F3 subdomain folds into a three-dimensional integrin-binding structure that resembles, at least globally, the integrin-binding structure of talin, our study has revealed that the first NPYX (Tyr747 in the β3 integrin tail) site in the central region of the β integrin cytoplasmic domains, which is crucial for the formation of the talin-β integrin complex, is not required for the formation of the MIG-2-β integrin complex. Thus, although it is likely that the MIG-2- and talin-binding interfaces share certain common residues, there must be a structural difference in the precise integrin-binding modes between MIG-2 and talin. For example, the β6-β7 loop in the talin F3 subdomain, which is responsible for recognizing integrin Tyr747 (38), appears to differ from that in the MIG-2 FERM F3 subdomain. The latter appears to make significantly less contact with the NPYX region of the integrin tail in our model.

At the cellular level, one important function of the MIG-2/integrin interaction revealed by this study is that this interaction is essential for recruiting MIG-2 to FAs. It is interesting to compare the MIG-2/integrin interaction with the MIG-2/migfilin interaction. Migfilin is another MIG-2-binding protein that is also found at FAs (20–22). However, unlike integrin binding, migfilin binding is not required for MIG-2 localization to FAs (20). Instead, the interaction of MIG-2 with migfilin recruits migfilin to FAs (20), which in turn facilitates the localization of certain migfilin-binding FA proteins such as VASP to the adhesion sites (23). Thus, integrins, MIG-2, migfilin, and VASP appear to localize to FAs in a sequential fashion. This sequential localization mechanism is probably utilized by some other, but clearly not all, FA components during the assembly of FAs. Certain FA proteins, notably PINCH, integrin-linked kinase, and α-parvin, are first assembled into a multicomponent protein complex and then simultaneously localize to FAs (29, 45). Through both mechanisms, a large number of components are recruited to FAs, where they regulate a variety of cellular processes.

In general, integrin-mediated cell-ECM adhesion can be regulated through two distinct mechanisms. The first one is through regulation of integrin activation. Recent studies have demonstrated that binding of talin-H to β integrin tails plays a key role in integrin activation (for recent reviews, see Refs. 7–10). Since Horwitz et al. (46) discovered that talin interacts with integrins more than 2 decades ago, >20 β integrin tail-
binding proteins have been identified (reviewed in Ref. 47). Interestingly, among all the cytoplasmic β integrin-binding proteins that had been tested, only talin had been shown to be able to promote integrin activation (24, 41, 48). The results presented in this study demonstrate that MIG-2 can also play a role in this process. Although this is clearly an exciting finding, several lines of evidence suggest that the role of MIG-2 in integrin activation is not identical to that of talin. First, the extent to which MIG-2 promotes integrin activation appears to be smaller compared with talin-H. This could be due to the relatively low expression level of GFP-MIG-2 (compared with GFP-talin-H) in our experiments. However, despite our efforts (we performed seven transfection experiments), we have not been able to further increase the expression level of GFP-MIG-2 in CHO cells. Alternatively (but not necessarily mutually exclusively), the relatively weak effect of MIG-2 on integrin activation could be an intrinsic property of MIG-2.

A second and perhaps even more fundamental difference is that the MIG-2-binding site in the β3 integrin tail is not identical to the talin-binding site. The first NPXY (Tyr247 in the β3 integrin tail) site located in the central region of the β integrin tails is crucial for talin binding. However, this site is not required for MIG-2 binding (Figs. 1C and 4A, lane 5). Although the precise mode by which MIG-2 binds to β integrin tails remains to be determined, it is important to note that MIG-2 lacks several key residues in the S1–S2 loop that are crucial for interacting with the integrin membrane proximal region. For example, Leu325 in the S1–S2 loop of talin-H is vital for interacting with the β3 integrin membrane proximal region (39). However, this residue is not present in MIG-2 (Fig. 2, A and C). Previous studies have shown that a proline substitution mutation at Ser752, which is found in a variant of Glanzmann thrombasthenia, suppresses αIIbβ3 integrin activation (49, 50). The S752P point mutation does not impair talin-H binding (28). Interestingly, preliminary studies suggest that the S752P point mutation impairs the binding of MIG-2 to the β3 integrin tail.4 Thus, MIG-2 appears to interact with the membrane distal region rather than the membrane proximal region in the β3 integrin tail. It is widely accepted that interactions of talin-H with the membrane proximal region and consequently separation of the membrane proximal “clasp” are final intracellular steps in integrin activation (7–9, 11–13). On the other hand, the membrane distal region appears to regulate integrin activation indirectly (28, 51). On the basis of these considerations, we propose that MIG-2 promotes integrin activation through an indirect mechanism. We are currently investigating the three-dimensional structure of the MIG-2/β3 integrin tail complexes, which should help to shed light on the mechanism through which MIG-2 promotes integrin activation.

A third difference between talin and MIG-2 is that, although talin has been proposed to serve as a common activator of integrins in the final intracellular step of integrin activation (41), the role of MIG-2 in integrin activation appears to be context-dependent. For example, MIG-2-null colon cancer cells can adhere to the ECM, suggesting that MIG-2 is not absolutely required for integrin activation in these cells. Consistent with this, the expression of MIG-2 in the MIG-2-null colon cancer cells fails to significantly increase the initial cell-ECM adhesion, a process that is controlled primarily by integrin ligand-binding activity (15, 16).

The second mechanism by which cell-ECM adhesion can be regulated is through cytoskeletal strengthening (14–16). The results presented in this work suggest that the MIG-2/integrin interaction plays a prominent role in this process. This is based on two lines of evidence. First, quantitative centrifugation cell adhesion assays showed that overexpression of MIG-2, but not of the integrin binding-defective MIG-2 mutant, in SK-LMS-1 leiomyosarcoma and RKO colon cancer cells substantially enhanced the cytoskeletal strengthening phase, but not the cytoskeletal-independent initial phase, of cell-collagen adhesion. Second, immunofluorescence microscopic analyses showed that overexpression of MIG-2, but not of the integrin binding-defective MIG-2 mutant, enhanced the formation of FAs. Thus, the MIG-2/integrin interaction likely represents a key element in the cellular machinery that controls the cytoskeletal strengthening of cell-ECM adhesion.

It has been well established, by both theoretic considerations and experimental studies, that cell motility is favored by an intermediate level of cell-ECM adhesion (25, 26). Consistent with this model, we have found that overexpression of MIG-2, which enhances cell-ECM adhesion, reduces the motility of leiomyosarcoma and colon cancer cells. These results suggest that the MIG-2-mediated cytoskeletal strengthening of cell-ECM adhesion likely serves as an “adhesive brake” for the movement of these cells. Loss of this adhesive brake could contribute to high motile behavior of certain diseased cells. Interestingly, large-scale RNA profiling studies have shown that the MIG-2 mRNA level is reduced in colon cancer cells (see harvester.embl.de/harvester/Q96A/Q96AC1.htm and genome-www.stanford.edu/nci60)(43, 44). We have confirmed in this study that the MIG-2 protein level is indeed diminished in a number of colon cancer cell lines. Notably, introducing this adhesive brake (i.e. MIG-2) into MIG-2-null colon cancer cells results in an increase in cell-ECM adhesion and a reduction of cell motility. Because expression of the integrin binding-defective MIG-2 mutant (Q614A/W615A) in MIG-2-null colon cancer cells did not significantly alter colon cell-ECM adhesion and motility, integrin binding appears to be essential for MIG-2-mediated regulation of colon cell-ECM adhesion and motility.

Acknowledgments—We thank Drs. Tong-Chuan He and Bert Vogelstein for the pAdTrack-CMV and pAdEasy-1 vectors and Drs. Lin Zhang and Craig C. Garner for the colon cancer cells.

REFERENCES

1. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–599
2. Hemler, M. E., and Lobb, R. R. (1995) Curr. Opin. Hematol. 2, 61–67
3. Burridge, K., and Chrzanowska-Wodnicka, M. (1996) Annu. Rev. Cell Dev. Biol. 12, 463–518
4. Howe, A., Aplin, A. E., Alahari, S. K., and Juliano, R. L. (1998) Curr. Opin. Cell Biol. 10, 220–231
5. Geiger, B., Bershadsky, A., Pankov, R., and Yamada, K. M. (2001) Nat. Rev.
MIG-2/Integrin Interaction in Cell Adhesion and Migration

6. Hynes, R. O. (2002) Cell 110, 673–687
7. Campbell, I. D., and Ginsberg, M. H. (2004) Trends Biochem. Sci. 29, 429–435
8. Qin, J., Vinogradova, O., and Plow, E. F. (2004) PLoS Biol. 2, e169
9. Nayal, A., Webb, D. J., and Horwitz, A. F. (2004) Curr. Opin. Cell Biol. 16, 94–98
10. Critchley, D. R. (2005) Biochem. Soc. Trans. 33, 1308–1312
11. Vinogradova, O., Velyvis, A., Velyviene, A., Hu, B., Haas, T., Plow, E., and Qin, J. (2002) Cell 110, 587–599
12. Lauffenburger, D. A., and Horwitz, A. F. (1996) Cell 87, 318–326
13. Kim, M., Carman, C. V., and Springer, T. A. (2003) Science 301, 1720–1725
14. Lotz, M. M., Burdsgaard, C. A., Erickson, H. P., and McClay, D. R. (1989) J. Cell Biol. 109, 1795–1805
15. Yamada, K. M. (2003) in Current Protocols in Cell Biology (Bonifacino, J. S., Dasso, M., Harford, J. B., Ginsberg, M. H., and Yamada, K. M., eds) Vol. 1, pp. 9.0.1–9.0.9, John Wiley & Sons, Inc., New York
16. McClay, D. R., and Hertzler, P. L. (2003) in Current Protocols in Cell Biology (Bonifacino, J. S., Dasso, M., Harford, J. B., Lippincott-Schwartz, J., and Yamada, K. M., eds) Vol. 1, pp. 9.2.1–9.2.10, John Wiley & Sons, Inc., New York
17. Rogalski, T. M., Mullen, G. P., Gilbert, M. M., Williams, B. D., and Moore, S. C., and Wu, C. (2005) Proc. Natl. Acad. Sci. U. S. A. 101, 4094–4099
18. McClay, D. R., and Hertzler, P. L. (2003) in Current Protocols in Cell Biology (Bonifacino, J. S., Dasso, M., Harford, J. B., Lippincott-Schwartz, J., and Yamada, K. M., eds) Vol. 2, 1795–1805
19. Yamada, K. M. (2003) in Current Protocols in Cell Biology (Bonifacino, J. S., Dasso, M., Harford, J. B., Lippincott-Schwartz, J., and Yamada, K. M., eds) Vol. 2, 1795–1805
20. Tu, Y., Wu, S., Shi, X., Chen, K., and Wu, C. (2003) Cell 113, 37–47
21. Wu, C. (2005) J. Cell Sci. 118, 659–664
22. Gkretsi, V., Zhang, Y., Tu, Y., Chen, K., Stolz, D. B., Yang, Y., Watkins, S. C., and Wu, C. (2005) J. Cell Sci. 118, 679–710
23. Zhang, Y., Tu, Y., Gkretsi, V., and Wu, C. (2006) J. Biol. Chem. 281, 12397–12407
24. Kloecker, S., Major, M. B., Calderwood, D. A., Ginsberg, M. H., Jones, D. A., and Beckerle, M. C. (2004) J. Biol. Chem. 279, 6824–6833
25. Hutenlocher, A., Sandborg, R. R., and Horwitz, A. F. (1995) Curr. Opin. Cell Biol. 7, 697–706
26. Lauffenburger, D. A., and Horwitz, A. F. (1996) Cell 84, 359–369
27. Li, J., Mayne, R., and Wu, C. (1999) J. Cell Biol. 147, 1391–1397
28. Ma, Y.-Q., Yang, J., Pesho, M. M., Vinogradova, O., Qin, J., and Plow, E. F. (2006) Biochemistry 45, 6656–6662
29. Zhang, Y., Guo, L., Chen, K., and Wu, C. (2002) J. Biol. Chem. 277, 318–326
30. Guo, L., and Wu, C. (2002) FASEB J. 16, 1298–1300
31. Kolls, J., Peppel, K., Silva, M., and Beutler, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 215–219
32. Bauer, J. S., Schreiner, C. L., Gancotti, F. G., Ruoslahti, E., and Juliano, R. L. (1992) J. Cell Biol. 116, 477–487
33. Fukuda, T., Chen, K., Shi, X., and Wu, C. (2003) J. Biol. Chem. 278, 51234–51335
34. Schwede, T., Kopp, J., Guex, N., and Peitsch, M. C. (2003) Nucleic Acids Res. 31, 3381–3385
35. Brungger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
36. Roussel, A., and Cambillau, C. (1991) TURBO-FRODO in Silicon Graphics Geometry, Silicon Graphics, Mountain View, California
37. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA
38. Garcia-Alvarez, B., de Pereda, J. M., Calderwood, D. A., Ulmer, T. S., Critchley, D., Campbell, I. D., Ginsberg, M. H., and Liddington, R. C. (2003) Mol. Cell 11, 49–58
39. Wegener, K. L., Partridge, A. W., Han, J., Pickford, A. R., Liddington, R. C., Ginsberg, M. H., and Campbell, I. D. (2007) Cell 128, 171–182
40. Calderwood, D. A., Zent, R., Grant, R., Rees, D. J., Hynes, R. O., and Ginsberg, M. H. (1999) J. Biol. Chem. 274, 28071–28074
41. Tadokoro, S., Shattil, S. J., Eto, K., Tai, V., Liddington, R. C., de Pereda, J. M., Ginsberg, M. H., and Calderwood, D. A. (2003) Science 302, 103–106
42. Calderwood, D. A., Shattil, S. J., and Ginsberg, M. H. (2000) J. Biol. Chem. 275, 22607–22610
43. Ross, D. T., Scherf, U., Eisen, M. B., Perou, C. M., Rees, C., Spellman, P., Iyer, V., Jeffrey, S. S., Van de Rijn, M., Waltham, M., Peramenschikov, A., Lee, J. C., Lashkari, D., Shalon, D., Myers, T. G., Weinstein, J. N., Botstein, D., and Brown, P. O. (2000) Nat. Genet. 24, 227–235
44. Su, A. I., Welsh, J. B., Sapinoso, L. M., Dimitrov, P., Lapp, H., Schultz, P. G., Powell, S. M., Moskaluk, C. A., Frierson, H. F., Jr., and Hamilton, J. G., and Yamada, K. M., eds) Vol. 1, pp. 9.2.1–9.2.10, John Wiley & Sons, Inc., New York