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Filamin A mediates HGF/c-MET signaling in tumor cell migration

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Deregulated hepatocyte growth factor (HGF)/c-MET axis has been correlated with poor clinical outcome and drug resistance in many human cancers. Identification of novel regulatory mechanisms influencing HGF/c-MET signaling may therefore be necessary to develop more effective cancer therapies. In our study, we show that multiple human cancer tissues and cells express filamin A (FLNA), a large cytoskeletal actin-binding protein, and expression of c-MET is significantly reduced in human tumor cells deficient for FLNA. The FLNA-deficient tumor cells exhibited poor migrative and invasive ability in response to HGF. On the other hand, the anchorage-dependent and independent tumor cell proliferation was not altered by HGF. The FLNA-deficiency specifically attenuated the activation of the c-MET downstream signaling molecule AKT in response to HGF stimulation. Furthermore, FLNA enhanced *c-MET* promoter activity by its binding to SMAD2. The impact of FLNA deficiency on c-MET expression and HGF-mediated cell migration in human tumor cells was confirmed in primary mouse embryonic fibroblasts deficient for *Flna*. These data suggest that FLNA is one of the important regulators of c-MET signaling and HGF-induced tumor cell migration.

The hepatocyte growth factor (HGF) promotes cell migration, proliferation, invasion and survival in many human organ malignancies including liver, lung, prostate and pancreas carcinomas *via* binding to its receptor, the MET receptor tyrosine kinase.[1] Increasing evidence suggests that disruption of normal cell–cell adhesion *via* c-MET signaling endows primary tumor cells with invasive and metastatic potential by increasing cell motility.[2] In virtually all types of solid tumors, c-MET is activated by overexpression, amplification, mutation and/or a ligand-dependent autocrine/paracrine loop.[3] Hyperactivation of c-MET signaling is thought to contribute to proliferative, invasive or angiogenic cancer phenotypes and have been correlated with poor clinical outcomes and drug resistance in cancer patients.[4] In human cancers, *c-MET* germline missense, activating or somatic mutations are found,[5] but the most frequent occurrence is the aberrant expression of c-MET associated with metastatic phenotype.[4] Furthermore, it is well known that human tumor cells express c-MET and are susceptible to c-MET-mediated cellular migration and invasion both *in vitro* and *in vivo*.[5] Experimental activation of the HGF/c-MET autocrine loop *in vitro*[6] or in transgenic animals[7] generates invasive tumors. However, at this point, little is known about how c-MET signaling is completely regulated.

Filamin A (FLNA) is a large cytoskeletal protein that stabilizes delicate three-dimensional actin networks and links them to cellular membranes.[8] The actin cytoskeleton is essential for the dynamic regulation of cellular morphology and locomotion in response to external stimuli. In addition to filamentous actin, FLNA interacts with numerous intracellular molecules,[9] among which many FLNA interacting partners are regulators of cell motility. The functional importance of FLNA in regulating cell motility both directly and indirectly proposed a potential role in the metastasis of malignant tumor cells. Recently, the intracellular level of FLNA was found to be increased in peripheral hepatic cholangiocarcinoma[10] and the perturbed FLNA function may contribute to the

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aggressiveness of pancreatic cancer in patients.[11] The plasma level of a FLNA variant appears to be a specific and sensitive marker for patients with metastatic breast cancer and high-grade astrocytoma.[12] The studies in different cancer cell lines including human lung[13] and breast cancer,[14] squamous cell carcinoma[15] and glioma cells[16] suggested that FLNA may mediate the effects of growth factors in cancer cell migration and invasion. In addition, FLNA is overexpressed coincidently with some oncogenes and growth factors, such as c-MET in activated cancer cells.[15] To determine the biological role of this unknown coexpression of FLNA and c-MET may advance our knowledge in tumor biology to eventually develop new therapeutic approaches to treat human cancers.

In our study, we tested if FLNA is expressed in human cancers in which HGF/c-MET signaling has a decisive role for clinical survival and if FLNA has an impact on HGF/c-MET signaling that mediates tumor cell migration. To approach these issues, we studied expression of FLNA in human cancer tissues and cells, and analyzed HGF/c-MET signaling and migration of FLNA-deficient human melanomas and mouse fibroblasts. To determine how FLNA regulates c-MET signaling, we studied downstream signaling molecules and investigated the consequence of the interaction of FLNA with SMAD2 on the c-MET promoter activity. Our results point a novel function of a cytoskeletal protein regulating c-MET activation, which may be exploited to prevent HGF-driven cancer progression and metastasis.

Material and Methods

Immunohistochemistry

Paraffin-embedded tissue microarray samples containing multiple organ adenocarcinomas (Oligene GmbH, Berlin, Germany) were immunohistochemically stained with an antibody against FLNA (Chemicon International, Billerica, MA) as described previously.[9]

Cell culture and transient transfections

HepG2 hepatocarcinoma cell line was cultured in DMEM, H69 human small lung cancer cell line was cultured in RPMI-1640/RPMI 1640 with HEPES (1:1), BON human pancreatic carcinoid tumor cell line was cultured in DMEM/F12, and PC-3 prostate cancer cell line was cultured in RPMI 1640 without HEPES. All media were supplemented with 10% FBS, 2 mM glutamine and antibiotics, whereas H69 and PC-3 culture media were supplemented with additional 1 mM sodium pyruvate and 2.5 g/L glucose.

M2, a human melanoma cell line ($M2^{FLNA-}$) deficient for both *FLNA* mRNA and protein,[17] and its subline, that has been stably transfected to express FLNA ($M2^{FLNA+}$ and ATCC, Washington D.C.), were cultured in EMEM (Invitrogen, San Diego, CA) supplemented with 10% of FBS and containing 100 U/ml penicillin and 100 μg/ml streptomycin sulfate. To maintain FLNA expression in $M2^{FLNA+}$ cells, 0.3 mg/ml of G418 (Calbiochem, San Diego, CA) was added. Transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. $M2^{FLNA+}$ cells were incubated with 50 μM of the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (Invitrogen) for 30 min. *Flna*-deficient ($Flna^{o/-}$) and *wild-type* ($Flna^{o/+}$) MEFs were cultured in DMEM low glucose (PAA Laboratories GmbH, Pasching, Austria) supplemented with 10% FBS and containing 100 U/ml penicillin and 100 μg/ml streptomycin sulfate, 1× NEAA, 2 mM L-glutamine and 100.8 μM β-mercaptoethanol.[18] We used the nomenclature $Flna^{o/-}$ for *Flna*-deficient and $Flna^{o/+}$ for *wild-type* MEFs as the *Flna* gene is located in the X chromosome.

Reverse transcriptase-PCR

RNA was extracted using RNeasy Mini kit (Qiagen, Germantown, MD) according to the manufacturer. RNA samples were treated with DNase (Invitrogen) followed by the generation of cDNA using High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA). *c-MET* transcript was amplified with forward primer 5′-CTGGGCACCGAAAGATAAACC-3′ and reverse primer 5′-TGGCACCAAGGAAAATGTGATG-3′. *18S* internal control transcript was amplified with forward primer 5′-AGATCAAAACCAACCCGGTGA-3′ and reverse primer 5′-GGTAAGAGCATCGAGGGGGC-3′.

Immunoblotting

To prepare whole cell protein extracts, cells were washed with phosphate-buffered saline (PBS) and lysed with CelLytic M Cell Lysis Reagent (Sigma, St. Louis, MO) supplemented with Protease and Phosphatase Inhibitor Cocktail (Complete-Mini; Roche Biochemicals, Penzberg, Germany). Lysates were cleared by centrifugation for 15 min at $2 \times 10^4 g$ at 4°C. Protein concentration was determined by BCA Protein Assay Kit (Thermo Scientific, Waltham, MA).

Protein lysates were separated by SDS-PAGE and blotted onto a polyvinylidene difluoride membrane. Blocking was performed in TBS-0.1% Tween with 5% nonfat milk, followed by incubation with antibodies against FLNA (Chemicon International), c-MET, AKT, phosphorylated AKT (p-AKT), ERK1/2, phosphorylated ERK1/2 (p-ERK1/2), SMAD2, phosphorylated SMAD2 (p-SMAD2) (Cell Signaling Technology, Danvers, MA), GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) or Actin (Sigma) in TBS-0.1% Tween with 5% nonfat milk. After several washes with TBS-0.5% Tween, the membranes were incubated with anti-mouse IgG-horseradish peroxidase conjugate (Amersham Biosciences, Buckinghamshire, UK) in TBS-Tween with 5% nonfat milk. After several washes, proteins were visualized using ECL or Super ECL Reagents (Amersham Biosciences) according to the manufacturer's recommendations.

Migration and invasion assay

Cells were seeded on the upper chamber of the BioCoat Control Inserts (BD Biosciences, San Jose, CA) and the ability of cells to migrate through a micropore nitrocellulose

filter (8-μm thick, 8 μm pores in diameter) as a criterion for chemotactic stimuli was measured.[19] Medium supplemented with 2% serum with or without 80 ng/ml HGF was added to the lower chambers. Approximately 2.5×10^4 cells were seeded in each upper chamber. M2 cells were incubated at 37°C for 24 hr and migrated cells were fixed and stained with SNABB-DIFF Kit (Labex AB, Sweden).

MEFs were incubated at 37°C for 4 hr and migrated cells were fixed and stained with DAPI. Cells that migrated through the filter were counted using a light or fluorescence microscope. Triplicates of each sample are represented as mean ± SD data. For invasion assay, the same strategy was applied but the BioCoat Growth Factor Reduced Matrigel Invasion Chamber (BD Biosciences) was used instead and cells were incubated for 24 hr.

Colony-forming assay

Twelve-well cell culture plates were coated with 1 ml/well of 0.6% seaplaque agarose in serum-free culture medium. Approximately 2×10^3 cells were suspended in 1 ml of 0.4% agarose in culture medium containing 2% serum supplemented with or without 40 ng/ml HGF and seeded into each well. Cells were incubated at 37°C for 10 days and colonies were counted from nine optical fields from each well under a phase contrast microscope.

Proliferation assay

Cells cultured in medium containing 2% serum supplemented with or without 40 ng/ml HGF on 6-well plates were trypsinized and counted in a counter (Beckman Coulter). The results are given as mean ± SD values at 24, 48 and 72 hr in triplicates at each observation time.

Immunoprecipitation

The cells were lysed with PBS with 0.5% Triton X-100 supplemented with protease and phosphatase inhibitor cocktail (Roche Biochemicals). Precleared cell lysates were incubated with anti-FLNA antibody (Chemicon International) overnight at 4°C. Proteins were precipitated with 50 μl of 50% protein G-sepharose slurry (Amersham Biosciences). After three washes with PBS buffer supplemented with 0.5% Triton X-100, the precipitated proteins were analyzed by SDS-PAGE followed by immunoblotting assays as described above.

Luciferase assay

Transient transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In a *c-MET* reporter assay, $M2^{FLNA-}$ cells were cotransfected with *c-MET*-driven firefly luciferase reporter plasmid[20] and pRL-SV40 encoding Renilla luciferase gene as an internal control of transfection efficiency. In single or combined overexpression experiments of *SMAD2* and *FLNA*, $M2^{FLNA-}$ cells were transfected with 1 μg of pSMAD2 or pFLNA. Transfection of carrier DNA pCMX (mock-transfection) was performed to keep total DNA concentration at 2 μg. Firefly luciferase activity was measured and normalized to Renilla luciferase activity. Data are presented as luciferase activity relative to mock-transfected M2 cells.

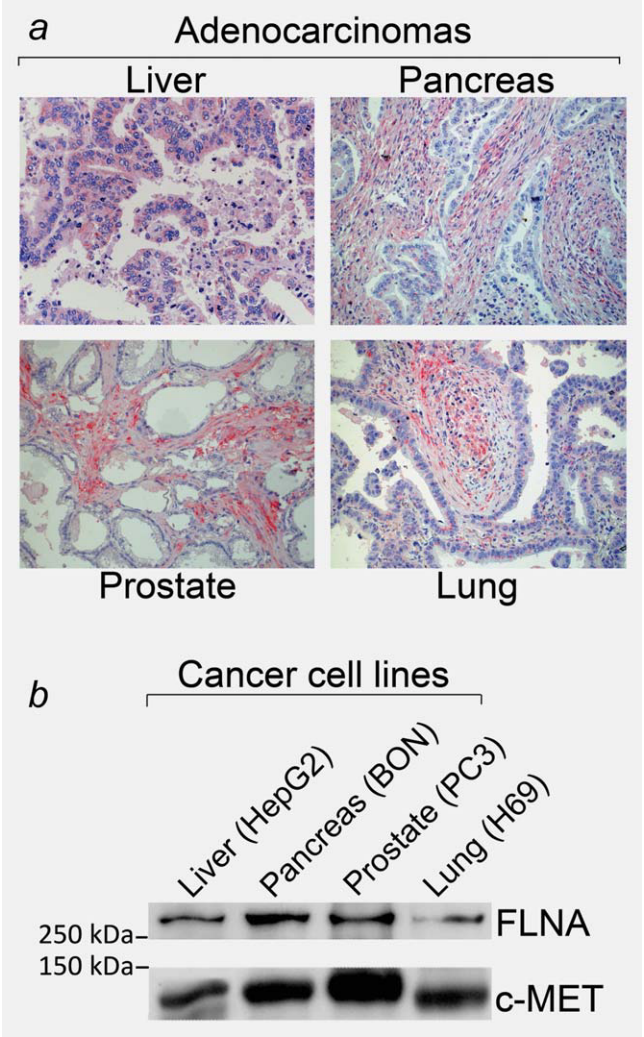


Figure 1. Human cancers express FLNA *in vivo* and *in vitro*. (*a*) Immunohistochemical detection of FLNA in tissue microarray samples of human liver, pancreas, prostate and lung adenocarcinomas. Magnifications ×200. (*b*) Immunoblots demonstrating coexpression of FLNA and c-MET in human liver (HepG2), pancreas (BON), prostate (PC3) and lung (H69) cancer cell lines.

Results

FLNA is expressed in human cancers in which c-MET signaling is crucial

To detect FLNA expression, we immunohistochemically stained a human tissue microarray containing adenocarcinomas (Fig. 1*a*). In normal organs, FLNA was strongly expressed particularly by smooth muscle cells in the arterial, venous and capillary vascular wall as well as in the muscular layers of organs with hole such as colon, esophagus and stomach (Supporting Information Fig. 1). Fibromuscular

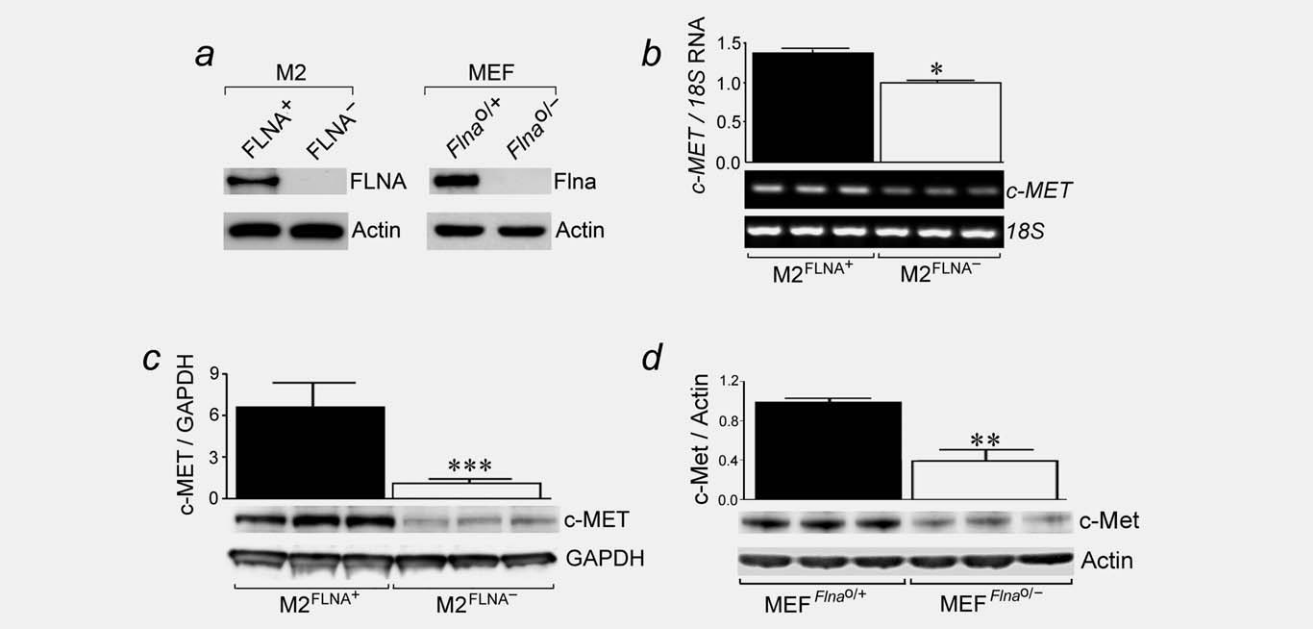


Figure 2. Lack of FLNA reduces c-MET expression. (*a*) Immunoblots demonstrating expression of FLNA in human melanoma cells (M2) expressing FLNA ($M2^{FLNA+}$) or deficient FLNA ($M2^{FLNA-}$), and in wild-type ($Flna^{o/+}$) and *Flna*-deficient ($Flna^{o/-}$) mouse embryonic fibroblasts (MEF). mRNA expression (*b*) and protein level of c-MET in $M2^{FLNA+}$ and $M2^{FLNA-}$ human melanoma cells (*c*), and in $Flna^{o/+}$ and $Flna^{o/-}$ MEF (*d*). Expression of *18S* mRNA, and GAPDH and Actin proteins served as internal controls. Mean ± SD data of triplicate experiments are given (Student's *t*-test, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$).

stromal cells under epithelium in normal tissues also expressed FLNA. However, FLNA expression was weak in epithelium of many normal organs, except the keratinocytes in the esophageal epithelium that expressed FLNA strongly. In adenocarcinoma tissues, both stromal and invaded adenocarcinoma cells strongly expressed FLNA in multiple organs including liver, pancreas, prostate, lung, kidney, breast, colon and ovary adenocarcinomas. Cancer cell lines obtained from the liver, pancreas, prostate and lung organs coexpressed FLNA and c-MET (Fig. 1*b*).

Reduced expression of c-MET in FLNA-deficient human melanoma cells

To study the role of FLNA on the c-MET signaling and function, we used two different cell types, human melanoma cells (M2) expressing or lacking FLNA and primary MEF isolated from wild-type or *Flna*-deficient mouse embryos (Fig. 2*a*). To determine if the relative levels of endogenous *c-MET* mRNA and c-MET protein were affected by FLNA deficiency, we performed RT-PCR and immunoblot analysis in $M2^{FLNA+}$ and $M2^{FLNA-}$ cells. We observed significantly lower levels of *c-MET* mRNA (Fig. 2*b*) and c-MET (140 kDa active form) protein in both $M2^{FLNA-}$ cells (Fig. 2*c*) and $Flna^{o/-}$ MEF (Fig. 2*d*).

Impaired HGF-induced cellular migration and invasion in FLNA-deficient human melanoma cells

In the absence of HGF, there were similar numbers of migrated M2 cells either expressing FLNA or lacking FLNA; however, in the presence of HGF, almost four times more $M2^{FLNA+}$ cells than $M2^{FLNA-}$ cells migrated (Fig. 3*a*). We also compared migration of $Flna^{o/+}$ and $Flna^{o/-}$ MEFs, and showed that fewer $Flna^{o/-}$ MEFs migrated compared with $Flna^{o/+}$ MEFs in the absence of HGF (Fig. 3*b*). In the presence of HGF, the number of migrated $Flna^{o/+}$ MEFs increased significantly, but the number of migrated $Flna^{o/-}$ MEFs remained lower than $Flna^{o/+}$ MEFs (Fig. 3*b*). There was no difference in the number of invaded M2 cells with or without FLNA in the absence of HGF; however, nearly three times more $M2^{FLNA+}$ cells than $M2^{FLNA-}$ cells invaded following stimulation with HGF (Fig. 3*c*). The proliferation of $M2^{FLNA+}$ and $M2^{FLNA-}$ cells was similar, and not affected by HGF stimulation up to 72 hr (Fig. 3*d*). $M2^{FLNA+}$ cells formed larger colonies in Matrigel (Figs. 3*e* and 3*f*), but the total number of colonies were similar between $M2^{FLNA+}$ and $M2^{FLNA-}$ cells (data not shown) and their number and size did not change in response to HGF (Fig. 3*f*).

c-MET signaling is impaired in FLNA-deficient human melanoma cells

To investigate the effect of FLNA deficiency on signaling molecules downstream of c-MET, we exposed $M2^{FLNA+}$ and $M2^{FLNA-}$ cells to HGF for up to 80 min and determined levels of p-AKT, p-ERK1 and p-ERK2. There was a similar basal level of p-AKT in both $M2^{FLNA+}$ and $M2^{FLNA-}$ cells (Fig. 2*a*). However, the level of p-AKT significantly increased in $M2^{FLNA+}$ cells but not in $M2^{FLNA-}$ cells in response to HGF

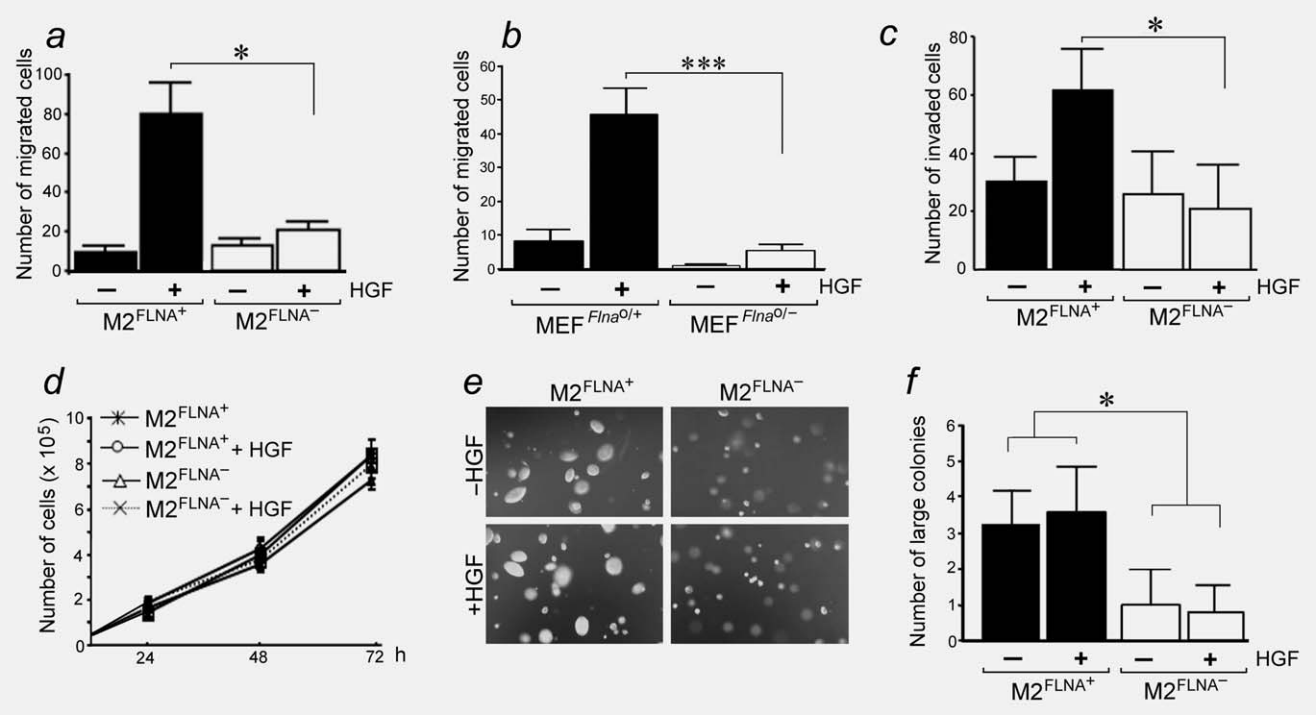


Figure 3. FLNA-deficiency in human melanoma cells impairs migration, invasion and colony formation in response to HGF stimulation. FLNA-expressing ($M2^{FLNA+}$) and FLNA-deficient ($M2^{FLNA-}$) M2 cells were assayed for migration (*a*), invasion (*c*) and proliferation (*d*) with or without HGF. (*b*) Normalized fold increases in the number of migrated $Flna^{o/+}$ and $Flna^{o/-}$ MEF cells in response to HGF. (*e*) Representative pictures of colony formation assay of $M2^{FLNA-}$ and $M2^{FLNA+}$ cells stimulated with or without HGF. (*f*) Number of large (>3 mm in diameter) $M2^{FLNA-}$ and $M2^{FLNA+}$ cell colonies in agar plate at day 10. Mean ± SD data of triplicate experiments are given (one-way ANOVA with Tukey's HSD as *post hoc* analysis, $*p < 0.05$ and $***p < 0.001$).

stimulation (Fig. 2*a*). In addition, the HGF-stimulated increase in p-AKT in $M2^{FLNA+}$ cells was abolished by administration of the PI3K inhibitor LY294002 (data not shown). Despite a tendency toward an initial increase 10 min after HGF stimulation, there were no changes at the levels of p-ERK1 and p-ERK2 between $M2^{FLNA+}$ and $M2^{FLNA-}$ cells in response to HGF stimulation (Figs. 4*b* and 4*c*).

FLNA and SMAD2 cooperatively regulate c-MET expression and function

We confirmed the physical interaction of FLNA with SMAD2 in a coimmunoprecipitation assay (Fig. 5*a*) as shown previously.[21] Actin positive control was included as a known interacting partner of FLNA. The level of phosphorylated SMAD2 was almost three times higher in $M2^{FLNA+}$ cells than in $M2^{FLNA-}$ cells (Fig. 5*b*). Similarly, levels of p-Smad2 were significantly lower in $Flna^{o/-}$ MEFs compared with $Flna^{o/+}$ MEFs (Fig. 5*c*). The reduced promoter activity of *c-MET* in $M2^{FLNA-}$ cells was significantly increased after transfection with plasmid vectors driving either *SMAD2* or *FLNA* alone or *SMAD2* and *FLNA* in combination (Fig. 5*d*).

Discussion

Our study describes a novel role for FLNA in the regulation of c-MET expression and cellular responses to HGF in both human tumor cells and mouse fibroblasts. We showed that FLNA deficiency leads to decreased *c-MET* mRNA and protein production, and suppresses cellular responses to HGF stimulation during cell migration and invasion. Furthermore, we showed that FLNA partially regulates this signaling by both influencing AKT phosphorylation and interacting with SMAD2. Thus, the presence of FLNA is important for c-MET/HGF signaling.

Melanomas that occur in *Hgf* transgenic mice have a striking correlation between high-metastatic potential and the levels of overexpressed MET.[22] We studied the activation of c-MET downstream signaling molecules AKT and ERK1/2 upon HGF stimulation in human melanoma cells. Activation of the PI3K/AKT pathway is associated with cell motility, whereas the ERK1/2 signaling pathway regulates cell proliferation and cell differentiation.[23] We found that human melanoma cells displayed sustained activation of AKT but only a transient activation of ERK1 in response to HGF. The FLNA-deficient cells lost this response regarding both AKT and ERK1/2 activation. This indicates that FLNA preferentially influences the AKT activation and thus HGF-mediated melanoma cell motility. Akt has been shown to mediate Ser^{2152} phosphorylation of FLNA in human breast cancer cells, which specifies a novel mechanism for mediating the effects of caveolin-1 and IGF-1-induced cancer cell

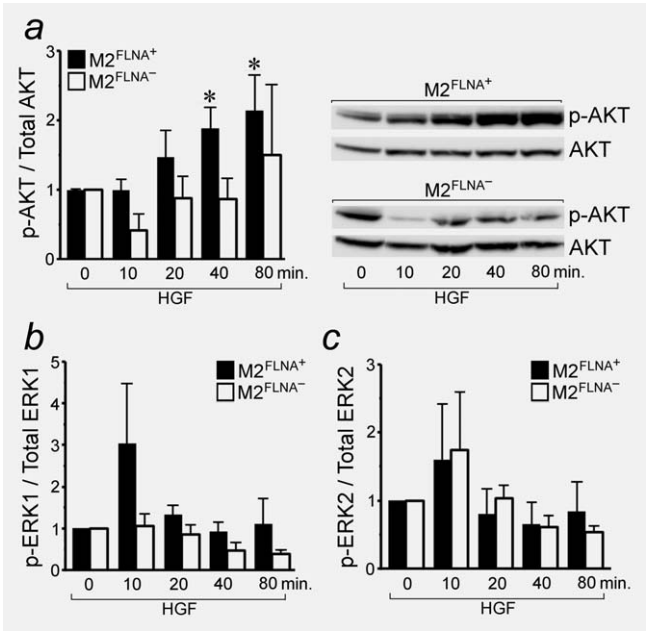


Figure 4. c-MET downstream AKT signaling is impaired in FLNA-deficient human melanoma cells in response to HGF stimulation. FLNA-expressing ($M2^{FLNA+}$) and FLNA-deficient ($M2^{FLNA-}$) human melanoma cells were incubated with 40 ng/ml HGF for up to 80 min. Ratios of phosphorylated AKT (*a*), ERK1 (*b*) and ERK2 (*c*) relative to their respective total level are quantified following densitometric analysis of immunoblots. A representative immunoblot for the time course of AKT phosphorylation in $M2^{FLNA+}$ and $M2^{FLNA-}$ cells. Mean ± SD data of triplicate experiments are given (one-way ANOVA with Tukey's HSD as *post hoc* analysis, $*p < 0.05$).

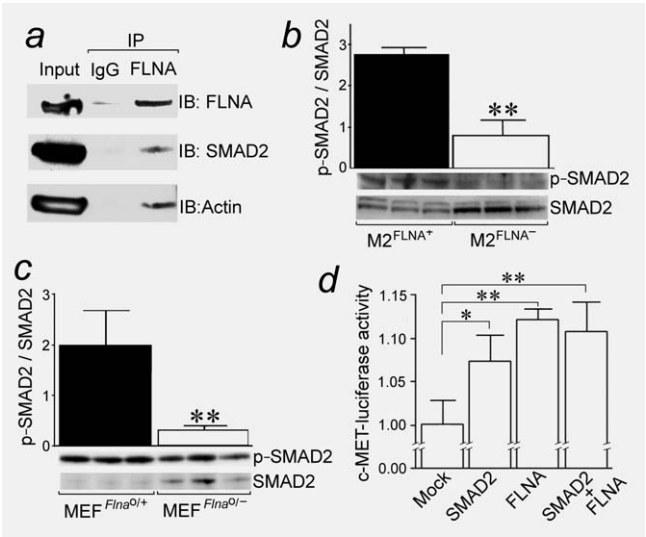


Figure 5. SMAD2 signaling is impaired in FLNA-deficient human melanoma cells. (*a*) Detection of SMAD2 as a FLNA-interacting protein by coimmunoprecipitation (IP). Mouse IgG was used as a negative control in IP, whereas actin, a known FLNA-binding protein, was included as a positive control. Ratios of phosphorylated to total SMAD2 in human melanoma ($M2^{FLNA+}$ and $M2^{FLNA-}$) cells (*b*), and MEF ($Flna^{o/+}$ and $Flna^{o/-}$) (*c*) are quantified following densitometric analysis of immunoblots. (*d*) *c-MET* promoter-driven luciferase reporter activity in $M2^{FLNA-}$ cells cotransfected with plasmid vectors expressing either no transgene (Mock), *SMAD2* and *FLNA* alone, or *SMAD2* and *FLNA* in combination. Mean ± SD data of triplicate experiments are given [Student's *t*-test (*b*, *c*) and one-way ANOVA with Tukey's HSD as *post hoc* analysis (*d*), $*p < 0.05$ and $**p < 0.01$].

migration.[14] Thus, the FLNA-mediated activation of AKT by HGF may reciprocally act on FLNA and promote its function. In correspondence with the differential activation of AKT and ERK1/2, FLNA deficiency impairs specifically HGF-mediated cellular migration and invasion, but has little impact in anchorage-dependent cell growth, suggesting that migration and growth may be separable events mediated by different pathways and mechanisms. The anchorage-independent growth as shown by the colony formation of melanoma cells is impaired in FLNA deficiency, implying that FLNA is critical for the acquisition of transformed phenotype. However, this effect seems to be independent of HGF/c-MET signaling as addition of HGF has no impact on the colony formation of both FLNA sufficient and deficient tumor cells. This could probably be regulated by other interacting partners of FLNA. For instance, FLNA has been shown to recruit Rac to the plasma membrane[24] and the Rac plasma membrane targeting is sufficient to drive proliferation in the absence of substrate anchorage.[25]

Multiple mechanisms regulate nuclear transport of transcription factors. However, little is known about the involvement of cytoskeletal proteins in classic nuclear functions such as transcription. Interestingly, FLNA appears to bind SMAD2 and plays an important role in SMAD-mediated signaling.[21] Using the same FLNA-deficient human melanoma cell line, Sasaki *et al.* suggested that defective TGF-β signaling was due to impaired receptor-induced serine phosphorylation of SMAD2.[21] Ligand-activated TGF-β receptors induce phosphorylation of receptor-regulated SMADs, which then accumulate in the nucleus to participate in target gene transcription, in collaboration with SMAD-interacting proteins. c-MET is a SMAD target gene as the *c-MET* promoter contains a putative SMAD-binding element.[20] In addition, transfection of tumor cells with TGF increased c-MET expression.[26] By summarizing these findings together, we questioned whether FLNA regulates c-MET activity *via* its interaction with SMAD2. Our studies indicate that FLNA-deficient cells have a significantly lower level of activated SMAD2 and that transfection of these cells with *SMAD2* partly restores *c-MET* promoter activity. Thus, induction of *c-MET* promoter activity following interaction of FLNA with SMAD2 may be one of the various mechanisms regulating HGF-mediated migration in these melanoma cells.

In addition to its direct role in promoting tumor cell growth and invasion, HGF signaling through the c-MET receptor, which is also expressed by endothelial cells, further

facilitates tumor metastasis by promoting hemangiogenesis directly and lymphangiogenesis indirectly.[27] Flna deficiency in mice leads to abnormal endothelial organization and aberrant adherens junctions in developing blood vessels, indicating an essential role of FLNA in endothelial function.[28] Interestingly, FLNB, a homologous protein of FLNA, plays a key role in VEGF-induced endothelial cell migration in the angiogenic process through its interaction with Rac-1 and Vav-2.[29] These findings raise an intriguing question whether the c-MET expression and signaling and HGF-mediated angiogenesis in tumors are dependent on the endothelial expression of FLNA.

In conclusion, FLNA appears to be an important regulator of c-MET signaling. This supports the concept that the cytoskeleton, including actin-binding proteins such as FLNA, is intimately involved in the regulation of cellular signaling and transcription.[30] Thus, regulation of c-MET signaling by FLNA represents another potential mechanism in HGF-mediated oncogenic responses and may be a new molecule for drug target in therapeutic efforts to block metastatic processes in cancer.

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