Biochemical and Biophysical Research Communications 428 (2012) 173–178

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# MYBPH inhibits NM IIA assembly via direct interaction with NMHC IIA and reduces cell motility

Yasuyuki Hosono [a], Jiro Usukura [b], Tomoya Yamaguchi [a], Kiyoshi Yanagisawa [a], Motoshi Suzuki [a], Takashi Takahashi [a,*]

[a] Division of Molecular Carcinogenesis, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466-8550, Japan
[b] Division of Integrated Project, EcoTopia Science Institute, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

ARTICLE INFO

*Article history:*
Received 3 October 2012
Available online 12 October 2012

*Keywords:*
MYBPH
Lung cancer
Non-muscle myosin IIA
Non-muscle myosin heavy chain
Migration
Myosin assembly

ABSTRACT

Actomyosin filament assembly is a critical step in tumor cell migration. We previously found that myosin binding protein H (MYBPH) is directly transactivated by the TTF-1 lineage-survival oncogene in lung adenocarcinomas and inhibits phosphorylation of the myosin regulatory light chain (RLC) of non-muscle myosin IIA (NM IIA) via direct interaction with Rho kinase 1 (ROCK1). Here, we report that MYBPH also directly interacts with an additional molecule, non-muscle myosin heavy chain IIA (NMHC IIA), which was found to occur between MYBPH and the rod portion of NMHC IIA. MYBPH inhibited NMHC IIA assembly and reduced cell motility. Conversely, siMYBPH-induced increased motility was partially, yet significantly, suppressed by blebbistatin, a non-muscle myosin II inhibitor, while more profound effects were attained by combined treatment with siROCK1 and blebbistatin. Electron microscopy observations showed well-ordered paracrystals of NMHC IIA reflecting an assembled state, which were significantly less frequently observed in the presence of MYBPH. Furthermore, an *in vitro* sedimentation assay showed that a greater amount of NMHC IIA was in an unassembled state in the presence of MYBPH. Interestingly, treatment with a ROCK inhibitor that impairs transition of NM IIA from an assembly-incompetent to assembly-competent state reduced the interaction between MYBPH and NMHC IIA, suggesting that MYBPH has higher affinity to assembly-competent NM IIA. These results suggest that MYBPH inhibits RLC and NMHC IIA, independent components of NM IIA, and negatively regulates actomyosin organization at 2 distinct steps, resulting in firm inhibition of NM IIA assembly.



## 1. Introduction

Cell migration is a critical step in tumor invasion and metastasis, while the contractile motion of cancer cells is associated with continuous structural transitions between assembled and disassembled states of actomyosin bundles [1–3]. Non-muscle myosin II (NM II), a major component of the actomyosin cytoskeleton, is comprised of 2 non-muscle myosin heavy chain (NMHCs), 2 myosin essential light chains (ELCs), and 2 myosin regulatory light chains (RLCs). In human cells, 3 NMHCs (IIA, IIB, and IIC) are encoded by distinct genes (MYH9, MYH10, and MYH14, respectively) and constitute the NM II isoforms, which are named NM IIA, NM IIB, and NM IIC, respectively [4,5]. Accumulating evidence indicates that NM II members, especially NM IIA, play crucial roles in cancer cell migration via bivalent binding to and linking of actin filaments [6–8].

One of the major activators of the NM II assembly is Rho kinase 1 (ROCK1), a downstream effector of RhoA, which phosphorylates RLC and induces subsequent unfolding of NM II into an assembly-competent form. This assembly-competent NM II is capable of NM II dimer formation via assembly of NMHC [9,10]. It has also been shown that RLC phosphorylation is crucially involved in cell morphogenesis and motility, as well as cancer invasion and metastasis [11–14]. In contrast, little is known about the regulatory mechanisms of the subsequent NMHC IIA assembly steps, while previous reports have focused on regulatory phosphorylation of NMHC by protein kinase C (PKC), casein kinase II (CK II), and transient receptor potential melastatin 7 (TRPM7) at the C-terminus of the NMHC [15,16].

TTF-1 is a lineage-specific transcription factor required for the development and the physiological functions of peripheral lung [17–19]. TTF-1 is also expressed in a major fraction of lung adenocarcinomas, which appears to reflect their derivation from the terminal respiratory unit [18,19]. We and others previously identified that TTF-1 also plays a role as a lineage-survival oncogene and exhibits focal amplification in lung adenocarcinomas [20–23]. In addition, we recently found that TTF-1 induces expression of the receptor tyrosine kinase-like orphan receptor 1 (ROR1), which in turn sustains a favorable balance between prosurvival PI3K-AKT

* Corresponding author. Fax: +81 52 744 2457.
*E-mail address:* tak@med.nagoya-u.ac.jp (T. Takahashi).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.036

and pro-apoptotic p38 signaling [24]. However, TTF-1 expression is paradoxically known to be associated with favorable prognosis in lung adenocarcinoma patients [25], which can be explained, at least in part, by our recent discovery that myosin binding protein H (MYBPH) is directly transactivated by TTF-1 and inhibits RLC phosphorylation via direct interaction with ROCK1, which in turn reduces cell motility and metastasis [26].

Here, we report findings showing that MYBPH has another mode of inhibitory activity in regulation of NM IIA through direct binding to NMHC IIA, suggesting the existence of dual roles of MYBPH for firmly imposing inhibition of NM IIA assembly.

## 2. Materials and methods

### 2.1. Materials

Antibodies, reagents, PCR primers, siRNAs and recombinant proteins are summarized in Supplementary Tables 1–3.

### 2.2. Immunoprecipitation–Western blot analysis (IP–WB)

Cells ($2 \times 10^7$) were lysed with modified RIPA buffer containing 50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 20 mM β-glycerophosphate (pH 7.6), 50 mM NaF, 1 mM $Na_3VO_4$, and 150 mM NaCl, supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche) and incubated with each antibody (5 μl) overnight at 4 °C, followed by addition of 80 μl of 50% protein G Sepharose (GE Healthcare) and subsequent incubation for 1 h. The immunoprecipitates were analyzed by Western blotting. In the input lane, a 1/100 amount of total cell lysate was applied to samples used in the immunoprecipitation experiments. In some experiments, NCI-H441 cells were treated with Y-27632 (5 μM) for 15 min before being harvested.

### 2.3. Negative staining and electron microscopy

The rod portions (0.66 μM) of GST-tagged NMHC IIA, and His-tagged MYBPH-wt or His-tagged MYBPHΔ-(1 + 2 + 3) were incubated in 100 μl of reaction mixture containing 10 mM Tris–HCl (pH 7.5), 0.1 mM EGTA, 1 mM EDTA, and 2.5 mM $MgCl_2$, supplemented with Complete Protease Inhibitor Cocktail Tablets in the presence of 150 mM NaCl overnight at 4 °C. For negative staining, aliquots of protein solution were placed on copper grids covered with carbon film and keep still for about 1 min to allow for adsorption of the molecules on the grids, after which the grids were washed with several drops of 2% uranyl acetate solution. Excess staining solution remaining on the grids was quickly absorbed with filter papers and dried. Images were obtained using a Hitachi H-7600 electron microscope (access 100 kV) onto film at a magnification of 50,000× and further processed with Adobe PHOTOSHOP software (Adobe Systems).

### 2.4. NMHC IIA sedimentation assay

The rod portions (0.66 μM) of GST-tagged NMHC IIA, and His-tagged MYBPH-wt or His-tagged MYBPHΔ-(1 + 2 + 3) were incubated in 100 μl of reaction mixture containing 10 mM Tris–HCl (pH 7.5), 0.1 mM EGTA, 1 mM EDTA, and 2.5 mM $MgCl_2$, supplemented with Complete Protease Inhibitor Cocktail Tablets in the presence of various concentrations of NaCl overnight at 4 °C. After ultracentrifugation at 186,000*g* for 60 min at 4 °C in a TLA 55 ultracentrifuge (Beckman), the supernatants were subjected to Western blot analysis. Western blot images of triplicate experiments were analyzed using ImageJ software with the input NMHC IIA utilized as a normalization control.

Other materials and methods are given in the Supplementary data.

## 3. Results and discussion

### 3.1. Combined treatment with siROCK1 and non-muscle myosin inhibitors inhibited cell motility increased by siMYBPH treatment

We recently showed that MYBPH inhibits ROCK1, and reduces cell motility and metastasis [26]. To better understand the functions of MYBPH in actomyosin organization, we treated MYBPH-silenced NCI-H441 cells with an NM II inhibitor blebbistatin [27]. Interestingly, increased cell motility of MYBPH-silenced NCI-H441 cells was partially suppressed by either siROCK1 or blebbistatin treatment, while combined treatment with those exhibited more profound effects than each alone (Fig. 1A). Another NM II inhibitor, 2,3-butanedione monoxime (BDM) [28], showed similar results (Fig. 1B). These findings suggest that MYBPH affects the assembly process of NM II not only at the ROCK1-mediated myosin regulatory light chain (RLC) phosphorylation step, but also via another as yet unidentified mechanism.

### 3.2. MYBPH binds to rod portion of NMHC IIA

It is well known that assembly of NMHC IIA is a crucial step toward actomyosin organization, because of its requirement for facilitating cell migration activity [4–8]. Therefore, we speculated that MYBPH somehow inhibits the NMHC IIA assembly process. To test this, we first examined the interaction between MYBPH and NMHC IIA using immunoprecipitation–Western blotting

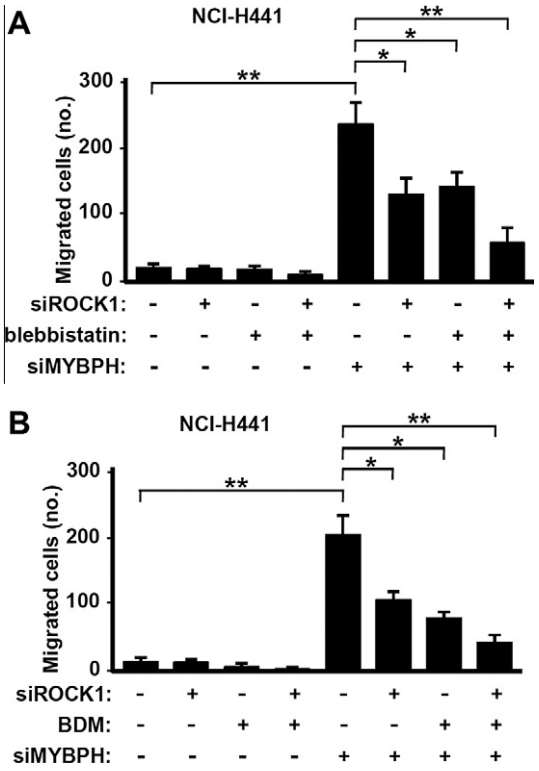


**Fig. 1.** Combined treatment with both siROCK1 and non-muscle myosin inhibitors inhibits increased cell motility induced by siMYBPH. (A) Motility assay in NCI-H441 cells co-treated with si-ROCK1 and the non-muscle myosin-specific inhibitor blebbistatin [27], as compared to each alone. Bars, mean ± SD; $^{*}P < 0.05$; $^{**}P < 0.005$. (B) Motility assay in NCI-H441 cells co-treated with si-ROCK1 and pan-myosin inhibitor 2,3-butanedione monoxime (BDM) [28], as compared to each alone. Bars, mean ± SD; $^{*}P < 0.05$; $^{**}P < 0.001$.

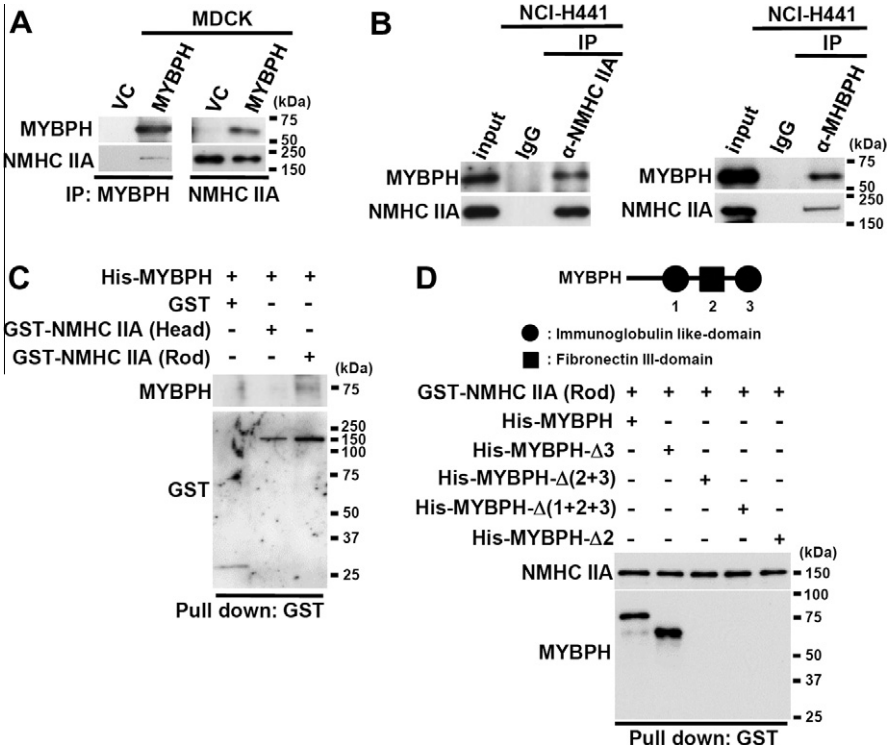


**Fig. 2.** MYBPH binds to the rod portion of NMHC IIA. (A) Immunoprecipitation–Western blot (IP–WB) analysis in MDCK cells expressing exogenous MYBPH. VC, vector control. (B) Top panel: findings of IP–WB analysis using anti-NMHC IIA antibody in H441 cells expressing endogenous MYBPH. Bottom panel: IP–WB analysis using anti-MYBPH antibody. (C) *In vitro* protein–protein binding assay of purified proteins of MYBPH and rod portion of NMHC IIA. The binding assay was performed as previously described [26]. (D) Top panel: schematic representation of domain organization of MYBPH. Bottom panel: *in vitro* protein–protein binding assay between MYBPH and NMHC IIA.

(IP–WB) analysis, and found that MYBPH bound to NMHC IIA in MYBPH-over-expressing MDCK cells, as well as NCI-H441 cells that endogenously express MYBPH (Fig. 2A and B). To assess the specificity of this interaction, an *in vitro* protein–protein binding assay using recombinant proteins was performed, which clearly demonstrated that MYBPH directly interacts with the rod portion of NMHC IIA (Fig. 2C). We also investigated which domain of MYBPH is important for its binding to the rod portion of NMHC IIA using various deletion mutants. The fibronectin type III domain of MYBPH was shown to be required for MYBPH binding to NMHC IIA (Fig. 2D), which appears to be in agreement with its structural resemblance to MYBPC [29].

### 3.3. MYBPH inhibits NMHC IIA assembly

To examine the effect of MYBPH on NMHC IIA assembly, we performed electron microscopy analysis, and visualized the assembled structures of NMHC IIA rod fragments in the presence of either wild-type MYBPH or an MYBPH-Δ (1 + 2 + 3) mutant. The rod-portion of NMHC IIA formed long and well-ordered paracrystal structures in the presence of an MYBPH-Δ (1 + 2 + 3) mutant, which lacks a capacity to bind to NMHC IIA, whereas those paracrystals were poorly developed in the presence of wild-type MYBPH. (Fig. 3A). To confirm that MYBPH inhibits NMHC IIA assembly, we also performed sedimentation assays under various salt concentrations using the rod portion of NMHC IIA, and either wild-type MYBPH or the MYBPH-Δ (1 + 2 + 3) mutant. A greater amount of unassembled NMHC IIA was observed in the presence of the wild-type than with the mutant (Fig. 3B). These results indicated that MYBPH inhibited the assembly of NMHC IIA through direct interaction. Phosphorylation of NMHC IIA on its tail region has been shown to cause NMHC IIA disassembly [5], suggesting its potential involvement. However, MYBPH did not appear to be involved in that process, since the phosphorylation status of NMHC IIA was not affected by knockdown of MYBPH in NCI-H441 cells or its over-expression in MDCK cells (Fig. 3C).

### 3.4. MYBPH has higher affinity to assembly-competent NMHC IIA monomer

Upon RLC phosphorylation, assembly-incompetent NM IIA unfolds and changes to an assembly-competent form, which is a prerequisite prior to forming bipolar NM IIA filaments and consequential linking of actin filaments [4,5]. IP–WB analysis showed reduced interaction between MYBPH and NMHC IIA in NCI-H441 cells treated with the ROCK inhibitor Y-27323, which impairs transition of NM IIA from an assembly-incompetent to -competent state (Fig. 3D), suggesting that MYBPH has a higher affinity to assembly-competent NM IIA. In order to examine whether MYBPH preferentially interact with the monomer form of NMHC IIA and inhibit its assembly, we performed *in vitro* binding assays using purified proteins of MYBPH and the rod portion of NMHC IIA under low and high salt concentration conditions, in which NMHC IIA is presumed to be in a predominantly dimer and monomer state, respectively. Our results demonstrated that MYBPH binds to NMHC IIA more efficiently in high as compared to low salt concentrations (Fig. 3E). Thus, MYBPH has a higher affinity to the assembly-competent and monomer forms of NMHC IIA.

### 3.5. Organization of peripheral actomyosin bundles induced by MYBPH knockdown counteracted by simultaneous treatment with non-muscle myosin inhibitors

MYBPH knockdown induces co-localization of NMHC IIA with the peripheral actin bundles, forming peripheral actomyosin bundles in NCI-H441 cells [26]. The present findings showed that

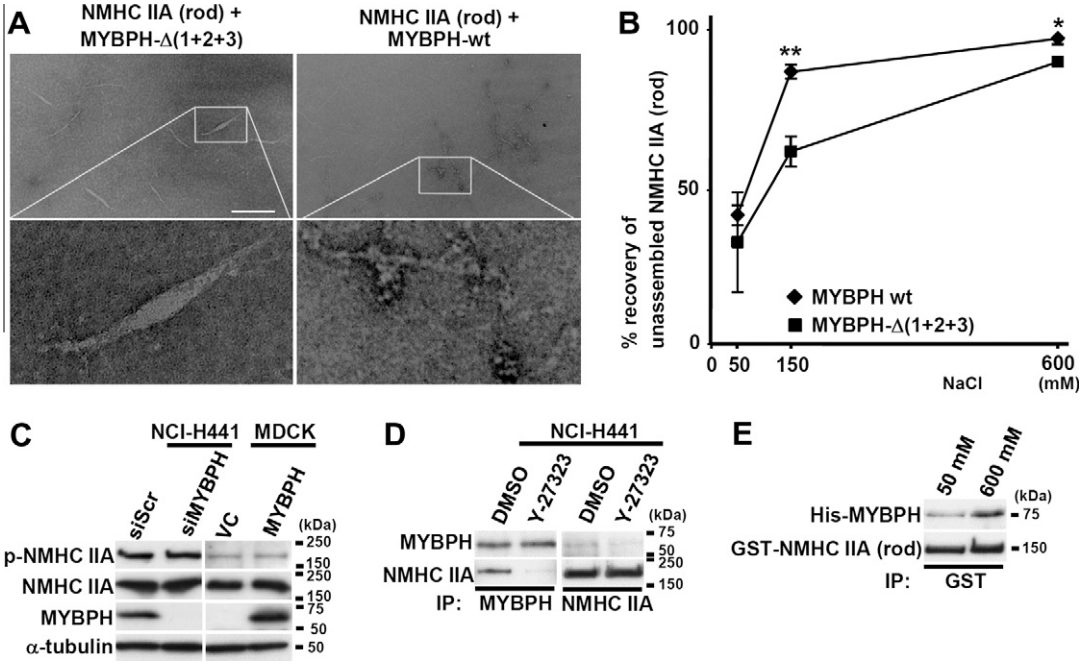


**Fig. 3.** MYBPH inhibits NMHC IIA assembly and has higher affinity to assembly-competent NMHC IIA monomer. (A) Electron microscopy analysis. Bar indicates 200 nm. (B) Sedimentation assay using rod portion of NMHC IIA in presence of wt-MYBPH. Bars, mean ± SD; $^{*}P < 0.01$; $^{**}P < 0.005$. (C) Western blot analysis in HCI-H441 cells knocked down for MYBPH or MDCK cells overexpressing MYBPH. (D) IP–WB analysis in NCI-H441 cells treated with ROCK inhibitor Y-27632. (E) *In vitro* protein–protein binding assay in various salt conditions.

siMYBPH-induced formation of the peripheral actomyosin bundles was significantly counteracted by simultaneous treatment with blebbistatin or BDM (Fig. 4A, high power field; Supplementary Fig. 1, low power field). Using a three-dimensional Matrigel invasion assay, we previously showed that ROCK1 inhibition clearly cancelled siMYBPH-induced single cell migration [26]. To examine whether NMHC IIA inhibition similarly affects siMYBPH-induced single cell migration, we treated NCI-H441 cells with siMYBPH and/or blebbistatin. We found that while siMYBPH induced single cell migration in accordance with our previous report, siMYBPH-treated NCI-H441 cells did not revert to the collective migratory phenotype in the presence of blebbistatin (Fig. 4B). In addition, it has been proposed that collective cell migration requires decreased ROCK1-driven actomyosin activity at the cell–cell junction in conjunction with sustainment of CDC42-dependent actomyosin activity around the outside surface of cell clusters [30,31]. Therefore, it is possible that cancelation of siMYBPH-induced single cell migration by the ROCK1 inhibitor alone may have been caused by distinct actomyosin inhibition patterns between ROCK1 inhibitor- and blebbistatin-treated cells, the latter of which inhibits actomyosin in both the cell–cell junction and on the cell cluster surface.

### 3.6. MYBPH may negatively regulate actomyosin organization at 2 distinct steps

We previously reported that MYBPH is a transcriptional target of the TTF-1 lineage-survival oncogene and plays a crucial role in inhibition of cancer cell motility, invasion, and metastasis [26]. While it was shown to be imposed through inhibition of RLC phosphorylation via physical interaction with and inhibition of ROCK1, the present findings further demonstrate that MYBPH has an additional binding molecule, namely NMHC IIA. MYBPH binds to the rod portion of NMHC IIA and inhibits its assembly, which is thought to take place following acquisition of assembly-competence conferred through RLC phosphorylation by ROCK1 [9,10]. This implies that MYBPH inhibits 2 independent components of NM IIA, *i.e.*, RLC and NMHC IIA, and thereby negatively regulates actomyosin organization at 2 distinct steps (Fig. 4C). Along this line, the additive effects of combined treatment with siROCK1 and blebbistatin on siMYBPH-induced increased motility may result from residual basal level competence, as there is compelling evidence showing that the transition between assembly-incompetent and -competent NMHC state is in equilibrium at a physiological ionic level (150 mM NaCl, pH 7.0) even in the absence of RLC [32]. Thus, this dual step inhibition of NM IIA assembly by MYBPH may play a role as a possible mechanism for imposing firmer inhibition.

### 3.7. Physiological and pathological implication of MYBPH inhibition at NMHC IIA assembly step

The NM IIA-attributed functions for regulating actomyosin organization are thought to be involved in development and progression of various types of cancer [6–8], while the involvement of RLC phosphorylation and RLC kinases such as ROCK1 in cancer progression is well established [11–14]. Nevertheless, to date very few regulators of NMHC IIA have been reported. S100A4, also called MTS1, mediates NMHC IIA disassembly via direct interaction, while casein kinase II (CK II) also disassembles NMHC IIA through phosphorylation of NMHC IIA on its tail region [15,33]. The present results showed that MYBPH binding to NMHC IIA does not affect this phosphorylation. Taken together, our findings add MYBPH to the very short list of physiological inhibitors of NMHC IIA assembly.

In addition to its effects on cancer progression, NMHC IIA has been found to play important roles in a wide range of disease states such as MYH9-related disorders, which exhibit a combination of different phenotypic features including large platelets and thrombocytopenia [34,35]. Defects in NMHC IIA assembly due to mutations in the rod portion of NMHC IIA are thought to be major causes of MYH9-related disorders. Thus, we consider it interesting

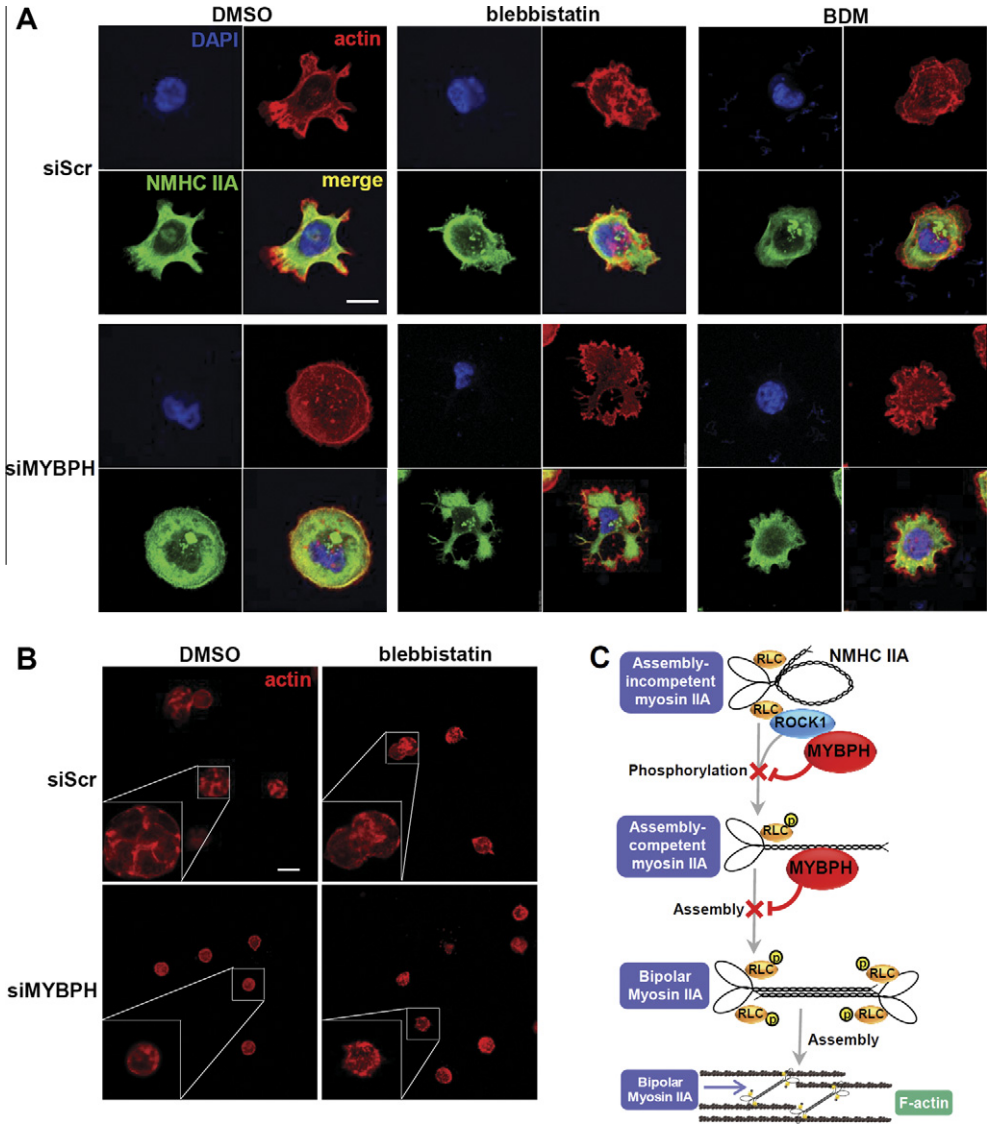


**Fig. 4.** siMYBPH-induced peripheral actomyosin bundle formation is counteracted by simultaneous treatment with non-muscle myosin inhibitors. (A) Immunofluorescence staining for actin (red) and NMHC IIA (green) in blebbistatin- or BDM-treated NCI-H441 cells. Immunofluorescence staining was performed as previously described [26]. Bar indicates 10 μm. (B) Three-dimensional Matrigel invasion assay in NCI-H441 cell treated with siMYBPH and/or blebbistatin. Three-dimensional Matrigel invasion assays were performed as previously described [26]. White bar indicates 50 μm. (C) Schematic diagram of multifaceted inhibitory roles of MYBPH in actomyosin organization at 2 distinct steps.

to search for MYBPH alterations in cases with similar disease phenotypes without NMHC IIA mutations.

In summary, our results demonstrate that MYBPH inhibits the assembly of NMHC IIA through direct binding to assembly-competent NMHC IIA, suggesting that this activity may in turn contribute to suppression of cancer invasion and metastasis together with its ROCK1 inhibitory function [26]. The dual roles of MYBPH in NM IIA inhibition comprise an intriguing mechanism to impose firm NM IIA inhibition. The present findings also provide clues for better understanding of the molecular mechanisms involved in inhibition of cancer invasion and metastasis by TTF-1 through transcriptional activation of MYBPH, as well as for better prognosis for TTF-1-positive lung adenocarcinoma patients.

## Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research on Innovative Areas from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, as well as a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science (JSPS). Y.H. was supported by a research fellowship of the Foundation for Promotion of Cancer Research.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.10.036.

## References

[1] D.A. Lauffenburger, A.F. Horwitz, Cell migration: a physically integrated molecular process, Cell 84 (1996) 359–369.

[2] P. Friedl, K. Wolf, Tumour-cell invasion and migration: diversity and escape mechanisms, Nat. Rev. Cancer 3 (2003) 362–374.

[3] A. Hall, The cytoskeleton and cancer, Cancer Metastasis Rev. 28 (2009) 5–14.

[4] M.A. Conti, R.S. Adelstein, Nonmuscle myosin II moves in new directions, J. Cell Sci. 121 (2008) 11–18.

[5] M. Vicente-Manzanares, X. Ma, R.S. Adelstein, A.R. Horwitz, Non-muscle myosin II takes centre stage in cell adhesion and migration, Nat. Rev. Mol. Cell Biol. 10 (2009) 778–790.
[6] V. Betapudi, L.S. Licate, T.T. Egelhoff, Distinct roles of nonmuscle myosin II isoforms in the regulation of MDA-MB-231 breast cancer cell spreading and migration, Cancer Res. 66 (2006) 4725–4733.
[7] Y. Huang, P. Arora, C.A. McCulloch, W.F. Vogel, The collagen receptor DDR1 regulates cell spreading and motility by associating with myosin IIA, J. Cell Sci. 122 (2009) 1637–1646.
[8] S. Medjkane, C. Perez-Sanchez, C. Gaggioli, E. Sahai, R. Treisman, Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis, Nat. Cell. Biol. 11 (2009) 257–268.
[9] S. Etienne-Manneville, A. Hall, Rho GTPases in cell biology, Nature 420 (2002) 629–635.
[10] K. Riento, A.J. Ridley, Rocks: multifunctional kinases in cell behaviour, Nat. Rev. Mol. Cell Biol. 4 (2003) 446–456.
[11] K. Itoh, K. Yoshioka, H. Akedo, M. Uehata, T. Ishizaki, S. Narumiya, An essential part for Rho-associated kinase in the transcellular invasion of tumor cells, Nat. Med. 5 (1999) 221–225.
[12] E. Sahai, C.J. Marshall, ROCK and Dia have opposing effects on adherens junctions downstream of Rho, Nat. Cell Biol. 4 (2002) 408–415.
[13] S. Wilkinson, H.F. Paterson, C.J. Marshall, Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion, Nat. Cell Biol. 7 (2005) 255–261.
[14] C.C. Wong, C.M. Wong, F.C. Ko, L.K. Chan, Y.P. Ching, J.W. Yam, I.O. Ng, Deleted in liver cancer 1 (DLC1) negatively regulates Rho/ROCK/MLC pathway in hepatocellular carcinoma, PLoS One 3 (2008) e2779.
[15] N.G. Dulyaninova, V.N. Malashkevich, S.C. Almo, A.R. Bresnick, Regulation of myosin-IIA assembly and Mts1 binding by heavy chain phosphorylation, Biochemistry 44 (2005) 6867–6876.
[16] Z.H. Li, A.R. Bresnick, The S100A4 metastasis factor regulates cellular motility via a direct interaction with myosin-IIA, Cancer Res. 66 (2006) 5173–5180.
[17] S. Kimura, Y. Hara, T. Pineau, P. Fernandez-Salguero, C.H. Fox, J.M. Ward, F.J. Gonzalez, The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary, Genes Dev. 10 (1996) 60–69.
[18] Y. Yatabe, T. Mitsudomi, T. Takahashi, TTF-1 expression in pulmonary adenocarcinomas, Am. J. Surg. Pathol. 26 (2002) 767–773.
[19] T. Takeuchi, S. Tomida, Y. Yatabe, T. Kosaka, H. Osada, K. Yanagisawa, T. Mitsudomi, T. Takahashi, Expression profile-defined classification of lung adenocarcinoma shows close relationship with underlying major genetic changes and clinicopathologic behaviors, J. Clin. Oncol. 24 (2006) 1679–1688.
[20] H. Tanaka, K. Yanagisawa, K. Shinjo, A. Taguchi, K. Maeno, S. Tomida, Y. Shimada, H. Osada, T. Kosaka, H. Matsubara, T. Mitsudomi, Y. Sekido, M. Tanimoto, Y. Yatabe, T. Takahashi, Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1, Cancer Res. 67 (2007) 6007–6011.
[21] J. Kendall, Q. Liu, A. Bakleh, A. Krasnitz, K.C. Nguyen, B. Lakshmi, W.L. Gerald, S. Powers, D. Mu, Oncogenic cooperation and coamplification of developmental transcription factor genes in lung cancer, Proc. Natl. Acad. Sci. USA 104 (2007) 16663–16668.
[22] B.A. Weir, M.S. Woo, G. Getz, S. Perner, L. Ding, R. Beroukhim, W.M. Lin, M.A. Province, A. Kraja, L.A. Johnson, K. Shah, M. Sato, R.K. Thomas, J.A. Barletta, I.B. Borecki, S. Broderick, A.C. Chang, D.Y. Chiang, L.R. Chirieac, J. Cho, Y. Fujii, A.F. Gazdar, T. Giordano, H. Greulich, M. Hanna, B.E. Johnson, M.G. Kris, A. Lash, L. Lin, N. Lindeman, E.R. Mardis, J.D. McPherson, J.D. Minna, M.B. Morgan, M. Nadel, M.B. Orringer, J.R. Osborne, B. Ozenberger, A.H. Ramos, J. Robinson, J.A. Roth, V. Rusch, H. Sasaki, F. Shepherd, C. Sougnez, M.R. Spitz, M.S. Tsao, D. Twomey, R.G. Verhaak, G.M. Weinstock, D.A. Wheeler, W. Winckler, A. Yoshizawa, S. Yu, M.F. Zakowski, Q. Zhang, D.G. Beer, Wistuba II, M.A. Watson, L.A. Garraway, M. Ladanyi, W.D. Travis, W. Pao, M.A. Rubin, S.B. Gabriel, R.A. Gibbs, H.E. Varmus, R.K. Wilson, E.S. Lander, M. Meyerson, Characterizing the cancer genome in lung adenocarcinoma, Nature 450 (2007) 893–898.
[23] K.A. Kwei, Y.H. Kim, L. Girard, J. Kao, M. Pacyna-Gengelbach, K. Salari, J. Lee, Y.L. Choi, M. Sato, P. Wang, T. Hernandez-Boussard, A.F. Gazdar, I. Petersen, J.D. Minna, J.R. Pollack, Genomic profiling identifies TITF1 as a lineage-specific oncogene amplified in lung cancer, Oncogene 27 (2008) 3635–3640.
[24] T. Yamaguchi, K. Yanagisawa, R. Sugiyama, Y. Hosono, Y. Shimada, C. Arima, S. Kato, S. Tomida, M. Suzuki, H. Osada, T. Takahashi, NKX2-1/TITF1/TTF-1-Induced ROR1 is required to sustain EGFR survival signaling in lung adenocarcinoma, Cancer Cell 21 (2012) 348–361.
[25] V.K. Anagnostou, K.N. Syrigos, G. Bepler, R.J. Homer, D.L. Rimm, Thyroid transcription factor 1 is an independent prognostic factor for patients with stage I lung adenocarcinoma, J. Clin. Oncol. 27 (2009) 271–278.
[26] Y. Hosono, T. Yamaguchi, E. Mizutani, K. Yanagisawa, C. Arima, S. Tomida, Y. Shimada, M. Hiraoka, S. Kato, K. Yokoi, M. Suzuki, T. Takahashi, MYBPH, a transcriptional target of TTF-1, inhibits ROCK1, and reduces cell motility and metastasis, EMBO J. 31 (2012) 481–493.
[27] A.F. Straight, A. Cheung, J. Limouze, I. Chen, N.J. Westwood, J.R. Sellers, T.J. Mitchison, Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor, Science 299 (2003) 1743–1747.
[28] L.P. Cramer, T.J. Mitchison, Myosin is involved in postmitotic cell spreading, J. Cell Biol. 131 (1995) 179–189.
[29] E. Flashman, C. Redwood, J. Moolman-Smook, H. Watkins, Cardiac myosin binding protein C: its role in physiology and disease, Circ. Res. 94 (2004) 1279–1289.
[30] C. Gaggioli, S. Hooper, C. Hidalgo-Carcedo, R. Grosse, J.F. Marshall, K. Harrington, E. Sahai, Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells, Nat. Cell Biol. 9 (2007) 1392–1400.
[31] C. Hidalgo-Carcedo, S. Hooper, S.I. Chaudhry, P. Williamson, K. Harrington, B. Leitinger, E. Sahai, Collective cell migration requires suppression of actomyosin at cell–cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6, Nat. Cell Biol. 13 (2011) 49–58.
[32] K.M. Trybus, S. Lowey, Conformational states of smooth muscle myosin. Effects of light chain phosphorylation and ionic strength, J. Biol. Chem. 259 (1984) 8564–8571.
[33] E.J. Kim, D.M. Helfman, Characterization of the metastasis-associated protein, S100A4. Roles of calcium binding and dimerization in cellular localization and interaction with myosin, J. Biol. Chem. 278 (2003) 30063–30073.
[34] J.D. Franke, F. Dong, W.L. Rickoll, M.J. Kelley, D.P. Kiehart, Rod mutations associated with MYH9-related disorders disrupt nonmuscle myosin-IIA assembly, Blood 105 (2005) 161–169.
[35] Y. Zhang, M.A. Conti, D. Malide, F. Dong, A. Wang, Y.A. Shmist, C. Liu, P. Zerfas, M.P. Daniels, C.C. Chan, E. Kozin, B. Kachar, M.J. Kelley, J.B. Kopp, R.S. Adelstein, Mouse models of MYH9-related disease: mutations in nonmuscle myosin II-A, Blood 119 (2012) 238–250.