Biochemical and Biophysical Research Communications 444 (2014) 382–386

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

FBI1/Akirin2 promotes tumorigenicity and metastasis of Lewis lung carcinoma cells

Yuko Komiya, Hirotada Akiyama, Ryuji Sakumoto, Fumio Tashiro *

Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan

ARTICLE INFO

Article history:
Received 8 January 2014
Available online 24 January 2014

Keywords:
14-3-3β
14-3-3 beta interactant 1/Akirin2
Anchorage-independent growth
Tumorigenicity
Metastasis

ABSTRACT

The 14-3-3 family of proteins regulates various signaling pathways involved in cell cycle, apoptosis, stress response, and malignant transformation. We previously demonstrated that the β isoform of the 14-3-3 protein promotes cell growth and tumorigenicity of rat K2 hepatocellular carcinoma cells. We identified fourteen-three-three beta interactant 1 (FBI1)/Akirin2 as a binding partner of 14-3-3β and showed that the complex of these proteins promotes tumorigenicity and metastasis of K2 cells. In addition, we demonstrated that FBI1/Akirin2 downregulation shortened the duration of MAPK activity. Because 14-3-3β and FBI1/Akirin2 overexpression is observed in various cancer cell lines, 14-3-3β-FBI1/Akirin2 oncogenic function should be elucidated in different types of cancer. In this study, we used LLC1 Lewis lung carcinoma cells as a model. We established FBI1/Akirin2 knockdown cell clones through transfection of an antisense FBI1/Akirin2 expression vector and assessed the capacity for cell growth *in vitro* and tumorigenicity and metastasis *in vivo*. FBI1/Akirin2 downregulation decreased anchorage-independent growth, whereas the growth rate in monolayer culture was not affected. Moreover, an *in vivo* assay in nude mice showed that FBI1/Akirin2 overexpression is required for LLC1 tumor growth and metastasis. These results suggest that FBI1/Akirin2 plays an important role in oncogenesis of LLC1 lung carcinoma cells, and this protein may also serve as an oncogene in other cancers.

1. Introduction

The 14-3-3 family of proteins is highly conserved and ubiquitously expressed [1,2]. At least 7 isoforms (β, γ, ε, σ, δ, τ, and η) have been identified in mammals. The 14-3-3 family members form a homo- or heterodimer and interact with target proteins. The interaction with target proteins alters enzyme activity, subcellular localization, or complex formation of the target. A large number of signaling molecules have been identified as binding partners of 14-3-3 proteins. It is known that 14-3-3 proteins regulate various signaling pathways such as those involved in cell cycle, metabolism, malignant transformation, and apoptosis [3,4]. We previously discovered that, among the 7 isoforms, β has an oncogenic function in aflatoxin B_1-induced rat hepatocellular carcinoma K2 cells [5]. The 14-3-3β protein is overexpressed in K2 cells compared with untransformed rat liver tissue. 14-3-3β downregulation through transfection with an antisense *14-3-3β* expression vector inhibits cell growth, tumorigenicity, and angiogenesis of K2 cells. Furthermore, we observed that 14-3-3β is overexpressed in various types of cancer cell lines. These results demonstrate the oncogenic function of 14-3-3β.

During functional analysis of 14-3-3β, we identified a novel binding partner of 14-3-3β, fourteen-three-three beta interactant 1 (FBI1), and characterized the oncogenic function of FBI1 in K2 cells [6]. Since the *FBI1* gene is also known as *Akirin2*, we refer to this gene as *FBI1/Akirin2* in this study. *Akirins* are a recently identified gene family, and these genes are highly conserved from metazoan to mammalian species [7]. Akirin1 was first identified in *Drosophila* as an important participant in innate immune response [8]. In mice, several lines of evidence show that Akirin 1 performs a function in skeletal myogenesis [9]. In our previous screening of proteins binding to 14-3-3β, we isolated FBI1/Akirin2 as a functional partner of 14-3-3β and demonstrated that 14-3-3β and FBI1/Akirin2 complex functions as a transcriptional suppressor [6]. The 14-3-3β-FBI1/Akirin2 complex binds to the promoter region of mitogen-activated protein kinase (MAPK) phosphatase 1 (MKP-1). MKP-1 dephosphorylates extracellular signal-regulated kinases (ERKs) and functions as a negative feedback factor of the MAPK pathway [10]. FBI1/Akirin2 downregulation increases MKP-1 expression, resulting in a shortened activation period of ERK1/2. FBI1/Akirin2 knockdown cells express a low level of cyclin

Abbreviations: LLC, Lewis lung carcinoma; MKP, MAPK phosphatase; ECM, extracellular matrix.

* Corresponding author.
E-mail address: ftashir@rs.noda.tus.ac.jp (F. Tashiro).

http://dx.doi.org/10.1016/j.bbrc.2014.01.064
0006-291X/

D1 compared with parental K2 cells. Furthermore, FBI1/Akirin2 knockdown selectively inhibits anchorage-independent growth in soft agar, whereas cell growth in monolayer culture is not inhibited in K2 cells. FBI1/Akirin2 knockdown cells exhibit slower tumor growth and less metastasis in an *in vivo* mouse model. Taken together, these results demonstrate that FBI1/Akirin2 and 14-3-3β play an important role in K2 cell oncogenesis.

Computational analysis shows that 14-3-3 binding sites of FBI1/Akirin2 are highly conserved among several species [7]. In addition, our previous results showed that *FBI1/Akirin2* is overexpressed in various cancer cell lines, including rat hepatoma cells, glioblastoma cells, and pheochromocytoma cells.

Therefore, in the present study, we hypothesized that FBI1/Akirin2 has an universal oncogenic function in various cancers. To test our hypothesis, in this study, we used Lewis lung carcinoma cells (LLC1) as a model and analyzed the effect of FBI1/Akirin2 downregulation on cell growth *in vitro* and tumorigenicity and metastasis *in vivo*.

2. Materials and methods

2.1. Stable transfection

The mouse *FBI1/Akirin2* gene was isolated using reverse-transcription polymerase chain reaction (RT-PCR), and the amplified fragment was inserted in the antisense orientation into the pcDNA3 vector (pcDNA3-AS-FBI1/Akirin2). The detailed information on the vector construct is available upon request. LLC1 cells were obtained from ATCC and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). LLC1 cells were transfected with either pcDNA3-AS-FBI1/Akirin2 or the empty pcDNA3 vector using the DOTAP transfection reagent (Roche, Mannheim, Germany), according to the manufacturer's instructions. After 2 weeks of the selection in 1 mg/mL G418 (Wako, Tokyo, Japan), resistant clones were expanded and tested for the expression level of endogenous and antisense *FBI1* RNA using Northern blotting.

2.2. RNA isolation and Northern blotting

Total RNA was prepared from pcDNA3-AS-FBI1/Akirin2-transfected clones and from mouse lung tissue using the acidic guanidine thiocyanate–phenol–chloroform method. Northern blotting was performed as previously described [5]. Specific mRNA expression levels were quantified using the Image J software (NIH, USA).

2.3. A growth assay and an in vitro colonization assay

Cells (1×10^3) were seeded in 24-well plates, containing DMEM supplemented with 10% FCS, and grown for various periods. The cell number was counted using a TATAI hemocytometer. For the soft agar assay, cells (8×10^3) were suspended in 0.3% agar medium, containing 10% FCS, and layered on a 0.5% agar-coated 35-mm dish and grown for 2 weeks. The colonies formed were stained with 0.25% 1-*p*-iodophenyl-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT; Sigma, St. Louis, MO) for 24 h, and the number of colonies (>0.07 mm in diameter) was counted.

2.4. ERK1/2 activation

Cells were serum-starved for 12 h before stimulation with the 10% FCS–DMEM medium. At various time points after the serum stimulation, cells were lysed in TNM buffer [20 mM Tris–HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM Na_3VO_4, 25 mM NaF, 0.1% Nonidet P-40], and the lysates were subjected to Western blotting. Anti-phospho-ERK1/2 (1:2000, Cell Signaling Technology, Beverly, MA) and anti-ERK1/2 (1:2000, Upstate, Lake Placid, NY) antibodies were used for immunostaining.

2.5. Tumorigenicity and lung metastasis assays

The cells (10^6 per 200 μL of PBS per flank) were subcutaneously injected into 5-week-old athymic mice (BALB/c Jcl *nu/nu*, Clea Japan, Tokyo, Japan). After 28 days, the tumor volume was measured and calculated as (short axis^2 × long axis)/2. For the assay of lung metastasis, the cells (10^6 per 200 μL of the serum-free medium) were injected into the tail vein of nude mice. After 3 weeks, metastatic foci formed in the lungs were analyzed. Mouse care and handling conformed to the National Institutes of Health guidelines for animal research. The experimental protocols were approved by the Tokyo University of Science Animal Care and Use Committee.

2.6. The wound healing assay

When the cells reached confluence, the cell layer was scratched using a sterilized pipette tip. Detached cells were washed out with PBS. To observe the cell migration process, photographs were taken using a phase contrast microscope at different time points. The migration speed of cells was determined using time lapse analysis (Leica AS MDW, Heidelberg, Germany).

2.7. Statistical analysis

The data were calculated as mean ± standard error of the mean (SE). The differences between 2 groups were analyzed using Student's *t* test, and *p* value of <0.01 was considered statistically significant.

3. Results

3.1. Establishment of antisense FBI1 LLC1 cell lines

To clarify the oncogenic function of FBI1/Akirin2 in LLC1 lung carcinoma cells, we analyzed the effect of *FBI1/Akirin2* mRNA downregulation on the growth ability of the cells *in vitro* and *in vivo*. First, we analyzed the *FBI1/Akirin2* mRNA expression level in LLC1 cells and mouse lung tissue. As shown in Fig. 1A, *FBI/Akirin2* mRNA expression in mouse lung tissue was barely detectable. In contrast, LLC1 cells strongly expressed *FBI1/Akirin2*, and the expression level was similar to that in K2 hepatocellular carcinoma cells. LLC1 cells also expressed 14-3-3β mRNA (data not shown). To reduce *FBI1/Akirin2* expression level, we transfected an antisense

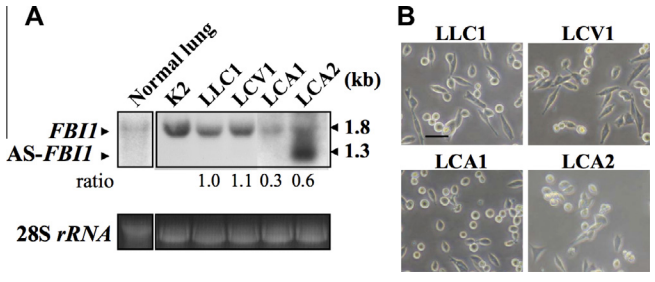

Fig. 1. Establishment of antisense *F BI1/Akirin2*-transfected LLC1 lung carcinoma cells. (A) Characterization of antisense *FBI1/Akirin2* cDNA expression vector-transfected LLC1 cells using Northern blotting. Total RNA was extracted from healthy mouse lung tissue, K2 cells, LLC1 cells, empty vector-transfected LCV1, and antisense *FBI1/Akirin2* cDNA-transfected LCA1 and LCA2 cells and subsequently analyzed using Northern blotting. (B) Morphological changes in the transfectant clones. Cells were cultivated in monolayer culture and photographed using a phase contrast microscope. The scale bar is 50 μm.

FBI1/Akirin2 (AS-FBI1/Akirin2) expression vector into LLC1 cells and established stably transfected clones (LCA1 and LCA2). The empty pcDNA3 vector was transfected and the established LCV1 clone was used as a control. Northern blotting revealed that endogenous *FBI1* mRNA expression levels in LCA1 and LCA2 cells were diminished to 28% and 60% of the parental LLC1 cell level, respectively, whereas the expression level in LCV1 cells was similar to that of parental LLC1 cells (Fig. 1A). In addition, the LCA1 and LCA2 clones exhibited round morphology, whereas parental LLC1 and control LCV1 cells exhibited typical fibroblast morphology (Fig. 1B).

3.2. FBI1/Akirin2 downregulation suppressed anchorage-independent growth and shortened the duration of ERK1/2 activation

To examine whether FBI1/Akirin2 downregulation influences LLC1 cell proliferation, the transfectants were grown in monolayer culture and in soft agar medium and the growth ability was analyzed. As shown in Fig. 2A, in monolayer culture, there was no significant difference in the growth rate among the transfectant clones. In contrast, the colony formation ability of LCA1 and LCA2 cells was dramatically reduced in soft agar: to 50% and 5% of that of parental LLC1 cells, respectively (Fig. 2B and C). These results suggested that FBI1/Akirin2 selectively controlled the anchorage-independent growth of LLC1 cells, as previously observed in K2 cells. We previously demonstrated that FBI1/Akirin2 promotes sustained ERK1/2 activity by suppressing MKP-1 expression [6]. To examine whether FBI1/Akirin2 has the same effect on MAPK signaling in LLC1 cells, the phosphorylated form of ERK1/2 was analyzed using Western blotting after serum stimulation. Five minutes after serum stimulation, ERK1/2 were robustly phosphorylated, and there was no difference in the phosphorylation level among all clones. Nevertheless, 30 min after serum stimulation, the phosphorylated ERK1/2 was less abundant in LCA1 and LCA2 cells, whereas it was still abundant in LLC1 and LCV1 cells (Fig. 2D). This result suggested that FBI1/Akirin2 overexpression is necessary for sustained ERK1/2 activation.

3.3. Transfection with an antisense FBI1/Akirin2 construct significantly reduced LLC1 cell tumorigenicity and metastasis

Since the ability of anchorage-independent growth is related to aggressiveness of tumors, we subsequently assessed the effect of FBI1/Akirin2 downregulation on tumorigenicity and metastasis *in vivo*. The transfectant cells were subcutaneously injected into the flank of nude mice, and the size of the tumors was estimated after 3 weeks. Although all cell lines developed tumors in all mice, the tumor size of LCA1 and LCA2 cells was significantly smaller than that of LLC1 and LCV1 cells (Fig. 3A and B). The tumor size of LCA1 and LCA2 cells was reduced to 30% of that of the parental LLC1 cells. LLC1 cells are known as highly metastatic cells when cells are injected into the tail vain of nude mice. Using this model, we assessed the capacity for metastasis of the transfectant clones. The cells were injected into the tail vain of nude mice, and lung metastasis was assessed after 3 weeks. LLC1 and LCV1 cells developed a large number of metastatic foci in lungs, and the weight of lungs was almost 3 times that of control (serum-free–medium injected) mouse lungs (Fig. 3C and D). Although the weight of the lungs of LCA1- and LCA2-injected mice was slightly higher compared with the control, there were no visible metastatic foci. These results showed that FBI1/Akirin2 promoted tumorigenicity and metastasis of the LLC1 cell line.

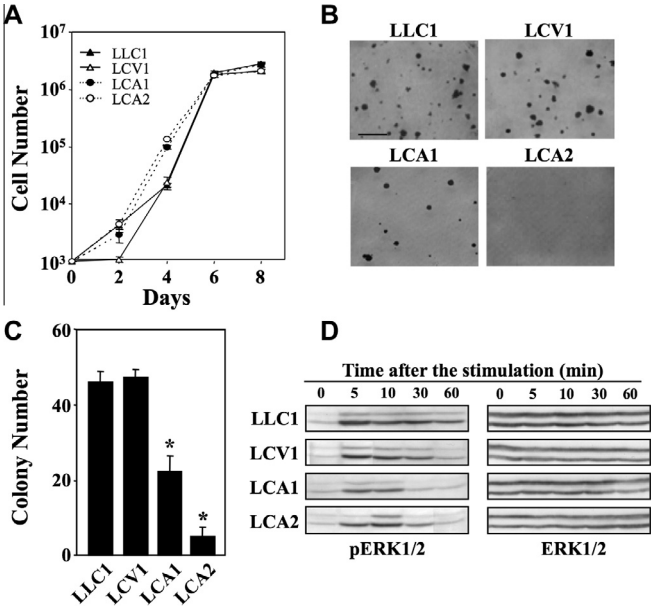

Fig. 2. *FBI1/Akirin2* mRNA downregulation inhibited anchorage-independent growth and shortened the duration of ERK1/2 activity. (A) The growth rate of the transfectant clones in monolayer culture. Cells were grown in monolayer culture for various periods, and the number of cells was counted. (B and C) The growth ability in a semisolid medium. Cells (8×10^3) were grown in a 35-mm culture dish, containing soft agar for 3 weeks. Subsequently, the colonies formed were photographed (B) and counted (C) after staining with INT. The scale bar is 5 mm. The data are shown as mean ± SE of triplicate experiments (separate culture dishes; $^*p < 0.01$ compared with parental LLC1 cells). (D) The cells were cultured in a serum-free medium for 12 h and stimulated with a medium, containing 10% FCS. At various time points after the stimulation, the cells were lysed and subjected to Western blotting. The right panels show data on phosphorylated ERK1/2, and the left panels show the total level of ERK1/2 proteins.

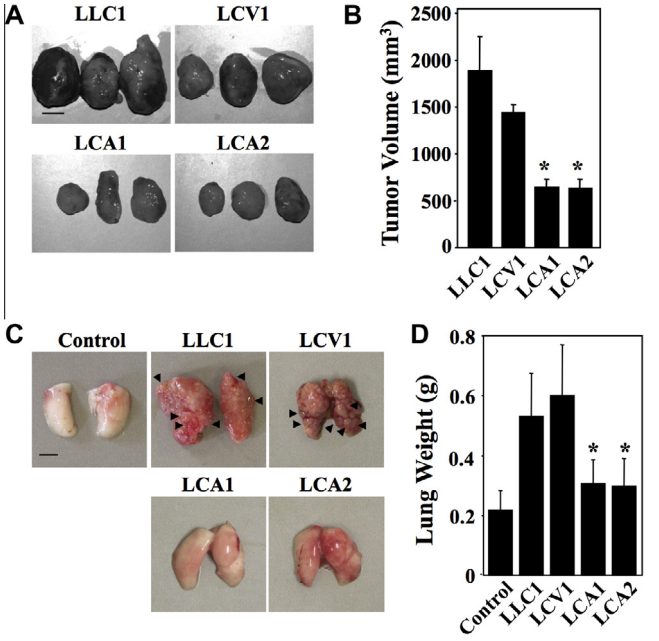

Fig. 3. *FBI1/Akirin2* mRNA downregulation inhibited tumorigenicity and metastasis. (A and B) Effects of *FBI1/Akirin2* mRNA downregulation on tumorigenicity of LLC1 lung carcinoma cells. Cells were subcutaneously injected into the flank of nude mice (10^6 cells per flank). After 28 days, the resulting tumors were removed and photographed (A). The scale bar is 10 mm. (B) The average volume of tumors developed in the flank of 3–5 mice was measured and calculated as mean ± SE ($^*p < 0.01$ compared with the parental LLC1 cell line). (C) Suppression of lung metastasis of intravenously injected LLC1 cells by forced expression of antisense *FBI1/Akirin2* RNA. Cells (10^6) were injected into the tail vein of nude mice. After 3 weeks, the lungs were removed and weighed. Control mice were injected with a serum-free medium. (D) The average weight of the lungs was measured and calculated as mean ± SE ($^*p < 0.01$ compared with the parental cell line).

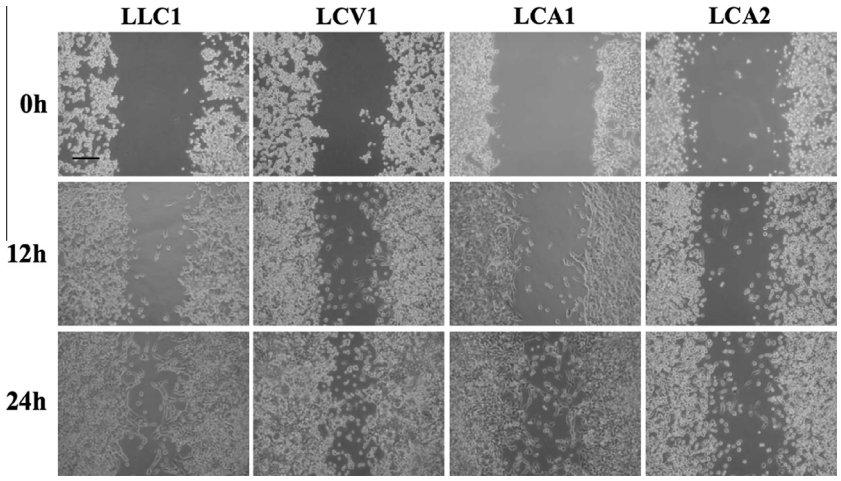

Fig. 4. Effects of *FBI1/Akirin2* mRNA downregulation on the motility of LLC1 cells. The wound-healing properties of LLC1 cells and antisense *FBI1/Akirin2* transfectants. The cells were grown to confluence in monolayer culture and subsequently scratched with a pipette tip. The wound areas were photographed using a phase contrast microscope after 0, 12, and 24 h. The scale bar is 100 μm.

3.4. Effects of FBI1 downregulation on LLC1 cell motility

Cancer metastasis is a multistep processes, and cell motility is one of the key properties involved in migration into surrounding tissues. During cell migration, cells continuously alter their shape by dynamically changing the actin cytoskeleton [11]. As shown in Fig. 1B, antisense *FBI1/Akirin2*-expressing cells are round, suggesting that FBI1/Akirin2 may have an effect on the cell spreading and migration. Accordingly, we analyzed the effect of the antisense *FBI1/Akirin2* construct on LLC1 cell motility in a wound healing assay. As shown in Fig. 4, LLC1 and LCV1 cells migrated into the scratched area in a time-dependent manner and healed over 80% of the wound area after 24 h. In contrast, in LCA1 and LCA2 cell culture, the wound areas were closed only 40% after 24 h. The migration speed during the first 2 h was analyzed using a time-lapse system. The migration speed of LLC1 and LCV1 cells was 22 and 17 μm/h, respectively. The migration speed of LCA1 cells was almost a half (10 μm/h). Moreover, the migration of LCA2 cells was hardly detectable for the first 2 h. These results indicated that FBI1/Akirin2 downregulation decreased the cell motility.

4. Discussion

We previously reported that 14-3-3β and its binding partner FBI1/Akirn2 have an oncogenic function in rat hepatocellular carcinoma K2 cells [5,6]. In the present study, we expanded our research of FBI1/Akirin2 to another cancer model, LLC1 Lewis lung carcinoma cells. LLC1 cells express relatively high levels of *FBI1/Akirin2* compared with untransformed lung tissue. Our results show that FBI1/Akirn2 knockdown in LLC1 cells significantly suppresses anchorage-independent growth *in vitro* and tumor growth and metastasis *in vivo* in a mouse model. Furthermore, FBI1/Akirin2 suppression shortened the duration of ERK1/2 activation and reduced cell motility. These results suggest that FBI1/Akirin2 performs an oncogenic function in lung cancer and hepatocellular carcinoma.

The MAPK pathway is activated by numerous growth factors and regulates various pathways in cancer cells, such as those involved in cell-cycle progression, apoptosis resistance, cell migration, invasion, and metastasis. The mutations that activate MAPK can constitutively transform cells [12]. Furthermore, it is known that the duration of ERK1/2 activation is key to signal transduction into specific downstream cascades [13,14]. In fibroblasts, sustained ERK1/2 activation induces cyclin D1 transcription and G1/S transition of the cell cycle. In contrast, transient activation cannot induce cyclin D1 [15]. We previously reported that FBI1/Akirin2 knockdown K2 cells show shortened ERK1/2 activity and reduced cyclin D1 expression. As expected, in LLC1 cells in the present study, FBI1/Akirin2 knockdown shortens the duration of ERK1/2 activity after serum stimulation. In addition, antisense *FBI1/Akirin2* transfection inhibits anchorage-independent growth but not anchorage-dependent growth in monolayer culture. Under anchorage-independent conditions, cells lose the supportive signals from the extracellular matrix (ECM), and detachment from ECM leads to subsequent phosphorylation/dephosphorylation and inactivation of the survival signals. The MAPK pathway is one of the pathways downstream of ECM-related signaling. FBI1/Akirin2 may be able to compensate for the lack of survival signals from ECM through prolonged activation of ERK1/2, thereby promoting colony formation and tumorigenicity of K2 and LLC1 cells.

Our study shows that FBI1/Akirin2 downregulation significantly reduces lung metastasis. Cancer metastasis is a multistep process that requires invasion of surrounding tissue, intravasation into blood or lymphatic vessels, survival in the circulation, extravasation to a secondary tissue, and proliferation in a new microenvironment. FBI1/Akirin2 appears to promote metastasis in at least 2 ways: anchorage-independent growth and motility. When untransformed epithelial cells lose the interaction with ECM, cells immediately undergo apoptosis, which is referred to as anoikis. In contrast, cancer cells are able to survive without survival signals from ECM in an anchorage-independent manner, and the anoikis resistance is important for successful metastatic dissemination [16–18]. During metastasis, cancer cells need to migrate into surrounding tissue [19]. FBI1/Akirin2 knockdown cells exhibit rounded cell morphology. This feature may indicate that FBI1/Akirin2 affects the cell spreading on a substrate. In addition, the motility of FBI1/Akirin2 knockdown cells significantly diminished compared with parental LLC1 cells and an empty-vector control. These results demonstrate that LLC1 cells may require the high level of FBI1/Akirin2 expression for anchorage-independent growth and motility, which result in an aggressive metastatic phenotype of tumor cells.

It should be noted that we observed some differences between K2 and LLC1 cells in the effects of the FBI1/Akirin2 knockdown. In K2 cells, matrix metalloproteinase (MMP) 2 and MMP-9 were downregulated by the AS-FBI1/Akirin2 transfection, whereas this effect was undetectable in LLC1 transfectants (data not shown). Although proteolysis is important for the invasion step, there are

some reports showing that cancer cells can invade the surrounding tissue without strong proteolysis. Those cells migrate through amoeboid-like movements and squeeze themselves into the narrow space in ECM [20,21]. It appears that high cellular motility is crucial for the metastatic ability of LLC1 cells, whereas MMP expression is more important for K2 cell metastasis. These differences may reflect the diversity of FBI1/Akirin2 target genes in each cell line. In fact, our microarray data indicate that antisense *FBI1/Akirin2* transfection changed the expression level of at least 26 genes over 2-fold compared with that in parental K2 cells. In addition, we recently reported that one of the candidate genes from the microarray data, BCAM, is transcriptionally suppressed by FBI1/Akirin2, and the reduction in BCAM expression correlates with tumorigenicity and the metastatic ability of K2 cells [22]. These data suggest that FBI1/Akrin2 has multiple targets and may utilize different downstream targets to promote tumorigenicity and metastasis, depending on a genetic background of cancer cells.

In conclusion, we demonstrated that FBI1/Akirin2 performs an oncogenic function in LLC1 Lewis lung carcinoma cells and rat hepatocellular carcinoma K2 cells. FBI1/Akirin2 promotes anchorage-independent and tumor growth, which are likely mediated by sustained ERK1/2 activity. FBI1/Akirin2 also promotes cell migration, leading to metastasis. Although further investigation is required, these results suggest that FBI1/Akirin2 may perform an universal oncogenic function in various cancers. Further research into the downstream signaling of FBI1/Akirin2 may give us some clues to new therapeutic targets in lung and hepatocellular carcinomas.

Acknowledgments

This work was supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology Grants-in-Aid for Scientific Research (Grant numbers 70089332, 23701104, and 25870775).

References

[1] M.J. van Hemert, H.Y. Steensma, G.P. van Heusden, 14-3-3 Proteins: key regulators of cell division, signaling and apoptosis, Bioessays 23 (2001) 936–946.
[2] A. Aitken, 14-3-3 and its possible role in co-ordinating multiple signaling pathways, Trends Cell Biol. 6 (1996) 341–347.
[3] A.K. Freeman, D.K. Morrison, 14-3-3 Proteins: diverse functions in cell proliferation and cancer progression, Semin. Cell Dev. Biol. 22 (2011) 681–687.
[4] A.K. Gardino, M.B. Yaffe, 14-3-3 Proteins as signaling integration points for cell cycle control and apoptosis, Semin. Cell Dev. Biol. 22 (2011) 688–695.
[5] A. Sugiyama, Y. Miyagi, Y. Komiya, N. Kurabe, C. Kitanaka, N. Kato, Y. Nagashima, Y. Kuchino, F. Tashiro, Forced expression of antisense 14-3-3beta RNA suppresses tumor cell growth in vitro and in vivo, Carcinogenesis 24 (2003) 1549–1559.
[6] Y. Komiya, N. Kurabe, K. Katagiri, M. Ogawa, A. Sugiyama, Y. Kawasaki, F. Tashiro, A novel binding factor of 14-3-3beta functions as a transcriptional repressor and promotes anchorage-independent growth, tumorigenicity, and metastasis, J. Biol. Chem. 283 (2008) 18753–18764.
[7] D.J. Macqueen, I.A. Johnston, Evolution of the multifaceted eukaryotic akirin gene family, BMC Evol. Biol. 9 (2009) 34.
[8] A. Goto, K. Matsushita, V. Gesellchen, L. El Chamy, D. Kuttenkeuler, O. Takeuchi, J.A. Hoffmann, S. Akira, M. Boutros, J.M. Reichhart, Akirins are highly conserved nuclear proteins required for NF-kappaB-dependent gene expression in drosophila and mice, Nat. Immunol. 9 (2008) 97–104.
[9] X. Chen, Z. Huang, H. Wang, G. Jia, G. Liu, X. Guo, R. Tang, D. Long, Role of akirin in skeletal myogenesis, Int. J. Mol. Sci. 14 (2013) 3817–3823.
[10] Y. Chu, P.A. Solski, R. Khosravi-Far, C.J. Der, K. Kelly, The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation, J. Biol. Chem. 271 (1996) 6497–6501.
[11] A. Wells, J. Grahovac, S. Wheeler, B. Ma, D. Lauffenburger, Targeting tumor cell motility as a strategy against invasion and metastasis, Trends Pharmacol. Sci. 34 (2013) 283–289.
[12] J. Downward, Targeting RAS signaling pathways in cancer therapy, Nat. Rev. Cancer 3 (2003) 11–22.
[13] C.J. Marshall, Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation, Cell 80 (1995) 179–185.
[14] L.O. Murphy, J. Blenis, MAPK signal specificity: the right place at the right time, Trends Biochem. Sci. 31 (2006) 268–275.
[15] L.O. Murphy, S. Smith, R.H. Chen, D.C. Fingar, J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, Nat. Cell Biol. 4 (2002) 556–564.
[16] S.M. Frisch, R.A. Screaton, Anoikis mechanisms, Curr. Opin. Cell Biol. 13 (2001) 555–562.
[17] S.M. Frisch, H. Francis, Disruption of epithelial cell-matrix interactions induces apoptosis, J. Cell Biol. 124 (1994) 619–626.
[18] P.J. Reddig, R.L. Juliano, Clinging to life: cell to matrix adhesion and cell survival, Cancer Metastasis Rev. 24 (2005) 425–439.
[19] J. Kassis, D.A. Lauffenburger, T. Turner, A. Wells, Tumor invasion as dysregulated cell motility, Semin. Cancer Biol. 11 (2001) 105–117.
[20] E. Sahai, C.J. Marshall, Differing modes of tumor cell invasion have distinct requirements for Rho/ROCK signaling and extracellular proteolysis, Nat. Cell Biol. 5 (2003) 711–719.
[21] K. Wolf, I. Mazo, H. Leung, K. Engelke, U.H. von Andrian, E.I. Deryugina, A.Y. Strongin, E.B. Brocker, P. Friedl, Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis, J. Cell Biol. 160 (2003) 267–277.
[22] H. Akiyama, Y. Iwahana, M. Suda, A. Yoshimura, H. Kogai, A. Nagashima, H. Ohtsuka, Y. Komiya, F. Tashiro, The FBI1/Akirin2 target gene, BCAM, acts as a suppressive oncogene, PLoS One 8 (2013) e78716.