Clinics in Oncology

Research Article
Published: 01Jun, 2016

Over-Expression of Cofilin-1 Suppressed Mobility of Lung Cancer Cells is Associated with Down-Regulation of SNAIL-1 and Induction of *Let-7*

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Abstract

Metastatic lung cancer means the spread of cancer from the primary site to nearby structures or distant organs. Epithelial-mesenchymal transition (EMT) is an important mechanism to be associated with metastasis. Suppression of EMT may prevent the cancer metastasis. We previously found that over-expression of cofilin-1, an actin binding protein belongs to the actin depolymerizing factor (ADF)/cofilin family leads to morphological change and inhibition of invasion of human non-small cells lung cancer (NSCLC). This effect is associated with up-regulation of the tumor suppressive *let-7* microRNA through TWIST-1 transcription factor, an important biomarker of EMT. Here we investigated whether other EMT related molecules would be affected by over-expressed cofilin-1. Over-expression of cofilin-1 in human H1299 lung cancer cells also suppressed SNAIL-1 transcription factors, but E-cadherin and N-cadherin were not significantly affected. Importantly, over-expression of cofilin-1 induced *let-7* could be suppressed by enforced expression of SNAIL-1, suggesting that EMT related transcription factors can be suppressed by over-expressed cofilin-1 to induce *let-7* expression. However, over-expression of cofilin-1 may not suppress EMT. To monitor the effects of cofilin-1 and *let-7* on lung cancer migration *in vivo*, we established a multiple reporter genes transduced lung cancer cell line that can be detected using the reporter gene imaging. The cofilin-1 induced *let-7* was suppressed by transfection of locked nucleic acid (LNA) to inhibit *let-7*. Compared to normal lung cancer cells, over-expression of cofilin-1 suppressed the lung cancer migration, but simultaneously transfection of LNA recovered their migration ability to lungs in small animals. Taken together, over-expression of cofilin-1 can suppress the invasion and migration of lung cancer cells through up-regulation of *let-7 in vitro* and *in vivo*. Additionally, cofilin-1 may regulate EMT related transcription factors but not the whole EMT mechanism.

Keywords: Cofilin-1; SNAIL-1; *let-7*; EMT; Reporter gene imaging; Locked nucleic acid

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Received Date: *03 May 2016*
Accepted Date: *27 May 2016*
Published Date: *01 Jun 2016*

Citation:
Wang CY, Tsai CH, Chang CY, Liao MJ, Liu RS, Lee YJ. Over-Expression of Cofilin-1 Suppressed Mobility of Lung Cancer Cells is Associated with Down-Regulation of SNAIL-1 and Induction of Let-7. Clin Oncol. 2016; 1: 1015.

Introduction

According to American Cancer Society's estimation, lung cancer is the second most common cancer type independent of sex. However, lung cancer is the leading cause of death in cancer patients worldwide [1]. More than 85% of lung cancer belongs to non-small cells lung cancer (NSCLC) that includes several subtypes, such as adenocarcinoma, squamous cell carcinoma, large carcinoma and less commonly found adenosquamous carcinoma. Metastasis is the primary cause of lung cancer death, including bone and brain metastasis. Therefore, suppression of metastasis is important for tumor control.

Actin cytoskeleton is formed by Rho small GTPase signaling pathway that can form different types of actin architectures for cell morphology, attachment and migration. This signaling mediates the activity of cofilin-1, a non-muscle is form of actin depolymerizing factor (ADF)/cofilin family member to accelerate the actin dynamics [2,3]. Over-expression of cofilin-1 may disrupt the balance of actin dynamics and lead to obstacle of cell motility. However, the underlying mechanisms remain to be addressed.

Metastasis is strongly associated with the epithelial –mesenchymal transition (EMT). EMT is a process of cell morphological change that allows cancer penetrating through the vessel and traveling to distant organs for regrowth [4,5]. Several markers of EMT with the property of transcription factors have been widely reported, including Twist Basic Helix-Loop-Helix Transcription Factor 1 (TWIST-1), Zinc finger protein SNAI1 (SNAIL-1) and SNAI2 (Slug), Zinc finger E-box-binding homeobox 1/2 (ZEB1 and ZEB2) [6,7]. E-cadherin is responsible for cell-cell adhesion and is usually down-regulated by these transcription factors followed by the emergence of mesenchymal shapes [8]. N-cadherin is up-regulated to balance the down-regulated E-cadherin for altered cell adhesion [9]. These transcription factors also promote anti-apoptosis, angiogenesis, chromosomal instability, and are generally regarded oncogenes. Interestingly, recent reports showed that TWIST-1 can suppress the expression of tumor suppressive lethal-7 (*let-7*) microRNA, which can further regulate *Ras* and high mobility group A2 (HMGA2) oncogenes [10-12]. Additionally, low *let-7* level significantly correlates to the postoperative death [13]. However, it is unclear whether different EMT-related markers will influence the expression of *let-7* or not.

Because over-expression of cofilin-1 would disrupt the actin dynamics, it is speculated that cell migration should be affected. Previously, we have found that over-expressed cofilin-1 could suppress the expression of TWIST-1 in NSCLC cells [14]. The *let-7* family members were subsequently up-regulated, and *let-7b* and *let-7e* exhibited most significant up-regulation. Because TWIST-1 is involved in EMT, it is interesting to investigate whether other EMT related molecules can regulate the expression of *let-7* microRNA. In this study we further examined the expression of SNAIL-1 and E-cadherin after over-expression of cofilin-1 in lung cancer cells. Additionally, we used reporter gene imaging to monitor whether knockdown of *let-7* in cofilin-1 over-expressing lung cancer cells would recover the migration ability in small animals. This study would establish a signaling pathway between cofilin-1 and *let-7* that regulate lung cancer migration *in vitro* and *in vivo*.

Materials and Methods

Cell culture

Human lung cancer H1299 cells with *tet*-on inducible cofilin-1 over-expression cell line (HCOXP) and reporter genes harboring HCOXP cells (HCOXP-3R) were maintained in *Dulbecco's Modified Eagle's* medium (*DMEM*) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin solution, 2mM L-glutamine (Sigma-Aldrich Co, St. Louis, MO, USA), and 0.1mg/ml hygromycine B (Invitrogen, Carlsbad, CA, USA). HCOXP cells were maintained in a humidified incubator with 5% CO_2 and 37°C by passaged every 48 hours.

Western blot analysis and antibodies

The procedure of protein extraction, gel running and electro-transferring has been described previously [15]. The primary antibodies used in this study include: anti-cofilin-1, anti- ser3-phospho-cofilin-1, anti-Twist-1, anti-TWIST-1 (Genetex Inc., Irvine, CA, USA), anti-SNAIL-1, anti-cofilin-1 (Genetex Inc., Irvine, CA, USA), anti-phospho-cofilin-1 (Santa Cruz Biotechnology Inc., Dallas, TX, USA), anti-E-cadherin, anti-N-cadherin, anti-HSV1-tk (Santa Cruz Biotechnology Inc., Dallas, TX, USA) and anti- GAPDH (Sigma-Aldrich Co, St. Louis, MO, USA) antibodies.

Quantification of *let-7*

To measure *let-7* microRNA levels before and after cofilin-1 over-expression, quantitative PCR (qPCR) of targeted miRNA was used. In brief, complementary DNA (cDNA) was generated from 5 μg total RNA using SuperScript II reverse transcriptase (Life-Technologies Co, Carlsbad, CA, USA). The cDNA products were then mixed in the Fast SYBR Green Master Mix (Life-Technologies Co, Carlsbad, CA, USA) and subjected to the Step One Plus Real-Time PCR System (Life-Technologies Co, Carlsbad, CA, USA) according to the manufacturer's instructions. The stem loop primers used for *let-7b* and *let-7e* were 5'-GTCGTATCCAGTG CAGGGTCCGAGGTATTCGCACTGGATACGACAACcac-3' and 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGC ACTGGAT ACGACAACTAT-3', respectively. The forward primers of qPCR used for *let-7b* and *let-7e* were 5'- GCCGCTTGAGGTAGTAGGTTGT-3' and 5'-GCCGCTTGAGGtAGGAGGTTGT-3', respectively. The universal reverse primer was used for both *let-7*: 5'-CCAGTGC AGGGTCCGAGGT-3'. For internal control, the vertebrate U6 small nuclear RNA was amplified using the primer set: 5'-CGCTTCGGC AGCACATATAC-3' and 5'-TTCACGAATTTGCGTGTCAT-3'. Establishment of HCOXP-3R cells and reporter gene validations – LT-3R plasmid, a multicistronic lentiviral construct was used to establish HCOXP-3R cells for expressing firefly luciferase (fLuc), green fluorescent protein (GFP) and herpes simplex virus type 1-thymidine kinase (HSV1-tk) reporter genes [16]. This plasmid was co-transfected with pCMV-ΔR8.91 plasmid and pMD.G plasmid into the 293T packaging cell line to produce virion soup using the calcium phosphate precipitation method. Ultracentrifugation was used to concentrate the virion soup, which was added to HCOXP cell culture for infection. After infection, cells were sorted by the fluorescence-activated cell sorting (FACS, FACSAria, BD Biosciences, San Jose, CA, USA) based on the GFP emitted fluorescent signals. The obtained stable clone was named HCOXP-3R cells, in which the GFP expression was visualized using the fluorescent microscope. The fLuc activity and expression of HSV1-tk protein were determined by the luciferase assay and Western blot analysis as described before [16].

Knockdown of *let-7* – Chemically modified locked nucleic acid (LNA™, Exiqon, Los Angeles, CA, USA) was used to silence the expression of *let-7* microRNA. In brief, 30nM LNA was transfected into HCOXP-3R cells using the JetPEI transfection reagent (Polyplus-transfection, SA, Illkirch, France). The sequences of LNA for targeting on *let-7*b and *let-7*e were 5'-ACCACACAACCTACTACCTC-3' and 5'-ACTATACAACCTCCTACCTC-3', respectively.

Luciferase assay – The expression of luciferase activity in cells with over-expressed cofilin-1 and co-transfected LNA were determined using the *in vitro* luciferase assay. Cells were cultured in 12-well plates and lyzed by passive lysis buffer and then added with 5-fold diluted reporter assay buffer (50mM glycylglycin, 1M magnesium sulfate, 10mg/ml bovine serum albumin, and 0.5M EDTA) mixed with 100mM adenosine 5'-triphosphate disodium salt (Sigma-Aldrich Co., St. Louis, MO, USA), 1M dithiothreitol and 50mM D-luciferin luciferin (Promega Co., Madison, WI, USA) transferring to a 96-wel black plate. The luminescent signals were detected using a multimode microplate reader (TECAN, Switzerland).

In vitro invasion assay – Cells were trypsinized and five thousand cells were seeded in transwells coated with Matrigel (BD biosciences, San Jose, CA, USA) in serum-free DMEM. Each transwell was placed in a 24-well dish containing DMEM with 10% FBS. After 48 hours of

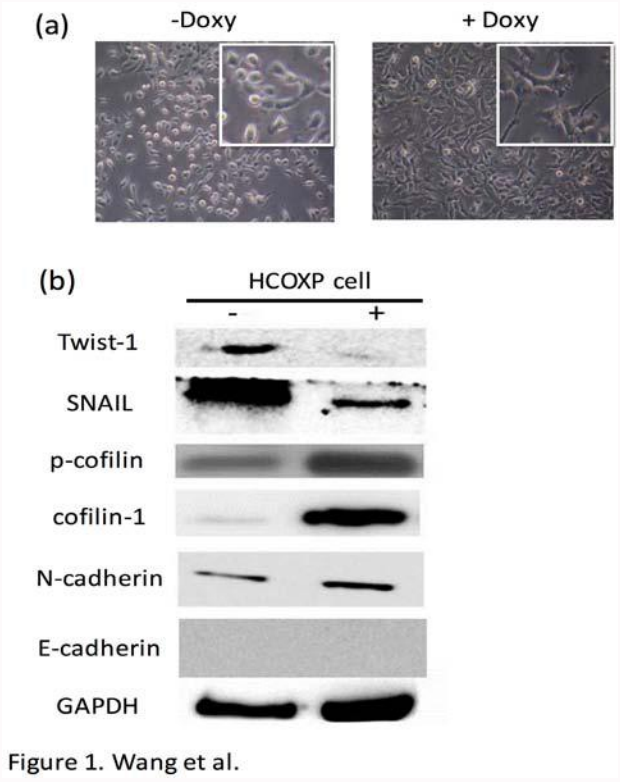

Figure 1: Effects of over-expressed cofilin-1 on EMT related molecules. (a) Morphological change induced by over-expression of cofilin-1. Insets are enlarged photos of normal cells and cofilin-1 over-expressing cells. (b) Western blot analysis for detection of EMT related molecules in response to over-expression of cofilin-1.

incubation, the transwells were cleaned with a cotton stub, fixed using 4% paraformaldehyde followed by crystal violate (1.25% in ethanol) staining for 30 minutes. The transwells were then rinsed, visualized and counted under a bright-field microscope.

Experimental metastasis animal model and bioluminescent imaging

HCOXP-3R cells ($1x10^6$) were injected into the nude mice via tail veins. After injection, the animals were subjected to IVIS-50 imaging system (Caliper Co, Hopkinton, MA, USA) to detect the bioluminescent signals *in vivo*. Before imaging, the animals were i.p. injected with 150mg/kg D-luciferin (VivoGlo Luciferin, Promega Corp., Madison, WI, USA) and anesthetized with 1% isofluorane for 15 minutes. The images were acquired using the bundled software. The animal studies have been approved by Institutional Animal Care and Use Committee (IUCAC No. 1021208) of National Yang-Ming University.

Statistic analysis

Each datum represented means ± S.D., and the results were analyzed by student's *t*-test between two samples. For multiple samples, one-way analysis of variance (ANOVA) was used for statistic analysis. In both conditions, $p < 0.05$ was regarded significance. The analysis and plots were executed using Sigmaplot 10.0 software (Systat Software, Inc, a Jose, CA, USA).

Results

Effects of cofilin-1 over-expression on EMT related molecules

HCOXP cells are derived from H1299 lung cancer cells harboring a *tet*-on gene expression system for over-expression of cofilin-1. Because induction of cofilin-1 expression in these cells will lead to apparent morphological change likes EMT, we investigated whether over-expression of cofilin-1 would influence the EMT related molecules (Figure 1A). TWIST-1, SNAIL-1, N-cadherin and E-cadherin were examined after over-expression of cofilin-1 using the Western blot analysis. Although TWIST-1 and SNAIL-1 were down-regulated by over-expressed cofilin-1, E-cadherin and N-cadherin levels were not changed significantly (Figure 1B). Over-expressed cofilin-1 can be phosphorylated on the serine-3 as described before. Therefore, these data suggest that cofilin-1 would influence the expression of TWIST-1 and SNAIL-1 transcription factors but not the whole EMT related biomarkers.

Over-expression of cofilin-1 induced let-7 up-regulation was suppressed by SNAIL-1

Previously, we have found that over-expression of cofilin-1 can induce *let-7* microRNA through suppression of TWIST-1 expression [14]. Over-expression of TWIST-1 can counteract the induced *let-7* microRNA by over-expressed cofilin-1. Here we investigated whether SNAIL-1 transcription factor can also regulate *let-7* or not. In HCOXP cells, induction of cofilin-1 expression could induce *let-7*b and *let-7*e that have been reported to be most responsive to over-expressed cofilin-1 (Figure 2A). We next showed that in doxycycline treated HCOXP cells, transfection of pCDH-SNAIL-1 construct could restore the SNAIL-1 level in these cells (Figure 2B). This treatment led to suppression of *let-7*b and *let-7*e induced by over-expressed cofilin-1 (Figure 2C). Therefore, down-regulation of SNAIL-1 by over-expressed cofilin-1 is also involved in regulate the expression of *let-7* microRNA.

Characterization of HCOXP-3R cells responding to knockdown

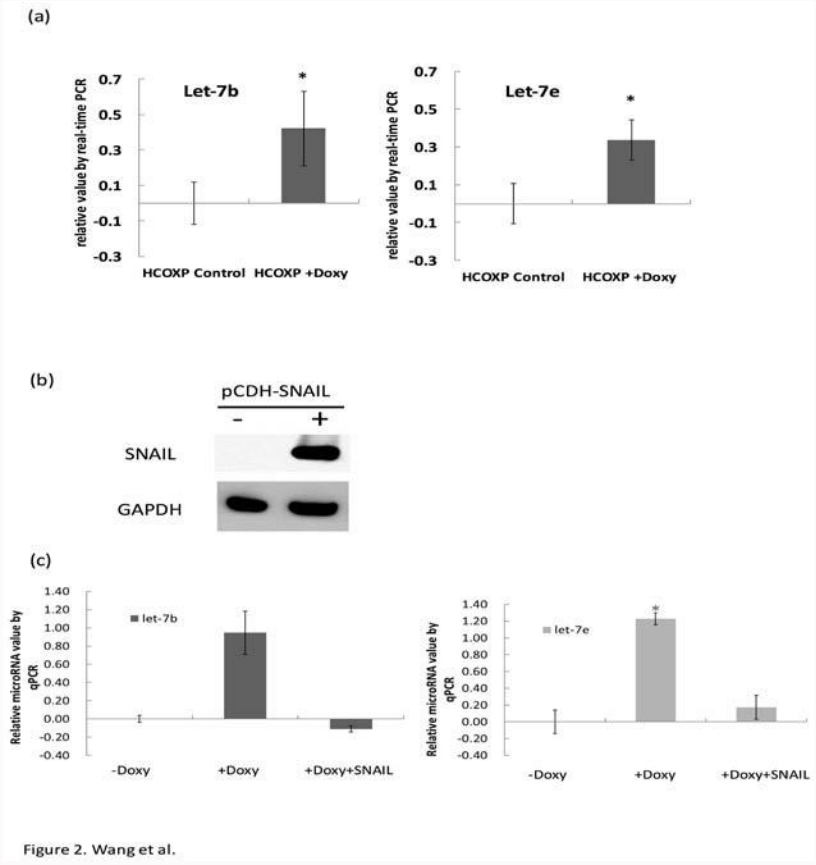

Figure 2: Effects of SNAIL-1 on suppression of *let-7* induced by over-expression of cofilin-1. (a) Quantification of *let-7b* and *let-7e* using qPCR before and after over-expression of cofilin-1. (b) Transfection of pCDH-SNAIL-1 to HCOXP cells followed by Western blot analysis. (c) Over-expression of SNAIL-1 suppressed the expression of *let-7b* and *let-7e* induced by cofilin-1.

of *let-7* after over-expression of cofilin-1 – We have previously established a lentiviral-based multicistronic reporter construct including green fluorescent protein gene (GFP), firefly luciferase (fLuc), and herpes virus type 1 – thymidine kinase (HSV1-tk) gene, which are used for examination of transfection efficiency, bioluminescent imaging, and radionuclide based imaging *in vivo*, respectively. Here we transduced this construct into HCOXP cells that have not been examined before. The obtained stable cells were named HCOXP-3R cells. The expression of GFP was examined using the fluorescent microscope (Figure 3A). The activity of fLuc was determined using the luciferase assay with luciferin substrate (Figure 3B). The expression of HSV1-tk was examined using Western blot analysis (Figure 3C). This novel stable lung cancer cell line would be used for in vivo tracking under different conditions. We next compared the cell viability and invasive ability in HCOXP-3R cells before and after over-expression of cofilin-1. The luciferase activity of HCOXP-3R cells were inhibited by induced cofilin-1 expression, but co-treatment of LNA also recovered the luciferase activity (Figure 3D). HCOXP-3R cells invading through the matrigel coated transwells were further compared by above conditions. The results showed that over-expression of cofilin-1 could suppress the invasion, but the effects were compromised after treatment of *let-7* targeting LNA (Figure 3E). Thus, the *in vivo* effect of over-expressed cofilin-1 on lung cancer was subsequently examined using this novel stable clone.

Bioluminescent imaging of lung accumulation by HCOXP-3R cells responding to cofilin-1 over-expression and LNA treatment – We next investigated whether migration of HCOXP-3R cells to lungs will be affected by over-expressed cofilin-1 and co-treated LNA. HCOXP-3R cells were either treated with doxycycline to induce cofilin-1 expression or transfected with LNAs followed by doxycycline treatment. These cells ($1x10^6$ each) were then separately i.v. injected into nude mice. After injection, the mice were subjected to IVIS-50 system and imaged for the bioluminescent signals immediately. Compared to untreated cells, over-expression of cofilin-1 apparently inhibited the migration of HCOXP-3R cells to lungs (Figure 4A and B). However, suppression of *let-7*b or *let-7*e by LNAs could recover the lung migration of cofilin-1 over-expressing cells (Figure 4C and D). The photons flux in chest of each group was also semi-quantified according to the analytic software of IVIS system (Figure 4E). Therefore, the effects of cofilin-1 signaling pathways on lung cancer growth and metastasis *in vivo* would be easily examined using the HCOXP-3R cells in the future.

Discussion

Previously, over-expression of cofilin-1 was found to induce *let-7* microRNA in human H1299 lung cancer cells. This signaling pathway was mediated by TWIST-1 rather than other *let-7* regulators, that is, LIN28B and c-Myc [14]. Because TWIST-1 is known to be one of the important markers of EMT, it is of interest to investigate whether over-expression of cofilin-1 induced *let-7* is caused by suppression of EMT. Although SNAIL-1 was also down-regulated by over-expressed cofilin-1, another EMT markers E-cadherin and N-cadherin were not significantly affected under this condition. The basal level of E-cadherin in H1299 cells were barely detected as reported

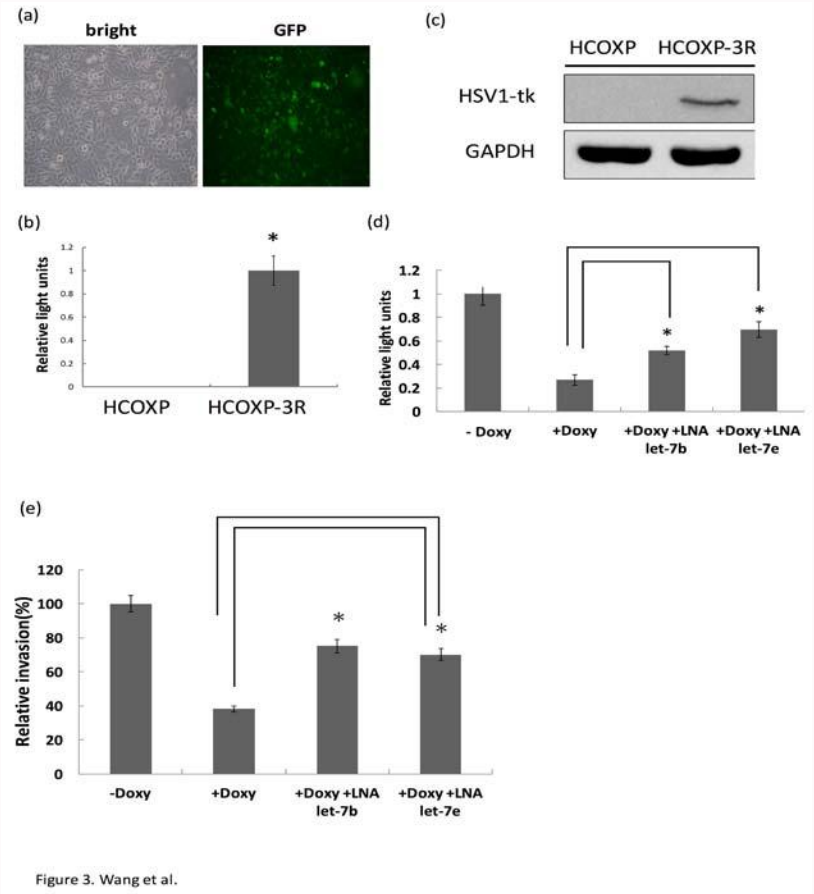

Figure 3: Effects of knockdown of *let-7* in HCOXP cells with co-expressed reporter genes. (a) Fluorescent microscope for visualizing GFP. (b) Luciferase assay for detecting the luciferase activity. (c) Detection of HSV1-tk protein by Western blot analysis. (d) Comparison of cell viability by detecting the luciferase activity in cofilin-1 over-expressing cells before and after transfection of LNA. (e) Comparison of *in vitro* invasion ability according to above conditions. *: p<0.05.

previously [17], while suppression of TWIST-1 and SNAIL-1 by over-expressed cofilin-1 did not up-regulate E-cadherin. N-cadherin was also not reduced. Therefore, the current data suggest that cofilin-1 may influence certain EMT related molecules rather than the EMT phenomenon.

The expression of *let-7* has been reported to be regulated by LIN28A/B, c-Myc and TWIST-1 during cancer development [12,18,19]. As a tumor suppressor, up-regulation of *let-7* leads to suppression of tumor metastasis and cancer growth. Interestingly, a recent report showed that SNAIL-1 can temporarily bind to *let-7* promoters and reduce its expression for efficient reprogramming of fibroblasts [20]. Here we found that *let-7*b and *let-7*e induced by over-expressed cofilin-1 could be suppressed by transfection of SNAIL-1. This result suggests that the SNAIL-1 regulated *let-7* expression also plays a role in lung cancer cells. Like TWIST-1, SNAIL-1 also mediates the cofilin-1 regulated *let-7* expression. How over-expression of cofilin-1 leads to down-regulation of TWIST-1 and SNAIL-1 but not E-cadherin is unclear and may be important to be studied in the future.

The most critical experiments in this report were the first time to demonstrate the cofilin-1/*let-7* signaling pathway would affect the migration of lung cancer cells to lungs *in vivo*. To this end, we used the reporter gene imaging to track the positions of cancer cells in the nude mice. The multicistronic reporter gene construct, so called LT-3R has been used in tracking the growth of glioblastomas *in vivo* [16]. Here we transduced this construct to HCOXP cells, which were subsequently i.v. injected into small animals. This experimental metastasis model has been used for investigating the cancer cells migration to lungs in different cancer types [21,22]. The LT-3R plasmid transduced HCOXP cells exhibited similar phenotypes with parental HCOXP cells, including cell growth and growth suppression by over-expressed cofilin-1. Because reporter genes only express in viable cells [23], the luciferase assay should be sufficient to demonstrate the effects of over-expressed cofilin-1 on suppression of cell viability. Transfection of *let-7* targeted LNA in cofilin-1 over-expressing HCOXP-3R cells led to recovery of luciferase expression, suggesting that *let-7* is important for mediating the effects of cofilin-1 on cell growth and viability. Furthermore, the cellular invasive ability suppressed by over-expressed cofilin-1 was also recovered by LNA. These *in vitro* studies support that HCOXP-3R cells can used as a surrogate to monitor the behaviors of HCOXP cells *in vivo*.

To investigate the cancer metastasis in small animals, both "experimental metastasis" (intravascular injection of cells) and "spontaneous metastasis" (orthotopic injection of cells) are usually applied [24]. Because lung cancer model was used here, we first examined whether over-expression of cofilin-1 could affect the migration of these cells to lungs via i.v. injection, and whether LNA would reverse the cofilin-1 effects as found in *in vitro* studies. The data showed that the *in vivo* study was consistent with *in vitro* effects of cofilin-1 over-expression. However, we could not detect the migration of HCOXP-3R cells out of lungs because the bioluminescent signals

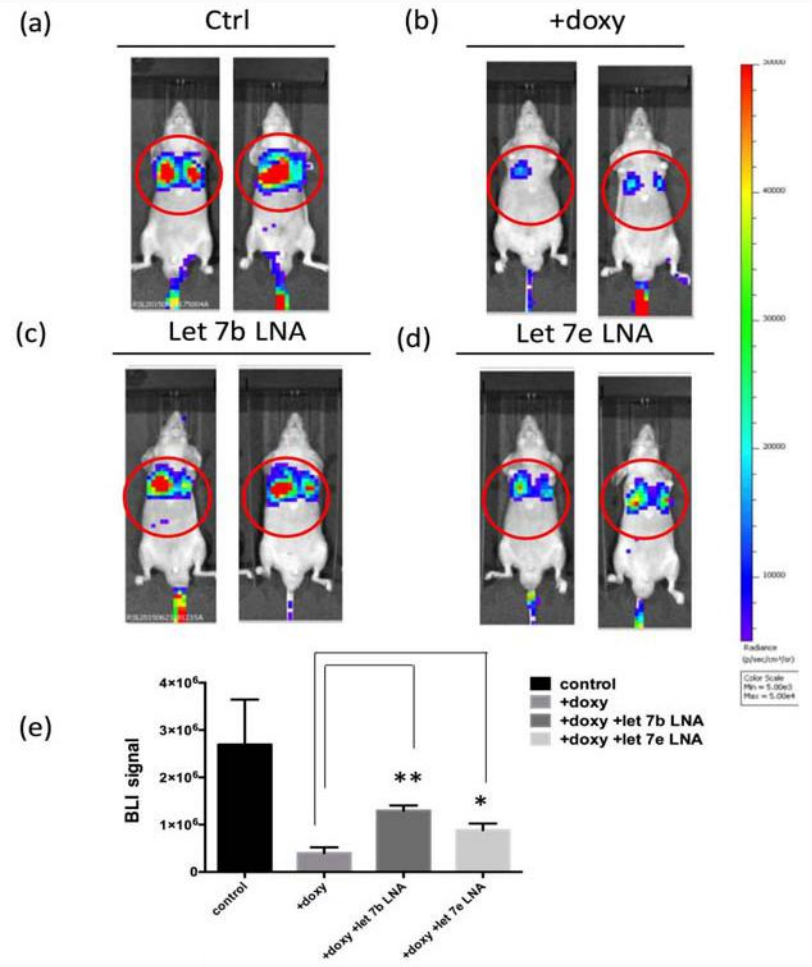

Figure 4: *In vivo* imaging of HCOXP-3R cells distribution through intravenous injection into nude mice. (a) Untreated HCOXP-3R cells. (b) Doxycycline treated HCOXP-3R cells. (c) *Let-7b* and (d) *let-7e* targeted LNA transfected HCOXP-3R cells that have been treated with doxycycline. (e) The regions of interest (ROI) of each group were marked by red circles and quantified for the BLI signals. *: $p < 0.05$. **: $p < 0.01$ (N=4).

were disappeared on next day (data not shown). This should not due to the loss of reporter genes because the expression of reporter genes in HCOXP-3R cells remains detectable after one month of continuous culturing. The current data suggest that over-expression of cofilin-1 may inhibit the migration of HCOXP-3R cells to lungs, but suppression of *let-7* in these cells will compromise this effect. Whether different levels of bioluminescent signals detected in lungs represents reduced extravasation of HCOXP -3R cells after over-expression of cofilin-1 is of interest to further investigate.

In summary, this is the first study showing that over-expressed cofilin-1 can inhibit the migration of lung cancer *in vivo*, to the best of our knowledge. We also demonstrate that cofilin-1 induced *let-7* is required for inhibition of migration because knockdown of *let-7* by LNA can reverse this effect. Although down-regulation of TWIST-1 has been previously reported to be required for cofilin-1 mediated up-regulation of *let-7*, SNAIL-1 transcription factor is also involved in this signaling pathway. However, it appears that EMT is not the primary role to be ablated by over-expressed cofilin-1. Taken together, the *in vivo* evidence of cofilin-1/*let-7* pathway on controlling the lung cancer migration would be important for clinical consideration of molecular targeting therapy.

Acknowledgement

This study was supported by a united grant of Cheng-Hsin General Hospital and National Yang-Ming University (103F003C05), and the Ministry of Science and Technology of Taiwan (102-2628-B-010-012-MY3).

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69-90.
2. Carlier MF, Laurent V, Santolini J, Melki R, Didry D, Xia GX, et al. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. J Cell Biol. 1997; 136: 1307-1322.
3. Mizuno K. Signaling mechanisms and functional roles of cofilin phosphorylation and dephosphorylation. Cell Signal. 2013; 25: 457-469.
4. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol. 2006; 172: 973-981.
5. Popper HH. Progression and metastasis of lung cancer. Cancer Metastasis Rev. 2016; 35: 75-91.
6. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014; 15: 178-196.
7. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009; 119: 1429-1437.
8. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion.

Cancer Metastasis Rev. 2009; 28: 15-33.

9. Wheelock MJ, Shintani Y, Maeda M, Fukumoto Y, Johnson KR. Cadherin switching. J Cell Sci. 2008; 121: 727-735.

10. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev. 2007; 21: 1025-1030.

11. Wang X, Cao L, Wang Y, Wang X, Liu N, You Y. Regulation of let-7 and its target oncogenes (Review). Oncol Lett. 2012; 3: 955-960.

12. Yang WH, Lan HY, Huang CH, Tai SK, Tzeng CH, Kao SY, et al. RAC1 activation mediates Twist1-induced cancer cell migration. Nat Cell Biol. 2012; 14: 366-374.

13. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res. 2004; 64: 3753-3756.

14. Tsai CH, Lin LT, Wang CY, Chiu YW, Chou YT, Chiu SJ, et al. Over-expression of cofilin-1 suppressed growth and invasion of cancer cells is associated with up-regulation of let-7 microRNA. Biochim Biophys Acta. 2015; 1852: 851-861.

15. Lee YJ, Sheu TJ, Keng PC. Enhancement of radiosensitivity in H1299 cancer cells by actin-associated protein cofilin. Biochem Biophys Res Commun. 2005; 335: 286-291.

16. Lin LT, Chiou SH, Lee TW, Liu RS, Hwang JJ, Chang CH, et al. A comparative study of primary and recurrent human glioblastoma multiforme using the small animal imaging and molecular expressive profiles. Mol Imaging Biol. 2013; 15: 262-272.

17. L. Asnaghi, W.C. Vass, R. Quadri, P.M. Day, X. Qian, R. Braverman, A.G. Papageorge, D.R. Lowy, E-cadherin negatively regulates neoplastic growth in non-small cell lung cancer: role of Rho GTPases, Oncogene, 29 (2010) 2760-2771.

18. Chang TC, Zeitels LR, Hwang HW, Chivukula RR, Wentzel EA, Dews M, et al. JT: Lin-28B transactivation is necessary for Myc-mediated let-7 repression and proliferation. Proc Natl Acad Sci U S A. 2009; 106: 3384-3389.

19. Wang T, Wang G, Hao D, Liu X, Wang D, Ning N, et al. Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors and its effects on the hallmarks of cancer. Mol Cancer. 2015; 14: 125.

20. Unternaehrer JJ, Zhao R, Kim K, Cesana M, Powers JT, Ratanasirintrawoot S, et al. The epithelial-mesenchymal transition factor SNAIL paradoxically enhances reprogramming. Stem Cell Reports. 2014; 3: 691-698.

21. Kjønniksen I, Storeng R, Pihl A, McLemore TL, Fodstad O. A human tumor lung metastasis model in athymic nude rats. Cancer Res. 1989; 49: 5148-5152.

22. Rashid OM, Nagahashi M, Ramachandran S, Dumur CI, Schaum JC, Yamada A, et al. Is tail vein injection a relevant breast cancer lung metastasis model? J Thorac Dis. 2013; 5: 385-392.

23. Shin JH, Chung JK, Kang JH, Lee YJ, Kim KI, So Y, et al. Noninvasive imaging for monitoring of viable cancer cells using a dual-imaging reporter gene. J Nucl Med .2004; 45: 2109-2115.

24. Price JE. Spontaneous experimental metastasis models: nude mice. Methods Mol Biol. 2014; 1070: 223-233.