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MicroRNA-101 down-regulates sphingosine kinase 1 in colorectal cancer cells

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ABSTRACT

MicroRNAs (miRs) dysregulation is a general feature of colorectal cancer (CRC) and other solid tumors, and is associated cancer progression. In the current study, we demonstrate that microRNA-101 (miR-101) inhibits CRC cells probably through down-regulating sphingosine kinase 1 (SphK1). Our results showed that exogenously expressing miR-101 inhibited CRC cell (HT-29 and HCT-116 lines) growth *in vitro*. At the molecular level, miR-101 dramatically down-regulated SphK1 mRNA and protein expression, causing pro-apoptotic ceramide production in above CRC cells. On the other hand, inhibition of miR-101 through expressing antagomiR-101 increased SphK1 expression to down-regulate ceramide level in HT-29 cells. miR-101 expression increased the *in vitro* anti-CRC activity of conventional chemo-agents: paclitaxel and doxorubicin. CRC cells with SphK1-shRNA knockdown showed similar phenotypes as the miR-101-expressed CRC cells, presenting with elevated level of ceramide and high sensitivity to paclitaxel or doxorubicin. *In vivo*, HCT-116 xenograft growth in severe combined immuno-deficient (SCID) mice was dramatically inhibited by over-expressing miR-101. Further, miR-101 enhanced paclitaxel-induced anti-HCT-116 activity *in vivo*. Together, these results indicate that miR-101 exerts its anti-CRC activities probably through down-regulating SphK1.

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1. Introduction

Colorectal cancer (CRC) causes severe cancer-related mortalities around the world [1–3]. Over the past decades, significant improvements have been achieved in surgery and/or chemotherapy for CRC. However, the overall survival (OS) of CRC has not been dramatically improved, especially for those patients with advanced or metastatic CRCs [1–4]. One reason is molecular heterogeneity of CRCs, which hinders the uniform application of specific molecularly-targeted agents [1–3]. Further, the application of conventional and novel cancer chemotherapy is limited in CRC cells exhibiting pre-existing and/or acquired resistances [5].

Sphingolipid metabolites have emerged as key players in a number of cancerous processes [6]. Among them, sphingosine-1-phosphate (S1P) is known to promote cell survival and proliferation, while ceramides and sphingosine could induce cell apoptosis [6]. Sphingosine kinase 1 (SphK1) catalyzes the phosphorylation of sphingosine to S1P, thus decreasing ceramide and sphingosine while increasing S1P [6,7]. *In vivo* and *in vitro* studies show that SphK1 regulates cancer cell survival, proliferation, transformation, as well as apoptosis prevention, chemo-resistance and angiogenesis [6,7]. SphK1 is often over-expressed/over-activated in CRC [8] and many other cancers [6,7]. On the other hand, inhibitors of SphK1 lead to tumor suppression [6,7].

MicroRNAs (miRs) are shown to regulate gene expression either at the translational or post-transcriptional levels [9,10]. These 19–24 nucleotide single-stranded noncoding RNAs could inhibit

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targeted mRNAs translation with partial complementarity in their 3′ untranslated regions (UTRs) [9,10]. miRs dysregulation has been recognized as a general feature of CRC [11,12], contributing to cancer progression and/or oncogenic activities [13]. Our previous study demonstrated that expression of microRNA-451 (miR-451) in HT-29 CRC cells in-activated AMP-activated protein kinase (AMPK) signaling, causing mTOR complex 1 (mTORC1) activation, and cell migration/proliferation [14]. In the current study, we found that miR-101-mediated inhibition of CRC cells is associated with SphK1 down-regulation.

2. Material and methods

2.1. Cell culture

Human CRC HT-29 cells and HCT-116 cells were maintained in DMEM/RMPI medium (Sigma, St. Louis, MO), supplemented with a 10% FBS (Sigma), Penicillin/Streptomycin (1:100, Sigma) and 4 mM L-glutamine (Sigma, St. Louis, MO), in a CO_2 incubator at 37 °C [14].

2.2. Chemicals and reagents

Paclitaxel and doxorubicin were purchased from Sigma (St. Louis, MO). All antibodies utilized in this study were obtained from Cell Signaling Technology (Beverly, MA).

2.3. Methyl thiazol tetrazolium (MTT) assay of cell viability

Cell viability was assessed using the MTT assay as described [14–16].

2.4. Colonies formation assay

CRC cells with or without miR transfection ($1*10^4$) were suspended in 1 ml of DMEM containing 0.25% agar (Sigma), 5% FBS. The cell suspension was then added on the top of a pre-solidified 100 mm culture dish. After 10 days of incubation, the number of colonies were fixed, stained and manually counted.

2.5. Histone-DNA enzyme-linked immunosorbent assay (ELISA) assay

Cell apoptosis was examined by Histone-DNA ELISA PLUS kit (Roche Applied Science, Shanghai, China) according to the manufacturer's protocol [14–16].

2.6. Cellular ceramide assay

The ceramide level was analyzed by the same method described in Ref. [17], and was valued as fmol by nmol of phospholipid (PL).

2.7. Western blots

were performed as previously described [14–16]. All blots in this study were subjected to different exposures: from 10 s to 10 min. Blot intensity was quantified by ImageJ software (NIH) after normalization to corresponding loading controls.

2.8. Constructs and transfection

MiR-101 precursor, with the sequence of:5′-TGCCCTGGCTC AGTTATCACAGTGCTGATGCTGTCTATTCTAAAGGTACAGTACTGTGA-TAACTGAAGGATGGCA-3' [18] was sub-cloned into pSuper-puro-GFP vector to generate miR-101 expression construct. AntagomiR-101 and antagomiR-control were purchased from Ambion (Shanghai, China). The human SphK1-shRNA-puromycin construct was purchased from Santa Cruz Biotech (Santa Cruz, CA). For transfection, cells were seeded in a 6-wells plate at 50% confluence. After 24 h, CRC cells were transfected using Lipofectamine 2000 transfection reagent (Invitrogen, USA) according to the manufacturer's instructions (0.15 μg construct per transfection). After 12 h of incubation at 37 °C, transfection medium was replaced with 2 ml of complete medium containing 2% FBS. Puromycin (1.0 μg/ml, Sigma) was added after transfection to select stable cells (6 days). Afterward, cells were lysed for analyzing miR-101 or SphK1.

2.9. RNA extraction and real-time PCR

Total RNA was prepared using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Real Time-PCR assay was performed. Briefly, 1 μl of diluted reverse transcription (RT) product was utilized as template for a 10 ml PCR. The PCR reaction mixture contains 1 × SYBR Master Mix (Applied Biosystem, Foster City, CA), 1 μg RNA and 200 nM primers. An ABI Prism 7500 Fast Real-Time PCR system (Foster City, CA, USA) was used for PCR reactions. The following primers were used: *GAPDH,* forward: 5′- TGC ACC ACC AAC TGC TTA-3′; reverse: 5′- GGA TGC AGG GAT GAT GTT C-3′ [19]. MiR-101: forward: 5′- CGG CGG TAC AGT ACT GTG ATA A-3′, reverse: 5′- CTG GTG TCG TGG AGT CGG CAA TTC-3' (Universal stem-loop primer) [18]. *SphK1*, forward, 5′-CGC CGC AGG GAA TGA CAC C-3′, and reverse, 5′-GCC TGT CCC CCC AAA GCA TAA C-3′ [20]. After amplification, melt curve analysis was performed to analyze product melting temperature. *GAPDH* gene was chosen as the reference gene for normalization, and the $2^{-\Delta\Delta Ct}$ method was applied to quantify targeted mRNA change within samples.

2.10. Tumor xenografts

As reported in our previous studies [21], male severe combined immuno-deficient (SCID) mice were implanted s.c. with HCT-116 cells ($2*10^6$ cells) with or without miR-101 transfection. When the xenografted tumors reached the average volume of 100 mm^3, the mice were treated with or without paclitaxel (1.5 mg/kg, i.v.), with 8 mice per group. Paclitaxel was administered *i.v.* once daily, for a total of 14 days. Tumor volume was recorded every 5 days, calculated using the following formula: $\pi/6 \times$ larger diameter $\times$ (smaller diameter)^2. Mice body weight was also recorded. After 30 days, HCT-116 xenografts were isolated through surgery, and expression of miR-101 and SphK1 was analyzed through the above methods. All studies were done in accordance with the standards of ethical treatment approved by the Institutional Animal Care and Use Committee (IACUC) and Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.11. Immunohistochemistry (IHC) staining

The staining was performed on cryostat sections (3 μm) of xenograft tissues according to standard methods. We incubated slides in the appropriate dilutions of primary antibody (anti-SphK1, 1:100) and subsequently stained them with horseradish peroxidase (HRP)-coupled secondary antibody (Santa Cruz). We visualized peroxidase activity using 3-amino-9-ethyl-carbazol (AEC) and counterstained tissues with MAYER'S hemalaun solution (Merck).

2.12. Statistical analysis

The values in the figures were expressed as the means ± standard deviation (SD). Statistical analysis was

performed by ANOVA (SPSS 16.0). Values of $P < 0.05$ were considered as statistically different.

3. Results

3.1. MiR-101 inhibits CRC cell growth

We first tested the potential role of miR-101 on CRC cell growth. Two well-established CRC cell lines, HT-29 and HCT-116, were utilized. MiR-101 was introduced into above CRC cells through transfection of the construct (see Material and Methods), and stable cells were selected through puromycin. As shown in Fig. 1A and B, the expression level of miR-101 was dramatically elevated in stable cells transfected with miR-101. In both cell lines, miR-101 over-expression dramatically inhibited cancer cell growth, tested by viable cell counting (Fig. 1C and D) and colonies formation assay (Fig. 1E and F). In both cell lines, the effect of miR-101 was significant, resulting in over 60% of growth inhibition (Fig. 1C and D). As expected, the empty vector (pSuper-puro) had almost no effect on growth of both lines (Fig. 1A–F). Thus, these results indicate that miR-101 inhibits CRC cell growth.

3.2. MiR-101 down-regulates SphK1 and increases intracellular ceramide level in CRC cells

SphK1 plays an important role in CRC progression [8], and is important for CRC cell survival and growth [8]. Next, we tested the effect of miR-101 on SphK1 expression in CRC cells. Results from the real-time PCR assay showed that miR-101 dramatically decreased SphK1 mRNA expression in both HT-29 cells and HCT-116 cells (Fig. 2A and B). Correspondingly, SphK1 protein was also depleted by miR-101 in CRC cells (Fig. 2C and D). As expected, SphK1 shRNA (shSphK1) also dramatically decreased SphK1 mRNA and protein expression in HT-29 cells and HCT-116 cells (Fig. 2A–D). One important consequence of SphK1 depletion is ceramide accumulation. Thus, we examined the intracellular ceramide level in above cells. Results showed that ceramide level was elevated in stable CRC (HT-29 or HCT-116) cells transfected with miR-101 or shSphK1 (Fig. 2E and F). The potential effect of antagomiR-101, the miR-101 inhibitor [22], was also tested in colon cancer cells. As shown in Fig. 2G, over-expression of antagomiR-101 downregulated endogenous miR-101 expression in HT-29 cells. As a consequence, mRNA and protein expression of SphK1 was increased (Fig. 2)H and I, and the ceramide level was decreased (Fig. 2J). Similar results were also observed in HCT-116 cells (Data not shown). These results indicate that miR-101 down-regulates SphK1 to facilitate ceramide accumulation in CRC cells.

3.3. MiR-101 expression increases the activity of paclitaxel and doxorubicin in vitro

Ceramide could significantly augment the anti-tumor activity of conventional chemotherapeutic agents [23,24]. For example, C6

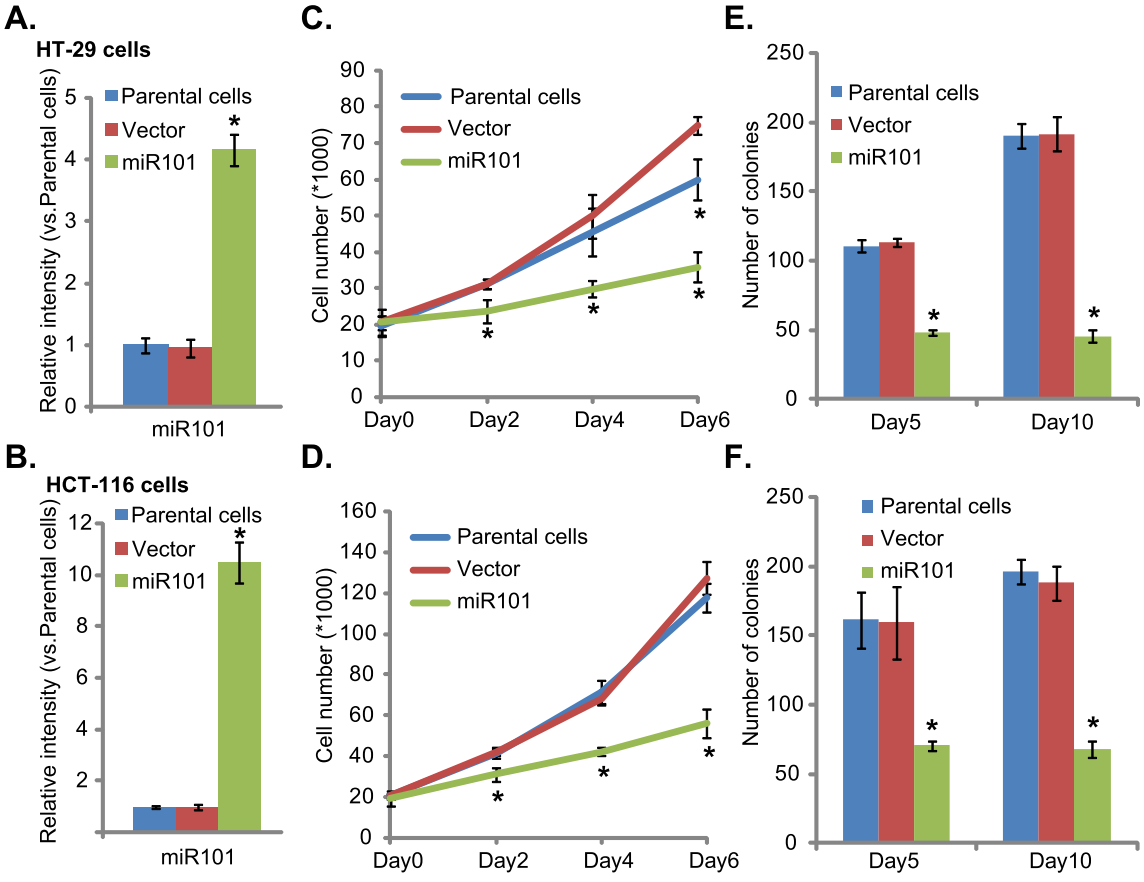

Fig. 1. ***MiR-101 inhibits CRC cell growth***-Stable HT-29 cells or HCT-116 cells, transfected with miR-101 construct or the empty vector (pSuper-puro, Vector), as well as their parental cells, were subjected to real-time PCR assay for analyzing miR-101 expression (**A** and **B**). Exactly same number (20,000) of above HT-29 cells or HCT-116 cells were cultured in growth medium (with 10% FBS) for indicated time, cell number was counted every 2 days (**C** and **D**). Above cells were also subjected to colonies formation assay, and number of colonies at day 5 and day 10 was counted manually (**E** and **F**). All experiments in this figure were repeated five times, and similar results were always obtained. *$P < 0.05$ vs. parental cells.

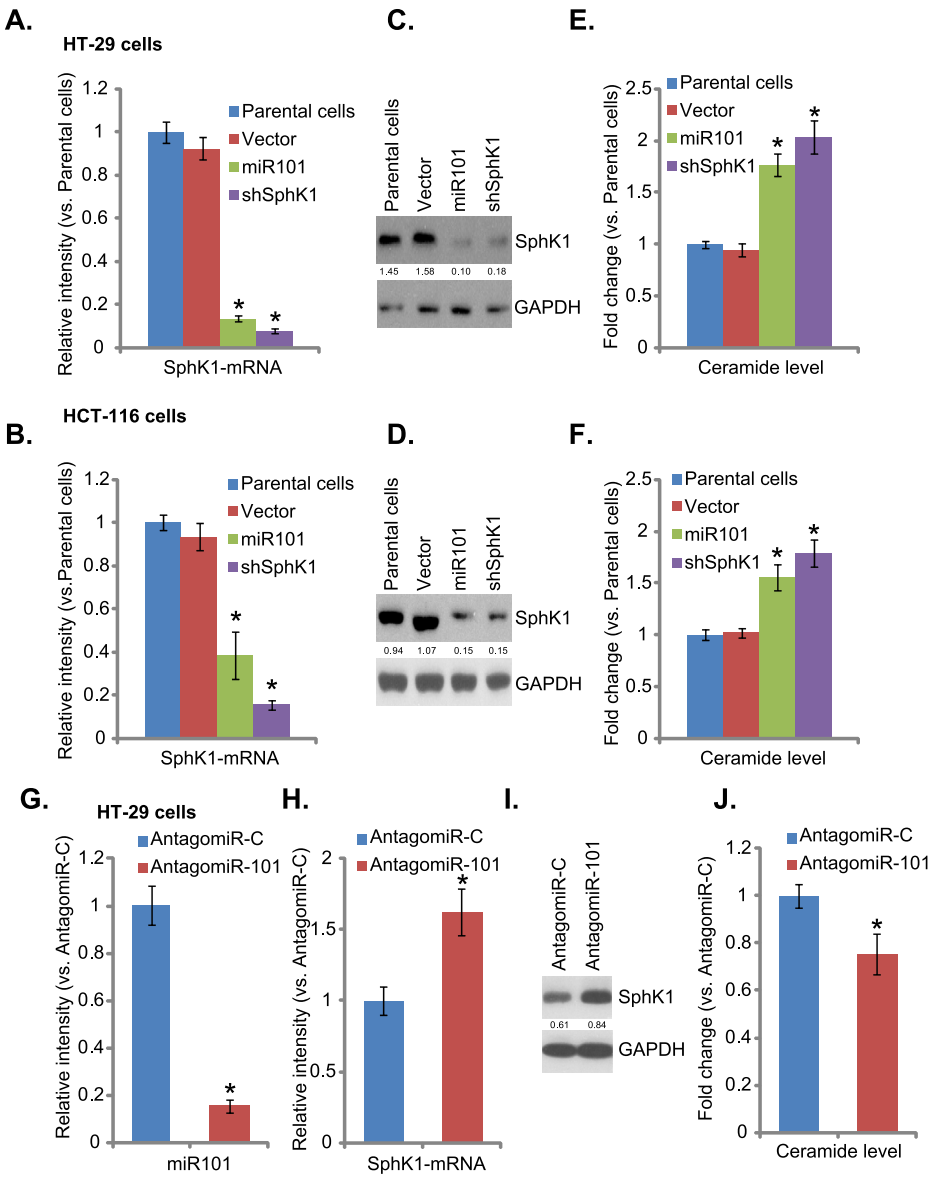

Fig. 2. ***MiR-101 down-regulates SphK1 and increases intracellular ceramide level in CRC cells*****-**Stable HT-29 cells or HCT-116 cells transfected with miR-101 construct, SphK1-shRNA construct (shSphK1), or the empty vector (pSuper-puro, Vector), as well as their parental cells, were subjected to real-time PCR assay (**A**–**B**) or Western blot assay (**C**–**D**) to test mRNA or protein expression of SphK1. For both assay, GAPDH was utilized as an internal control (**A**–**D**). The intracellular ceramide level in above cells was also analyzed as described (**E** and **F**). Levels of miR-101 (**G**), SphK1 mRNA (**H**), SphK1 protein (**I**) and ceramide (**J**) in HT-29 cells expressing antagomiR-101 or antagomiR-control (antagomiR-C) were shown. GAPDH was utilized as an internal control (**G**–**I**). Quantitative results were normalized to the value of parental cells (for **A-F**), or the antagomiR-C expressing cells (For **G-J**). All panels in figure were repeated three times, and similar results were always obtained. $^{*}P < 0.05$ vs. parental cells (for **A-F**), or antagomiR-C expressing cells (For **G-J**).

ceramide, a short chain cell permeable ceramide, dramatically enhanced the activity of multiple anti-cancer drugs, including paclitaxel [25], doxorubicin [26] and histone deacetylase inhibitors (HDACi) [27]. Thus, we tested the effect of miR-101 on paclitaxel- or doxorubicin-induced activity in human CRC cells. Results showed that miR-101-transfected stable HT-29 cells were significantly more sensitive to paclitaxel or doxorubicin than the parental cells (Fig. 3A and B). Paclitaxel or doxorubicin induced substantial viability reduction (Fig. 3A, tested by MTT assay) and apoptosis (Fig. 3B, tested by Histone-DNA ELISA assay) in stable HT-29 cells with miR-101 over-expression. Further, stable HT-29 cells with shSphK1 showed similar phenotypes as miR-101-expressed cells, and were hyper-sensitive to paclitaxel or doxorubicin (Fig. 3A and B). Similar results were also observed in HCT-116 cells (Data not shown). Together, these results indicate that miR-101 down-regulates SphK1 to increase the sensitivity of conventional chemo-drugs (paclitaxel or doxorubicin) *in vitro*.

3.4. MiR-101 inhibits HCT-116 growth and increases paclitaxel activity in vivo

At last, we tested the role of miR-101 *in vivo*. Mice HCT-116 xenograft model was applied [21]. Tumor growth curve results in Fig. 4A showed that *in vivo* growth of stable HCT-116 cells transfected with miR-101 was slower than the parental HCT-116 cells ($P < 0.05$). More importantly, paclitaxel-induced anti-HCT-116 activity *in vivo* was dramatically enhanced by miR-101 over-expression (Fig. 4A). Note that no significant mice body weight change

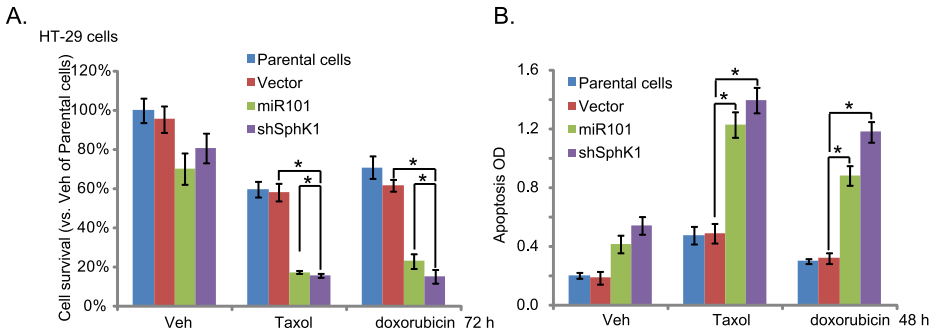

Fig. 3. ***MiR-101 expression increases the activity of paclitaxel and doxorubicin in cultured CRC cells***-Same number of stable HT-29 cells transfected with miR-101 construct, SphK1-shRNA construct (shSphK1), or the empty vector (pSuper-puro, Vector), as well as their parental cells were stimulated with paclitaxel (Taxol, 0.5 μg/ml), doxorubicin (0.25 μg/ml) or vehicle control (Veh, 0.1% DMSO), cell viability was analyzed by MTT assay (**A**, 72 h), and cell apoptosis was tested by Histone-DNA apoptosis ELISA plus assay (**B**, 48 h). All experiments in this figure were repeated five times, and similar results were always obtained. $^{*}P < 0.05$ vs. parental cells.

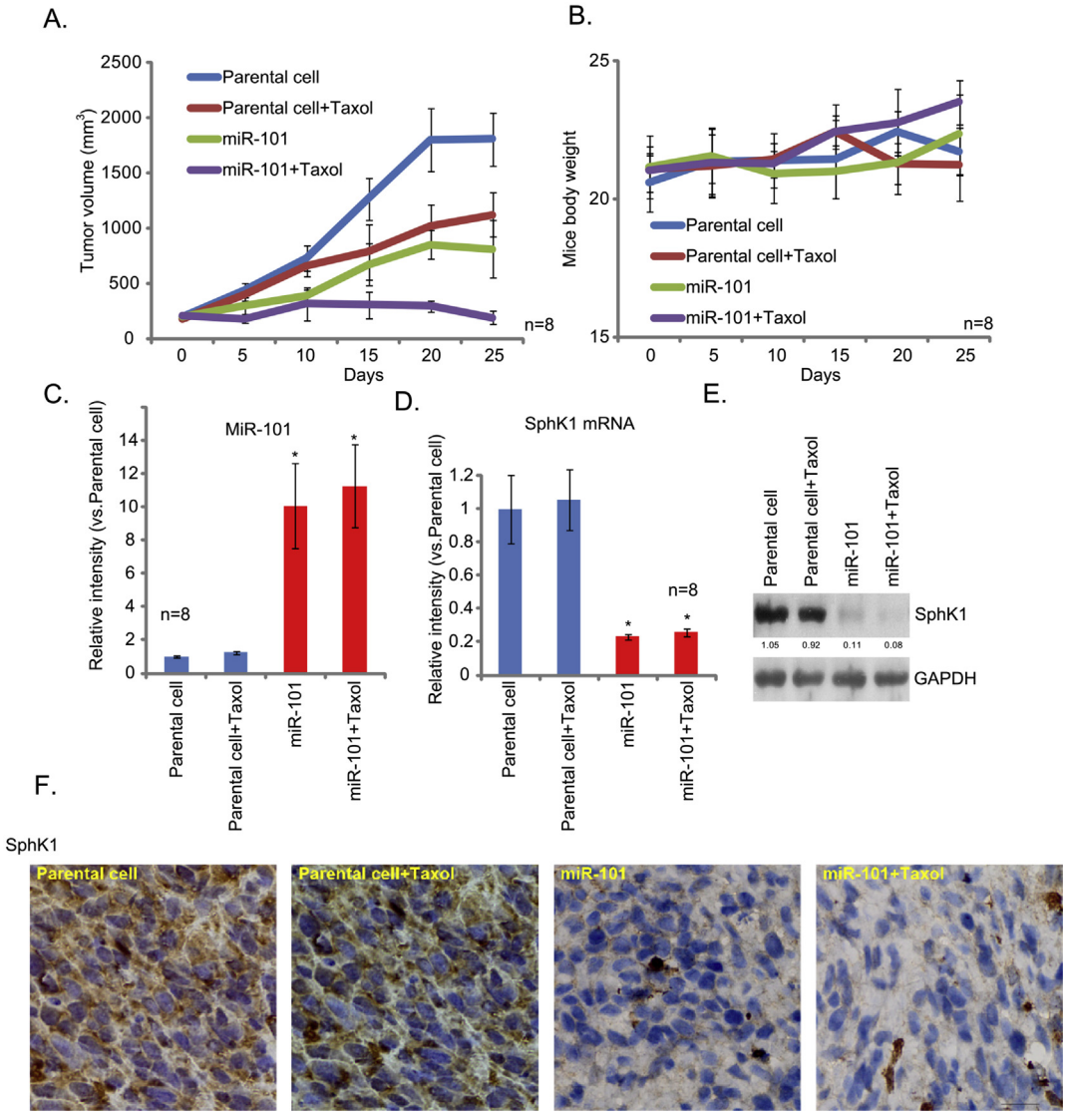

Fig. 4. ***MiR-101 inhibits HCT-116 growth and increases paclitaxel activity in vivo***-The growth curve of stable miR-101-expressed HCT-116 cells or control parental HCT-116 cells in SCID mice administrated with or without Paclitaxel (Taxol, 1.5 mg/kg, *i.v.*, daily, 14 days) was shown (**A**, every 5 days), mice body weight was also recorded (**B**, every 5 days). At day 25, HCT-116 xenografts were isolated through surgery, and expressions of miR-101 and SphK1 mRNA in fresh tumor xenografts were tested by real-time PCR (**C** and **D**), and SphK1 protein expression was tested by Western blots (**E**) or IHC staining (**F**). *In vivo* experiments were repeated twice, and similar results were obtained. Bar = 20 μm (**F**) $^{*}p < 0.05$ vs. parental cells.

was observed between groups over the experiment duration (Fig. 4B), nor apparent toxicity was noticed. Results in Fig. 4C confirmed miR-101 over-expression in corresponding xenografts. Accordingly, the SphK1 mRNA and protein expressions were reduced in above xenografts (Fig. 4D and E). IHC images in Fig. 4F further confirmed SphK1 downregulation by miR-101 in isolated tumors. Note that paclitaxel by itself had no effect on miR-101 or SphK1 expression (Fig. 4C–F). Together, these *in vivo* studies further confirm the effect of miR-101 on SphK1 expression and paclitaxel-induced activity against CRC cells.

4. Discussions

Studies from different groups have showed the important roles of SphK1 in promoting cancer progression, including proliferation, transformation, as well as metastasis, chemo-resistance and angiogenesis [6,7]. Meanwhile, it has been shown that SphK1 is over-expressed in CRCs, which is associated with poor prognosis [8,28]. SphK1 inhibitors, alone or in combination with conventional anti-cancer agents, were tested in several CRC models both *in vivo* and *in vitro*, and showed promising results [8,28]. MiR-101, on the other hand, was found to be downregulated in CRCs [29]. Based on these information and the results of this study, we speculate that over-expression of SphK1 in human CRCs could be a consequence of miR-101 downregulation, and miR-101-mediated anti-CRC activity could be due to its effect on SphK1.

MiR-101 has been shown to act as an important tumor suppressor in various human cancers including prostate cancers, liver cancers and CRCs. It level is often downregulated in various cancers. Several pro-cancer proteins ("oncogenes") have been identified thus far as miR-101-targeted genes [18,29–31]. For example, Chiang et al., show that miR-101 decreases expression of enhancer of zeste homolog 2 (EZH2) and EED, two subunits of PRC2 complex, in human HepG2 hepatoma cells [18]. Further, genomic loss of miR-101 leads to overexpression of histone methyltransferase EZH2 in several human cancers [32]. In CRCs, miR-101 downregulation promotes Wnt/β-catenin signaling pathway activation and cancer malignancy [33].

In the current study, we provided evidence to support that SphK1 could be an important target protein of miR-101. We found that exogenously expressing miR-101 dramatically down-regulated SphK1 mRNA and protein expression in CRC cells. Further, ceramide level in miR-101-expressed cells was increased. On the other hand, inhibition of miR-101 through antagomiR-101 increased SphK1 expression, but reduced ceramide accumulation in HT-29 cells. Importantly, CRC cells with SphK1 shRNA knockdown showed similar phenotypes as the miR-101-expressed CRC cells. Both manipulations dramatically increased the chemo-sensitivity of paclitaxel or doxorubicin against CRC cells. In summary, the results of this study showed that miR-101 exerts its anti-CRC activities probably through down-regulating SphK1, and reduced miR-101 observed in CRCs might be an important cause of chemo-resistance and other cancerous behaviors.

Conflict of interests

No conflict of interests were stated.

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References

[1] H.J. Schmoll, A. Stein, Colorectal cancer in 2013: towards improved drugs, combinations and patient selection, Nat. Rev. Clin. Oncol. 11 (2014) 79–80.
[2] E.J. Kuipers, T. Rosch, M. Bretthauer, Colorectal cancer screening–optimizing current strategies and new directions, Nat. Rev. Clin. Oncol. 10 (2013) 130–142.
[3] A. Brouquet, B. Nordlinger, Metastatic colorectal cancer outcome and fatty liver disease, Nat. Rev. Gastroenterol. Hepatol. 10 (2013) 266–267.
[4] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, CA Cancer J. Clin. 64 (2014) (2014) 9–29.
[5] M. Liscovitch, Y. Lavie, Cancer multidrug resistance: a review of recent drug discovery research, IDrugs 5 (2002) 349–355.
[6] D. Shida, K. Takabe, D. Kapitonov, S. Milstien, S. Spiegel, Targeting SphK1 as a new strategy against cancer, Curr. Drug Targets 9 (2008) 662–673.
[7] M. Vadas, P. Xia, G. McCaughan, J. Gamble, The role of sphingosine kinase 1 in cancer: oncogene or non-oncogene addiction? Biochim. Biophys. Acta 1781 (2008) 442–447.
[8] T. Kawamori, T. Kaneshiro, M. Okumura, S. Maalouf, A. Uflacker, J. Bielawski, Y.A. Hannun, L.M. Obeid, Role for sphingosine kinase 1 in colon carcinogenesis, FASEB J. 23 (2009) 405–414.
[9] H. Du, L. Guo, F. Fang, D. Chen, A.A. Sosunov, G.M. McKhann, Y. Yan, C. Wang, H. Zhang, J.D. Molkentin, F.J. Gunn-Moore, J.P. Vonsattel, O. Arancio, J.X. Chen, S.D. Yan, Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease, Nat. Med. 14 (2008) 1097–1105.
[10] A.L. Huang, M.C. Ostrowski, D. Berard, G.L. Hager, Glucocorticoid regulation of the Ha-MuSV p21 gene conferred by sequences from mouse mammary tumor virus, Cell 27 (1981) 245–255.
[11] S. Muhammad, K. Kaur, R. Huang, Q. Zhang, P. Kaur, H.O. Yazdani, M.U. Bilal, J. Zheng, L. Zheng, X.S. Wang, MicroRNAs in colorectal cancer: Role in metastasis and clinical perspectives, World J. Gastroenterol. 20 (2014) 17011–17019.
[12] A.V. Orang, A. Barzegari, MicroRNAs in colorectal cancer: from diagnosis to targeted therapy, Asian Pac J. Cancer Prev. 15 (2014) 6989–6999.
[13] S. Wach, E. Nolte, A. Theil, C. Stohr, T.R. T, A. Hartmann, A. Ekici, B. Keck, H. Taubert, B. Wullich, MicroRNA profiles classify papillary renal cell carcinoma subtypes, Br. J. Cancer 109 (2013) 714–722.
[14] M.B. Chen, M.X. Wei, J.Y. Han, X.Y. Wu, C. Li, J. Wang, W. Shen, P.H. Lu, MicroRNA-451 regulates AMPK/mTORC1 signaling and fascin1 expression in HT-29 colorectal cancer, Cell. Signal 26 (2014) 102–109.
[15] M.B. Chen, W.X. Shen, Y. Yang, X.Y. Wu, J.H. Gu, P.H. Lu, Activation of AMP-activated protein kinase is involved in vincristine-induced cell apoptosis in B16 melanoma cell, J. Cell. Physiol. 226 (2010) 1915–1925.
[16] M.B. Chen, Y. Zhang, M.X. Wei, W. Shen, X.Y. Wu, C. Yao, P.H. Lu, Activation of AMP-activated protein kinase (AMPK) mediates plumbagin-induced apoptosis and growth inhibition in cultured human colon cancer cells, Cell. Signal 25 (2013) 1993–2002.
[17] L. Gong, B. Yang, M. Xu, B. Cheng, X. Tang, P. Zheng, Y. Jing, G.J. Wu, Bortezomib-induced apoptosis in cultured pancreatic cancer cells is associated with ceramide production, Cancer Chemother. Pharmacol. 73 (2014) 69–77.
[18] C.W. Chiang, Y. Huang, K.W. Leong, L.C. Chen, H.C. Chen, S.J. Chen, C.K. Chou, PKCalpha mediated induction of miR-101 in human hepatoma HepG2 cells, J. Biomed. Sci. 17 (2010) 35.
[19] M.J. Dehoux, R.P. van Beneden, L. Fernandez-Celemin, P.L. Lause, J.P. Thissen, Induction of MafBx and Murf ubiquitin ligase mRNAs in rat skeletal muscle after LPS injection, FEBS Lett. 544 (2003) 214–217.
[20] M. Yamanaka, D. Shegogue, H. Pei, S. Bu, A. Bielawska, J. Bielawski, B. Pettus, Y.A. Hannun, L. Obeid, M. Trojanowska, Sphingosine kinase 1 (SPHK1) is induced by transforming growth factor-beta and mediates TIMP-1 up-regulation, J. Biol. Chem. 279 (2004) 53994–54001.
[21] M.B. Chen, X.Y. Wu, G.Q. Tao, C.Y. Liu, J. Chen, L.Q. Wang, P.H. Lu, Perifosine sensitizes curcumin-induced anti-colorectal cancer effects by targeting multiple signaling pathways both in vivo and in vitro, Int. J. Cancer 131 (2012) 2487–2498.
[22] Z. Shen, L. Chen, X. Yang, Y. Zhao, E. Pier, X. Zhang, Y. Xiong, Downregulation of Ezh2 methyltransferase by FOXP3: new insight of FOXP3 into chromatin remodeling? Biochim. Biophys. Acta 1833 (2013) 2190–2200.
[23] C.F. Lin, C.L. Chen, Y.S. Lin, Ceramide in apoptotic signaling and anticancer therapy, Curr. Med. Chem. 13 (2006) 1609–1616.
[24] M.T. Dimanche-Boitrel, A. Rebillard, E. Gulbins, Ceramide in chemotherapy of tumors, Recent Pat. Anticancer Drug Discov. 6 (2011) 284–293.
[25] C.H. Wu, C. Cao, J.H. Kim, C.H. Hsu, H.J. Wanebo, W.D. Bowen, J. Xu, J. Marshall, Trojan-horse nanotube on-command intracellular drug delivery, Nano Lett. 12 (2012) 5475–5480.
[26] C. Ji, B. Yang, Y.L. Yang, S.H. He, D.S. Miao, L. He, Z.G. Bi, Exogenous cell-permeable C6 ceramide sensitizes multiple cancer cell lines to Doxorubicin-

induced apoptosis by promoting AMPK activation and mTORC1 inhibition, Oncogene 29 (2010) 6557–6568.
[27] Q.Y. Zhu, Z. Wang, C. Ji, L. Cheng, Y.L. Yang, J. Ren, Y.H. Jin, Q.J. Wang, X.J. Gu, Z.G. Bi, G. Hu, Y. Yang, C6-ceramide synergistically potentiates the anti-tumor effects of histone deacetylase inhibitors via AKT dephosphorylation and alpha-tubulin hyperacetylation both in vitro and in vivo, Cell. Death Dis. 2 (2011) e117.
[28] T. Kawamori, W. Osta, K.R. Johnson, B.J. Pettus, J. Bielawski, T. Tanaka, M.J. Wargovich, B.S. Reddy, Y.A. Hannun, L.M. Obeid, D. Zhou, Sphingosine kinase 1 is up-regulated in colon carcinogenesis, FASEB J. 20 (2006) 386–388.
[29] A. Strillacci, C. Griffoni, P. Sansone, P. Paterini, G. Piazzi, G. Lazzarini, E. Spisni, M.A. Pantaleo, G. Biasco, V. Tomasi, MiR-101 downregulation is involved in cyclooxygenase-2 overexpression in human colon cancer cells, Exp. Cell. Res. 315 (2009) 1439–1447.
[30] L.B. Frankel, J. Wen, M. Lees, M. Hoyer-Hansen, T. Farkas, A. Krogh, M. Jaattela, A.H. Lund, microRNA-101 is a potent inhibitor of autophagy, EMBO J. 30 (2011) 4628–4641.
[31] L.L. Fu, X. Wen, J.K. Bao, B. Liu, MicroRNA-modulated autophagic signaling networks in cancer, Int. J. Biochem. Cell. Biol. 44 (2012) 733–736.
[32] S. Varambally, Q. Cao, R.S. Mani, S. Shankar, X. Wang, B. Ateeq, B. Laxman, X. Cao, X. Jing, K. Ramnarayanan, J.C. Brenner, J. Yu, J.H. Kim, B. Han, P. Tan, C. Kumar-Sinha, R.J. Lonigro, N. Palanisamy, C.A. Maher, A.M. Chinnaiyan, Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer, Science 322 (2008) 1695–1699.
[33] A. Strillacci, M.C. Valerii, P. Sansone, C. Caggiano, A. Sgromo, L. Vittori, M. Fiorentino, G. Poggioli, F. Rizzello, M. Campieri, E. Spisni, Loss of miR-101 expression promotes Wnt/beta-catenin signalling pathway activation and malignancy in colon cancer cells, J. Pathol. 229 (2013) 379–389.