LNMICC promotes nodal metastasis of cervical cancer by reprogramming fatty acid metabolism

Chunliang Shang¹, Wei Wang¹, Yuandong Liao¹, Yili Chen¹, Tianyu Liu¹, Qiqiao Du¹, Jiaming Huang¹, Yanchun Liang¹, Junxiu Liu¹, Yunhe Zhao¹, Luyan Guo¹, Zheng Hu¹*, and Shuzhong Yao¹*

¹ Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University, Zhongshan Second Road 58, Guangzhou 510080, People’s Republic of China.

* Correspondence: Shuzhong Yao, Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University, Zhongshan Second Road 58, Guangzhou 510080, People’s Republic of China. Email: yszlfy@163.com or Zheng Hu, Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University, Zhongshan Second Road 58, Guangzhou 510080, People’s Republic of China. Email: huzheng1998@163.com

Running Title: LNMICC promotes lymph node metastasis in cervical cancer.

Key words: lymph node metastasis; fatty acid metabolism; long non-coding RNA; cervical cancer.

Conflict of Interest: The authors declare no potential conflicts of interest.
Abstract

Cancer spread to lymph nodes (LN) predicts poor survival but underlying mechanisms remain little understood. In this study, we show that overexpression of the long non-coding RNA LNMICC associates with LN metastasis of primary cervical cancer, where it serves as an independent high-risk factor in patient survival. Functional investigations demonstrated that LNMICC promoted LN metastasis by reprogramming fatty acid metabolism, by recruiting the nuclear factor NPM1 to the promoter of the fatty acid binding protein FABP5. We also found that the pro-metastatic effects of LNMICC were directly targeted and suppressed by miR-190. Our results establish a new mechanism of LN metastasis and highlight LNMICC as a candidate prognostic biomarker and therapeutic target in cervical cancer.
Introduction

Cervical cancer is the second most common cancer among females worldwide, with approximately 527,600 new cases and 265,700 deaths in 2012 [1]. Pelvic lymph node (LN) metastasis is a critical independent prognostic factor and one of the leading causes of cervical cancer death [2]. However, there is still no effective and reliable means to prevent or control LN metastasis. It has been well recognized that the spread of cervical cancer cells to lymph nodes is not a simple physical or mechanical process but a multi-step and multi-factor complex process, and complex biological mechanisms participate in this event [3]. Thus, this fact underscores the urgency and importance of understanding the molecular mechanisms of LN metastasis.

In LN metastasis, lymphangiogenesis is an important initial step and essential event [4]. In the meantime, cervical cancer cells acquire the driving force to erode the extracellular matrix and the motility to extravasate into the newly developed lymphatic vessels through the activation of epithelial to mesenchymal transition (EMT), which is a biological process enabling polarized epithelial cell changes to a mesenchymal-like phenotype [5]. The crosstalk between lymphatic endothelial cells (LEC) and cervical cancer cells is a key process in metastasis progression. However, it is currently unclear how to effectively prevent the formation of lymphangiogenesis and inhibit the occurrence of EMT in cervical cancer cells.

The reprogramming of cellular energy metabolism, which support the unrestricted proliferation and metastatic progression of cancer cells, is widely accepted to be an emerging hallmark of cancer [6]. The Warburg effect, which is an increased uptake of glucose and the switch to aerobic glycolysis, is the most commonly observed metabolic phenotype in cancer cells [7]. However, it is evidently clear that the molecular mechanisms involved in carcinogenesis are not the same as those promoting cancer cell metastasis. Growing evidence indicates that the Warburg effect may contribute to the early stages of cancer progression. Instead, the reprogramming of fatty acid (FA) metabolism in cancer cells may provide a selective advantage toward the metastatic process [8, 9]. FAs are the major building blocks for energy metabolism and represent the essential components of the signal transduction network of biological membranes. De novo fatty-acid synthesis is one of the special metabolic phenotypes prevalent in cancer cells [10]. Fatty acid-binding proteins (FABPs), as indispensable carriers of FA uptake and transport, are proved to be critical central regulators of FA metabolism and inflammatory pathways [11]. FABP5 is a small (15 kDa) member of the cytoplasmic FABP family and exhibits high affinity binding of long-chain FAs. There is now convincing evidence that FABP5 plays a crucial role in cancer progression, invasion and metastasis through the induction of EMT and the regulation of angiogenic responses [12, 13]. However, it remains largely unknown how cervical cancer cells cope with the reprogramming of FA metabolism, thus resulting in LN metastasis.

Long non-coding RNAs (lncRNAs) are a novel class of transcripts longer than 200 nucleotides without protein-coding potential. LncRNAs, which are emerging as pivotal regulation factors of cell biological behavior, can alter tumor growth kinetics,
promote lymphangiogenesis and distant metastasis of cancer by interacting with RNA, DNA, or proteins [14, 15]. In recent years, the expression of certain lncRNAs has been frequently reported to be functionally important for cervical cancer, including lincRNA-p21 [16], HOXA11-AS [17], HOTAIR [18] and ANRIL [19]. In our previous study, we identified a lncRNA termed LNMICC (lncRNA associated with LN metastasis in cervical cancer) (GenBank: HG501394.1, ENST00000518880.1) that is upregulated in cervical cancer tissues with LN metastasis compared to its expression in those without LN metastasis [20]. However, to date, the exact mechanisms through which lncRNAs regulate LN metastasis in cervical cancer remain elusive.

Current evidence demonstrates that lncRNAs play both pro- and anti-metastatic roles via the regulation of EMT and angiogenesis as well as binding to certain metastatic factors [21]. In addition, lncRNAs, as key transcriptional regulators of central metabolic pathways, can provide proliferating advantages through significantly enhancing the biosynthesis of FA [22]. This strong evidence led us to speculate whether LNMICC promotes LN metastasis in cervical cancer through the FABP5-mediated reprogramming of FA metabolism.

In the present study, we first demonstrated that LNMICC was a valuable prognostic predictor of cervical cancer. More importantly, we found that LNMICC targeted the FABP5 promoter region through directly interaction with NPM1, thus mediating fatty acid metabolism reprogramming and finally promoting the process of lymphangiogenesis and EMT in cervical cancer. Meanwhile, our data also indicated that the function of LNMICC could be suppressed by miR190 via directly targeting the miRNA-binding site in cervical cancer cells. Taken together, our findings provide novel insights into the mechanism of LN metastasis in cervical cancer.

Methods

Clinical specimens

For in situ hybridization (ISH), a total of 211 paraffin-embedded tissues of cervical cancer collected from January 2005 to December 2010 were obtained from the archives of the pathology department at the First Affiliated Hospital of Sun Yat-sen University. For total RNA isolation, another 40 patients from Jun 2014 to March 2016 were recruited. Based on their postoperative pathological examinations, 40 cases were respectively assigned into two groups: pelvic LN metastasis group and non-pelvic LN metastasis group. The specimens were immediately frozen in liquid nitrogen and stored at -80°C until later RNA extraction. All enrolled cervical cancer patients were matched from stage Ia2 to IIa2 with available follow-up data and underwent radical hysterectomy and lymphadenectomy. None of the patients were treated with radiotherapy or chemotherapy before surgery. Consent was informed and written consent forms were obtained from each patient. 20 normal uterine cervical tissues as controls were collected from women who underwent hysterectomy for non-malignant conditions. The study was approved by the Institutional Review Board of First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). Patient studies were conducted in accordance with ethical guideline: Declaration of Helsinki.
Cell culture

The human cervical cancer lines SiHa, CaSki, ME180, MS751, HeLa and HeLa229 were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured according to their guidelines in a humid atmosphere with 5% CO\textsubscript{2} at 37°C. Human lymphatic endothelial cells (HLECs) were obtained from ScienCell Research Laboratories (USA) and maintained in the recommended Endothelial Cell Medium (ECM; ScienCell, San Diego, CA, USA). The cell lines were tested for authenticity in 2015 using short tandem repeat (STR) genotyping and screened for mycoplasma contamination (e-Mycoplasm PCR Detection Kit; iNtRON). Primary normal cervical epithelial cells were isolated from 10 above-mentioned normal uterine cervical specimens. The detailed procedure is provided in the Supplementary Methods.

Assessment of protein-coding potential

A combination of an in vitro translation assay and a protein-coding potential assessment tool (http://lilab.research.bcm.edu/cpat/index.php) was used to evaluate the protein-coding potential of transcripts.

In situ hybridization (ISH), quantitative real-time reverse transcription PCR (qRT-PCR), gel electrophoresis, Western blot and immunohistochemistry (IHC)

LNMICC expression was detected in paraffin-embedded tissues by ISH. The ISH probe used for detecting LNMICC-labeled digoxin was designed as 5‘DigN-AATCCTCTAGCTCAACTCAGGT-3‘DigN and synthesized by Takara Biotech Co. (Dalian, China). The ISH procedure used is described in the Supplementary Methods. RNA extraction, complementary DNA synthesis, and qRT-PCR reactions were performed as described in the Supplementary Methods. Gel electrophoresis, Western blot and IHC staining were performed as described in the Supplementary Methods. The primer sequences used in this study are listed in Supplementary Table 1. Antibodies and working concentrations are presented in Supplementary Table 2.

Plasmid, lentivirus construction and cell transfection

Detailed descriptions of plasmid, siRNA and shRNA lentiviral vector construction and cell transfection can be found in the Supplementary Methods and in Supplementary Table 1.

Cell migration and invasion assays, endothelial cell tube formation assay, cell proliferation assay and flow cytometry assays of cell apoptosis

Wound-healing and transwell invasion assays were performed to evaluate cell migration and invasion ability, respectively, as described in the Supplementary Methods. Lymphangiogenesis was assessed by an endothelial cell tube formation assay. Cell proliferation and cell apoptosis were analyzed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) assay and flow cytometry assay, respectively, according to the protocol described in the
Supplementary Methods.

Quantification of neutral lipids, triglycerides and phospholipids
The lipophilic fluorescence dye BODIPY 493/503 (Invitrogen) was employed for monitoring the neutral lipid accumulation in cervical cancer cells as described in the Supplementary Methods. For quantitative estimation of triglycerides (TAGs) or phospholipids (PLs), the EnzyChromTM Triglyceride Assay Kits (BioAssay Systems, Hayward, CA, USA) and EnzyChromTM Phospholipid Assay Kits (BioAssay Systems, Hayward, CA, USA) were used according to the protocols.

Isolation of cytoplasmic and nuclear RNA
Cytoplasmic and nuclear RNA isolation was performed using the PARIS™ Kit (Invitrogen) according to the manufacturer's instructions. RNA extracted from each of the fractions was analyzed by qRT-PCR to determine the levels of nuclear control transcript (HOTAIR, U6), cytoplasmic control transcript (GAPDH, β-actin), and LNMICC.

Truncating mutation and dual luciferase reporter assay
The FABP5 promoter was truncated by 100 bp and cloned into a pGL3-basic vector and sequenced. The luciferase activity was measured using the Dual-Luciferase assay system (Promega). The detailed procedure is described in the Supplementary Methods.

RNA pull-down assay, mass spectrometry and RNA immunoprecipitation (RIP)
Detailed descriptions of the RNA pull-down assay, mass spectrometry and RIP procedure can be found in the Supplementary Methods.

Chromatin immunoprecipitation (ChIP) assay
Chromatin fragments were immunoprecipitated with an anti-NPM1 antibody (10306-1-AP, Proteintech). The chromatin acquired prior to immunoprecipitation was amplified as the input. Normal human immunoglobulin G (IgG) was used as a negative control, and the Anti-Histone H3 antibody was used as a positive control. The qRT-PCR was performed using SYBRVR Premix Ex TaqTM (Takara, Dalian, China). The primer sequences are listed in Supplementary Table 1.

In vivo tumor growth and metastasis assays and hematoxylin-eosin (HE) staining
Nude mice were maintained under SPF conditions in the Department of Sun Yat-sen University Animal Centered according to the institution's guidelines. All animal studies were approved by the Animal Ethical and Welfare Committee of Sun Yat-sen University. The detailed procedures used to establish the subcutaneous xenograft tumor model and the xenograft mice metastatic model and those used for the HE staining are described in the Supplementary Methods.

Statistical analyses
All statistical analyses were performed using SPSS software package (version 13.0, SPSS, Inc.) and Prism5.0 software (GraphPad, La Jolla, CA, USA). The data are presented as the mean±S.D. of at least three independent experiments. The independent sample t-test was used for comparing groups for statistical differences. The Kaplan-Meier method was used for disease-free survival (DFS) and overall survival analysis, and significance was determined by log-rank test. A multivariate Cox proportional hazard model analysis was performed to identify independent prognostic factors of cervical cancer. Multivariate logistic regression was analyzed to identify the independent risks related to LN metastasis of cervical cancer. The relationships between LNMICC expression level and clinicopathological characteristics were tested by the χ² test or Fisher’s exact test. Correlations between LNMICC and FABP5 or miR190 were analyzed by Spearman rank correlation analysis. Results were considered statistically significant at \( p < 0.05 \).

Results

LNMICC is upregulated in cervical cancer with LN metastasis and predicts poor prognosis

To explore whether LNMICC exerted an impact on LN metastasis, we first investigated the mRNA levels of LNMICC in cervical cancer tissues. The qRT-PCR results showed that LNMICC was more highly expressed at the primary site of cervical cancers with LN metastasis than in those without LN metastasis and in normal uterine cervical tissues (Fig. 1A). We then detected LNMICC expression in six cervical cancer cell lines and primary normal cervical epithelial cells using qRT-PCR and gel electrophoresis. Surprisingly, the expression level of LNMICC was significantly higher in cervical cancer cells derived from metastatic sites, including MS751, ME180 and CaSki, than cervical cancer cells derived from primary sites, including SiHa, HeLa, HeLa229, or primary normal cervical epithelial cells. (Fig. 1B and Supplementary Fig. S1A). These results suggest that LNMICC upregulation may promote LN metastasis in cervical cancers.

To further investigate the role of LNMICC in cervical cancer, 211 cases were examined with ISH. The patients were divided into two groups based on LNMICC expression in tumor tissues: the High-LNMICC group (ISH score ≤4) and the Low-LNMICC group (ISH score >4). The results showed that 85.1% (40/47) of primary sites of cervical cancers with LN metastasis had high LNMICC expression (Fig. 1C, Supplementary Fig. S1B and Supplementary Table 3). Then, we analyzed the relationship between LNMICC expression and the clinicopathological characteristics of cervical cancer. A higher LNMICC expression level significantly correlated with tumor size (\( p=0.0361 \)), stromal invasion (\( p=0.001 \)), lymphovascular space invasion (\( p<0.0001 \)), lymph node metastasis (\( p=0.0003 \)), recurrence (\( p=0.0014 \)) and vital status at follow-up (\( p<0.0001 \)) (Supplementary Table 3). Furthermore, Kaplan-Meier survival curves and log-rank test analyses showed that patients with high LNMICC expression had significantly decreased overall survival (OS) and disease-free survival (DFS) (Fig. 1D, E). In a xenograft mouse LN metastatic model,
the group of mice harboring stable LNMICC-knockdown (LNMICC-KD) cells exhibited improved survival (Fig. 1F, G). In addition, univariate and multivariate analysis suggested that LNMICC expression (95% CI: 1.136-5.932; \( p = 0.024 \)), tumor size (95% CI: 1.132-3.170; \( p = 0.015 \)), lymphovascular space invasion (95% CI: 5.302-22.415; \( p = 0.0001 \)) and LN metastasis (95% CI: 1.795-6.949; \( p < 0.0001 \)) were independent prognostic factors for the OS of cervical cancer patients (Supplementary Table 4). To determine whether LNMICC is a high-risk factor for pelvic LN metastasis, a multivariate logistic regression analysis was performed and revealed that LNMICC, lymphovascular space invasion and parametrial infiltration were independently associated with pelvic LN metastasis in patients with cervical cancer (Supplementary Table 5).

Additionally, the coding potential of LNMICC was calculated using the protein-coding potential assessment tool (CPAT). The results showed that LNMICC does not have a typical protein-coding ORF that is longer than 300 nt [23], and the coding probability of LNMICC is very low (Supplementary Fig. S2A). Then, the full sequence of LNMICC was cloned into the eukaryotic expression vector pcDNA3.1 with C-terminal Flag tag. GAPDH with C-terminal Flag tag was used as positive control. Western blot analysis showed that the GAPDH with Flag-tag group could detect the expression of Flag-tagged protein, while the LNMICC Flag-tagged group did not show expression of this protein (Supplementary Fig. S2B). Collectively, these observations confirmed that LNMICC had no coding capability.

**LNMICC promotes cervical cancer metastasis and lymphangiogenesis in vitro**

To demonstrate the potential effects of LNMICC for promoting metastasis in cervical cancer, we performed gain- and loss-of-function experiments in cervical cancer cells. According to LNMICC expression in a variety of cervical cancer cell lines (Fig. 1B), the SiHa and HeLa229 cell lines were selected for overexpression and knockdown of LNMICC. We constructed an MS751 cell line derived from a metastatic LN of a cervical cancer patient with LNMICC overexpression and down-regulation. The qRT-PCR assay was used to confirm the efficiencies of overexpression and interference (Fig. 2A and Supplementary Fig. S3A). Transwell and wound-healing assays indicated that LNMICC overexpression increased the invasion and migration ability of SiHa and MS751 cells, whereas LNMICC knockdown significantly decreased the invasion and migration ability of HeLa229 and MS751 cells, respectively (Fig. 2B, C). Furthermore, we investigated the effect of LNMICC on the tube formation of HLECs, which is crucial for tumor LN metastasis. Compared with those of the corresponding control groups, the culture supernatants of LNMICC-overexpressing cells significantly promoted HLEC tube formation. In contrast, LNMICC knockdown abolished the effects of HeLa229 and MS751 cell-culture supernatants in promoting tube formation (Fig. 2D). Because the LN metastasis burden positively correlates with primary tumor size [24], we further determined whether LNMICC promotes cervical cancer cell proliferation and tumor growth. MTT assays showed that LNMICC knockdown decreased the proliferative capacity of HeLa229 and MS751 cells (Supplementary Fig. S3B, C). Consistent with
decreased cell proliferation, the percentage of apoptotic cells was markedly increased following siLNMICC treatment relative to that in control groups, as determined using flow cytometry (Supplementary Fig. S3D).

In addition, we analyzed the effects of LNMICC on the EMT process using Western blot. The results showed a significant down-regulation of E-Cadherin and claudin-1 and upregulation of snail, N-Cadherin and vimentin after LNMICC overexpression in SiHa and MS751 cells. LNMICC knockdown had the opposite effect in HeLa229 and MS751 cells (Fig. 2E, F). Vascular endothelial growth factor (VEGF)-C signaling, as the key lymphangiogenic pathway, is crucially involved in lymphangiogenesis [25]. Western blot analysis revealed that the protein level of VEGF-C was markedly increased in response to LNMICC overexpression, whereas LNMICC knockdown inhibited VEGF-C expression in cancer cells (Fig. 2E, F). Taken together, these data indicated that LNMICC in cervical cancer cells contributed to lymphangiogenesis and LN metastasis.

**LNMICC activates fatty acid metabolism in cervical cancer cells.**

Currently, the pivotal role of FA metabolism in cancer metastasis has been highlighted [26], and IncRNAs have been demonstrated to regulate FA metabolism via different mechanisms [27]. Therefore, we further examined whether LNMICC was linked to altered FA metabolism in cervical cancer. According to the expression level of LNMICC from GSE26511, we estimate that LNMICC participates in the linolenic acid metabolism classified as omega-3 FA metabolism with NES values of 1.7127879 (p<0.001 Fig. 3A). Interestingly, the multivariate regression analysis revealed that BMI ≥25 (kg/cm²) was independently associated with pelvic LN metastasis in patients with cervical cancer (Supplementary Table 5), and higher LNMICC expression level significantly correlated with BMI (p=0.0232) (Supplementary Table 3). Furthermore, Kaplan-Meier survival analysis showed that BMI ≥25 (kg/cm²) indicated a poorer OS and DFS (Fig. 3B, C). These findings led us to believe that LNMICC may be involved in reprogramming of FA metabolism in cervical cancer. To evaluate the effects of LNMICC on lipid content in cervical cancer cell lines, the levels of intracellular TAG and PLs were measured. Our data showed that LNMICC knockdown induced significantly decreased levels of intracellular TAG and PLs in HeLa229 and MS751 cells, whereas overexpression of LNMICC increased the levels of TAG and PL in SiHa and MS751 cells (Fig. 3D-G). These results were further supported by cellular staining with the lipophilic fluorescence dye BODIPY 493/503, which indicated that LNMICC knockdown decreased the intracellular contents of neutral lipids in HeLa229 and MS751 cells, while LNMICC overexpression had the opposite effect in SiHa and MS751 cells (Fig. 3H, I).

To demonstrate the role of LNMCC in the deregulation of FA metabolism in cervical cancer, we examined the effects of LNMICC on several key FA metabolic enzymes, including FASN, ACC1, ACOX1, CPT1A and FABP5, in cervical cancer cells. Consequently, significant down-regulation of FASN, ACC1 and FABP5 and upregulation of ACOX1 and CPT1A protein and mRNA levels were observed compared with the levels in controls in HeLa229 and MS751 cells after LNMICC
knockdown (Fig. 3J and Supplementary Fig. S4A-D). LNMICC overexpression reverted the expression of key FA metabolic enzymes in SiHa and MS751 cells (Fig. 3K and Supplementary Fig. S4E-H). To provide further evidence, we measured the expression of these FA metabolic enzymes in cervical cancer tissues with different LNMICC levels using IHC staining, and obtained consistent results (Fig. 3L). Thus, we conclude that LNMICC promotes the reprogramming of FA metabolism in cervical cancer.

**LNMICC promotes lymph node metastasis though FABP5-mediated FA metabolism in vitro and in vivo**

Although the mode of action for IncRNAs remains to be fully understood, some IncRNAs exert their functions through interacting with proteins in the nucleus, whereas others are reported to modulate mRNA stability and translation in the cytoplasm [28, 29]. According to ISH, LNMICC was located primarily in the nucleus, which was confirmed by nuclear/cytoplasm fractionation (Fig. 4A), and this result suggests that LNMICC may exert its biological function in the nucleus. As IncRNAs have been shown to exert cis-regulatory effects on nearby genes [30], we examined whether LNMICC would affect the expression of its in cis genes. Bioinformatics analysis revealed that LNMICC is located upstream of the FABP5 gene with high degree of sequence conversation, and no overlapping region was found at the RNA level in UCSC genome database (Supplementary Fig. S5A). Therefore, we first measured the expression of LNMICC and FABP5 in 40 fresh cervical cancer tissues. Scatter plot analysis showed a positive correlation between the mRNA levels of LNMICC and FABP5 (r=0.902, p<0.001) (Fig. 4B), which was confirmed by TCGA datasets (r=0.902, p<0.001) (Supplementary Fig. S5B). These data and those in Supplementary Fig. S5C-D indicate that FABP5 was regulated by LNMICC.

Giving the positional relationship of LNMICC and FABP5 on the genome, we then detected whether LNMICC bound to the promoter of the FABP5 gene. We analyzed a 1-kb locus upstream from the transcription start sites (TSSs) of the FABP5 gene. Through truncating mutation and a dual luciferase reporter assay, we identified (approximately) a -400 to -200-bp segment of FABP5 promoter as a sufficient binding site for LNMICC (Fig. 4C).

To further investigate the mechanism through which LNMICC enhanced the transcription of FABP5, we performed RNA pull-down assays to identify potential proteins binding to LNMICC. The LNMICC-bound complex was separated by SDS-PAGE and then subjected to mass spectrometry (Fig. 4D). NPM1 was the only transcription factor in the binding targets of LNMICC that was identified (Supplementary Fig. S5E), and the results were further confirmed by Western blotting (Fig. 4E). RIP assays were also performed using antibodies against NPM1 in cell extracts from HeLa229 and MS751 cells, and the results showed that LNMICC was detected by qRT-PCR in NPM1-immunoprecipitated RNAs in both HeLa229 and MS751 cells (Fig. 4F). As verified in Figure 4C, we found the putative NPM1-binding sites within 400 bp upstream of the transcriptional start of FABP5. ChIP-qPCR assays revealed that NPM1 directly bound to the predicted binding sites of the FABP5
promoter (Fig. 4G). These data indicated that LNMICC recruits NPM1 to activate FABP5 transcription.

To assess the crosstalk between LN metastasis and FA metabolism, we sought to determine whether FABP5 had a key role in LNMICC-induced LN metastasis in vitro and in vivo. First, we investigated the effects of FABP5 on lipogenesis in SiHa and MS751 cells. As expected, silencing of FABP5 by siRNA dramatically reversed the lipogenesis-promoting effects of LNMICC overexpression on the content of intracellular TAG, PLs and the intensity of BODIPY staining in this system (Fig. 4H-J and Supplementary Fig. S5F). Furthermore, the invasion and migration assay data showed that FABP5 down-regulation remarkably reversed the LNMICC-induced increase in cell invasion and migration in SiHa and MS751 cells (Supplementary Fig. S6A-C). Additionally, the results from the endothelial cell tube-formation assay were consistent with those of the invasion assay. Knockdown of FABP5 expression in LNMICC-overexpressed cells inhibited the tube formation of HLECs (Supplementary Fig. S6D-F). Then, we explored whether LNMICC promotes EMT and lymphangiogenesis through modulating FABP5. As shown in Supplementary Figure S6G, the expression levels of snail, N-Cadherin and vimentin were significantly decreased after FABP5 down-regulation in LNMICC-overexpressed cells. However, the expression levels of E-Cadherin and claudin-1 significantly increased using the same processing conditions. Meanwhile, FABP5 inhibition after LNMICC overexpression in SiHa and MS751 cells led to reduced VEGF-C expression (Supplementary Fig. S6G).

Furthermore, we selected the high-LNMICC-expressing cell lines MS751 and HeLa229 as representatives for establishing stable LNMICC-KD cells to develop xenograft mouse metastatic models. Notably, the specimens from the foot pads of mice in the control group contained more fat vacuoles than those in the corresponding LNMICC-KD group (Fig. 5A), which suggests that lipids metabolism may facilitate the metastasis of cervical cancer cells. The subcutaneous xenograft tumor model showed that FABP5 overexpression could partially accelerate tumor growth and the increase in tumor size/weight caused by LNMICC knockdown (Fig. 5B-D). Importantly, the incidence of LN metastasis dramatically declined in the LNMICC-KD groups, and FABP5 overexpression could reverse this trend in a xenograft mouse LN metastatic model (Fig. 5E). Moreover, the results from the xenograft mouse lung metastatic model were consistent with those of the LN metastasis model (Fig. 5F). These results were pathologically confirmed with HE staining. Taken together, these data suggest that LNMICC may facilitate LN metastasis by directly modulating FABP5-mediated reprogramming of FA metabolism.

**LNMICC is a direct target of miR190**

Emerging evidence suggests that miRNAs have the potential to promote the degeneration of IncRNA [31]. To further explore the regulatory mechanism of the IncRNA expression pattern, miRcode [32] software was used to predict the potential complementary base pairing between LNMICC and miRNAs. We transfected MS751
cells with mimics of all predicted miRNAs, and miR190 had the most significant effect on LNMICC expression (Fig. 6A). Therefore, we selected miR190 for further study. QRT–PCR showed that, whereas miR190 was inhibited in SiHa and MS751 cells, LNMICC expression showed the opposite trend in these cell lines (Supplementary Fig. S7A). However, there was no obvious difference in miR190 levels after LNMICC overexpression or knockdown (Supplementary Fig. S7B, C). Additionally, compared with the high-metastatic MS751 cells, the low-metastatic SiHa cells displayed higher expression levels of miR190 (Fig. 6B). A significant inverse correlation between LNMICC and miR190 was also detected in 40 fresh cervical cancer tissues (Fig. 6C). These results suggest that LNMICC might be the downstream effector of miR190, which might be involved in LN metastasis in cervical cancer.

To confirm whether miR190-mediated LNMICC regulation occurs through direct targeting of the predicted miRNA binding site in the LNMICC sequence (Fig. 6D), a dual luciferase reporter assay was performed. As shown in Figure 6D, co-transfection of SiHa and MS751 cells with psicheck2- LNMICC -WT vector and miR190 mimic significantly reduced luciferase reporter activity. Conversely, these repressive effects were abrogated by site-directed mutagenesis in the LNMICC sequence. It is well known that miRNAs bind their targets and mediate translational repression and/or RNA degradation functions in an Ago2-dependent manner [33]. RIP experiments showed that the LNMICC and miR190 were detected in Ago2 immunoprecipitates from SiHa and MS751 cells, which indicates that miR190 interacts with LNMICC in a sequence-specific manner (Fig. 6E). Collectively, these results revealed that miR190 exerts inhibitory effects on LNMICC expression by directly binding to LNMICC.

Next, we transfected SiHa and MS751 cells with miR190 mimic and LNMICC plasmid vector to investigate the effects of miR190 on FA metabolism and metastasis mediated by LNMICC. Then, decreased FASN, ACC1 and FABP5 expression by miR190 mimics was restored to basal levels by the LNMICC plasmid vector treatment, and the high expression of CPT1A and ACOX1 was also inhibited (Fig. 6F). Additionally, we found that miR190 mimics could efficiently offset the effects of accelerating lipogenesis of LNMICC overexpression on SiHa and MS751 cells, as revealed by both quantification of PLs (Fig. 6G), TAG (Fig. 6H) and neutral lipids (Fig. 6I). In addition, the restoration of miR190 by miR190 mimics transfection in SiHa and MS751 cells increased E-cadherin and claudin-1 and decreased snail, N-Cadherin and vimentin, which reversed the LNMICC-stimulated mesenchymal cell phenotype (Fig. 6J). Meanwhile, restoration of miR190 after LNMICC stimulation in SiHa and MS751 cells resulted in decreased VEGF-C expression (Fig. 6J). Moreover, we validated that FABP5 siRNA could abrogate the effects of LNMICC in inducing cell invasion and migration and abolished LNMICC-mediated HLEC tube formation (Supplementary Fig. S7D-F). Those observations suggest that the effects of LNMICC overexpression on the acceleration of lipogenesis and LN metastasis could be diminished by miR190, in accordance with the suppression of LNMICC expression by miR190.
Discussion

The lymphatic system is the most common route of distant metastasis for cervical cancer. A better understanding of the molecular mechanisms underlying LN metastasis may assist in identifying patients at high risk for mortality and may provide an effective clinical treatment of cervical cancer. However, little attention has been paid to understanding why cervical cancers are generally more prone to metastasize to lymph node. In this study, we explored the crucial role of crosstalk between lncRNA and FA metabolism in promoting LN metastasis, providing new insight into the interaction between metabolism and metastasis (Fig. 7).

A growing body of literature suggests that lncRNAs serve as potential biomarkers and are involved in the development and progression of cervical cancer [34]. However, the biological role of lncRNAs in LN metastasis remains unknown. In agreement with our previous results [20], we discovered that LNMICC was frequently upregulated in the primary site of cervical cancers with LN metastasis, and its expression independently correlated with LN metastasis. Our data showed that high LNMICC expression was associated with poor patient outcomes, which highlights its applicability as a novel promising prognostic biomarker for cervical cancer. In vitro and in vivo studies showed that LNMICC overexpression promoted cervical cancer cell invasion, migration and lymphangiogenesis and increased the rate of LN metastasis in xenograft animal models. Accordingly, we have good reason to believe that high LNMICC expression creates a favorable environment for LN metastasis in cervical cancer.

Emerging evidence confirms that lncRNAs function as competing endogenous RNAs (ceRNAs) to sponge miRNAs [35, 36]. In the present study, we used luciferase assays to indicate that miR190 directly binds to LNMICC. Furthermore, RIP assays verified that LNMICC and miR190 were incorporated into a RNA-induced silencing complex (RISC) complex using the Ago2 antibody, which is a key component of the RISC complex. Although no complementary element was predicted between miR190 and FABP5 (downstream RNA of LNMICC), this does not mean that a LNMICC-associated ceRNA network is nonexistent. In contrast, the alignment between the LNMICC and miR190 is not very specific, because at least 6 miRNAs were predicted to form complementary base pairings with LNMICC. Moreover, miR190 may also share some miRNA response elements (MRE) with several protein-coding genes, such as ACOX1 and ACC1, as predicted by MiRanda (Supplementary Fig. S7G). Interestingly, our results showed that miR190 can regulate the expression of ACOX1 and ACC1 though LNMICC. This result provides strong evidence for us to further explore the role of ceRNA regulatory networks in LN metastasis. In a previous study [37], miR190 was found to be the only miRNA identified by a genome-wide association study in the loci associated with LN metastasis of breast cancer. Our findings support the notion that miR190 functions as a metastasis suppressor, significantly reducing invasiveness, migration and lymphangiogenesis by regulating LNMICC expression.

It has been well-documented that metastatic cancer cells require FA for providing
energy, macromolecules for membrane synthesis, and lipid signals, which are critical for metastatic colonization in distant organs. As reported in previous studies, the FA receptor CD36 is a marker of metastasis-initiating cells and leads cancer cells to have a higher propensity to metastasize LN in a lipid metabolism-dependent manner [8, 38]. More interestingly, IncRNAs have been shown to be involved in lipid metabolism in cancer. For example, the IncRNA HULC could facilitate lipid metabolism through a miR9-mediated RXRA signaling pathway in hepatoma cells [27]. In this study, we wondered whether LNMICC could also modulate FA metabolism to promote LN metastasis in cervical cancer. Through RNA pull-down and ChIP assays, we identified FABP5 as a central regulator of lipid metabolism that directly interacted with LNMICC by recruiting NPM1. Additionally, FABP5-induced reprogramming of FA metabolism of cervical cancer cells was critical for promoting LN metastasis, indicating effective crosstalk between HLECs and cervical cancer cells. Surprisingly, we found that BMI ≥25 (kg/cm²) was independently associated with pelvic LN metastasis and was associated with poor OS in our clinical data, which further supported these conclusions.

Cancer cells are prone to disseminate through the lymphatic system during the metastatic spread of cervical cancer. However, it is not clear what drives cancer cells to choose the lymphatic system for migration. Mikael C. Karlsson et al demonstrated that EMT promotes the migration of breast cancer through the lymphatic system to lymph nodes and that HLECs secrete chemokines to attract EMT cells [39, 40]. Similar to those results, we found that LNMICC overexpression could induce EMT in cervical cancer cells and activate the expression of VEGF-C to promote lymphangiogenesis via FABP5-mediated reprogramming of FA metabolism. Consistent with our study, Takanori Ohata et al indicated that FABP5 facilitated the invasiveness and metastasis of hepatocellular carcinoma through the induction of EMT [13]. Interestingly, these findings reveal that the crosstalk between EMT and FA metabolism will be a novel breakthrough in exploring the mechanism of LN metastasis.

In conclusion, LNMICC as a promising prognostic biomarker for cervical cancer, promotes LN metastasis through the reprogramming of FA metabolism by recruiting the NPM1 to the FABP5 promoter, and its biological function can be suppressed by miR190. Thus, our findings provide new insights into the mechanism of FA metabolism in the LN metastasis of cervical cancer and add a promising new target for the development of novel anti-metastasis therapeutics.

**Acknowledgments:**
This work was supported by grants from the National Natural Science Foundation of China (No. 81672561) (S.Z. Yao); the Natural Science Foundation of Guangdong Province, China (No. 2015A030313073) (S.Z. Yao); and the Science and Technology Program of Guangzhou, China (No. 201510010289/1563000183) (S.Z. Yao).

The authors thank Dr. Junfeng Zhu and Dr. Ying Tuo (Department of Pathology, the First Affiliated Hospital, Sun Yat-sen University) for help with the pathological diagnoses and guidance.
References:

[1]. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A., Global cancer statistics, 2012. CA: A Cancer Journal for Clinicians, 2015; 65:87-108.

[2]. Obrzut B, Semczuk A, Naróg M, Obrzut M, Król P, Prognostic Parameters for Patients with Cervical Cancer FIGO Stages IA2-IIB: A Long-Term Follow-Up. Oncology, 2017 May 3 [Epub ahead of print].

[3]. Karaman, S, M. Detmar, Mechanisms of lymphatic metastasis. Journal of Clinical Investigation, 2014; 124:922-8.

[4]. Alitalo, A, M. Detmar, Interaction of tumor cells and lymphatic vessels in cancer progression. Oncogene, 2012; 31:4499-508.

[5]. Kalluri, R, R.A. Weinberg, The basics of epithelial-mesenchymal transition. Journal of Clinical Investigation, 2009; 119:1420-1428.

[6]. Hanahan, D, R.A. Weinberg, Hallmarks of Cancer: The Next Generation. 2011; 144:646 - 674.

[7]. Cairns, R.A., I.S. Harris, T.W. Mak, Regulation of cancer cell metabolism. Nature Reviews Cancer, 2011; 11:85-95.

[8]. Pascual G, Avgustinova A, Mejetta S, Martín M, Castellanos A, Attolini CS, et al., Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature, 2017; 541:41-45.

[9]. Nath, A, C. Chan, Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. Scientific Reports, 2016; 6:18669.

[10]. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr, Cellular Fatty Acid Metabolism and Cancer. Cell Metabolism, 2013; 18: 153-161.

[11]. Storch, J, A.E. Thumser, Tissue-specific Functions in the Fatty Acid-binding Protein Family. Journal of Biological Chemistry, 2010; 285: 32679-32683.

[12]. Yu CW, Liang X, Lipsky S, Karaaslan C, Kozakewich H, Hotamisligil GS, et al., Dual role of fatty acid-binding protein 5 on endothelial cell fate: a potential link between lipid metabolism and angiogenic responses. Angiogenesis, 2016; 19: 95-106.

[13]. Ohata T, Yokoo H, Kamiyama T, Fukai M, Aiyama T, Hatanaka Y, et al., Fatty acid-binding protein 5 function in hepatocellular carcinoma through induction of epithelial-mesenchymal transition. Cancer Med, 2017; 6:1049-1061.

[14]. Dhamija, S, S. Diederichs, From junk to master regulators of invasion: IncRNA functions in migration, EMT and metastasis. Int J Cancer, 2016; 139: 269-80.

[15]. Schmitt, A.M, H.Y. Chang, Long Noncoding RNAs in Cancer Pathways. Cancer Cell, 2016; 29: 452-463.

[16]. Yoon JH, Abdelmohsen K, Srikanthan S, Yang X, Martinson JL, De S, et al., LncRNA-p21 suppresses target mRNA translation. Mol Cell, 2012; 47:648-55.

[17]. Kim HJ, Eoh KJ, Kim LK, Nam EJ, Yoon SO, Kim KH, et al., The long noncoding RNA HOXA11 antisense induces tumor progression and stemness maintenance in cervical cancer. Oncotarget, 2016; 7:83001-83016.

[18]. Sharma S, Mandal P, Sadhukhan T, Roy Chowdhury R, Ranjan Mondal N, Chakravarty B, et al., Bridging Links between Long Noncoding RNA HOTAIR and...
HPV Oncoprotein E7 in Cervical Cancer Pathogenesis. Sci Rep, 2015; 5:11724.

[19]. Zhang JJ, Wang DD, Du CX, Wang Y, Long Noncoding RNA ANRIL Promotes Cervical Cancer Development By Acting as a Sponge of miR-186. Oncol Res, 2017 May 22. [Epub ahead of print].

[20]. Shang C, Zhu W, Liu T, Wang W, Huang G, Huang J, et al., Characterization of long non coding RNA expression profiles in lymph node metastasis of early-stage cervical cancer. Oncol Rep, 2016; 35:3185-97.

[21]. Serviss, J.T, P. Johnsson, D. Grandå R, An emerging role for long non-coding RNAs in cancer metastasis. Frontiers in Genetics, 2014; 5:234.

[22]. Hung CL, Wang LY, Yu YL, Chen HW, Srivastava S, Petrovics G, et al., A long noncoding RNA connects c-Myc to tumor metabolism. Proceedings of the National Academy of Sciences, 2014; 111:18697-18702.

[23]. Slavoff SA, Mitchell AJ, Schwaid AG, Cabili MN, Ma J, Levin JZ, et al., Peptidomic discovery of short open reading frame-encoded peptides in human cells. Nature Chemical Biology, 2012; 9:59-64.

[24]. Cairns, R.A, R.P. Hill, Acute hypoxia enhances spontaneous lymph node metastasis in an orthotopic murine model of human cervical carcinoma. Cancer Res, 2004; 64:2054-61.

[25]. Ji H, Cao R, Yang Y, Zhang Y, Iwamoto H, Lim S, et al., TNFR1 mediates TNF -α-induced tumour lymphangiogenesis and metastasis by modulating VEGF-C-VEGFR3 signalling. Nature Communications, 2014; 5:4944.

[26]. Luo X, Cheng C, Tan Z, Li N, Tang M, Yang L, et al., Emerging roles of lipid metabolism in cancer metastasis. Molecular Cancer, 2017; 16:76.

[27]. Cui M, Xiao Z, Wang Y, Zheng M, Song T, Cai X, et al., Long Noncoding RNA HULC Modulates Abnormal Lipid Metabolism in Hepatoma Cells through an miR-9-Mediated RXRA Signaling Pathway. Cancer Research, 2015; 75:846-857.

[28]. Mercer, T.R, J.S. Mattick, Structure and function of long noncoding RNAs in epigenetic regulation. Nature Structural & Molecular Biology, 2013; 20:300-307.

[29]. Huarte, M., The emerging role of IncRNAs in cancer. Nature Medicine, 2015; 21: 1253-1261.

[30]. Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, et al., Long Noncoding RNAs with Enhancer-like Function in Human Cells. Cell, 2010; 143: 46-58.

[31]. Yoon, J., K. Abdelmohsen, M. Gorospe, Functional interactions among microRNAs and long noncoding RNAs. Seminars in Cell & Developmental Biology, 2014; 34: 9-14.

[32]. Jeggari, A., D.S. Marks, E. Larsson, miRcode: a map of putative microRNA target sites in the long non-coding transcriptome. Bioinformatics, 2012; 28: 2062-2063.

[33]. Filipowicz, W., S.N. Bhattacharyya, N. Sonenberg, Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Reviews Genetics, 2008; 9:102-114.

[34]. Cui, X., X. Jing, X. Wu, The prognostic value of long non coding RNAs in cervical cancer: A meta-analysis. Oncotarget, 2017 May 4. [Epub ahead of print]
[35]. Ma MZ, Zhang Y, Weng MZ, Wang SH, Hu Y, Hou ZY, et al., Long Noncoding RNA GCASPC, a Target of miR-17-3p, Negatively Regulates Pyruvate Carboxylase-Dependent Cell Proliferation in Gallbladder Cancer. Cancer Res, 2016; 76:5361-71.

[36]. Tay, Y., J. Rinn, P.P. Pandolfi, The multilayered complexity of ceRNA crosstalk and competition. Nature, 2014; 505:344-52.

[37]. Chu HW, Cheng CW, Chou WC, Hu LY, Wang HW, Hsiung CN, et al., A novel estrogen receptor-microRNA 190a-PAR-1-pathway regulates breast cancer progression, a finding initially suggested by genome-wide analysis of loci associated with lymph-node metastasis. Human Molecular Genetics, 2013; 23: 355-367.

[38]. Li, Z, Y. Kang, Lipid Metabolism Fuels Cancer’s Spread. Cell Metabolism, 2017; 25:228 - 230.

[39]. Karlsson MC, Gonzalez SF, Welin J, Fuxe J, Epithelial - mesenchymal transition in cancer metastasis through the lymphatic system. Molecular Oncology, 2017; 11: 781 - 791.

[40]. Pang MF, Georgoudaki AM, Lambut L, Johansson J, Tabor V, Hagikura K, et al., TGF-β1-induced EMT promotes targeted migration of breast cancer cells through the lymphatic system by the activation of CCR7/CCL21-mediated chemotaxis. 2016; 35: 748.
Figure Legends

Fig. 1. LNMICC is overexpressed in cervical cancer and associated with poor prognosis. (A) The qRT-PCR results showed that LNMICC was more highly expressed at the primary site of cervical cancers with LN metastasis than in those without LN metastasis or normal uterine cervical tissues (n=20/group). (B) LNMICC expression in six cervical cancer cell lines with different metastatic potentials. NCC represents primary normal cervical epithelial cells. (C) ISH analysis of LNMICC in specimens of normal uterine cervical tissues, cervical cancers with LN metastasis and cervical cancers without LN metastasis. Kaplan-Meier survival curves showed poor overall survival (D) and disease-free survival (E) with high expression of LNMICC. (F-G) Kaplan-Meier analysis of mice injected with MS751 or HeLa229 cells into the foot pad (n=10/group). (*p<0.05; **p<0.01, ***p<0.001).

Fig. 2. LNMICC promotes lymphangiogenesis and cervical cancer cells metastasis via EMT and VEGF-C, respectively. (A) The qRT-PCR results showed the efficiencies of knockdown of LNMICC in MS751 and HeLa229 cells. (B) The migration abilities were explored by a wound-healing analysis of MS751 and SiHa cells transfected with an LNMICC overexpression plasmid (left), and MS751 and HeLa229 cells transfected with two LNMICC-siRNAs (right). Original magnification: 100x. (C) Transwell assays were performed to investigate the effects of LNMICC on the invasion abilities of MS751, SiHa (left), HeLa229 (right) cell lines. Original magnification: 100x. (D) HLECs were pretreated with the culture medium supernatants of the cervical cancer cells to investigate the effects of LNMICC on the tube formation of HLECs. Representatives are shown (left). Total branch points and average length per field were calculated (right). Scale bars, 100 μm. Western blot analysis of EMT marker (snail, E-Cadherin, N-Cadherin, claudin-1 and vimentin) and VEGF-C in indicated cells with overexpression (E) or knockdown (F) of LNMICC. GAPDH was used as a loading control. (*p<0.05; **p<0.01).

Fig. 3. Effects of LNMICC expression on the intracellular contents of lipids and key lipid metabolic enzymes in cervical cancer cells. (A) Identification of gene sets enriched in phenotypes correlated with LNMICC (236271_at) by GSEA using GSE26511 data. Kaplan-Meier survival curves showed poor disease-free survival (B) and overall survival (C) with BMI $\geq$ 25 (kg/cm$^2$). Cellular content of phospholipids (D, E) and triglycerides (F, G) were detected in the indicated cells. (H, I) The neutral lipids content was detected by double staining with BODIPY 493/503 dye and Hoechst in the indicated cells. Scale bars, 16 μm. (J-K) Western blot analysis for protein levels of the key lipid metabolic enzymes FASN, ACC1, ACOX1, CPT1A and FABP5 in the indicated cells. (L) Representative IHC images of the lipid metabolic enzymes FASN, ACC1, ACOX1, CPT1A and FABP5 in human cervical cancer tissues. Original magnification: 200x. (*p<0.05; **p<0.01).

Fig. 4. LNMICC activates FA metabolism by recruiting NPM1 to enhance
**FABP5 transcription.** (A) The assay of nuclear/cytoplasm fractionation showing nuclear localization of LNMICC in MS751 and HeLa229 cells. (B) Scatter plot analysis of the correlation between mRNA expression levels of LNMICC and FABP5 in 40 fresh cervical cancer tissues. (C) Different loci of the FABP5 promoter were constructed into pGL3 vectors and subjected to luciferase reporter assays in MS751 and HeLa229 cells. (D) SDS-PAGE analysis with silver staining showed the proteins were pulled down by LNMICC or its antisense RNA from indicated cells. The peptides were detected by subsequent mass spectrometry. (E) Another independent RNA pull-down experiment with cell extract. Specific bands were identified by Western blot assays using a corresponding antibody. (F) The RIP assays showed the LNMICC retrieved by the NPM1 antibody in MS751 and HeLa229 cells. The qRT-PCR products were analyzed with gel electrophoresis (left). The mRNA levels were graphed (right). (G) Localization of the NPM1 to FABP5 promoter in MS751 and HeLa229 cells, which were analyzed by ChIP. H3 served as a positive control. (H) The neutral lipid content was detected by double staining with BODIPY 493/503 dye and Hoechst in the indicated cells. Scale bars, 16 μm. The levels of phospholipids (I) and triglycerides (J) were measured in the indicated cells. (*p<0.05; **p<0.01)

**Fig. 5.** LNMICC facilitated growth and metastasis in an FABP5-mediated manner in vivo. (A) The fat vacuoles were enriched in tissues from the foot pads of mice. (B) The representative pictures of dissected tumors from nude mice transplanted with stable LNMICC-KD or LNMICC-KD/FABP5-OE cells. (C) Subcutaneous tumor growth curves of mice in different treatment groups were depicted. (D) The average weight of tumors at the time the animals were sacrificed in the experimental groups. (E) The representative pictures of HE staining of lymph nodes in different parts of mice (left) and the rate of LN metastasis (right). Subcapsular sinuses dilatation followed by accumulation of tumor cells was identified as metastatic lymph nodes. Original magnification: 100x. (F) The representative images of HE staining of the lungs (left) and the rate of lung metastasis (right) in mice with different treatment cells. (*p<0.05; **p<0.01)

**Fig. 6.** LNMICC is a direct target of miR190. (A) The qRT-PCR analysis of LNMICC expression in MS751 cells transfected with miRNAs mimics, which were predicted by miRcode. (B) The expression levels of miR190 in SiHa and MS751 cell lines with different metastatic potentials. (C) Scatter plot analysis of the correlation between mRNA expression levels of LNMICC and miR190 in 40 fresh cervical cancer tissues. (D) Schematic of wild-type and mutant psicheck2-LNMICC reporter constructs (left). Dual luciferase reporter assay of SiHa and MS751 cells co-transfected with the psicheck2- LNMICC -WT or -MUT reporter plasmids and miR190 mimic (right). (E) The RIP assays showed the LNMICC and miR190 were immunoprecipitated by an Ago2 antibody in SiHa and MS751 cells. The qRT-qPCR products were analyzed with gel electrophoresis (left). The mRNA levels were graphed (right). (F) Western blot analysis for protein levels of the key lipid metabolic enzymes FASN, ACC1, ACOX1, CPT1A and FABP5 in the indicated cells.
content of phospholipids (G) and triglycerides (H) were detected in the indicated cells. (I) The content s of neutral lipids were detected by double staining with BODIPY 493/503 dye and Hoechst in the indicated cells. Scale bars, 16 μm. (J) Western blot analysis for protein levels of EMT markers (snail, E-Cadherin, N-Cadherin, claudin-1 and vimentin) and VEGF-C in the indicated cells (*p<0.05; **p<0.01).

**Fig. 7.** A proposed mechanistic schematic as to how LNMICC is inhibited by miR190 and promotes lymph node metastasis though FABP5-mediated FA metabolism.
Figure 1

A

B

C

D

E

F

G
Figure 3

A. Enrichment plot: KEGG_LINOLEIC_ACID_METABOLISM

B. Disease-free survival (%)

C. Overall survival (%)

D. Phospholipid (mM/10^6 cells)

E. Phospholipid (mM/10^6 cells)

F. Triglyceride (mM/10^6 cells)

G. Triglyceride (mM/10^6 cells)

H. Image of cells

I. Image of cells

J. Western blot images

K. Table of gene expression

L. Image of tissue sections

Downloaded from cancerres.aacrjournals.org on July 22, 2018. © 2017 American Association for Cancer Research.
**Figure 5**

---

**A**

- **LNM model**
- **NC**
- **LNMCC-KD**

**B**

- **MS751**
- **LNMCC-KD**
- **LNMCC-KD + FABP5-OE**
- **NC**
- **HeLa229**
- **LNMCC-KD**
- **LNMCC-KD + FABP5-OE**

**C**

- **MS751**
- **HeLa229**

**D**

- **Tumor weight (mg)**
- **MS751**
- **HeLa229**

**E**

- **Lymph node (LN)**
- **Positive LN**
- **Negative LN**

---

**F**

- **Lung**
- **NC**
- **LNMCC-KD**
- **LNMCC-KD + FABP5-OE**

---

*Note: Figures A, B, C, D, E, and F depict various comparisons and analyses related to tumor behavior and metastasis in different cell lines and conditions.*
Figure 6

A

B

C

D

E

F

G

H

I

J

Downloaded from cancerres.aacrjournals.org on July 22, 2018. © 2017 American Association for Cancer Research.
Figure 7

Lymph Node Metastasis

lymphangiogenesis

VEGF-C

up-regulate

EMT

FABP5

NPM1

promoter

Modulation

Fatty acid metabolism Reprogramming

ACC1

FASN

CPT1A

ACOX1

LNMICC

Ago2

miR190
LNMICC promotes nodal metastasis of cervical cancer by reprogramming fatty acid metabolism

Chunliang Shang, Wei Wang, Yuandong Liao, et al.

Cancer Res  Published OnlineFirst December 11, 2017.