RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2022.154

ScienceAsia 49 (2023): **177–183**

Role of autophagy-mediated miR-18a in breast cancer progression *in vitro*

Ming Sun[a,*], **Haijian Guo**[b]

[a] Department of Ultrasound, Affiliated Hospital of Nantong University, Nantong 226006 China
[b] Department of Cardiology, Affiliated Hospital of Nantong University, Nantong 226006 China

[*]Corresponding author, e-mail: chrisming9887@163.com

Received 29 Mar 2022, Accepted 4 Oct 2022
Available online 5 Dec 2022

ABSTRACT: MiR-18a-5p (miR-18a) is a potential biomarker of breast cancer risk in several clinically relevant studies. However, role of miR-18a in breast cancer progression remains unclear. Therefore, we sought to determine the effect of miR-18a in MCF-7 cell proliferation, migration and invasion under the condition of inflammation-related stimulus. In our study, lipopolysaccharide (LPS) increased miR-18a expression in MCF-7 cells. Anti-miR-18a lentivirus significantly inhibited MCF-7 cell proliferation, migration and invasion induced by LPS. Anti-miR-18a lentivirus also significantly increased apoptosis cells. Moreover, LPS exposure promoted the MCF-7 autophagy with the increased expression of LC3B-II. 3-Methyladenine (MA), an autophagy inhibitor, repressed the cell proliferation induced by LPS. Finally, we identified that alteration of miR-18a expression was mediated by autophagy in LPS-exposed MCF-7 cells. These findings reveal an interesting regulation underlying inflammatory-induced breast cancer progression *in vitro*, which may be beneficial to understand the pathogenesis in tumor microenvironment.

KEYWORDS: breast cancer, MiR-18a, autophagy, inflammation

INTRODUCTION

Breast cancer is the most common malignant tumor with the highest incidence in women. Surgery combined with radiotherapy and chemotherapy is the first choice for breast cancer, which significantly prolongs the survival of patients [1]. With the spread of new treatment strategies and methods, breast cancer has been one of the most effective solid tumors and the five-year survival rate for breast cancer is more than 80%. However, the incidence of breast cancer increases with years, especially in the eastern coastal areas in China. Existing reports have shown that there are many factors leading to breast cancer, such as genetic factor, diets and environmental factor [2]. Whatever the pathogenic mechanism, it seems to be closely related to inflammation.

In the early 19th century, cancers were thought to develop from inflammation, and their histological patterns were very similar. Both cancer tissue and inflammatory tissue are composed of mesenchymal cells and angiogenic cells. The biggest difference between the two is that cancer tissue cannot be recovered, while inflammatory tissue will eventually disappear [3]. Inflammation is a critical symbol of cancer that substantially promote cancer development and progression. There is growing evidence that systemic inflammation and local immune response play a positive role in cancer progression and survival of patients with cancer [4]. Lipopolysaccharide (LPS) recognized as a good tool for inducing inflammation was successfully promote cell viability and progression of multiple cancer cell lines *in vitro* [5]. Both clinical and basic medical studies have shown that inflammation is an adverse factor in promoting cancer development and progression. However, the molecular mechanism of cancer development and progression induced by inflammation is not well understood.

MicroRNAs (miRNAs) are one type of non-coding RNAs with the capability modulating target mRNA expression at the post-transcriptional level. Extensive studies show the potential effect of miRNAs on the occurrence and development of breast cancer [6]. Some miRNAs are significantly associated with clinical prognosis and diagnostic indicators, which is a good theoretical foundation to improve the treatment of breast cancer [7, 8]. MiR-18a-5p (miR-18a) is a potential biomarker of breast cancer risk in several clinically relevant studies [9, 10]. Although the role of other miRNAs in breast cancer is well known, whether miR-18a involves in inflammation-induced breast cancer progression is not clearly understood. In this study, we demonstrated that miR-18a was upregulated in LPS-exposed MCF-7 cells, a human breast cancer cell line, and inhibition of miR-18a repressed MCF-7 cell proliferation, migration and invasion. We further investigated that autophagy was involved in regulating abnormal miRNA expression in LPS-exposed MCF-7 cells. Therefore, our data revealed an interesting mechanism that autophagy-mediated miR-18a involves in breast cancer progression *in vitro*.

MATERIALS AND METHODS

Cell culture and LPS administration

MCF-7 cells, a human breast cancer cell line, were purchased from China center for type culture collec-

tion and cultured in DMEM (C11995500, Gibco, New York, USA) with 10% fetal bovine serum (FBS, 13011-8611, Tianhang Biotech, Hangzhou, China) and 1% penicillin-streptomycin (C100C5, NCM Biotech, Suchow, China). Cell culture condition was maintained in an incubator (5% CO_2, 37 °C). LPS was obtained from Sigma-Aldrich (L2630, Shanghai, China) and dissolved in sterile water for storage referring to manufacturer's instructions. MCF-7 cells were exposed to 100 ng/ml LPS for 48 h in DMEM with FBS.

3-Methyladenine (3-MA) and actinomycin D treatment

MCF-7 cells were pretreated with 3-MA (3 mM, M9281, Sigma-Aldrich) for 1 h and then exposed to LPS. Actinomycin D (2 mg/ml, GC16866, GLPB, California, USA) was added into the cell culture medium to block transcription and then MCF-7 cells were treated with 3-MA (3 mM) simultaneously.

Lentivirus infection

MCF-7 cells were plated in the 24-well plates and infected by GFP lentivirus carrying the puromycin resistance gene (HanBio, Shanghai, China) at MOI of 5 with polybrene for 48 h. Then, MCF-7 cells stably expressing the GFP signal were screened by puromycin for cell migration experiment. Similarly, miR-18a/anti-miR-18a lentivirus (HanBio) infected MCF-7 cells at MOI of 5 with polybrene for 48 h and control group was infected by vector lentivirus.

Cell counting Kit-8 (CCK8) assay

MCF-7 cells (1×10^4/ml) were plated in the 96-well plates for 12 h in DMEM with FBS. Then, MCF-7 cells were treated with LPS for 48 h and CCK8 regent (HY-K0301, MedChemExpress, New Jersey, USA) was used to detect cell viability. The intensity of absorbed light was measured at 480 nm using a microplate reader. Values in each group were calculated according to absorbency index to exhibit a percentage of the control group.

Cell apoptosis assay

Flow cytometry staining was performed to evaluate cell apoptosis using a cell apoptosis rapid detection kit (C11061, RiboBio, Guangzhou, China). Experimental procedures were referring to manufacturer instruction for adherent cell. Briefly, common medium was removed and caspase3/7 staining medium was added into each well at 37 °C for 45 min. Then, samples were analyzed by flow cytometry.

Transwell assay

Cell invasion was performed by transwell assay using a chamber consisting of Transwell membrane filter inserts (3422, Corning, New York, USA). Chambers were precoated with matrix adhesive and MCF cells of 5×10^4 were seeded into upper chambers with FBS-free DMEM for 48 h. DMEM with 10% FBS was added into lower chambers. MCF-7 cells on the lower surface of the filter were stained with 0.4% crystal violet. The numbers of invading cells were counted under a light microscope from five fields in a single chamber of three samples and data were normalized to control group.

Wound healing assay

Cell migration was evaluated by wound healing assay as previous study [11]. Briefly, cultured medium was changed into DMEM without FBS when MCF-7 cells were incubated to 75% confluence in 24-well plate. Cell-free straight lines were created by a 200-μl pipette scratching on the bottom of wells. Cell migration images were captured by fluorescence microscope. Image J software was used to measure the distance of cell migration.

Real-time PCR (qPCR)

Total RNA was extracted by Trizol reagent (R401-01, Vazyme, Nanjing, China) from MCF-7 cells. Extracted RNA was firstly deleted gDNA and subsequently reversely transcribed into cDNA using a miRNA 1st strand cDNA synthesis kit (MR101-01, Vazyme) and a strand cDNA synthesis kit (R212-01, Vazyme). MiR-18a expression was detected by qPCR using miRNA universal SYBR qPCR master mix (MQ101-01, Vazyme) and mRNA expression was detected using SYBR green master mix (Q121-02, Vazyme). Method of 2(-ÎŤĤŤCT) was conducted to calculate the relative expression of miR-18a. The following primers were employed: miR-18a (forward: 5′-GATAGCAGCACAGAAATATTGGC-3′, reverse: 5′-GTGCAGGGTCCGAGGT-3′); U6 (forward: 5′-GCGCGTCGTGAAGCGTTC-3′, reverse: 5′-GTGCAG GGTCCGAGGT-3′); IL-6 mRNA (forward: 5′-CCACCG GGAACGAAAGAGAA-3′, reverse: 5′-GAGAAGGCAAC TGGACCGAA-3′); GAPDH mRNA (forward: 5′-ACCAT CTTCCAGGAGCGAGAT-3′, reverse: 5′-GGGCAGAGAT GATGACCCTTT-3′).

Western blot

MCF-7 cells were lysed in ice-cold lysis buffer and protein concentration was measured using a BCA protein assay kit (P0012S, Beyotime, Shanghai, China). MCF-7 cell samples of 30 μg proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, proteins were transferred to polyvinylidene difluoride membranes. After blocking nonspecific signal with 5% nonfat milk, membranes were incubated with primary antibody at 4 °C overnight. Horseradish peroxidase-conjugated second antibody was used to amply the protein signal. Blot images were captured by chemiluminescence imaging system and protein band densitometry was evaluated by Image J software. Details of primary antibodies obtained from Proteintech, Wuhan, China, are: Bax

(50599-2); Bcl-2 (12789-1); LC3B (14600-1); GAPDH (60004-1).

Statistical analysis

All data are presented as the mean ± SD. The data were analysed using GraphPad Prism 6 Software and significance was performed using Student's *t*-tests. $p < 0.05$ was considered as significant significance.

RESULTS

Anti-miR-18a suppressed LPS-induced breast cancer progression *in vitro*

MiR-18a expression was measured in MCF-7 cells with LPS treatment. Compared to control group, LPS treatment notably elevated the expression of miR-18a (Fig. 1A). To demonstrate the role of miR-18a in breast cancer progression, we observed the MCF-7 cell proliferation, migration and invasion induced by LPS after miR-18a inhibitor treatment. LPS treatment increased the MCF-7 cell proliferation, and administration of anti-miR-18a suppressed the cell proliferation induced by LPS (Fig. 1B). Similarly, anti-miR-18a treatment inhibited the cell migration and invasion induced by LPS (Fig. 1C-D). These results implied that LPS promoted breast cancer progression via miR-18a *in vitro*.

Anti-miR-18a regulated inflammatory response and apoptosis in MCF-7 cells

Having determined the effect of miR-18a on cell proliferation, we next proceeded to detect inflammation-related and apoptosis-related protein expression in MCF-7 cells with LPS treatment. Compared to control group, IL-6 mRNA expression was increased in LPS group, and this elevated expression of IL-6 mRNA expression induced by LPS was inhibited by anti-miR-18a treatment (Fig. 2A). The ratio of Bcl-2 and Bax was increased in MCF-7 cells with LPS treatment. MiR-18a inhibitory repressed the elevation in ratio of Bcl-2 and Bax induced by LPS treatment (Fig. 2B). Moreover, anti-miR-18a increased the percent of activated caspase3/7 positive cells compared with LPS group (Fig. 2C). These results suggested that inhibition of miR-18a could repress inflammatory response and promote MCF-7 cell apoptosis.

Autophagy promoted miR-18a expression in MCF-7

Autophagy has been demonstrated to play a critical role in breast cancer progression. Next, we examined the LC3B expression, a golden marker of autophagy, in the exposure of LPS. Compared with control group, LC3B-II expression was increased in MCF-7 cells with LPS treatment (Fig. 3A). Administration of 3-MA, an autophagy inhibitor, significantly decreased LC3B-II expression compared with LPS group (Fig. 3A). To explore the relationship between autophagy and miR-18a, we detected miR-18a expression in MCF-7 with 3-MA treatment. Compared with LPS group, 3-MA treatment decreased miR-18a expression (Fig. 3B). Actinomycin D was applied to inhibit transcription and decreased miR-18a expression in a time-dependent manner (Fig. 3C). 3-MA still reduced miR-18a expression in MCF-7 with actinomycin D treatment (Fig. 3D), which suggested that the regulation of autophagy on miR-18a expression was at the post-transcriptional level.

Autophagy regulated breast cancer progression via miR-18a

Having identified the effect of autophagy on miR-18a expression, we further investigated whether autophagy regulated breast cancer progression via miR-18a *in vitro*. Administration of 3-MA suppressed MCF-7 cell proliferation and this effect was rescued by miR-18a overexpression (Fig. 4A). In migration assay, miR-18a overexpression significant inhibited the decreased migration capability induced by 3-MA (Fig. 4B). Moreover, decreased invasion capability induced by 3-MA was reversed by miR-18a expression (Fig. 4C).

DISCUSSION

Inflammation is a common property of all diseases forming mutually reinforcing relationships, which contributes to the exacerbation of human diseases. Although chronic inflammation extremely increases cancer risk, how inflammation promotes cancer progression is still not well understood. Recently, the concept of tumor microenvironment was proposed and the role of immune inflammation in tumor was further confirmed [12, 13]. A murine model of lung cancer was established by tobacco carcinogen nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in combination with pseudomonas aeruginosa LPS, which directly showed the promotive effect of LPS on cancer [14]. In our study, LPS exposure promoted MCF-7 cell proliferation, migration and invasion, showing a promotive effect on cancer progression *in vitro*.

Recently, miRNAs become one type of star molecules in human disease research, especially in cancer. Growing clinic studies have demonstrated that it is possible for miRNAs to be potential tools and targets for novel diagnostic approach [15, 16]. Our study exhibited the crucial role of miR-18a in LPS-induced breast cancer progression, showing a similar function of miR-18a in cancer progression induced by diverse factors. MiR-18a plays a positive role in breast cancer and promotes epithelial-mesenchymal transition to accelerate migration and invasion [17]. Higher miR-18a may result in poor prognosis by activating Wnt pathway in ER-positive breast cancer [18]. Similarly, we observed that miR-18a was elevated in MCF-7 cells after exposure to LPS and anti-miR-18a application suppressed breast cancer progression and cell survival *in vitro*. Moreover, anti-miR-18a administration increased the expression of pro-apoptotic pro-

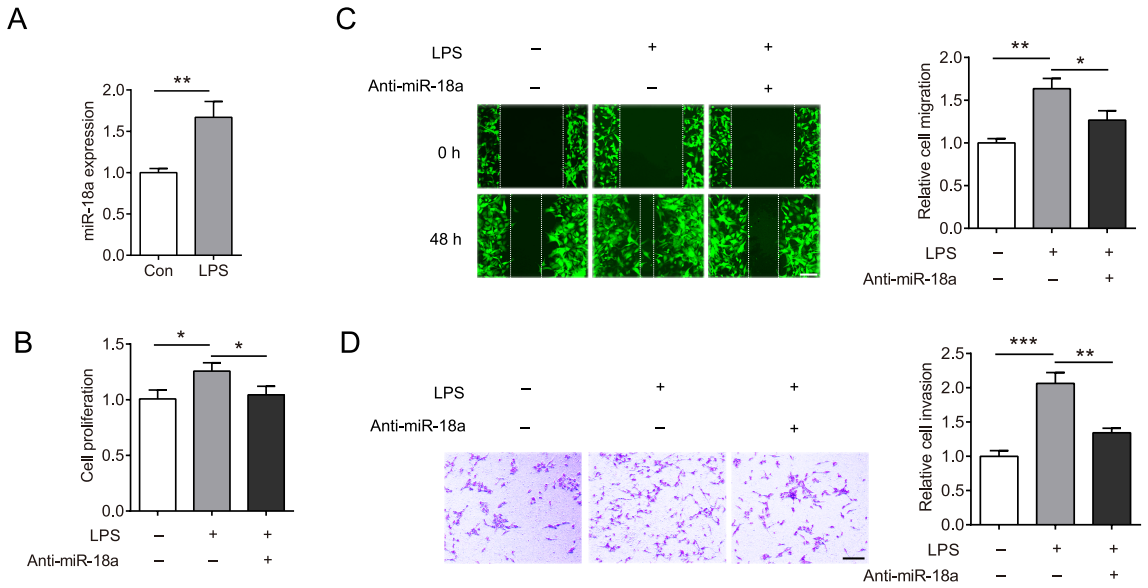

Fig. 1 The effect of miR-18a on LPS-induced breast cancer progression *in vitro*. (A) The expression of miR-18 in MCF-7 cells after LPS exposure. (B) Cell proliferation, as evaluated by CCK8 in LPS exposure with/without anti-miR-18a treatment. (C) Cell migration, as examined by wound healing assay in LPS exposure with/without anti-miR-18a treatment. Scale bar: 200 μm. (D) Cell invasion, as examined by transwell assay in LPS exposure with/without anti-miR-18a treatment. Scale bar: 200 μm. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. Con, control; LPS, lipopolysaccharide.

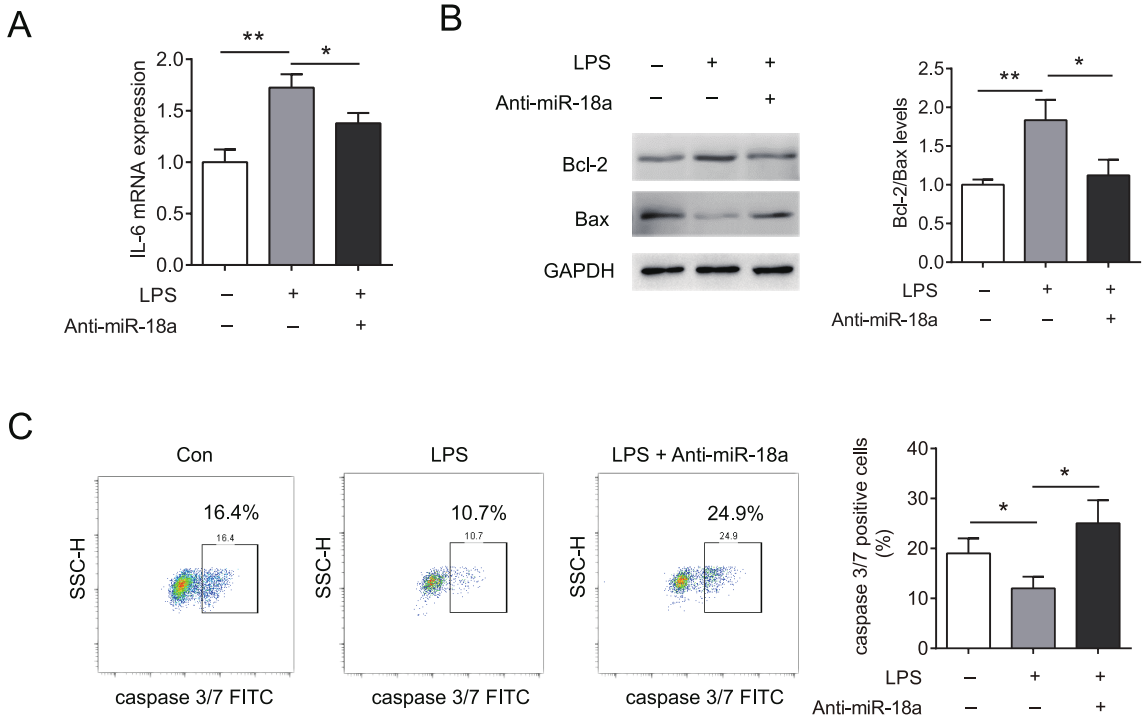

Fig. 2 Anti-miR-18a repressed inflammatory response and promoted MCF-7 cell apoptosis. (A) The expression of IL-6 mRNA, as examined by real-time PCR in LPS exposure with/without anti-miR-18a treatment. (B) Representative images, showing the expression of Bcl-2 and Bax in LPS exposure with/without anti-miR-18a treatment. Ratio of Bcl-2 and Bax, was shown by bar graph. (C) Representative images, showing the percent of activated caspase3/7 positive cells performed by flow cytometry analysis. $^{*}p < 0.05$ and $^{**}p < 0.01$. LPS, lipopolysaccharide; SSC-H, side scatter height; FITC, fluorescein isothiocyanate.

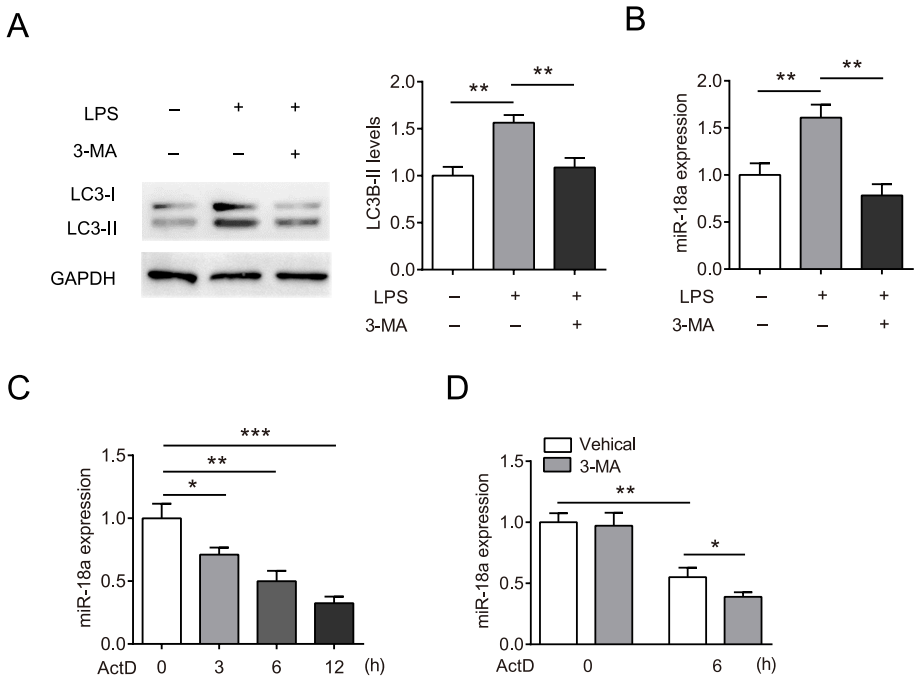

Fig. 3 Autophagy inhibited miR-18a degradation. (A) Representative images, showing the expression of LC3B in LPS exposure with/without 3-MA treatment. Relative LC3B-II expression was shown by bar graph. (B) The expression of miR-18a in MCF-7 cells in LPS exposure with/without 3-MA treatment. (C) The expression of miR-18a in MCF-7 cells after actinomycin D treatment. (D) The expression of miR-18a in MCF-7 cells with 3-MA and actinomycin D treatment. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. LPS, lipopolysaccharide; 3-MA, 3-Methyladenine; ActD, actinomycin D.

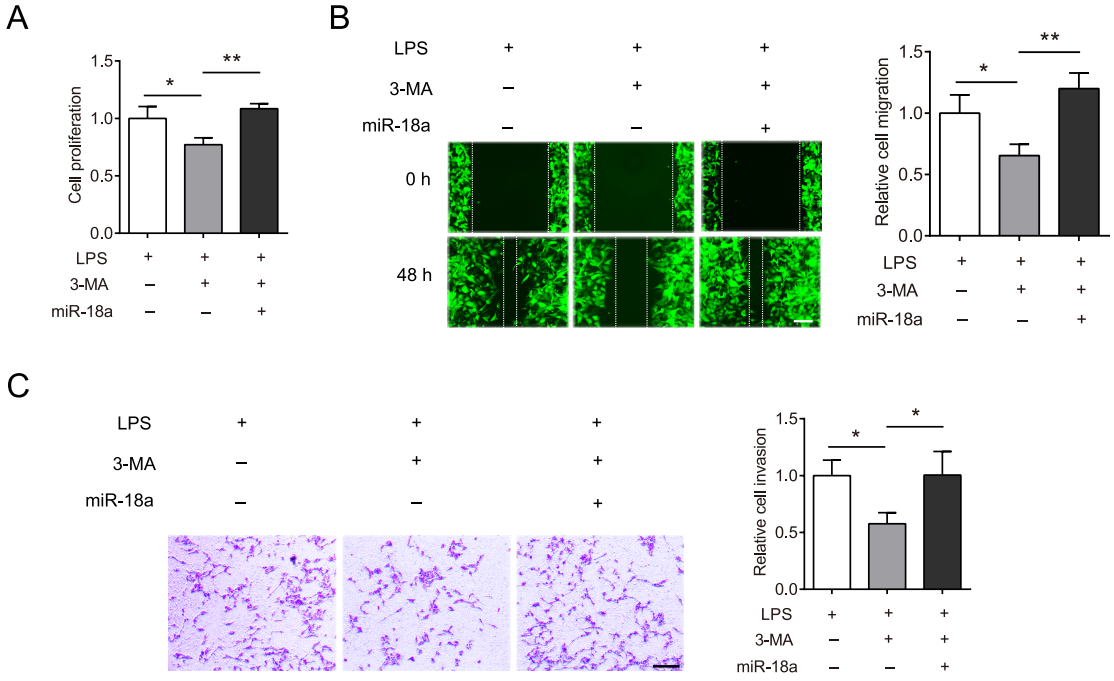

Fig. 4 Autophagy-miR-18a axis participated in breast cancer progression. (A) Cell proliferation, as evaluated by CCK8 in LPS exposure with/without 3-MA and miR-18a treatment. (B) Cell migration, as examined by wound healing assay in LPS exposure with/without 3-MA and miR-18a treatment. Scale bar: 200 μm. (C) Cell invasion, as examined by transwell assay in LPS exposure with/without 3-MA and miR-18a treatment. Scale bar: 200 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$. LPS, lipopolysaccharide; 3-MA, 3-Methyladenin.

tein Bax and decreased the expression of anti-apoptotic protein Bcl-2, which is consistent with the result of cell proliferation. Due to elevated miR-18a expression, LPS exposure promoted MCF-7 cell proliferation at a lower level of apoptosis. The above facts show that miR-18a could play a harmful role in many types of breast cancer. Thus, investigating the mechanism that cells remove miRNAs, when they are no longer needed, is key to fully understand how and when they function.

A large number of literatures have identified miRNAs as upstream regulators targeting mRNAs to affect cell autophagy level. Overexpression of miR-106a-5p inhibits BTG3 action and accelerates malignant phenotype via autophagy decline in nasopharyngeal carcinoma [19]. MiR-145-3p exerts a tumor suppressive action and enhances bortezomib sensitivity in multiple myeloma via autophagy by targeting HDAC4 [20]. MiR-10a-5p was lowly expressed as a tumor suppressor gene in oral carcinoma tissues and suppressed the metastasis of oral carcinoma cells *in vitro* by targeting PIK3CA via the PI3K/AKT/mTOR pathway [21]. These studies identify not only the relationship between miRNAs and autophagy, but also the antitumor effect of autophagy. However, higher level of autophagy-related protein (BECN1) could be a critical reason for paclitaxel resistance in ovarian cancer [22], which suggests that autophagy plays different roles in different tumor drug therapy. Cancer cells have abnormal basal metabolism levels, which means that autophagy needs to be activated to maintain cell basal metabolism and meet growth needs [23, 24]. There are also many cases of excessive autophagy or drug-induced autophagy promoting tumor cell death [25, 26]. Thus, the role of autophagy in inflammation-induced breast cancer progression is still unclear.

In addition to the complicated effect of autophagy, there is almost no report about autophagy-mediated miRNA expression in human cancer. Recently, a study on autophagy in yeast has attracted great attention that proves selective mRNA degradation by rapamycin-induced autophagy [27]. In our study, we applied 3-MA to inhibit autophagy of MCF-7 cells and examined miR-18a expression. Interestingly, 3-MA administration significantly reduced miR-18a expression in MCF-7 cells, suggesting a possibility that autophagy deficiency inhibited transcription of pri-miR-18a or autophagy deficiency promoted miR-18a degradation. To exclude the potential effect of autophagy on DNA transcription, MCF-7 cells were treated with actinomycin D to inhibit DNA transcriptive progression. When DNA transcription is inhibited by actinomycin D, miR-18a expression was decreased in a time-dependent manner, conforming to the rule of RNA degradation. Contrary to the previous content on the effect of autophagy in yeast, application of 3-MA in MCF-7 cells promoted the degradation of miR-18a. Autophagy is an important way for cells to maintain metabolism by degrading broken and senescent organelles, as well as dysfunctional proteins [28]. Based on the above facts, we analyzed that the degradation of miR-18a by autophagy is not a direct process, but may depend on a certain protein. These results indicated that autophagy positively regulated miR-18a expression in inflammation-induced breast cancer progression *in vitro*.

CONCLUSION

In summary, we showed that miR-18a was a key factor in LPS-induced breast cancer progression. The increased expression of miR-18a induced by LPS was mediated by elevated autophagy. Our findings provided an interesting regulation underlying LPS-induced breast cancer progression, which may be beneficial to understand the mechanism of breast cancer.

Acknowledgements: This study is funded by Nantong Municipal Science and Technology Plan Guiding Project (MSZ19106).

REFERENCES

1. Thorat MA, Balasubramanian R (2020) Breast cancer prevention in high-risk women. *Best Pract Res Clin Obstet Gynaecol* **65**, 18–31.
2. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, Shi W, Jiang J, et al (2017) Risk factors and preventions of breast cancer. *Int J Biol Sci* **13**, 1387–1397.
3. Coussens LM, Werb Z (2002) Inflammation and cancer. *Nature* **420**, 860–867.
4. Diakos CI, Charles KA, McMillan DC, Clarke SJ (2014) Cancer-related inflammation and treatment effectiveness. *Lancet Oncol* **15**, 493–503.
5. Tezcan G, Garanina EE, Alsaadi M, Gilazieva ZE, Martinova EV, Markelova MI, Arkhipova SS, Hamza S, et al (2020) Therapeutic potential of pharmacological targeting nlrp3 inflammasome complex in cancer. *Front Immunol* **11**, 607881.
6. McGuire A, Brown JA, Kerin MJ (2015) Metastatic breast cancer: The potential of mirna for diagnosis and treatment monitoring. *Cancer Metastasis Rev* **34**, 145–155.
7. Kahraman M, Roske A, Laufer T, Fehlmann T, Backes C, Kern F, Kohlhaas J, Schrors H, et al (2018) Microrna in diagnosis and therapy monitoring of early-stage triple-negative breast cancer. *Sci Rep* **8**, 11584.
8. Yu L, Zhao J, Gao L (2018) Predicting potential drugs for breast cancer based on mirna and tissue specificity. *Int J Biol Sci* **14**, 971–982.
9. Shidfar A, Costa FF, Scholtens D, Bischof JM, Sullivan ME, Ivancic DZ, Vanin EF, Soares MB, et al (2017) Expression of mir-18a and mir-210 in normal breast tissue as candidate biomarkers of breast cancer risk. *Cancer Prev Res (Phila)* **10**, 89–97.
10. Luengo-Gil G, Garcia-Martinez E, Chaves-Benito A, Conesa-Zamora P, Navarro-Manzano E, Gonzalez-Billalabeitia E, Garcia-Garre E, Martinez-Carrasco A, et al (2019) Clinical and biological impact of mir-18a expression in breast cancer after neoadjuvant chemotherapy. *Cell Oncol (Dordr)* **42**, 627–644.
11. Liu Z, Wang Y, Dou C, Xu M, Sun L, Wang L, Yao B, Li Q, et al (2018) Hypoxia-induced up-regulation of vasp

promotes invasiveness and metastasis of hepatocellular carcinoma. *Theranostics* **8**, 4649–4663.
12. Han S, Wang W, Wang S, Yang T, Zhang G, Wang D, Ju R, Lu Y, et al (2021) Tumor microenvironment remodeling and tumor therapy based on m2-like tumor associated macrophage-targeting nano-complexes. *Theranostics* **11**, 2892–2916.
13. Palliyage GH, Ghosh R, Rojanasakul Y (2020) Cancer chemoresistance and therapeutic strategies targeting tumor microenvironment. *ScienceAsia* **46**, 639–649.
14. Liu CH, Chen Z, Chen K, Liao FT, Chung CE, Liu X, Lin YC, Keohavong P, et al (2021) Lipopolysaccharide-mediated chronic inflammation promotes tobacco carcinogen-induced lung cancer and determines the efficacy of immunotherapy. *Cancer Res* **81**, 144–157.
15. Lu TX, Rothenberg ME (2018) Microrna. *J Allergy Clin Immunol* **141**, 1202–1207.
16. Paskeh MDA, Mirzaei S, Orouei S, Zabolian A, Saleki H, Azami N, Hushmandi K, Baradaran B, et al (2021) Revealing the role of mirna-489 as a new onco-suppressor factor in different cancers based on pre-clinical and clinical evidence. *Int J Biol Macromol* **191**, 727–737.
17. Zhang N, Zhang H, Liu Y, Su P, Zhang J, Wang X, Sun M, Chen B, et al (2019) Srebp1, targeted by mir-18a-5p, modulates epithelial-mesenchymal transition in breast cancer via forming a co-repressor complex with snail and hdac1/2. *Cell Death Differ* **26**, 843–859.
18. Nair MG, Prabhu JS, Korlimarla A, Rajarajan S, P SH, Kaul R, Alexander A, Raghavan R, et al (2020) Mir-18a activates wnt pathway in er-positive breast cancer and is associated with poor prognosis. *Cancer Med* **9**, 5587–5597.
19. Zhu Q, Zhang Q, Gu M, Zhang K, Xia T, Zhang S, Chen W, Yin H, et al (2021) Mir106a-5p upregulation suppresses autophagy and accelerates malignant phenotype in nasopharyngeal carcinoma. *Autophagy* **17**, 1667–1683.
20. Wu H, Liu C, Yang Q, Xin C, Du J, Sun F, Zhou L (2020) Mir145-3p promotes autophagy and enhances bortezomib sensitivity in multiple myeloma by targeting hdac4. *Autophagy* **16**, 683–697.
21. Tao R, Wang F, Feng XK, Yang WD (2022) Mir-10a-5p inhibits the migration and invasion of human oral carcinoma cells by targeting pik3ca through pi3k/akt/mtor pathway. *ScienceAsia* **48**, 538–544.
22. Zhang SF, Wang XY, Fu ZQ, Peng QH, Zhang JY, Ye F, Fu YF, Zhou CY, et al (2015) Txndc17 promotes paclitaxel resistance via inducing autophagy in ovarian cancer. *Autophagy* **11**, 225–238.
23. Poillet-Perez L, White E (2019) Role of tumor and host autophagy in cancer metabolism. *Genes Dev* **33**, 610–619.
24. Yang MC, Wang HC, Hou YC, Tung HL, Chiu TJ, Shan YS (2015) Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. *Mol Cancer* **14**, 179.
25. Wang Y, Xiong H, Liu D, Hill C, Ertay A, Li J, Zou Y, Miller P, et al (2019) Autophagy inhibition specifically promotes epithelial-mesenchymal transition and invasion in ras-mutated cancer cells. *Autophagy* **15**, 886–899.
26. Li GM, Li L, Li MQ, Chen X, Su Q, Deng ZJ, Liu HB, Li B, et al (2021) Dapk3 inhibits gastric cancer progression via activation of ulk1-dependent autophagy. *Cell Death Differ* **28**, 952–967.
27. Makino S, Kawamata T, Iwasaki S, Ohsumi Y (2021) Selectivity of mrna degradation by autophagy in yeast. *Nat Commun* **12**, 2316.
28. White E, Mehnert JM, Chan CS (2015) Autophagy, metabolism, and cancer. *Clin Cancer Res* **21**, 5037–5046.