M2 macrophages contribute to cell proliferation and migration of breast cancer

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Abstract

Background: Breast cancer is a kind of malignant tumor that severely threatens women's health and life worldwide. Macrophages have been reported to mediate tumor progression, while the potential mechanism still needs further identification.

Methods: Human monocytic cell line THP-1 was used to induce M2-macrophage. Real-time PCR and western blot were performed to determine gene expression in mRNA and protein level, respectively. Cell proliferation was determined using MTT assays, while cell migration was detected based on the scratch wound healing assays.

Results: The supernatant medium of M2-macrophages incubated breast cancer cells showed increased cell proliferation and reduced expression of IRF-7. Overexpression of IRF-7 reversed the increased level of M2-macrophage induced cell proliferation and migration. The supernatant medium of M2-macrophages incubation promoted miR-1587 expression in breast cancer cells. miR-1587 overexpression promoted cell proliferation and migration of breast cancer. In addition, miR-1587 knockdown suppressed cell proliferation and migration that induced by M2-macrophages. miR-1587 targets IRF-7 to regulate its expression. Knockdown of IRF-7 reversed the effects of miR-1587 knockdown on cell proliferation and migration.

Conclusion: Collectively, this study revealed that miR-1587/IRF-7 mediated the mechanism of M2-macrophages-induced breast cancer progression, and this would shed light on the further clinical therapy of breast cancer.

Introduction

Breast cancer is the most common cancer in women, with the second leading cause of cancer-related death. Annually, more than 1.8 million women were diagnosed with breast cancer worldwide, and most of them are diagnosed as no metastatic disease ⁽¹⁾. While the late diagnosis results to poor prognosis and a high mortality rate. The biological behaviors of breast cancer are early recurrence, rapid malignant progression, as well as short overall survival time, which seriously threaten patients' health and life. Despite the improvement on detection and treatment, the mortality rate still high. Therefore, to understand the pathobiology of breast cancer progression would be more important on reducing mortality rate and increasing patients' survival.

Tumor microenvironment (TME) is a complex interaction among various cells, such as tumor cells, immune cells, endothelial cells, and fibroblasts. Tumor-associated macrophages (TAMs), accounting for 50–80% of interstitial cells, are the important components of TME ⁽²⁾. TAMs mediate immune response by inhibiting T-cell proliferation and preventing expressing immunosuppressive molecules, which could eliminate tumor cells, and promote tumor growth and survival ^(3, 4). Additionally, TAMs are correlated with the poor prognosis of breast cancer ⁽⁵⁾. TAMs can be classified into classical pathway-activated

macrophages (M1-type) and alternative pathway-activated macrophages (M2-type). M1-type TAMs mainly show an anti-cancer effect, while M2-type mainly manifest the opposite⁽⁶⁾. TAMs in tumor tissues tend to polarize into the M2 phenotype, which produces anti-inflammatory factors, and contributes to tumor cells immune escape, further promotes tumor progression. Therefore, to identify the mechanism of M2 on breast cancer progression seems to be important.

Type I interferons (IFNs) are the main defensive factors on the innate immune response when during the viral infection, and thus identified as therapeutic agents viral infection^(7,8). The expression of IFNs is mediated by pattern recognition receptors (PRRs), and further leads to the phosphorylation of IFN regulatory factor 7 (IRF7) and IRF3⁽⁹⁾. It has been reported that IRF7 activation and expression can be regulated by various mechanisms in transcriptional, translational and posttranslational level⁽¹⁰⁾. What's more, IRF7 activity and stability are also regulated by its phosphorylation, ubiquitination, SUMOylation and acetylation⁽¹⁰⁾. In addition, IRF7 expression is induced by viral infections⁽¹¹⁾. Recently, IRF7 is identified to mediate cancer progression and inflammatory disorders^(12,13). And the progression of breast cancer can be controlled by IRF7^(14,15).

In the present study, we found that M2 stimulation could suppress IRF7 expression, which promotes cell proliferation and migration. Afterwards, the potential mechanism underlying IRF7 on breast cancer progression was explored. We showed that miR-1587 targets IRF7 to regulate M2-induced breast cancer progression.

Material And Methods

Cell culture

The human monocytic cell line THP-1 and human breast cancer cell line MDA-MB-231 were purchased from American Type Culture Collection (ATCC) and cultured in the Dulbecco's modified Eagles' medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO₂. To induce M2-type macrophages, THP-1 were pretreated with phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) for 18 h, and then cultured by IL-4 (25 ng/mL) for 3 d. For the following experiments, both M2-type macrophages and THP-1 cells were planted in the 96-well plates with 2×10^5 cells/mL, 200 μ L/well. After 24 h, the supernatant (100 μ L) was collected to culture MDA-MB-231 cells for 24 h.

Real-time PCR

Total RNA was isolated from cells using Trizol reagent (Invitrogen) and quantified by 10% agarose gel electrophoresis. The extracted RNA was used to reverse-transcribe into cDNA. For real-time PCR, cDNA was used as the template, and SYBR Green Real-time PCR Master Mix (Toyobo, Japan) was used for the experiments based on an ABI 7500 real-time PCR system (Applied Biosystems). The mRNA expression was assessed using $2^{-\Delta\Delta C_t}$ method. GAPDH served as internal control.

Western blot

Cells were treated by RIPA buffer and centrifuged at 15,000 g under 4°C for 15 min. The collected protein concentration was determined by BCA protein assay kit (Invitrogen). After separated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the protein was transferred onto PVDF and incubated with the primary antibodies of Arg-1, CD86, CD206, iNOS, IRF5, IRF6, IRF7, IRF9 and GAPDH (Abcam) at 4°C for 24 h. After washed by PBS for 3 times, the PVDF was incubated with the secondary antibody (Beyotime, Shanghai, China) at room temperature for 1 h. The protein bands were visualized by ECL methods. GAPDH served as internal control.

Cell proliferation

Cell proliferation was determined using Cell Counting Kit-8 (CCK-8, Sangon Biotech, Shanghai, China). Briefly, cells were planted at a 96-well plate with the concentration of 10^3 cells per well. After 24 h, the cells were incubated with CCK-8 (10 μ L) solution at 37 °C for 3 h. A microplate reader was employed to detect the absorbance at 490 nm (Bio-Rad, CA, USA).

Cell migration

For determination cell migration, scratch wound healing assays were performed. Briefly, cells were seeded in a 6-well plate with the concentration at 10^5 cells per well. After 25 h, cells were spread across the plate, and 200 μ L micropipette tip was used to scratch through the cells. After washing by PBS, cells were cultured at 37°C with 5% CO₂. After 24 h, cell migration was photographed with an inverted microscope (40 × magnifications, Olympus, Japan).

Cell transfection

The inhibitor and the mimic of miR-1587 and their negative control were purchased from Sangon Biotech (Shanghai, China). The pcDNA-IRF7, si-IRF7 and their negative control were purchased from RiboBio (Guangzhou, China). After reached to 70% influence, cells were transfected with the Lipofectamine 3000 (Invitrogen) according to the manufacturer's instruction. After 24 h, the transfection efficiency was determined.

Dual-luciferase reporter assay

The fragment of IRF7 3'UTR containing the predicted binding sites of miR-1587 wild-type (WT) or mutant (MUT) was amplified and cloned into pmirGLO luciferase vector (Promega, USA). Next, the miR-1587 mimic or its negative control was co-transfected with the reporter plasmid into HEK293 cells. After 48 h, the luciferase activity was determined using a dual-luciferase reporter assay system (Promega, USA) following the manufacturer's instruction.

Animals

A total of 18 female BALB/c nude mice (6-weeks old) were purchased from Vital River (Beijing, China). Mice were raised under standard condition at 25°C, and 12-12 h dark-light cycles. All mice were randomly divided into 3 groups (n=6 in each group), including control, M2 and M2+miR-1587(-). In control group, mice were subcutaneously injected MDA-MB-231 cells (5×10^6 cells/200 μ L PBS/mouse). In the M2 group, MDA-MB-231 cells were co-cultured with the supernatant medium of M2-type macrophages for 24 h, and then subcutaneously injected into mice. In the M2+miR-1587 (-) group, MDA-MB-231 cells were transfected with anti-miR-1587 and then co-cultured with the supernatant medium of M2-type macrophages for 24 h, after that, the cells were subcutaneously injected into mice. After 7 d, the supernatant medium of M2-type macrophage was injected into tumor (100 μ L/mouse), and the injection was performed every three days. Tumor volumes were measured once per week for 4 weeks. Four weeks later, all mice were euthanasia, and tumor tissues were collected for determination of miR-1587 and IRF7 expression.

The study was approved by the Ethics Committee of Huai'an Second People's Hospital and the Affiliated Huai'an Hospital of Xuzhou Medical University, and all experiments were handled in accordance with the animal care and use guidelines.

Statistical analysis

All experiments were repeated for three independent times. Statistical analysis was performed based on the SPSS 20.0 and GraphPad prism 8.0 software. Data were presented as means $\pm$ SD. Statistical difference between two groups was analyzed using Student t-test, while one-way analysis (ANOVA) was conducted to determine difference among three or more groups. $P < 0.05$ was considered as statistically significant difference.

Results

M2-type macrophages reduce IRF7 expression in breast cancer cells

In order to examine the effect of M2 macrophages on IRF7 expression, the macrophages (Ma) were prior induced by PMA using THP-1, subsequently, IL-4 was administered to induce M2 macrophages (M2). First, we examined M2-type marker Arg-1, CD86, and CD206, as well as M1-type marker iNOS expression, and we found that the expression levels of Arg-1, CD86, and CD206 were significantly increased in IL-4 stimulated Ma in comparing with Ma, while iNOS expression showed no significantly difference between two groups (Figure 1a), indicating M2-type macrophages were successfully induced by IL-4. Next, the supernatant medium of both M2-type macrophages and THP-1 cells were collected to culture breast cancer cells MDA-MB-231, and we found that supernatant medium of M2-type macrophages promoted cell proliferation (Figure 1b). In addition, the expression of IRF5, IRF6 and IRF7 was strongly decreased, while IRF9 was increased in MDA-MB-231 that stimulated with the supernatant medium of M2-type macrophages in comparing with control, and IRF7 showed significant difference between the two groups (Figure 1c and d). Thus, IRF7 was considered as a candidate to mediate the breast cancer progression.

IRF7 overexpression suppresses M2 macrophage-induced cell proliferation and migration

To identify the role of IRF7 on M2 macrophage stimulated breast cancer cells behaviors, MDA-MB-231 cells were divided into 4 groups, including control, M2, M2+NC, and M2+IRF7(+). The cells in control group did not receive any treatment. Cells in M2 group received the treatment of supernatant medium of M2 macrophage. IRF7 overexpressed plasmid was constructed to induce the expression of IRF7 in MDA-MB-23 cells that treated with the supernatant medium of M2-macrophage, and considered as M2+IRF7(+) group, while M2+NC were MDA-MB-23 cells received the NC of IRF7(+) and then treated with the supernatant medium of M2 macrophage. We found that the supernatant medium of M2 macrophage treatment significantly suppressed IRF7 expression, which was abolished by IRF7 overexpression (Figure 2a). The supernatant medium of M2 macrophage treatment induced MDA-MB-231 cell proliferation and migration, while IRF7 overexpression seemed to abolish its effects (Figure 2b and c). Taken together, the results demonstrate IRF7 mediates breast cancer progression.

The increased miR-1587 expression promotes cell proliferation and migration

MiR-1587 has been identified to mediate the tumorigenicity of glioma ⁽¹⁶⁾. An online prediction showed that miR-1587 has binding site with IRF7 3'UTR. To explore the potential mechanism of IRF7 on breast cancer progression, we selected miR-1587 as a potential candidate on IRF7 regulation. Interestingly, we found that the miR-1587 expression was higher in MDA-MB-231 cells that treated with the supernatant medium of M2-macrophage rather than that in MDA-MB-231 cells without any treatment (Figure 3a). The transfection efficiency of miR-1587 overexpression was determined using real-time PCR (Figure 3b). miR-1587 overexpression induced cell proliferation and migration of MDA-MB-231 cells (Figure 3c and d). Additionally, miR-1587 knockdown seemed to reduce miR-1587 expression that induced by the supernatant medium of M2 macrophage (Figure 3e). What's more, cell proliferation and migration of MDA-MB-231 cells that induced by the supernatant medium of M2 macrophage was also abolished by miR-1587 knockdown (Figure 3f and g). All those results demonstrate that miR-1587 mediates the behaviors of M2 macrophages-treated MDA-MB-231.

IRF7 is a target of miR-1587

To identify the interaction between miR-1587 and IRF7, MDA-MB-231 cells were overexpressed miR-1587, and we found that IRF7 mRNA expression was increased in comparing with NC group, while knockdown of miR-1587 simultaneously suppressed IRF7 mRNA expression (Figure 4a). Western blot analysis resulted to a consistent result on IRF7 mRNA expression (Figure 4b). Based on an online prediction, we found that miR-1587 has potential binding sites with IRF7 3'UTR (Figure 4c). Next, the luciferase reporter plasmids containing WT-IRF7 3'-UTR or MUT-IRF7 3'-UTR were co-transfected with miR-1587 mimic into HEK293 to determine the regulatory pattern of miR-1587 on IRF7 expression, and the results showed that miR-1587 mimic reduced the relative luciferase activity of Luciferase reporter plasmids containing IRF7 3'-UTR (Fig. 4d). All those results demonstrated that miR-1587 targets IRF7 to suppress its expression.

MiR-1587 dedicates cell progression by down-regulating IRF7 in breast cancer

We further evaluated the interaction between miR-1587 and IRF7 on breast cancer progression. As it is presented in the figure 5a, in comparing with MDA-MB-231 cells without any treatment, the supernatant medium of M2 macrophage treatment suppressed IRF7 expression in MDA-MB-231 cells, while miR-1587 knockdown showed more efficient on inducing it. In addition, miR-1587 knockdown suppressed cell proliferation and migration that induced by M2, however, they were abolished by IRF7 knockdown (Figure 5b and c). Next, the in vivo experiments were employed to verify the role of miR-1587 and IRF7 on tumor progression. As presented in figure 5d, mice subcutaneously injected MDA-MB-231 cells that pretreated with the supernatant medium of M2 macrophage showed the biggest tumor volume, which was suppressed by miR-1587 knockdown, and the xenograft tumor at 28 day were also presented (Figure 5d). The tumor tissues were collected, with the results that M2 treated mice showed higher expressions of miR-1587 and lower expression of IRF7, while they were abolished by miR-1587 knockdown (Figure 5e and f).

Discussion

It was accepted that the TAMs in cancer contribute to tumor progression by enhancing the capacity of tumor invasion, metastasis, drug-resistance, angiogenesis, as well as immune escape ^(17–20). MicroRNAs (miRNAs) are single-stranded RNA molecules that mediated gene expression at posttranscriptional level in various processes. It has been reported that miRNAs mediate TAMs functions. For example, miR-148b regulates TAMs infiltration in hepatocellular carcinoma ⁽²¹⁾. TAM-derived exosomes carried miR-501-3p promotes pancreatic ductal adenocarcinoma ⁽²²⁾. In the present study, we found miR-1587 promotes breast cancer progression by mediating TAMs.

MiR-1587 function has been reported to be regulated by the induction of G-quadruplex formation and dimerization ⁽²³⁾. Glioma-associated mesenchymal stem cells-derived exosomal miR-1587 contributes to the tumorigenicity ⁽¹⁶⁾. miR-1587 targets ZEB2 to regulate cell proliferation, migration, apoptosis, and autophagy in keloid fibroblasts ⁽²⁴⁾. In the present study, we found that the supernatant medium of M2 macrophage treated breast cancer cells showed high level of miR-1587, and miR-1587 overexpression contributes to the cell proliferation and migration of breast cancer cells, indicating that miR-1587 mediates the TAMs function of breast cancer. What's more, just as the preliminary report, exosomes enriched miR-1587 mediates glioma progression ⁽¹⁶⁾. We found that miR-1587 is enriched in breast cancer cells that pretreated with the supernatant of M2 macrophages, and further promoted it progression. However, whether miR-1587 is enriched in the M2 macrophages still needs further identification.

Interferon regulatory factors (IRFs) are reported to mediate cell differentiation, apoptosis, cell cycle, and immune response ^(25, 26). Recent studies have shown that IRF1, IRF6, IRF7, and IRF8 served as cancer suppressor genes and mediated breast cancer cell activity ^(27, 28). IRF7 is an important family member of IRFs, and acts as a transcription factor to manipulate IFN expression, as well as interferon stimulating response genes. The phosphorylated IRF7 that induced by TBK1/IKK can be transported to the nucleus,

and then participated to IRF3 mediated biological processes under virus infection ⁽²⁵⁾. The IRF7 expression has been studied in various cancers. For example, the up-regulated IRF7 mediated cell migration and invasion of non-small cell lung cancer. The elevated IRF7 expression is associated with poor prognosis of patients with renal cell carcinoma ⁽²⁹⁾. In addition, the down-regulated IRF7 is correlated with the lower overall survival of gallbladder carcinoma ⁽³⁰⁾. In the breast cancer, up-regulation of IRF7 in the treated cancer cells promoted resistance to chemotherapy ⁽¹⁴⁾. In the present study, we found that IRF7 was decreased in the breast cancer cells that treated by the supernatant medium of M2-macrophage, indicating that IRF7 mediated TAMs function in breast cancer. Interestingly, miR-1587 has binding sites with IRF7 3'UTR, combining with the luciferase assays, we concluded that miR-1587 targets IRF7 to regulate its expression.

Breast cell behaviors, such as cell proliferation, migration, invasion, and apoptosis have been considered in tumor progression study. In the present study, we found that the supernatant medium of M2 macrophage promoted tumor progression by inducing cell proliferation and migration, which might due to that M2 could produce anti-inflammatory factors, and induce immune escape. In addition, the supernatant medium of M2 macrophage treatment promoted miR-1587, but suppressed IRF7 expression, and miR-1587 knockdown abolished the effect of M2, while knockdown of IRF7 reversed the effect of miR-1587 knockdown on cell proliferation and migration. The in vivo experiments verified the role of miR-1587 on tumor growth. Those results indicated that miR-1587/IRF7 mediates M2 macrophage induced breast cancer progression.

Conclusion

Taken together, the results in the present study demonstrated that M2 could induce breast cancer cell proliferation and migration, as well as the down-regulated IRF7. MiR-1587 targets IRF7 to regulate breast cancer cell proliferation and migration. MiR-1587/IRF7 mediates M2 macrophage induced cell proliferation and migration of breast cancer. This study may provide a promising biomarker for further breast cancer therapy.

Declarations

Ethics approval and consent to participate

This study was approved by the Huai'an Second People's Hospital and the Affiliated Huai'an Hospital of Xuzhou Medical University.

Consent for publication

The study was undertaken with the consent of the Huai'an Second People's Hospital and the Affiliated Huai'an Hospital of Xuzhou Medical University.

Availability of data and material

Not available.

Competing interests

All authors declare no conflict of interest.

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Author contribution

Xiaoyu Zhang, Daoyuan Tu , Jin Dou , and Mingkao Wang conceived the conception and design. Daoyuan Tu , Jin Dou , and Mingkao Wang conducted the experiments. Daoyuan Tu and Jin Dou collected the data. Mingkao Wang analyzed the data. Xiaoyu Zhang revised the manuscript.

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Not available.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424.
2. Meng Z, Zhang R, Wang Y, Zhu G, Jin T, Li C, Zhang S. (2019) miR-200c/PAI-2 promotes the progression of triple negative breast cancer via M1/M2 polarization induction of macrophage, *Int Immunopharmacol*, 106028.
3. Aras S, Zaidi MR. TAMEless traitors: macrophages in cancer progression and metastasis. *Br J Cancer*. 2017;117:1583–91.
4. Zhao X, Qu J, Sun Y, Wang J, Liu X, Wang F, Zhang H, Wang W, Ma X, Gao X, Zhang S. (2017) Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature, *Oncotarget* 8, 30576–30586.
5. Choi J, Gyamfi J, Jang H, Koo JS. The role of tumor-associated macrophage in breast cancer biology. *Histol Histopathol*. 2018;33:133–45.
6. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14:399–416.
7. Alberti A, Benvegna L. Management of hepatitis C. *J Hepatol*. 2003;38(Suppl 1):104–18.
8. Festi D, Sandri L, Mazzella G, Roda E, Sacco T, Staniscia T, Capodicasa S, Vestito A, Colecchia A. Safety of interferon beta treatment for chronic HCV hepatitis. *World J Gastroenterol*. 2004;10:12–6.

9. Akira S, Uematsu S, Takeuchi O. (2006) Pathogen recognition and innate immunity, *Cell* 124, 783–801.
10. Ning S, Pagano JS, Barber GN. IRF7: activation, regulation, modification and function. *Genes Immun.* 2011;12:399–414.
11. Ko YA, Chan YH, Liu CH, Liang JJ, Chuang TH, Hsueh YP, Lin YL, Lin KI. (2018) Blimp-1-Mediated Pathway Promotes Type I IFN Production in Plasmacytoid Dendritic Cells by Targeting to Interleukin-1 Receptor-Associated Kinase M, *Front Immunol* 9, 1828.
12. Ling T, Weng GX, Li J, Li C, Wang W, Cao L, Rao H, Ju C, Xu LG. TARBP2 inhibits IRF7 activation by suppressing TRAF6-mediated K63-linked ubiquitination of IRF7. *Mol Immunol.* 2019;109:116–25.
13. Rajagopalan D, Tirado-Magallanes R, Bhatia SS, Teo WS, Sian S, Hora S, Lee KK, Zhang Y, Jadhav SP, Wu Y, Gan YH, Karnani N, Benoukraf T, Jha S. TIP60 represses activation of endogenous retroviral elements. *Nucleic Acids Res.* 2018;46:9456–70.
14. Lan Q, Peyvandi S, Duffey N, Huang YT, Barras D, Held W, Richard F, Delorenzi M, Sotiriou C, Desmedt C, Lorusso G, Ruegg C. Type I interferon/IRF7 axis instigates chemotherapy-induced immunological dormancy in breast cancer. *Oncogene.* 2019;38:2814–29.
15. Touati N, Tryfonidis K, Caramia F, Bonnefoi H, Cameron D, Slaets L, Parker BS, Loi S. Correlation between severe infection and breast cancer metastases in the EORTC 10994/BIG 1 – 00 trial: Investigating innate immunity as a tumour suppressor in breast cancer. *Eur J Cancer.* 2017;72:95–102.
16. Figueroa J, Phillips LM, Shahar T, Hossain A, Gumin J, Kim H, Bean AJ, Calin GA, Fueyo J, Walters ET, Kalluri R, Verhaak RG, Lang FF. Exosomes from Glioma-Associated Mesenchymal Stem Cells Increase the Tumorigenicity of Glioma Stem-like Cells via Transfer of miR-1587. *Cancer Res.* 2017;77:5808–19.
17. Liu H, Wang J, Zhang M, Xuan Q, Wang Z, Lian X, Zhang Q. Jagged1 promotes aromatase inhibitor resistance by modulating tumor-associated macrophage differentiation in breast cancer patients. *Breast Cancer Res Treat.* 2017;166:95–107.
18. Tevis KM, Cecchi RJ, Colson YL, Grinstaff MW. Mimicking the tumor microenvironment to regulate macrophage phenotype and assessing chemotherapeutic efficacy in embedded cancer cell/macrophage spheroid models. *Acta Biomater.* 2017;50:271–9.
19. Li F, Kitajima S, Kohno S, Yoshida A, Tange S, Sasaki S, Okada N, Nishimoto Y, Muranaka H, Nagatani N, Suzuki M, Masuda S, Thai TC, Nishiuchi T, Tanaka T, Barbie DA, Mukaida N, Takahashi C. Retinoblastoma Inactivation Induces a Protumoral Microenvironment via Enhanced CCL2 Secretion. *Cancer Res.* 2019;79:3903–15.
20. Banerjee S, Lin CF, Skinner KA, Schiffhauer LM, Peacock J, Hicks DG, Redmond EM, Morrow D, Huston A, Shayne M, Langstein HN, Miller-Graziano CL, Strickland J, O'Donoghue L, De AK. Heat shock protein 27 differentiates tolerogenic macrophages that may support human breast cancer progression. *Cancer Res.* 2011;71:318–27.

21. Ke M, Zhang Z, Cong L, Zhao S, Li Y, Wang X, Lv Y, Zhu Y, Dong J. MicroRNA-148b-colony-stimulating factor-1 signaling-induced tumor-associated macrophage infiltration promotes hepatocellular carcinoma metastasis. *Biomed Pharmacother.* 2019;120:109523.
22. Yin Z, Ma T, Huang B, Lin L, Zhou Y, Yan J, Zou Y, Chen S. Macrophage-derived exosomal microRNA-501-3p promotes progression of pancreatic ductal adenocarcinoma through the TGFBR3-mediated TGF-beta signaling pathway. *J Exp Clin Cancer Res.* 2019;38:310.
23. Tan W, Yi L, Zhu Z, Zhang L, Zhou J, Yuan G. (2018) Hsa-miR-1587 G-quadruplex formation and dimerization induced by NH₄(+), molecular crowding environment and jatrorrhizine derivatives, *Talanta* 179, 337–343.
24. Hou Z, Fan F, Liu P. (2019) BTXA regulates the epithelial-mesenchymal transition and autophagy of keloid fibroblasts via modulating miR-1587/miR-2392 targeted ZEB2, *Biosci Rep* 39.
25. Chen YJ, Li J, Lu N, Shen XZ. Interferon regulatory factors: A key to tumour immunity. *Int Immunopharmacol.* 2017;49:1–5.
26. Tamura T, Yanai H, Savitsky D, Taniguchi T. The IRF family transcription factors in immunity and oncogenesis. *Annu Rev Immunol.* 2008;26:535–84.
27. Xu HF, Huang TJ, Yang Q, Xu L, Lin F, Lang YH, Hu H, Peng LX, Meng DF, Xie YJ, Tan L, Qian CN, Huang BJ. Candidate tumor suppressor gene IRF6 is involved in human breast cancer pathogenesis via modulating PI3K-regulatory subunit PIK3R2 expression. *Cancer Manag Res.* 2019;11:5557–72.
28. Luo X, Xiong X, Shao Q, Xiang T, Li L, Yin X, Li X, Tao Q, Ren G. (2017) The tumor suppressor interferon regulatory factor 8 inhibits beta-catenin signaling in breast cancers, but is frequently silenced by promoter methylation, *Oncotarget* 8, 48875–48888.
29. Lin L, Cai J. (2020) Circular RNA circ-EGLN3 promotes renal cell carcinoma proliferation and aggressiveness via miR-1299-mediated IRF7 activation, *J Cell Biochem.*
30. Goeppert B, Truckenmueller F, Ori A, Fritz V, Albrecht T, Fraas A, Scherer D, Silos RG, Sticht C, Gretz N, Mehrabi A, Bewerunge-Hudler M, Pusch S, Bermejo JL, Dietrich P, Schirmacher P, Renner M, Roessler S. Profiling of gallbladder carcinoma reveals distinct miRNA profiles and activation of STAT1 by the tumor suppressive miRNA-145-5p. *Sci Rep.* 2019;9:4796.

Figures

Figure 1

M2-macrophage promotes breast cancer cell proliferation and suppresses IRF7 expression. THP-1 cells were pretreated with PMA for 18 h to induce macrophage (Ma), and then cultured by IL-4 for 3 d to induced M2-macrophage (M2), (a) M2-type marker Arg-1, CD86, and CD206, as well as M1-type marker iNOS expression profiles were determined using western blot. (b) Breast cancer cells MDA-MB-231 were incubated with the supernatant medium of M2 or Ma, cell proliferation was determined using MTT assay. (c and d) The dysregulated IRFs in MDA-MB-231 that incubated with the supernatant medium of M2 were measured by real-time PCR and western blot. * $p < 0.05$, ** $p < 0.01$ vs control; # $p < 0.05$ vs Ma.

Figure 2

Overexpressed IRF7 attenuates the effects of M2-macrophage on breast cancer cell proliferation and migration. Breast cancer cells MDA-MB-231 were incubated with nothing, or the supernatant medium of M2 or/and IRF7 overexpression. (a) IRF7 mRNA and protein expression was determined using real-time PCR and western blot, respectively. (b) Cell proliferation was determined using MTT assays. (c) Cell migration was measured using scratch wound healing assays. ** $p < 0.01$ vs control; # $p < 0.05$, ## $p < 0.01$ vs M2+NC.

Figure 3

Role of miR-1587 overexpression on M2-induced cell proliferation and migration. (a) miR-1587 expression in MDA-MB-231 that pretreated with nothing or the supernatant medium of M2-macrophage was determined by real-time PCR. (b) Transfection efficiency of miR-1587 overexpression in MDA-MB-231 cells was determined using real-time PCR. (c) Cell proliferation of MDA-MB-231 cells under miR-1587 overexpression was determined by MTT. (d) Cell migration of MDA-MB-231 cells under miR-1587 overexpression was determined by scratch wound healing assays. * $p < 0.05$, ** $p < 0.01$ vs NC. Breast cancer cells MDA-MB-231 were pretreated with the supernatant medium of M2-macrophage or/and miR-1587 overexpression, (e) miR-1587 expression was determined by real-time PCR. (f) Cell proliferation was measured using MTT assays. (g) Cell migration was determined using scratch wound healing assays. * $p < 0.05$, ** $p < 0.01$ vs M2+NC.

Figure 4

IRF7 is a target of miR-1587. MiR-1587 overexpression and knockdown on IRF7 expression was determined using real-time PCR(a), and western blot (b). (c) Online prediction showed that miR-1587 has binding sites with IRF7 3'UTR. (d) Relative luciferase assays were conducted to determine the interaction between miR-1587 and IRF7. * $p < 0.05$, ** $p < 0.01$ vs NC.

Figure 5

IRF7 knockdown attenuates the effects of miR-1587 knockdown in vitro and in vivo. (a) Breast cancer cells MDA-MB-231 were pretreated with nothing or the supernatant medium of M2-macrophage or/and miR-1587 knockdown, IRF7 protein expression was determined using western blot. Breast cancer cells MDA-MB-231 were pretreated with the supernatant medium of M2-macrophage or/and miR-1587 knockdown or/and IRF7 knockdown, (b) cell proliferation was detected using MTT assay. (c) Cell migration was measured using scratch wound healing assays. * $p < 0.05$, ** $p < 0.01$ vs control; # $p < 0.05$, ## $p < 0.01$ vs M2+NC. Mice were determined into three groups ($n = 6$ in each group), including control, M2 and M2+miR-1587 (-). In control group, mice were subcutaneously injected MDA-MB-231 cells (5×10^6 cells/200 μ l PBS/mouse). (d) Tumor volume was detected once a week for 4 weeks. (e) miR-1587 expression in the tumor tissues was determined using real-time PCR. (f) IRF7 protein expression in the tumor tissues was determined using western blot. ** $p < 0.01$ vs control; # $p < 0.05$, ## $p < 0.01$ vs M2.