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MiR-29a promotes cell proliferation and EMT in breast cancer by targeting ten eleven translocation 1

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ABSTRACT

Increasing evidence has shown that microRNAs played an important role in regulating carcinogenesis. However, the role of miR-29a in breast cancer is still unclear. Herein, we showed that miR-29a was significantly up-regulated in breast cancer as compared with non-tumor tissues. Moreover, the up-regulation of miR-29a was significantly correlated with tumor metastasis and shorter overall survival in breast cancer patients. Knockdown of miR-29a in breast cancer cell lines inhibited cell proliferation and migration. Furthermore, data from bioinformatic analysis validated by dual-luciferase reporter gene assay showed that ten eleven translocation 1 (TET1) was a direct target of miR-29a, and over-expression of TET1 inhibited cell proliferation and migration which could be induced by the up-regulation of miR-29a. TET1 silencing promoted cell growth and migration in breast cancer. MiR-29a over-expression had the same effect. MiR-29a targets TET1, down regulates its expression and thus promotes EMT in breast cancer. Altogether, we demonstrate that miR-29a acts as a tumor activator by targeting TET1 and induces cell proliferation and EMT in breast cancer.

1. Introduction

Breast cancer is one of the tumors with highest malignant potential worldwide and millions of new cases are diagnosed each year. Despite the improvement in diagnosis and surgical treatment, the prognosis of breast cancer patients was poor because of recurrence and remote metastasis after operation [1]. The treatment responses are variable depending on breast cancer subtypes. Patients with estrogen receptor negative-ER (−), progesterone receptor negative-PGR (−) and the human EGF receptor 2 negative-HER2 (−) breast cancer had the worst prognosis [2]. Though these risk factors had been widely studied, the mechanism involved in carcinogenesis was unclear [3,4]. Thus, investigation of mechanisms underlying breast cancer cell proliferation and metastasis is very necessary.

MicroRNAs (miRNAs) are small, single-stranded, non-coding RNAs which degrade mRNA or inhibit mRNA translation through binding to 3′-untranslated region (3′-UTR) of target mRNA [5]. It has been reported that >60% protein translation was regulated by miRNAs. MiRNAs regulate many biological processes and play an important role in modulating cell cycle, phenotype, cell migration and differentiation [6–8]. It has been widely recognized that aberrant miRNAs expression is associated with cell malignant transformation [9]. Dysregulation of miRNAs expression exists in many kinds of tumors and involved in the initiation, development and progression stages [10,11].

High-throughput technology help people identify series of miRNAs that are involved in the carcinogenesis of breast cancer [12–14]. MiR-29a has been found to be up-regulated in many kinds of tumors including prostate cancer, esophageal cancer and colorectal cancer [15–17]. Epithelial-Mesenchymal Transition (EMT) is critical for tumor metastasis and high expression of miR-29a has been reported to induce EMT in various tumor cells [18,19]. But there are few studies about miR-29a in breast cancer patients. Although the role of miR-29a has been deeply studied in many kinds of tumors, whether it can induce EMT or other malignant transformation in breast cancer still remains unknown.

TET1 is a member of the ten eleven translocation (TET) family, which converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), thus inducing CpG island demethylation in specific gene promoter [20]. TET1, which acts as a tumor suppressor, is often absent in tumor tissues including colon cancer, gastric cancer, hepatic cancer and breast cancer [21–23]. Depressed expression of TET1 could induce cell migration and promote tumor metastasis through EMT [24,25]. The mechanism of TET1 down-regulation in tumor cells is unclear. Miao Sun. et al. reported that down-regulation of TET1 is due to the hypermethylation status of its promoter [26]. Kuang-Hsiang Chuang et al. found that miR-494 could target 3′UTR of TET1 and inhibit TET1 protein expression [25]. In this study, we found that TET1 was a direct

Abbreviations: CCK-8, cell counting kit-8; ER, estrogen receptor; HER2, human EGF receptor 2; TET1, ten eleven translocation 1.

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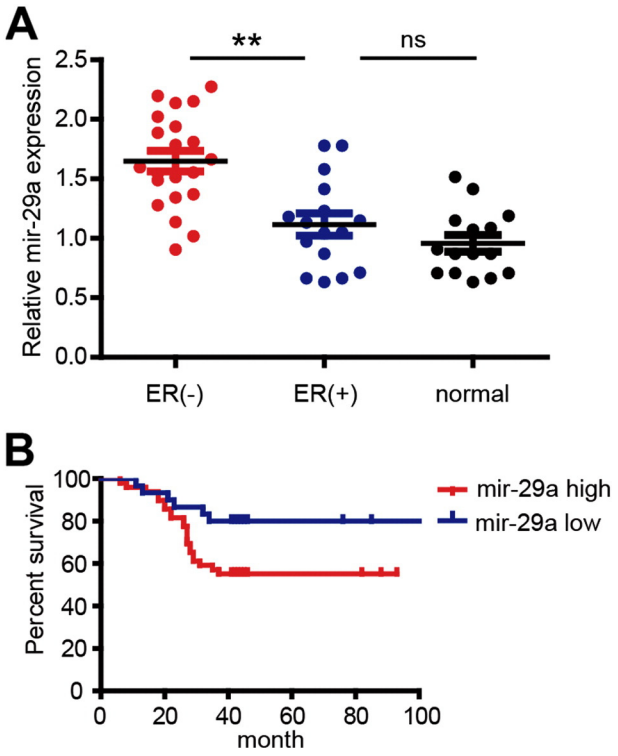

Fig. 1. MiR-29a is up-regulated in ER$^-$ breast cancer and is correlated with poor survival. (A) MiR-29a expression was significantly up-regulated in ER (−) breast compared with ER (+) and adjacent non-tumor tissues. (B) Breast cancer patients with high miR-29a expression had shorter overall survival.

target of miR-29a. The up-regulation of miR-29a decreased TET1 expression and induced accelerated cell proliferation and EMT *in vitro. In vivo* experiment also showed similar results. Collectively, in this study we demonstrated TET1 was a direct target of miR-29a, and down-regulation of TET1 by miR-29a induced EMT and tumor growth in breast cancer.

2. Methods

2.1. Tissues

79 formalin-fixed and paraffin-embedded (FFPE) breast cancer samples were collected for IHC analysis. 60 pairs of tumor and matched non-tumor tissues for PCR were collected from patients with breast cancer who underwent surgery in Zhejiang Provincial People's Hospital. None of the patients received any chemotherapy or radiotherapy prior to operation. Written informed consents were obtained from patients. This study was approved by the ethics committee of Zhejiang Provincial People's Hospital.

2.2. Cell culture

All human breast cancer cell lines were purchased from American Type Culture Collection (ATCC). All cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and maintained at 37 °C containing 5% CO_2.

2.3. Cell proliferation and migration

Cell proliferation was detected by CCK-8 method and colony formation assay. 0.5×10^4 cells per well were seeded in 96-well plates and OD450 of CCK-8was measured on day 0, 1, 2, 3, 4. 500 breast cancer cells per well were seeded in 6-well plates. After two weeks, colonies were fixed with methanol containing 0.2% crystal violet and colony number was counted. For migration assay, 800 μl medium with 10% fetal bovine serum was added to the lower chamber, while 200ul serum-free medium containing 10^5 cells was added to the upper chamber. After 24 h, chambers were fixed and stained with 0.2% Crystal violet and cell number was determined under microscope.

2.4. Cell cycle

Cell cycle was detected by flow cytometry. Cells were harvested and fixed in 70% pre-cold ethanol at 4 °C over night and then stained with propidium iodide containing RNase in the dark at 37 °C for 30 min. After staining, flow cytometry was used for cell cycle detection.

2.5. RNA extraction and qPCR

Total RNA was extracted by TRIzol reagent (Invitrogen, USA) according to the manufacturer's instruction. To detect miR-29a expression, total RNA was treated following the manufacturer's protocol of the First-Strand Synthesis kit (TaKaRa Bio, Japan). To detect TET1 expression, total RNA was converted into cDNA by reverse transcription kit (Promega, Madison, USA). qPCR was performed using SYBR Green Mix (ABI, CA, USA). Comparative Ct method was used to calculate expression levels. U6 small nuclear RNA and GAPDH were used as endogenous control for miR-29a and TET1 respectively. Primers used are as follows: TET1 Forward-CGCTACGAAGCACCTCTCTTA, Reverse-CTTGCATTGGAA CCGAATCATTT; miR-29a TAGCACCATCTGAAATCGGTTA; miR-200a CATCTTACCGGACAGTGCTGGA; miR-200b CATCTTACTGGGCAGCAGCA TTGGA; miR-200c CGTCTTACCCAGCAGTGTTTGG.

2.6. Constructs

MiR-29a mimics (miR-29a), miR-29a negative control (miR-NC), miR-29a inhibitor (anti-miR-29a), miR-29a inhibitor negative control (anti-miR-NC) were purchased from RiboBio (RiboBio Co., Guangzhou, China). shRNA used to knock down TET1 (TET1 KD) and lentivirus used to stably overexpress miR-29a in breast cancer cells were purchased from Genechem Company (Shanghai, China). Cells were transfected by lentivirus according to the manufacturer's instruction. Lipofectamine-RNAi MAX (Invitrogen) was used for transfection. 5×10^4 breast cancer cells in 12-well plate were transfected with miRNA duplex. 48 h after transfection, cells were collected for the following experiments. The sequences of miR-29a mimics (miR-29a),

Table 1
Correlationship between miR-29a expression level and clinico-pathological data.

Clinico-pathological parameters	MiR-29a expression		*p* value
	Low ($n = 35$)	High ($n = 44$)	
Age (years)			0.705
≤60	19	22	
≥60	16	22	
Gender			0.653
Male	12	13	
Female	23	31	
Tumor size (cm)			0.017*
≤5 cm	23	17	
≥5 cm	12	27	
Local invasion			0.051
T1, T2	17	12	
T3, T4	18	32	
Lymph node metastasis			0.000*
No	22	4	
Yes	13	40	
TNM stage			0.000*
I, II	28	4	
III, IV	7	40	
ER			0.024*
Negative	15	30	
Positive	20	14	

* $p < 0.05$

Table 2
Target sequences.

	Sense: ACUGAUUUCUUUUGGUGU UCAG Antisense: GCACCAUCUGAAAUCG GUUA
MiR-29a mimics (miR-29a)	UU
MiR-29a inhibitor (anit-miR-29a)	UAACCGAUUUCAGAUGGUGCUA
MiR-29a negative control (miR-NC)	Sense: UUCUCCGAACGUGUCACG UTT Antisense: ACGUGACACGUUCGGA GAATT
MiR-29a inhibitor negative control (anti-miR-NC)	CAGUACUUUUGUGUAGUACAA
TET1 shRNA	CCTTGATAGAATCACTCAGTT

miR-29a negative control (miR-NC), miR-29a inhibitor (anti-miR-29a), miR-29a inhibitor negative control (anti-miR-NC) and TET1 shRNA were listed in Table 2.

2.7. Western Blot

Western Blot was performed as previously described. Anti-CyclinD1, anti-P21, anti-GAPDH and secondary antibodies were purchased from Cell Signaling Biotechnology (MA, USA). Anti-TET1 antibody was purchased from GeneTex (GeneTex, USA).

2.8. Luciferase assay

MCF-7 cells were cultured in 24-well plates. The pGL3 vector containing wild type or mutant 3′UTR of TET1, Renilla vector and miR-29a mimics or miR-NC were co-transfected into cells. After 48 h, luciferase activity was measured by dual-luciferase reporter assay system (Promega, USA). Luciferase activity was normalized to corresponding Renilla luciferase activity. All experiments were performed three times.

2.9. 5-hmC detection

Dot bolt was used to detect 5-hmC content in treated cell lines. Genomic DNA was extracted using DNA isolation kit (Tiangen, Beijin, China), denatured in 0.1 M NaOH, treated at 95 °C for 10 min and immediately cooled on ice. DNA was then spotted on nylon membrane and baked at 80 °C for 2 h, blocked with 5% non-fat milk, incubated with anti-5-hmC and anti-5-mC antibody (Active Motif, USA) at 4 °C over night. The membrane was then incubated with HRP-labeled anti-mouse secondary antibody. Signal was detected by ECL kit (Pierce, USA).

2.10. Xenograft model

Six week old male BALB/c nude mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed under specific pathogen-free conditions. 1×10^6 MCF-7 cells, in which miR-29a was stably over-expressed and 1×10^6 MDA-453 cells in which miR-29a was stably knocked down by lentivirus were subcutaneously injected into the right flank of nude mice. Tumor volume was measured every 5 days. All mice were sacrificed after 35 days. Tumor grafts from nude mice were fixed for IHC analysis. Tumor volume was calculated using the formula: tumor volume $= (W + L)/2 \times W \times L \times 0.5236$. All the animal experiments were carried out according to the guide for the Care and Use Laboratory Animals of Zhejiang Provincial People's Hospital.

2.11. Statistical analysis

SPSS 19.0 software was used for statistical analysis. The Pearsonχ^2 test was used to analyze the relationship between miR-29a expression and clinic-pathological parameters. Kaplan–Meier method was used for analysis of overall survival. In Kaplan–Meier analysis, the mean expression level of miR-29a was selected as the cut-off value. Patients with miR-29a higher than mean expression were classified into the miR-29a high expression group, while those with miR-29a lower than

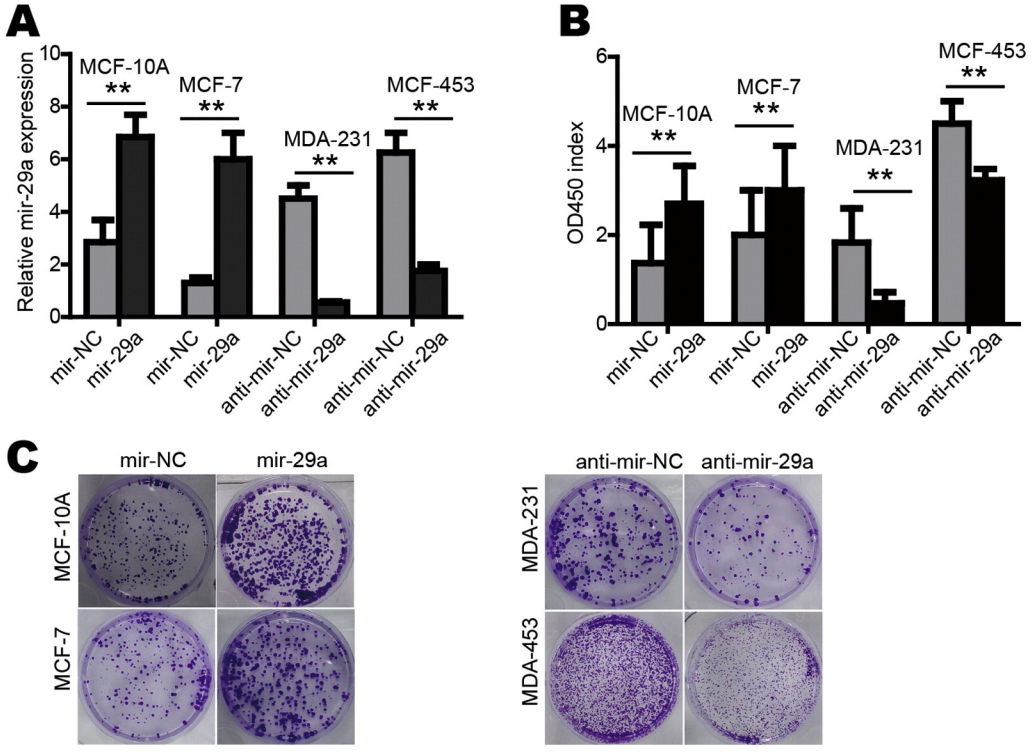

Fig. 2. MiR-29a promotes breast cancer proliferation *in vitro*. (A) Knockdown or over-expression efficiency of miR-29a in different cell lines. (B) Cell proliferation measured by CCK-8 method. MiR-29a over-expression promoted cell growth in MCF-10A and MCF-7, while miR-29a knockdown decreased cell growth in MDA-231 and MDA-453. (C) MiR-29a over-expression increased colony formation in MCF-10A and MCF-7, while miR-29a knockdown decreased colony number in MDA-231 and MDA-453.

mean expression were classified into the miR-29a low expression group. The relation between TET1 and miR-29a was analyzed by Spearman's correlation. Other experiments were assessed by the Student's *t*-test. Values were considered statistically significant when $P < 0.05$.

3. Results

3.1. MiR-29a is up-regulated in ER (−) breast cancer and correlated with poor survival

We detected miR-29a level in tumor tissues and non-tumor tissues in breast cancer patients. As shown in Fig. 1A, miR-29a expression was significantly up-regulated in ER (−) breast cancer tissues as compared with ER (+) breast cancer tissues and non-tumor tissues ($p < 0.05$). Furthermore, the Kaplan-Meier analysis showed that patients with high miR-29a had poorer prognosis than those with low miR-29a expression (Fig. 1B). The clinico-pathological parameters also showed significant correlation between elevated expression of miR-29a and tumor size, lymph node metastasis, TNM stage and ER expression, but there was no correlation between miR-29a expression and age, gender and local invasion (Table 1). These results indicate that miR-29a plays a vital role in breast cancer progression.

3.2. MiR-29a over-expression promotes breast cancer proliferation both in vitro

We first used cell proliferation assay to detect the biological role of miR-29a in breast cancer. As shown in Fig. 2A, miR-29a was significantly up-regulated after transfection with miR-29a mimics, while miR-29a inhibitor down regulated miR-29a. Proliferation assay measured by CCK-8 showed accelerated cell proliferation mediated by up-regulation of miR-29a while down-regulation of miR-29a inhibited cell growth (Fig. 2B). Furthermore, miR-29a over-expression induced increased colony counts as compared with miR-NC, while miR-29a knockdown had decreased colony counts as compared with anti-miR-NC (Fig. 2C).

3.3. MiR-29a regulates cell cycle and promotes migration in breast cancer

To investigate the mechanism underlying the effects of miR-29a on breast cancer cell proliferation, we analyzed cell cycle by flow cytometry. As shown in Fig. 3A, the percentage of cells in G1/G0 phase was

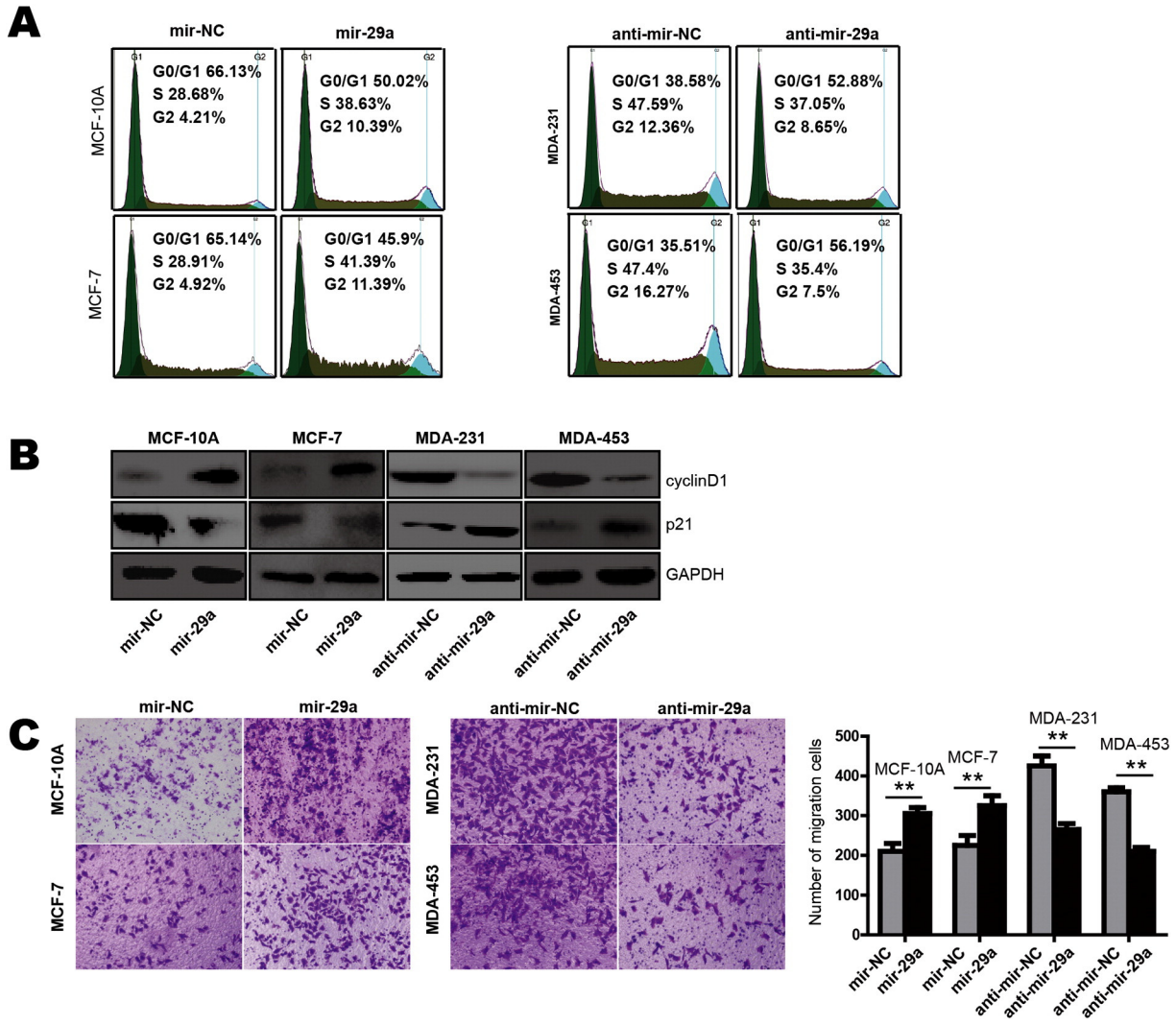

Fig. 3. MiR-29a regulates cell cycle and promotes migration in breast cancer. (A) MiR-29a over-expression increased the percentage of cells in G2 phase and miR-29a knockdown induced more cells in G0/G1 phase. (B) MiR-29a over-expression increased CyclinD1 and decreased p21, while miR-29a knockdown decreased CyclinD1 and increased p21. (C) MiR-29a over-expression increased cell migration, while miR-29a knockdown inhibited cell migration.

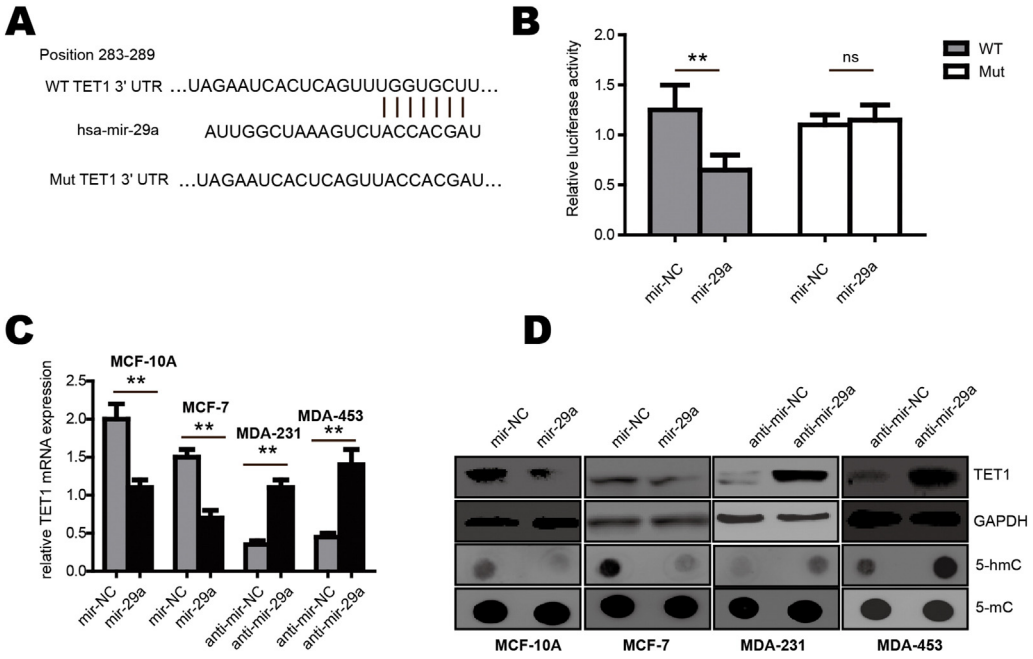

Fig. 4. TET1 is a direct target of miR-29a. (A) Structure of human TET1 3′-UTR containing wild type and mutant miR-29a binding sites. (B) Relative luciferase activity of wild type and mutant TET1 3′-UTR. (C and D) RT-PCR and Western Blot showed miR-29a over-expression induced lower TET1 level and decreased 5-hmC content, while miR-29a knockdown increased TET1 level and 5-hmC content.

significantly decreased and that in G2 phase was increased when miR-29a was over-expressed in MCF-10A and MCF-7 cells. When miR-29a was knocked down in MDA-231 and MDA-453, more cells were arrested in G1/G0 phase. Moreover, we found that miR-29a up-regulated cyclinD1 and down regulated p21 (Fig. 3B). These results indicate that miR-29a promotes cell proliferation through modulating cell cycle.

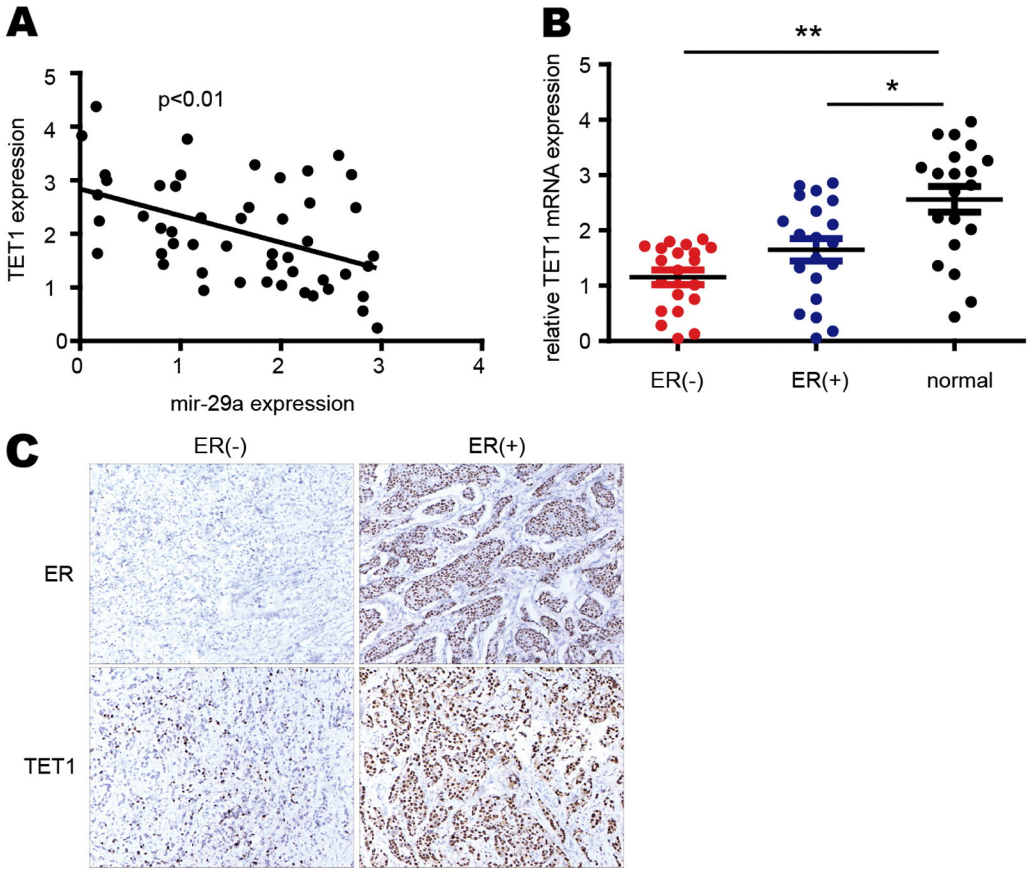

Fig. 5. TET1 inversely correlates with miR-29a levels. (A) RT-PCR result showed inverse correlation between TET1 and miR-29a. (B) TET1 expression was down-regulated in ER (−) breast cancer tissues compared with ER (+) and adjacent non-tumor tissues. (C) IHC analysis of ER and TET1 in breast cancer tissues.

To demonstrate the role of miR-29a in promoting migration, we performed transwell assay by using breast cancer cell transfected with miR-29a mimics and miR-29a inhibitor. We observed enhanced cell migration after transfection with miR-29a mimics, while miR-29a inhibitor decreased cell migration potential (Fig. 3C). These results indicate that miR-29a promotes migration of breast cancer cells.

3.4. TET1 is a direct target of miR-29a

To investigate the mechanism by which miR-29a executed its function, we attempted to find the target of miR-29a. Among the targets predicted by MiRanda, TargetScan and PicTar, we selected TET1 based on its recognized involvement in carcinogenesis and EMT process. To validate that TET1 is a direct target of miR-29a, we performed

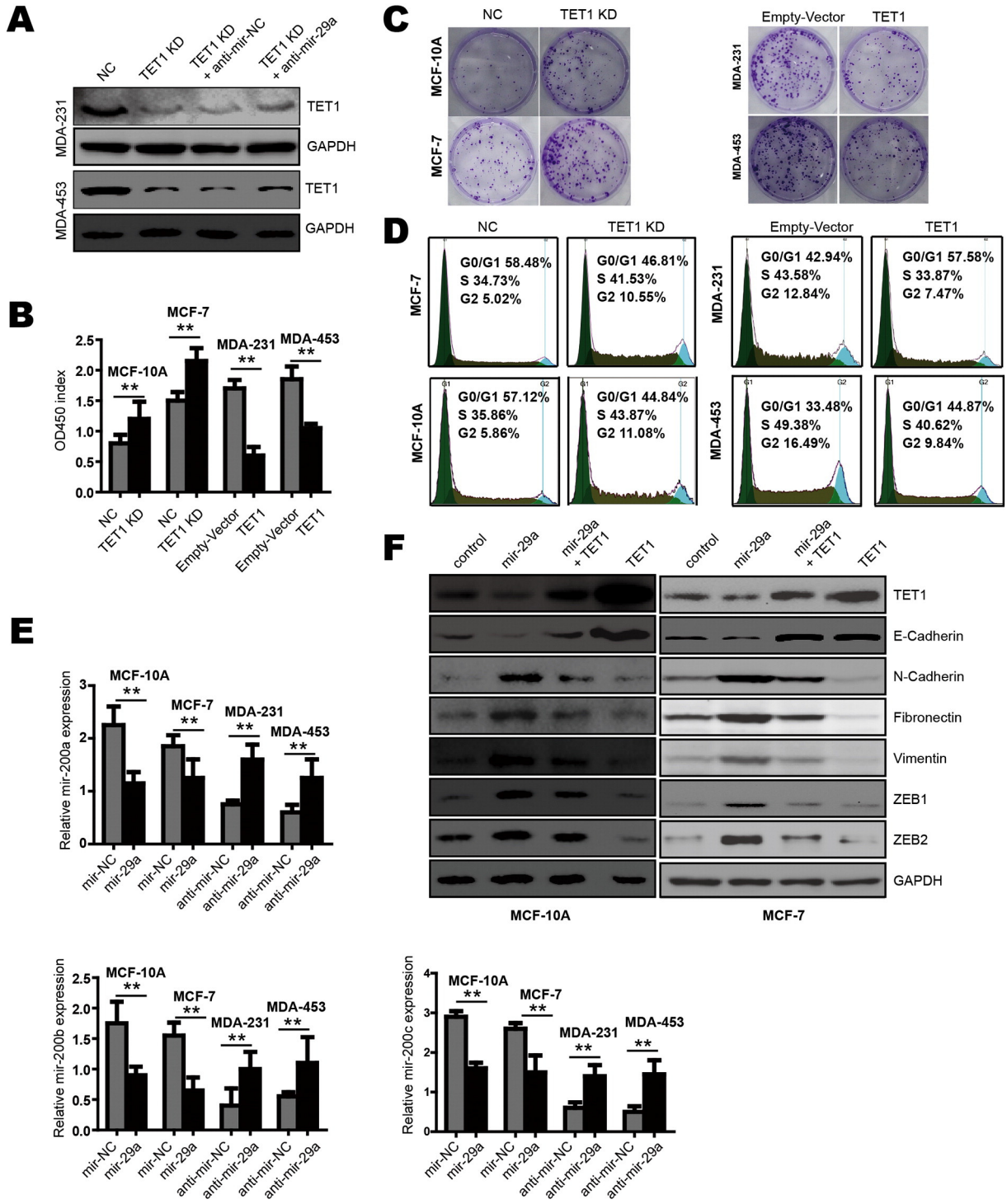

Fig. 6. MiR-29a induces EMT through TET1 and miR-200 family. (A) miR-29a could regulate TET1 level. (B) CCK-8 method showed TET1 knockdown increased cell proliferation, while TET1 over-expression decreased cell proliferation. (C) Colony formation assay showed TET1 knockdown increased cell proliferation, while TET1 over-expression inhibited cell growth. (D) TET1 knockdown increased cell percentage in G2 phase, while TET1 over-expression induced cell arrest in G0/G1 phase. (E) MiR-29a over-expression down-regulated miR-200 family, while miR-29a knockdown up-regulated miR-200 family level. (F) Western Blot showed miR-29a could induce EMT while TET1 inhibited the EMT process.

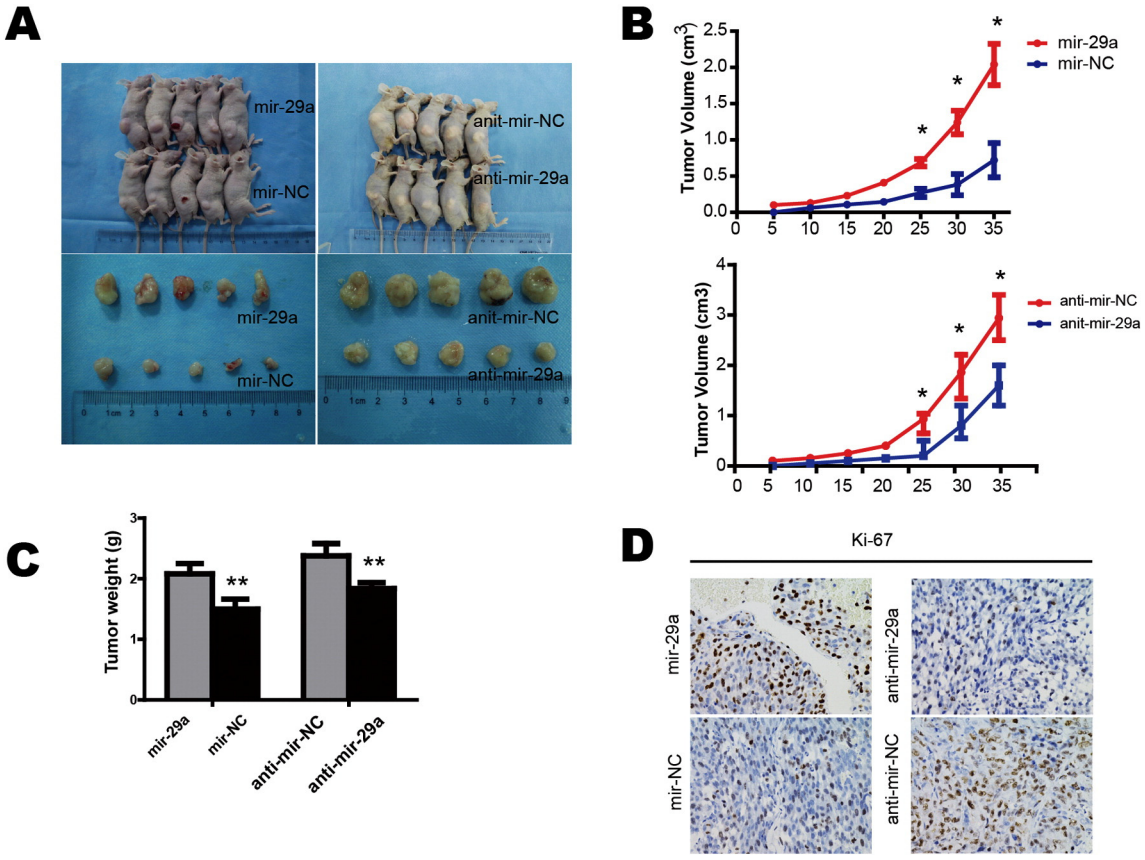

Fig. 7. MiR-29a promotes breast cancer proliferation *in vivo*. (A, B and C) MiR-29a over-expression in MCF-7 induced bigger and heavier tumor *in vivo*, while miR-29a knockdown in MDA-231 decreased tumor volume and weight. (D) IHC analysis showed miR-29a over-expression increased Ki-67 positive cells, while miR-29a knockdown decreased Ki-67 positive cells.

dual-luciferase assay. Wild-type and mutant 3′-UTR of TET1 were respectively harbored into reporter plasmid. The results showed that the luciferase activity of wild type TET1 3′UTR was significantly decreased by miR-29a while the activity of mutant TET1 3′UTR was not changed by miR-29a (Fig. 4A and B). The results of qPCR and Western Blot also showed up-regulation of endogenous TET1 by miR-29a knockdown, while miR-29a over-expression decreased TET1 level and 5-hmC content (Fig. 4C and D). These data indicate that miR-29a directly targets 3′UTR of TET1 and down regulates TET1 mRNA and protein level in breast cancer cells.

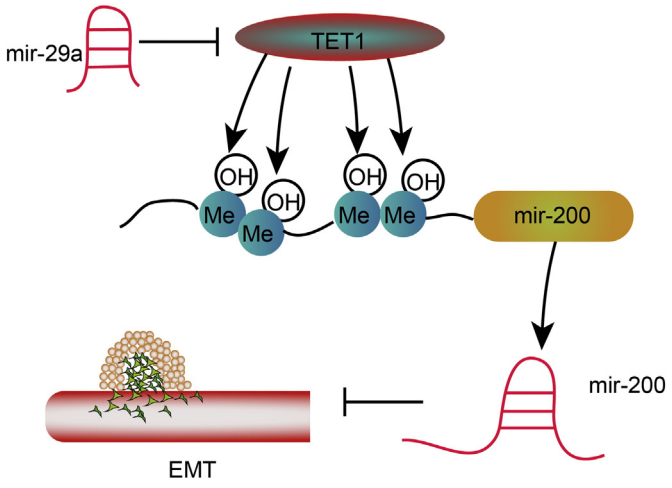

Fig. 8. Schematic presentation of miR-29a and TET1 in EMT.

3.5. TET1 inversely correlates with miR-29a levels

To further validate the correlation between miR-29a and TET1, we detected levels of miR-29a and TET1 in breast cancer tissues and adjacent non-tumor tissues. As shown in Fig. 5A, we observed significant negative correlation between miR-29a and TET1 in breast cancer patients. Furthermore, we observed that the down-regulation of TET1 was more obvious in ER (−) breast cancer tissues (Fig. 5B), while IHC analysis also showed that TET1 expression was higher in ER (+) breast cancer tissues (Fig. 5C). These results demonstrate the inverse correlation between miR-29a and TET1 in breast cancer and further confirmed the regulation of endogenous TET1 by miR-29a.

3.6. MiR-29a targeted TET1 contributes to cell proliferation and EMT

TET1 acts as a tumor suppressor in various kinds of tumors. As shown in Fig. 6A, Western Blot confirmed that miR-29a knockdown could partially recover down-regulated TET1 expression induced by TET1 shRNA in MDA-231 and MDA-453 cells. As miR-29a could influence cell proliferation and cell cycle, we also knocked down or over expressed miR-29a target gene TET1 to observe its biological function. We observed that TET1 knockdown increased while its over-expression inhibited cell proliferation (Fig. 6B, C and D). It has been reported that depressed TET1 expression mediated by miRNA could induce EMT in hepatocellular carcinoma (HCC) through down-regulation of miR-200 family [25]. In our study, we observed decreased TET1 expression and enhanced cell migration mediated by miR-29a, so we hypothesized that mi-29a could also promote EMT by targeting TET1 and down-regulating miR-200 family in breast cancer. As shown in Fig. 6E, we observed decreased levels of miR-200 family induced by miR-29a over-expression in MCF-10A and MCF-7 cells, while miR-29a

knockdown in MDA-231 and MDA-453 cells increased miR-200 family expression. The results of Western Blot also showed that miR-29a led to low expression of E-Cadherin and high expression of N-Cadherin, fibronectin and vimentin. But TET1 over-expression inhibited EMT process in MCF-10A and MCF-7 cells (Fig. 6F). Collectively, these results suggested that miR-29a targeted TET1 and induced cell proliferation and EMT in breast cancer.

3.7. MiR-29a promotes breast cancer growth in vivo

To investigate the oncogenic effect of miR-29a *in vivo*, we generated a xenograft model. As shown in Fig. 7A, B and C, after miR-29a was over-expressed in MCF-7 cells, bigger tumor volume and heavier tumor weight were observed in miR-29a over-expression group, while miR-29a knockdown in MDA-453 cells reduced tumor volume and tumor weight. Ki-67 in tumors from nude mice also indicated that miR-29a over-expression accelerated cell growth (Fig. 7D). These results demonstrate that miR-29a is a tumor activator both *in vitro* and *in vivo*.

4. Discussion

Aberrant expression of miRNAs has been found in various malignant tumors. Many researchers reported that miR-29a acted as a tumor suppressor and is often absent during tumorigenesis of prostate cancer, gastric cancer, lung cancer, *etc.* However, some previous reports have shown the oncogenic feature and high expression of miR-29a in breast cancer. Zhong S et al. found that high expression of miR-29a was associated with drug-resistance in breast cancer [27]. Choghaei E et al. reported the anti-apoptosis function of miR-29a in breast cancer cells [28]. These controversial results of miR-29a in tumors indicated that miR-29a played different roles based on cancer types. In our study, we found that miR-29a was significantly up-regulated in ER (−) breast cancer as compared with normal tissues, indicating that miR-29a was implicated in the progression of breast cancer.

Up-regulation of miR-29a was further found to be correlated with poorer prognosis in breast cancer patients by Kaplan-Meier analysis. And the clinicopathological parameters also showed significant association between high expression of miR-29a and local invasion, lymph node metastasis and TNM stage. This suggested that miR-29a may be a novel biomarker for diagnosis and prognosis prediction in breast cancer patients.

The ability of tumor growth and metastasis is critical for tumor progression and mirRNAs are closely involved in this process. Our *in vitro* and *in vivo* data indicated the oncogenic effect of miR-29a in promoting breast cancer cell growth and metastasis. As is known, cell proliferation is regulated by cell cycles and dysregulated cell cycling can induce aberrant cell growth. In this study, we found that miR-29a accelerated cell cycle progression by modulating cyclinD1 and p21.

To investigate into the mechanism by which miR-29a enhances malignant transformation of cancer cells, we identified the target gene of miR-29a. TET1, a member of the ten eleven translocation (TET) family, regulated gene expression by modulating the methylation status of specific gene promoter. In the present study, we found TET1 was a direct target gene of miR-29a. Elevated expression of miR-29a in breast cancer cells decreased TET1 level and induced EMT. Although TET1, as a tumor suppressor, is always absent in breast cancer, the mechanism by which TET1 is down-regulated remains unknown. Miao S et al. reported that the hypermethylation status in TET1 promoter may contribute to its down-regulation in breast cancer [26]. It has been reported that TET1 could be modulated by miRNAs such as miR-494 [25]. In our study, we found a negative correlation between miR-29a and TET1 expression. Furthermore, we validated that TET1 was regulated by miR-29a through luciferase assay. We also found miR-29a could down regulated 5-hmC and miR-200 family through TET1, which promoted carcinogenesis and EMT. But there was also limitation in our study. Whether TET1 expression could be regulated by other miRNAs except miR-29a or other biological process such as ubiquitin was absolutely unknown. Therefore, the regulation of TET1 in breast cancers needs further investigation.

Collectively, in this study we demonstrated the oncogenic effect of miR-29a in breast cancer and identified its target gene TET1 (Fig. 8). We clarified that miR-29a-induced acceleration of cell proliferation and metastasis was mediated by TET1 in breast cancer. These results suggest that miR-29a may be a new biomarker for diagnosis as well as a therapeutic target for breast cancer.

Conflict of interest

All authors declared no conflict of interest.

Transparency document

The transparency document associated with this article can be found, in online version.

Acknowledgement

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References

[1] C.G. Victora, R. Bahl, A.J. Barros, G.V. Franca, S. Horton, J. Krasevec, S. Murch, M.J. Sankar, N. Walker, N.C. Rollins, Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect, Lancet 387 (2016) 475–490.
[2] A. Cinkaya, M. Akin, A. Sengul, Evaluation of treatment outcomes of triple-negative breast cancer, J. Cancer Res. Ther. 12 (2016) 150–154.
[3] M. Saveria Rotundo, T. Galeano, P. Tassone, P. Tagliaferri, mTOR inhibitors, a new era for metastatic luminal HER2-negative breast cancer? A systematic review and a meta-analysis of randomized trials, Oncotarget (2016).
[4] E. Laas, P. Mallon, F.P. Duhoux, A. Hamidouche, R. Rouzier, F. Reyal, Low concordance between gene expression signatures in ER positive HER2 negative breast carcinoma could impair their clinical application, PLoS One 11 (2016) e0148957.
[5] N. Valeri, C. Braconi, P. Gasparini, C. Murgia, A. Lampis, V. Paulus-Hock, J.R. Hart, L. Ueno, S.I. Grivennikov, F. Lovat, A. Paone, L. Cascione, K.M. Sumani, A. Veronese, M. Fabbri, S. Carasi, H. Alder, G. Lanza, R. Gafa, M.P. Moyer, R.A. Ridgway, J. Cordero, G.J. Nuovo, W.L. Frankel, M. Rugge, M. Fassan, J. Groden, P.K. Vogt, M. Karin, O.J. Sansom, C.M. Croce, MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer, Cancer Cell 25 (2014) 469–483.
[6] R. Venkatadri, T. Muni, A.K. Iyer, J.S. Yakisich, N. Azad, Role of apoptosis-related miRNAs in resveratrol-induced breast cancer cell death, Cell Death Dis. 7 (2016) e2104.
[7] F. Peng, Y. Zhang, R. Wang, W. Zhou, Z. Zhao, H. Liang, L. Qi, W. Zhao, H. Wang, C. Wang, Z. Guo, Y. Gu, Identification of differentially expressed miRNAs in individual breast cancer patient and application in personalized medicine, Oncogenesis 5 (2016) e194.
[8] F. Qu, C.B. Li, B.T. Yuan, W. Qi, H.L. Li, X.Z. Shen, G. Zhao, J.T. Wang, Y.J. Liu, MicroRNA-26a induces osteosarcoma cell growth and metastasis via the Wnt/beta-catenin pathway, Oncol. Lett. 11 (2016) 1592–1596.
[9] M.V. Iorio, C.M. Croce, microRNA involvement in human cancer, Carcinogenesis 33 (2012) 1126–1133.
[10] Y.X. Song, Z.Y. Yue, Z.N. Wang, Y.Y. Xu, Y. Luo, H.M. Xu, X. Zhang, L. Jiang, C.Z. Xing, Y. Zhang, MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation, Mol. Cancer 10 (2011) 1.
[11] Z. Zhang, W. Zheng, J. Hai, MicroRNA-148b expression is decreased in hepatocellular carcinoma and associated with prognosis, Med. Oncol. 31 (2014) 984.
[12] Y.Y. Chang, W.H. Kuo, J.H. Hung, C.Y. Lee, Y.H. Lee, Y.C. Chang, W.C. Lin, C.Y. Shen, C.S. Huang, F.J. Hsieh, L.C. Lai, M.H. Tsai, K.J. Chang, E.Y. Chuang, Deregulated microRNAs in triple-negative breast cancer revealed by deep sequencing, Mol. Cancer 14 (2015) 36.
[13] A. Gamez-Pozo, J. Berges-Soria, J.M. Arevalillo, P. Nanni, R. Lopez-Vacas, H. Navarro, J. Grossmann, C.A. Castaneda, P. Main, M. Diaz-Almiron, E. Espinosa, E. Ciruelos, J.A. Fresno Vara, Combined label-free quantitative proteomics and microRNA expression analysis of breast cancer unravel molecular differences with clinical implications, Cancer Res. 75 (2015) 2243–2253.
[14] A. Fiannaca, M. La Rosa, L. La Paglia, R. Rizzo, A. Urso, Analysis of miRNA expression profiles in breast cancer using biclustering, BMC Bioinforma. 16 (Suppl. 4) (2015) S7.
[15] M.L. Zhi, Z.J. Liu, X.Y. Yi, L.J. Zhang, Y.X. Bao, Diagnostic performance of microRNA-29a for colorectal cancer: a meta-analysis, Genet. Mol. Res. 14 (2015) 18018–18025.
[16] F. Ahmed, T. Shiraishi, R.L. Vessella, P. Kulkarni, Tumor necrosis factor receptor associated factor-4: an adapter protein overexpressed in metastatic prostate cancer is regulated by microRNA-29a, Oncol. Rep. 30 (2013) 2963–2968.

[17] C. Liu, P. Duan, B. Li, C. Huang, Y. Jing, W. Yan, miR-29a activates Hes1 by targeting Nfia in esophageal carcinoma cell line TE-1, Oncol. Lett. 9 (2015) 96–102.
[18] C.A. Gebeshuber, K. Zatloukal, J. Martinez, miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis, EMBO Rep. 10 (2009) 400–405.
[19] J.W. Rostas III, H.C. Pruitt, B.J. Metge, A. Mitra, S.K. Bailey, S. Bae, K.P. Singh, D.J. Devine, D.L. Dyess, W.O. Richards, J.A. Tucker, L.A. Shevde, R.S. Samant, microRNA-29 negatively regulates EMT regulator N-myc interactor in breast cancer, Mol. Cancer 13 (2014) 200.
[20] Y. Kudo, K. Tateishi, K. Yamamoto, S. Yamamoto, Y. Asaoka, H. Ijichi, G. Nagae, H. Yoshida, H. Aburatani, K. Koike, Loss of 5-hydroxymethylcytosine is accompanied with malignant cellular transformation, Cancer Sci. 103 (2012) 670–676.
[21] S.G. Jin, Y. Jiang, R. Qiu, T.A. Rauch, Y. Wang, G. Schackert, D. Krex, Q. Lu, G.P. Pfeifer, 5-Hydroxymethylcytosine is strongly depleted in human cancers but its levels do not correlate with IDH1 mutations, Cancer Res. 71 (2011) 7360–7365.
[22] L.L. Kodach, R.J. Jacobs, J. Heijmans, C.J. van Noesel, A.M. Langers, H.W. Verspaget, D.W. Hommes, G.J. Offerhaus, G.R. van den Brink, J.C. Hardwick, The role of EZH2 and DNA methylation in the silencing of the tumour suppressor RUNX3 in colorectal cancer, Carcinogenesis 31 (2010) 1567–1575.
[23] C.H. Hsu, K.L. Peng, M.L. Kang, Y.R. Chen, Y.C. Yang, C.H. Tsai, C.S. Chu, Y.M. Jeng, Y.T. Chen, F.M. Lin, H.D. Huang, Y.Y. Lu, Y.C. Teng, S.T. Lin, R.K. Lin, F.M. Tang, S.B. Lee, H.M. Hsu, J.C. Yu, P.W. Hsiao, L.J. Juan, TET1 suppresses cancer invasion by activating the tissue inhibitors of metalloproteinases, Cell Rep. 2 (2012) 568–579.
[24] X. Hu, L. Zhang, S.Q. Mao, Z. Li, J. Chen, R.R. Zhang, H.P. Wu, J. Gao, F. Guo, W. Liu, G.F. Xu, H.Q. Dai, Y.G. Shi, X. Li, B. Hu, F. Tang, D. Pei, G.L. Xu, Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming, Cell Stem Cell 14 (2014) 512–522.
[25] K.H. Chuang, C.L. Whitney-Miller, C.Y. Chu, Z. Zhou, M.K. Dokus, S. Schmit, C.T. Barry, MicroRNA-494 is a master epigenetic regulator of multiple invasion-suppressor microRNAs by targeting ten eleven translocation 1 in invasive human hepatocellular carcinoma tumors, Hepatology (2015).
[26] M. Sun, C.X. Song, H. Huang, C.A. Frankenberger, D. Sankarasharma, S. Gomes, P. Chen, J. Chen, K.K. Chada, C. He, M.R. Rosner, HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 9920–9925.
[27] S. Zhong, W. Li, Z. Chen, J. Xu, J. Zhao, MiR-222 and miR-29a contribute to the drug-resistance of breast cancer cells, Gene 531 (2013) 8–14.
[28] E. Choghaei, G. Khamisipour, M. Falahati, B. Naeimi, M. Mossahebi-Mohammadi, R. Tahmasebi, M. Hasanpour, S. Shamsian, Z.S. Hashemi, Knockdown of microRNA-29a changes the expression of heat shock proteins in breast carcinoma MCF-7 cells, Oncol. Res. 23 (2016) 69–78.