Let-7c-5p inhibits cell proliferation and induces cell apoptosis by targeting *ERCC6* in breast cancer

XIAONAN FU[1], XUAN MAO[1], YUXIANG WANG[1], XIANFENG DING[1] and YONGFENG LI[2]

[1]College of Life Sciences, Zhejiang Sci-Tech University, [2]Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, P.R. China

Received March 26, 2017; Accepted July 7, 2017

DOI: 10.3892/or.2017.5839

Abstract. In this study, we found that let-7c-5p expression was clearly downregulated in breast cancer tissues compared with that of corresponding adjacent tissues. Furthermore, overexpression of let-7c-5p in MCF-7 breast cancer cells could significantly inhibit cell proliferation and induce cell apoptosis. The target genes of let-7c-5p were predicted by the way of bioinformatics, and validated by dual luciferase reporter assay and western blotting demonstrating that excision repair cross complementing 6 (ERCC6) gene was a direct target. Collectively, the present study suggested that let-7c-5p acted as a tumor suppressor in breast cancer possibly by negatively regulating ERCC6, which took an important part in nucleotide excision repair and it may provide a new potential strategy for breast cancer therapy.

Introduction

Breast cancer is one of the most common malignant diseases among women both in developed and developing countries, accounting for 29% of new cancer cases and 14% of cancer-associated deaths among American women (1). In China, the incidence of breast cancer is lower than in America, but during the past decade, the incidence has been increasing markedly, especially in rural areas (2). Therefore, more effective methods for breast cancer diagnosis and treatments are urgently needed.

MicroRNAs (miRNAs) are endogenous non-coding RNAs that function as gene regulators mainly by binding to the 3'UTR of their target mRNAs, inducing mRNAs degradation or translation repression (3). Biological processes, including cell proliferation, differentiation, apoptosis and senescence are controlled by miRNAs. Aberrant expression of miRNAs is associated with a variety of cancers, such as lung cancer, gastric cancer, and breast cancer (4-6). Let-7 was originally discovered in *Caenorhabditis elegans*, controlling the timing of stem-cell division and differentiation (7). Subsequently, let-7 and its family members have been found playing important roles in tumor suppression. Akao *et al* reported that let-7 might suppress the growth of human colon cancer cells and reduced oncogene *RAS* and *c-myc* expression (8). Esquela-Kerscher *et al* found that let-7 could inhibit the growth of lung cancer cell lines, as well as the growth of lung cancer cell xenografts in mice (9). Let-7c-5p, a member of let-7 family, is downregulated in prostate cancer, overexpression of let-7c-5p suppresses androgen receptor expression and leads to inhibition of prostate cancer cell proliferation (10). Nwaeburu *et al* found that forced expression of let-7c-5p by quercetin could activate Numbl expression and resulted in suppression of pancreatic cancer progression (11). Some other studies suggest that let-7c-5p functions as a anti-oncogene in human non-small cell lung cancer, hepatocellular carcinoma and colorectal cancer (12-14).

Although let-7c-5p has been studied in several cancers, its function and molecular mechanism in breast cancer remain to be further identified. Here, we measured let-7c-5p expression in breast cancer tissues and corresponding adjacent tissues, and examined the effects of let-7c-5p on human breast cancer cell proliferation and apoptosis. Moreover, for the first time, we found that *ERCC6* was a target of let-7c-5p in breast cancer.

Materials and methods

Tissues collection. Nine paraffin-embedded breast cancer and corresponding adjacent tissue samples were collected from Zhejiang Cancer Hospital (Hangzhou, China). This study was approved by the ethics committee of the Zhejiang Sci-Tech University (Hangzhou, China) and Zhejiang Cancer Hospital. All the samples were stored at 4˚C until RNA extraction.

RNA extraction and qRT-PCR. Total RNA was extracted from paraffin-embedded tissues using Recover All™ Total Nucleic Acid Isolation (Ambion, Austin, TX, USA) following the manufacturer's instructions. RNA concentrations were measured by the NanoDrop ND-2000 spectrophotometer

Correspondence to: Professor Xianfeng Ding, College of Life Sciences, Zhejiang Sci-Tech University, Xiasha Road #2, Hangzhou, Zhejiang 310018, P.R. China
E-mail: bdd114@163.com

Dr Yongfeng Li, Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022, P.R. China
E-mail: liyf@zjcc.org.cn

Key words: let-7c-5p, breast cancer, proliferation, apoptosis, ERCC6

Table I. Primers for qRT-PCR.

Primer	Sequence (5'-3')
let-7c-5p stem-loop	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACAACCAT
let-7c-5p	F: GAGGTAGTAGGTTGTATGGTTG R: GCAGGGTCCGAGGTATTC
ERCC6	F: CAATAGTCTGCCTCCCCACCCC R: CAACTTCTCGTTCCTCAACACATC
GAPDH	F: TGCCAAATATGATGACATCAAGAA R: GGAGTGGGTGTCGCTGTTG

F, forward; R, reverse.

Table II. Primers for construction of recombinant plasmids.

Primer	Sequence (5'-3')
Wild-type 3'UTR	F: ACAACATTGCTTCCTA R: TCAATCCAAGTATTTTCTCC
Mutant 3'UTR-1	F: ACAACATTGCTTCCTA R: AAGTTTTAATTCACATCATGCAAACAA
Mutant 3'UTR-2	F: TGTTTGCATGATGTGAATTACAACTT R: TCAATCCAAGTATTTTCTCC

F, forward; R, reverse.

(NanoDrop Technologies, Wilmington, DE, USA). RNA reverse transcription was performed with 100 ng of total RNA. Expression of let-7c-5p, ERCC6 and internal control GAPDH were detected using SYBR Premix Ex Taq™ II (Takara, Dalian, China). qRT-PCR was performed on the Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method, the expression of let-7c-5p and ERCC6 was normalized with GAPDH. Primers for qRT-PCR are listed in Table I.

Cell culture and transfection. The MCF-7 human breast cancer cell line was obtained from Zhejiang Sci-Tech University, cultured in DMEM-high glucose medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), at 37˚C in a 5% CO_2 humidified incubator (HF90, Heal Force, Hong Kong). Mimics for miR-NC (5'-CAGUAC UUUUGUGUAGUACAA-3') and let-7c-5p (5'-UGAGGUAG UAGGUUGUAUGGUU-3') were purchased from GenePharma (Shanghai, China). Reverse transfection was performed in this study using GeneTran™ III High Efficiency Transfection Reagent (Biomiga, Inc., San Diego, CA, USA) according to the manufacturer's recommendations.

MTT assay. MCF-7 cells were seeded in 96-well plates at approximately 4000 cells per well and transfected with miR-NC or let-7c-5p mimics. MTT (Sigma, St. Louis, MO, USA) was used to examine the effects of let-7c-5p on cell proliferation at 24, 48 and 72 h post-transfection. At each time, 20 μl MTT solution was added to each well, and incubated at 37˚C for 4 h, the formazan produced by viable cells was dissolved in 150 μl dimethylsulfoxide (DMSO). The cell numbers were evaluated by reading the absorbance at 490 nm (15).

Cell apoptosis assay. Cells were seeded in 6-well plates at approximately $3x10^5$ cells per well and transfected with miR-NC or let-7c-5p mimics. All cells were harvested at 24 h post-transfection, washed with cold PBS for twice, stained with FITC and PI using BD Pharmingen FITC Annexin V™ apoptosis detection kit I (BD Biosciences, San Jose, CA, USA) following the manufacturer's instructions. Flow cytometry analysis was finished on BD Accuri C6 flow cytometer with C6 software.

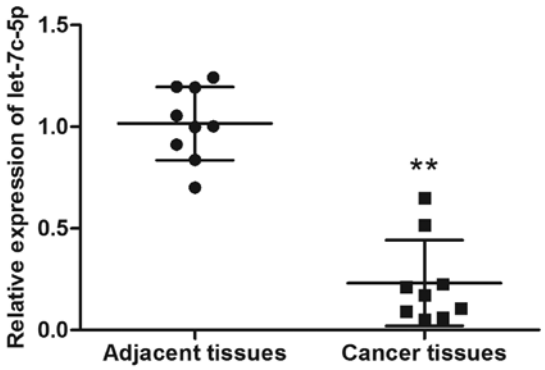

Figure 1. Expression of let-7c-5p in breast cancer tissues and corresponding adjacent tissues. The let-7c-5p expression level was detected by qRT-PCR, normalized with GAPDH. Let-7c-5p expression was greatly lower in cancer tissues than in adjacent tissues. $^{**}P<0.01$.

Dual luciferase reporter assay. The potential target genes of let-7c-5p were predicted using the algorithms of TargetScan (http://www.targetscan.org/vert_71/), PicTar (http://pictar.mdc-berlin.de/) and starBase (http://starbase.sysu.edu.cn/). ERCC6 was taken as a potential target gene of let-7c-5p. There was only one potential complementary site for let-7c-5p in the 3'UTR of ERCC6 mRNA, and the wild-type 3'UTR fragment containing putative binding site was amplified by PCR and cloned into the *Xba*I and *Not*I sites downstream of *Renilla* luciferase vector pRL-TK (Promega, Beijing, China), the recombinant plasmid was named pRL-ERCC6-wt. The putative let-7c-5p binding site was deleted by overlap-extension PCR, and the recombinant plasmid was named pRL-ERCC6-mut. Primers are listed in Table II, constructs were verified by sequencing.

For dual luciferase reporter assay, cells were seeded in 24-well plates in triplicate and cotransfected with the firefly luciferase vector pGL3 and recombinant plasmid pRL-ERCC6-wt or pRL-ERCC6-mut, together with mimics for either miR-NC or let-7c-5p at a final concentration of 20 nM. After 48 h, luciferase activities were measured using the dual luciferase reporter assay system (Promega). *Renilla* luciferase activity was normalized with firefly luciferase activity.

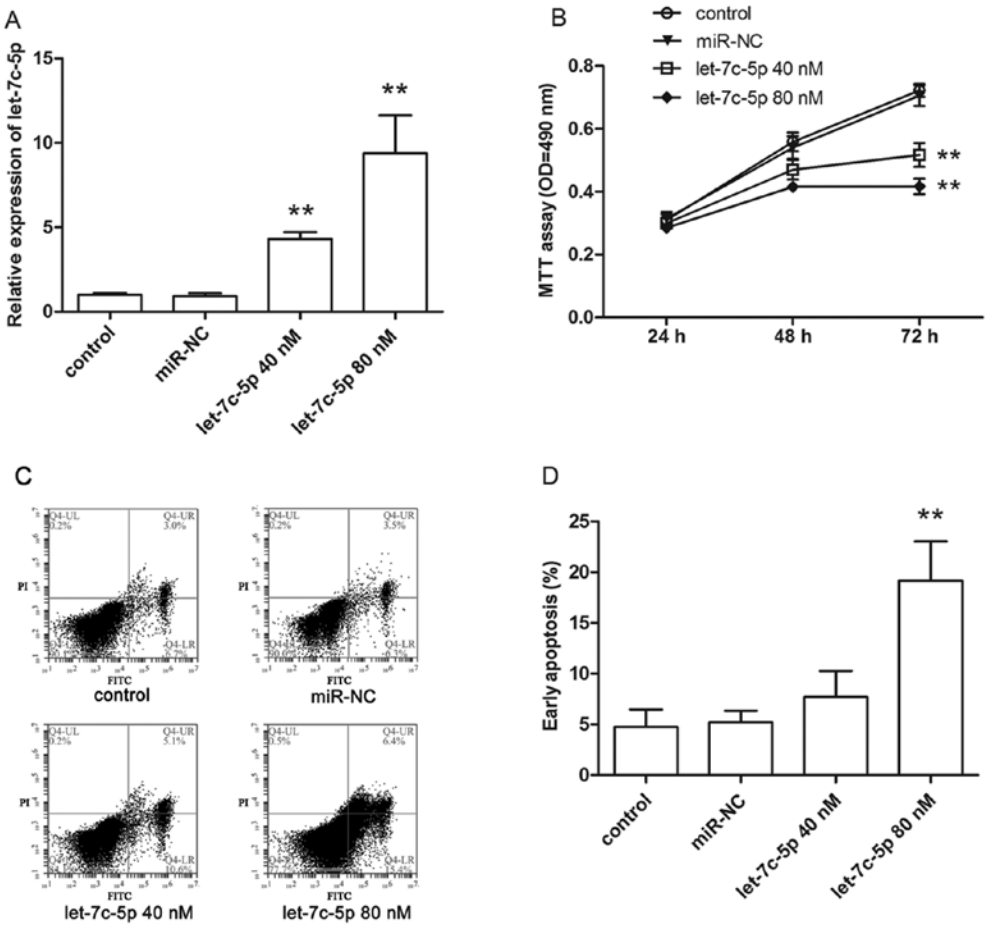

Figure 2. The effects of let-7c-5p on cell proliferation and apoptosis in breast cancer. (A) Let-7c-5p expression in MCF-7 cells was enhanced by transfection with let-7c-5p mimics at a final concentration of 40 or 80 nM. (B) Overexpression of let-7c-5p inhibited proliferation of MCF-7 cells. (C) Analysis of cell apoptosis by flow cytometry with FITC and PI staining. Early stage apoptotic cells are presented in the lower right quadrant. (D) Cell apoptosis was significantly induced by let-7c-5p. $^{**}P<0.01$.

Western blotting. Cells were harvested at 48 h post-transfection and lysed in RIPA buffer on ice. Total proteins were separated by 10% SDS-PAGE, electroblotted onto PVDF membranes. Membranes were blocked with 5% non-fat milk powder for 2 h at room temperature and incubated overnight at 4˚C with a polyclonal antibody: anti-ERCC6 antibody (1:2000 dilution; Abcam, Cambridge, MA, USA) or anti-GAPDH antibody (1:2000 dilution; HuaBio, China). After washing three times with TBS-T, membranes were incubated with a secondary antibody anti-rabbit HRP-conjugate (1:2000 dilution; HuaBio) for 2 h at room temperature (16). Antibody detection was performed with ECL Western Blotting Substrate (Solarbio, Beijing, China), and photos were taken using the Tanon 5500 imaging system (Tanon, Shanghai, China).

Statistical analysis. Statistical analysis was performed using SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA). All data were presented as mean ± standard deviation (SD). Statistical significance was tested by Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Reduced expression of let-7c-5p in breast cancer tissues. As shown in Fig. 1, expression of let-7c-5p was significantly decreased in the nine breast cancer tissues, indicating that let-7c-5p could potentially serve as a biomarker for breast cancer.

Overexpression of let-7c-5p inhibits MCF-7 cell proliferation and induces cell apoptosis. To determine the transfection efficiency, cells were harvested after transfection with 40 or 80 nM let-7c-5p mimics for 24 h. Total RNA was isolated from cells using the traditional TRIzol method. qRT-PCR was performed to measure the expression of let-7c-5p. As shown in Fig. 2A, let-7c-5p expression was enhanced significantly by let-7c-5p mimics.

To examine the effects of let-7c-5p on MCF-7 cell proliferation *in vitro*, MTT assay was performed at 24, 48 and 72 h post-transfection, the cell growth curve showed that the proliferation ability of transfected cells was reduced remarkably compared to control group cells (Fig. 2B).

Additionally, we explored whether cell apoptosis could be induced by let-7c-5p. After transfection for 24 h, cells were harvested, and cell apoptosis was detected by flow cytometer. As shown in Fig. 2C, early stage apoptotic cells are presented in the lower right quadrant, a higher rate of apoptosis was observed in transfected cells, especially in those transfected with 80 nM let-7c-5p mimics (Fig. 2D).

ERCC6 is a direct target gene of let-7c-5p in breast cancer. TargetScan, PicTar and starBase were used to predict the

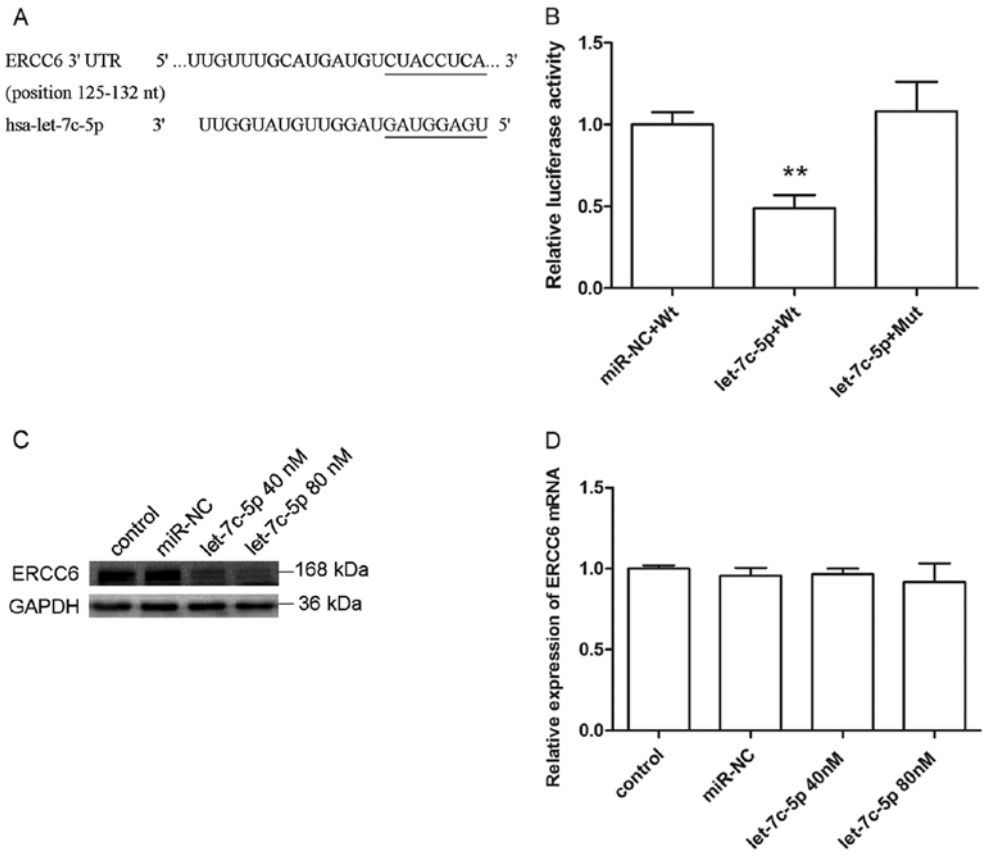

Figure 3. Let-7c-5p targets *ERCC6* in MCF-7 cells. (A) Let-7c-5p binding site in 3'UTR of *ERCC6* mRNA predicted by bioinformatics. (B) *ERCC6* was a direct target gene of let-7c-5p. MCF-7 cells were co-transfected with internal control plasmid pGL3 and recombinant plasmid pRL-ERCC6-wt or pRL-ERCC6-mut along with miR-NC or let-7c-5p mimics, relative luciferase activity was analyzed at 48 h post-transfection. $^{**}P<0.01$. (C) Western blot analysis of ERCC6 protein expression. ERCC6 protein expression was suppressed by let-7c-5p. (D) qRT-PCR analysis of *ERCC6* mRNA expression. *ERCC6* mRNA expression was not inhibited significantly.

target genes of let-7c-5p, and the intersection of the prediction results from the three algorithms showed that there were 41 candidate genes with at least one binding site of let-7c-5p (Table III). Of these genes, *ITGB3* and *MAP4K3* have been verified as targets of let-7c-5p in human non-small cell lung cancer. In this experiment, we aimed to find a new target of let-7c-5p in breast cancer. *ERCC6*, which has been reported to be associated with cancer risk, was selected as a putative target gene.

The let-7c-5p binding sites in 3'UTR of *ERCC6* mRNA were predicted by TargetScan, as a result, there was only one potential complementary sequence for let-7c-5p at nt 125-132 (Fig. 3A). To validate whether let-7c-5p targets the 3'UTR of *ERCC6* mRNA, we used the firefly luciferase vector pGL3 as an internal control plasmid, and co-transfected pGL3 and recombinant plasmid pRL-ERCC6-wt or pRL-ERCC6-mut along with miR-NC or let-7c-5p mimics into MCF-7 cells. Luciferase activities were measured after co-transfection for 48 h, as shown in Fig. 3B, dual luciferase reporter assay demonstrated that the *Renilla* luciferase activity of pRL-ERCC6-wt, which contained the wild-type 3'UTR fragment of *ERCC6*, was decreased by approximately 50% by let-7c-5p, compared to the miR-NC group. In addition, the luciferase activity of pRL-ERCC6-mut, in which the predicted binding site has been deleted, was not suppressed significantly. These results showed that *ERCC6* was a direct target gene for let-7c-5p in breast cancer.

To better understand the effects of let-7c-5p on ERCC6, we evaluated the *ERCC6* mRNA and protein expression level in MCF-7 cells after treatment with let-7c-5p mimics for 48 h. As shown in Fig. 3C and D, protein expression of ERCC6 was clearly inhibited by let-7c-5p, but the mRNA expression of *ERCC6* did not change significantly. These findings suggested that let-7c-5p regulated ERCC6 mainly through suppression of ERCC6 protein accumulation, but not affecting the mRNA level.

Discussion

A large number of studies suggest that miRNAs are involved in multiple human cancers, some are oncogenes, like miR-183, which is over-expressed in synovial sarcoma, rhabdomyosarcoma and colon cancer, targets the tumor suppressor gene *EGR1* and contributes to cell migration in these tumor types (17). Some other miRNAs may act as tumor suppressor genes, such as miR-100, which is found to reduce colon cancer cell motility and growth by targeting *Lgr5 in vitro* (18). MiRNA let-7c-5p acts as a cancer suppressor in different ways, such as preventing early cancer progression through repressing HMGA2 expression (19), inhibiting migration and invasion of human non-small cell lung cancer and colorectal cancer (13,14), and inducing cell apoptosis and disrupting cell cycle in human hepatocellular carcinoma cells (12). As for breast cancer,

Table III. Potential target genes of let-7c-5p predicted by TargetScan, PicTar and starBase.

Potential target genes	Position
ADRB2	chr5:148207936-148207943
AHCTF1	chr1:247003542-247003549
APBB3	chr5:139937904-139937911
BACH1	chr21:30717135-30717142
CD200R1	chr3:112641981-112641988
CDV3	chr3:133307221-133307228
CLDN12	chr7:90043275-90043282
COIL	chr17:55016284-55016291
COL1A1	chr17:48262068-48262074
DMD	chrX:31138095-31138102
E2F6	chr2:11585579-11585586
ERCC6	chr10:50666729-50666736
FGD6	chr12:95474757-95474764
FNIP1	chr5:130979161-130979168
FRAS1	chr4:79462429-79462436
GAN	chr16:81412340-81412347
GNPTAB	chr12:102140912-102140919
GOLT1B	chr12:21670278-21670285
HAND1	chr5:153854615-153854622
INTS2	chr17:59943299-59943306
IRS2	chr13:110408191-110408198
ITGB3	chr17:45388633-45388639
KLHDC8B	chr3:49213851-49213858
LRIG2	chr1:113666778-113666785
LRIG3	chr12:59266314-59266321
MAP4K3	chr2:39476696-39476703
MED8	chr1:43850492-43850499
MLL2	chr12:49414882-49414889
NAP1L1	chr12:76441001-76441008
NLK	chr17:26522778-26522784
PRPF38B	chr1:109243302-109243309
RANBP2	chr2:109401057-109401064
RNF20	chr9:104324767-104324773
RRM2	chr2:10269649-10269656
SEMA4C	chr2:97526289-97526296
SLC20A1	chr2:113421361-113421367
SLC35D2	chr9:99083376-99083383
TMEM2	chr9:74298636-74298643
WDR37	chr10:1176192-1176199
ZCCHC3	chr20:280315-280321
ZFYVE26	chr14:68214907-68214914

let-7c-5p is downregulated in both tissues and serum of the patients, and the expression of let-7c-5p is affected by post-menopausal status (20). Higher expression level of let-7c-5p is reported to be correlated with better clinical prognosis of patients with estrogen receptor-positive breast cancer (21), thus we hypothesize that let-7c-5p may play a suppressive part in breast cancer.

In this study, we also confirmed that let-7c-5p expression was decreased in breast cancer tissues compared with that of matched adjacent tissues, moreover, we found that overexpression of let-7c-5p could inhibit breast cancer cell proliferation and induce cell apoptosis significantly, these observations authenticated the hypothesis that let-7c-5p acted as a tumor suppressor in breast cancer. Furthermore, we found that let-7c-5p could bind to the 3'UTR of *ERCC6* mRNA. However, miRNAs regulate their target mRNAs in different ways, if the miRNAs have perfect or near-perfect complementarity to the 3'UTR of target mRNAs, the target mRNAs will be destroyed by miRNA, in this situation, both the mRNA and protein levels will be affected. On the other hand, the miRNAs have partial complementary sites in the 3'UTR of target mRNAs, the inhibition of protein accumulation will be direct, but the mRNA level may not be affected significantly (22,23). Herein, let-7c-5p has partial complementarity to the 3'UTR of *ERCC6* mRNA, as a result, ERCC6 protein expression was suppressed but *ERCC6* mRNA expression was not inhibited obviously by let-7c-5p in MCF-7 cells.

ERCC6, also known as CSB, is a chromatin remodeling factor. The protein encoded by *ERCC6* gene is a DNA-binding protein, which has important activity in nucleotide excision repair (24). It has been identified that mutations in *ERCC6* gene are associated with the rare disease Cockayne syndrome type B (25). In recent years, *ERCC6* is also showed to be involved in cancers. Ma *et al* unraveled some genetic variants of *ERCC6* jointly taking part in the lung cancer development among Chinese people (26). Chiu *et al* and Liu *et al* also reported that *ERCC6* polymorphisms were associated with the increased risk of gastric cancer and oral cancer (27,28).

Chromosomal instability is observed in most cancers including breast cancer. In cancer cells with high levels of chromosomal instability and replication stress, DNA damage occurs more frequently, and accumulation of DNA damage results in cell malfunction and cell apoptosis (29,30). So more DNA repair mechanisms need to be activated to correct the damage and maintain the activity of cancer cells (31,32). In the current study, we find that suppression of ERCC6 protein expression by let-7c-5p is related with reduced growth ability of breast cancer cells and higher rate of apoptosis, indicating ERCC6 may be involved in DNA damage repair in breast cancer and reduced ERCC6 protein level leads to DNA damage accumulation.

Many clinical anticancer drugs target DNA directly (33), such as cisplatin, which interferes with DNA replication and induces DNA damage by binding to DNA and forming DNA-cisplatin adducts. However, cisplatin resistance is a serious problem in cancer treatment, and activation of DNA repair mechanism is one of the main mechanisms involved in cisplatin resistance (34). The expression of excision repair cross-complementation group 1 (ERCC1), which participates in nucleotide excision repair, can be induced by cisplatin treatment, and increased excision of DNA-cisplatin adducts contributes to cisplatin resistance (35). As a DNA-binding protein, ERCC6 is also likely to be associated with anti-cancer drugs resistance via the nucleotide excision repair.

Nevertheless, this potential mechanism of ERCC6 has not been verified, thus more studies on ERCC6 are still needed.

In conclusion, our study suggested that let-7c-5p was a crucial contributor to cell apoptosis and inhibition of cell proliferation in breast cancer, partially through targeting ERCC6. These findings may provide a new potential strategy for breast cancer treatment.

Acknowledgements

This study was supported by the Natural Science Foundation of Zhejiang Province (LY15C050002 and LQ17H160013). This study was also supported by the National Natural Science Foundation of China (81402100), the Foundation of Health and Family Planning Commission of Jiangsu Province (Q201408) and the Social Development Foundation of Zhejiang (SH2016031, SH2014026).

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7-30, 2016.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
3. Filipowicz W, Bhattacharyya SN and Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat Rev Genet 9: 102-114, 2008.
4. Chen LT, Xu SD, Xu H, Zhang JF, Ning JF and Wang SF: MicroRNA-378 is associated with non-small cell lung cancer brain metastasis by promoting cell migration, invasion and tumor angiogenesis. Med Oncol 29: 1673-1680, 2012.
5. Guo B, Liu L, Yao J, Ma R, Chang D, Li Z, Song T and Huang C: miR-338-3p suppresses gastric cancer progression through a PTEN-AKT axis by targeting P-REX2a. Mol Cancer Res 12: 313-321, 2014.
6. Shi Y, Luo X, Li P, Tan J, Wang X, Xiang T and Ren G: miR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ. Cancer Lett 358: 27-36, 2015.
7. Roush S and Slack FJ: The let-7 family of microRNAs. Trends Cell Biol 18: 505-516, 2008.
8. Akao Y, Nakagawa Y and Naoe T: let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biol Pharm Bull 29: 903-906, 2006.
9. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG and Slack FJ: The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle 7: 759-764, 2008.
10. Nadiminty N, Tummala R, Lou W, Zhu Y, Zhang J, Chen X, eVere White RW, Kung HJ, Evans CP and Gao AC: MicroRNA let-7c suppresses androgen receptor expression and activity via regulation of Myc expression in prostate cancer cells. J Biol Chem 287: 1527-1537, 2012.
11. Nwaeburu CC, Bauer N, Zhao Z, Abukiwan A, Gladkich J, Benner A and Herr I: Up-regulation of microRNA let-7c by quercetin inhibits pancreatic cancer progression by activation of Numbl. Oncotarget 7: 58367-58380, 2016.
12. Zhu X, Wu L, Yao J, Jiang H, Wang Q, Yang Z and Wu F: MicroRNA let-7c inhibits cell proliferation and induces cell cycle arrest by targeting CDC25A in human hepatocellular carcinoma. PLoS One 10: e0124266, 2015.
13. Han HB, Gu J, Zuo HJ, Chen ZG, Zhao W, Li M, Ji DB, Lu YY and Zhang ZQ: Let-7c functions as a metastasis suppressor by targeting MMP11 and PBX3 in colorectal cancer. J Pathol 226: 544-555, 2012.
14. Zhao B, Han H, Chen J, Zhang Z, Li S, Fang F, Zheng Q, Ma Y, Zhang J, Wu N, *et al*: MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. Cancer Lett 342: 43-51, 2014.
15. Mao Y, Wu S, Zhao R and Deng Q: MiR-205 promotes proliferation, migration and invasion of nasopharyngeal carcinoma cells by activation of AKT signalling. J Int Med Res 44: 231-240, 2016.
16. Wu Q, Jin H, Yang Z, Luo G, Lu Y, Li K, Ren G, Su T, Pan Y, Feng B, *et al*: MiR-150 promotes gastric cancer proliferation by negatively regulating the pro-apoptotic gene EGR2. Biochem Biophys Res Commun 392: 340-345, 2010.
17. Sarver AL, Li L and Subramanian S: MicroRNA miR-183 functions as an oncogene by targeting the transcription factor EGR1 and promoting tumor cell migration. Cancer Res 70: 9570-9580, 2010.
18. Zhou MK, Liu XJ, Zhao ZG and Cheng YM: MicroRNA-100 functions as a tumor suppressor by inhibiting Lgr5 expression in colon cancer cells. Mol Med Rep 11: 2947-2952, 2015.
19. Park SM, Shell S, Radjabi AR, Schickel R, Feig C, Boyerinas B, Dinulescu DM, Lengyel E and Peter ME: Let-7 prevents early cancer progression by suppressing expression of the embryonic gene HMGA2. Cell Cycle 6: 2585-2590, 2007.
20. Li XX, Gao SY, Wang PY, Zhou X, Li YJ, Yu Y, Yan YF, Zhang HH, Lv CJ, Zhou HH, *et al*: Reduced expression levels of let-7c in human breast cancer patients. Oncol Lett 9: 1207-1212, 2015.
21. Sun X, Xu C, Tang SC, Wang J, Wang H, Wang P, Du N, Qin S, Li G, Xu S, *et al*: Let-7c blocks estrogen-activated Wnt signaling in induction of self-renewal of breast cancer stem cells. Cancer Gene Ther 23: 83-89, 2016.
22. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.
23. Pillai RS, Bhattacharyya SN and Filipowicz W: Repression of protein synthesis by miRNAs: How many mechanisms? Trends Cell Biol 17: 118-126, 2007.
24. Selby CP and Sancar A: Human transcription-repair coupling factor CSB/ERCC6 is a DNA-stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. J Biol Chem 272: 1885-1890, 1997.
25. Ghai SJ, Shago M, Shroff M and Yoon G: Cockayne syndrome caused by paternally inherited 5 Mb deletion of 10q11.2 and a frameshift mutation of ERCC6. Eur J Med Genet 54: 272-276, 2011.
26. Ma H, Hu Z, Wang H, Jin G, Wang Y, Sun W, Chen D, Tian T, Jin L, Wei Q, *et al*: ERCC6/CSB gene polymorphisms and lung cancer risk. Cancer Lett 273: 172-176, 2009.
27. Chiu CF, Tsai MH, Tseng HC, Wang CL, Tsai FJ, Lin CC and Bau DT: A novel single nucleotide polymorphism in ERCC6 gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol 44: 582-586, 2008.
28. Liu JW, He CY, Sun LP, Xu Q, Xing CZ and Yuan Y: The DNA repair gene ERCC6 rs1917799 polymorphism is associated with gastric cancer risk in Chinese. Asian Pac J Cancer Prev 14: 6103-6108, 2013.
29. Leung HW, Wu CH, Lin CH and Lee HZ: Luteolin induced DNA damage leading to human lung squamous carcinoma CH27 cell apoptosis. Eur J Pharmacol 508: 77-83, 2005.
30. Kaina B: DNA damage-triggered apoptosis: Critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol 66: 1547-1554, 2003.
31. Minocherhomji S, Ying S, Bjerregaard VA, Bursomanno S, Aleliunaite A, Wu W, Mankouri HW, Shen H, Liu Y and Hickson ID: Replication stress activates DNA repair synthesis in mitosis. Nature 528: 286-290, 2015.
32. Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, *et al*: Replication stress links structural and numerical cancer chromosomal instability. Nature 494: 492-496, 2013.
33. Kasyanenko N: DNA as a target for anticancer drugs based on the coordination compounds of metals. FEBS J 280: 89-89, 2013.
34. O'Grady S, Finn SP, Cuffe S, Richard DJ, O'Byrne KJ and Barr MP: The role of DNA repair pathways in cisplatin resistant lung cancer. Cancer Treat Rev 40: 1161-1170, 2014.
35. Zhu H, Luo H, Zhang W, Shen Z, Hu X and Zhu X: Molecular mechanisms of cisplatin resistance in cervical cancer. Drug Des Devel Ther 10: 1885-1895, 2016.