Inhibition of miR-214 attenuates the migration and invasion of triple-negative breast cancer cells

YI ZHANG1*, ZHIJING ZHAO1*, SIQI LI1, LIYING DONG1, YAN LI1, YING MAO1, YING LIANG1, YUN TAO2 and JUNFENG MA1

1Thyroid-Breast Surgery Department, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101; 2Clinical Skill Center, Kunming Medical University, Kunming, Yunnan 650500, P.R. China

Received May 3, 2018; Accepted November 21, 2018

Correspondence to: Dr Junfeng Ma, Thyroid-Breast Surgery Department, The Second Affiliated Hospital of Kunming Medical University, 374 Diamian Road, Wuhua, Kunming, Yunnan 650101, P.R. China
E-mail: majunf_jfm@163.com

*Contributed equally

Key words: triple negative breast cancer, miR-214, α1-antitrypsin, PI3K/Akt/mTOR pathway

Abstract. Triple-negative breast cancer (TNBC) is a subtype of breast cancer. MicroRNA (miR)-214 is closely associated with controlling the development of tumor cells; therefore, in the present study, the target gene and effects of miR-214 on TNBC cells were explored. Luciferase activity was examined by luciferase reporter assay. The viability, invasion and migration of MDA-MB-231 TNBC cells were measured using Cell Counting kit-8, Transwell and wound-healing assays, respectively. The expression levels of various factors were determined using reverse transcription-quantitative polymerase chain reaction and western blotting. The results demonstrated that the expression levels of miR-214 were higher and the levels of α1-antitrypsin (α1-AT) were lower in TNBC tissues compared with in normal tissues. Subsequently, α1-AT was revealed to be a target of miR-214. Furthermore, inhibition of miR-214 decreased cell viability, invasion and migration, enhanced the expression of E-cadherin and tissue inhibitor of metalloproteinases-2, and reduced the expression of metastatic tumour antigen 1 and matrix metalloproteinase-2. Inhibition of miR-214 also significantly downregulated the phosphorylation of protein kinase B (Akt) and mammalian target of rapamycin (mTOR), and markedly downregulated that of phosphoinositide 3-kinase (PI3K); however, the expression levels of total PI3K, Akt and mTOR remained stable in all groups. Taken together, these findings suggested that miR-214 targeting α1-AT may be a potential mechanism underlying TNBC development.

Introduction

Cancer is the second most common disease that threatens human health and mortality globally (1). Among women, breast cancer (BC) has the highest incidence of all malignant tumors (2). Triple-negative BC (TNBC) is a subtype of BC, which accounts for 10-20% of cases of BC globally. TNBC is characterized by negative expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, and is considered an independent clinicopathological type of cancer that is characterized by strong invasiveness (3-5). Metastasis is a critical biological hallmark of malignant tumors and is the leading cause of mortality among patients with TNBC (6‑8); however, the specific process and underlying mechanism remains unclear. Therefore, further study of the mechanism underlying TNBC metastasis and exploration of its effective prevention are of great significance in terms of improving the survival rate and quality of life for patients.

MicroRNAs (miRNAs/miRs) are non-coding single-stranded RNA molecules, which consist of ~22 nucleotides (9). The role of miRNAs is fulfilled by regulating the expression of target genes, which is regulated by complementary binding of the miRNA to the 3′-untranslated region (3′UTR) of the target gene mRNA (10,11). miRNAs may act as oncogenes or tumor suppressor genes in the development of cancer (12-15). Numerous studies have reported that the abnormal expression of miRNAs is closely associated with the development of various tumor types (13-18), and miRNAs are expected to be a novel molecular target for the diagnosis and treatment of cancer. The abnormal expression of miR-214 contributes to the formation of various human tumors, including ovarian cancer, colorectal cancer, gastric cancer and BC (19-22). Furthermore, miR-214 is closely associated with regulation of the development of tumor cells, including cell growth, apoptosis and metastasis (21,23-25); however, at present, the role and target of miR-214 in TNBC is not fully understood.

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signal
transduction pathway is one of the most important signaling pathways in cells. This pathway serves a vital role in regulating cell proliferation, apoptosis, differentiation and metabolism by affecting the activation state of several downstream effector molecules (26-28). In recent years, abnormal activation of the PI3K/Akt/mTOR pathway has been detected in numerous human malignant tumors, and activation of this pathway may be a critical factor leading to the proliferation and metastasis of tumor cells (29-31).

In the present study, the target gene of miR-214 in TNBC was analyzed using the microRNA.org website. In addition, the effects of miR-214 on the growth and metastasis of TNBC cells, and the associated pathways were analyzed.

Materials and methods

Tissue collection. Between June 2016 and November 2017, 37 TNBC, 37 non-TNBC BC and 37 normal tissues were collected from patients with TNBC (38-55 years), patients with n-TNBC (35-53 years) and normal control individuals (all female, 36-52 years) who were diagnosed and subjected to mastectomy at the Second Affiliated Hospital of Kunming Medical University (Kunming, China). All patients provided written informed consent and allowed their tissues to be used for research purposes. The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University.

Cell culture. The MDA-MB-231 human TNBC cell line was purchased from Cobioer Biosciences Co., Ltd. (Nanjing, China). The cells were cultured in RPMI-1640 medium (Huayueyang Biotechnology Co., Ltd., Beijing, China) supplemented with 10% fetal bovine serum (FBS; Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China), and a mixture of penicillin (100 µg/ml) and streptomycin (100 U/ml; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) in a 95% humidified incubator (Sanyo Electric Co., Ltd.; Panasonic Corporation, Kadoma, Japan) containing 5% CO₂ at 37°C.

Cell transfection. miR-214 mimics, miR-214 inhibitor and miRNA negative control (NC) were purchased from Vigen Biosciences (Rockville, MD, USA). The miRNA sequences were as follows: miR-214 mimics, 5′-UGACGGACAGACG GACGACA-3′; miRNA inhibitor, 5′-ACUGCCUGUCCUGUC CUGCUGU-3′; and miRNA NC, 5′-UCUACUUCUUCAGG AGGUUGUA-3′. Cell transfection with miR-214 mimics (100 pmol), inhibitor (100 pmol) or miRNA NC (100 pmol) was performed when the confluence of MDA-MB-231 cells reached ~60%. Transfection was conducted using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Santa Clara, CA, USA) at 37°C for 24 h; a QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) was used to obtain α1-AT-3’UTR mutant. The cells were lysed using 1X passive lysis buffer at room temperature for 15 min and the suspension was transferred to the black enzyme plate. LARII (100 µl) and Stop&Glo® reagent (100 µl) were then added to the plates at room temperature, and luciferase activity normalized to Renilla luciferase was immediately measured using a GloMax® Discover Multimode Microplate Reader (GM3000; Promega Corporation).

RT-qPCR analysis. Total RNA was extracted from cells and tissues using RNA extraction kit (Takara Biotechnology Co., Ltd., Dalian, China). Subsequently, 1 µg RNA was used to synthesize cDNA using the SuperScript® Vilo™ cDNA synthesis kit (Thermo Fisher Scientific, Inc.). The RT reaction conditions were as follows: 30°C for 10 min, 42°C for 30 min and 95°C for 5 min. cDNA was then amplified using Fast SYBR® Green Master Mix (Thermo Fisher Scientific, Inc.). The qPCR reaction conditions were as follows: 95°C for 10 sec, followed by 40 cycles at 95°C for 5 sec and 60°C for 30 sec, and a final step at 75°C for 1 min. Primer sequences are listed in Table I. U6 and β-actin were used as internal controls. mRNA expression levels were quantified using the 2^-ΔΔCt method (32).

Western blot analysis. Total proteins were lysed from cells and tissues using radioimmunoprecipitation assay buffer (Beijing Solarbio Science & Technology Co., Ltd.), and protein concentrations were determined using bichinchoninic acid protein assay (Thermo Fisher Scientific, Inc.). Proteins (1 µg/µl; 10 µg/lane) were separated by 12% SDS-PAGE and were transferred onto polyvinylidene fluoride membranes (Hangzhou RENO Membrane Technology, Co., Ltd., Hangzhou, China, www.renomem.com). Subsequently, 5% non-fat milk was used to block the membranes at room temperature for 1.5 h. The membranes were then hybridized to anti-α1-AT (cat. ab179443, 1:1,000; Abcam, Cambridge, MA, USA), anti-E-cadherin (cat. ab15148, 1:800; Abcam), anti-tissue inhibitor of metalloproteinases-2 (TIMP2; cat. no. ab180630, 1:1,000; Abcam), anti-metastatic tumour antigen 1 (MTA1; cat. no. ab751, 1:1,000; Abcam), anti-matrix metalloproteinase-2 (MMP2; cat. no. ab32028, 1:1,000; Abcam), anti-phosphorylated (p) -PI3K (cat. no. ab182651, 1:1,000; Abcam), anti-matrix metalloproteinase-2 (MMP2; cat. no. ab37150, 1:1,200; Abcam), anti-matrix metalloproteinase-2 (MMP2; cat. no. ab37150, 1:1,200; Abcam), anti-matrix metalloproteinase-2 (MMP2; cat. no. ab32028, 1:1,000; Abcam) and anti-β-actin (cat. no. ab8227, 1:600; Abcam) at 4°C for 24 h. Subsequently, the membranes were hybridized to the corresponding secondary antibodies (Abcam): Horseradish peroxidase (HRP)-conjugated donkey anti-mouse immunoglobulin (IgG) H&L (cat. no. ab6820, 1:7,000), HRP-conjugated rabbit anti-mouse IgG H&L (cat. no. ab6728, 1:7,000) and HRP-conjugated goat anti-rabbit IgG H&L (cat. no. ab6721, 1:7,000) at 37°C for 60 min. The blots were detected by enhanced chemiluminescence.
detection reagent (Shanghai Yeasen Biotechnology Co., Ltd., Shanghai, China). VisionWorks® LS Image Acquisition and Analysis software version 7.0 (UVP, LLC, Phoenix, AZ, USA) was used to quantify band intensities.

Cell Counting kit-8 (CCK-8) assay. Cell viability was assessed by CCK-8 (Dalian Meilun Biotechnology Co., Ltd., Dalian, China). Briefly, cells were seeded in a 96-well plate (3x10^4 cells/well) at 37˚C for 24 h. Subsequently, cells were exposed to PBS (control), miRNA NC or miR-214 inhibitor at 37˚C for 6, 12 and 24 h. CCK-8 reagent (10 µl) was then added to the cells, which were incubated for a further 4 h at 37˚C. The optical density value was then assessed at 450 nm using a microplate reader (SMR16.1; Uscn Life Sciences, Inc., Wuhan, China).

Wound-healing assay. Cells were seeded in a 6-well plate (6x10^4 cells/well) and were cultured for 24 h. The cells were then exposed to PBS (control), or were transfected with miRNA NC or miR-214 inhibitor. Subsequently, the cells were scratched using a 200-µl pipette tip (Thermo Fisher Scientific, Inc.). Following culturing at 37˚C for 24 h, the cells were observed under a DSX100 optical microscope (Olympus Corporation, Tokyo, Japan).

Transwell assay. Matrigel (200 µg/ml, 100 µl; BD Biosciences, Franklin Lakes, NJ, USA) was added to the upper Transwell chamber at room temperature until it solidified, after which, cells were added to the upper chamber. The medium supplemented with 15% FBS was added to the lower chamber. The transfected cells were centrifuged at 5,000 x g for 20 min at 4˚C, and the cell suspension (2x10^5 cells/ml) was cultured in the upper chamber at 37˚C for 24 h. Crystal violet (0.1%; Shanghai Gefan Biotechnology Co., Ltd., Shanghai, China) was used to stain the cells for 20 min. Finally, cells were observed under a DSX100 optical microscope (Olympus Corporation).

Statistical analysis. All data are presented as the mean ± standard error of mean, as determined by Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Data were analyzed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). The differences among groups were determined by one-way analysis of variance, followed by Dunnett's test. Each experiment was performed in triplicate and repeated three times. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of miR-214 and α1-AT among patients with BC. RT-qPCR was used to analyze the expression levels of miR-214 and α1-AT in normal, n-TNBC and TNBC tissues. In n-TNBC and TNBC tissues, the expression levels of miR-214 were higher and the mRNA expression levels of α1-AT were lower than in normal tissue (P<0.01; Fig. 1A and 1B). In addition, according to miRanda, α1-AT was predicted to possess an miR-214-binding site (Fig. 1C). Subsequently, it was revealed that the luciferase activity of α1-AT-3'UTR was reduced in cells transfected with miR-214 mimics; however, miR-214 mimics did not affect the luciferase activity of α1-AT-3'UTR mut (Fig. 1D). Furthermore, the expression levels of α1-AT were decreased following upregulation of miR-214 via transfection with miR-214 mimics (Fig. 1E-G). These results suggested that α1-AT may be a target of miR-214.
Suppression of miR-214 attenuates the viability of MDA-MB-231 cells. In order to examine the effects of a miR-214 inhibitor on MDA-MB-231 cells, the expression levels of miR-214 and cell viability were detected by RT-qPCR and CCK-8 assay, respectively. The results of RT-qPCR revealed that the expression levels of miR-214 were significantly decreased in cells transfected with miR-214 inhibitor (Fig. 1H). In addition, the miR-214 inhibitor markedly suppressed cell viability at 12 and 24 h (Fig. 1I).

Suppression of miR-214 reduces the migratory and invasive abilities of MDA-MB-231 cells. Wound-healing and Transwell assays were conducted to study the effects of a miR-214 inhibitor on the migratory and invasive abilities of MDA-MB-231 cells. As the wound-healing assay results indicated, in cells transfected with a miR-214 inhibitor, the relative wound width was markedly increased (Fig. 2A). The results of the Transwell assay indicated that the miR-214 inhibitor significantly decreased the relative invasion rate of cells (Fig. 2B).

Suppression of miR-214 regulates migration-associated factors in MDA-MB-231 cells. RT-qPCR and western blotting were performed to study the effects of miR-214 inhibition on the expression of migration-associated factors. As shown in Fig. 3A-D, the mRNA expression levels of MTA1 and MMP2 were decreased, whereas the mRNA expression levels of E-cadherin and TIMP2 were increased in cells transfected with a miR-214 inhibitor. In addition, alterations in the protein expression levels of E-cadherin, TIMP2, MTA1 and MMP2 were consistent with alterations in the mRNA expression levels (Fig. 3E).

Suppression of miR-214 inhibits the PI3K/Akt/mTOR signaling pathway in MDA-MB-231 cells. In order to investigate the effects of the miR-214 inhibitor on signaling in MDA-MB-231 cells, the PI3K/Akt/mTOR signaling pathway was examined by western blotting. The results revealed that transfection with the miR-214 inhibitor markedly downregulated phosphorylation of PI3K, Akt and mTOR. However, the expression levels of total ZHANG et al: THE ROLE OF miR-214 IN TNBC
PI3K, Akt and mTOR remained stable in the various groups (Fig. 4). The proportions of p-Akt/Akt and p-mTOR/mTOR were significantly reduced by the miR-214 inhibitor (Fig. 4).

Discussion

Increasing evidence has demonstrated that miR-214 is aberrantly expressed and its function is altered in various types of tumor; in particular, low miR-214 expression has been detected in liver cancer and ovarian carcinoma (21,33), whereas high miR-214 expression has been observed in BC, gastric cancer and pancreatic cancer (20,24,34). Notably, Kalniete et al (20) detected high expression levels of miR-214 in patients with TNBC. Similar to this previous study, the present data demonstrated that miR-214 was highly expressed in TNBC tissues.

α1-AT is a member of the serine protease inhibitor superfamily, which is dysregulated in lung cancer, prostate cancer and BC (35,36). Previous studies have reported that α1-AT may act as a tumor suppressor in BC cells (37,38). The present data revealed that the expression levels of α1-AT were decreased in TNBC tissues. According to microRNA.org, α1-AT was identified as a target gene of miR-214. In addition, when MDA-MB-231 cells were transfected with miR-214 mimics, the expression levels of α1-AT were downregulated. Therefore, these findings suggested that α1-AT may be a target gene of miR-214, and that miR-214 may exert its effect on TNBC through targeting α1-AT.

Cell proliferation, invasion and migration are known to be essential processes associated with tumor development. Wang et al (39) revealed that miR-214 increases the invasive capacity of BC cells. Zhang et al (40) also reported that miR-214 silencing decreases the proliferation of nasopharyngeal carcinoma cells. Furthermore, Xin et al (41) demonstrated that miR-214 facilitates the metastasis of gastric cancer cells. The present study explored the effects of miR-214 on the viability, invasion and migration of TNBC cells. The results demonstrated that inhibition of miR-214 significantly decreased the viability, migration and invasion of MDA-MB-231 cells.

E-cadherin is an intercellular adhesion molecule, and downregulation of E-cadherin decreases intercellular adhesion (42). The main function of MMPs is to degrade and reshape the dynamic balance of the extracellular matrix. A previous study reported that when tumor cell metastasis is inhibited, the levels of E-cadherin are upregulated and the levels of MMPs are downregulated (43). TIMPs are tissue inhibitors of the MMP family, and MTAs are also a class of critical tumor metastasis-associated genes. It has been reported that tumor necrosis factor α suppresses radiation-induced cell metastasis in neuroblastoma via increasing TIMP2 and E-cadherin levels, and decreasing MTA-2 and MMP levels (44). Therefore, this study examined the effects of miR-214 on the migration/invasion-associated factors using RT-qPCR and western blotting. It was revealed that miR-214 silencing decreased cell migration and invasion via upregulating E-cadherin and TIMP2, and downregulating MTA1 and MMP2.

Previous studies have demonstrated that miR-214 has a role in regulating the PI3K/Akt/mTOR pathway under various pathological conditions (25,45,46). Liu et al (25) reported that miR-214 controls the PI3K/Akt pathway via targeting phosphatase and tensin homolog in oophoroma. Zhao et al (46)
also indicated that miR-214 expedited osteoclastogenesis via regulating PI3K/Akt signaling. Li et al (45) demonstrated that miR-214 mediates the PI3K/Akt/mTOR signaling pathway in rat muscle atrophy. Therefore, it was hypothesized that miR-214 may mediate the PI3K/Akt/mTOR pathway in TNBC. As expected, knockdown of miR-214 suppressed the PI3K/Akt/mTOR pathway in MDA-MB-231 cells.

In conclusion, this study demonstrated that miR-214 was highly expressed in TNBC tissues, and that α1-AT was a target gene for miR-214. Silencing of miR-214 markedly suppressed the proliferation, migration/invasion of MDA-MB-231 cells by upregulating E-cadherin and TIMP2, and downregulating MTA1 and MMP2. Furthermore, miR-214 silencing inhibited the PI3K/Akt/mTOR pathway. These findings indicated that miR-214 targeting α1-AT may be a potential mechanism underlying TNBC development.

Acknowledgements

Not applicable.
Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions
JM made substantial contributions towards the design of the study. YT collected breast cancer and normal tissues. YaI performed gene expression experiments in tissues. YM performed target prediction for miR-214. YaL performed cell transfections. LD performed viability, migration and invasion assays. SL performed RT-qPCR assays. ZZ performed western blotting. YZ analyzed the data and drafted the manuscript. All authors read and approved of the final version of the manuscript.

Ethics approval and consent to participate
All patients provided written informed consent and allowed their tissues to be used for research purposes. The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University.

Patient consent for publication
All participants provided written informed consent prior to the present study.

Competing interests
The authors declare that they have no competing interests.

References
1. Wu J, Goyal L, Nipp R, Wo J, Qadan M and Upptot RN: The Tipping Point: Key oncologic imaging findings resulting in critical changes in the management of malignant tumors of the gastrointestinal tract. Curr Probl Diagn Radiol 48: 61-74, 2019.
2. DeSantis C, Ma J, Bryan L and Jemal A: Breast cancer statistics, 2013. CA Cancer J Clin 64: 52-62, 2014.
3. Dunne M, Dou YN, Drake DM, Spence T, Gontijo SML, Wells PG and Allen C: Hyperthermia-mediated drug delivery induces biological effects at the tumor and molecular levels facilitating improved cisplatin efficacy in triple negative breast cancer. J Control Release 282: 35-45, 2018.
4. Park IH, Kong SY, Kwon Y, Kim MK, Sim SH, Joo J and Park JH: Radiol 82: 506-510, 2017.
5. Farah M, Nagarajan P, Torres-Cabala CA, Curry JL, Amaria RN, Lee KS: Phase I/II clinical trial of everolimus combined with gemcitabine/cisplatin for metastatic triple-negative breast cancer. J Control Release 282: 35-45, 2018.
6. Liu Y, Lin J, Zhai S, Sun C, Xu C, Zhou H and Liu H: MicroRNA-214 suppresses ovarian cancer by targeting β-Catenin. Cell Physiol Biochem 45: 1654-1662, 2018.
7. Wang X, Zhang H, Bai M, Ning T, Ge S, Deng T, Liu R, Zhang L, Yin G and Bu Y: Exosomes serve as nanoparticles to deliver anti-miR-214 to reverse chemoresistance to cisplatin in gastric cancer. Mol Ther 26: 774-783, 2018.
8. Chen X, Wang YW, Zhu WJ, Li Y, Liu L, Yin G and Gao P: A four-microRNA signature predicts lymph node metastasis and survival in breast cancer. J Exp Clin Cancer Res 37: 122-132, 2018.
9. Li HL, Liang S, Cui JH and Han GY: Targeting of GSK-3β by miR-214 to facilitate gastric cancer cell proliferation and apoptosis. Eur Rev Med Pharmacol Sci 22: 127-134, 2018.
10. Liu J, Chen W, Zhang H, Liu T and Zhao L: miR-214 targets the PTEN-mediated PI3K/Akt signaling pathway and regulates cell proliferation and apoptosis in ovarian cancer. Oncol Lett 14: 5711-5718, 2017.
11. Costa RLB, Han HS and Gradjashir WJ: Targeting the PI3K/AKT/mTOR pathway in triple-negative breast cancer: A promising review. Breast Cancer Res Treat 169: 397-406, 2018.
12. Ramakrishnan V and Kumar S: PI3K/AKT/mTOR pathway in multiple myeloma: From basic biology to clinical promise. Leuk Lymphoma 1-11, 2018.
13. Simioni C, Martelli AM, Zauli G, Vitale M, McCubrey JA, Capitanio S and Neri LM: Targeting the phosphatidylinositol 3-kinase/Akt/mTOR axis to delay the onset of osteosarcoma in B-lineage acute lymphoblastic leukemia: An update. J Cell Physiol 233: 6440-6454, 2018.
14. Du L, Li X, Zhen L, Chen W, Mu L, Zhang Y and Song A: Everolimus inhibits breast cancer cell growth through PI3K/AKT/mTOR signaling pathway. Mol Med Rep 17: 7163-7169, 2018.
30. Yeh YH, Hsiao HF, Yeh YC, Chen TW and Li TK: Inflammatory interferon activates HIF-1α-mediated epithelial-to-mesenchymal transition via PI3K/AKT/mTOR pathway. J Exp Clin Cancer Res 37: 70, 2018.

31. Zhang H, Xu HL, Wang YC, Lu ZY, Yu XF and Sui DY: 20(S)-Protopanaxadiol-induced apoptosis in MCF-7 breast cancer cell line through the inhibition of PI3K/AKT/mTOR signaling pathway. Int J Mol Sci 19: pi: E1053, 2018.

32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Method 25: 402-408, 2001.

33. Fan Y, Qian X and Zhang C: UG SNP rs11904020 in 3'UTR of STAT3 regulated by miR-214 promotes hepatocellular carcinoma development in Chinese population. Tumour Biol 37: 14629-14635, 2016.

34. Kuninty PR, Bojmar L, Tjomsland V, Larsson M, Storm G, Ostman A, Sandstrom P and Prakash J: MicroRNA-199a and -214 as potential therapeutic targets in pancreatic stellate cells in pancreatic tumor. Oncotarget 7: 16396-16408, 2016.

35. El-Akawi ZJ, Al-Hindawi FK and Bashir NA: Alpha-1 antitrypsin (alpha1-AT) plasma levels in lung, prostate and breast cancer patients. Neuro Endocrinol Lett 29: 482-484, 2008.

36. García-Orad A, Arizti P, Durán L, Urcelay B and De Pancorbo MM: Alpha-1 antitrypsin phenotypes among breast cancer patients in the Basque population. Hum Hered 44: 203-208, 1994.

37. Zhao C, Sun W, Zhang P, Ling S, Li Y, Zhao D, Peng J, Wang A, Li Q, Song J, et al: miR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. RNA Biol 12: 343-353, 2015.

38. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.