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ORIGINAL ARTICLE

Long non-coding RNA PCAT29 regulates the growth, migration and invasion of human triple-negative breast cancer cells

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Summary

Purpose: *A number of studies have provided concrete evidence about the role of Long noncoding RNAs (LncRNAs) in the development and progression of cancer. As such LncRNAs are believed to exhibit the potential to be used as therapeutic targets for the treatment of cancer. This study was undertaken to investigate the role and therapeutic implication of LncRNA PCAT29 in triple-negative breast cancer.*

Methods: *breast cancer cell lines MDA-MB-231, MDA-MB-436, BT20, HCC70 and HCC38 and non-cancer cell line MB157 were used in this study. Gene expression analysis was performed by qRT-PCR. Cell proliferation was monitored by MTT and colony formation assays. Apoptosis was detected by annexin V/propidium iodide (PI) assay. Cell migration and Invasion was detected by wound heal and transwell assays.*

Results: *The expression of LncRNA PCAT29 was significantly suppressed in the breast cancer tissues and the triple-negative breast cancer cell lines. PCAT29 overexpression caused inhibition of the proliferation rate and colony formation of the MDA-MB-231 cells. The proliferation of MD-MB-231 cells was inhibited by apoptotic cell death which was accompanied by elevation of Bax and depletion of Bcl-2 expression. The wound healing assay showed that PCAT29 caused remarkable inhibition of the MDA-MB-231 cell migration. The transwell assay showed that PCAT29 overexpression resulted in 65% inhibition of the MDA-MB-231 cell invasion.*

Conclusion: *PCAT29 regulates the proliferation, migration and invasion of breast cancer cells and may point to a novel therapeutic target in triple-negative breast cancer.*

Key words: *triple-negative breast cancer, apoptosis, migration, invasion, LncRNAs, PCAT29*

Introduction

Breast cancer is one of the frequently detected in women accounting for significant mortality across the world. Annually, 1.3 million new cases of breast cancer and 0.4 million deaths are reported due to breast cancer [1]. Global breast cancer statistics has shown that the incidence of breast cancer is increasing significantly. Around 18% increase was observed for breast cancer incidence and mortality from 2008 to 2012. In US, 1 in every 8 women will develop breast cancer during her life [2]. It is believed that by 2050 there will be 3.2 million new cases of breast cancer annually [3]. Triple-negative breast cancers constitute approximately 15% of all the breast cancers and are characterized by lack of expression of oestrogen, progesterone and ERBB2 receptors [4]. The incidence of triple-negative breast cancer has been shown to be comparatively higher in premenopausal African and African-American women [5]. Additionally, the people with BRCA1 mutation are at higher risk of triple-negative breast cancer [6]. This type of breast cancer is highly aggressive and has very bad prognosis as compared

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to other types of breast cancer. The treatment options for triple-negative breast cancer are limited and there are very high chances of relapses after treatment [7]. Therefore, there is pressing need to identify novel therapeutic targets and subsequently develop drugs for these targets that can allow efficient treatment of triple-negative breast cancer patients. Recently, many studies have provided strong evidence that long non-coding RNAs (LncRNAs) may exhibit therapeutic implications in the treatment of human diseases including cancer [8]. The LncRNA PCAT29 has been shown to act as a tumor suppressor in different types of cancers [9]. Nonetheless, its therapeutic implications have not been explored in triple-negative breast cancer. This study was therefore undertaken to investigate the role and therapeutic implications of LncRNA PCAT29 in triple-negative breast cancer

Methods

Tissue samples and cell lines

Snap-frozen breast cancer tissues and adjacent normal tissues were collected from Department of Thyroid and Breast Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The triple-negative human breast cancer cell lines MDA-MB-231, MDA-MB-436, BT20, HCC70 and HCC38 and non-cancer cell line MB157 were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) containing penicillin (100 U/mL), streptomycin (100 U/mL) (Sigma-Aldrich, St. Louis, MO, USA), and 10% fetal bovine serum (FBS; Gibco) at 37°C in 5% CO_2.

Expression analysis

RNA was extracted from the breast cancer tissues and cell lines with the help of the TRIzol reagent and then subsequently purified by RNeasy Mini Kit (Qiagen, Hilden, Germany). The miScript Reverse Transcription Kit (Qiagen) was then used to synthesize cDNA from the total RNA. Thereafter, cDNA was amplified by using SYBR Premix Ex *Taq*™ (TaKaRa, Otsu, Shiga, Japan). The cycling conditions were as follows: 94°C for 25 sec, followed by 38 cycles of 95°C for 20 sec, and 57°C for 30 sec. The expression was estimated by $2^{-\Delta\Delta Ct}$ method and actin was used as internal control.

Cell transfection

The pcDNA-PCAT29 and NC were purchased from RiboBio (Guangzhou, China). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was then used to perform the transfections in accordance with the manufacturer's guidelines. As the MDA-MB-231 and CAMA-1 cells reached 80% confluence, the appropriate concentrations of pcDNA-PCAT29 or NC was transfected into these cells.

Cell proliferation assay

The triple-negative MDA-MB-231 breast cancer cells were seeded in 96-well culture plates with approximately 2500 cells/well. The viability of the cells was evaluated at different time intervals by Vybrant MTT Cell Proliferation Assay (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. Finally, the optical density (OD) was measured at 570 nm with a spectrophotometer.

Apoptosis assay

The transfected MDA-MB-231 cells were cultured for 24 h at 37°C and then fixed with ethanol (70%) for 20 min. The cells were then washed with phosphate buffered saline (PBS) and subsequently stained with a solution of acridine orange (AO)/ethidium bromide (EB). Finally, the cells were examined under microscope to detect the induction of apoptosis. For determination of the percentage of apoptosis the transfected cells were stained with annexin V/PI and subsequently examined by flow cytometer.

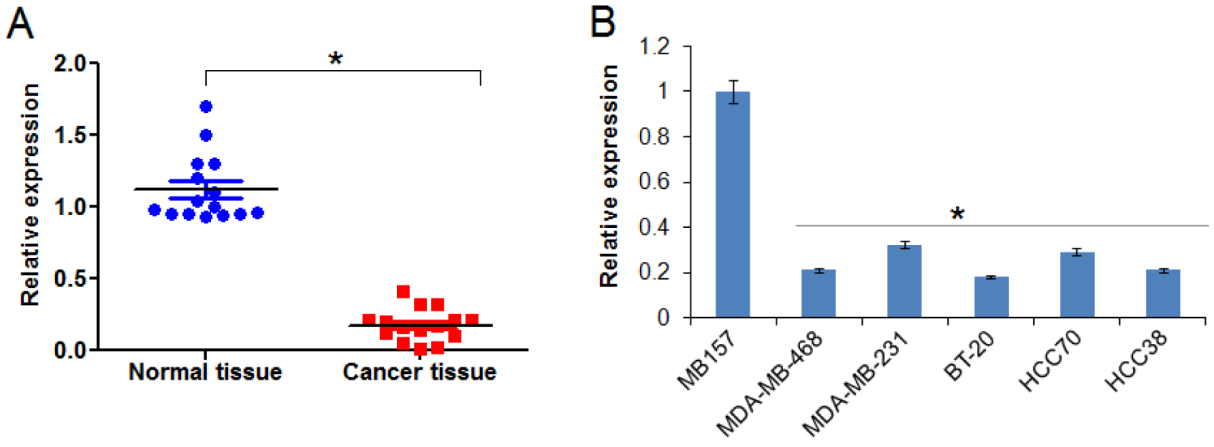

Figure 1. A: Expression of lncRNA PCAT29 in normal and breast cancer tissues. **B:** Expression of PCAT29 in normal MB157 and triple-negative breast cancer cell lines as determined by the qRT-PCR. The experiments were performed in triplicate and expressed as mean ± SD (*p<0.05).

Wound healing assay

The transfected MDA-MB-231 and CAMA-1 cells were placed in 12-well plates with approximately 1×10^5 cells per well. A scratch was made by a sterile pipette tip 24 h after transfection. The cells were PBS-washed and fresh Dulbecco's modified Eagle's medium (DMEM) was added. Following 24-h incubation at 37°C, the cells were fixed with methanol. The initial wound width and final wound width were determined from photomicrographs.

Cell invasion assay

Transwell chambers with Matrigel were employed to monitor the invasion of the MDA-MB-231. In brief, the cells were transfected with appropriate constructs and 48 h posttransfection the cells were harvested and suspended in fresh DMEM. Two hundred µL of the cell suspension containing approximately 5×10^4 cells was placed onto the upper compartment, and a fresh 500 µL DMEM was placed in the lower compartment. After 24 h cells present at the upper compartment were removed by swabbing while cells that invaded to the lower surface were fixed with 3.7% paraformaldehyde for 10 min and subsequently stained with 0.05% crystal violet. Finally, 10 random fields were selected to determine the invasion under light microscope.

Western blotting

The breast cancer tissues and cell lines were lysed with RIPA lysis buffer and the protein concentration in each sample was measured by Bradford assay. Equal concentrations of the proteins from each sample were loaded on 10% SDS polyacrylamide gels (SDS-PAGE), followed by shifting to polyvinylidene fluoride membranes. Blocking of the membrane was then performed by fat-free milk (5%) in a mixture of tris-buffered saline (TBS) and polysorbate 20. This was followed by incubation with the primary antibody for 24 h at 4°C. Subsequently, secondary antibody was added at 25°C for about 2 h. The bands of interest were finally observed by chemiluminescence assay.

Results

The PCAT29 is downregulated in breast cancer

Gene expression levels of the PCAT29 were assessed in the breast cancer cell lines and the breast cancer tissues by the qRT-PCR. In all the breast cancer tissues, PCAT29 was significantly downregulated. The downregulation was found to up to 11-fold relative to normal tissues (Figure 1). Additionally, the expression of PCAT29 was also determined in triple-negative breast cancer cell lines which showed that PCAT29 was downregulated in all the breast cancer cell lines up to 7-fold in compassion with the normal cells.

The PCAT29 inhibits the proliferation of triple negative breast cancer cells

The triple-negative MDA-MB-231 breast cancer cell line was transfected with pcDNA-PCAT29 and NC and the overexpression of PCAT29 was validated by qRT-PCR (Figure 2). We observed that PCAT29 mimics transfection caused up to 5.8-fold upregulation of the PCAT29 in the MDA-MB-231 cells. The PCAT29 overexpressing breast cancer cells were then subjected to MTT assay and the viability was measured at different time intervals. We observed that PCAT29 overexpression in the MDA-MB-231 cells caused a time-dependent decrease in the viability as

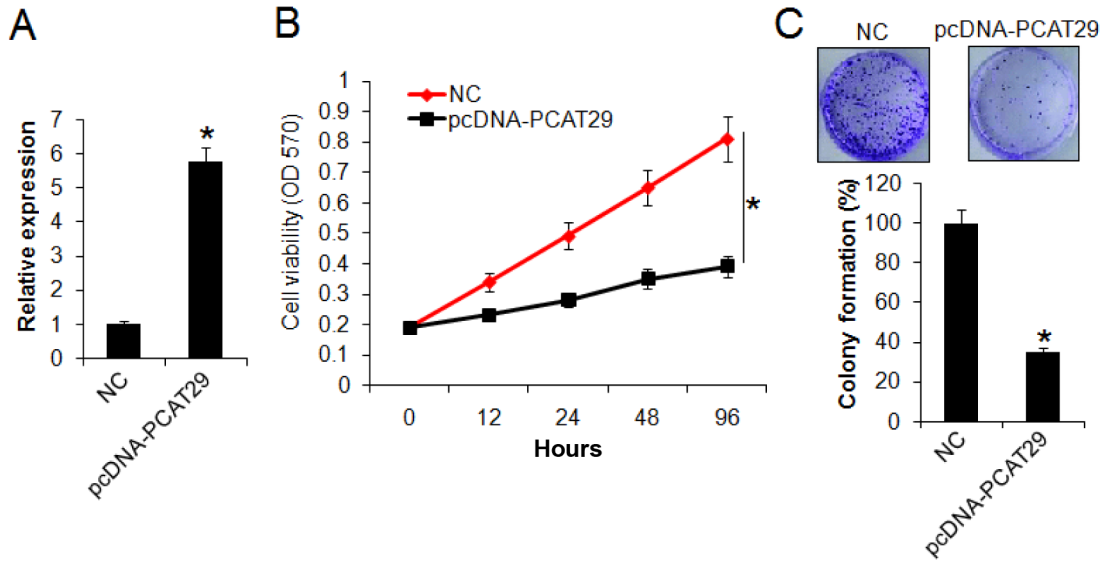

Figure 2. A: Expression of PCAT29 in NC or pcDNA-PCAT29 transfected MDA-MB-231 cells as determined by qRT-PCR. **B:** MTT assay showing the viability of the NC or pcDNA-PCAT29 transfected MDA-MB-231 cells. **C:** Colony formation assay of the NC or pcDNA-PCAT29 transfected MDA-MB-231 cells. The experiments were performed in triplicate and expressed as mean ± SD (*p<0.05).

compared to the NC transfected cells. We also found that PCAT29 overexpression resulted in inhibition of colony formation by the MDA-MB-231 cells.

The PCAT29 inhibits induces apoptosis in triple negative breast cancer cells

The underlying mechanism for the decrease in the viability of the triple-negative MDA-MB-231 breast cancer cells was revealed by the AO/EB staining wherein we found that PCAT29 overexpression triggered nuclear fragmentation of the MDA-MB-231 cells (Figure 3). In the annexin V/PI staining, we observed that PCAT29 overexpression increased the percentage of apoptosis in the triple-negative breast cancer cells. We also observed that PCAT29 overexpression caused elevation of the Bax and depletion of the Bcl-2 expression in MDA-MB-231 cells.

The PCAT29 suppresses the migration and invasion of the breast cancer cells

The wound healing assay showed that PCAT29 overexpression suppressed the migration of MDA-

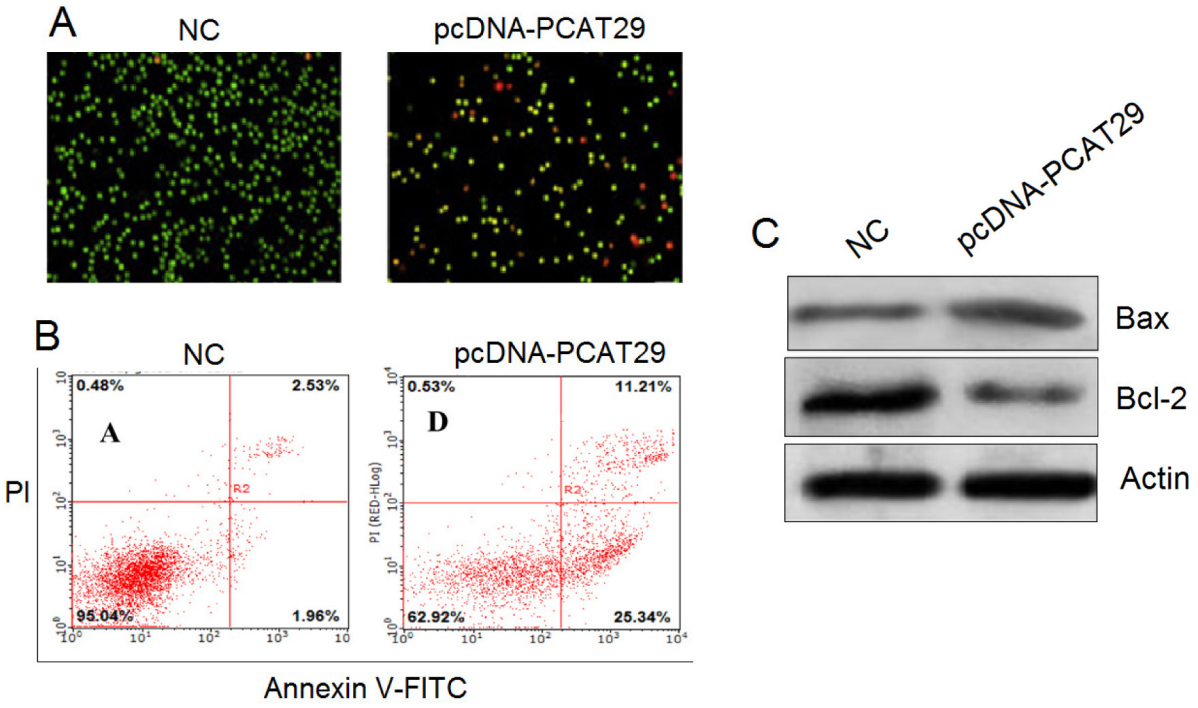

Figure 3. A: AO/EB staining showing overexpression of PCAT29 induces apoptosis in MDA-MB-231 cells. **B:** Annexin V/PI staining of the NC or pcDNA-PCAT29 transfected MDA-MB-231 cells showing the percentage of apoptotic cells. **C:** Western blot showing increased expression of Bax and decreased expression of Bcl-2 in NC or pcDNA-PCAT29 transfected MDA-MB-231 cells. The experiments were performed in triplicate.

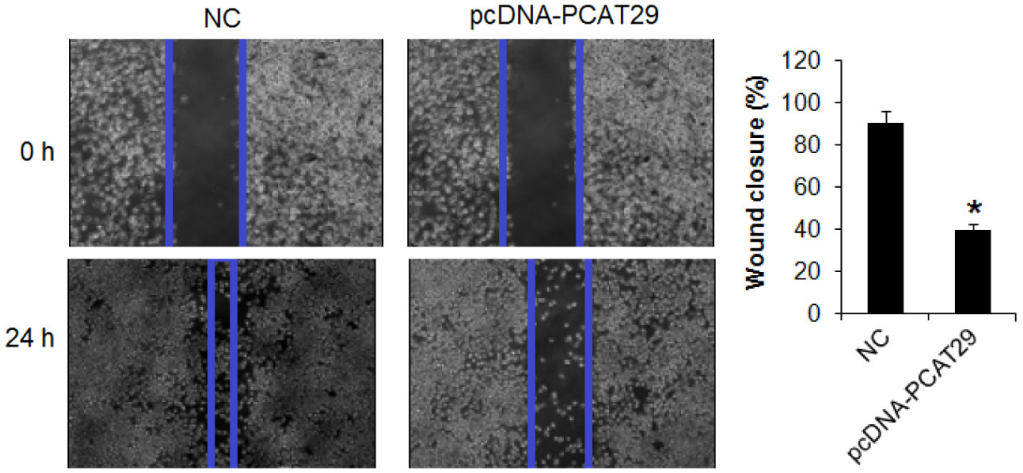

Figure 4. Wound healing assay showing inhibition of migration of the NC or pcDNA-PCAT29 transfected MDA-MB-231 cells. The experiments were performed in triplicate and expressed as mean±SD. *p<0.05.

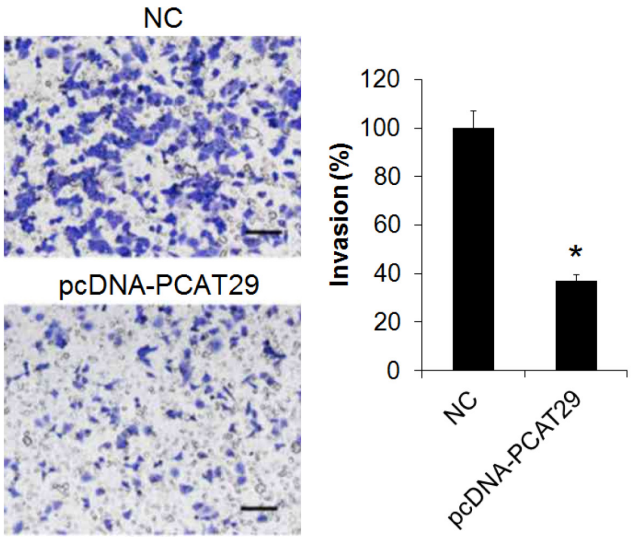

Figure 5. Transwell assay showing the invasion of the NC or pcDNA-PCAT29 transfected MDA-MB-231 cells. The Figure depicts that overexpression of PCAT29 inhibits the invasion of MDA-MB-231 cells. The experiments were performed in triplicate and expressed as mean±SD. *p<0.05.

MB-231 cells (Figure 4). The wound closure was found to be around 37% in PCAT29 overexpressing MDA-MB-231 as compared to around 88% in the NC transfected MDA-MB-231 cells. The transwell assay showed that the invasion of the breast cancer cells was also suppressed (Figure 5). The invasion of the MDA-MB-231 was suppressed by 65% relative to the control.

Discussion

LncRNAs have drawn huge attention for their wide array of cellular and physiological functions [10]. Recent studies have proved that the expression of many LncRNAs is dysregulated in cancer, indicating that these LncRNAs may also have a role in the development of cancers [11]. The LncRNA PCAT2 9 has been shown to act as tumor suppressor, for example PCAT29 has been shown to suppress the growth of prostate cancer [12]. In the present study we found that PCAT29 is significantly suppressed in the breast cancer tissues as well as in the triple-negative breast cancer cell lines. Nonetheless, PCAT29 overexpression caused a remarkable decrease in the proliferation rate and the colony formation of the MDA-MB-231 triple-negative breast cancer cells. These observations are also consistent with previous studies wherein PCAT29 has been found to suppress the proliferation of renal carcinoma [13]. The underlying mechanism for the decrease in the proliferation rate of the MDA-MB-231 cancer cells was elucidated by carrying out the AO/EB and annexin V/PI staining which showed that PCAT29 overexpression led to induction of apoptosis of the MDA-MB-231 cells. We also found that the induction of apoptosis in triple-negative breast cancer cells was also accompanied by elevation of Bax and depletion of Bcl-2 expression. Previous studies have shown that several LncRNAs halt the growth of cancer cells via induction of apoptosis. For instance, LncRNA OIP5-AS1 has been reported to control the apoptosis induction in bladder cancer cells [14]. Similarly, the induction of apoptosis in prostate cancer cells is regulate by the LncRNA PART1 [15]. In yet another study, LncRNA LINC00460 has been found to control the induction of apoptosis in colorectal cancer cells by modulating the expression of KLF2 and CUL4A [16]. For the metastasis of cancers, the initial step is the migration and invasion of the cancer cells to the neighboring tissues [17]. In this study, we also examined the effect of PCAT29 overexpression on the migration and invasion of the MDA-MB-231 triple-negative breast cancer cells and found that PCAT29 overexpression caused a considerable decline in the proliferation and migration of the MDA-MB-231 cells. A previous study has also shown that PCAT29 reduces the renal cancer cell migration and invasion [13]. Additionally, many of the LncRNAs have been shown to regulate the migration and invasion of the cancer cells, for instance, LncRNA HULC suppresses the migration and invasive potential of osteosarcoma cells [18]. Similarly, the migration and invasion of the bladder cancer cells is suppressed by LncRNA MEG3 [19]. In another study, LncRNA H19 has been found to control the migration and invasion of melanoma cells [20].

Conclusion

The findings of the present study revealed that LncRNA PCAT29 is downregulated in breast cancer tissues and triple-negative breast cancer cells. Functional analysis revealed that PCAT29 acts a tumor suppressor in triple-negative breast cancer cells and suppresses proliferation via induction of apoptosis. PCAT29 also suppressed the migration and invasion of the triple-negative breast cancer cells and all these may indicate a novel therapeutic target in triple-negative breast cancer.

Conflict of interests

The authors declare no conflict of interests.

References

1. Tao Z, Shi A, Lu C, Song T, Zhang Z, Zhao J. Breast cancer: epidemiology and etiology. Cell Biochem Biophys 2015;72:333-8.
2. McPherson K, Steel C, Dixon JM. Breast cancer-epidemiology, risk factors, and genetics. BMJ 2000;321:624-8.
3. Kelsey JL. Breast cancer epidemiology: summary and future directions. Epidemiol Rev 1993;15:256-63.
4. Dent R, Trudeau M, Pritchard KI et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13:4429-34.
5. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med 2010;363:1938-48.
6. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007;109:25-32.
7. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007;8:235-44.
8. Evans JR, Feng FY, Chinnaiyan AM. The bright side of dark matter: lncRNAs in cancer. J Clin Invest 2016;126:2775-82.
9. Sakurai K, Reon BJ, Anaya J, Dutta A. The lncRNA DRAIC/PCAT29 locus constitutes a tumor-suppressive nexus. Mol Cancer Res 2015;13:828-38.
10. Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015;21:1253.
11. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer discovery. 2011 Oct 1;1(5):391-407.
12. Malik R, Patel L, Prensner JR et al. The lncRNA PCAT29 inhibits oncogenic phenotypes in prostate cancer. Mol Cancer Res 2014;12:1081-7.
13. Sun X, Luo LH, Zhong KZ, Li JR. LncRNA PCAT29 suppresses cell proliferation, invasion, and migration in renal carcinoma by regulating FLOT1. Clin Surg Res Commun 2018;2:34-41.
14. Wang Y, Shi F, Xia Y, Zhao H. LncRNA OIP5-AS1 predicts poor prognosis and regulates cell proliferation and apoptosis in bladder cancer. J Cell Biochem 2019;120:7499-505.
15. Sun M, Geng D, Li S, Chen Z, Zhao W. LncRNA PART1 modulates toll-like receptor pathways to influence cell proliferation and apoptosis in prostate cancer cells. Biol Chem 2018;399:387-95.
16. Lian Y, Yan C, Xu H et al. A novel lncRNA, LINC00460, affects cell proliferation and apoptosis by regulating KLF2 and CUL4A expression in colorectal cancer. Mol Ther-Nucl Acids 2018;12:684-97.
17. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003;3:362.
18. Kong D, Wang Y. Knockdown of lncRNA HULC inhibits proliferation, migration, invasion, and promotes apoptosis by sponging miR-122 in osteosarcoma. J Cell Biochem 2018;119:1050-61.
19. Feng SQ, Zhang XY, Fan HT, Sun QJ, Zhang M. Upregulation of LncRNA MEG3 inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells. Neoplasma 2018;65:925.
20. Liao Z, Zhao J, Yang Y. Downregulation of lncRNA H19 inhibits the migration and invasion of melanoma cells by inactivating the NF κB and PI3K/Akt signaling pathways. Mol Med Rep 2018;17:7313-8.