Int J Clin Exp Pathol 2015;8(8):9052-9061
www.ijcep.com /ISSN:1936-2625/IJCEP0011038

Original Article

Macrophage infiltration promotes invasiveness of breast cancer cells via activating long non-coding RNA UCA1

Shuzheng Chen[1], Chuxiao Shao[1], Min Xu[1], Jiansong Ji[1], Yanru Xie[1], Yongliang Lei[2], Xiaoguang Wang[2]

[1]Lishui Central Hospital & Zhejiang University Lishui Hospital, Lishui, Zhejiang, China; [2]Lishui Center for Disease Control and Prevention, Lishui, Zhejiang, China

Received June 3, 2015; Accepted July 21, 2015; Epub August 1, 2015; Published August 15, 2015

Abstract: There is now considerable evidence supporting the view that macrophage infiltration is playing a critical role in the proliferation and progression of breast cancer but the underlying molecular mechanisms remain largely unknown. To this end, using long non-coding RNA (lncRNA) expression profiling, we examined changes in lncRNA expression in breast cancer cells treated with conditioned medium (CM) from cultured human THP-1 macrophages. We found that treatment with macrophage CM induced the expression of numerous lncRNAs, including urothelial cancer associated 1 (UCA1). Knockdown of UCA1 using shRNA inhibited AKT phosphorylation and abolished invasiveness of tumor cells induced by macrophage CM. Consistent with these results; we further showed that UCA1 level was significantly enhanced in human primary breast tumors and correlated with advanced clinical stage, supporting its role in promoting carcinogenesis and progression of breast cancer. Together, these results suggest that macrophage could promote invasiveness of breast cancer cells by enhancing expression of lncRNA UCA1.

Keywords: Macrophage, breast cancer, UCA1

Introduction

A malignant tumor is comprised of tumor cells and the tumor microenvironment. It is now clear that the tumor microenvironment plays a critical role in regulating the fate and behavior of tumor cells. Among all cell types associated with the tumor microenvironment, tumor-associated macrophages (TAMs) are the major component of the leucocytic infiltration in tumors [1, 2]. It is well established that TAMs could enhance tumor progression by promoting tumor invasion, migration and angiogenesis. For example, cytokines, chemokines, and growth factors secreted by the inflammatory cells might also induce malignant transformation and/or promote tumor cell survival by regulating expression of genes involved in apoptosis, proliferation and cell-cycle control [3-5]. Free radicals produced by host leukocytes might cause DNA damage and induce somatic mutations in tumor cells. Indeed, a large variety of TAMs have been frequently observed to be associated with breast tumor, and their interaction with the surrounding tumor cells has just begun to be unveiled.

Derived from circulating monocytic precursors, TAMs are recruited within the tumor microenvironment by tumor-derived signals and differentiate into mature macrophages. They could change their properties in response to different signals and display a phenotypic plasticity with two major types of macrophages, M1 and M2, which have antitumorigenic or protumorigenic functions, respectively [6, 7]. In most cases, activated macrophages act in an inflammatory microenvironment are M2 type and promote tumorigenesis. It has also been suggested that a dynamic switching from M1 phenotype to M2-like one might occur during tumor development. Previous studies indicate that macrophage infiltration is significantly correlated with metastasis and poor prognosis in breast cancer. After polarized with specific inducers, macrophages could produce a distinctive set of cytokines and chemokines such as Tumor necrosis factor (TNFα) and Transforming Growth Factor β (TGF-β) [7], which may enhance the activity of several transcription factors such as Transducer and Activator of Transcription 3 (STAT-3) and downstream signal cascade including Protein Kinase B Alpha (AKT) signaling and

Extracellular-signal-Regulated Kinases (ERK) pathways, to control expression of target genes involved in tumor development and progression [8-10].

Long non-coding RNAs (lncRNAs) are evolutionarily conserved non-coding RNAs that are longer than 200 nucleotides in length [11, 12]. They are playing critical roles in co-transcriptional and post-transcriptional regulation of protein-coding genes expression. Growing evidence suggests that deregulated expression of lncRNAs is closely linked to tumor progression and drug resistance in different tumors [9, 13-15]. Although recent studies have identified many molecular changes in tumor cells triggered by tumor microenvironment, however, how macrophage filtration regulates expression of lncRNAs in breast cancer remains unknown. Here we ask whether macrophage filtration could facilitate breast tumor carcinogenesis by regulating expression of lncRNAs with oncogenic or tumor suppressing functions. To this end, we mimicked a tumor microenvironment by co-culturing breast cancer cells with conditioned medium from differentiated THP-1 macrophages. Changes in expression profiling of 90 major lncRNAs in these cells were then examined using LncProfiler qPCR Array. We identify UCA1 as the most significantly induced lncRNA by macrophage CM in breast cancer cell lines. Further functional assay support an important link between UCA1 and macrophage-induced invasiveness of breast cancer cells via regulating AKT signaling.

Materials and methods

Differentiation of THP-1 monocytes to macrophages

THP-1 monocytes were seeded at a density of 40,000 cells/cm^2 onto 6-well plates (BD Biosciences, Franklin Lakes, NJ, USA) in RPMI-1640 medium (Life Technologies, Inc., Rockville, MD) containing 10% FCS and 10 mM HEPES. Cells were induced to differentiate into macrophages with 100 ng/ml 12-O-tetradecanoylphorbol-13-acetate (TPA, Sigma-Aldrich, St. Louis, MO, USA) for differentiation. After 24 h, differentiated cells were thoroughly washed 5 times with fresh medium and then cultured for 24 h in 3 ml RPMI-1640 medium containing 10% FCS and 10 mM HEPES. After centrifuge, the supernatant of the culture containing factors released from macrophage was collected as macrophage conditioned medium (macrophage CM).

LncRNA expression profiling

Total RNA was prepared from MCF-7 cells treated with macrophage conditioned medium or ordinary medium using Trizol (Invitrogen, Carlsbad, CA, USA). The expression of 90 lncRNA was performed using the LncProfiler™ qPCR Array Kit (System Biosciences, Mountain View, CA). Three biological replicates were performed for each group. LncRNA expression profiling and data analysis were performed according to the manufacturer's instructions.

Reverse transcription-PCR

Total RNA from cells was isolated using Trizol (Invitrogen, Carlsbad, CA, USA). 200 ng of RNA was reverse-transcribed to cDNA using SuperScript First Strand cDNA System (Invitrogen, Carlsbad, CA, USA) and gene specific primers. PCR was performed using a S1000™ Thermal Cycler (BIO-RAD Laboratories, Inc., Hercules, CA).

The following primers were used: UCA1: F 5'-CTCTCCATTGGGTTCACCATTC-3'; R 5'-GCGGCAGGTCTTAAGAGATGAG-3'. 18 s RNA: F 5'-GTAACCCGTTGAACCCCATT-3'; R 5'-CCATCCAATCGGTAGTAGCG-3'. F, forward primer; R, reverse primer.

Quantitative real-time PCR

Total RNA from cells or tissues was isolated using Trizol (Invitrogen, Carlsbad, CA, USA). 200 ng of RNA was reverse-transcribed to cDNA using SuperScript First Strand cDNA System (Invitrogen, Carlsbad, CA, USA) and gene specific primers. Quantitative real-time PCR was performed using a CFX96 Connect system (BIO-RAD Laboratories, Inc., Hercules, CA) and SYBR Green Supermixes (BIO-RAD Laboratories, Inc., Hercules, CA). The gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control. A dissociation curve was produced and relative gene expression was calculated by using the $2^{-\Delta\Delta CT}$ method. Experiments were performed in triplicates.

The following PCR primers were used: UCA1: F 5'-CTTCTGCATAGGATCTGCAATCAG-3'; R 5'-TTTTGTCCCCATTTTCCATCATACG-3'. lincRNA-ROR: F 5'-AGGAAGCCTGAGAGTTGGC-3'; R 5'-CTCAGTGGGGAAGACTCCAG-3'. YRNA-1: F 5'-GCTGGTCCG-

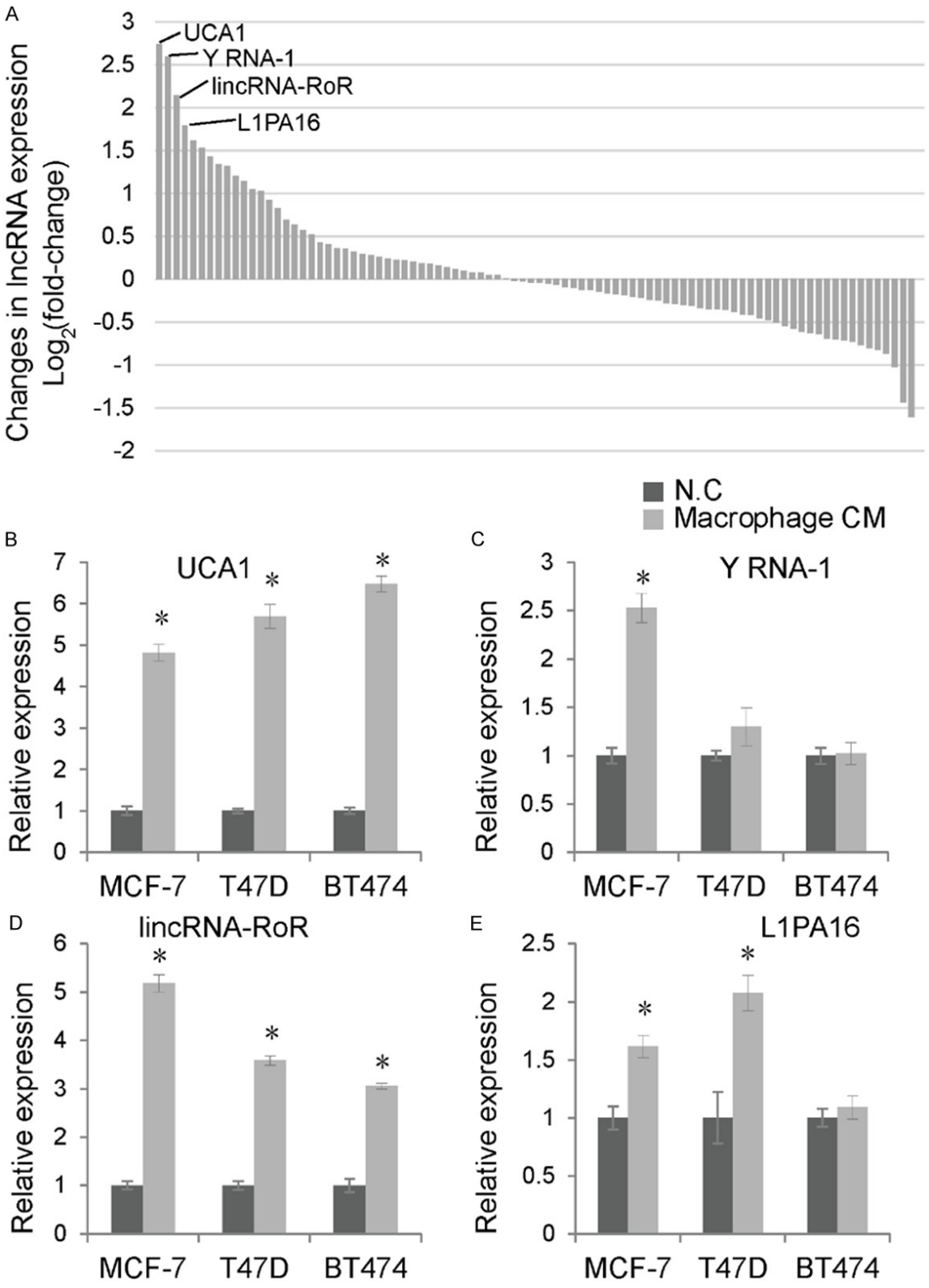

Figure 1. Changes in lncRNA expression profile in response to macrophage CM treatment. (A) Changes in expression of 90 lncRNAs between MCF-7 cells treated with macrophage CM or control medium for 24 h. Fold change were log2 transformed and the data presents the average from three biological replicates. Macrophage-induced changes of four most significantly induced lncRNA, including (B) UCA1, (C) Y RNA-1, (D) lincRNA-RoR and (E) L1PA16 were validated in MCF-7 cells as well as two additional breast cancer cell lines, T47D and BT474. Data represent the mean ± s.d. of three biological replicates; *P < 0.05. Statistical analysis was performed by t-test.

Table 1. Significantly changed lncRNAs by treatment with macrophage CM in MCF-7 cells

LncRNA	Fold Change	Adjusted *P*-value
UCA1	6.66	8.52E-03
Y RNA-1	6.05	9.80E-04
LINCRNA-ROR	4.43	1.67E-03
L1PA16	3.47	4.36E-03
EGOA	2.89	2.75E-02
HOTAIRM1	2.53	1.19E-02
ZEB2NAT	2.49	1.02E-02
21A	2.31	8.25E-03
Nespas	2.21	1.57E-02
HAR1A	2.07	3.89E-02
AIR	1.77	1.39E-02
HULC	1.62	2.91E-03
H19 UPSTREAM	0.65	8.91E-03
SAF	0.64	2.99E-02
HOXA3AS	0.61	1.02E-02
PRINS	0.49	1.02E-02
ALPHA 250	0.37	2.91E-03
LINCRNA-SFMBT2	0.33	1.31E-02

Expression of lncRNAs of MCF-7 cells treated with macrophage CM or control medium for 24 h were measured using lncRNA qPCR array with three biological replicates. Differentially expressed lncRNAs were identified using t-test followed by multiple comparison correction using Benjamini-Hochberg procedure. LncRNAs with fold change > 1.5 and adjusted *p*-value < 0.05 was considered significantly changed.

AAGGTAGTGAG-3'; R 5'-AAGTGCAGTAGTGAGAAGGGG-3'. L1PA16: F 5'-CCCCACATAGCTTCCAGTGT-3'; R 5'-CAACTTGAGGCCATCATCCT-3. GAPDH: F 5'-AGGTCGGAGTCAACGGATTTG-3'; R 5'-GTGATGGCATGGACTGTGGT-3'. F, forward primer; R, reverse primer.

UCA1 knockdown and overexpression

Sequences of siRNA targeting UCA1 and the non-specific control were as previously described (ref). They were converted into shRNA cassettes and cloned into the pRNAT-U6.1/Neo vector. ShRNA cassettes used:

pRNAT-U6.1/Neo-shUCA1 (UCA1 KD) 5'-gatccGTTAATCCAGGAGACAAAGAttcaagagaTCTTTGTCTCCTGGATTAAtttttttggaaa-3'; pRNAT-U6.1/Neo-CTL (control KD) 5'-gatccTTCTCCGAACGTGTCACGTttcaagagaACGTGACACGTTCGGAGAAttttttggaaa-3'.

ShRNA constructs were transfected into MCF-7 or T47D cells using lipofectamine2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h cells was selected with 200 μg/ml neomycin (G418) (Invitrogen, Carlsbad, CA, USA) for 3 weeks, and knockdown efficiency was confirmed by RT-PCR and real-time PCR.

Cell proliferation assay

Cells were seeded in the 96-well plate at a concentration of 3×10^3 cells per well and incubated for 24 h. Then the medium was changed to macrophage CM or control medium and cells were incubated for up to 24 h. Cell growth viability was measured using Vybrant MTT Cell Proliferation Assay Kit (Invitrogen, Carlsbad, CA, USA). Absorbance was then recorded using an Elx800 Reader (Bio-Tek Instruments Inc., Winooski, VT). Three biological replicates were obtained for each sample.

Cell Invasion assay

The invasion assay was conducted using Transwell cell culture chambers (Millipore Corp., Billerica, MA). The invading cells were counted at 400 × magnification in 10 different fields for each insert. The experiment was repeated three times. For AKT inhibitors study, cells were treated with 20 μM PI3 Kinase Inhibitor LY294002 (Cell Signaling Technology, Beverly, MA) in the cell culture medium.

Tumor tissues

A total of 71 patients who were diagnosed as breast cancer and had undergone resection in Lishui Central Hospital & Zhejiang University Lishui Hospital from 2011 to 2013 were included in this study. None of these patients had received preoperative radiotherapy or chemotherapy prior to surge. Tumor and adjacent normal tissues were snap-frozen in liquid nitrogen and stored at -80°C until used for RNA extraction. The project protocol was approved by the Institutional Review Board of Lishui Hospital. Informed consent for clinical research was obtained from each patient and all experiments were performed in accordance with relevant guidelines.

Results

Changes in lncRNA expression profile induced by macrophage CM

In this study, we focused our work on the changes in lncRNA expression induced by macropage.

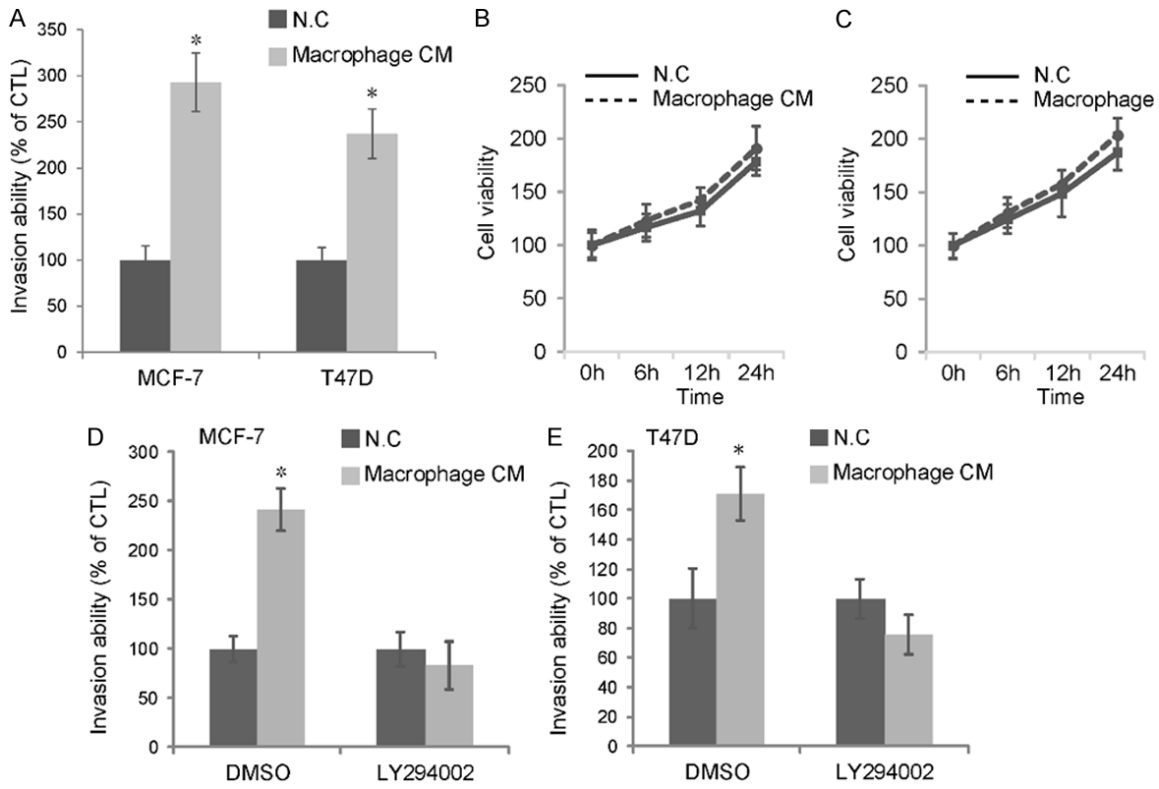

Figure 2. Macrophage CM activates cancer cell invasion via AKT signaling. A. MCF-7 or T47D cells were treated with macrophage CM or control medium (N.C) for 24 h and the invasion ability was obtained with transwell assay. Data represent the mean ± s.d. of three biological replicates. B. MCF-7 or C. T47D cells were treated with macrophage CM or control medium for up to 24 h and cell viability was measured using MTT assay. Data represent the mean ± s.d. of three biological replicates. D. MCF-7 or E. T47D cells were treatment with macrophage CM containing 20 μM PI3 Kinase Inhibitor LY294002 or DMSO, respectively, and the invasion ability was obtained with transwell assay. Data represent the mean ± s.d. of three biological replicates.

To mimick an inflammatory microenvironment, we exposed breast cancer cell MCF-7 to macrophage CM, which contained the soluble factors derived from THP-1 macrophages, for 24 h. MCF-7 cells treated with the same medium without macrophages were used as a control. We then used LncProfiler qPCR Array to profile in expression of 90 major lncRNAs with three biological replicates (**Figure 1A**). Differentially expressed lncRNAs were identified using t-test followed by multiple comparison correction using Benjamini-Hochberg procedure. This allowed us to identify 12 significantly upregulated lncRNAs and 6 significantly downregulated lncRNAs following macrophage CM treatment with a fold change greater than 1.5 fold (**Table 1**).

We then validated changes of four most significantly induced lncRNAs by macrophage CM, namely, UCA1, Y-RNA-1, lincRNA-RoR and L1PA16 in two additional breast cancer cell lines T47D and BT474. As shown in **Figure 1B-E**, expression of UCA1 and lincRNA-RoR were consistently enhanced in all three cell lines. Notably, recent studies have shown that UCA1 is significantly up-regulated in several human cancers [16], including bladder cancer, breast cancer and liver cancer, and is linked to enhanced proliferation and drug resistance of tumor cells [8, 17, 18]. This suggested that cytokine/chemokine produced by macrophage may induce the expression of a subset of lncRNAs to promote the proliferation and progression of breast cancer cells. We thus focused our efforts on UCA1 in the following functional studies.

Macrophage CM promotes breast cancer invasion

We then examined whether macrophage CM may enhance proliferation or invasiveness of breast cancer cells in vitro. Two breast cancer cell lines (MCF-7 and T47D) were treated with macrophage CM for 24 h and their invasion and

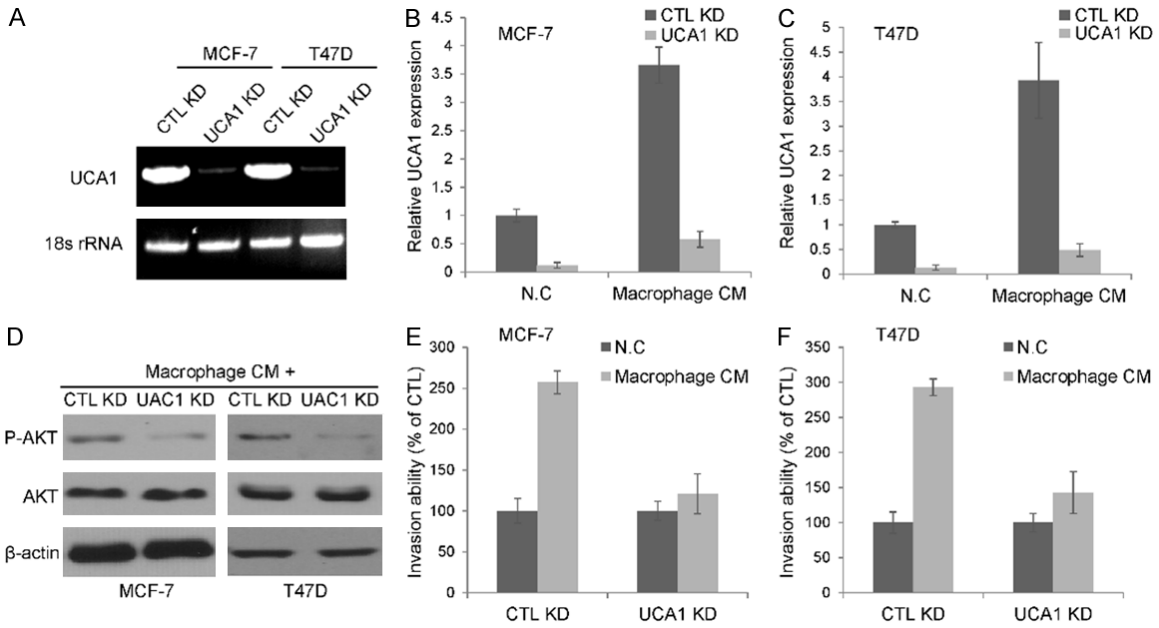

Figure 3. UCA1 knockdown abolished macrophage-induced invasion of breast cancer cells. A. Semiquantitative RT-PCR analysis of UCA1 expression in MCF-7 and T47D cells with stable UCA1 or control knockdown. B. Quantitative real-time PCR analysis of UCA1 expression in MCF-7 or C. T47D cells with stable UCA1 or control knockdown, treated with macrophage CM or control medium, respectively. Data represent the mean ± s.d. of three biological replicates. D. Western-blot analysis of AKT, phosphorylated AKT and β-actin in MCF-7 or T47D cells with stable knockdown of UCA1 or control treated with macrophage CM for 24 h. E. Relative invasion ability of MCF-7 or F. T47D cells with stable UCA1 knockdown or control knockdown treated with macrophage CM or control medium, respectively. Data represent the mean ± s.d. of three biological replicates.

proliferation abilities were examined, respectively. Significantly, both MCF-7 and T47D cells treated with macrophage CM showed increased number of invading cells as compared with controls (**Figure 2A**, $P < 0.001$, t-test). However, no significant change in cell proliferation was observed during this time window (**Figure 2B** and **2C**), suggesting that enhanced invasiveness of the tumor cells were not associated with their proliferation ability. Consistent with previous findings that macrophages may stimulate invasion of gastric and colorectal cancer through activation of AKT signaling in vivo [16, 19, 20], inhibition of AKT signaling using LY294002 impaired the function of macrophage CM to promote invasion of MCF-7 and T47D cells (**Figure 2D** and **2E**). These results established that the soluble factors derived from macrophages may activate AKT signaling to mediate invasion of breast cancer cells.

UCA1 knockdown abolished invasiveness of breast cancer cells induced by macrophage CM

To investigate the function of UCA1 in mediating macrophage-induced invasiveness, we established stable UCA1 knockdown cell lines using shRNA. As shown in **Figure 3A**, reverse transcription polymerase chain reaction (RT-PCR) result showed that shRNA-mediated silencing resulted in dramatic reduction in UCA1 mRNA expression. This result is validated by quantitative real-time PCR (q-PCR), that UCA1 expression in both cell lines were reduced by about 90% under normal condition (**Figure 3B** and **3C**). As expected, following treatment with macrophage CM, UCA1 abundance was potently elevated in both MCF-7 and T47D cells, while only slight increase was observed in stable knockdown cell lines (**Figure 3B**).

We then examined whether UCA1 was required for activation of AKT signaling in cells activated by macrophage CM. In line with results in bladder tumor cells [8, 18], knockdown of UCA1 in both MCF-7 and T47D cells resulted in potent decrease in AKT phosphorylation (**Figure 3C**). Moreover, UCA1 knockdown resulted in significantly reduced invasiveness of both cell lines treated with macrophage CM. This is consistent with established role of UCA1 in regulating bladder cancer metastasis and supports the

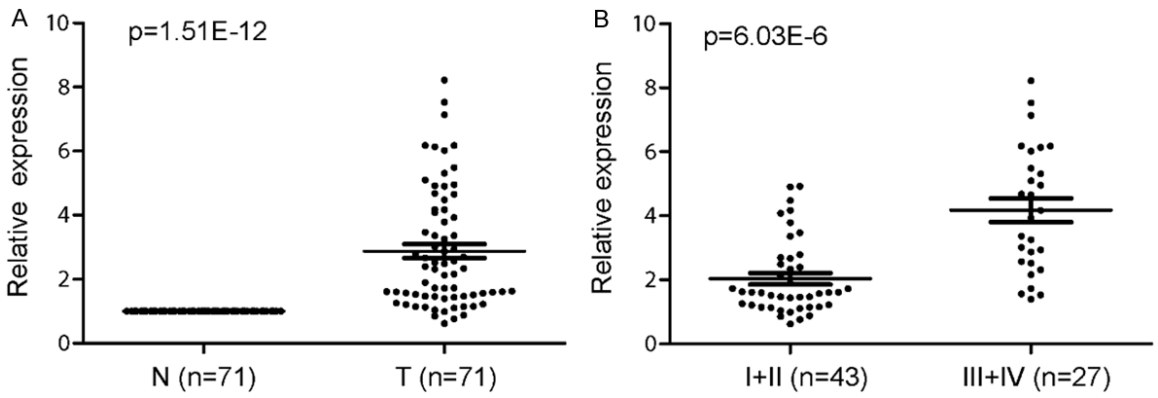

Figure 4. Enhanced expression of UCA1 in breast tumor tissues. A. UCA1 expression was examined by quantitative real-time PCR in 71 pairs of breast tumor tissues (T) and adjacent normal tissues (N). Expression of UCA1 in normal tissues was normalized to 1 to control for patient-patient variations, and UCA1 expression in each tumor was normalized based on the corresponding adjacent normal tissue. *P*-value was calculated using paired student's t-test. B. Relative expression of UCA1 in 43 stage I + II tumors vs. 27 stage III + IV tumors. *P*-value was calculated using student's t-test.

hypothesis that macrophage infiltration may promote invasiveness of tumor cells via activating UCA1.

Enhanced expression of UCA1 in breast tumors

To further investigate the role of UCA1 in vivo, we performed quantitative PCR (q-PCR) to examine the expression of UCA1 in 71 pairs of human primary breast tumors and the corresponding adjacent non-tumorous tissues. As shown in **Figure 4A**, consistent with previous studies, UCA1 expression was significantly enhanced in breast tumors as compared with normal tissues (P = 1.51E-12, paired student's t-test). Of note, high UCA1 expression was associated with advanced clinical stage of the disease (**Figure 4B**, P = 6.03E-6, student's t-test), supporting the notion that UCA1 plays a pivotal role in the progression of breast cancer.

Discussion

Breast cancer is a good example of inflammation-related cancer which represents a paradigm of the crosstalk between tumor-immune microenvironment and tumorigenesis. The existence of activated inflammatory cells in tumor tissue is well established and they may influence various aspects of carcinogenesis.

Macrophage is a major component of leukocyte infiltration in the majority of tumors, which produces a myriad of factors to promote tumor growth and invasiveness. The major focus of this study was to investigate the influence of tumor microenvironment on expression of lncRNAs in breast cancer cells and how this is able to affect tumor progression. To this end, we mimicked a tumor microenvironment by exposing breast cancer cells to macrophage CM and used LncRNA qPCR array to identify differentially expressed lncRNAs.

With this systematic approach, we identified several lncRNAs that are significantly induced by macrophage CM. We identified UCA1 as the most significantly induced lncRNA by macrophage CM in various breast cell lines. Previous studies suggest that UCA1 is an oncogenic lncRNA highly expressed in bladder transitional cell carcinoma [18, 19, 21, 22]. It regulates cell cycle progression of bladder cancer cells via PI3K-AKT signaling pathway [8] and may increase the cisplatin resistance of bladder cancer cells by enhancing the expression of Wnt6 [19]. There is also evidence showing that lncRNA UCA1 promotes breast tumor growth by suppression of p27 [23]. Enhanced expression of UCA1 was likewise observed in colorectal cancer, which is reported to have marked influence on proliferation, apoptosis and cell cycle progression [20]. Consistent with these studies, we observed that the expression of UCA1 in breast tissues was conspicuously higher than that observed in pair-matched adjacent normal tissues. Our functional assay also supports the role of UCA1 as an oncogenic lncRNA and highlighted its function in mediating macrophage-induced tumor invasion.

As a proof-of-concept, we only focused on the function of UCA1 in the current study. However, other lncRNAs induced by macrophage CM may also play critical roles in tumorigenesis of breast cancer. Linc-ROR is highly expressed in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [24]. It may facilitate maintaining the high expression of stem cell factors OCT4, SOX2 and Nanog via function as a molecular sponge for mir-145 [24]. There is also evidence supporting its role as p53 repressor in response to DNA damage. Recent studies show that linc-RoR expression is induced by transforming growth factor b (TGF-β) and modulates chemotherapy resistance in tumor cells [9]. This is consistent with our data as TGF-β is also released by macrophage in tumor microenvironment. Another induced lncRNA, SOX2OT, is reported to play a key role in regulating SOX2 expression in breast cancer and is involved in cancer stem cell maintenance [25]. It would be interesting to examine whether macrophage could induce these lncRNAs to mediate proliferation and drug resistance in breast cancer cells in the future.

We recognize, however, that our approach has several limitations. First, given the complexity of the signals in microenvironment in determining macrophage subtype and function, a mixed phenotype-expressing macrophage population may exist in vivo. Accordingly, our in vitro system is simplified as only one kind of macrophage cell was used. We plan to test the effect of more macrophage cell types as well as patient-derived macrophages. Second, some signaling transduction might require direct interaction between macrophage and the target tumor cells. However, in our assay breast cancer cells were treated with only soluble factors in the conditioned medium derived from macrophages. It would be interesting to examine the lncRNA expression profiles of tumor cell lines directly co-cultured with macrophages. Third, treatment with macrophage CM may remodel gene expression profiling and signal transduction in the target tumor cells in a highly dynamic manner. As gene expression is regulated at multiple levels with various compensation mechanisms [26-32], some lncRNAs might not change their expression in a timely manner in response to macrophage stimuli. In this study, however, we didn't perform a time course analysis. In the future it would be interesting to examine how different lncRNAs may response to CM treatment overtime differently [33]. Last but not least, our lncRNA profiling is limited to the 90 lncRNA available in the qPCR array. Further genome-wide studies using, e.g., RNA-Seq, may largely expand the lncRNA expression profile and identify more novel lncRNAs that are regulated by tumor microenvironment and linked to tumor progression [33, 34].

Nevertheless, despite all these limitations, to our knowledge our analysis is the first systematic study that links inflammation to lncRNA expression profile in tumor cells. As a proof-of-concept, we provide robust evidence that tumor microenvironment could regulate tumor development and progression via remodeling of lncRNAs expression. As the roles of more and more lncRNA in regulating carcinogenesis are discovered, we suggest that this connection may be widespread and these lncRNAs provides promising new targets for therapeutic intervention for breast cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaoguang Wang, Lishui Center for Disease Control and Prevention, Lishui, Zhejiang, China. E-mail: xgwangls@gmail.com

References

[1] Takanami I, Takeuchi K and Kodaira S. Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis. Oncology 1999; 57: 138-142.

[2] Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J and Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 1996; 56: 4625-4629.

[3] Leonardi GC, Candido S, Cervello M, Nicolosi D, Raiti F, Travali S, Spandidos DA and Libra M. The tumor microenvironment in hepatocellular carcinoma (review). Int J Oncol 2012; 40: 1733-1747.

[4] Yang JD, Nakamura I and Roberts LR. The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets. Semin Cancer Biol 2011; 21: 35-43.

[5] Deng WJ, Nie S, Dai J, Wu JR and Zeng R. Proteome, phosphoproteome, and hydroxyproteome of liver mitochondria in diabetic rats at early pathogenic stages. Mol Cell Proteomics 2010; 9: 100-116.

[6] Gordon S and Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5: 953-964.
[7] Hao NB, Lu MH, Fan YH, Cao YL, Zhang ZR and Yang SM. Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012; 2012: 948098.
[8] Yang C, Li X, Wang Y, Zhao L and Chen W. Long non-coding RNA UCA1 regulated cell cycle distribution via CREB through PI3-K dependent pathway in bladder carcinoma cells. Gene 2012; 496: 8-16.
[9] Takahashi K, Yan IK, Kogure T, Haga H and Patel T. Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer. FEBS Open Bio 2014; 4: 458-467.
[10] Yin S, Yang J, Lin B, Deng W, Zhang Y, Yi X, Shi Y, Tao Y, Cai J, Wu CI, Zhao G, Hurst LD, Zhang J, Hu L and Kong X. Exome sequencing identifies frequent mutation of MLL2 in non-small cell lung carcinoma from Chinese patients. Sci Rep 2014; 4: 6036.
[11] Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
[12] Mattick JS. The genetic signatures of noncoding RNAs. PLoS Genet 2009; 5: e1000459.
[13] Taft RJ, Pang KC, Mercer TR, Dinger M and Mattick JS. Non-coding RNAs: regulators of disease. J Pathol 2010; 220: 126-139.
[14] Perez DS, Hoage TR, Pritchett JR, Ducharme-Smith AL, Halling ML, Ganapathiraju SC, Streng PS and Smith DI. Long, abundantly expressed non-coding transcripts are altered in cancer. Hum Mol Genet 2008; 17: 642-655.
[15] Yin S, Deng W, Hu L and Kong X. The impact of nucleosome positioning on the organization of replication origins in eukaryotes. Biochem Biophys Res Commun 2009; 385: 363-368.
[16] Li JY, Ma X and Zhang CB. Overexpression of long non-coding RNA UCA1 predicts a poor prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 7938-7944.
[17] Tsang WP, Wong TW, Cheung AH, Co CN and Kwok TT. Induction of drug resistance and transformation in human cancer cells by the noncoding RNA CUDR. RNA 2007; 13: 890-898.
[18] Wang Y, Chen W, Yang C, Wu W, Wu S, Qin X and Li X. Long non-coding RNA UCA1a(CUDR) promotes proliferation and tumorigenesis of bladder cancer. Int J Oncol 2012; 41: 276-284.
[19] Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F and Liu Y. Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. FEBS J 2014; 281: 1750-1758.
[20] Han Y, Yang YN, Yuan HH, Zhang TT, Sui H, Wei XL, Liu L, Huang P, Zhang WJ and Bai YX. UCA1, a long non-coding RNA up-regulated in colorectal cancer influences cell proliferation, apoptosis and cell cycle distribution. Pathology 2014; 46: 396-401.
[21] Wang F, Ying HQ, He BS, Pan YQ, Deng QW, Sun HL, Chen J, Liu X and Wang SK. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. Oncotarget 2015; 6: 7899-917.
[22] Lopez MF, Krastins B, Sarracino DA, Byram G, Vogelsang MS, Prakash A, Peterman S, Ahmad S, Vadali G, Deng W, Inglessis I, Wickham T, Feeney K, Dec GW, Palacios I, Buonanno FS, Lo EH and Ning M. Proteomic signatures of serum albumin-bound proteins from stroke patients with and without endovascular closure of PFO are significantly different and suggest a novel mechanism for cholesterol efflux. Clin Proteomics 2015; 12: 2.
[23] Huang J, Zhou N, Watabe K, Lu Z, Wu F, Xu M and Mo YY. Long non-coding RNA UCA1 promotes breast tumor growth by suppression of p27 (Kip1). Cell Death Dis 2014; 5: e1008.
[24] Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X and Liu H. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013; 25: 69-80.
[25] Askarian-Amiri ME, Seyfoddin V, Smart CE, Wang J, Kim JE, Hansji H, Baguley BC, Finlay GJ and Leung EY. Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer. PLoS One 2014; 9: e102140.
[26] Lei H, Zhai B, Yin S, Gygi S and Reed R. Evidence that a consensus element found in naturally intronless mRNAs promotes mRNA export. Nucleic Acids Res 2013; 41: 2517-2525.
[27] Yin S, Wang P, Deng W, Zheng H, Hu L, Hurst LD and Kong X. Dosage compensation on the active X chromosome minimizes transcriptional noise of X-linked genes in mammals. Genome Biol 2009; 10: R74.
[28] Yin S, Deng W, Zheng H, Zhang Z, Hu L and Kong X. Evidence that the nonsense-mediated mRNA decay pathway participates in X chromosome dosage compensation in mammals. Biochem Biophys Res Commun 2009; 383: 378-382.
[29] Wang P, Yin S, Zhang Z, Xin D, Hu L, Kong X and Hurst LD. Evidence for common short natural trans sense-antisense pairing between transcripts from protein coding genes. Genome Biol 2008; 9: R169.
[30] Chan CT, Deng W, Li F, DeMott MS, Babu IR, Begley TJ and Dedon PC. Highly Predictive

Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. Chem Res Toxicol 2015; 28: 978-988.
[31] Chan CT, Pang YL, Deng W, Babu IR, Dyavaiah M, Begley TJ and Dedon PC. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. Nat Commun 2012; 3: 937.
[32] Biswas SK and Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010; 11: 889-896.
[33] Ohno S, Inagawa H, Dhar DK, Fujii T, Ueda S, Tachibana M, Suzuki N, Inoue M, Soma G and Nagasue N. The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. Anticancer Res 2003; 23: 5015-5022.
[34] Medrek C, Ponten F, Jirstrom K and Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer 2012; 12: 306.