**PDZRN4-mediated colon cancer cell proliferation and dissemination is regulated by miR-221-3p**

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**Background:** Suppression of PDZRN4 expression in colon cancer tissues may be associated with elevated levels of the microRNA 221 (miR-211). To uncover potential targets for treatment, the present study investigated PDZRN4 in colon cancer development, and explored the role of miR-221-3p in the regulation of PDZRN4.

**Methods:** RNA expression arrays were searched in the NCBI database, and PDZRN4 (PDZ domain containing ring finger 4) was selected as a potential downregulated gene in colon cancer. PDZRN4 mRNA and protein in colon cancer and matched normal tissues were analyzed. The proliferation and dissemination of HCT116 cells overexpressing PDZRN4 was assessed via functional assays. Bioinformatics analysis and luciferase reporter assay were applied to determine the regulatory link between miR-221-3p and PDZRN4 mRNA.

**Results:** There was significantly less PDZRN4 mRNA and PDZRN4 protein in colon cancer tissue compared with normal tissues. HCT116 cells overexpressing PDZRN4 were less able to disseminate relative to the control. Expression of PDZRN4 was directly inhibited by miR-221-3p. Knockout of miR-211-3p was associated with attenuated proliferation and dissemination of HCT116 cells.

**Conclusions:** PDZRN4 may function as a tumor suppressor and is downregulated in colon cancer tissues, possibly due to dysregulation via miR-221-3p. This study provides new insight into colon cancer development.

**Keywords:** PDZ domain containing ring finger 4 (PDZRN4); colon cancer; microRNA; 3'-untranslated region (3'-UTR); mRNA; proliferation

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**Introduction**

Despite rapid advances in diagnostic and treatment strategies, colon cancer still ranks high among commonly diagnosed cancers, and its incidence is rising (1). In China, the incidence in men and women is 16.9 and 11.6 per 100,000, respectively (2). Colon cancer is closely associated with other diseases such as diabetes and obesity, making it a major public health risk (3,4). Despite the rapid increase in newly diagnosed patients, the etiology and mechanism of colon cancer development remains unclear.

The gene PDZRN4 (PDZ domain containing ring finger 4) belongs to the ligand of numb protein-X (LNX) family and is dysregulated in multiple diseases, including radiation-induced papillary thyroid carcinoma, rectal adenocarcinoma, and hepatocellular carcinoma (5,6). In human liver cancer cell lines, the ectopic expression of PDZRN4 inhibited cancer cell proliferation, as well as plate colony formation and anchorage-independent colony...
formation (7). Analysis of gene expression data indicates that the gene PDZRN4 is downregulated in colon cancer. In the present study, we investigated the role of PDZRN4 in colon cancer development.

MicroRNAs (miRNAs) are a small noncoding RNA that, by binding to a coding sequence or 3'-untranslated region (UTR), can interfere with mRNA translation or stability, changing expression of the gene. Depending on its downstream gene, miRNAs can function as either tumor suppressors or oncogenes. Because miRNAs have such roles in the development of cancers (8-10), we wondered whether miRNAs may be implicated in the differential expression of PDZRN4 in colon cancer.

Specifically, miR-221 is a putative regulator in tumor development (11-13), and according to open access data in the National Center for Biotechnology Information (NCBI) database its levels are usually elevated in colon cancer (GSE101502). In addition, higher level of miR-221 in colon cancer tissues is associated with poor prognosis (14,15). The expression of miR-221 is regulated by multiple regulators, including KRAS, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT3 (signal transducer and activator of transcription 3), and KRAP (Ki-ras-induced actin-interacting protein), while KRAP-regulated miR-221 expression could only be seen in a 3D culture, and NF-κB and STAT3 activation is, in turn, manipulated by miR-221 expression (16,17).

It is possible that miRNAs can have multiple targets, and the link between miR-221 and PDZRN4 remains unclear. To contribute information toward the diagnosis and treatment of colon cancer, the present study explored the potential regulation of PDZRN4 by miR-221-3p.

Methods

Ethics statement

The Institutional Ethics Committee of Second Affiliated Hospital of Soochow University (Suzhou, 215004, Jiangsu Province, China) reviewed and authorized the clinical section of this research.

Colon cancer tissue samples

Twenty pairs of colon cancer tissues and adjacent normal tissues were collected from patients who received treatment in our hospital. None of the patients received radiotherapy or chemotherapy before surgery (Table S1).

All diagnoses were obtained via pathological examination. All examinations and evaluations of clinical samples were conducted using paired normal tissue as a control.

Cell line

Cells of the human colon cancer cell line, HCT116 were purchased from the American Type Culture Collection and cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) at 37 °C, 5% CO2.

293T cells were previously preserved in our lab and cultured in Dulbecco’s modified Eagle’s medium (Gibco, Grand Island, NY, USA) containing 10% FBS and 2 mM L-glutamine at 37 °C, 5% CO2.

Cells were observed with a microscope (Olympus, Tokyo, Japan) and green fluorescent protein (GFP) was observed with a fluorescence microscope.

Plasmids

The pLX-PDZRN4 plasmid was constructed by inserting the PDZRN4 coding sequence into the lentiviral expression vector, pLX-IRES-ZsGreen1. The pLX-miR-221-3p sponge was constructed by referring to a published article (18).

Immunohistochemistry (IHC)

The cancer tissues and paired adjacent normal tissues obtained from patients during surgery were processed for staining with hematoxylin and eosin and IHC. All the samples were formalin-fixed, paraffin-embedded, and immunostained with PDZRN4 antibody (ThermoFisher Scientific, Waltham, MA, USA). Quantification was conducted by counting the positive cells per field. Data is shown as mean ± standard deviation (SD).

Western blot

Cells were lysed with sodium dodecyl sulfate (SDS) lysis buffer (Beyotime, China) and denaturalized in a water bath at 100 °C. The prepared protein was resolved via SDS-PAGE, transferred to a polyvinylidene difluoride membrane from Millipore, and incubated with PDZRN4 primary antibody and HRP-Goat Anti-Rabbit IgG (H + L) (MultiSciences, Hangzhou, China). Protein levels were detected with a peroxide LumiGLO reagent (Cell Signaling
Technology, 7003). Quantification of the relative protein levels was conducted according to the ratio of gray-value to the corresponding internal references. A representative graph was constructed and protein was quantified based on five pairs of clinical samples or three independent experiments and shown as mean ± SD.

**Reverse transcription followed by real-time quantitative PCR (RT-qPCR)**

RNA was extracted in accordance with the instructions for TRIzol reagent use (GENEray, Shanghai, China). The total RNA was subjected to reverse transcription using a kit from GENEray, Shanghai, China. Finally, cDNA was used for qPCR analysis. The RNA levels were normalized to internal references, and shown as mean ± SD (for primers, Table 1).

**Fluorescence-activated cell sorting (FACS, flow cytometry)**

The rate of GFP-positive cells was determined using Accmri C6 (BD, San Jose, CA, USA). Cells were digested into single cells and determined by the GFP channel of Accmri C6. GFP-positive cells and the total cell number were counted automatically. The percentage of positive cells was calculated as positive cells number/total cell number ×100%.

**Cell proliferation assay using cell-counting kit 8 (CCK8)**

Two thousand cells were suspended in 100-μL culture medium and seeded per well in a 96-well plate; 8 plates were seeded. Each plate was collected daily after seeding, 10 μL CCK8 solution (MultiSciences, Hangzhou, China) was added, and measurements were performed using a microplate reader at 450 nm; the first measurement was done a few hours after seeding. The relative proliferation rate is shown as mean ± SD of reads at 450 nm.

**Plate clone formation assay**

One hundred cells were suspended in 2 mL culture medium in a 6-well plate. The medium was replenished every 2 days. The cell culture was observed daily until visible clones had formed. The culture medium was carefully removed and clones were stained with crystal violet solution. Data is shown as mean ± SD of numbers of clones per field.

**Migration and invasion assay**

Cells were seeded to the upper chamber of Millicell hanging cell culture inserts (Merck Millipore, Darmstadt, Germany). Culture medium without FBS and complete culture medium, were added, respectively, to the upper and lower chambers of the culture wells. Cells that had migrated to the lower surface of the filter were fixed with 70% methanol and then stained with 0.5% crystal violet solution.

For the invasion assay, hanging cell culture inserts were pre-coated with diluted Matrigel an hour before cell seeding. The cells that migrated or invaded the lower surface of the wells were counted. Data are shown as mean ± SD. Student's t-test was performed when comparing data between groups.

**Wound-healing assay**

Cells were seeded to six-well culture plates and cultured overnight. By the second day, the culture medium was carefully removed and medium pipette tips were used to scratch the monolayer cell culture. Floating cells were carefully removed and fresh medium was added to the cell culture.

Graphs were constructed at the time of the scratch (0 hour) and 12 hours after treatment.

The percentage of scratch remaining after wounding was calculated as: (measurement at 12 hours/measurement at 0 hour) ×100%. The percentage of wound closure was calculated as: 100% − percentage of wound remaining. The percentage of wound closure is shown as mean ± SD.

**Statistical analysis**

The data in this study are shown as mean ± SD. The comparison of means between two groups was conducted using Student's t-test. P<0.05 was considered significant; *P<0.05, **P<0.01 and ***P<0.001.
Results

**PDZRN4 is downregulated in colon cancer at both the mRNA and protein level**

In the initial phase of this investigation, mRNA array data obtained from NCBI was analyzed (GEO accession: GSE75970 and GSE74604). We compared gene expression levels in both the array and screened differentially expressed genes having a 2-fold difference in quantity between colon cancer tissues and normal tissues (Figures S1, S2). After searching studies and reports concerning differentially expressed genes, we found PDZRN4, downregulated in both arrays, had never been studied in colon cancer, and was not well illustrated in almost any disease.

The levels of PDZRN4 protein in 5 pairs of colon cancer tissues and matched normal tissues were compared using IHC and western blot (Figure 1A, B, C, D). Both analyses showed lower amounts of PDZRN4 protein in the colon cancer tissues than in the normal tissues. In addition, RT-qPCR of 20 pairs of samples showed less PDZRN4 mRNA in the cancer samples (Figure 1E). These results suggest that PDZRN4 is suppressed at both the mRNA and translated levels in colon cancer.

**Ectopic expression of PDZRN4 attenuated HCT116 cell's tumorigenesis**

To determine if PDZRN4 may be a regulator in colon cancer development, the gene was overexpressed in HCT116 cells (Figure 2A). The FACS, western blot, and RT-qPCR results confirmed the successful overexpression of PDZRN4 (Figure 2B, C, D, respectively).
Figure 2 Overexpression of PDZRN4 inhibited the proliferation, migration, and invasion of HCT116 cells. (A) HCT116 cells were transduced with lentiviruses (middle) pLVX empty vector and (right) pLVX-PDZRN4. Representative images of (left) untreated cells, pLVX empty vector, or pLVX-PDZRN4 transduced cells were taken under (Phase, top) light microscope and (GFP, bottom) fluorescent microscope (100×). (B) Percentages of cells treated as in Figure 2A tested positive for GFP using FACS. (C) Western blot of PDZRN4 proteins in cells treated as in Figure 2A. (D) mRNA levels of PDZRN4 in cells treated as in Figure 2A. ***P<0.001 for Student’s t-test. (E) Proliferation rates of cells treated as in Figure 2A by cell proliferation assay. *P<0.05, ***P<0.001 for Student’s t-test. (F) Representative images for the clone formation assay of cells treated as in Figure 2A. (G) Numbers of clones formed (Figure 2F). *P<0.05, **P<0.01 for Student’s t-test. (H) Percentage of cells treated as in Figure 2A in different cell cycle phases. (I) Percentages of cells in different cell phases. ***P<0.001 for Student’s t-test. (J) Representative images for transwell migration and invasion of cells treated as in Figure 2A (100×). (K) Number of migrated and invaded cells per field. ***P<0.001 for Student’s t-test. (L) Representative images of wound healing assay for cells treated as in Figure 2A (×100) ***P<0.001 for Student’s t-test.
To investigate how PDZRN4 regulates cancer cell activities, the cells overexpressing PDZRN4 were then applied for functional assays. The cells overexpressing PDZRN4 proliferated less compared with the control cells, as measured by the cell proliferation (Figure 2E) and plate clone formation (Figure 2EG) assays. A study of the cell cycle of the transformed cells showed that fewer of those overexpressing PDZRN4 reached S phase, compared with the control. This suggests that there was less DNA replication in the overexpressing cells (Figure 2H,I).

Tumor cells often have an enhanced ability to disseminate. In the present study, cells with overexpressed PDZRN4 were much less able to disseminate via migration and invasion (Figure 2J,K), as also indicated by the wound healing assay (Figure 2L,M). Altogether, these data suggest that PDZRN4 is suppressed in colon cancer, and this suppression promotes cancer cell proliferation and dissemination. Thus, PDZRN4 may function as a tumor suppressor.

With this supposition, the regulation of PDZRN4 expression in colon cancer was investigated.

**PDZRN4 mRNA is a direct target of miR-221-3p**

Since PDZRN4 expression was dysregulated at both the mRNA and protein levels, we investigated whether it may be regulated by miRNAs. Thus, data from the noncoding RNA expression profile (GEO accession: GSE101501) was explored for implications in colon cancer. Using the databases RNAhybrid, TargetScanHuman, miRWalk, and miR, the following miRNAs listed as upregulated in colon cancer tissues in the databases were selected and investigated for binding potential with the PDZRN4 3’-UTR: miR-153-3p, miR-5195-3p, miR-371a-3p, miR-133a-5p, miR-221-3p, miR-31-5p, miR-373-3p, miR-615-5p, miR-582-3p, and miR-301b-3p. To determine the association of these miRNAs with PDZRN4, PDZRN4 was measured after knocking out each miRNA respectively. It was found that, among these miRNAs, transfection with the miR-221-3p inhibitor was associated with dramatically higher levels of both protein PDZRN4 and mRNA PDZRN4 (Figure 3A,B).

To verify binding further, luciferase report assays were conducted. The results showed inhibition of luciferase activity, indicating that miR-221-3p directly binds to the 3’-UTR of PDZRN4 mRNA (Figure 3C).

Further investigation revealed that, compared with normal tissues, miR-221-3p is overexpressed in colon cancer tissues (Figure 3D). At this point in the investigation, to confirm the presence of a regulation network, the miR-221-3p and PDZRN4 3’-UTR vector was transfected into 293T cells. The luciferase report assay showed that luciferase activity was dose-dependent with miR-221-3p (Figure 3E), and this was true also for PDZRN4 protein based on the western blot (Figure 3F). These results indicated the direct inhibition of PDZRN4 expression by miR-221-3p.

To specify the binding sequence, we mutated several bases in both miR-221-3p and the PDZRN4 3’-UTR (Figure 3G). According to the dual luciferase assay, mutation in either miRNA or the PDZRN4 3’-UTR attenuated regulation (Figure 3H), and western blot was consistent with this (Figure 3I). These data indicated that the mutant regions of miR-221-3p and the PDZRN4 3’-UTR are the binding sites of this miR-3’ UTR regulation system (Figure 3G).

**Knockdown of miR-221-3p restrained tumorigenesis of HCT116 cells**

To support that miR-221-3p-regulated PDZRN4 expression interfered with the activity of colon cancer cells, miR-221-3p was knocked down by transducing HCT116 cells with a miRNA sponge. Observation and FACS detection of red fluorescent protein revealed that miR-221-3p was successfully dysregulated in HCT116 cells (Figure 4A,B). In addition, consistent with the results of miRNA overexpression, the levels of PDZRN4 protein were comparable with that observed prior to knockdown of miR-221-3p (Figure 4C).

We then conducted more assays of phenotype. Both the cell proliferation (Figure 4D) and plate clone formation (Figure 4E,F) assays indicated that suppression of miR-221-3p expression lowered the proliferation of HCT116 cells. In addition, observations of the cell cycle showed inhibition of cell cycle progression (Figure 4G,H). Together, these data indicate that miR-221-3p functions in regulating HCT116 cell proliferation.

Moreover, the transwell migration, invasion, and wound-healing assays showed intense inhibition of cancer cell dissemination after treatment with the miR-221-3p sponge (Figure 4I,J,K,L). Together, these data indicate that miR-221-3p modulated the dissemination of colon cancer HCT116 cells.

**Discussion**

Gene dysregulation is a feature of cancer development. Various dysregulated genes may function as either tumor
Figure 3 An overexpressed miRNA in colon cancer, miR-221-3p, directly targets PDZRN4. (A) Transfection of different miRNA inhibitors in 293T cells regulates PDZRN4 protein levels. (B) PDZRN4 mRNA in cells treated as in Figure 3A. ***P<0.001 for Student's t-test. (C) Luciferase activity was detected in HCT116 cells co-transfected with different miRNAs. ***P<0.001 for Student's t-test. (D) miR-221-3p is upregulated in colon cancer tissues. ***P<0.001 for Student's t-test. (E) miR-221-3p regulated luciferase activity in a dose-dependent manner. ***P<0.001 for Student's t-test. (F) miR-221-3p inhibited PDZRN4 expression in a dose-dependent manner. (G) Schematic illustration of the putative seed sequences of miR-221-3p complementary with PDZRN4 3'-UTR, and mutagenesis of binding sites in the 3'-UTR of PDZRN4 and miR-221-3p. (H) Luciferase activity was analyzed in HEK 293T cells co-transfected with miR-221-3p or miR-221-3p Mut, and PDZRN4 3'-UTR or PDZRN4 3'-UTR Mut. *P<0.05 for Student's t-test. (I) miR-221-3p failed to regulate endogenous PDZRN4 expression. HCT116 cells were transfected with NC, miR-221-3p mimic, or miR-221-3p mut and western blot was conducted 48 hours after transfection.
Figure 4 Knockout of miR-221-3p expression attenuated the proliferation, migration, and invasion of HCT116 cells. (A) HCT116 cells were transduced with a pLVX empty vector or miR-221-3p sponge lentivirus. Representative images of untreated cells (left) and transduced cells were taken under (Phase, top) light microscope, and (red fluorescent protein, bottom) fluorescent microscope (100×). (B) Percentage of cells treated as in Figure 4A tested positive for GFP by FACS. (C) PDZRN4 protein in HCT116 cells treated as in Figure 4A. (D) Proliferation of cells treated as Figure 4A by cell proliferation assay. ***P<0.001 for Student's t-test. (E) Representative images taken for the clone formation of cells treated as Figure 4A. (F) Numbers of clones formed in Figure 4E. **P<0.01 for Student's t-test. (G) Percentage of cells treated as Figure 4A in different cell cycle phase. (H) Percentages of cells in different cell phases. *P<0.05, **P<0.01, ***P<0.001 for Student's t-test. (I) Representative images for transwell migration and invasion of cells treated as Figure 4A (100×). (J) Number of migrated and invaded cells per field. ***P<0.001 for Student's t-test. (K) Representative images of wound healing assay for cells treated as Figure 4A (×100). (L) Percentages of wound closure. ***P<0.001 for Student's t-test.
suppressors or oncogenes. Thus, we searched and analyzed gene expression profiles, and chose an inhibited gene in colon cancer for further investigation, i.e., PDZRN4. The PDZRN4 mRNA and PDZRN4 protein levels in colon cancer tissues were much lower than that of the adjacent normal tissues. Overexpressed PDZRN4 in the colon cancer cell line arrested cell cycle, inhibited cell proliferation, and attenuated migration and invasion. This suggests that PDZRN4 protein in colon cancer tissues is negatively associated with cancer cell dissemination, and PDZRN4 may function as a tumor suppressor in colon cancer development.

Furthermore, we found that PDZRN4 translation was inhibited by an overexpressed onco-miRNA, miR-221-3p. The luciferase report assay showed a direct association between miR-221-3p and the 3'-UTR of PDZRN4 mRNA. In addition, the cell proliferation and plate clone formation assays showed that as the miR-221-3p level subsided, so did the proliferation and dissemination of the colon cancer cell. This indicates that miR-221-3p-PDZRN4 has an important role in regulating colon cancer development, in which miR-221-3p inhibits the translation of PDZRN4 and thereby promotes the proliferation and dissemination of colon cancer cells.

In this study, the low number of clinical samples and time allowed limited sufficient inspection PDZRN4 expression in colon cancer tissues. Whether PDZRN4 protein is a viable biomarker of colon cancer requires further investigation.

**Conclusions**

This study investigated the expression of PDZRN4 in colon cancer tissues, its potential function in the development of colon cancer, and its inhibition by its upstream regulator, miR-221-3p. Three main conclusions are highlighted. First, according to the gene expression profile and detection in clinical colon cancer samples, PDZRN4 mRNA and PDZRN4 protein are downregulated in colon cancer. Second, overexpression of PDZRN4 in the colon cancer cell line and the phenotype assays indicated that PDZRN4 functions to attenuate cell proliferation, migration, and invasion. Thirdly, miR-211-3p inhibited PDZRN4 translation by binding to its mRNA 3'-UTR. The knockdown of miR-211-3p dramatically reduced the proliferation, migration, and invasion of colon cancer HCT116 cells.

These discoveries suggest that PDZRN4 may be a potential target for defeating colon cancer, but more investigations are necessary.

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**Footnote**

**Conflicts of Interest:** Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.07.12). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Institutional Ethics Committee of Second Affiliated Hospital of Soochow University (Suzhou, 215004, Jiangsu Province, China) reviewed and authorized the clinical section of this research. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all patients.

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© Translational Cancer Research. All rights reserved. Transl Cancer Res 2019;8(4):1289-1300 | http://dx.doi.org/10.21037/tcr.2019.07.12
### Table S1 Patient information enrolled in research

| Patient No. | Gender | Age, years | Histological type                  | LNM  | Tumor size, cm³  |
|-------------|--------|------------|-----------------------------------|------|-----------------|
| PT 1        | F      | 64         | Mucinous adenocarcinoma           | POS  | 3.5×4.5×1.5     |
| PT 2        | M      | 75         | Canalicular adenoma               | POS  | 8.0×5.5×2.5     |
| PT 3        | F      | 83         | Signet-ring cell carcinoma        | POS  | 7.0×4.5×2.0     |
| PT 4        | M      | 79         | Mucinous adenocarcinoma           | POS  | 5.0×4.0×2.3     |
| PT 5        | M      | 63         | Canalicular adenoma               | POS  | 3.5×2.0×1.0     |
| PT 6        | F      | 37         | Canalicular adenoma               | POS  | 5.0×4.5×2.0     |
| PT 7        | F      | 85         | Canalicular adenoma               | NEG  | 4.0×4.0×1.0     |
| PT 8        | M      | 51         | Signet-ring cell carcinoma        | POS  | 6.0×2.5×2.0     |
| PT 9        | F      | 84         | Canalicular adenoma               | NEG  | 10.0×5.0×4.5    |
| PT 10       | M      | 73         | Canalicular adenoma               | NEG  | 5.5×3.5×2.0     |
| PT 11       | M      | 74         | Canalicular adenoma               | POS  | 4.0×3.3×1.5     |
| PT 12       | F      | 55         | Canalicular adenoma               | NEG  | 1.1×0.9×0.5     |
| PT 13       | F      | 58         | Canalicular adenoma               | POS  | 6.5×4.0×3.0     |
| PT 14       | F      | 74         | Mucinous adenocarcinoma           | POS  | 3.5×2.0×1.5     |
| PT 15       | M      | 64         | Polypoid adenocarcinoma           | POS  | 3.0×2.2×1.6     |
| PT 16       | M      | 60         | Canalicular adenoma               | POS  | 4.3×3.2×1.5     |
| PT 17       | F      | 70         | Canalicular adenoma               | POS  | 8.0×5.0×2.5     |
| PT 18       | F      | 58         | Canalicular adenoma               | POS  | 6.0×3.5×2.0     |
| PT 19       | M      | 71         | Canalicular adenoma               | POS  | 3.2×3.0×0.8     |
| PT 20       | M      | 78         | Canalicular adenoma               | POS  | 6.0×4.0×4.0     |

F, female; LNM, lymph node metastasis; M, male; NEG, negative; POS, positive; PT, patient.
VIP  PPP1R14A  ADAMTS8  CNRIP1  PTN  CCL21
REPG1  MMP9  PPP2R2B  FERM72  SGCE  PGL2
MYH11  PTGS1  NEXN  DDR2  ANTR2  KCNMB1
DES  SPARC1L1  MIE1S1  MIE52  ANGPTL1  PDE5A
C7  LMOD1  SYNCP  PRUNE2  HSP93  SPEG
PGM6  LRPR1  SYT4  PODN  TSHZ3  GDNF2
CNN1  STMN2  GPMA  PODM3  FCR1A  AFF3
MYOM1  RUND3B8  ADHNE1  SGCA  SMARC03  GNAO1
CJD  STDX2  JAM2  ATP2B4  LMD03  PTCH1D1
RBPM52  CDH19  SMYD1  RERG  EIF4E3  POPDC2
ATP1A2  MYOT  SLC3A9  GREM2  TBC1D9  SMTN
FHL1  RGM1  PRRT2  EB1F  ANK581  MRRN1
CLEC3B  SETBP1  FOSB  CASZ1  ARMX1  TAGLN
MAMDC2  ACAEB1  ANA9K  PLN  CAP2  TACR2
ABC8  DACK3  SLC25A23  TAGLN3  ARQPRF  CYBD1
DPT  SNPX  SLT2  ELAVL4  BNC2  ANK2
NPTX1  FXYD6  LVE1  MAE  SLCOA16  FRX1
PPH4  GNG7  TEF  MASPF1  RPM1  COLEC12
SCARA5  LMS2  PLEKHD1  HSPA2  LRFRP1  TEDD1
HAND1  CD209  CITED2  KCNQ5  ARROC4  CACN1H
RN150  NNT  CTNPFR  ETFDH  REEP1  M6P1
CASQ2  GSN  PALM  MAP1B  FM12  GPR162
HSPB6  PLP1  ZSCAN18  EMP3  OPTN  JPH2
PLAC9  ADH1B  SC9B  AGTR1  LDB3  SLT3
CXCL12  SGC2  PCP4  ADAMTS1  OLML1  FOLR2
ADH1A  PI16  CBX7  TSPAN18  ZNF671  SLCO2A17
LRNR2  FNPB1  RINK1  KIAA1683  ADAMTS13  A2M
FAM107A  PCCO1E2  PDZD4  CSF1R  VPR2  ADCA9
CKB  FLNA  BCHE  STBD1  SCUBE2  TPM1
POE6A  PEDE78  NLGN1  REEP2  PMP22  C8X6
ACTG2  FXYD1  SORBS2  OLML2A  GFRA3  MAD8
SIMA6A  IFGBP6  HTR4  SPECC1L  XGB  DIKI1C
MYLK  FOXO2  PDZRN4  GSTM2  LG4  RASGRP2
SCGN  TSC22D3  KIAA0513  AXI1  SYNGR1  UCHL1
C2orf40  TIMM90  TCN2  GSTM5  TGFBR11  DENND1B
PNCK  PEG3  SNAP91  ARHGEF37  SALL2  CELF2
POK4  SORBS1  GLPR2  RGL1  DAAAM2  RCD51
GPX3  THBS4  MIA47  MYOC  LIN00341  CSPG4
CRYAB  CLIP3  RELN  GLG3  NDE1  CLMP
HSPB8  MGP  PTH1R  FAM188A2  ROR2  CBLN2
NPF  STAB1  KCNMA1  F13A1  KCNH2  EML1
METTL7A  XRK4  TGFBR3  LRCH2  PPR2R3A  DAOAH2
TECAL2  KPI1A  BOC  KIT  CHOOL  LPP
AB3B3P  LIFR  P5G11  TRM9  SGCD  CHST15
MFA4  FAM46B  PDGFR4  PTPF  FGFR13  KCNAP3
GP8A2  CPXM2  KANK2  SSBP2  CCDC136  SNAP25
ZBTB16  CSRIP1  MEF2C  TNFSF12  ANKRD55  GYPC
SODR  COX7A1  CT5orf52  KCNAS  SCBP  OGN
GABARP1L1

**Figure S1** Upregulated genes in both arrays (by acronym).
Figure S2 Downregulated genes in both arrays.