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RESEARCH PAPER

miR-200c inhibits invasion and migration in human colon cancer cells SW480/620 by targeting ZEB1

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Abstract MicroRNAs are a class of ≈22-nt noncoding single-strand RNAs regulating gene expression postscriptionally. Metastasis caused poor prognosis in colorectal cancer patients and half of the patients developed metastatic lesions when admission. Here we investigated the possible roles of microRNAs in regulating metastasis in the paired colon cancer cells SW480 and SW620. Among those dysregulated microRNAs, miR-200c was speculated to inhibit metastasis by targeting ZEB1. Overexpression of miR-200c was concurrent with downregulation of ZEB1 mRNA and protein. Functional assays demonstrated that modulation of miR-200c with mimics or inhibitors changed potential of metastasis in SW480/620 cancer cells in vitro. Taken together, our study demonstrated that miR-200c inhibits metastatic ability by targeting ZEB1 in colon cancer cells SW480/620 and suggested that modulation of miR-200c could serve as therapeutic tool for inhibiting metastasis in colorectal cancer.

Keywords Colorectal cancer · Metastasis · miR-200c

Colorectal cancer is the third leading cause of cancer death worldwide. About half of the patients developed metastatic lesions when admission [1]. Metastasis causes poor prognosis in colorectal cancer patients. Its mechanisms are multifaceted and extremely intricate. It is very important for us to explore metastatic mechanisms in colorectal cancer, which may provide new targets for treatment of metastasis. The SW480 cancer cell was originated from primary colon cancer in a 50-year-old male patient while the SW620 cancer cell was originated from metastatic lymph node in the same patient. SW620 cancer cell has higher potential of metastasis than SW480 cancer cell [2–4]. In this study, we explored the possible metastatic mechanisms of action by microRNA(s) in these two colon cancer cell lines with different metastatic potential.

MicroRNAs are a class of ≈22-nt noncoding single-strand RNAs. The initial products of microRNAs, pri-microRNAs, are cleaved by Drosha into pre-microRNAs in the nuclear compartment. After being transported into cytoplasm by Exportin-5, pre-microRNAs are cleaved by Dicer into the mature microRNAs. Through base-pairing to the 3′ untranslated region, microRNAs negatively regulate mRNA targets at the posttranscriptional level. In this way, they are crucial players participating in many cellular processes [5–8]. In malignancy, microRNAs can act as tumor suppressor genes (for example, miR-15a and miR-16-1 in chronic lymphocytic leukemia [9] and in prostate cancer [10], let-7 family members in lung cancer [11], and miRNA-34b/c in colorectal cancer cells [12]) or oncogenes (for example, miR-155 in lymphoma [13], miR-17-92 in lung cancer [14], and miR-21 in colorectal cancer [15]). MicroRNAs can also act on the multiple steps of metastasis [16]. MiR-10b stimulates cell motility by translation repression of HOXD10 in breast cancer [17]. Huang et al. [18] found that miR-373 and miR-520c promotes breast cancer cell migration and invasion by suppression of CD44. On the other hand, microRNAs can act as metastasis suppressors. Lower expression of miR-335 and miR-126 is significantly associated with poor metastasis-free survival or relapse in breast cancer patients [19]. They suppress

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metastasis by repressing the expression of a transcription factor, SOX4.

The aim of our study is to explore the candidate microRNA(s) playing a role in regulating the metastatic ability in colon cancer.

Materials and methods

Cancer cell line

Human colon cancer cell lines SW480 and SW620 (Cell Bank, Chinese Academy of Sciences, Shang Hai, P. R. China), were cultured in Leibovitz L-15 medium (Invitrogen, Carlsbad, CA) supplemented with 10 % fetal calf serum (Gibco BRL, Grand Island, NY) at 37 °C in a humidified incubator containing 5 % CO_2.

MicroRNA microarray

Total RNA was extracted from the two cancer cell lines by using Trizol (Invitrogen, Carlsbad, CA). Then the sample was sent to CapitalBio Corporation (Beijing, P. R. China) to analyze the expression of microRNAs. According to the manufacturer's protocol, one microgram of purified total RNA was labeled by using the FlashTag kit (Genisphere, Hatsfiled, PA). The FlashTag labeling process began with a Ploy(A) tailing reaction by using the Ploy(A) polymerase and ATP. A biotinylated 3′-DNA dendrimer, which is a branched structure of single and double-stranded DNA conjugated with approximately 15 biotins, was ligated to the target RNA. These RNAs were then hybridized to the GeneChip microRNA Array (Affymetrix, Santa Clara, CA). Hybridization was performed at 48 °C with rotation for 16 h (Affymetrix GeneChip Hybridization Oven 640). The GeneChip microRNAs arrays were washed and stained (streptavidin–phycoerythrin) on an Affymetrix Fluidics Station 450 followed by scanning on a GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA). The hybridization data were analyzed by GeneChip Operating software (GCOS). A global scaling procedure was performed to normalize the data by using microRNA QC Tool software (Affymetrix, Santa Clara, CA). In a comparison analysis, we applied Significant Analysis of Microarray software (SAM) to identify significantly differentially expressed genes.

Real-time quantitative RT-PCR

Total RNA was extracted from cells using Trizol total RNA isolation reagent (Invitrogen, Carlsbad, CA). Expression of microRNAs was quantified by Taqman microRNA assays (Applied Biosystems, Foster City, CA) as described [20]. All of the PCR products were analyzed on 3 % agarose gels. The relative expression of microRNAs was calculated by using the $^{\Delta\Delta}$Ct method and normalized to H-5S rRNA. All of the RT-PCRs were repeated triplicately. The primers were designed in Primer express 2.0 software and listed in Table 1.

Table 1 The primers used for stem-loop RT-PCR for miR-200c

Primer	Sequence
miR-200c RT	5′-GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTCCATC-3′
miR-200c forward	5′-AGCGGTAATACTGCCGGGTA-3′
miR-200c reverse	5′-GTGCAGGGTCCGAGGT-3′
H-5S rRNA forward	5′-CGCCCGATCTCGTCTGAT-3′
H-5S rRNA reverse	5′-GGTCTCCCATCCAAGTACTAACCA-3′

In TargetScan data base [21], miR-200c has two positions which are connected with ZEB (zinc-finger-enhancer binding protein)1 3′-UTR (Fig. 1). We wondered if miR-200c plays a role in the regulation of metastasis in colorectal cancer by targeting ZEB1.

Quantitative RT-PCR

In parallel, 2 μg RNA samples were reverse-transcribed to cDNA by using reverse transcriptase. The sequences of the ZEB1 and internal reference 18s rRNA were shown in the Table 2. Quantitative PCR was performed by using the SYBR Green PCR Master Mix (TOYOBO Corp., Osaka-fu, Japan) in ABI PRISM® 7500 Sequence Detection

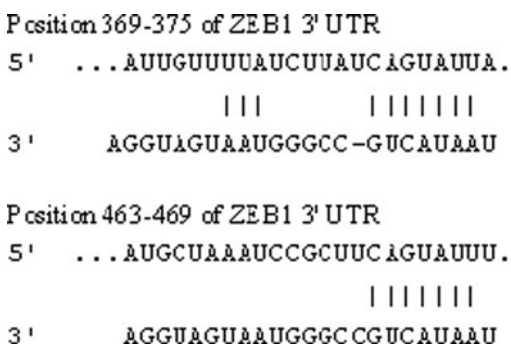

Fig. 1 miR-200c has two positions which are connected with ZEB1 3′-UTR

Table 2 The primers used for quantitative RT-PCR for ZEB1

Primer	Sequence
ZEB1 forward	5′-ACCTCTTCACAGGTTGCTCCT
ZEB1 reverse	5′-AGTGCAGGAGCTGAGAGTCA
18s rRNA forward	5′-CCTGGATACCGCAGCTAGGA
18s rRNA reverse	5′-GCGGCGCAATACGAATGCCCC

System (Applied Biosystems, Foster City, CA). The reaction mixture contained 5.0 μl cDNA, 0.5 μl forward and reverse primers, 10 μl SYBR Green PCR Master Mix (2×), and 4 μl dH_2O, in a final volume of 20 μl. The reaction conditions were as following: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 15 s, 60 °C for 15 s, 72 °C for 32 s.

Western blot

Cells were in 6-well plates for 72 h. Then cells were harvested and homogenized with lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.5, 1 % NP40, 1 % deoxycholate, 0.1 % SDS, protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Proteins from cells were resolved by 10 % SDS-PAGE gel (Invitrogen, Carlsbad, CA) and transferred to the nitrocellulose membrane (Millipore, Bedford, MA). The membrane was incubated with Homo Sapiens polyclonal antibody specific to ZEB1 (Invitrogen, Carlsbad, CA) or mouse anti-actin (1:5,000; Abcam, Cambridge, United Kingdom) and visualized by chemiluminescence (ECL, Amersham, Freiburg, Germany).

Transfection

Lipofectamine™ RNAiMAX (Invitrogen, Carlsbad, CA) was used to transfect SW620 cancer cells with the miR-200c mimic (RIBOBIO Co. LTD, Guang Zhou, P. R. China) according to the manufacturer's instruction, while it was used to transfect SW480 cancer cells with the miR-200c inhibitor (RIBOBIO Co. LTD, Guang Zhou, P. R. China), and with their respective negative control. Cells were plated in 6 cm diameter cell culture dishes to 60 % confluence. For each dish, 1.25 μl of miR-200c mimic or inhibitor (20 μm) or negative control were soluted in 100 μl Opti-MEM medium. Two microliters of Lipofectamine™ RNAiMAX were mixed with Opti-MEM medium. Then the transfection complex were added to the cells in one dish and incubated for 4 h at 37 °C in a CO_2 incubator and transferred to RPMI1640 medium with 10 % FBS for 6 h. After transfected with microRNA-control for 6 h, the cells were observed under fluorescent microscopy.

Migration and invasion assay in vitro

Migration assay in vitro was performed by using BD BioCoat Matrigel invasion chambers (Bectron Dickinson Labware, Bedford, MA) with polyethylene terephthalate-filters with 6 wells, 8 μm pore size. Briefly, 100 μl of serum free medium containing 1×10^5 starved post-transfection cells were seeded into the upper compartment and 600 μl medium containing 10 % FBS was added to the lower compartment as chemoattachment. After 24 h of incubation at 37 °C in 5 % CO_2, filters were fixed and stained. The filters were gently rinsed with de-ionized water and nonmigrating cells on the upper surface of the filter were completely removed by cotton swab. The number of migration cells on the lower surface of the

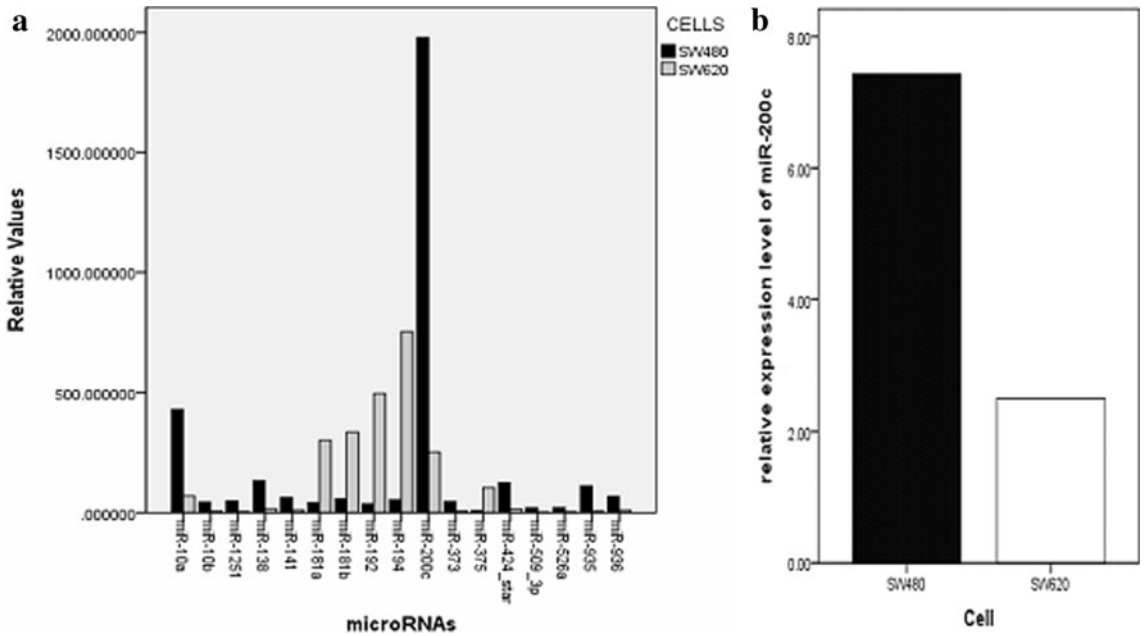

Fig. 2 **a** The relative expression of microRNAs in SW620 compared with SW480 cancer cell by microarray. The relative expression of miR-200c is downregulated more than 7 times in SW620 compared with SW480 cancer cell by microarray. **b** The relative expression of miR-200c is downregulated more than three times in SW620 compared with SW480 cancer cell by stem-loop RT-PCR analysis

Table 3 microRNAs differentially expressed between SW480 and SW620 cancer cells

miRNAs	More than twice up- or down-regulation in SW620	Relative detection value	Related function in literature
Down-regulation in SW620			
hsa-miR-10b	7.77	44.72485	Promotor of tumor invasion and metastasis [18]
hsa-miR-373	7.356	45.80452	Promotor of tumor invasion and metastasis [19]
hsa-miR-141	5.74	62.5456	Prediction of advanced colorectal cancer and poor prognosis [30, 31]
hsa-miR-10a	6.20	430.8929	Promotors of tumor invasion and metastasis [27–29]
hsa-miR-138	7.90	133.014	Downregulation of miR-138 leads to overexpression of human telomerase protein in anaplastic thyroid cancer cell line [32, 33]
hsa-miR-200c	7.80	1979.443	Suppressor of migration and invasion [22–24]
hsa-miR-936	6.67	67.8378	No description[a]
hsa-miR-424_star	7.966	124.9801	No description[a]
hsa-miR-526a	10.20	21.45972	No description[a]
hsa-miR-1251	12.11	48.23552	No description[a]
hsa-miR-935	16.30	111.4357	No description[a]
hsa-miR-509-3p	22.63	20.07031	No description[a]
Up-regulation in SW620			
hsa-miR-194	14.00	753.2983	Suppressor of metastasis [34–36]
hsa-miR-375	12.49	104.5445	Suppressor of metastasis [37, 38]
hsa-miR-192	13.79	496.8511	Suppressor of metastasis [39]
hsa-miR-181a	7.15	301.0816	Tumor suppressor gene [40–42]
hsa-miR-181b	5.75	336.2941	Tumor suppressor gene [40–42]

[a] No description in literature

membrane was then counted under microscope. Invasion assay was done by using chambers coated with matrigel basement membrane matrix.

Statistical analysis

Data from different experiments are presented as mean ± standard deviation (SD). Differences of number of cancer cells in invasion and migration assay between different groups were compared using paired-samples *T* test. *p* value less than 0.05 was considered statistically significant.

Results

Differentially expressed microRNAs between SW480 and SW620 cancer cell lines

Comprehensive microRNAs expression profiling was assayed between these two cell lines. The result showed that 12 microRNAs were down-regulated and 5 microRNAs up-regulated more than fivefold in SW620 cell compared to its parental cell line SW480 cell, respectively (Fig. 2a, Table 3). The relative microchip signal of miR-200c in SW480 was highest compared with other microRNAs. MiR-200c has already been shown to inhibit metastasis in some kinds of tumors [22–24]. It was selected for further study (The reasons are described in detail in "Discussion" section).

In accordance with microarray data, the result of real-time quantitative RT-PCR showed that the relative expression of miR-200c is downregulated more than three times in SW620 compared with SW480 cancer cell (Fig. 2b).

miR-200c represses ZEB1 expression posttranscriptionally

To investigate the relationship between miR-200c and ZEB1, we enhanced miR-200c in SW620 cancer cell and inhibited it in SW480 cancer cell. The transfection efficiency was determined by counting fluorescent cells and total cells from six random fields for each condition. The transfection efficiency was approximately 78 % in SW620 cancer cells (Fig. 3) and approximately 86 % in SW480 cancer cells (Fig. 4). RT-PCR results showed that miR-200c was markedly elevated in mimics transfected SW620 cancer cells, while the protein level and mRNA level of

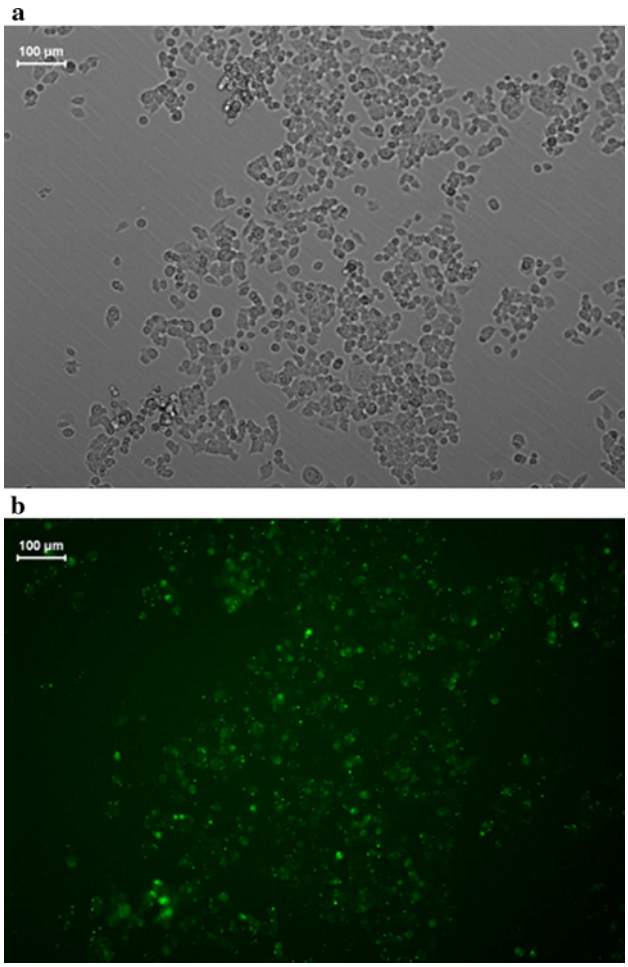

Fig. 3 **a** SW620 cancer cells transfected with microRNA-control-FAM under bright field (×200). **b** SW620 cancer cells transfected with microRNA-control-FAM under fluorescent field (×200)

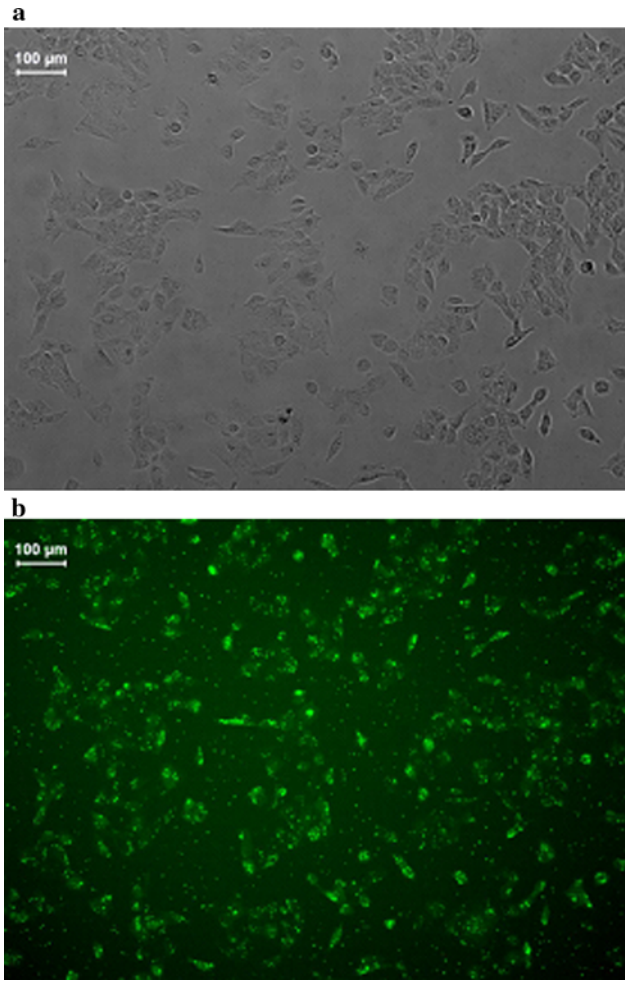

Fig. 4 **a** SW480 cancer cells transfected with microRNA-control-FAM under bright field (×200). **b** SW480 cancer cells transfected with microRNA-control-FAM under fluorescent field (×200)

ZEB1 was lowered (Fig. 5). In inhibitor transfected SW480 cancer cells, miR-200c was markedly lowered, while the protein level and mRNA level of ZEB1 was elevated (Fig. 6).

miR-200c inhibits migration and invasion in SW480 and SW620 cancer cell

To validate the involvement of miR-200c dysregulation in migration and invasion, functional analysis was performed to test the effects of miR-200c. In the migration assay, the migrating cells of mimics transfected SW620 cancer cells were significantly lower than the control group ($p = 0.0016$) and primary SW620 cancer cells ($p = 0.0027$) (Table 4; Figs. 7, 8). In the invasion assay, the invasing cells of mimics transfected SW620 cancer cells were significantly lower than the control group ($p = 0.00596$) (Table 5; Figs. 9, 10) and primary SW620 cancer cells ($p = 0.00056$).

We transfected SW480 cancer cells with miR-200c inhibitor. The migrating transfected SW480 cancer cells were significantly higher than the control group ($p = 0.00318$) and primary SW480 cancer cells ($p = 0.00053$) (Table 6; Figs. 11, 12). The invasing cells were significantly higher than the control group ($p = 0.045$) (Table 7; Figs. 13, 14) and primary SW620 cancer cells ($p = 0.047$).

Those results indicated that ZEB1 is a functional target of miR-200c in SW480 and SW620 colon cancer cells, and that over-expression of miR-200c down-regulates expression of ZEB1 posttranscriptionally and inhibits the potential of metastasis in SW620 cancer cells, while inhibition of miR-200c up-regulates ZEB1 posttranscriptionally and enhances the potential of metastasis in SW480 cancer cells.

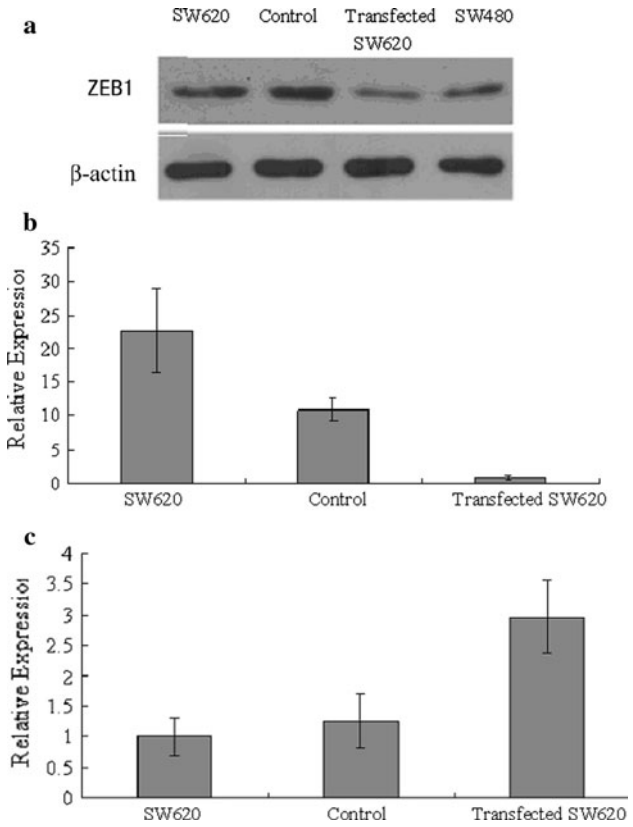

Fig. 5 **a** Protein level of ZEB1 was lower in SW480 cancer cells than in SW620 cancer cells; Protein level of ZEB1 was lowered in transfected SW620 cancer cells by Western blot. **b** mRNA level of ZEB1 was lowered in transfected SW620 cancer cells by RT-PCR. **c** miR-200c was elevated in transfected SW620 cancer cells

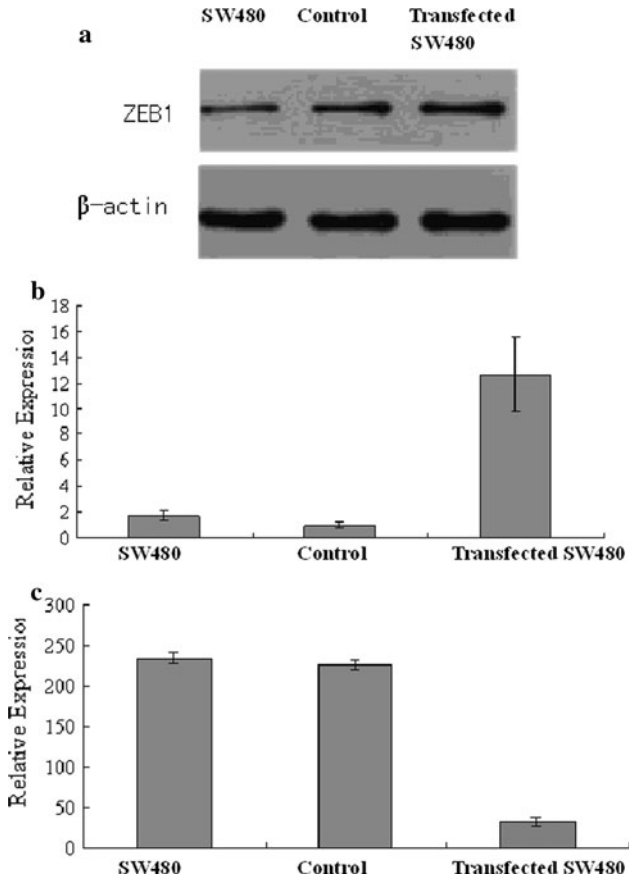

Fig. 6 **a** Protein level of ZEB1 was elevated in transfected SW480 cancer cells by Western blot. **b** mRNA level of ZEB1 was elevated in transfected SW480 cancer cells by RT-PCR. **c** miR-200c was lowered in transfected SW480 cancer cells

Discussion

Recently, some studies have described altered microRNAs expression which affects metastasis in breast cancer, colorectal cancer and glioma [17, 18, 25, 26]. Researches on microRNAs interfering metastasis are considered to offer novel therapeutical approaches for cancer. Our present data demonstrates that the involvement miR-200c in inhibiting metastasis in colon cancer cells might add to the evidences that microRNAs could serve as potential targets for suppressing tumor metastasis.

Firstly, a subset of microRNAs was found to be differentially expressed in SW480/SW620 cancer cells by genechip microarray. Among those 12 up-regulated microRNAs in SW480 cancer cells (lower potential of metastasis), miR-373 [19], miR-10b [18], miR-10a [27–29] were reported as promotors of tumor invasion and metastasis. High expression of miR-141 predicted advanced colorectal cancer and poor prognosis [30, 31]. So these four microRNAs were not selected. MiR-138 is overexpressed in thyroid cancer [32]. Downregulation of miR-138 leads to

Table 4 Migration cell numbers in SW620 different groups

Number of migration cells	1	2	3	4	5	6	7	8	Mean	SD	*p* value
SW620	398	422	378	425	378	185	258	196	330	100.63	0.0027*
Control	331	364	423	371	398	476	210	328	363	78.663	0.0016**
Mimics transfected SW620	115	162	141	161	135	98	250	149	151	45.49	

* The difference between miR-200c mimics transfected SW620 cancer cells and SW620 was significant (*t* test, *p* value 0.0027)

** The difference between miR-200c mimics transfected SW620 cancer cells and control group was significant (*t* test, *p* value 0.0016)

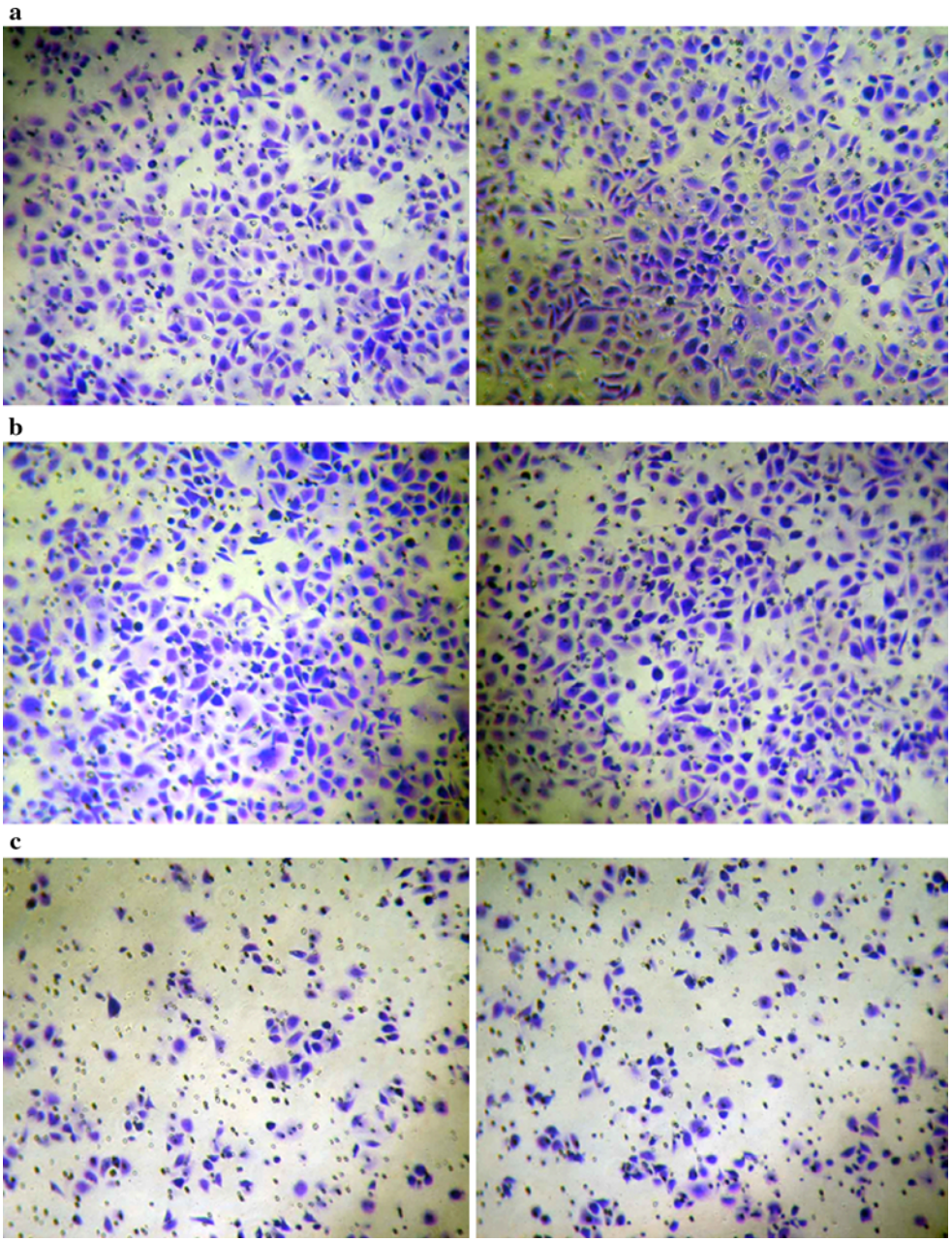

Fig. 7 **a** Migration of SW620 cancer cells, **b** control group, and **c** mimics transfected SW620 cancer cells

overexpression of human telomerase protein in anaplastic thyroid cancer cell lines [33]. MiR-936, miR-424_star, miR-526a, miR-1251, miR-935, and miR-509-3p were not reported in literature. The function of these seven microRNAs in metastasis is not clear.

Among those five up-regulated microRNAs in SW620, miR-194 has been shown to be a suppressor of metastasis in endometrial cancer, liver cancer and gastric cancer [34–36]. MiR-375 can inhibit tumor growth and metastasis in oesophageal squamous cell carcinoma by repressing insulin-like growth factor 1 receptor [37] and inhibit metastasis in squamous cervical cancer by targeting

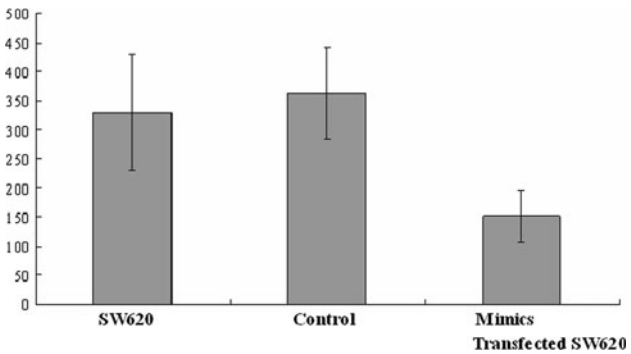

Fig. 8 Number of migration cells in SW620 different groups

Table 5 Invasion cell numbers in SW620 different groups

Number of invasion cells	1	2	3	4	5	6	7	8	Mean	SD	*p* value
SW620	241	246	172	154	189	263	110	198	197	51.82	0.00056*
Control	178	182	183	202	187	126	230	182	183	28.93	0.00596**
Mimics transfected SW62	110	115	125	115	113	156	67	65	108	29.88	

* The difference between miR-200c mimics transfected SW620 cancer cells and SW620 was significant (*t* test, *p* value 0.00056)

** The difference between miR-200c mimics transfected SW620 cancer cells and control group was significant (*t* test, *p* value 0.00596)

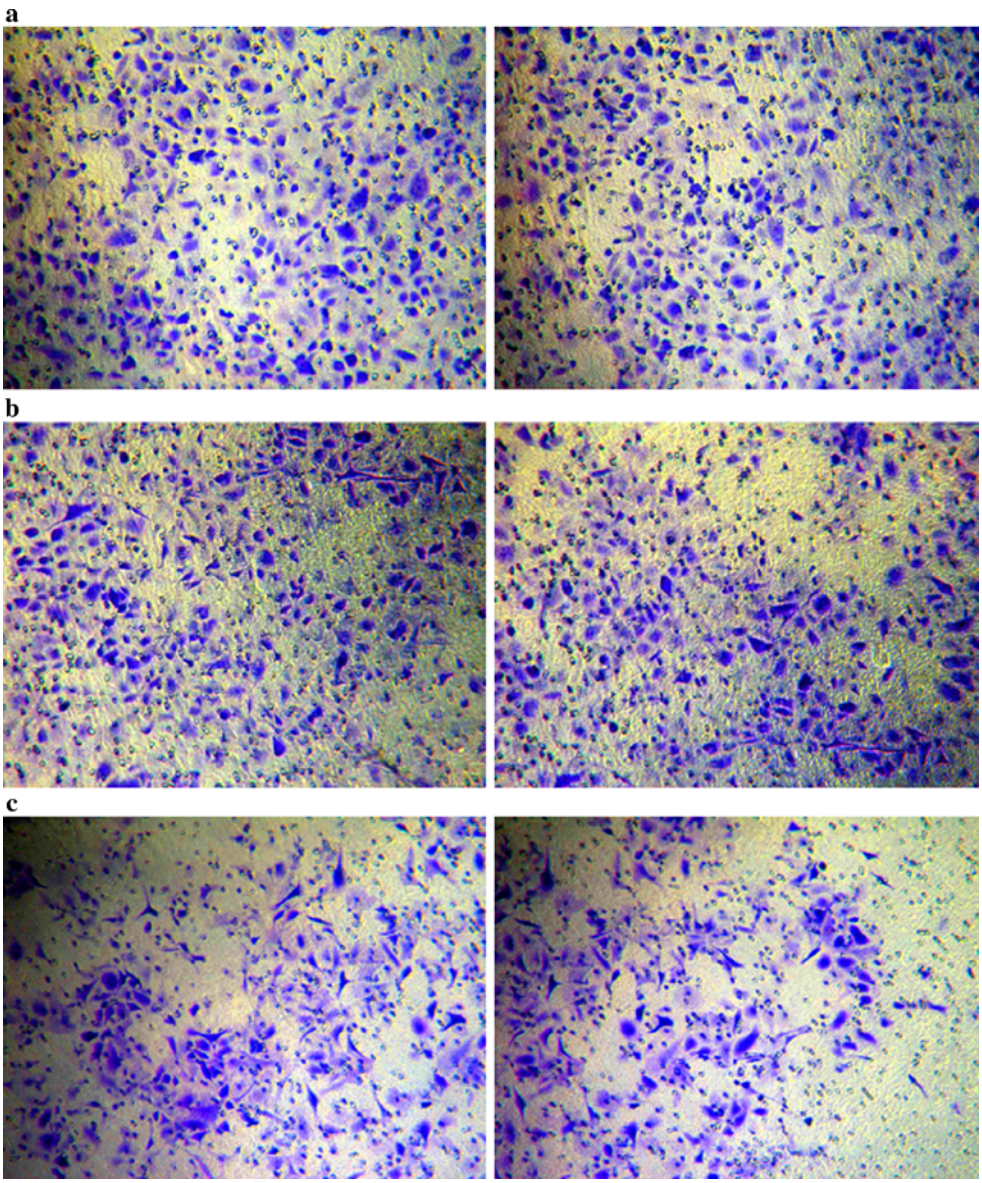

Fig. 9 **a** Invasion of SW620 cancer cells, **b** control group, and **c** mimics transfected SW620 cancer cells

transcription factor SP1 [38]. MiR-192 family members are up-regulated by p53 then they can inhibit metastasis by repressing epithelial mesenchymal transition (EMT) through ZEB2 [39]. Since SW620 cancer cell has higher potential of metastasis, they may not play roles in modulation of metastasis in these two paired colon cancer cell

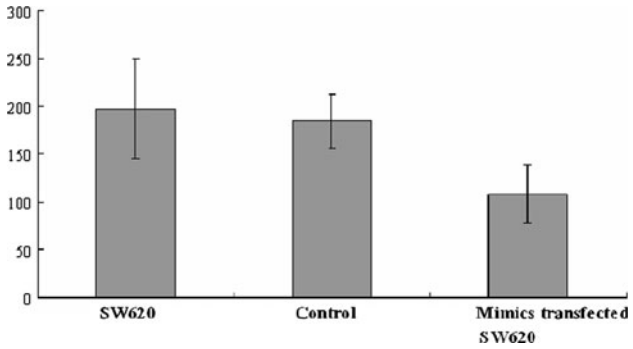

Fig. 10 Number of invasion cells in SW620 different groups

lines and were not included as research targets. MiR-181a and miR-181b act as tumor suppressor gene [40–42], but their effects on metastasis are not clear at present and further studies are needed.

Compared with other microRNAs, the relative microchip signal of miR-200c in SW480 was highest and the difference was 7.8 times (Fig. 2; Table 3). MiR-200c has been well characterized as metastasis association in many kinds of tumors. In breast cancer, melanoma cancer and pancreatic cancer, it suppresses tumor migration and invasion [22–24]. Downregulation of miR-200 is observed in relapsers of stage I ovarian epithelial cancer more than in non-relapers [43]. Ceppi reported that loss of miR-200c expression induces an aggressive, invasive and chemoresistant phenotype in non-small cell lung cancer [44]. So, we selected miR-200c as research target among these microRNAs. The quantitative RT-PCR results were in accordance with microarray results.

Secondly, we wanted to find the target gene of miR-200c. In TargetScan data base [21], miR-200c has two positions connecting with ZEB1 3′-UTR. It was reported that ZEB1 expression was reduced by miR-200 family members in lung cancer cell A549 [45]. MiR-200c was negatively correlated with ZEB1, thus inhibiting EMT process [46, 47]. EMT is a cellular programme converting polarized immotile epithelial cells into a more mesenchymal mobile phenotype [48, 49] and has been reported in breast, ovarian and esophageal cancer models [50–52]. In colorectal cancer, activation of EMT is crucial for invasion and migration of cancer cells [53, 54]. The typical apical-basal axis of epithelial is composed of adherence junctions, desmosomes, tight junctions and gap junctions with a luminal layer on their basal surface which can limit the cell migration and invasion. After stimulated by some intracellular factors, the epithelial-derived tumor cells have the characteristic of mesenchyme and detach from the junctions, adhere to the peripheral cells and migrate. They express proteases which can allow them to pass through basement membrane and migrate [55]. ZEB1 is a crucial inducer in EMT. It has been shown to promote tumor invasion and migration by E-cadherin gene silencing in cancer [56, 57]. Overexpression of miR-200c leads to translational inhibition of ZEB1 which induces EMT in cells [45, 46].

Then, we wondered if miR-200c played a role in the regulation of metastasis in these two colon cancer cells by targeting ZEB1. By western blotting, we observed the expression of ZEB1 in SW620 is higher than in SW480 cancer cells. Exogenous transfection of miR-200c mimics into SW620 cancer cell lead to lower expression of ZEB1 and lower mRNA, while miR-200c inhibitor transfection lead to higher expression of ZEB1 and higher mRNA in SW480 cancer cell. These data provide further evidence that miR-200c can regulate the expression of ZEB1 in mRNA level.

Thirdly, we performed functional analysis after regulating ZEB1 expression in SW620/480 cancer cell with miR-200c mimics/inhibitor. In invasion test, the mean number of mimics transfected SW620 cancer cell per field was 108 (SD 29.88), and the difference was significant compared with control group and primary SW620 cancer cell. The mean number of inhibitor transfected SW480 cancer cell per field was 23 (SD 9.14) and the difference was significant compared with control group and primary SW480 cancer cell. In migration test, the mean number of mimics transfected SW620 cancer cell per field was 151 (SD 45.49), and the difference was significant compared with control group and primary SW620 cancer cell. The mean number of inhibitor transfected SW480 cancer cell per field was 160 (SD 21.37) and the difference was significant compared with control group and primary SW480

Table 6 Migration cell numbers in SW480 different groups

Number of migration cells	1	2	3	4	5	6	7	8	Mean	SD	*p* value
SW480	90	93	72	100	89	165	124	91	103	28.94	0.00053*
Control	83	88	97	85	78	60	152	96	92	26.75	0.00318**
Inhibitor transfected SW480	159	186	145	142	155	196	135	165	160	21.37	

* The difference between miR-200c inhibitor transfected SW480 cancer cells and SW480 was significant (*t* test, *p* value 0.00053)

** The difference between miR-200c inhibitor transfected SW480 cancer cells and control group was significant (*t* test, *p* value 0.00318)

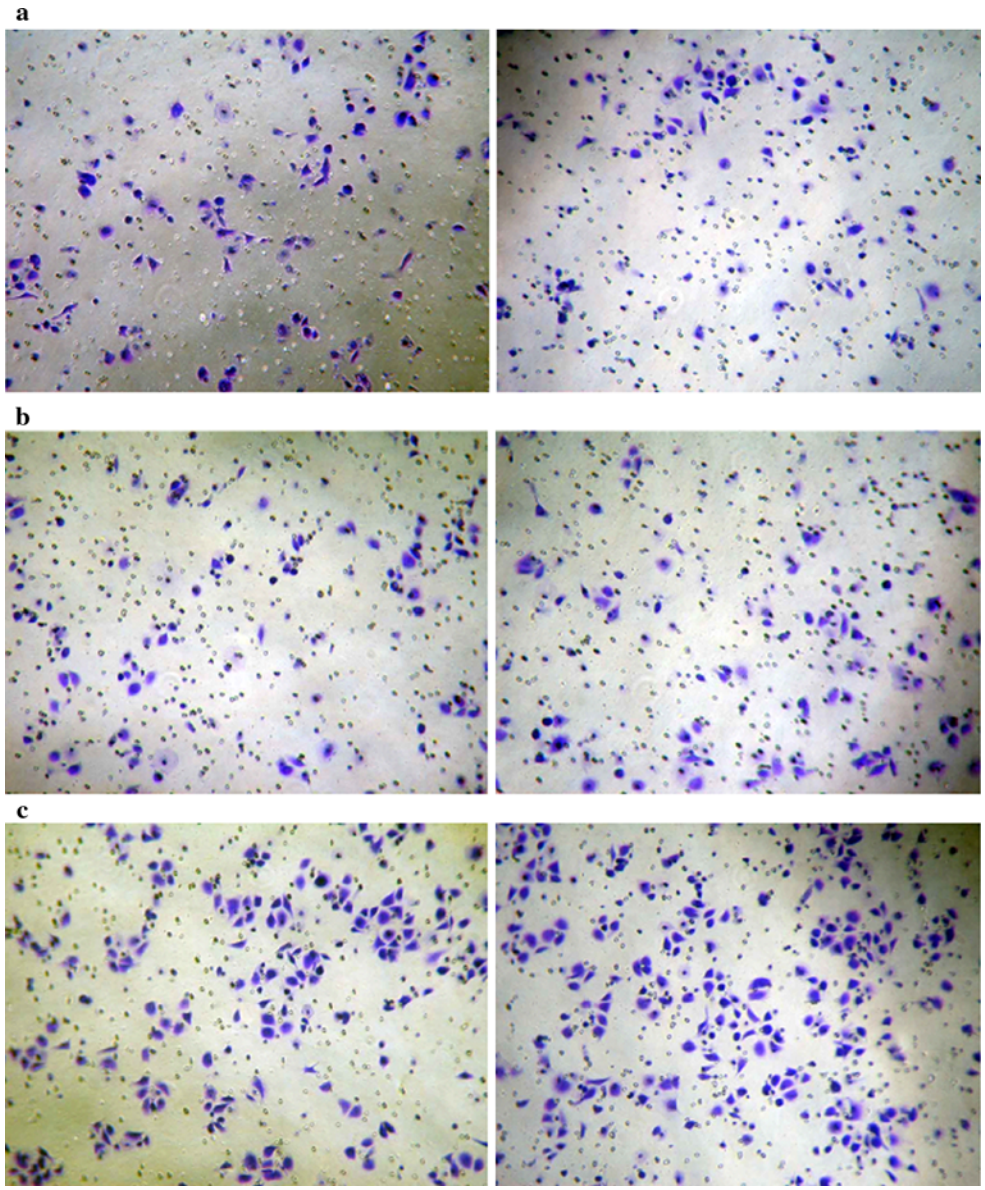

Fig. 11 **a** Migration of SW480 cancer cells, **b** control group, and **c** inhibitor transfected SW480 cancer cells

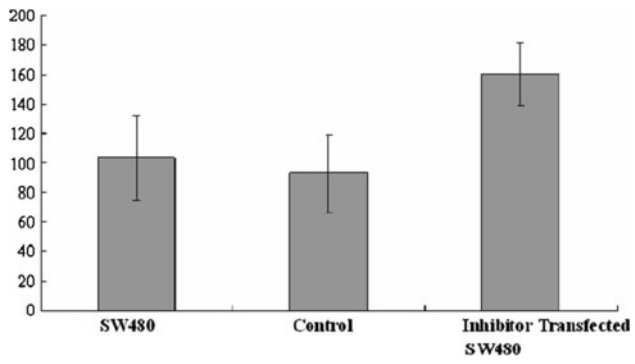

Fig. 12 Number of migration cells in SW480 different groups

cancer cell. These showed us that miR-200c in SW620/480 cancer cell is of functional significance since we demonstrated that modulation of its expression can alter the invasion and migration ability of cells in metastasis assays.

Taken together, our study demonstrated that miR-200c inhibits metastatic ability by targeting ZEB1 in colon cancer cells SW480/620 and suggested that modulation of miR-200c with inhibitors or mimics could serve as therapeutic tool for inhibiting metastasis in colorectal cancer. Our data provided a new insight into the development of miRNA-based cancer gene therapy for advanced colorectal cancer. Future research to assess the roles of miR-200c in clinical context is warranted.

Table 7 Invasion cell numbers in SW480 different groups

Number of invasion cells	1	2	3	4	5	6	7	8	Mean	SD	*p* value
SW480	16	11	12	13	15	5	7	20	12	4.838	0.047*
Control	25	20	16	16	12	7	3	11	14	7.046	0.045**
Inhibitor transfected SW480	27	32	12	23	25	36	18	10	23	9.14	

* The difference between miR-200c inhibitor transfected SW480 cancer cells and SW480 was significant (*t* test, *p* value 0.047)

** The difference between miR-200c inhibitor transfected SW480 cancer cells and control group was significant (*t* test, *p* value 0.045)

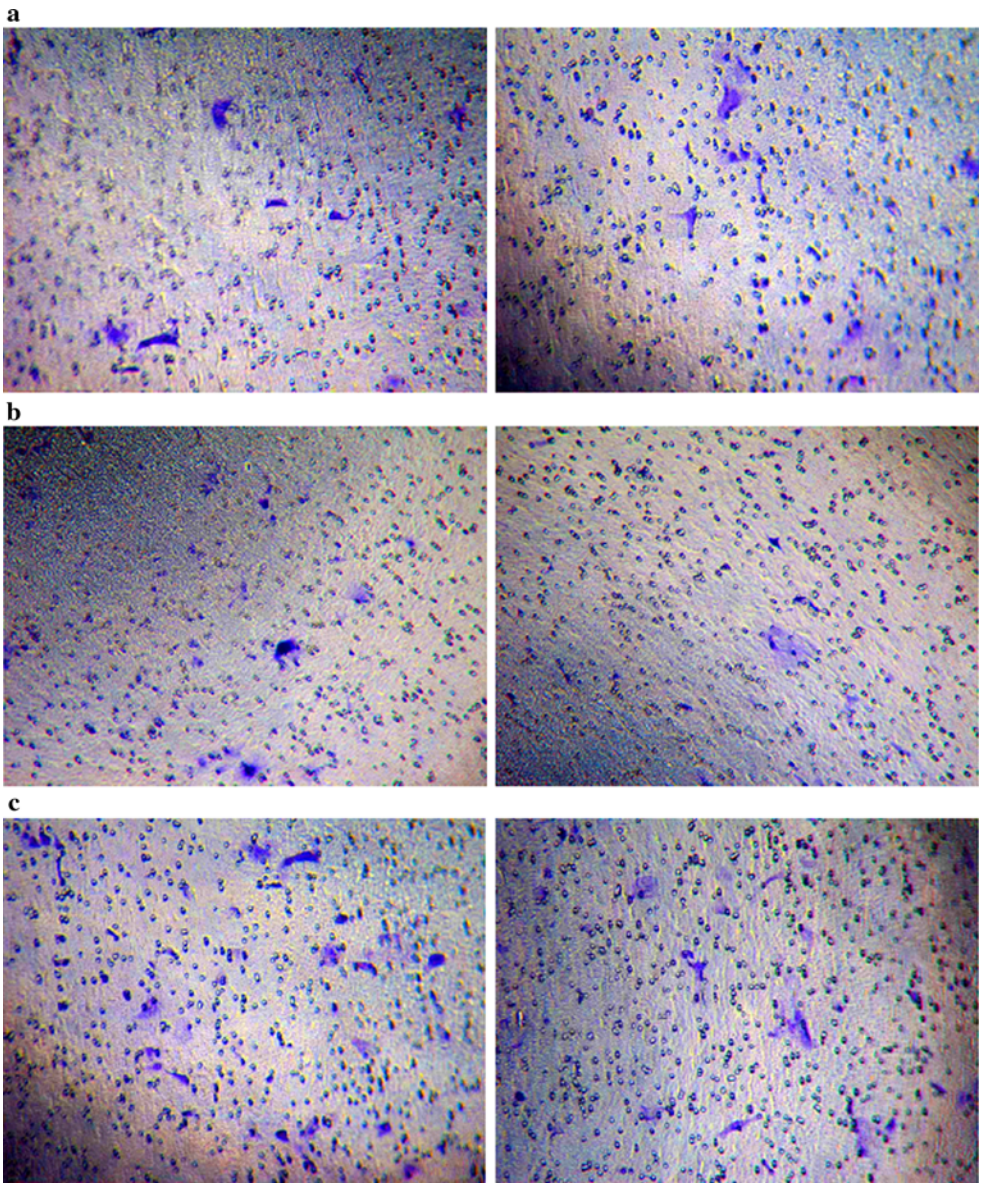

Fig. 13 **a** Invasion of SW480 cancer cells, **b** control group, and **c** inhibitor transfected SW480 cancer cells

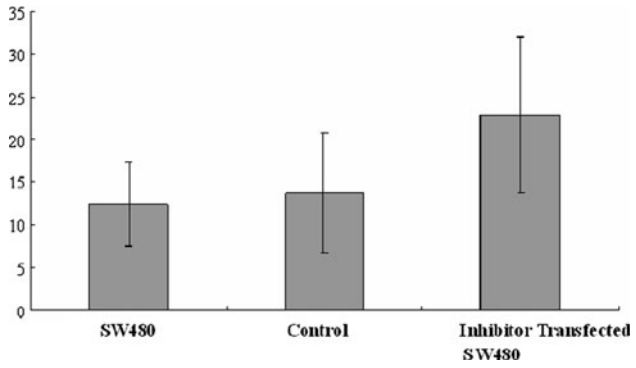

Fig. 14 Number of Invasion cells in SW480 different groups

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