microRNA-200c modulates the epithelial-to-mesenchymal transition in human renal cell carcinoma metastasis

XUEGANG WANG[1], XUANYU CHEN[1], RONG WANG[1], PEI XIAO[2], ZHENGHONG XU[2], LI CHEN[1], WEIWEI HANG[1], ANMING RUAN[1], HONGMEI YANG[2] and XIAOPING ZHANG[1]

[1]Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022; [2]Department of Pathogen Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China

Received February 11, 2013; Accepted March 11, 2013

DOI: 10.3892/or.2013.2530

Abstract. microRNAs (miRNAs) play essential roles in several physiological and pathological processes, including tumor metastasis. Metastasis is associated with poor prognosis in renal carcinoma patients and almost 20-30% of patients present with distant metastasis at the time of diagnosis. The aim of the present study was to investigate the possible roles of miR-200c in regulating metastasis and to identify its target genes in renal cell carcinoma (RCC). Among the miRNAs downregulated in our tissue specimen microarray, miR-200c was downregulated significantly. Functional assays demonstrated that restoration of miR-200c significantly inhibited the migration and invasion of SN12-PM6 and 786-0 cells *in vitro*. Genome-wide gene expression analysis and TargetScan database studies showed that ZEB1, which has been shown to promote tumor invasion and migration through E-cadherin gene silencing, is a promising candidate target gene of miR-200c. Overexpression of miR-200c in SN12-PM6 and 786-0 cells was concurrent with downregulation of ZEB1 and upregulation of E-cadherin mRNA and protein. In addition, miR-200c affected the protein expression of p-Akt and Akt. Thus, our study demonstrated that miR-200c decreases the metastatic ability of renal carcinoma cells by upregulating E-cadherin through ZEB1 and that modulating the expression of miR-200c could influence Akt protein levels. We therefore concluded that there is an Akt-miR-200c-E-cadherin axis in the epithelial-to-mesenchymal transition process in RCC.

Correspondence to: Professor Xiaoping Zhang, Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Road, Wuhan, Hubei 430022, P.R. China
E-mail: xiaoping.zhang2008@gmail.com

Professor Hongmei Yang, Department of Pathogen Biology, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, P.R. China
E-mail: macy.yang88@gmail.com

Key words: renal cell carcinoma, miR-200c, metastasis, epithelial-to-mesenchymal transition

Introduction

microRNAs (miRNAs) constitute a class of 20-22-nt noncoding single-strand RNA that are initially synthesized by RNA polymerase II as long primary transcripts, which are subsequently capped and polyadenylated. These precursors of miRNAs, pri-miRNAs, are cleaved by Drosha into pre-miRNAs and transported out of the nucleus by exportin-5. Pre-miRNAs are further processed by Dicer in the cytoplasm to yield mature miRNAs. Through base pairing to the 3' untranslated region (3'UTR) of mRNA, miRNAs negatively regulate gene expression post-transcriptionally to induce suppression of translation or degradation of multiple mRNAs. They are thus crucial players that participate in numerous key cellular processes such as cell growth, differentiation and death (1-3). In malignancy, functional characterization has revealed a role for miRNAs as oncogenes or tumor-suppressor genes (4). The contribution of miRNAs to the malignant progression of human tumors was recently investigated. Malignant tumor progression shares several characteristics with the process of epithelial-to-mesenchymal transition (EMT), which facilitates tissue remodeling during embryonic development and is considered an essential early step in tumor metastasis. In EMT, epithelial tumor cells are stimulated by extracellular cytokines to lose their epithelial polarity and gain mesenchymal phenotypes with increased migratory and invasive capabilities (5,6). One of the molecular hallmarks driving this transition is the functional loss of E-cadherin, which is a cell adhesion protein and a major constituent of adherens junctions, and is thought to be a suppressor of migration/invasion during carcinoma progression (7,8). Recent studies have indicated that the expression of the miRNA-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is markedly downregulated in cells that have undergone EMT. By serving as a powerful regulator of the EMT, miR-200c can maintain the epithelial phenotype of tissues by suppressing expression of the E-cadherin transcription factor ZEB1 in some types of cancer (9-13).

Renal cell carcinoma (RCC) is the third most common urological cancer after prostate and bladder cancer, and it accounts for approximately 3% of adult malignancies and 90-95% of neoplasms arising from the kidney (14,15). Previous studies have shown that almost 20-30% of patients

have distant metastasis when diagnosed and that metastasis is associated with poor prognosis. The mechanisms underlying metastasis are multifaceted and intricate. It is therefore important to explore metastatic mechanisms in RCC as these may provide new targets for treating metastasis. In this study, we investigated the possible metastatic mechanisms of action of miR-200c in 2 RCC cell lines: the 786-0 cancer cell line, derived from primary human RCC in a 58-year-old male patient, and the SN12-PM6 cancer cell line, obtained from lung metastases produced in nude mice (16,17).

Materials and methods

Clinical specimens. All primary RCC tissues and normal renal tissues were surgically resected at Wuhan Union Hospital. Tissue samples were immediately frozen in liquid nitrogen and then stored in a deep freezer at -80˚C. Histopathological diagnoses were performed according to the World Health Organization (WHO) classification system. Informed consent was obtained from each patient, and the use of tissue samples for all experiments was approved by the Clinical Research Ethics Committee of Wuhan Union Hospital.

miRNA microarray and analysis. A miRNA microarray platform was used to determine the expression profiles of miRNA in 5 paired RCCs and normal renal tissues. The array used was the commercially available G4471A Human miRNA Microarray (Agilent Technologies, Santa Clara, CA, USA), which consists of 961 probes for 851 human miRNAs, and is based on Sanger miRBase release 12.0. The arrays were washed and scanned using a laser confocal scanner (G2565BA; Agilent Technologies) according to the manufacturer's instructions. The fluorescence intensity was calculated using the Feature Extraction software (Agilent Technologies). Differentially expressed miRNAs were identified using a filter based on a 2-fold change in expression, combined with ANOVA analysis ($P<0.01$).

Lentivirus production and transduction. We used the lentiviral vector pGCSIL-GFP (GeneChem Co., Ltd., Shanghai, China) to construct the hsa-miR-200c lentiviral expression vector pGCSIL-GFP-hsa-miR-200c as well as the lentiviral vector pGCSIL-GFP-negative as a negative control. For 6-well transduction in SN12-PM6 and 786-0 cells, cells were plated at a density of 150,000/well in a 6-well dish. When the cells were 50% confluent, they were transduced with recombinant lentivirus vectors at a multiplicity of infection (MOI) of 30 and 10, respectively, and supplemented with 5 μg/ml polybrene from GeneChem Co., Ltd. The cells were then collected for western blot analysis, quantitative RT-PCR (qRT-PCR), and migration and invasion assays. The SN12-PM6 cell line stably expressing miR-200c was termed SN12-PM6 miR-200c; the negative control cell line was termed SN12-PM6 miR-Ctr. We also generated 786-0 miR-200c and 786-0 miR-Ctr cells.

Cell culture and transfection. All cell lines were maintained in DMEM medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco-BRL, Grand Island, NY, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2. Lipofectamine™ 2000 (Invitrogen) was used to transfect SN12-PM6 miR-200c and 786-0 miR-200c with the antagomiR-200c (RiboBio Co., Ltd., Guangzhou, China) according to the manufacturer's instructions.

Transwell migration and invasion assays. For transwell migration assays, 20,000 cells were plated in the top chamber onto a non-coated membrane (24-well insert; pore size, 8 μm; Corning-Costar, Corning, NY, USA). For invasion assays, 20,000 cells were plated in the top chamber onto a Matrigel-coated membrane. Each well was freshly coated with 60 μg of Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) prior to the invasion assay. In both assays, cells were plated in medium without serum or growth factors, and medium supplemented with serum was used as a chemoattractant in the lower chamber. The cells were incubated for 24 h in the migration and the invasion assay, and cells that did not migrate or invade through the pores were removed with a cotton swab. Cells on the lower surface of the membrane were fixed with methanol and stained with crystal violet. The cells migrating or invading through the membrane were counted with a Nikon Eclipse TE2000-S (Nikon, Japan) at x100 magnification in 4 random fields/well.

Quantitative RT-PCR analysis. Total RNA was extracted using TRIzol (Invitrogen), and 1-μg RNA samples were reverse transcribed to cDNA by using reverse transcriptase M-MLV (Invitrogen). qRT-PCR was performed using the SYBR-Green PCR master mix (Invitrogen) on a Roche LightCycler 480 System (Roche Diagnostics, Germany). For analysis of miRNA expression by qRT-PCR, reverse transcription and PCR were conducted using a Bulge-Loop™ miRNA qPCR Primer Set for hsa-miR-200c and U6 snRNA (RiboBio Co., Ltd.) according to the manufacturer's instructions. Other primers used in qRT-PCR are described in Table I. GAPDH and U6 snRNA were used as endogenous controls.

Western blot analysis. Whole-cell lysates were prepared using RIPA buffer (Beyotime, Shanghai, China) containing a cocktail of protease inhibitors and phosphatase inhibitors (Roche Applied Science, Indianapolis, IN, USA). Equal amounts of protein sample (40-60 μg) were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore, Bedford, MA, USA) using the Invitrogen semidry transfer system and then incubated with the specific primary antibody overnight at 4˚C. The membranes were then washed and subsequently incubated with a secondary antibody conjugated to horseradish peroxidase (HRP). The protein detected was then visualized using enhanced chemiluminescence. The following antibodies were used for western blotting: anti-β-actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-E-cadherin and anti-ZEB1 (both from Bioworld, Minneapolis, MN, USA), anti-Akt and anti-p-Akt (both from Cell Signaling Technology, Beverly, MA, USA). The blotted proteins were detected and quantified using ChemiDoc-XRS+ (Bio-Rad, USA).

Statistical analysis. Data from different experiments are presented in terms of mean ± standard deviation (SD) values. Differences among different groups with respect to the

Table I. Primers used for quantitative RT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ZEB1	TGCTCCCTGTGCAGTTACACCTT	CCAGACTGCGTCACATGTCTTTGA
E-cadherin	GTCCTGGGCAGAGTGAATTT	CATCTGTGCCCACTTTGAAT
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG

number of cancer cells in the invasion and migration assays were compared using the paired-samples t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Downregulated miRNAs in RCC and normal renal tissues. A comprehensive miRNA expression profile was obtained for the 5 paired RCC and normal renal tissues. Fig. 1A shows miRNAs that were downregulated in tumors relative to normal tissues: 12 miRNAs were downregulated >4-fold in tumor tissues, as listed in Table II. miR-200c was one of the most strongly downregulated miRNAs in tumor tissues, which was expected, as it had already been shown to inhibit metastasis in some types of tumors. We further examined the expression of miR-200c in 20 more pairs of RCC and normal human specimens to validate the microarray data and found that the data for 19 of 20 pairs were consistent with the microarray data in that miR-200c expression was significantly lower in RCCs than in normal kidney tissues (Fig. 1B). Therefore, miR-200c was selected for further study.

Establishment of stably transduced RCC cell lines. To investigate the effect of miR-200c in tumor cells, SN12-PM6 and 786-0 cells were transduced with pGCSIL-GFP-hsa-miR-200c to establish cell lines stably expressing miR-200c. The transduction efficiency was determined by counting fluorescent cells and total cells from 6 random fields for each condition. The transduction efficiency was ~88% in SN12-PM6 cells and ~96% in 786-0 cells (Fig. 2A). qRT-PCR indicated that compared to the control, SN12-PM6 miR-200c cells and 786-0 miR-200c cells had 26.4-fold and 18.1-fold higher miR-200c expression, respectively (Fig. 2B).

miR-200c inhibits migration and invasion in SN12-PM6 and 786-0 cancer cells. 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 test, the mean number of SN12-PM6 cells expressing miR-200c per field was 30, which was significantly different from that for the control group. The mean number of 786-0 cells expressing miR-200c per field was 21, which was also significantly different from the control group. In the invasion test, the mean number of SN12-PM6 and 786-0 cells expressing miR-200c per field was 45 and 20, respectively, which was significantly different from the number for the control group (Fig. 3A). To further explore the role of miR-200c and to determine whether inhibition of miR-200c could reverse the inhibition of cellular migration and invasion produced by overexpression, loss-of-function analyses

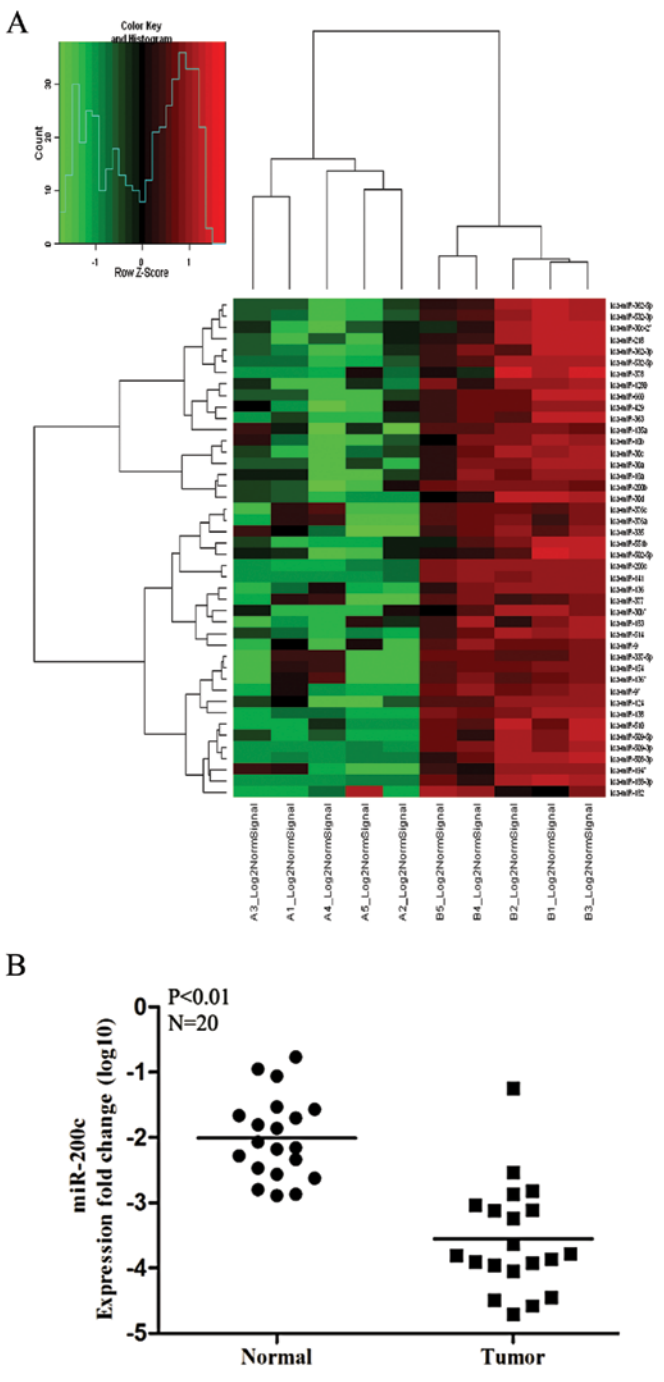

Figure 1. Downregulation profile of miRNAs in 5 pairs of RCC and normal renal tissues, and miR-200c downregulated in human RCC specimens. (A) The tree was generated by hierarchical cluster analysis of 851 miRNAs expressed in 10 cases. Rows, miRNAs; columns, cases. The significantly downregulated miRNAs are listed in Table II. (P<0.01, fold-change>4) (B) miR-200c was confirmed in 20 pairs of RCC and normal human specimens by qRT-PCR.

Table II. Downregulated miRNAs in tumors.

miRNA	P-value	Fold-change
hsa-miR-141	4.11E-08	103.990700
hsa-miR-200c	4.35E-06	99.787830
hsa-miR-138	1.14E-06	22.829310
hsa-miR-514	5.95E-04	18.366480
hsa-miR-509-3p	3.74E-04	8.679166
hsa-miR-9*	9.41E-04	8.520466
hsa-miR-508-3p	0.003473	7.809710
hsa-miR-124	0.004609	7.624603
hsa-miR-510	8.37E-05	6.083151
hsa-miR-218	0.001076	5.091851
hsa-miR-509-5p	0.001346	4.811123
hsa-miR-363	6.94E-04	4.533517

were performed. We transfected cells stably expressing miR-200c with antagomiR-200c, which resulted in significant reduction of miR-200c levels in SN12-PM6 miR-200c and 786-0 miR-200c cells. In the migration and invasion tests, the mean number of inhibitor-transfected SN12-PM6 cells expressing miR-200c per field was 188 and 176, and the mean number of inhibitor-transfected 786-0 cells expressing miR-200c per field was 178 and 156, respectively, which was significantly different from the control group (Fig. 3B). These findings indicate that the migration and invasion ability of SN12-PM6 and 786-0 cells can be negatively modulated by miR-200c.

Expression of ZEB1 is negatively regulated by miR-200c in RCC cell lines. The E-cadherin transcriptional repressor ZEB1 has previously been implicated in EMT and tumor metastasis. EMT occurs during tumor progression and confers invasive and metastatic properties to cancer cells. ZEB1 has been proposed as a putative target of miR-200c on the basis of *in silico* miRNA target prediction programs (TargetScan, PicTar, miRanda) and studies in other types of cancer (18,19). Our results also showed that miR-200c levels were markedly elevated in SN12 miR-200c and 786-0 miR-200c cells, whereas the mRNA and protein level of ZEB1 decreased. Conversely, inhibition of miR-200c expression resulted in upregulation of ZEB1. These observations indicate that ZEB1 is negatively regulated by miR-200c at the posttranscriptional level. To further examine whether miR-200c overexpression-induced inhibition of EMT-related genes actually inhibits the EMT phenotype, we evaluated the expression of E-cadherin. Overexpression of miR-200c significantly increased E-cadherin mRNA and protein expression, whereas anti-miR-200c produced the opposite result (Fig. 4A and B).

Next, we analyzed whether miR-200c, ZEB1 and E-cadherin were correlated in RCC cells and human cancer tissues. We measured the expression of miR-200c, ZEB1, and E-cadherin in SN12-PM6 cells, 786-0 cells, and 20 pairs of human RCC tissues by qRT-PCR. The expression of miR-200c was inversely correlated with that of ZEB1 but positively correlated with that of E-cadherin (Fig. 4C).

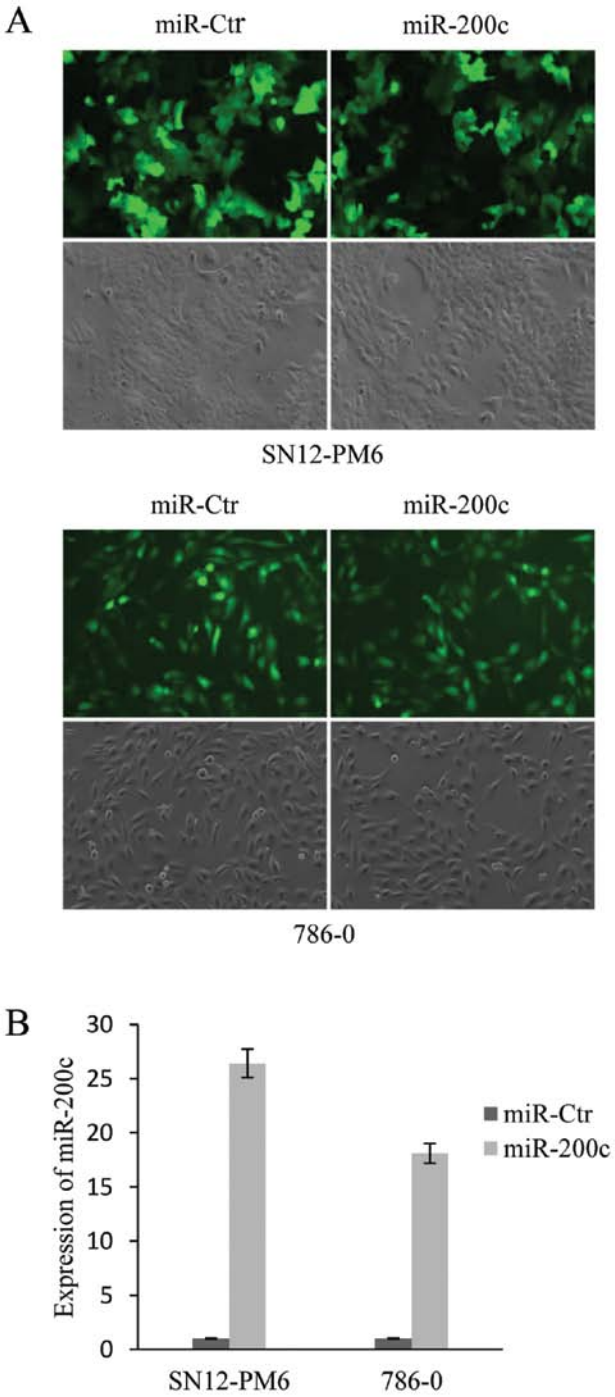

Figure 2. SN12-PM6 and 786-0 cells were transduced with a lentivirus expressing miR-200c or miR-Ctr. (A) Phase contrast and GFP expression under a fluorescent microscope (x200). (B) Expression levels of miR-200c after lentivirus transduction.

miR-200c is involved in the regulation of p-Akt and Akt protein expression. Akt is involved in a number of basic cellular processes, including EMT. Akt strongly represses transcription of the E-cadherin gene and internalization/sequestration of E-cadherin in perinuclear organelles to induce EMT (20,21). We found that overexpression of miR-200c in SN12-PM6 and 786-0 cells was able to inhibit the protein expression of p-Akt and Akt and that blockade of miR-200c could reverse this result (Fig. 5A).

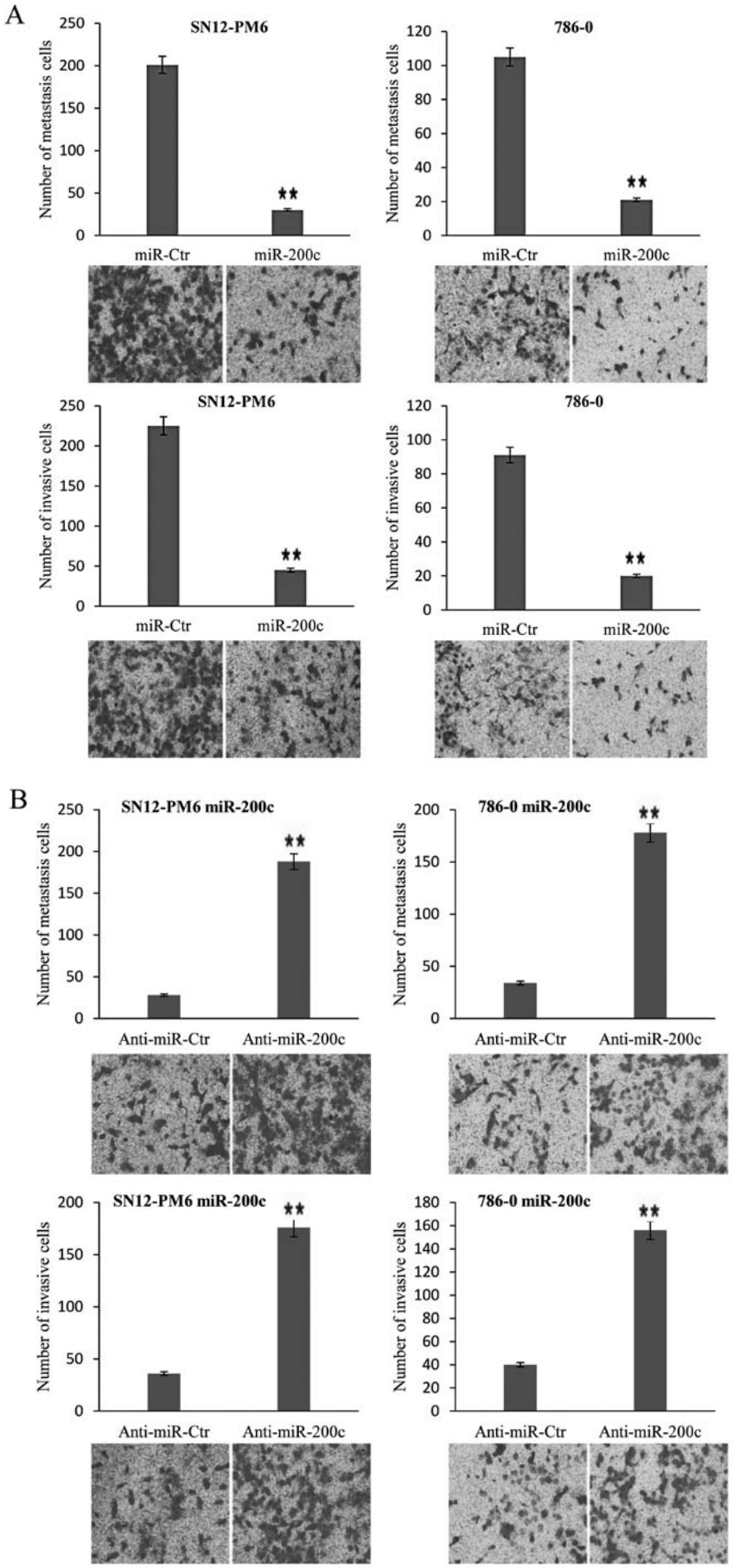

Figure 3. Enforced expression of miR-200c inhibits metastasis and invasion in RCC cells. (A) Migration and invasion assay of SN12-PM6 and 786-0 cells with stable miR-200c expressing lentivirus. (B) Migration assay and invasion assay of SN12-PM6 miR-200c and 786-0 miR-200c cells transfected with antagomiR-200c and control. (x100 in four different fields per filter; $^{**}P<0.01$ compared with the negative control).

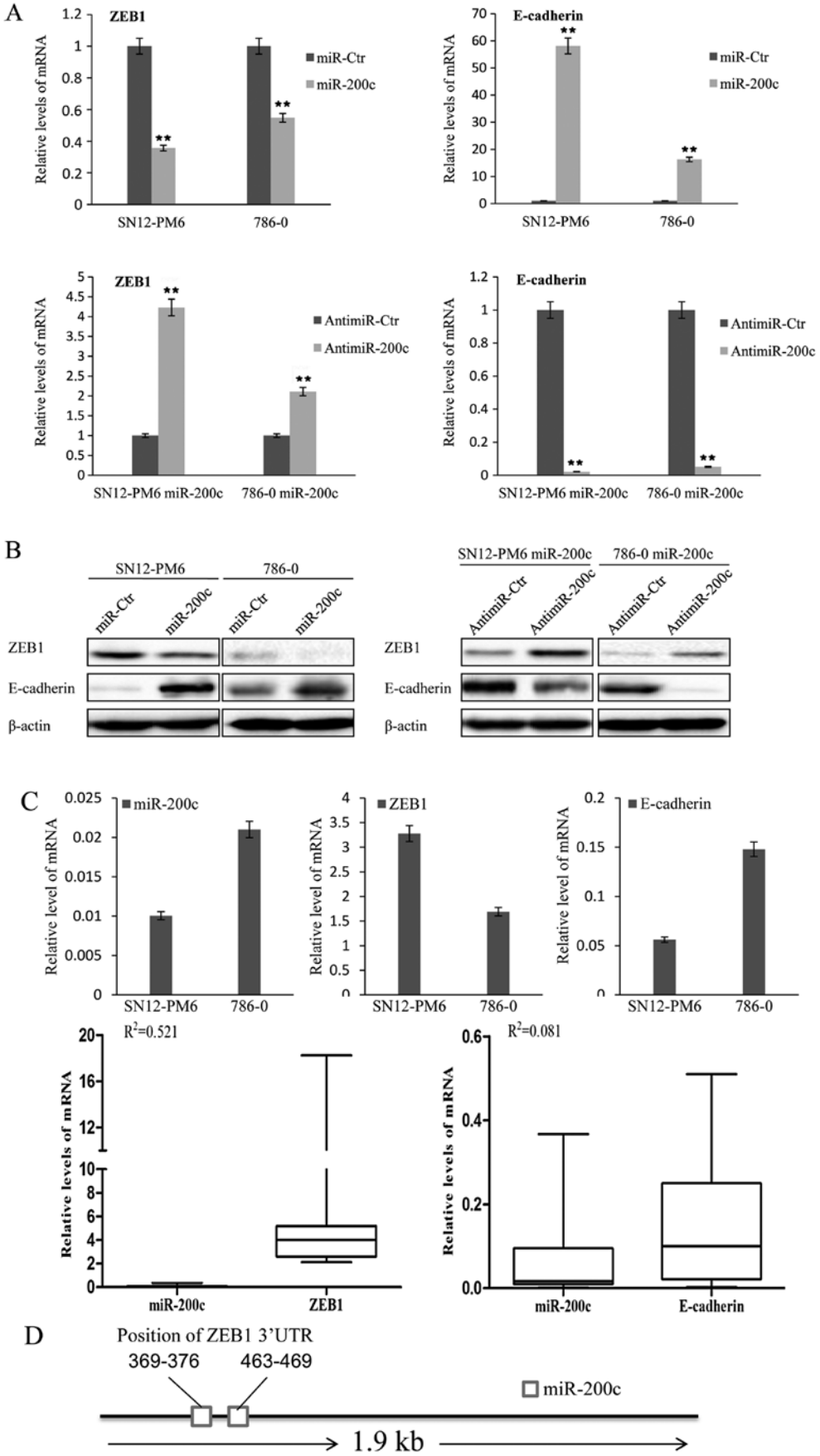

Figure 4. miR-200c targets transcriptional repressor ZEB1 to enhance E-cadherin expression. (A) mRNA expression of ZEB1 and E-cadherin following overexpression of miR-200c. ZEB1 and E-cadherin mRNA expression after 48 h transfected antagomiR-200c. (B) Protein expression of ZEB1 and E-cadherin after miR-200c overexpression and antimiR-200c. (C) The relative expression of miR-200c, ZEB1 and E-cadherin in SN12-PM6 and 786-0 cells. miR-200c level was inversely correlated with ZEB1 expression but positively correlated with E-cadherin in human RCC specimens. (D) miR-200c has two positions binding with ZEB1 3'UTR. (**P<0.01 compared with the negative control).

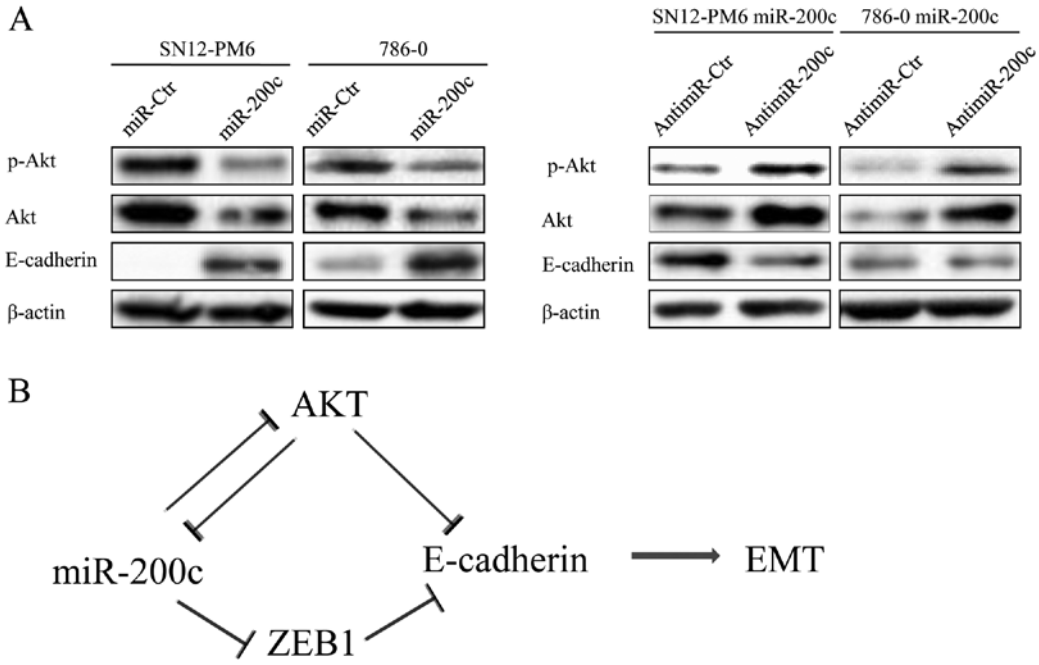

Figure 5. miR-200c decreases the protein expression of p-Akt and Akt in RCC cells. (A) Effect of miR-200c on protein expression of p-Akt and Akt in SN12-PM6 and 786-0 cells. (B) An assumed diagram of Akt-miR-200c-E-cadherin axis for EMT in RCC cells.

Discussion

Previous studies have linked the miR-200 family with the epithelial phenotype and the ZEB family. miR-200c is a member of the miR-200 family; this family has been associated with metastasis in several types of tumors, particularly through negative correlation with ZEB1, which inhibits EMT (9,22,23). In breast cancer, melanoma, and pancreatic cancer, miR-200c suppresses tumor migration and invasion (24-26). Nakada *et al* (27) reported that overexpression of miR-141 and miR-200c caused downregulation of ZFHX1B and upregulation of E-cadherin in 2 renal carcinoma cell lines. However, the functional involvement of miR-200c in EMT and tumor migration, as well as direct targeting of ZEB1 by miR-200c, has not been investigated in renal cell carcinoma (RCC) studies. In our tissue microarray, miR-200c in tumor tissue was downregulated significantly relative to other miRNAs. Therefore, we selected miR-200c as our research target.

First, we performed functional analysis in SN12-PM6 and 786-0 cells stably overexpressing miR-200c. EMT is an important process in which epithelial cells acquire mesenchymal fibroblast-like properties and show reduced intercellular adhesion and increased motility during development. Accumulating evidence points to a critical role of EMT during tumor progression and malignant transformation, as it endows cancer cells with invasive and metastatic properties (8,28,29). In our migration and invasion test, enforced expression of miR-200c in SN12-PM6 and 786-0 cells inhibited cell mobility and invasion activity. Blockade of expression of miR-200c in SN12-PM6 miR-200c and 786-0 miR-200c cells increased their invasion and migration abilities. We demonstrated that modulation of miR-200c expression can alter the invasion and migration abilities of SN12-PM6 and 786-0 cells in metastasis assays, indicating that miR-200c is of functional significance in RCC cells.

We then identified miR-200c as a suppressor of EMT through direct targeting of ZEB1, which is a well-known transcriptional repressor of E-cadherin. miRNA target prediction programs and previous studies have indicated that ZEB1 has 2 target sites for miR-200c (Fig. 4D), as confirmed by luciferase reporter assays (8,19,30). ZEB1 is a crucial inducer of EMT, which has been shown to promote tumor invasion and migration through E-cadherin gene silencing (9,12). In our present study, we analyzed whether miR-200c played a role in the regulation of metastasis in these 2 renal carcinoma cells by targeting ZEB1. Using quantitative RT-PCR (qRT-PCR), we observed that stable overexpression of miR-200c by an hsa-miR-200c lentivirus in SN12-PM6 and 786-0 cells led to decreased expression of ZEB1 and increased expression of E-cadherin, whereas blockade of miR-200c in SN12-PM6 miR-200c and 786-0 miR-200c cells led to restoration of ZEB1 expression and decreased E-cadherin expression. These results were observed at the protein level as well as the mRNA level. We also found that ZEB1 expression was inversely correlated with miR-200c expression in RCC cells and human RCC specimens. These data demonstrate that increased expression of miR-200c results in negative regulation of its gene target ZEB1, which regulates E-cadherin expression to trigger EMT in RCC cells.

Collectively, these findings suggest a potential tumor suppressor role for miR-200c, which is downregulated in human RCC, thereby leading to EMT and tumor cell invasion and metastasis. Loss of miR-200c is associated with an aggressive cancer cell phenotype. According to previous research and our data, Akt is frequently activated in human epithelial cancer and the PI3K/Akt pathway plays a pivotal role in RCC pathogenesis (31,32). Additionally, Akt has been found to regulate E-cadherin mRNA and protein and to induce EMT (20), and miR-200c regulates the expression of E-cadherin by targeting ZEB1 in some cancer cell lines, including RCC cell lines (12).

Finally, miR-200c is downregulated in human RCC specimens and regulates the protein level of Akt in RCC cell lines. We therefore consider that RCC metastasis may be under the control of the Akt-miR-200c-E-cadherin axis. A summary diagram that outlines this hypothesis is shown in Fig. 5B. Our future work will focus on the identification and functional characterization of additional direct miR-200c downstream targets and metastasis promoter proteins, as well as the specific mechanism underlying the regulation of miR-200c by Akt. This will improve our understanding of the events contributing to EMT and metastasis, leading to the development of novel therapeutic strategies for RCC.

Acknowledgements

This research was supported by National Natural Science Foundation of China (grant no. 30572139, 30872924 and 81072095), Program for New Century Excellent Talents in University from Department of Education of China (NCET-08-0223) and the National High Technology Research and Development Program of China (863 Program) (2012AA021101) to X.Z, and supported by National Natural Science Foundation of China (grant no. 2010MS011 and 31070142) to H.Y.

References

1. Bagga S, Bracht J, Hunter S, *et al*: Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122: 553-563, 2005.
2. Lim LP, Lau NC, Garrett-Engele P, *et al*: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433: 769-773, 2005.
3. Ambros V: microRNAs: tiny regulators with great potential. Cell 107: 823-826, 2001.
4. Zhang B, Pan X, Cobb GP and Anderson TA: microRNAs as oncogenes and tumor suppressors. Dev Biol 302: 1-12, 2007.
5. Bracken CP, Gregory PA, Khew-Goodall Y and Goodall GJ: The role of microRNAs in metastasis and epithelial-mesenchymal transition. Cell Mol Life Sci 66: 1682-1699, 2009.
6. Yang J and Weinberg RA: Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14: 818-829, 2008.
7. Bracken CP, Gregory PA, Kolesnikoff N, *et al*: A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res 68: 7846-7854, 2008.
8. Hur K, Toiyama Y, Takahashi M, *et al*: MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut: Jul 10, 2012 (Epub ahead of print).
9. Gregory PA, Bert AG, Paterson EL, *et al*: The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 10: 593-601, 2008.
10. Park SM, Gaur AB, Lengyel E and Peter ME: The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 22: 894-907, 2008.
11. Christoffersen NR, Silahtaroglu A, Orom UA, Kauppinen S and Lund AH: miR-200b mediates post-transcriptional repression of ZFHX1B. RNA 13: 1172-1178, 2007.
12. Hurteau GJ, Carlson JA, Spivack SD and Brock GJ: Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. Cancer Res 67: 7972-7976, 2007.
13. Mongroo PS and Rustgi AK: The role of the miR-200 family in epithelial-mesenchymal transition. Cancer Biol Ther 10: 219-222, 2010.
14. Gottardo F, Liu CG, Ferracin M, *et al*: Micro-RNA profiling in kidney and bladder cancers. Urol Oncol 25: 387-392, 2007.
15. White NM and Yousef GM: MicroRNAs: exploring a new dimension in the pathogenesis of kidney cancer. BMC Med 8: 65, 2010.
16. Saiki I, Naito S, Yoneda J, Azuma I, Price JE and Fidler IJ: Characterization of the invasive and metastatic phenotype in human renal cell carcinoma. Clin Exp Metastasis 9: 551-566, 1991.
17. Williams RD, Elliott AY, Stein N and Fraley EE: In vitro cultivation of human renal cell cancer. II. Characterization of cell lines. In Vitro 14: 779-786, 1978.
18. Chen ML, Liang LS and Wang XK: miR-200c inhibits invasion and migration in human colon cancer cells SW480/620 by targeting ZEB1. Clin Exp Metastasis 29: 457-469, 2012.
19. Burk U, Schubert J, Wellner U, *et al*: A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep 9: 582-589, 2008.
20. Grille SJ, Bellacosa A, Upson J, *et al*: The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res 63: 2172-2178, 2003.
21. Larue L and Bellacosa A: Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene 24: 7443-7454, 2005.
22. Korpal M, Lee ES, Hu G and Kang Y: The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 283: 14910-14914, 2008.
23. Hurteau GJ, Carlson JA, Roos E and Brock GJ: Stable expression of miR-200c alone is sufficient to regulate TCF8 (ZEB1) and restore E-cadherin expression. Cell Cycle 8: 2064-2069, 2009.
24. Ahmad A, Aboukameel A, Kong D, *et al*: Phosphoglucose isomerase/autocrine motility factor mediates epithelial-mesenchymal transition regulated by miR-200 in breast cancer cells. Cancer Res 71: 3400-3409, 2011.
25. Elson-Schwab I, Lorentzen A and Marshall CJ: MicroRNA-200 family members differentially regulate morphological plasticity and mode of melanoma cell invasion. PLoS One 5: e13176, 2010.
26. Yu J, Ohuchida K, Mizumoto K, *et al*: MicroRNA, hsa-miR-200c, is an independent prognostic factor in pancreatic cancer and its upregulation inhibits pancreatic cancer invasion but increases cell proliferation. Mol Cancer 9: 169, 2010.
27. Nakada C, Matsuura K, Tsukamoto Y, *et al*: Genome-wide microRNA expression profiling in renal cell carcinoma: significant down-regulation of miR-141 and miR-200c. J Pathol 216: 418-427, 2008.
28. Kang Y and Massague J: Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 118: 277-279, 2004.
29. Thiery JP and Morgan M: Breast cancer progression with a Twist. Nat Med 10: 777-778, 2004.
30. TargetScanHuman release 6.2. Available at: http://www.targetscan.org. Accessed Sep 1, 2012.
31. Porta C and Figlin RA: Phosphatidylinositol-3-kinase/Akt signaling pathway and kidney cancer, and the therapeutic potential of phosphatidylinositol-3-kinase/Akt inhibitors. J Urol 182: 2569-2577, 2009.
32. Testa JR and Bellacosa A: AKT plays a central role in tumorigenesis. Proc Natl Acad Sci USA 98: 10983-10985, 2001.