Hybrid-polymerase chain reaction to identify novel target genes of miR-134 in paclitaxel resistant human ovarian carcinoma cells

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Abstract. Increasing evidence has shown that miR-134 is involved in the promotion of tumorigenesis and chemoresistance. However, whether miR-134 participates in ovarian cancer chemoresistance and its functional targets still remains unclear. The objective of this study was to apply hybrid-polymerase chain reaction (PCR) to screen target genes of miR-134 in ovarian carcinoma paclitaxel resistant SKOV3-TR30 cells, and to provide a number of novel targets of miR-134 for further study of ovarian cancer paclitaxel resistance. The current study found that miR-134 was decreased in SKOV3-TR30 cells compared with the parental SKOV3 cell line. By applying hybrid-PCR, 8 putative target genes of miR-134 in SKOV3-TR30 cells were identified, including *C16orf72*, *PNAS-105*, *SRM*, *VIM*, *F-box protein 2*, *GAPDH*, *PRPF6* and *RPL41*. Notably, the target sites of *VIM* and *PRPF6* were not located in 3'untranslated region, but rather in the coding sequence region. By conducting a luciferase reporter assay, miR-134 was demonstrated to recognize the putative binding sites of these target genes including *VIM* and *PRPF6*. Transfecting SKOV3-TR30 cells with miR-134 mimic and performing reverse transcription-PCR in addition to western blot analysis confirmed that miR-134 regulates vimentin expression at a post transcriptional level. This finding provides a novel perspective for studying the mechanism of miR-134/mRNA interaction. In conclusion, this study was the first to apply an effective method of hybrid-PCR to screen putative target mRNAs of miR-134 in paclitaxel resistant SKOV3-TR30 cells and indicate that miR-134 may contribute to the induction of SKOV3-TR30 paclitaxel resistance by targeting these genes.

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Introduction

Ovarian cancer is the leading cause of mortality from gynecological cancers and the fifth most common cause of cancer-related mortality in females in the USA (1). Despite significant advances in surgical management and chemotherapy over the past two decades, the survival rate for ovarian cancer has not improved significantly. Paclitaxel-based chemotherapy is important in the therapy of newly diagnosed and recurrent ovarian cancer, however drug resistance may seriously impede the clinical effect (2,3). Therefore researchers are eager to understand how drug resistance develops in ovarian carcinoma.

MicroRNAs (miRNAs) are RNAs of ~19-23 nucleotides in length, and consist of a cluster of short non-protein-coding RNAs (4). Increasingly, studies have demonstrated that miRNAs are crucial in tumor cell response to chemotherapeutic drugs by acting as oncogenes and tumor suppressors (5-7). More and more novel miRNAs, including miR-200c, miR-125b, miR-214, have been reported to be associated with paclitaxel resistance in ovarian cancer (8-10).

MiR-134, which is located in the 14q32.31, was initially identified in cloning research of rat in 2002 (11); since this identification, reports have shown that abnormal expression of miR-134 is associated with tumor formation, cell proliferation and even chemoresistance. In 2012, Hirota *et al* (12) demonstrated that miR-134 expression was significantly decreased in lung cancer tissues, and that miR-134 affects the fluorouracil sensitivity of lung cancer by decreasing the expression of dihydropyrimidine dehydrogenase. The study by Guo *et al* (13) in 2010 revealed that in multi-resistant small cell lung cancer cell line H69AR, miR-134 expression was decreased significantly, and that increasing the expression of miR-134 in drug-resistant cells can significantly increase therapeutic sensitivity to cisplatin, etoposide and doxorubicin. However, there is currently no study with regard to miR-134 in ovarian carcinoma chemoresistance.

A conventional way to identify miRNA targets is by using bioinformatics, however different algorithms always produce divergent results (14-16). Additionally, miRNAs often exhibit a temporal or tissue-specific expression pattern, and their target mRNAs share this characteristic, therefore, prediction of targets by bioinformatic methods such as TargetScan, cannot be modulated by these factors when researchers seek

tissue and stage specific mRNA of a certain miRNA. For these reasons, in the current study, hybrid-polymerase chain reaction (PCR) (17), a rapid experimental approach for screening putative target mRNAs of any known miRNA, was conducted to identify target genes of miR-134 in SKOV3-TR30 cells. This study is the first study aiming to target mRNA for a certain miRNA in the study of ovarian cancer chemoresistance, as well as aiming to identify novel targets of miR-134 to elucidate how miR-134 participates in the formation of ovarian cancer paclitaxel resistance.

Materials and methods

Cell culture. The ovarian carcinoma cell line SKOV3 was provided by the Tumor Cell Bank of the Chinese Academy of Medical Sciences (Beijing, China). The paclitaxel-resistant ovarian carcinoma cell line, SKOV3-TR30 with a resistant ability 27-fold greater than its parental cell line, was derived from SKOV3 cell line and provided by The Obstetrics and Gynecology Hospital Affiliated to Zhejiang University (Hangzhou, China). SKOV3 cells were maintained in McCoy's 5A medium (Gibco Life Technologies, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco), 100 μg/ml penicillin (Hyclone, Logan, UT, USA) and 100 μg/ml streptomycin (Hyclone). SKOV3-TR30 cells were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum, 100 μg/ml penicillin, 100 μg/ml streptomycin and 30 nmol/l paclitaxel; paclitaxel was withdrawn 1 week prior to the experiment. All cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C. HEK293 cells were maintained in a Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum. Cells in the logarithmic phase of growth were used for all studies described, cultured in a humidified atmosphere of 5% CO_2 at 37°C.

RNA extraction and mRNA purification. RNA was obtained from the SKOV-3 and SKOV-3TR30 cells. Total RNA was isolated using Trizol agent (Qiagen, Shanghai, China) according to the manufacturer's instructions. RNA was dissolved in 40 μl RNase free H_2O and treated with the TURBO DNA-free™ kit (Ambion Life Technologies, Carlsbad, CA, USA). Additionally, the RNA quality was determined via ethidium bromide staining following agarose/formaldehyde gel electrophoresis.

Quantitative reverse transcription (qRT)-PCR for miR-134 expression. Total RNA from treated cells was extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and quantified using an ultraviolet spectrophotometer (UVP Inc., Upland, CA, USA) at a wavelength of 260 nm. For miR-134 qRT-PCR, cDNA was synthesized from 10 ng of total RNA using TaqManTM miRNA hsa-miR-134-specific primers (Applied Biosystems Life Technologies, Beijing, China) and a TaqManTM MicroRNA Reverse Transcription Kit (Applied Biosystems Life Technologies). qPCR was performed on the ABI PRISM 7900 Sequence Detection System (Applied Biosystems Life Technologies). U6 snRNA was used as an endogenous control. All reactions were performed in triplicate. The relative expression of miR-134 was normalized to U6 RNA using the $2^{-\Delta\Delta CT}$ method.

Hybrid-PCR. An miR-134 specific primer was designed for hybrid-PCR. The sequence of miR-134 is UGUGACUG-GUUGACCAGAGGGG. A reverse and complementary sequences of miR-134 were generated for the miR-134 hybrid primer and the primer sequence was 5'CCCCTCTGGTCRRC-CRGTCRC3'. The seed region of miR-134 was correspondingly located in the 3'-terminal of hybrid-primer. [As G:U pairs are allowed for the miRNA:mRNA duplexes, the primer was synthesized as a compatible primer. R in the primer represents adenine (A) or guanine (G)]. After reverse transcription using 3'-Full RACE Core Set kit (Takara Bio, Inc., Otsu, Japan), sequences between miR-134 binding sites and polyA signal were amplified with the hybrid-primer, the outer primer (5'-TACC GTCGTTCCACTAGTGATTT-3') and the inner primer (5'-CGCGGATCCTCCACTAGTGATTTCAC TATAGG-3') provided in the kit. To acquire the actual sequences of miR-134 putative target mRNAs, all hybrid-PCR products were harvested using the Qiaex® ‖ gel extraction kit (Qiagen), cloned into pMD 18-T vectors (TakaraBio, Inc.,) and transformed into *E. coli* to produce a pool which should contain partial sequences of putative mRNAs that miR-134 would bind to. Insertions were identified by PCR using M13 primers, and confirmed by electrophoresis on a 2% agarose gel to determine the size of inserted fragments in the pool. Clones observed in different sizes were selected and the corresponding plasmids were sequenced on an ABI 3730 automated sequencer (Applied Biosystems Life Technologies).

Sequences blast and analysis. In order to identify the putative target genes of miR-134, mRNA specific sequences located between the corresponding sequence of miR-134 hybrid primer and polyA structure were intercepted and analyzed using the online Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast).

Plasmid construction. MiR-134 putative binding sites within 200-300 bases of flanking sequences of each target gene were amplified from mRNA-derived cDNA and subsequently cloned into *Bam*HI and *Mlu*I sites of the multiple cloning regions of the luciferase reporter constructor pMIR (Ambion Life Technologies). The sequence predicted to express miR-134 was cloned into miRNA expression vector pSilencer4.1 (Ambion Life Technologies) at the *Bam*HI-*Hin*dIII sites. All primer sequences used in plasmid construction are listed in Table I. All constructs were confirmed by DNA sequencing.

Luciferase Reporter Assays. HEK293 cells were plated at $2x10^5$ cells per well in a 24-well plate 24 h before transfection. pMIR construct (200 ng) carrying the putative target sequence was co-transfected with miR-134 expression plasmid, pSilencer-miR-134 (pSilencer was used as normal control) and 200 ng control renilla plasmid, pRL-TK (Promega Corporation, Madison, WI, USA) into HEK293 cells using lipofectamine 2000 (Invitrogen Life Technologies). Cells were collected 24 h post transfection and luciferase activity levels were measured using the Dual Luciferase Assay System (Promega Corporation). Firefly Luciferase activity was normalized to renilla luciferase activity for each reaction. Transfected wells were analyzed in triplicate for each group.

Table I. Primers designed for constructing the plasmids used for luciferase reporter assays.

| Genes inserted	Primer sequences
C16orf72	F: 5'CCCAAGCTTTTGATAAAACGTGCCATT
	R: 5'GGACTAGTTTACGCTTCTACTGCTGA
SRM	F: 5'CCCAAGCTTATGAATAATAGCAGTTCT
	R: 5'GGACTAGTGGGAGATAGGTAGGAGTAGC
PNAS-105	F: 5'CCCAAGCTTGCGCCGCCCGCCCGCCCG
	R: 5'GGACTAGTTGAGGGGCAACAGAAGGCAG
VIM	F: 5'CCCAAGCTTGGAGCCCGCTGAGACTTGAA
	R:5'GGACTAGTAAAGATTTATTGAAGCAGAACC
F-box protein 22	F: 5'CCCAAGCTTCCTGGCGGAGGCCGGCCACC
	R: 5'GGACTAGTCTCTTCCTATGCAGGAAGAC
GAPDH	F: 5'CCCAAGCTTTGGTAAAGTGGATATTGTTG
	R: 5'GGACTAGTGTTGAGCACAGGGTACTT
PRPF6	F: 5'CCCAAGCTTGACGCGACGACGGCGACACT
	R: 5'GGACTAGTGCCTGTTCTGACACGAGACA
RPL41	F: 5'CCCAAGCTTGTGGAGGAAGAAGCGAATG
	R: 5'GGACTAGTTTTATGAGCAAGGTGGGT
miR-134	F: 5'CGCGGATCCTGTGAGGTGACGCTGGTG
	R: 5'CCCAAGCTTTCGTGGTGGATTCGCTTT
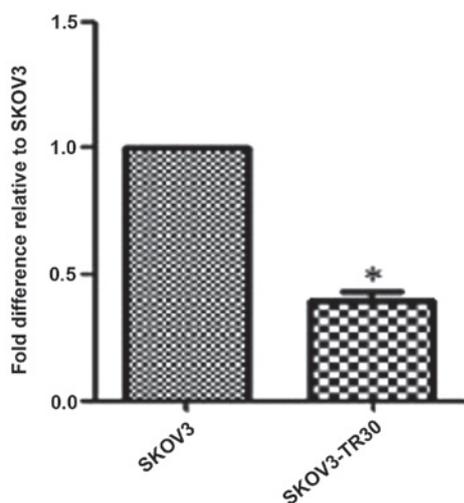
Figure 1. The expression level of miR-134 was significantly decreased in SKOV3-TR30 compared with SKOV3. Total RNA was extracted from SKOV3 and SKOV3-TR30 cells and expression level of miR-134 was quantified by quantitative reverse transcription-polymerase chain reaction normalized to the expression of U6. Data are presented as the mean ± standard deviation of three separated experiments (*P<0.05).

MicroRNA transfection. MiR-134 mimics and miRNA mimic negative control were chemically synthesized by RiboBio (Guangzhou, China). RNA oligonucleotides were transfected into cells at a final concentration of 50 nM using Lipofectamine 2000 according to the manufacturer's protocol.

Protein expression/western blotting. Proteins were harvested using RIPA lysis buffer (ThermoFisher Scientific, Waltham, MA, USA), diluted with buffer containing SDS (Life Technologies,

Grand Island, NY, USA) and denatured at 95°C for 10 min. Protein lysates were subjected to 10% Precise Tris-Glycine gel (Thermo-Fisher Scientific) for 75 min at 100 V and transferred onto a nitrocellulose membrane for 65 min at 100 V. Membranes were washed with Tris buffered saline (TBS; Sigma-Aldrich, St. Louis, MO, USA), blocked with 5% dried nonfat milk (Bio-Rad Laboratories, Hercules, CA, USA) in 1% tween-TBS and probed using a rabbit polyclonal antibody against vimentin (VIM; catalog no. ab45939; Abcam, Cambridge, UK; dilution, 1:1,000), with a rabbit anti-GAPDH antibody (catalog no. ab181603; Abcam; dilution, 1:2,000) as a visual loading control. Bands were visualized using a horseradish peroxidase-conjugated polyclonal goat anti-rabbit IgG secondary antibody (catalog no. ab136817; Abcam; dilution, 1:2,000) and enhanced chemiluminescence, and quantified using Chemi-Doc XRS imaging software version 2.0 (Bio-Rad Laboratories).

Statistical analysis. Statistical comparison between two groups was performed using Student's *t*-test. P<0.05 was considered to indicate a statistically significant difference. All data are presented as the mean and standard deviation from at least three separate experiments.

Results

MiR-134 is down-regulated in SKOV3-TR30 cells compared with in SKOV3. In our previous study we applied MicroRNA Gene Chip analysis and found that miR-134 had a significantly decreased expression in paclitaxel resistant SKOV3-TR30 cell line compared with in its parental human ovarian carcinoma SKOV3 cell line (18). In the current study, this result is further confirmed by using qRT-PCR. SKOV3 and SKOV3-TR30
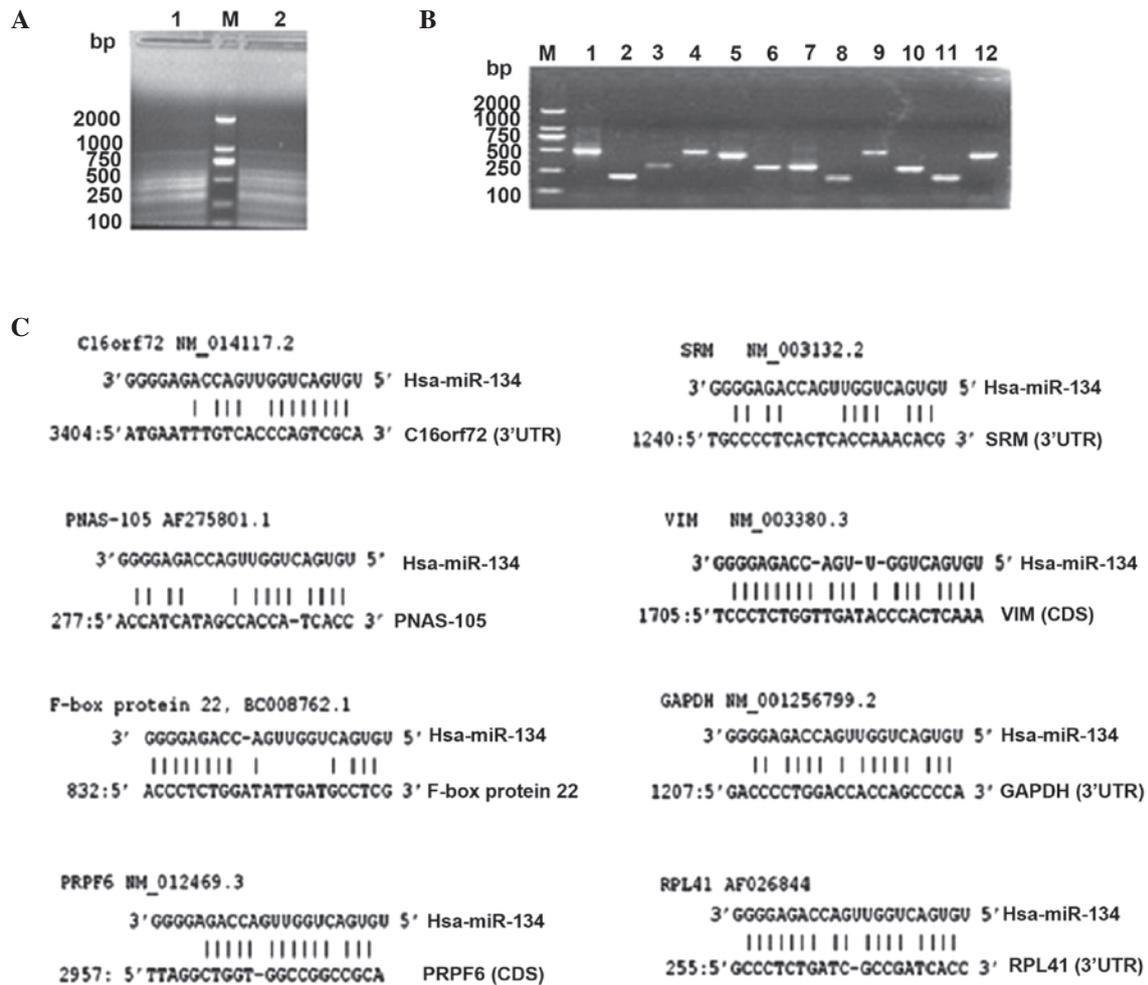
Figure 2. Hybrid-PCR was applied and 8 putative target mRNAs of miR-134 were identified in SKOV3-TR30 cells. (A) Hybrid-PCR was conducted as described. Products of hybrid-PCR were subjected to electrophoresis on 2% agarose gel with DL2000 alongside. Lines 1 and 2 show that the amplification products from hybrid-PCR were variable in length. M: DL2,000 marker. (B) All Hybrid-PCR products were purified and cloned into T-vector. After picking the positive colony forming units, M13 primers were used to identify the insertions in order to further sequence. Lines 1-12 are the elementary identification results of positive colony forming units which contain hybrid-PCR products. Lines 2.8.11 are the negative which represent the empty T vectors, while the remainder represent the positive clones, indicating target mRNAs of hybrid-PCR products were successfully inserted into the T-vector. M: marker DL2000. (C) Overall eight putative target mRNAs of hsa-miR-134 in SKOV3-TR30 cells were obtained. Putative binding sites of each gene were shown. Among them, target sites of vimentin and PRPF6 were not located in 3' untranslated region but rather in the coding region. PCR, polymerase chain reaction.

cells were analyzed to determine the miR-134 expression level, as shown in Fig. 1. miR-134 expression was significantly decreased in SKOV3-TR30 cells compared with SKOV3 cells, suggesting that the decreased expression of miR-134 may be associated with the paclitaxel resistance of SKOV3-TR30 cells (Fig. 1).

Hybrid-PCR may amplify and aid in the identification of the putative target mRNA of miR-134 in SKOV3-TR30 cells. To explore the mechanism of how miR-134 may cause paclitaxel resistance in SKOV3-TR30 cells, a newly established method hybrid-PCR, was applied (17) to identify the target mRNAs of miR-134 in SKOV3-TR30 cells. Hybrid-PCR was projected as semi-nested PCR using the hybrid-primer and the outer/inner primers homologous to the oligo dT-3 site adaptor primer. The products of amplification were variable in length (Fig. 2A). To acquire the actual sequences of miR-134 putative target mRNAs, all hybrid-PCR products (with different lengths shown in Fig. 2A and B) were harvested by gel extraction, cloned into T-vectors and then transformed into *E. coli*

for further selection and amplification. Positive colony forming units were picked for sequencing (Fig. 2B shows partial of the positive colony units). In total, 28 sequences were obtained successfully in the current study. Hybrid-primer sequences and polyA structure were confirmed for a complete extremity of mRNA. These 28 mRNA sequences located between hybrid-primer and polyA were intercepted and the BLAST tool was used to identify their host genes. Of the 28 sequences, 8 matched sequences in GenBank and their host mRNAs were identified successfully. The remaining 20 were not identified. Detailed information is reported in Table II and Fig. 2C.

Validation of predicted targets of miR-134 by luciferase reporter assays. The current study aimed to validate the interaction between miR-134 and the predicted target genes obtained from hybrid-PCR. The majority of studies have shown that miRNAs regulate target gene expression by binding to the sites located in the 3'untranslated regions (UTR) of mRNAs. In recent years, increasing evidence has demonstrated that miRNAs also bind in the coding region (CDS). As we previously found

Table II. 8 putative target mRNAs of miR-134 identified by Hybrid-PCR in SKOV3-TR30 cells.

| Putative target mRNA	Accession number
Homo sapiens chromosome 16 open reading frame 72 (C16orf72)	NM_014117.2
Homo sapiens PNAS-105 (PNAS-105)	AF275801.1
Homo sapiens spermidine synthase (SRM)	NM_003132.2
Homo sapiens vimentin (VIM)	NM_003380.3
Homo sapiens F-box protein 22(F-box protein 22)	BC008762.1
Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_001256799.2
Homo sapiens PRP6 pre-mRNA processing factor 6 homolog (PRPF6)	NM_012469.3
Homo sapiens ribosomal protein L41 mRNA(RPL41)	AF026844
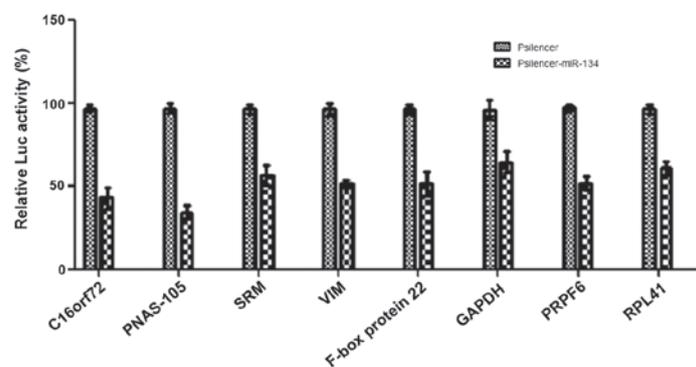
Figure 3. Dual luciferase assay used to validate targets of miR-134. HEK293T cells were transiently co-transfected with pSilencer-miR-134 or pSilencer negative control and pMIR-luciferase constructs containing the predicted binding sites from the eight indicated target genes. Luciferase activity was normalized to Renilla activity. The data plotted are the mean and standard deviation of three independent biological replicates with three technical replicates.
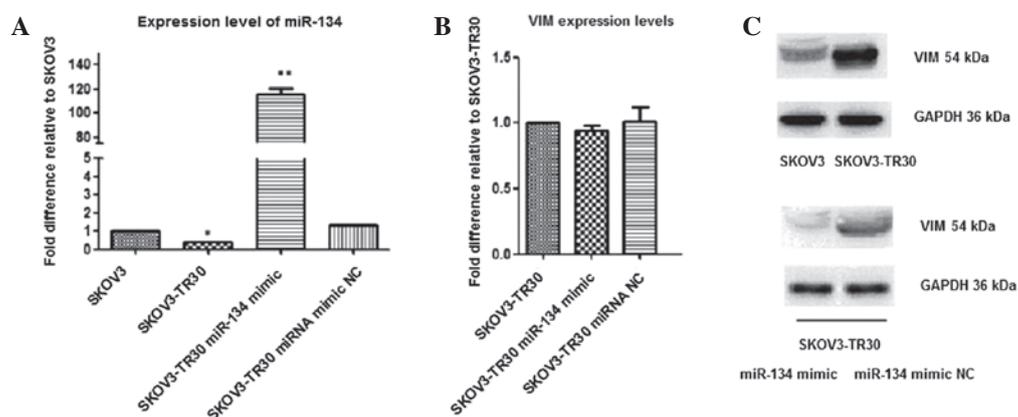

Figure 4. Overexpression of miR-134 inhibits VIM protein level in SKOV3-TR30 cells. (A) miR-134 expression in SKOV3-TR30 cells after transfection of miR-134 mimics was measured by qRT-PCR normalized to the expression of U6. The data are presented as the mean and SD of triplicate samples (*P<0.05, *P<0.01). The results were expressed as Log10 ($2^{-\Delta\Delta CT}$). (B) The analysis of the VIM mRNA level was performed in the above mentioned cells lines. Total RNA was extracted and subjected to real-time PCR to analyze the expression level of VIM. The data are presented as the mean and SD of triplicate samples. (C) Western blot analysis for endogenous VIM protein level using antibody against VIM in SKOV3 and SKOV3-TR30 cells and VIM in SKOV3-TRT30 cells transfected with miR-134 mimic and miRNA mimic negative control. GAPDH was used as internal control. Similar results were obtained in three independent experiments. VIM, vimentin; PCR, polymerase chain reaction; SD, standard deviation.

that miR-134 binds to the CDS of *VIM* and *PRPF6*, in the current study, we also determined whether or not the putative binding sequences located in the CDS represent functional target sites for miR-134. Putative binding sequences of each target gene were successfully amplified and cloned into luciferase reporter constructor pMIR (data not shown). Luciferase activity was measured in the presence of pSilencer-miR-134 or pSilencer negative control and normalized using Renilla activity. Compared with the pSilencer negative control group, co-transfection of Psilencer-miR-134 with pMIR containing candidate target sequences all led to a decrease in luciferase activity. These data demonstrate that the putative binding sites

that have been validated in the current study may be recognized by miR-134. Using this approach, we were able to validate all of the target genes predicted by hybrid-PCR (Fig. 3).

VIM is a direct target of miR-134 in SKOV3-TR30 cells. To further clarify whether the target mRNA protein levels were mediated through miR-134, *VIM* was selected for further investigation. Recently, it has been demonstrated that miRNAs can regulate target mRNAs by binding to the CDS (19-22). Notably, in the current study, 8 putative target mRNAs of has-miR-134 were obtained overall in SKOV3-TR30 cells, and among these, putative binding sites of *VIM* and *PRPF6* were not located in the 3'UTR, but rather in the CDS region. Additionally, studies have shown that VIM functions in cell adhesion, migration, survival, and cell signaling processes via dynamic assembly/disassembly in cancer cells (23,24). Therefore, in the current study *VIM* was selected to investigate whether miR-134 may target *VIM* in SKOV3-TR30 cells.

SKOV3-TR30 cells were transfected with miR-134 mimic (while using transfected miRNA mimc negative control as the control group) and subsequently RT-PCR and western blot analysis were performed to measure the VIM mRNA and protein levels. The results demonstrated that miR-134 had only a limited effect on the mRNA of the endogenous target VIM, however, the corresponding protein was affected more substantially. Combined with luciferase reporter assay (Fig. 4), the results indicate that *VIM* is likely to be direct target of miR-134 and that VIM protein was regulated at the post-transcriptional level in SKOV-TR30 cells.

Discussion

Among gynecological malignancies, ovarian carcinoma is the leading cause of cancer death worldwide (25) and the majority of cases are associated with recurrence and chemoresistance (26). Studies have shown that abnormal expression of certain miRNAs such as miR-200c , miR-125b, miRNA-31,miR-182 in ovarian cancer were associated with activated tumor cell proliferation, enhanced migration ability and may increase chemoresistance (8,9,27,28).

Increasing evidence has shown that miR-134 is involved in the promotion of tumorigenesis, chemoresistance and its expression is downregulated in a number of cancers. In our previous study following analysis by miRNA microarray, we reported for the first time that miR-134 is downregulated in ovarian cancer paclitaxel-resistant SKOV3-TR30 cells compared with the parental SKOV3 cells (18); in the current study we confirm this result by applying real-time PCR, as shown in Fig. 1.

miRNAs often show temporal and tissue-specific expression, which means that the differential expression of certain miRNA only occurs in a certain cell or at a particular stage; their target mRNA also exhibits this characteristic. However, the prediction of targets by bioinformatics methods such as TargetScan cannot accommodate these factors when users require tissue- and stage-specific mRNA of a certain miRNA. Hybrid-PCR, a recently reported tissue- and stage-specific method (17) was used in order to identify specific mRNA targets of miR-134 in ovarian cancer SKOV3-TR30 cells to gain an improved understanding of how miR-134 functions in ovarian carcinoma paclitaxel resistance.

By applying this method, 8 putative target genes of miR-134 were identified in SKOV3-TR30 cells, including *C16orf72*, *PNAS-105*, *SRM*, *VIM*, *F-box protein 2*, *GAPDH*, *PRPF6* and *RPL41*. Furthermore, by successfully constructing PMIR luciferase reporter plasmids of putative binding sites in these 8 mRNAs and applying luciferase reporter assays, the current results demonstrated that, compared with the pSilencer negative control group, co-transfection of Psilencer-miR-134 with pMIR containing the candidate target sequences all led to a decrease in luciferase activity. These data demonstrated that the putative binding sites that have been identified by hybrid-PCR may be recognized by miR-134. Subsequently, *VIM* was selected out of the 8 putative target genes to further investigate whether miR-134 regulates the protein expression level of the target genes. *VIM* was selected for the following reasons: Firstly, it is well known that VIM is an essential constituent of cytoskeletal proteins of mesenchymal cells and VIM is a marker of epithelial-mesenchymal transition (EMT) (29). Many studies have shown that EMT is critical for chemoresistance, particularly in ovarian cancer (30,31). Secondly, *VIM* has a putative miR-134 target site in its CDS region. A number of recent reports have shown that miRNAs also target the CDS of mammalian genes (32,33) and have a critical function in certain aspects of cancer cell biology, including in apoptosis. The results in the current study demonstrate that miR-134 mimic (compared with miRNA mimic negative control) was successfully transfected into SKOV3-TR30 cells and the protein level of VIM was significantly decreased in SKOV3-TR30 cells transfected with miR-134 mimic.

In summary, in this study demonstrated that miR-134 is downregulated in the ovarian carcinoma paclitaxel-resistant cell line SKOV3-TR30, compared with the parental SKOV3 cell line. By conducting hybrid-PCR, 8 putative targets of miR-134 were identified in SKOV3-TR30 cells, and by applying a luciferase reporter assay we further confirmed that hybrid-PCR may be used as a simple and effective method to screen putative target mRNAs of miR-134 in the study of ovarian cancer chemoresistance. By transfecting SKOV3-TR30 cells with miR-134 mimic and subsequently conducting RT-PCR and western blot experiments, this study presents the first evidence that *VIM* is regulated at the post-transcriptional level by miR-134, which directly targets *VIM* within its coding region; this finding provides an important and novel perspective for determining the complex mechanism of miRNA/mRNA interactions. Follow-up studies will investigate the mechanism of miR-134 in paclitaxel resistance in SKOV3-TR30 cells by regulating the target genes identified in this study, including VIM.

Acknowledgements

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