Gain-of-function microRNA screens identify miR-193a regulating proliferation and apoptosis in epithelial ovarian cancer cells

HARUO NAKANO1,2, YOJI YAMADA1, TATSUYA MIYAZAWA1 and TETSUO YOSHIDA1

1Biologics Research Laboratories, Kyowa Hakko Kirin Co., Ltd., Machida-shi, Tokyo 194-8533, Japan

Received January 31, 2013; Accepted March 19, 2013

DOI: 10.3892/ijo.2013.1896

Abstract. MicroRNAs (miRNAs) are a small class of non-coding RNAs that negatively regulate gene expression, and are considered as new therapeutic targets for treating cancer. In this study, we performed a gain-of-function screen using miRNA mimic library (319 miRNA species) to identify those affecting cell proliferation in human epithelial ovarian cancer cells (A2780). We discovered a number of miRNAs that increased or decreased the cell viability of A2780 cells. Pro-proliferative and anti-proliferative miRNAs include oncogenic miR-372 and miR-373, and tumor suppressive miR-124a, miR-7, miR-192 and miR-193a, respectively. We found that overexpression of miR-124a, miR-192, miR-193a and miR-193b inhibited BrdU incorporation in A2780 cells, indicating that these miRNAs affected the cell cycle. Overexpression of miR-193a and miR-193b induced an activation of caspase 3/7, and resulted in apoptotic cell death in A2780 cells. A genome-wide gene expression analysis with miR-193a-transfected A2780 cells led to identification of ARHGAP19, CCND1, ERBB4, KRAS and MCL1 as potential miR-193a targets. We demonstrated that miR-193a decreased the amount of MCL1 protein by binding 3'UTR of its mRNA. Our study suggests the potential of miRNA screens to discover miRNAs as therapeutic tools to treat ovarian cancer.

Introduction

MicroRNAs (miRNAs) are small non-coding RNAs of 20-22 nucleotides, and function to suppress the expression of target mRNAs by translation blockade and/or mRNA degradation (1,2). They are involved in many biological processes including cell proliferation, differentiation and apoptosis, and their dysregulation can contribute to the pathological state including cancer (1,3,4). Several groups have documented miRNA expression profiling in ovarian cancers using miRNA microarray and massive parallel sequencing technology (5-10). miR-93, miR-141, miR-200 and miR-214 are frequently upregulated whereas miR-100, miR-143, miR-145 and let-7 are downregulated in ovarian carcinomas compared with normal counterparts (5-9). Abnormal miRNA expression is due to DNA copy number amplification and deletion, epigenetic modification and/or the dysregulation of miRNA processing in cancer state (7,11,12). miR-214 upregulated in ovarian cancer can target PTEN tumor suppressor gene whereas down-regulated let-7 can target the RAS oncogene (8,13), suggesting that miRNAs may have a role as novel class of oncosenes or tumor suppressor genes in ovarian cancer (14).

Based on these findings, the clinical potential of miRNAs as cancer biomarkers and/or therapeutic agents is widely recognized and accepted (15). A single miRNA can regulate multiple mRNA transcripts that cooperatively work in cellular differentiation and function (16-19). The use of miRNA mimics or anti-miRNAs may represent powerful therapeutic tools to accomplish regression and/or re-differentiation of cancer by effectively targeting tumor suppressive or oncogenic genes with less toxicity (15,20). Indeed, a number of pre-clinical trials of miRNAs are currently in progress (21). In this study, we performed a gain of function screen using miRNA mimics library containing 319 miRNAs to identify miRNAs that can affect cell proliferation in A2780 ovary cancer cells. We found several anti-proliferative miRNAs including miR-124, miR-192 and miR-193 in A2780, suggesting that the potential of miRNA screens for discovering miRNAs as therapeutic tools to treat ovarian cancer.

Materials and methods

Cell culture. Human ovarian cancer cell line A2780 was obtained from Dr T. Tsuruo (22), and human colorectal cancer cell line DLD-1 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). A2780 and DLD-1 were cultured in RPMI-1640 (Gibco, Life Technologies, Carlsbad, CA, USA) containing 50 IU/ml penicillin and 50 µg/ml streptomycin (Gibco, Life Technologies), supplemented with 5% (A2780) or 10% (DLD-1) fetal bovine serum (FBS, JRH Biosciences, Lenexa, KS, USA) at 37°C in an atmosphere of 5% CO2.
miRNA library screening. A gain of function miRNA screen on cell viability was performed using A2780 as previously described (23). A2780 was seeded at 2,500 cells per well in 96-well plates the day before transfection. Synthetic miRNA mimic library (human Pre-miR™ miRNA precursor library-ver.1, Ambion, Applied Biosystems, Foster City, CA, USA) was screened using 50 nM in a duplicate. The library contained 319 miRNAs registered in miRBase ver. 7.1 (http://www.mirbase.org/). miRNA mimics were transfected using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocols. Pre-miR miRNA precursor molecule-negative control (13) (Ambion, Applied Biosystems) was used as a negative control for miRNA mimics. We confirmed transfection efficiency (>90%) using siControl TOX transfection control (50 nM, Dharmacon, Lafayette, CO, USA). After 3 days of transfection, the cell viability was measured using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) according to the manufacturer's instructions. Data were expressed as percentage of the negative control. Several miRNA hits were selected to assess reproducibility and dose-dependency (5, 25 and 50 nM).

Brdu incorporation assay. miRNA (25 nM)-transfected cells in 96-well format were harvested for one day, and then were incubated with 10 µM of 5'-bromo-2-deoxy-uridine (Brdu) for 2-4 h. The cells were fixed with cold ethanol/HCl, and the incorporated Brdu was detected using Brdu labeling and detection kit III (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instruction.

Caspase 3/7 activation assay. miRNA (25 nM)-transfected cells in 96-well format were harvested for 2 days, and then used to measure caspase 3/7 activity using Caspase-Glo 3/7 assay (Promega) according to the manufacturer's instruction.

RNA isolation and whole genome microarray. A2780 cells were transfected with miR-193a (Pre-miR miRNA precursor molecules, hsa-miR-193a-3p, Ambion, Applied Biosystems) or negative control miRNA (25 nM), and allowed to grow in the medium (RPMI-1640) for 10 h before RNA isolation. Total RNA was isolated using the RNeasy mini RNA isolation kit (Qiagen). Scanning microarray microarray analysis was carried out using the 44K platform. The integrity of the RNA was verified using an Agilent 2100 Bioanalyzer (1.8-2.0: Agilent Technologies, Palo Alto, CA, USA). Transcriptome microarray analysis was carried out using the 44K Whole Human Genome Microarray chip (Agilent Technologies) according to the manufacturer's instructions. Scanning microarray chips and processing data were done by Pharmafrontier Co., Ltd, Kyoto, Japan. Differentially expressed probe sets were identified with a fold change >1.5. Gene ontology (GO) pathway enrichment analysis was performed among genes differentially expressed after miR-193a transfection by SigTerm software (24). The downregulated genes with miR-193a transfection were compared with predicted miR-193a target genes searched by TargetScan (http://www.targetscan.org/). Over-representation of predicted miR-193a target genes within downregulated gene sets was assessed by SigTerm software.

Western blot analysis. miRNA or siRNA (25 nM)-transfected A2780 cells were lysed in radio immunoprecipitation assay (RIPA) buffer [50 mM Tris-HCl (pH 8.0), 150 mM sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate] supplemented with 1% of a protease inhibitor cocktail stock solution (set III, Roche Diagnostics GmbH) after 1 or 2 days transfection. The following pre-designed siRNA was used as a positive control: MCL1 siRNA (Hs_MCL1_6 HP validated siRNA, SI02781205, Qiagen GmbH, Hilden, Germany). Proteins (10 or 20 µg) were separated by SDS-PAGE. Upon electroblotting to polyvinylidene fluoride (PVDF) membrane (Immobilon-P, Millipore, Billerica, MA, USA), non-specific binding sites were blocked by incubation in TBST (Tris-buffered saline/0.05% Tween-20) containing 1% skim milk, and then incubated with rabbit polyclonal anti-MCL1 (1:200, S-19, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), or mouse monoclonal anti-α-tubulin (1:2,500, clone DM1A, Sigma, St. Louis, MO, USA) in blocking solution. After washing with TBST, the membrane was incubated with HRP-conjugated rabbit anti-mouse IgG secondary antibody (P0161, Dako, Glostrup, Denmark) or HRP-conjugated swine anti-rabbit IgG secondary antibody (P0217, Dako). Signals were detected using enhanced chemiluminescence (ECL) or ECL-plus reagent (Amersham™ GE Healthcare UK Ltd., Buckinghamshire, UK).

Luciferase miRNA target reporter assay. 3′-untranslated regions (UTRs) of MCL1 gene (1556 bp), containing predicted binding sites of miR-193a, were amplified by PCR from A2780 cDNA, and inserted into the pGL3 control vector (Promega) by using Xba-I site immediately downstream from the stop codon of Firefly luciferase. The following primers were used: MCL1 3′-UTR forward: CGGCTAGCGAAAAGCAAGTGG, reverse: GATT, MCL1-mutant-Primer 2: GGCCACTTTCCTGTTCTG; MCL1-mutant-Primer 1: AGCCAGGCAAGTCATAGAATT. GAPDH (137 bp) forward: ACTTTGTCAAGCTCATTTCCTG, reverse: CCTGGCACAGCTATCAAAAG; for detection: MCL1 (222 bp) forward: TCTAAGTGCTGACGGTTT, reverse: CAAGAGG, reverse: CGGCTAGCAGGGAGGGTCACTCA. The following primers were used: GAPDH (137 bp) forward: ACTTTGTCAAGCTCATTTCCTG, reverse: CTCTCTTCCTCCTTG. Luciferase miRNA mimics were transfected into DLD-1 cells were cultured in 96-well formats and co-transfected with 100 ng of pGL3 Firefly luciferase reporter vector, 20 ng of pRL-TK Renilla luciferase control vector (Promega) and 25 nM miRNA or negative control miRNA using Lipofectamine 2000. Firefly and Renilla luciferase activities were measured consecutively using the Dual-Luciferase Reporter Assay System (Promega) 24 h after transfection. All the experiments were done in triplicate and repeated at least twice on different days.

qRT-PCR. Total RNA was prepared from miRNA or siRNA (25 nM)-transfected cells 2 days after transfection using RNeasy mini kit (Qiagen), and then first strand cDNA was synthesized using SuperScript III (Life Technologies) according to the manufacturer's instruction. Real-time RT-PCR was performed using 7900 HT fast real-time PCR system (Applied Biosystems Inc., Foster City, CA, USA) with SYBR-Green as a reporter. The following primers were used for detection: MCL1 (222 bp) forward: TCTAAGTGCTGACGGTTT, reverse: CAAGAGG, reverse: CGGCTAGCAGGGAGGGTCACTCA. Deletion of the first 3 nucleotides corresponding miR-193a seed-region complementary site was inserted in mutant constructs using KOD-plus-Mutagenesis kit (Toyobo, Osaka, Japan), according to the manufacturer's protocol. The following primers were used for generation of mutant constructs: MCL1-mutant-Primer 1: AGGCTAGCGAAAAGCAAGTGG, reverse: CGGCTAGCAGGGAGGGTCACTCA, forward: AAAGCTACAGTTCAGGT. DLD-1 cells were cultured in 96-well formats and co-transfected with 100 ng of pGL3 Firefly luciferase reporter vector, 20 ng of pRL-TK Renilla luciferase control vector (Promega) and 25 nM miRNA or negative control miRNA using Lipofectamine 2000. Firefly and Renilla luciferase activities were measured consecutively using the Dual-Luciferase Reporter Assay System (Promega) 24 h after transfection. All the experiments were done in triplicate and repeated at least twice on different days.
Results

Effects of miRNA mimic library transfection on cell proliferation of A2780 cell line. To identify miRNAs that affect cell proliferation of ovarian cancer cells, we performed a gain of function screen using synthetic miRNA mimic library (319 miRNAs) for human epithelial ovary cancer cells (A2780). The library consists of miRNAs registered in early version of miRBase (ver. 7.1 in October, 2005, http://www.mirbase.org/), and many of them were expressed in ovarian normal and cancer tissues and cell lines (5). We detected cellular ATP to assess cell viability in miRNA (50 nM)-transfected cells 3 days after transfection. Frequency distribution indicated that broad ranges of miRNA mimic transfections affected the cell viability of A2780 (Fig. 1A). A total of 46 out of 319 miRNAs induced more than 50% changes in the cell viability of A2780 after 3 days transfection. Table I shows top 10 miRNAs that increased or decreased the cell viability of A2780. They included known oncogenic miRNAs such as miR-372 (cell viability, 187%) and miR-373 (165%), and tumor suppressive miRNAs such as miR-124a (28.3%), miR-7 (37.1%), miR-192 (36.6%) and miR-193a (29.7%) in several different cancer types (18,25-27). The seed family miRNAs that have the same sequences in seed region (2nd to 8th nucleotide) of miRNAs showed similar effects on cell viability.

Table I. Results of miRNA library screening.

| miRNAs that increased cell viability | miRNAs that decreased cell viability |
|-------------------------------------|-------------------------------------|
| miRNA | Cell viability (%) | miRNA | Cell viability (%) |
| miR-301 | 218 | miR-124a | 28.3 |
| miR-372 | 187 | miR-517c | 29.4 |
| miR-93 | 185 | miR-193a | 29.7 |
| miR-302b | 181 | miR-506 | 31.9 |
| miR-130a | 173 | miR-199a | 34.5 |
| miR-302d | 172 | miR-192 | 36.6 |
| miR-363 | 166 | miR-7 | 37.1 |
| miR-373 | 165 | miR-193b | 37.7 |
| miR-9* | 162 | miR-432 | 37.8 |
| miR-130b | 162 | miR-497 | 38.3 |

Data represent the cell viability in miRNA mimics (50 nM)-transfected cells 3 days after transfection. Data were expressed as a percentage of the negative control in an average of duplicates. Top 10 miRNAs that increased or decreased the cell viability are listed.
in A2780 cells. For example, miR-93/miR-302/miR-372/mir-373 seed family miRNAs (miR-93, miR-302b, miR-302d, miR-372, miR-373) were pro-proliferative, while miR-193 seed family miRNAs (miR-193a, miR-193b) were anti-proliferative (Table I). miR-200/miR-141 seed family miRNAs that are upregulated in ovarian cancer (5,6,10) had a little effect on the cell viability in A2780 cells (the cell viability; 97.9, 113, 92.0 and 101% with miR-200a, miR-200b, miR-200c and miR-141 transfection, respectively). miR-100, miR-143 and miR-145 that are down-regulated miRNAs in ovarian cancer (5,6,8) induced a 15-30% decrease in the cell viability of A2780 (the cell viability; 84.1, 81.8 and 73.1 with miR-100, miR-143 and miR-145 transfection, respectively). We are interested in miRNA mimics that decreased the cell viability of A2780 since these miRNA mimics themselves could have therapeutic potential to treat ovarian cancer. To further evaluate miRNA mimics on the inhibition of cell proliferation in A2780, we selected top 10 anti-proliferative miRNAs (miR-124a, miR-192, miR-193a and miR-193b) from the first screen, and examined the cell viability in A2780 cells transfected with different concentrations of miRNAs (5, 25, 50 nM). We confirmed results of our first screening at 50 nM, and found that miR-124a, miR-192, miR-193a and miR-193b induced a large decrease in the cell viability of A2780 even at 5 nM (Fig. 1B), indicating that these miRNAs had a profound anti-proliferative effect in A2780 cells. We examined whether miR-124a, miR-192, miR-193a and miR-193b affected DNA synthesis to inhibit cell proliferation in A2780 cells. One day after miRNA transfection, BrdU incorporation was examined to evaluate DNA synthesis in transfected cells. As shown in Fig. 2A, miR-124a, miR-192, miR-193a and miR-193b decreased an incorporation of BrdU compared with the negative control, indicating that these miRNAs induced the inhibition of DNA synthesis in A2780 cells. We next examined whether these miRNAs affected apoptotic pathway to inhibit cell proliferation in A2780 cells. We found that miR-193a and miR-193b but not miR-124a and miR-192 induced more than twofold increase in an activity of caspase 3/7, the effector of apoptotic pathway, in A2780 cells (Fig. 2B). The result indicated that miR-193a and miR-193b could induce the apoptotic cell death in A2780 cells. Actually, apoptotic cell debris was frequently observed in miR-193a-transfected A2780 cells (Fig. 2C, arrows).
downregulated 518 genes with predicted miR-193a target genes (142 genes) obtained by TargetScan (Fig. 3). This resulted in the match of 34 candidate miR-193a target genes, and they were significantly over-represented in the downregulated gene sets by using the SigTerm software. Table III showed 34 candidate miR-193a target genes obtained by our transcriptome analysis.

**Table II.** Twenty most significantly enriched (P<0.05) gene ontology (GO) pathways among downregulated genes after miR-193a transfection into A2780 cells.

| Term                                               | P-value |
|----------------------------------------------------|---------|
| Small GTPase regulator activity                    | 0.0022  |
| Ras guanyl-nucleotide exchange factor activity     | 0.0030  |
| Rho guanyl-nucleotide exchange factor activity     | 0.0052  |
| Regulation of Rho protein signal transduction      | 0.0060  |
| Blood vessel development                           | 0.0069  |
| Cytoplasmic vesicle part                           | 0.0072  |
| Vasculature development                            | 0.0080  |
| Post-Golgi vesicle-mediated transport              | 0.0080  |
| Protein localization                               | 0.0149  |
| Phospholipid transporter activity                  | 0.0155  |
| Regulation of small GTPase mediated signal transduction | 0.0156 |
| Guanyl-nucleotide exchange factor activity         | 0.0168  |
| GTase regulator activity                            | 0.0181  |
| Macromolecule localization                         | 0.0187  |
| Insulin receptor signaling pathway                  | 0.0193  |
| Guanyl kinase activity                              | 0.0196  |
| Early endosome                                      | 0.0213  |
| Neuron projection                                   | 0.0217  |
| Intracellular signaling cascade                     | 0.0225  |
| One-carbon compound metabolic process              | 0.0229  |

**Figure 3.** Transcriptome analysis with miR-193a-transfected A2780. Venn diagram to illustrate the relationship between the downregulated genes (10 h after miR-193a transfection) and predicted target genes by TargetScan. Predicted miR-193a target genes within the downregulated gene sets were significantly enriched by SigTerm software (P=1.54E-17).

**Table III.** Candidate miR-193a target genes downregulated in miR-193a-transfectants.

| Entrez gene ID | Symbol | Fold change |
|----------------|--------|-------------|
| 23119          | HIC2   | -6.20       |
| 10152          | ABI2   | -3.34       |
| 595            | CCND1  | -3.30       |
| 54756          | IL17RD | -3.20       |
| 10238          | WDR68  | -3.15       |
| 3925           | STN1   | -2.97       |
| 5323           | PLAG1  | -2.97       |
| 3845           | KRAS   | -2.76       |
| 4076           | CAPRIN1| -2.66       |
| 2066           | ERBB4  | -2.58       |
| 57704          | GBA2   | -2.45       |
| 84986          | ARHGAP19| -2.23     |
| 10620          | ARID3B | -2.17       |
| 7342           | UBP1   | -2.09       |
| 27242          | TNFRSF21| -2.07     |
| 4170           | MCL1   | -2.00       |
| 56262          | LRRC8A | -1.93       |
| 10160          | FARPI  | -1.91       |
| 57472          | CNOT6  | -1.91       |
| 23179          | RGL1   | -1.79       |
| 23341          | DNAJC16| -1.79       |
| 88455          | ANKRD13A| -1.70     |
| 4215           | MAP3K3 | -1.67       |
| 114991         | ZNF618 | -1.66       |
| 23492          | CBX7   | -1.64       |
| 23365          | ARHGEF12| -1.64     |
| 22883          | CLSTN1 | -1.61       |
| 9939           | RBM8A  | -1.60       |
| 54890          | ALKBH5 | -1.59       |
| 115            | ADCY9  | -1.57       |
| 4189           | DNAJB9 | -1.51       |
| 1173           | AP2M1  | -1.51       |
| 9962           | SLC23A2| -1.51       |
| 23384          | SPECC1L| -1.50       |

The candidate genes include ARHGAP19 (RhoGAP19), CCND1 (cyclin D1), ERBB4, KRAS and MCL1 that function in cell signaling, cell cycle and apoptotic pathway.

**MCL1** is a direct target gene of miR-193a in A2780 cells. From our results of transcriptome analysis, we focused on MCL1 gene as miR-193a targets, since MCL1 was an anti-apoptotic gene of BCL2 family (28), and therefore might contribute to miR-193a-induced cell death in A2780 cells. MCL1 3'UTR contains one potential target site of miR-193a and the site is conserved between human and mouse. To examine the regulation of miR-193a on MCL1 protein expression, we performed western blot analysis with miR-193a-transfected A2780 cells. Transfection of positive control MCL1 siRNA induced the decrease in endogenous MCL1 proteins in A2780 cells.
We demonstrated that overexpression of miR-193a decreased MCL1 proteins in A2780 cells (Fig. 4A). We next performed qRT-PCR with miR-193a-transfected cells to examine whether miR-193a affected MCL1 mRNA expression. We found that miR-193a induced about 50% decrease in MCL1 mRNA expression in A2780 cells (Fig. 4B). These results indicated that miR-193a can directly regulate the translation of MCL1 mRNAs, we constructed a luciferase reporter plasmid that inserted MCL1 3’UTR (around 1.5 kb) at the downstream of Firefly luciferase gene, and tested the luciferase activity. As shown in Fig. 4C, co-transfection of miR-193a and MCL1 3’UTR reporter vector induced around 40% reduction of the luciferase activity compared with co-transfection of the negative control miRNA and the reporter vector. The decrease of the luciferase activity was attenuated by using the mutant reporter vector deleting miR-193a seed region complementary sites in MCL1 3’UTR (Fig. 4C, MCL1-3’UTR-MU). These results indicated that MCL1 would be a direct target of miR-193a. We further examined whether the downregulation of endogenous MCL1 could induce apoptosis in A2780 cells. As shown in Fig. 4D, the transfection of MCL1 siRNA (25 nM) induced caspase 3/7 activation comparable with miR-193a transfection in A2780 cells (Fig. 4D), indicating that the downregulation of MCL1 by miR-193a could contribute to miR-193a-induced apoptosis in A2780 cells.

**Discussion**

Several studies reveal that global miRNA expression is dysregulated in ovarian cancer (5-10), and miRNAs may represent new targets for detection, diagnosis and therapy in ovarian cancer (14). However, functions of many miRNAs in ovarian cancer remain to be elucidated. In this study, we performed a gain-of-function screen using a miRNA mimic library...
(319 miRNA species) to identify those affecting cell proliferation in epithelial ovarian cancer cells (A2780). The library consists of miRNAs registered in early version of miRBase (ver. 7.1 in October, 2005, http://www.mirbase.org/), and many of them were expressed in ovarian normal and cancer tissues and cell lines (5). We discovered pro-proliferative miRNAs (miR-9*, miR-93, miR-130a, miR-130b, miR-301, miR-302b, miR-302d, miR-363, miR-372, miR-373), and anti-proliferative miRNAs (miR-7, miR-124a, miR-192, miR-193a, miR-193b, miR-193a*, miR-432*, miR-497, miR-506, miR-517c) in A2780 cells. By the same miRNA mimics library screening, we found that miR-93/miR-372/miR-373 and miR-124a were pro-proliferative and anti-proliferative, respectively, in DLD-1 colorectal cancer cells (23), suggesting consistent roles of these miRNAs on cell proliferation in ovary and colorectal cancer cells. The base-pairing between target miRNAs and the seed region (2nd to 8th nucleotides) of miRNA is important for miRNAs to function to regulate their target genes (2). The seed family miRNAs induced similar cellular phenotypes on cell proliferation in this study (ex. pro-proliferative miR-93, miR-302b, miR-302d, miR-372, miR-373 and anti-proliferative miR-193a, miR-193b), supporting the importance of the seed region of miRNA on its function. Our miRNA hits did not always correspond to dysregulated miRNAs reported in ovarian cancer (5-10), but included pro-proliferative miR-93 that was upregulated in primary ovarian carcinomas (6), supporting an oncogenic role of this miRNA in ovarian cancer. Our miRNA hits also included tumor suppressive miR-7, miR-124a, miR-192 and miR-193a in several cancer types (18,25-27), suggesting that these miRNAs could be tumor suppressive in ovarian cancer. Among our miRNA hits, we further characterized miR-124a, miR-192, miR-193a and miR-193b that induced a large decrease in the cell viability of A2780 cells. miR-124a and miR-192 induced a decrease in BrdU incorporation, indicating that these miRNAs affected cell cycle resulting in inhibition of DNA synthesis in A2780 cells. Inhibitory effects of miR-124a and miR-192 on cell cycle gene pathway are reported in several cancer cell lines. miR-124a targets cyclin dependent kinase 6 (CDK6), and thereby inhibits the phosphorylation of retinoblastoma (Rb) in HCT116 cells (29). miR-192 is upregulated by genotoxic stress in HCT116, A549 and U2OS cell lines bearing wild-type p53, and induces the cell cycle arrest by enhancing CDKN1A/p21 expression (18,30).

We showed that miR-193a and miR-193b inhibited BrdU incorporation and induced caspase 3/7 activation in A2780 cells, indicating that these miRNAs could affect cell cycle and apoptotic gene pathways. Our transcriptome analysis with miR-193a-transfected A2780 cells identified ARHGAP19, CCND1, ERBB4, KRAS, MCL1 as potential miR-193a target genes. We demonstrated that the translation of MCL1 proteins was suppressed by miR-193a, suggesting that anti-apoptotic MCL1 would be one of the target genes for miR-193a-induced cell death in A2780. Anti-proliferative and pro-apoptotic functions of miR-193 are reported in several cancer cell lines including MDA-MB-453 (breast cancer), Malme-3M, SKMEL-28, SKMEL-5 (melanoma), HO-1-N-1, HSC-2 (oral squamous cell carcinoma), 22Rv1 (prostate cancer), SK-Hep-1 (hepatocellular carcinoma) and Kasumi-1 (acute myeloid leukemia) (26,31-36). Consistent with our results, CCND1, KRAS and MCL1 are identified as miR-193 target genes (26,32,33,37).
25. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Godwin AK, Urban N, Drescher CW, Knudsen BS and Tewari M: Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA CDNA libraries. PLoS One 4: e5311, 2009.

24. Creighton CJ, Nagaraja AK, Hanash SM, Matzuk MM and Nakano H, Miyazawa T, Kinoshita K, Yamada Y and Yoshida T: MicroRNAs: A new class of regulators of ovarian carcinoma. Cancer 101: 1248-1258, 2008.

23. Nakano H, Miyazawa T, Kinoshita K, Yamada Y and Yoshida T: Functional screening identifies a microRNA, miR-491 that regulates cell proliferation and is down-regulated in glioblastoma. Cancer Res 68: 3566-3572, 2008.

22. Tsuruo T, Hamilton TC, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M and Lim L: Transcript targets of the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol 27: 2240-2252, 2007.

21. Wahid F, Shehzad A, Khan T and Kim YY: MicroRNAs: A new class of regulators of ovarian carcinoma. Cancer 101: 1248-1258, 2008.

20. Mishra PJ and Merlino G: MicroRNA reexpression as differentiation therapy in cancer. J Clin Invest 119: 2119-2123, 2009.

19. Tussilova P, Jirušová M, Srama J, Závada R and Pejchal K: MicroRNA-193b regulates c-Kit proto-oncogene and represses cell proliferation in acute myeloid leukemia. Leuk Res 35: 1226-1232, 2011.

18. Geoghegan CA, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

17. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA and Hannon GJ: A microRNA component of the p53 tumour suppressor network. Nature 447: 1130-1134, 2007.

16. Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M and Lim L: Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol 27: 2240-2252, 2007.

15. Garofalo M and Croce CM: microRNAs: Master regulators as potential therapeutic targets in cancer. Annu Rev Pharmacol Toxicol 51: 25-43, 2011.

14. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

13. Johnson SM, Grosshans H, Sangrajgar J, Byrom M, Jarvis R, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M and Lim L: Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol 27: 2240-2252, 2007.

12. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, Pennacchio LA, Cheng JF, Nick AM, Deavers MT, Mourad-Zeidan A, Wang H, Mueller P, Lennard ME, Gray JW, Mok S, Birrer MJ, Lopez-Berestein G, Coleman RL, Bartelli M, and Sood AK: Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med 359: 2641-2650, 2008.

11. Johnson SM, Grosshans H, Sangrajgar J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D and Slack FJ: RAS is repressed by the let-7 microRNA family. Cell 120: 635-647, 2005.

10. Di Silvestre P, Gaias M, Magnani S, Turrino M, Cavaletto G, Ribeiro de Almeida M, Tosi E, Tisi G, Voi M, Balbo D, Di Tomaso E, Croce CM and Lakhani SR: MicroRNA-193b regulates c-Kit proto-oncogene and represses cell proliferation in acute myeloid leukemia. Leuk Res 35: 1226-1232, 2011.

9. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

8. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

7. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

6. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

5. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

4. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

3. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

2. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.

1. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood W III, Becker KG and Morin PJ: MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 3: e2037, 2008.