MicroRNA Expression Profiling in Human Ovarian Cancer: miR-214 Induces Cell Survival and Cisplatin Resistance by Targeting PTEN


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Abstract

MicroRNAs (miRNA) represent a novel class of genes that function as negative regulators of gene expression. Recently, aberrant miRNA expression and its clinicopathologic significance in human ovarian cancer have not been well documented. Here, we show that several miRNAs are altered in human ovarian cancer, with the most significantly deregulated miRNAs being miR-214, miR-199a*, miR-200a, miR-100, miR-125b, and let-7 cluster. Further, we show the frequent deregulation of miR-214, miR-199a*, miR-200a, and miR-100 in ovarian cancers. Significantly, miR-214 induces cell survival and cisplatin resistance through targeting the 3′-untranslated region (UTR) of the PTEN, which leads to down-regulation of PTEN protein and activation of Akt pathway. Inhibition of Akt using Akt inhibitor, API-2/triciribine, or introduction of PTEN cDNA lacking 3′-UTR largely abrogates miR-214-induced cell survival. These findings indicate that deregulation of miRNAs is a recurrent event in human ovarian cancer and that miR-214 induces cell survival and cisplatin resistance primarily through targeting the PTEN/Akt pathway. [Cancer Res 2008;68(2):425–33]

Introduction

MicroRNAs (miRNA) are a class of 22-nucleotide noncoding RNAs, which are evolutionarily conserved and function as negative regulators of gene expression. Like conventional protein-coding miRNA, miRNAs are transcribed by RNA polymerase II, spliced, and polyadenylated (called primitive miRNA or pri-miRNA). However, unlike mRNA, the pri-miRNAs contain a stem-loop structure that can be recognized and excised by the RNA interference machinery to generate hairpin “precursor” miRNAs (pre-miRNA) that are ~70 nucleotides in animals or ~100 nucleotides in plants. Pre-miRNAs are cleaved by the cytoplasmic RNase III Dicer into a 22-nucleotide miRNA duplex: one strand (miRNA*) of the short-lived duplex is degraded, whereas the other strand serves as a mature miRNA. The mature miRNA then guides a complex called miRNA-containing ribonucleo-protein particles to the complementary site(s) in the 3′-untranslated region (UTR) of a target mRNA. Consequently, translation blockade or mRNA degradation will occur depending on whether it is partially matched or completely matched with the target genes, respectively (1). Moreover, the levels of individual miRNAs are dramatically changed in different cell types and different developmental stages, suggesting that miRNA plays a role in cell growth, differentiation, and programmed cell death (1, 2).

miRNAs are aberrantly expressed or mutated in human cancer, indicating that they may function as a novel class of oncogenes or tumor suppressor genes (3–9). The first evidence of involvement of miRNAs in human cancer came from molecular studies characterizing the 13q14 deletion in human chronic lymphocytic leukemia, which revealed two miRNAs, miR-15a and miR-16-1 (3). Subsequently, miRNA deregulation was detected in other human malignancies, including breast carcinoma (4, 5), primary glioblastoma (6, 7), lung cancer (8), papillary thyroid carcinoma (9), colon carcinoma (10), and pancreatic tumors (11, 12). For instance, the miR-17-92 cluster is up-regulated in B-cell lymphomas and lung cancer. miR-143 and miR-145 are down-regulated in colon carcinomas. A decrease in let-7 is detected in human lung carcinomas and restoration of its expression induces cell growth inhibition in lung cancer cells (13). The BIC gene, which contains the miR-155, is up-regulated in some Burkitt’s lymphomas and several other types of lymphomas (14, 15).

In this report, we show deregulation of several miRNAs in human ovarian cancer. The aberrant expression of miR-214, miR-199a*, miR-200a, and miR-100 was detected in a near or over half of ovarian cancers, especially in late-stage and high-grade tumors. Significantly, we showed that miR-214 negatively regulates PTEN by binding to its 3′-UTR, leading to inhibition of PTEN translation and activation of Akt pathway. Consequently, miR-214 induces cell survival and cisplatin resistance, which were overridden by either small-molecule Akt inhibitor or expression of PTEN cDNA lacking 3′-UTR.

Materials and Methods

Cell lines and human tissue samples. Human ovarian cancer cell lines and human immortalized ovarian surface epithelial (HIOSE) cell lines were described previously (16). HIOSE cells were grown in 199/MDCB 105 (1:1) medium (Sigma) supplemented with 5% fetal bovine serum. Frozen human primary ovarian tumor and normal ovarian tissues were obtained from the Tissue Procurement Facility at H. Lee Moffitt Cancer Center.

miRNA array and Northern blot analysis. Oligonucleotide arrays were printed with trimer oligonucleotide probes (antisense to miRNAs) specific for 515 human and mouse miRNAs on GeneScreen Plus (NEN) membranes, and miRNA expression profiling was performed and analyzed as previously described (7). Briefly, total RNAs were isolated from 10 HIOSE cell lines and 10 primary serous ovarian carcinomas with Trizol reagent (Invitrogen). Low-molecular weight RNAs were enriched from total RNAs using Microcon YM-100 columns (Millipore). The low-molecular weight RNAs were labeled with [γ-32P]ATP and then hybridized to the miRNA array. To ensure accuracy of the hybridizations, each labeled RNA sample was hybridized with three separate membranes. In addition, eight oligonucleotides with nonmatching any known miRNA were used as hybridization controls. Hybridization signals for each spot of the array and background values at 15
empty spots were measured. Raw data were further automatically processed in Microsoft Excel. Hybridization signals that failed to exceed the average background value by more than three SDs were excluded from analysis.

For Northern blot analysis, 20 μg RNA was separated on 15% denaturing polyacrylamide gel and then electroblotted onto a Zeta-Probe GT Blotting Membrane (Bio-Rad). Following transfer, the membrane was dried and UV cross-linked. The probes were prepared using the StarFire Oligonucleotide Labeling System (Integrated DNA Technologies) according to the manufacturer's protocol. The blots were hybridized overnight at 50 °C in a buffer containing 5 v/v SSC, 20 mmol/L Na2HPO4 (pH 7.2), 7% SDS, 1 × Denhardt's, and 0.2 mg/mL salmon sperm DNA and then washed with 1 × SSC/1% SDS buffer at 50 °C (13). The probe sequences are as follows: hsa-miR-199a*, 5′-AACCAATGTGACAGACTCAGTGA-3′; hsa-miR-214, 5′-CTGGCTCTGCTGT-3′; hsa-miR-100, 5′-CACAAGTCCGATCTACGGGT-3′; and hsa-miR-200a, 5′-ACATCGTTACCCAGCAGTGT-3′.

RNA protection assay and quantitative reverse transcription-PCR.
Expression of miRNAs was also analyzed by RNA protection or miRVana miRNA Isolation kit (Ambion). The reverse quantitative transcription-PCR miRNA detection assay. For RNase protection assay, enriched small RNA was purified using the microRNA isolation kit (Ambion) according to the manufacturer's instructions. After hybridization and RNase treatment, the double-strand products were resolved in a 15% polyacrylamide 8 mol/L urea buffer at 50 °C. Labeling System (Integrated DNA Technologies) according to the manufacturer's instructions. Statistical analysis was done using two-sample t test, assuming equal variance, and P value was calculated based on two-tailed test.

miRNA locked nucleic acid in situ hybridization of formalin-fixed, paraffin-embedded tissue section.
A miRNA locked nucleic acid (LNA) probe was prepared by 5′-end labeling with digoxigenin-didUTP terminal transferase using the Dig-3′-End Labeling Kit (Roche). Following denaturation and proteinase K digestion, slides were prehybridized for 1 h and then hybridized with 10 nmol/L miRNA LNA probe in a hybridization buffer (Roche) for 12 h. After three consecutive washes in 4 × SSC/50% formamide, 2 × SSC, and 0.1% SSC, sections were treated with a blocking buffer (Roche) for 1 h and incubated with anti-DIG-AP Fab fragments (Roche) for 12 h. Following wash for three times in 1 × maleic acid and 0.3% Tween 20 buffer, reactions were detected in a detection solution [100 mmol/L Tris-HCl (pH 9.5) and 100 mmol/L NaCl] in the presence of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega) and then visualized under a microscope.

Results
Frequent deregulation of miR-199a*, miR-214, miR-200a, and miR-100 in human ovarian cancer.
miRNA profiles have been reported in different types of tumors derived from different organs (3–12), including ovarian cancer (5). However, the frequency and pathobiological significance of aberrant miRNA expression in human ovarian cancer have not been well documented. We initially analyzed miRNA expression in 10 human ovarian epithelial tumors and 10 “normal” HIose cell line pools by hybridization of the array containing 515 miRNAs. After normalization of control oligos, the differential expression of miRNAs between ovarian tumors and normal ovarian surface epithelial cells was quantified using a phosphorimager. Thirty-six of the 515 miRNAs showed differential expression with P values derived from the nonparametric Wilcoxon/Kruskal-Wallis test being <0.05. Of the 14 miRNAs that changed >1-fold were confirmed by Northern blot and/or qRT-PCR analysis (Fig. 1A and B; data not shown). To further validate our results, miR-199a*, miR-214, miR-200a, and miR-100, four of the most differentially expressed miRNAs, were analyzed in 30 primary ovarian cancers (Table 1). At least normal ovarian cells, more than half of the primary tumors exhibited elevated levels of miR-199a* (53%, 16 of 30) and miR-214 (56%, 17 of 30) and downregulated miR-100 (76%, 23 of 30). Moreover, increased miR-200a was detected in 43% of primary ovarian carcinomas examined (Table 1). Further, although the number of specimens is relatively small, the deregulation of miR-199a*, miR-214, and miR-200a seems to be associated with high-grade and late-stage tumors (Table 2). These data suggest that alterations of these three miRNAs could be involved in ovarian cancer progression.

The miR-214 targets PTEN leading to activation of the Akt pathway.
Because miR-214 was one of the most frequently up-regulated miRNAs in the ovarian tumors (Table 2; Fig. 1) and has recently been shown to play an important role in zebrafish muscle development (19), we next examined its potential targets by searching the PicTar and miBase database as well as sequence alignment analysis using GCG version 11.1. Among the candidates targeted, 3′-UTR of human PTEN contains a putative region (nucleotides 3257–3264, NM_000314) that matches to the seed sequence of hsa-miR-214, which is also conserved in mouse and rat (Fig. 2A). To examine whether PTEN is indeed the target of miR-214, a miR-214-negative cell line HIose-80 (Figs. 2B and 3A) was transfected with pcDNA3.1/V5-His-Topo-miR-214. The cells transfected with pcDNA3.1/V5-His-Topo vector alone and pcDNA3.1/V5-His-Topo-miR-199a* were used as controls. Immunoblotting and


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RT-PCR analyses revealed that PTEN protein but not mRNA was considerably decreased in miR-214–transfected HIOSE-80 cells (Fig. 2B). In contrast, knockdown of miR-214 by 2′-O-methyl miR-214 in A2780CP cells, which express high levels of endogenous miR-214 (Fig. 3A), increased the protein level of PTEN (Fig. 2B). Further, the phosphorylation levels of Akt, a major target of PTEN (20), and Akt substrates glycogen synthase kinase 3β and p70S6K were elevated by ectopic expression of miR-214 and decreased by knockdown of miR-214 (Fig. 2B), suggesting that miR-214 targets the PTEN/Akt pathway.

To further show that PTEN is negatively regulated by miR-214, we constructed luciferase reporters with wild-type (pGL3-PTEN-3′-UTR) and mutated (pGL3-PTENmut-3′-UTR) 3′-UTR of PTEN (Fig. 2A). Both the wild-type and the mutant reporters were introduced into A2780CP (miR-214 positive) and HIOSE-80 (miR-214 negative) cells, respectively. Luciferase activity of the wild-type, but not mutant, PTEN-3′-UTR reporter was significantly suppressed in miR-214–positive A2780CP cells but not in miR-214–negative HIOSE-80 cells. Moreover, ectopic expression of miR-214 in HIOSE-80 cells inhibited the wild-type but not the mutated reporter activity (Fig. 2C).

Having observed that miR-214 negatively regulates PTEN in cell culture system, we asked if this regulation is seen in vivo. Inverse correlation of expression of PTEN and miR-214 was investigated in
primary ovarian tumor specimens. Of the 30 primary ovarian tumors examined, 13 exhibited down-regulation of PTEN and 17 had overexpression of tumors examined, 13 exhibited down-regulation of PTEN and 17 primary ovarian tumor specimens. Of the 30 primary ovarian tumors examined, 13 exhibited down-regulation of PTEN and 17 had overexpression of tumors examined, 13 exhibited down-regulation of PTEN and 17 primary ovarian tumors examined, 13 exhibited down-regulation of PTEN and 17

miR-214 is an antiapoptotic factor that is associated with cisplatin resistance. Because Akt is a major cell survival pathway and its activation plays a key role in multiple drug resistance, including cisplatin (20, 21), we next examined the effects of miR-214 on cell survival and cisplatin resistance. Figure 3A shows that expression levels of miR-214 are low in immortalized human surface epithelial cell lines HIOSE-80 and MCC-3 as well as A2780S and OV119 cells compared with other ovarian cancer cell lines examined. Because A2780S and OV119 cells are sensitive to cisplatin (22), we ectopically expressed miR-214 in these two cell lines and examined if expression of miR-214 renders the cells resistant to cisplatin-induced cell death. Following the transfection of pcDNA3.1/V5-His-Topo–miR-214 and G418 selection, stable pool cells were obtained and the expression of miR-214 was confirmed by qRT-PCR (Fig. 3B). The cells transfected with miR-214 and pcDNA3.1/V5-His-Topo vector were treated with cisplatin or vehicle DMSO. As shown in Fig. 3C and D, the expression of miR-214 confers the cells resistant to cisplatin-induced cell death, suggesting that miR-214 is an antiapoptotic factor.

Having shown an elevated level of miR-214 in cisplatin-resistant A2780CP cells (Fig. 3A), we next examined if knockdown of miR-214 is able to override cisplatin resistance. A2780CP cells were transfected with 2'-O-me-anti-miR-214. The cells transfected with 2'-O-me-scrubbed miR were used as control. Following 72 h of transfection, qRT-PCR analysis showed that level of miR-214 was significantly decreased in the cells treated with 2'-O-me-anti-miR-214 (Fig. 4A). Further, the cells were treated with cisplatin or vehicle DMSO. Cell viability analysis revealed that knockdown of miR-214 alone reduced cell survival ~20% in A2780CP cells. Moreover, blocking miR-214 expression considerably sensitized A2780CP cells to cisplatin-induced apoptosis (Fig. 4B and C). Taken collectively, these data indicate that miR-214 could play an important role in cisplatin resistance.

Akt inhibitor, API-2/triciribine/TCN, or introduction of PTEN cDNA lacking 3’-UTR reduces cell survival and CDDP resistance induced by miR-214. Because ectopic expression of miR-214 reduces PTEN expression leading to activation of Akt pathway (Fig. 2B and C) and inhibition of the cisplatin-induced cell death (Fig. 3C and D), we next reasoned that inhibition of Akt should override miR-214–induced cell survival and cisplatin resistance. We have previously identified a specific Akt inhibitor, API-2/triciribine, which is currently in clinical trial (17). To test this hypothesis, miR-214–transfected A2780S cells were treated with API-2/triciribine, in combination with or without cisplatin. The cells transfected with Topo vector were used as control. As shown in Fig. 5A, API-2 abrogated miR-214–activated Akt and significantly inhibited miR-214–induced cell survival and cisplatin resistance.

It has been documented that miRNAs negatively regulate the expression of their targets primarily through base-pairing interactions in the mRNA 3’-UTR, leading to mRNA degradation or translational

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<th>Table 1. Alterations of miRNA and tumor histopathology</th>
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*Intensity of signal is ≥2-fold compared with that of normal ovary and/or HIOSE cells.

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<th>Table 2. miRNA expression level and tumor grade and clinical stage</th>
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*Statistical analysis was done using two-sample t test and P value was calculated based on two-tailed test.
miRNA Profiling in Ovarian Cancer and miR-214 Targets PTEN

Figure 2. miR-214 negatively regulates PTEN through binding to 3'-UTR of the PTEN. A, sequence alignment of human miR-214 with 3'-UTR of PTEN. The seed sequence of miR-214 (top) matches 3'-UTR of PTEN (middle). Bottom, mutations of the 3'-UTR of PTEN for creating the mutant luciferase reporter construct. B, rows 1 to 4, miR-214 reduces PTEN protein but not mRNA levels. HOISE-80 cells (left) were transfected with pcDNA3.1/V5-His-Topo-miR-214, pcDNA3.1/V5-His-Topo-miR199a*, and vector alone and immunoblotted with indicated antibodies; rows 5 and 6, the expression of miR-214 and miR-199a* was determined by qRT-PCR; row 8, PTEN mRNA level was measured by RT-PCR. U6 (row 7) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; row 9) were used for controls. Middle and right, knockdown of miR-214 inducing PTEN expression. A2780CP cells were transfected with antisense 2'-O-me oligonucleotide targeting miR-214 at concentration of 150 pmol/L/well (6-well plate) with Lipofectamine 2000. Anti-miR199a* and scramble 2'-O-me oligonucleotide were used as controls. Middle, after incubation of 72 h, cells were lysed and immunoblotted with indicated antibodies; right, inhibition of miR-214 and miR-199a* expression by 2'-O-me oligonucleotide in A2780CP cells was shown by qRT-PCR. GSK3β, glycogen synthase kinase 3β. C, miR-214 inhibits wild-type but not mutated PTEN-3'-UTR reporter activity. miR-214-positive A2780CP cells (left) and miR-214-negative HOISE-80 cells (right) were transiently transfected with indicated plasmids. Following 36 h of incubation, cells were subjected to luciferase assay. Columns, mean of three independent experiments; bars, SD. D, rows 1 and 2, representative tumor and normal tissue lysates were analyzed by Western blot with indicated antibodies; row 3, expression of miR-214 was analyzed by qRT-PCR; row 4, U6 was used as a control.
inhibition, which depends on whether it is partially matched or completely matched with the target genes. Because miR-214 down-regulates PTEN through binding to 3’-UTR of PTEN mRNA (Fig. 2), ectopic expression of PTEN by transfection of the cDNA that only contains the coding region of PTEN should escape the regulation by miR-214 and thus attenuate or decrease miR-214 function. To this end, pcDNA-PTEN lacking 3’-UTR was introduced into miR-214–transfected A2780S cells and then treated with or without cisplatin for 24 h. As shown in Fig. 5B, expression of PTEN decreased Akt activation induced by miR-214 and sensitized the miR-214–A2780S cells to cisplatin-induced apoptosis. These results further indicate that the PTEN/Akt pathway is a major target of miR-214 and largely mediates miR-214 antiapoptotic function.

Although adjuvant chemotherapy with cisplatin achieves clinical response in ~80% of patients, the tumor recurs in most patients within 3 years following treatment due to the development of chemoresistance (23). Having shown that miR-214 is involved in cisplatin resistance in ovarian cancer cell lines, we next examined if miR-214 is involved in cisplatin resistance in patients with ovarian cancer (e.g., more frequent overexpression in chemoresistant/recurrent tumors than in sensitive/primary lesions). Among 30 primary ovarian tumors examined, 11 patients with recurrent (chemoresistant) ovarian cancer were readmitted at H. Lee Moffitt Cancer Center. miRNA LNA in situ hybridization (LNA-ISH) analysis revealed that miR-214 levels were low or undetectable in eight primary tumors but elevated in their recurrent lesions (Fig. 5C; data not shown). The remaining tumors expressed high level of miR-214 in both primary and recurrent tumors. These data further suggest that miR-214 plays an important role in chemoresistance.

**Discussion**

Profiles of miRNA have been reported in different types of human malignancy (3–12). Thus far, there is a miRNA DNA copy number study of human ovarian cancer in combination with breast cancer and melanoma using high-resolution array-based comparative genomic hybridization. In this study, a high proportion of genomic loci containing miRNA genes exhibited DNA copy number alterations in ovarian and breast cancers and melanoma (5). In the present report, we performed miRNA expression profiling in normal HIOSE and epithelial ovarian carcinomas and showed that up-regulation of miR-214, miR-199a*, and miR-200a and
down-regulation of miR-100 are recurrent events and that alterations of the first three miRNAs seem to be associated with late-stage and high-grade ovarian tumors (Table 2). This finding suggests that deregulation of miR-214, miR-199a*, and miR-200a could contribute to ovarian tumor progression rather than initiation.

Although members of the let-7 family, miR-21, miR-145, miR-221, and miR-155, are often deregulated in several cancers, including carcinomas of breast, lung, and colon (24), there are miRNAs deregulated in specific neoplasms. For example, miR-122a, a liver-specific miRNA, is down-regulated in hepatocellular carcinoma (25); miR-204 and miR-211 are up-regulated in insulinomas (26). Accumulated evidence shows that miRNA expression signatures correlate well with specific clinical cancer characteristics and can be used to classify normal and cancerous tissues as well as subtype
of malignancy (27–29). Therefore, miRNA signatures might be more effective than mRNA signatures in categorizing, detecting, and predicting the course of human cancers as well as in characterizing developmental origins of tumors (29). Our study showed different expression patterns of miRNAs between ovarian cancer and normal HIOSE cells. Whereas deregulation of let-7, miR-100, miR-200a, and miR-125 has been detected in other tumors, alterations of other miRNAs, including miR-424 and miR-494, were to the best of our knowledge only observed in ovarian cancer. Further investigation is required for evaluating these miRNAs as specific markers in ovarian tumors.

Previous studies have shown that miRNA could serve as "oncogene" or "tumor suppressor gene" and regulate different cellular processes by targeting hundreds of genes. We showed that miR-214 is highly expressed in the cisplatin-resistant A2780CP cell line compared with its corresponding cisplatin-sensitive cell line A2780S. Knockdown of miR-214 overrides cisplatin resistance in A2780CP cells, whereas ectopic expression of miR-214 renders A2780S and OV119 cells resistant to cisplatin-induced apoptosis. It has been well documented that constitutive activation of Akt contributes to chemoresistance in different types of tumors, including ovarian carcinoma (20). miR-214 blocks PTEN translation leading to activation of the Akt pathway (Fig. 2). These indicate that miR-214 plays an important role in cisplatin resistance by targeting PTEN/Akt pathway. Although alterations of several oncogenes (e.g., Ras, Src, and Bcl2) and tumor suppressor genes (e.g., p53, RB, and p16) are closely associated with chemoresistance, the involvement of miRNA in this process has not been

Figure 5. Inhibition of Akt or transfection of PTEN cDNA lacking 3’-UTR overrides miR-214–induced cell survival. A, Topo-miR-214 and vector stably transfected A2780S cells were treated with Akt inhibitor API-2/triciribine (10 μmol/L) and/or cisplatin (20 μmol/L). The cells treated with DMSO were used as control. After 24 h of treatment, cells were subjected to immunoblotting analysis with indicated antibodies (top) and assayed for caspase-3/7 activity (bottom). B, top, A2780S cells were stably transfected with indicated plasmids and assayed for expression of PTEN, phospho-Akt-S473, and total Akt; bottom, after treatment with or without cisplatin for 24 h, cells were examined for caspase-3/7 activity. C, LNA-ISH. miR-214 was labeled with digoxigenin-ddUTP using the Dig-3’-End Labeling kit and hybridized to paraffin sections of normal ovary (middle left) as well as a patient with primary and recurrent/cisplatin-resistant ovarian serous carcinoma (middle right and right). Left and middle, H&E staining. Unlike normal ovarian surface epithelial cells (middle left) and primary tumor (middle right), recurrent/cisplatin-resistant tumor (right) expresses high level of miR-214. OC, ovarian carcinoma.
documented. Thus, our study provided direct evidence that miRNA expression deregulation is of critical importance in chemoresistance of human ovarian cancer.

Mutation of PTEN has been detected only in endometrioid ovarian cancer (30). However, down-regulation of PTEN protein is frequently detected in serous and mucinous epithelial ovarian tumors (31). The mechanism of down-regulation of PTEN was thought to be promoter hypermethylation. However, the demethylation agent 5-aza-2’-deoxycytidine failed to restore PTEN protein expression, suggesting that PTEN is highly regulated at the translational level and that methylation of the PTEN gene plays a subordinate role in ovarian cancer (32). In the present study, we showed that PTEN is negatively regulated by miR-214 at the protein level and that down-regulation of PTEN largely correlates with elevated levels of miR-214 in ovarian cancer (Fig. 2D). Therefore, these data indicate that miR-214 could be a causal factor of the down-regulation of PTEN in human ovarian cancer.

In summary, our study suggests that deregulation of miR-214, miR-199a*, miR-200a, and miR-100 is a frequent event in ovarian cancer. Alteration of miR-214, miR-199a*, and miR-200a seems to be associated with tumor progression. Further, miR-214 induces cell survival and cisplatin resistance by targeting the PTEN/Akt pathway. Therefore, these miRNAs could play an important role in the pathogenesis of this malignancy and are potential targets for ovarian cancer intervention. Further investigations are required for characterization of miRNAs as prognostic and/or diagnostic markers in ovarian cancers by examining a large series of specimens as well as their in vivo role in ovarian tumor development by creating transgenic and/or knockout mice.

Acknowledgments


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References

Correction: miRNA Profiling in Ovarian Cancer and miR-214 Targets PTEN

In the article on miRNA profiling in ovarian cancer and miR-214 targets PTEN in the January 15, 2008 issue of Cancer Research (1), the first sentence of the section "Target in vitro reporter assay" on page 426 should read as follows: For luciferase reporter experiments, the 3'-UTR segments of PTEN predicted to interact with miR-214 were amplified by PCR from human genomic DNA and inserted into the MluI and HindIII sites of pMIR-Reporter (Ambion) immediately downstream from the stop codon of luciferase.

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