5-Fluorouracil and oxaliplatin modify the expression profiles of microRNAs in human colon cancer cells *in vitro*

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Abstract. MicroRNAs (miRNAs) have recently taken center stage in the field of human molecular oncology. Most of the chemotherapeutics are able to interfere with nucleic acid metabolism and gene expression. The purpose of this study was to determine how 5-fluorouracil (5-FU) and oxaliplatin (L-OHP) modify the expression profiles of miRNAs in HCT-8 and HCT-116 colon cancer cells and whether the pharmacodynamic mechanisms of the chemotherapeutics could rely in part on their influence on miRNA expression. The expression profiles of miRNAs were determined using a miRNA microarray containing 856 human miRNA probes. The expression of selected miRNAs was then validated by real-time RT-PCR. Fifty-six up- and 50 down-regulations of miRNA expression with statistical significance were identified in colon cancer cells following exposure to 5-FU or L-OHP compared to matched control cells. The down-regulations of miR-197, miR-191, miR-92a, miR-93, miR-222 and miR-1826, whose expression was significantly down-regulated in both cell lines after the treatment of one drug or in one cell line following exposure to either drug, were further validated. Analysis of the relevant literature indicated that, in line with the tumor suppressive activity of 5-FU and L-OHP, the six down-regulated miRNAs might function as oncogenes due to their overexpression in cancers, and some of them correlated with the poor prognosis and treatment-resistance of cancer. In conclusion, we identify the modification of miRNA expression profiles in colon cancer cells following exposure to 5-FU and L-OHP, and our results indicate that their pharmacodynamic mechanisms could rely in part on their influence on the down-regulated miRNA expression. Further studies are needed to determine whether these miRNAs and their target genes might potentially provide for novel molecular markers and act as novel targets for treatment by interference.

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Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide with over one million cases annually, and is also the fourth commonest cause of cancer death worldwide although the mortality rate has decreased slightly during the past decades (1). Chemotherapy remains the primary treatment for both resectable and advanced colorectal cancer to improve overall survival and quality of life for patients. The combination of 5-fluorouracil (5-FU), a classical anti-metabolite and oxaliplatin (L-OHP), a third-generation platinum, has an important role in either palliative or adjuvant chemotherapy of colorectal cancer. 5-FU plus L-OHP has been demonstrated to improve patient outcome in the metastatic setting (2), and it also has a clear role as an adjunct to surgery to improve survival in stage III and certain 'high-risk' stage II colorectal cancer patients (3).

MicroRNAs (miRNAs) are non-coding, single-stranded RNAs of ~22 nucleotides, derived from miRNA duplex complexes processed from larger pre-miRNAs by the RNase III enzyme Dicer (4). General mechanism of miRNA action in animal and human cells is the inhibition of translation after forming a complex called the RNA-interference-induced silencing complex (RISC) or the induction of mRNA cleavage (4,5). Although the precise biological role of miRNAs has not been entirely elucidated, it has been found that each miRNA can regulate the expression of hundreds of target genes. At present, several hundreds of mammalian miRNAs have been identified, and some of them have been shown to be involved in essential biological process such as differentiation, proliferation and apoptosis (6,7).

Over 52% of miRNAs are located in cancer-associated fragile chromosomal sites (8). Certain miRNAs may function as oncogenes and others as tumor suppressors (9). The levels of some miRNAs are frequently changed in colorectal cancer (10,11). Although the roles of miRNAs in colorectal cancer development are being extensively investigated and become

increasingly well defined, their involvement in tumor cell response to chemotherapy has been suggested by a few reports. Moreover, there are several examples of rapidly induced change of miRNA expression as a consequence of various external stimuli like hypoxia, 5-FU, and adriamycin (12-14). However, despite the extensive use of the combination of 5-FU and L-OHP in clinical practice, the molecular mechanisms for the antitumor effect have not been fully elucidated, and little is known about how miRNA genes are modified in human colon cancer cells following exposure to 5-FU and L-OHP.

We speculate that if the pharmacodynamic mechanisms of 5-FU and L-OHP could rely, at least in part, on their influence on miRNA expression since both of them are able to interfere with nucleic acid metabolism. If such an association is confirmed, it might provide a potential opportunity for miRNAs acting as novel targets for treatment by interference and biomarkers for selection of chemotherapeutic agents. To test this hypothesis, in the present study, we used microarray analysis to identify the comprehensive modification of miRNA expression profiles in HCT-8 and HCT-116 colon cancer cells following exposure to the two extensively used anticancer drugs, i.e., 5-FU and L-OHP. The down-regulations of miR-197, miR-191, miR-92a, miR-93, miR-222 and miR-1826, whose expression was significantly down-regulated in both cell lines after the treatment of one drug or in one cell line following exposure to either drug, were then validated using real-time RT-PCR. Analysis of the relevant literature indicated that the pharmacodynamic mechanisms of the chemotherapeutics could rely in part on their influence on miRNA expression, and both these miRNAs and their target genes might potentially provide for novel molecular markers and act as novel targets for treatment by interference.

Materials and methods

Cell lines and reagents. Human colon cancer cell lines HCT-116 and HCT-8 were purchased from Cell Center of Peking Union Medical College. HCT-116 and HCT-8 cells were maintained respectively in DMEM medium (Hyclone) and RPMI-1640 medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone), 2 mM L-Glutamine, and antibiotics. Both cell lines were grown at 37˚C in a humidified incubator with 5% CO_2. L-OHP and 5-FU were purchased from Sanofi Aventis (Shanghai, China) and Xdhelp (Shanghai, China), respectively.

In vitro chemosensitivity array. Cancer cells were seeded at a density of $2x10^3$ cells/well in 96-well microtiter plates and allowed to attach overnight. Freshly prepared 5-FU or L-OHP alone was then added with the final concentration ranging from 0 to 256 μM. After 72 h, cell viability was assessed using MTT array. The absorbance at 570 nm of each well was read on a spectrophotometer. Results were expressed in terms of the concentration required to inhibit cell growth by 50% relative to untreated cells (IC_{50}). IC_{50} values were calculated using Graphpad Prism software (Graphpad Software, Inc.). Three independent experiments were performed in quadruplicate, and the data reported represented the mean ± SD.

Drug treatment of cancer cells in vitro. Cancer cells were seeded at a density of $6x10^6$ cells/20 ml in 75-cm^2 flasks. On day 1 after seeding, freshly prepared 5-FU or L-OHP alone was added. The concentrations of L-OHP for HCT-8 and HCT-116 cells were 4.25 μM (1.5X IC_{50}) and 3.00 μM (2.5X IC_{50}), respectively, and the concentrations of 5-FU for both cell lines were 10.00 μM as described previously (15). Both chemotherapeutics-treated and control cells were further cultured at 37˚C for 24 h, removed from tissue culture, washed two times with iced PBS, and immediately processed for RNA extraction.

MicroRNA microarray analysis. Total RNA was extracted from both chemotherapeutics-treated and matched control cells using TRIzol reagent according to the manufacturer's protocol (Invitrogen). The concentration of total RNA was evaluated by measuring the absorbance at 260 nm. Microarray assay was performed by LC Sciences (Houston, TX). Total RNA (2-5 μg) was size-fractionated (<300 nucleotides) using a YM-100 Microcon centrifugal filter (from Millipore). Poly-A tails were added to the RNA sequences at the 3'-ends using a poly(A) polymerase, and nucleotide tags were then ligated to the poly-A tails. The tagged RNAs were hybridized overnight to the dual-channel microarray μParaFlo microfluidics chips (LC Sciences) using a micro-circulation pump (Atactic Technologies). The chip contained 856 mature human miRNA probes, together with controls. There were four spot replicates for each probe. Each detection probe consisted of a chemically modified nucleotide coding segment complementary to target miRNA (from sanger miRBase release 12.0, http://microrna.sanger.ac.uk/sequences/) and a spacer segment of polyethylene glycol to extend the coding segment away from the substrate. The probes were made by *in situ* synthesis using PGR (photogenerated reagent) chemistry. The hybridization melting temperatures were balanced by chemical expression of the detection probes. Hybridization used 100 μl 6X SSPE buffer (0.90 M NaCl, 60 mM Na_2HPO_4, 6 mM EDTA, pH 6.8) containing 25% formamide at 34˚C. After hybridization detection used fluorescence labeling using tag-specific Cy3 and Cy5 dyes. Hybridization images were collected using a laser scanner (GenePix 4000B, Molecular Device) and digitized using Array-Pro image analysis software (Media Cybernetics).

Data analysis. Data analysis involved normalization, data adjustment, t-test and clustering. Normalization was carried out using a cyclic LOWESS (Locally-weighted Regression) method to remove system related variations. Data adjustment included data filtering, Log_2 transformation, and gene centering and normalization. The data filtering removed miRNAs with (normalized) intensity values below a threshold value of 32 across all samples. t-test was performed between 'control' and 'test' sample groups. T-values were calculated for each miRNA, and p-values were computed from the theoretical t-distribution. MiRNAs with p-values below 0.01 were selected for cluster analysis. The clustering was done using hierarchical method and was performed with average linkage and Euclidean distance metric (16).

MicroRNA real-time RT-PCR. Total RNA was extracted from both chemotherapeutics-treated and matched control cells

Table I. The primers used for quantitative RT-PCR for miRNAs.

Primer	Sequence (5'-3')
miR-1826 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATTGCG
miR-1826 UP	GCATTGATCATCGACACTTCGA
miR-93 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCT
miR-93 UP	GCGCAAAGTGCTGTTCGTG
miR-222 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCAG
miR-222 UP	GCGGAGCTACATCTGGCT
miR-197 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTGGG
miR-197 UP	GCGTTCACCACCTTCTCC
miR-191 RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGCTG
miR-191 UP	GCGCAACGGAATCCCAAAA
miR-92a RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGGCC
miR-92a UP	CCGGGTATTGCACTTGTCC
U6 snRNA RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATATG
U6 snRNA UP	GCGCGTCGTGAAGCGTTC
DOWN	GTGCAGGGTCCGAGGT

using TRIzol reagent (Invitrogen). The concentration of total RNA was quantitated by measuring the absorbance at 260 nm. The primers were synthesized by Invitrogen (Table I). RNA was reversely transcribed to cDNA using TIANScript M-MLV reverse transcriptase (Tiangen Biotech Co., Ltd., Beijing) under the following conditions: 16˚C for 30 min, 42˚C for 45 min and 85˚C for 5 min. Real-time PCR was performed sequentially on an IQ5 instrument (Bio-Rad) using SYBR Premix Ex Taq (Perfect Real-Time) kit according to the manufacturer's protocol (Takara). The reactions were incubated at 95˚C for 10 sec, followed by 45 cycles of 95˚C for 5 sec and 60˚C for 30 sec. U6 snRNA was used as endogenous control. PCR was performed in triplicate. The relative expression of each miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method (17).

Databases. The miRNA sequences were obtained from miRBase (http://microrna.sanger.ac.uk). The target genes were predicted with miRanda (http://cbio.mskcc.org/mirnaviewer), PicTar (http://pictar.mdc-berlin.de/) and TargetScan (http: //www.targetscan.org/).

Results

The growth of colon cancer cells was suppressed by chemotherapeutics. The chemosensitivity of the colon cancer cells was assayed using MTT test. The growth of both HCT-8 and HCT-116 colon cancer cells was suppressed by the treatment of 5-FU or L-OHP for 72 h (Fig. 1). The IC_{50} values of 5-FU and L-OHP for HCT-8 cells were 8.21 and 2.89 μM, respectively, and the corresponding values for HCT-116 cells were 11.06 and 1.20 μM (Table II).

miRNA microarray analysis showed that miRNAs were differentially expressed after the treatment of chemotherapeutics. To identify the differentially expressed miRNAs in human colon cancer cells following exposure to chemothera-

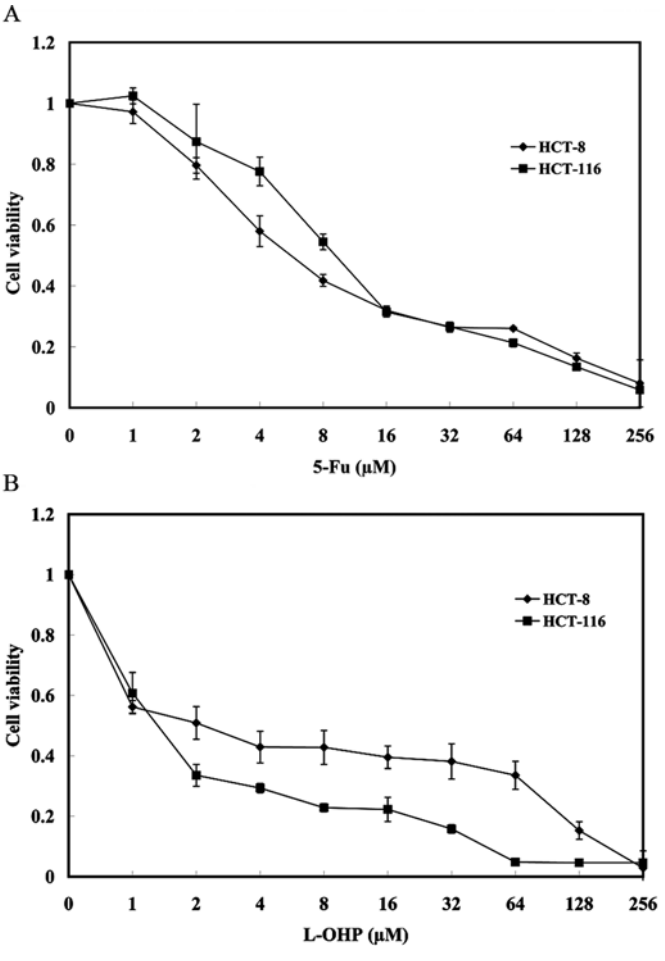

Figure 1. Chemosensitivity of HCT-8 and HCT-116 colon cancer cells towards 5-fluorouracil (5-FU) and oxaliplatin (L-OHP): 5-FU (A) and L-OHP (B). Cells were seeded at a density of $2x10^3$ cells/well in 96-well microtiter plates and allowed to attach overnight. Freshly prepared 5-FU or L-OHP alone was then added and cultured for an additional 72 h. Cell viability was assessed using MTT array. The absorbance at 570 nm of each well was read on a spectrophotometer. Three independent experiments were performed in quadruplicate, and the data reported represent the mean ± SD.

Table II. IC_{50} (72 h) of 5-fluorouracil (5-FU) and oxaliplatin (L-OHP) for HCT-8 and HCT-116 colon cancer cells.

	IC_{50} (72 h) (μM)	
Cell line	5-FU	L-OHP
HCT-8	8.21±0.55	2.89±0.01
HCT-116	11.06±1.16	1.20±0.21

peutics, we used comprehensive, human-specific miRNA microarray analysis of RNA extracted from chemotherapeutics-treated and matched control cells. From the microarray normalized values, the significant differences between the chemotherapeutics-treated and matched control cancer cells for a given detectable miRNA signal were calculated. The differentially expressed miRNAs with $p<0.01$ were analyzed using hierarchical clustering (Fig. 2). miRNAs (24 and 6) were significantly ($p<0.01$) up-regulated in HCT-8 cells following exposure to 5-FU and L-OHP for 24 h, respectively, and the corresponding numbers of up-regulated miRNAs were 18 and 8 in HCT-116 cells. The magnitude of increase of these miRNAs in chemotherapeutics-treated cells ranged from 0.25- to 3.66-fold (Log_2 scale) of control subjects. More than 10 miRNAs, such as miR-203 and miR-23a, were significantly up-regulated in both cell lines after the treatment of one drug or in one cell line following exposure to either drug (data not shown). In this study, the priority of further investigation was given to the significantly down-regulated miRNAs. There were 17 and 3 miRNAs significantly ($p<0.01$)

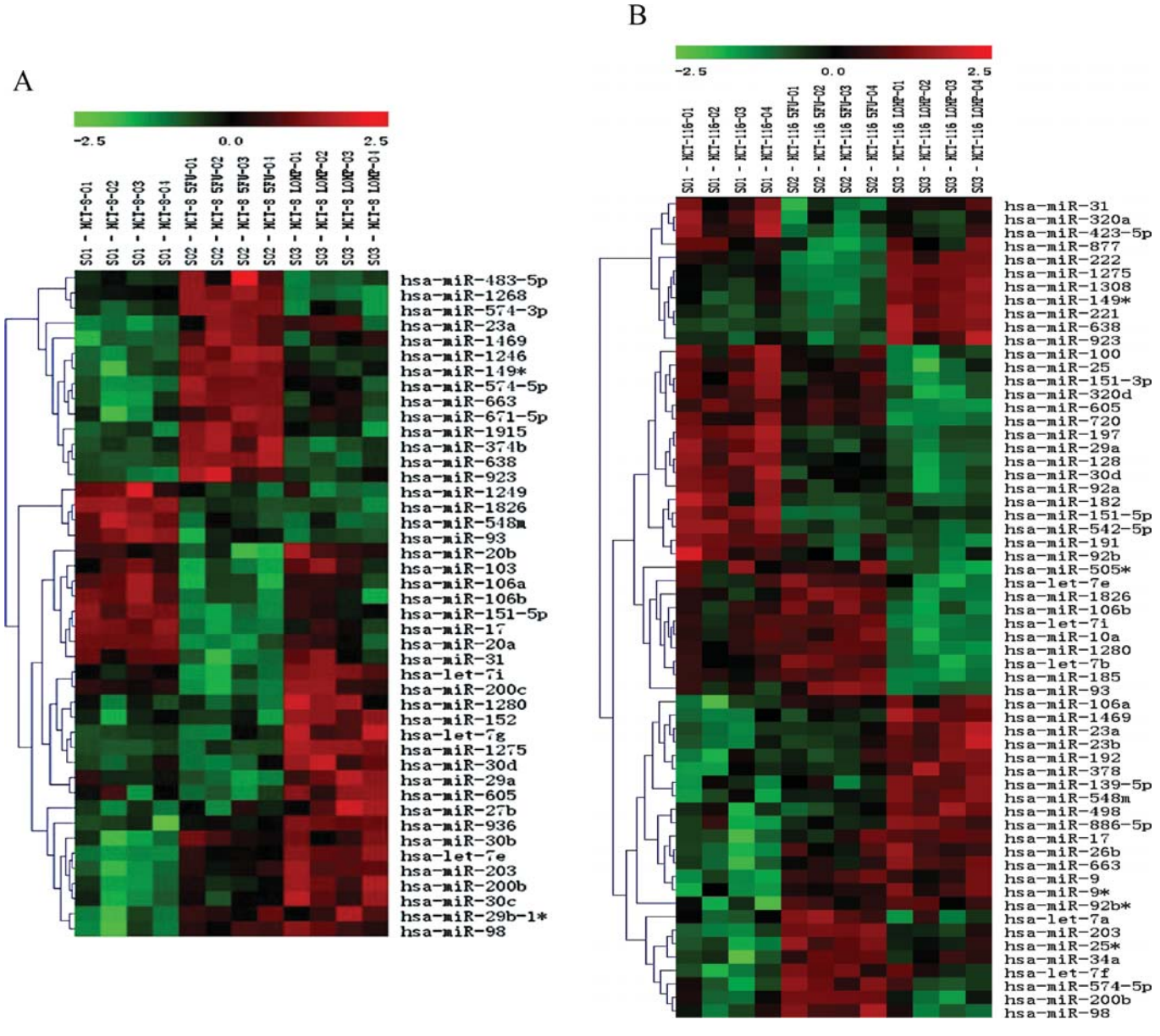

Figure 2. MiRNA gene expression profiles obtained from cluster analysis: HCT-8 cells (A) and HCT-116 cells (B). These miRNAs showed significant changes of expression between chemotherapeutics-treated and matched control cell lines (ANOVA function; $p<0.01$). Red indicates up-regulation; green, down-regulation; black, no change. The legend on the right indicates the miRNA represented in the corresponding row. The row code at the top represents the color scale of the Log_2 values.

Table III. Significantly ($p<0.01$) down-regulated miRNAs identified by microarray analysis in HCT-8 and HCT-116 colon cancer cells following exposure to 5-fluorouracil (5-FU) or oxaliplatin (L-OHP) for 24 h.

HCT-8 colon cancer cell line			
After exposure to 5-FU (10.00 μM)		After exposure to L-OHP (4.25 μM)	
microRNA	Fold change, Log_2 scale	microRNA	Fold change, Log_2 scale
miR-1826	-1.50	miR-1826	-1.47
miR-20a	-1.33	miR-93	-1.14
miR-17	-1.26	miR-151-5p	-0.34
miR-18a	-1.25		
miR-106b	-1.21		
miR-106a	-1.02		
miR-93	-0.86		
miR-24	-0.81		
miR-191	-0.78		
miR-151-5p	-0.69		
miR-320a	-0.67		
miR-10a	-0.57		
miR-222	-0.56		
miR-423-5p	-0.51		
miR-320c	-0.47		
miR-200c	-0.41		
miR-92a	-0.38		
HCT-116 colon cancer cell line			
After exposure to 5-FU (10.00 μM)		After exposure to L-OHP (3.00 μM)	
microRNA	Fold change, Log_2 scale	microRNA	Fold change, Log_2 scale
miR-197	-1.30	miR-197	-2.00
miR-1275	-1.25	miR-10a	-1.52
miR-423-5p	-0.69	miR-1280	-1.09
miR-128	-0.68	miR-128	-1.04
miR-151-5p	-0.61	miR-185	-0.79
miR-222	-0.46	miR-320d	-0.63
miR-31	-0.45	miR-25	-0.61
miR-15b	-0.44	let-7b	-0.59
miR-182	-0.39	miR-30d	-0.55
miR-320a	-0.38	miR-29a	-0.55
miR-29a	-0.37	miR-151-5p	-0.50
miR-191	-0.35	miR-191	-0.43
miR-1308	-0.33	miR-92a	-0.40
miR-92a	-0.31	miR-92b	-0.34
miR-320d	-0.30	let-7i	-0.39

down-regulated in HCT-8 cells following exposure to 5-FU and L-OHP for 24 h, respectively, and the corresponding numbers of down-regulated miRNAs were 15 for both in HCT-116 cells (Table III).

Valiadation of six down-regulated miRNAs by real-time RT-PCR. To confirm the results of the miRNA microarray analysis, we performed quantitative RT-PCR (qRT-PCR) for six selected down-regulated miRNAs using the same RNA extracted from chemotherapeutics-treated and matched control cells as used in microarray analysis. The six miRNAs, i.e. miR-197, miR-191, miR-92a, miR-93, miR-222 and miR-1826, were chosen because their expression was significantly down-regulated in both cell lines after the treatment of one

A

microRNA	Fold change Log_2 scale
HCT-8/5-Fu	
miR-1826	-1.50
miR-93	-0.86
miR-191	-0.78
miR-222	-0.56
miR-92a	-0.38
HCT-8/L-OHP	
miR-1826	-1.47
miR-93	-1.14
HCT-116/5-Fu	
miR-197	-1.30
miR-222	-0.46
miR-191	-0.35
miR-92a	-0.31
HCT-116/L-OHP	
miR-197	-2.00
miR-191	-0.43
miR-92a	-0.40

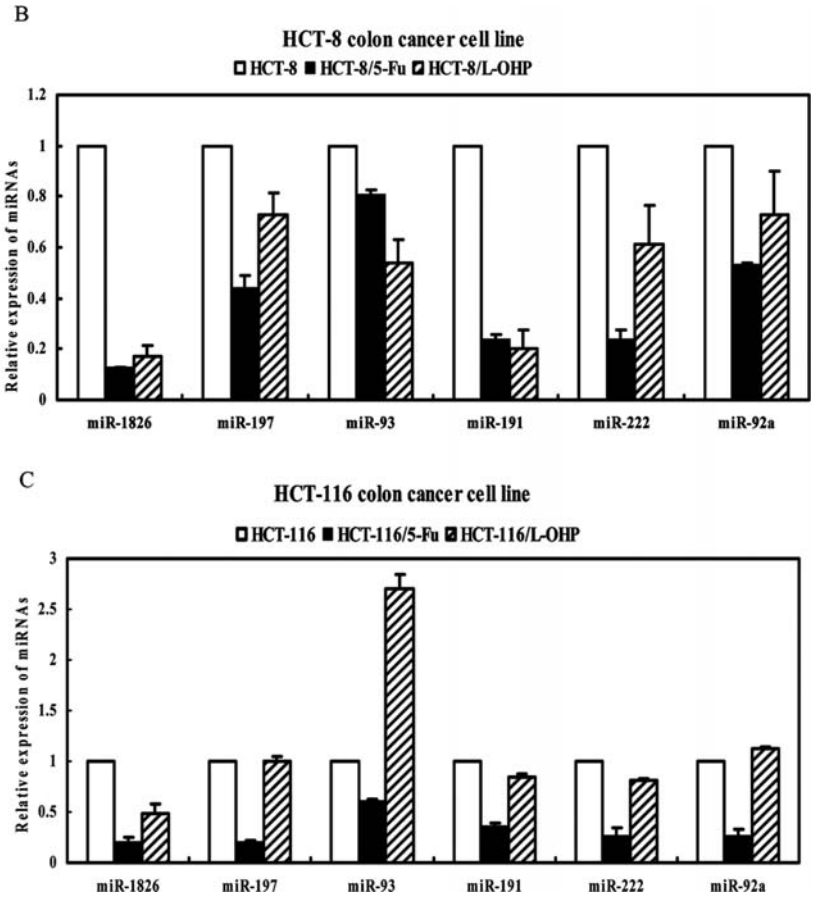

Figure 3. Validation of the down-regulations of miR-197, miR-191, miR-92a, miR-93, miR-222 and miR-1826, whose expression was significantly down-regulated in both cell lines after the treatment of one drug or in one cell line following exposure to either drug when analyzed by miRNA microarray analysis. (A) Fourteen down-regulations of miRNA expression identified by microarray analysis. (B) and (C) the results of quantative RT-PCR analysis. Triplicate assays were performed for each RNA sample and the relative amount of every miRNA was normalized to U6 snRNA. Data are shown as fold changes of miRNA levels in HCT-8 or HCT-116 colon cancer cell line following 24-h' exposure to 5-fluorouracil (5-FU) or oxaliplatin (L-OHP) (HCT-8/5-FU, HCT-8/L-OHP, HCT-116/5-FU and HCT-116/L-OHP) relative to matched control cell line (HCT-8 and HCT-116), which is set as 1 (mean ± SD).

drug or in one cell line following exposure to either drug when analyzed by miRNA microarray analysis. Moreover, priority was given to miRNAs that were subjected to subsequent functional analyses.

The qRT-PCR data showed that the expressions of all the six miRNAs were down-regulated in both cell lines after the treatment of 5-FU or L-OHP except for miR-93, miR-92a and miR-197 in HCT-116 cells following exposure to L-OHP (Fig. 3B and C). Twelve of the fourteen significant down-regulations identified by miRNA microarray analysis were validated after qRT-PCR, although miR-197 and miR-92a had been identified as down-regulated miRNAs in HCT-116 cells after the treatment of L-OHP by microarray analysis (Fig. 3).

Discussion

MiRNAs have recently taken center stage in the field of human molecular oncology. Even a small change in miRNA expression could cause a profound effect on gene expression of hundreds of mRNAs, and miRNAs have been increasingly recognized as key regulators in many biological systems. It was reasonable to hypothesize that antineoplastic drugs could alter miRNA gene expression pattern since most of them are able to interfere with nucleic acid metabolism and gene expression. Both 5-FU and L-OHP have an important role and are extensively used in colorectal cancer (2,3). However, the function of miRNAs in the pharmacodynamic mechanisms of the two chemotherapeutics remains largely a mystery.

In our study, using microarray analysis we identified 56 up-regulations and 50 down-regulations of miRNA expression with statistical significance in colon cancer cells following exposure to 5-FU or L-OHP. Then most of the down-regulations of six selected miRNAs (miR-197, -93, -92a, -222, -191 and -1826), whose expression was down-regulated in both cell lines after the treatment of one drug or in one cell line following exposure to either drug, were confirmed in qRT-PCR analysis. The qRT-PCR data also showed that all the expressions of these six miRNAs were down-regulated after the treatment of 5-FU or L-OHP except for miR-93, miR-92a and miR-197 in HCT-116 following exposure to L-OHP.

The biochemical function of these six miRNAs has been considered following analysis of the relevant literature. Most of them are miRNAs with well characterized cancer association. In line with the tumor suppressive activity of 5-FU and L-OHP, of particular interest is the observation that most of the six down-regulated miRNAs are overexpressed in cancers,

and some in colorectal cancer. The expression status of some miRNAs in cancers is correlated with clinical outcome. Some of their target genes are recognized as tumor suppressors.

miR-191 has been shown significantly overexpressed in colorectal tumors compared to normal colorectal samples (18). Up-regulation of miR-191 has also been detected in breast cancer (19). In acute myeloid leukemia (AML), patients with high expression of miR-191 have significantly worse overall and event-free survival than AML patients with low expression (20). Furthermore, PLCD1 and RNF139, two predicted candidate target genes of miR-191, are tumor suppressors in esophageal squamous cell carcinoma and human hereditary kidney cancer, respectively (21,22).

miR-93 is up-regulated in gastric cancer tissues compared to the corresponding normal tissues (23). It is also significantly overexpressed in the serum of ovarian cancer patients compared to controls (24). Higher expression of miR-93 is significantly correlated with a poor prognosis of serous ovarian carcinoma (25). miR-93 belongs to miR-106b-25 cluster. In gastric cancer, it is found that miR-93 suppresses p21, a Cip/Kip family member of Cdk inhibitors, and up-regulation of the miR-106b-25 cluster impairs the TGFß tumor suppressor pathway, interfering with the expression of p21 and Bim (26).

miR-92a is encoded by the miR-17-92 gene cluster. It is found to be overexpressed in medulloblastoma, primary chronic lymphocytic leukemia and the serum from ovarian cancer patients (27,28,24). In colorectal cancer, a recent Japan study (29) showed that the expression of miR-92 was significantly higher in colorectal cancer tissues than in normal tissues. Ng *et al* (30) found that the plasma level of miR-92 was significantly elevated in colorectal cancer patients and it differentiated colorectal caner from gastric cancer, inflammatory bowel disease and normal subjects. The elevation was detected not only in advanced stages but also in early stages of tumor, suggesting that this marker might be a potential non-invasive molecular marker for colorectal cancer screening.

miR-222 is among the most-studied miRNAs in human cancers. miR-222 has been shown to be up-regulated in gastric cancer, thyroid tumors, pancreatic cancer, childhood B-cell precursor acute lymphoblastic leukemia and hepatic cell cancers (23,31-34). The overexpression of miR-222 is found to be correlated with the resistance to hormone treatment and aggressiveness in prostate carcinoma (35,36). In breast cancer, miR-221/222 is associated with tamoxifen resistance, and the expression of miR-221 and miR-222 is significantly elevated in HER2/neu-positive primary human breast cancer tissues that are known to be resistant to endocrine therapy compared with HER2/neu-negative tissue samples (37). p27, a Cip/Kip family member of Cdk inhibitors, is a cell cycle inhibitor and tumor suppressor. miR-222 represses p27 expression in various cancers, such as gastric cancer (23), prostate carcinoma (35), thyroid papillary carcinoma (38) and breast cancer (37), and this repression promotes tumor cell proliferation.

miR-197 is identified as one of the up-regulated miRNAs in squamous cell carcinomas of the tongue compared to normal tissues (39). It is also differentially overexpressed in thyroid tumors vs. hyperplastic nodules (31).

Additionally, this study included a more up-to-date and comprehensive evaluation of human miRNAs, enabling the identification of several novel and less well-characterized miRNAs. No relevant reports about the relationship between miR-1826 and cancer have been found. We predicted its target genes, and found 147 candidates. The expressions of some target genes, such as SOX11 and TEGT, correlate with good prognosis of cancer (40,41). Worth of note is the predicted target gene RKIP (PEBP1), which is known as a metastasis suppressor (42). Chemotherapeutic agents have been shown to induce tumor apoptosis through induction of RKIP expression. In colorectal cancer patients, loss of RKIP expression is a marker of tumor progression and distant metastasis (43), and RKIP expression correlates with overall and disease-free survival (44).

Furthermore, the microarray data showed that there were variances in the profiles of the modified miRNAs either between the two cell lines following exposure to the same drug or between 5-FU and L-OHP in one cell line. These instances may be on account of the minor variances between either the chemosensitivities of the two cell lines or the pharmacodynamic mechanisms of 5-FU and L-OHP. The microarray analysis also found that miR-151-5p was down-regulated in both cell lines following exposure to either 5-FU or L-OHP, needing further investigation.

In conclusion, our preliminary study illustrates the modification of miRNA expression profiles in colon cancer cells following exposure to 5-FU and L-OHP. The down-regulations of miR-191, -222, -92a, -93 -197 and -1827 were validated by qRT-PCR. Analysis of the relevant literatures shows that the six down-regulated miRNAs may function as oncogenes due to their overexpression in cancers, and some of them correlate with poor prognosis and treatment-resistance. The findings indicate that the pharmacodynamic mechanisms of the tumor suppressive activity of these chemotherapeutics could rely in part on their influence on miRNA gene expression through certain pathways. Both these miRNAs and their target genes might potentially provide for novel molecular markers and act as novel targets for treatment by interference. The information may also be useful as a starting point to future studies to validate the predicated targeted genes in colon cancer cell lines and to observe the effects of antisense oligonucleotides against these miRNAs in colorectal cancer xenografts.

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