Supplementary Materials

Supplementary Methods

Samples and clinical data

In TCGA data, eighty-five percent of the samples are grade III tumors. All patients received a platinum-based chemotherapy and the tumor samples were obtained before treatment. The median progression-free survival and overall survival of the cohort are similar to those in previously published trials. Forty-five percent of the patients remained alive and 25% were free from disease at the time of last follow-up. Thirty-one percent of patients experienced disease progression within six months of completing platinum-based therapy. The follow-up time ranged from 0 to 179 months and the median follow-up was 30 months.

Cell lines, cell culture, reagents, and miRNA transfection

Human ovarian cancer cell lines (HeyA8, OVCA433, SKOV3) and HeLa were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).The authentication of all cell lines was done by the Characterized Cell Line Core Facility at The University of Texas MD Anderson Cancer Center (MDACC), Houston, TX by the STR Short Tandem Repeat (STR) Method. HeyA8, SKOV3, OVCA433 and HeLa cancer cells were cultivated with RPMI1640 medium supplemented with 10% FBS. The miR-506 mimic and negative control were obtained from Dharmacon (Chicago, IL). The anti-miR-506 LNA and negative control were obtained from EXIQON (Woburn, MA). Cells were seeded at 2×10^5 per well in 6-well plates and allowed to attach for at least 16 hours. MiR-506 mimic, anti-miR-506 LNA, or miR-Ctrl was transfected using LipofectamineRNAiMAX

(Invitrogen, Grand Island, NY) at a final concentration of 20 nM. Total RNA and protein were extracted 48 hours after transfection.

Real-time RT-PCR analysis and microarray gene expression analysis

Total RNA was isolated with an*mir*Vana^{TM}miRNAisolation kit (Ambion, Grand Island, NY). Reverse transcription was performed using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol. TaqMan real-time PCR assays for miR-506 were obtained from Applied Biosciences, Inc. (Grand Island, NY). Cyclophilin and β-actin were used as normalization controls. Data were analyzed by using the -2ΔΔct method.

Microarray experiments were carried out using whole human genome oligo arrays with 44k 60-mer probes (Agilent Technologies, Palo Alto, CA), using 500ng of total RNA starting material according to the manufacturer's protocol. Hybridized arrays were scanned with Agilent's dual laser-based scanner. Feature Extraction software version 8.0 (Agilent Technologies) was used to link a feature to a design file and to determine the relative fluorescence intensity between two samples. The microarray data are publicly available at GEO(accession number GSE50850).

Colony-formation assay

Twenty-four hours after transfection with 20nm miR-506,miR-Ctrl, or anti-miR-LNA, cells were harvested. Transfected cells were seeded in a 6-well plate (500 cells/well)and treated with cisplatin for 48 hours or olaparib for 7 days and then allowed to recover for 10-14 days, during which time the surviving cells spawned a

colony of proliferating cells. Colony formation was quantified by staining the cells with 0.1% crystal violet and counting surviving colonies containing >50 cells.

MTT assay

Twenty-four hours after transfection with 20nm miR-506,miR-Ctrl, or anti-miR-LNA,cells were seeded onto 96-well plates (1×10^3 cells/well) and treated with a titration of cisplatin or olaparib. The medium and drug were replenished at day 3 for olaparib treatment.After incubation for 5(cisplatin) or 7(olaparib) days, cell viability was estimated using the MTT reagent (Sigma Chemical, St. Louis, MO), and surviving fractions were calculated. Cell survival was calculated by normalizing the absorbance to that of untreated controls.

Western blot analysis

Primary β-actin antibody(goat) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal Rad51 antibody was purchased from Lab Vision Corporation (Fremont, CA). In brief, 30 μg of whole-cell lysate from each sample was loaded on a 10% polyacrylamide gel for electrophoresis; the membrane was blocked in 5% non-fat milk in 1× Tris-buffered saline solution (pH 7.4) containing 0.05% Tween-20 and probed with primary antibodies at a concentration of 1:1000 (for β-actin) or 1:200(for RAD51). The secondary antibodies were used at a concentration of 1:10,000. The proteins were visualized using the SuperSignal West Pico or SuperSignalFemtochemiluminescent substrate from Pierce (Rockford, IL).

Luciferase reporter assay

The 3′-UTR of *RAD51*containing the predicted binding site of miR-506 was amplified separately from normal fetal genomic DNA by PCR using the following specific primers: *RAD51*forward: GGCGAGCTCAGCTGAAGCTATGTTCGC;reverse: GGCTCTAGATTCACTACTAATAACTACTTTATTAG. The PCR products were cloned into the pmirGLO-control vector between the *Sac*I and *Xba*I sites in the correct direction. The consensus miR-506 binding sites in the 3′-UTR of *RAD51*were deleted by PCR using a QuikChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA). The following primers were used: forward: GTATGAAGTATCTTTGACATGAGGAATGACTTGGGTTTAAC;reverse: GTTAAACCCAAGTCATTCCTCATGTCAAAGATACTTCATAC. All clones were verified by DNA sequencing. For the luciferase reporter assay, subconfluentHeLa cells in 24-well plates were transfected with a triplicate repeat of pmirGLO reporter plasmid (0.5 μg) or *pmirGLO-RAD51*–3′-UTR and a miR-506 mimic, anti-miR-506, or miR-Ctrl (50 nM) and Lipofectamine 2000 (1μL; Invitrogen). Forty-eight hours after transfection, cells were subjected to lysis and luciferase activities were determined as for a dual-luciferase assay reporter system (Promega, Fitchburg, WI), according to the manufacturer's instructions.

Single-cell gel electrophoresis (comet) assay

Comet assay was performed per manufacturer’s instructions (Trevigen, Gaithersburg, MD). Proliferating HeyA8 cells were transfected with miR-Ctrl or miR-506 mimic. Twenty-four hours later, transfected cells were treated with 100 μM cisplatin for 1

hour, were allowed to repair for 18 hours, and then were analyzed by single-cell gel electrophoresis. Briefly, treated or untreated cells were collected, resuspended in ice-cold PBS at 1×10^5 cells per mL, mixed with low-melt agarose (1:10 ratio), and spread on frosted glass slides. After the agarose solidified, the slides were successively placed in lysis and alkaline solutions (Trevigen). Slides were then subjected to electrophoresis (1 V per cm of distance between electrodes) for 10 minutesin 1× TBE buffer, and cells were fixed with 70% (*v*/*v*) ethanol and stained with SYBRGold.DNA damage was quantified for 100 cells for each experimental condition by determining the tail moment, a function of both the tail length and intensity of DNA in the tail relative to the total DNA, using the Comet Score software (CASPLab). Gray level images were acquired under a laser scanning microscope. Differences between groups were analyzed by the Student *t*-test.

Double strand break–induced homologous recombination repair assay

A stable derivative of HeLa cells was established by transfection with pDR-*GFP* using Lipofectamine 2000 and selection in1.5 μg/mLpuromycin. Puromycin-resistant cells were cloned by limiting dilution in 96-well plates. Single colonies were plated in 12-well plates and transfected with cBAS to express the I-SceI endonuclease. Forty-eight hours after transfection, cells were trypsinized, and cells from each well (1×10^4) were counted by flow cytometry to select the clone with the highest GFP(+) rate. An average of three to four experiments were analyzedfinally yieldingHeLa-DR-GFP cells with a 9.72 ±1.13% GFP(+) rate. The reporter consists of two differentially mutated *GFP* genes oriented as direct repeats. Expression of I-SceI

generates a site-specific double-strand break within one of the mutated *GFP* genes, which, when repaired by gene conversion, results in a functional *GFP* gene;thus homologous recombination may be measured by the percentage of GFP-positive HeLa cells. Briefly, HeLa-DR-GFP cells were sequentially transfected with siRNA or miRNA mimic and the pCBASce vector. Two days later, GFP-positive cells were assayed by fluorescence-activated cell sorting (FACS) scan.

Immunofluorescence microscopy imaging

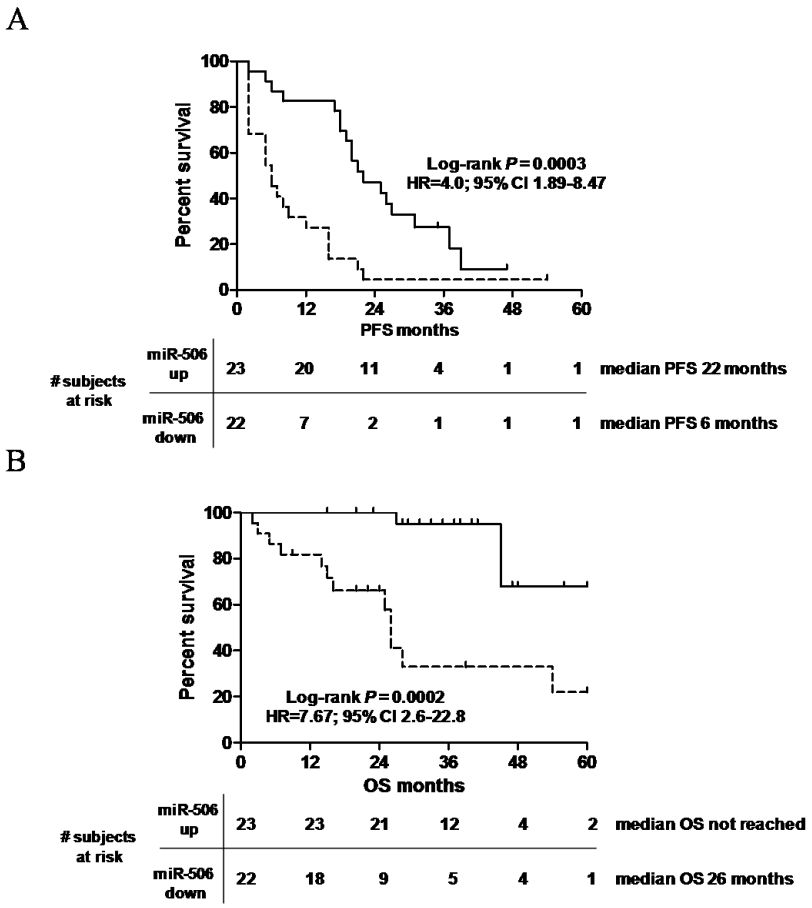
Cells were grown on coverslips and treated with 10μM cisplatin for 24hours. After treatment, cells were fixed with 1% paraformaldehyde for 20 minutes, blocked with 3% bovine serum albumin containing 0.1% Triton X-100 for 30 minutes, and incubated with antibodies against γH2AX (1:2000; Abcam, Cambridge, MA) or Rad51 (1:100; Santa Cruz Biotechnology) for 2 hours at room temperature. The cells were then incubated with species-specific Alexa597 (1:500, Invitrogen)–conjugated secondary antibody for 1hour at room temperature. Nuclei were counterstained with 4′6-diamidino-2-phenylindole (DAPI). Phase images were captured by a ZEISS HAL 100 microscope at a magnification of 200×. The fluorescence images were captured using a ZEISSAxioplan 2 microscope.

Immunohistochemical staining

Immunohistochemical staining was performed on tumor tissues from the mouse orthotopic model and tissue microarrays that included samples from 92 patients with serous ovarian cancer, both of which were assembled for our previous study [22]. Rabbit anti-human monoclonal antibody against RAD51 (1:250, sc8349;Santa Cruz

Biotechnology), and EnVision+System–HRP rabbit (Dako, Carpentaria, CA, USA)were used. RAD51-positive cells were definedas those with immunoreactivity in both cytoplasm andnucleus(mainly) and were quantified using a scoring systemof 0–9, multiplied by the intensity of the signal, andclassified by the percentage of positive cells (0, nosignal, 0%; 1, weak signal, <25%; 2, intermediatesignal, 25–50%; and 3, strong signal, >50%). miRNA*in situ* hybridization and RT–PCR had been performedin our previous study, and low and high miR-506expressionwas defined as scores of<6 and≥6,respectively [22].

Supplementary Figures

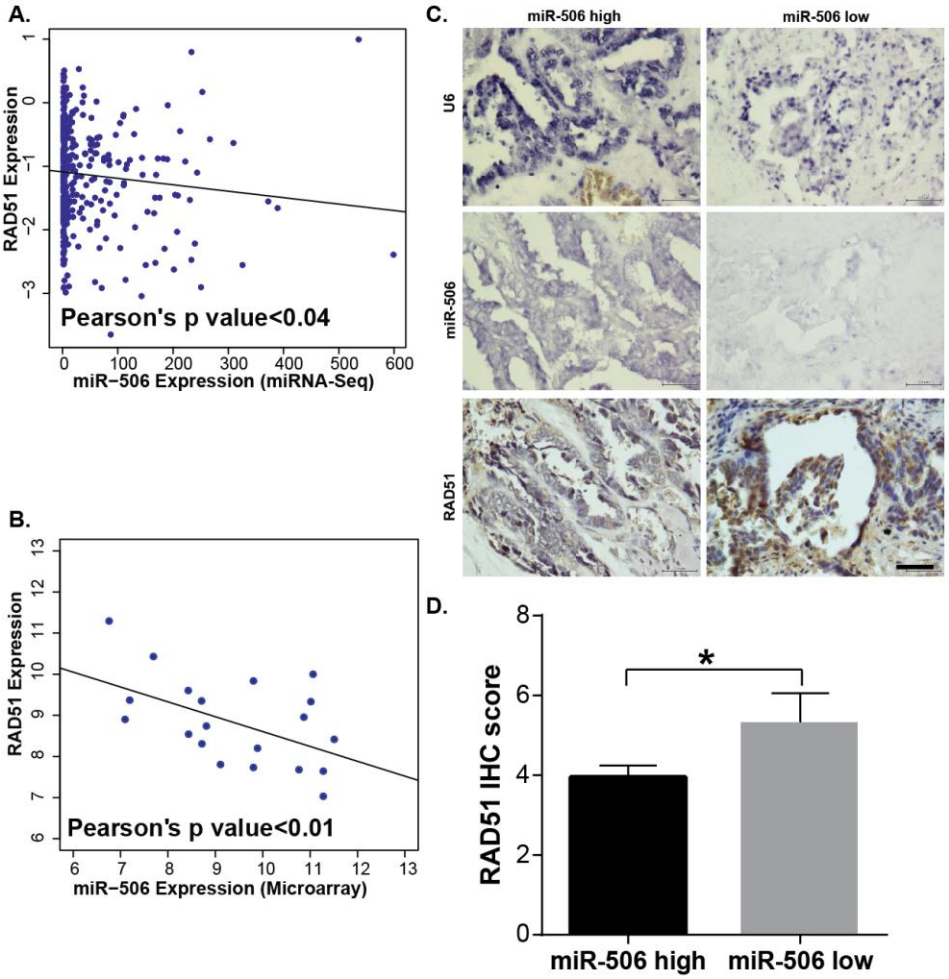


Supplementary Figure 1. miR-506 associated to patient's response to therapy and prognosis. Kaplan-Meier survival curves for PFS (**A**) and OS (**B**) of 45 consecutive EOC patients stratified for miR-506 below (dotted line) and above (continuous line) median expression.

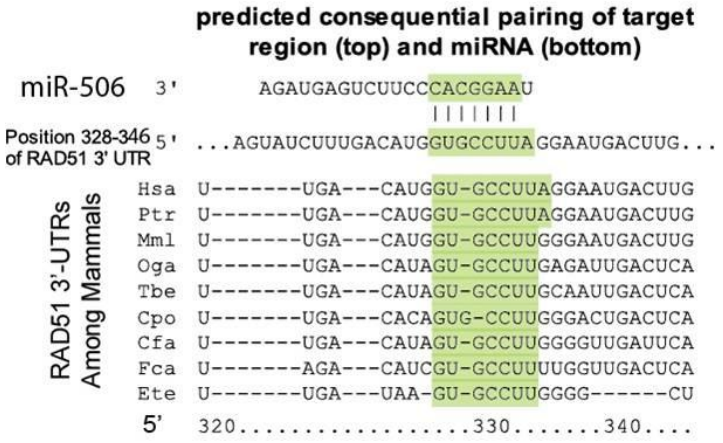
Multivariate Cox Model

	PFS		OS	
	HR (95% CI)	p	HR(95% CI)	p
miR506Status				
miR506_Low	1		1	
miR506_High	0.62(0.40-0.94)	0.02	0.52(0.34-0.91)	0.02
Stage				
II	1		1	
III	1.91(1.00-3.66)	0.03	2.32(0.94-5.71)	0.06
IV	2.22(1.11-4.66)	0.02	2.26(1.08-7.08)	0.03
Tumor Residual				
Macroscopic disease	1		1	
1-10 mm	1.67(1.18-2.35)	0	1.83(1.22-2.74)	0
10-20 mm	1.60(0.94-2.91)	0.08	1.70(0.96-3.02)	0.07
> 20 mm	1.45(0.93-2.15)	0.11	1.77(1.10-2.80)	0.02
Ethnic Background				
Ashkenazi Jewish	1		1	
Non-Ashkenazi	1.20(0.87-1.67)	0.21	1.12(0.77-1.78)	0.17
Age	1.00(0.99-1.01)	0.73	1.02(1.01-1.03)	0

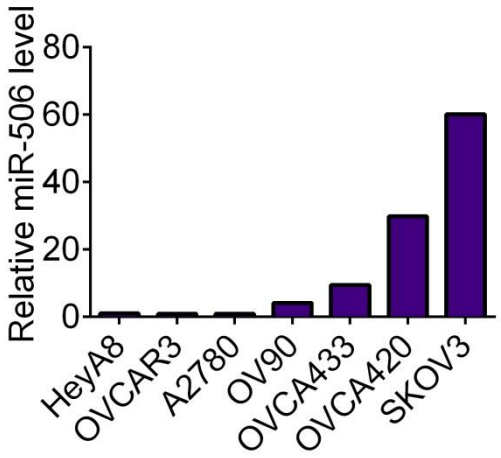
Supplementary Figure 2. Multivariable Cox Model of clinical relevant parameters in 468 ovarian cancer cases.



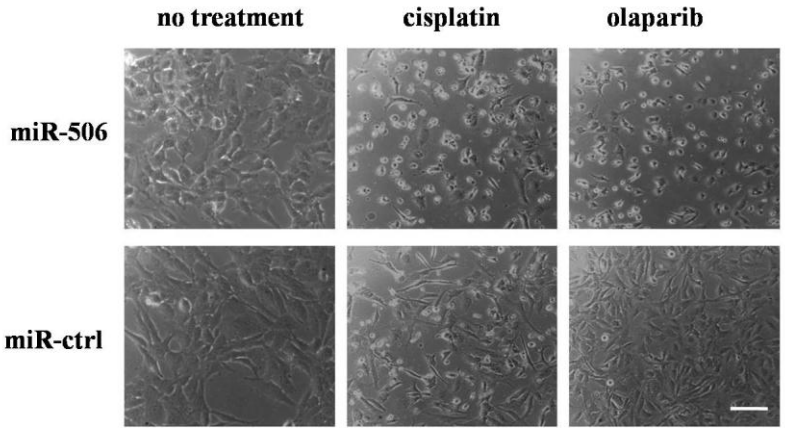
Supplementary Figure 3. There is an inverse correlation between miR-506 and RAD51 expression. A),B) Pearson's product-moment correlation curves of the levels of miR-506 from small RNA seq expression and RAD51 transcript from Agilent array in 445 cases TCGA dataset(A) and in 21 samples Bagnoli data(B), **C)** MiR-506 expression was inversely associated with RAD51 in serous ovarian cancers from Tianjin TMA human patients. Representative images of immunohistochemical staining for RAD51in cases expressing low or high levels of miR-506 are shown. Scale bar=200μM. **D)** Bar charts show the association between miR-506 level and expression of RAD51. The *x* axis represent relative expression of RAD51, as indicated by immunohistochemical staining (IHC); *$p = .037$, two-sided Student *t* test.



Supplementary Figure 4. TargetScan predicted that the *RAD51* 3′-UTR has one miR-506 binding site, which is highly conserved among different species. Green/yellow color band in the figure indicates the binding site (the sequence complementary to the seed sequence of miR-506). Abbreviations: Hsa: Human; Ptr: Chimpanzee; Mml: Rhesus; Oga: Bushbaby; Tbe: Threeshrew; Cpo: Guinea Pig; Cfa: Dog; Fca: Cat; Ete: Tenrec.



Supplementary F**igure 5. Relative levels of miR-506 in several ovarian cancer cell lines.** The level of miR-506 in HeyA8 was taken as 1.



Supplementary Figure 6. The morphological changes induced by miR-506 after treatment with cisplatin or olaparib in HeyA8 cells. Scale bar=50μM.