

Supplemental Information

Cisplatin sensitivity mediated by WEE1 and CHK1 is mediated by miR-155 and the miR-15 family

Lynn M. Pouliot, Yu-Chi Chen, Jennifer Bai, Rajarshi Guha, Scott E. Martin, Michael M. Gottesman, Matthew D. Hall

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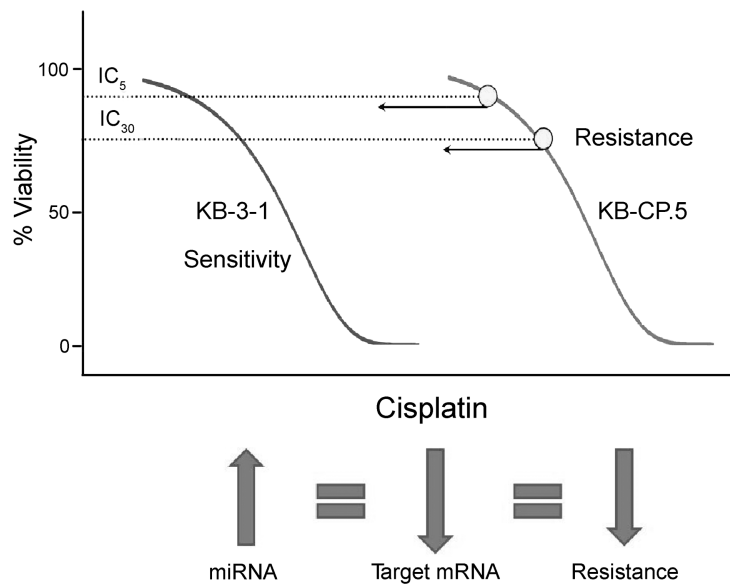


Figure S1. Schematic demonstrating the objective of the high throughput screen. Cells were incubated with each RNAi/mimic in the screen. CP-r KB-CP.5 cells were treated with cisplatin at a dose shown to kill 5% of the cell population (IC₅) and 30% of the cell population (IC₃₀) and examined for increased sensitivity to drug in the presence of each siRNA or miRNA mimic.

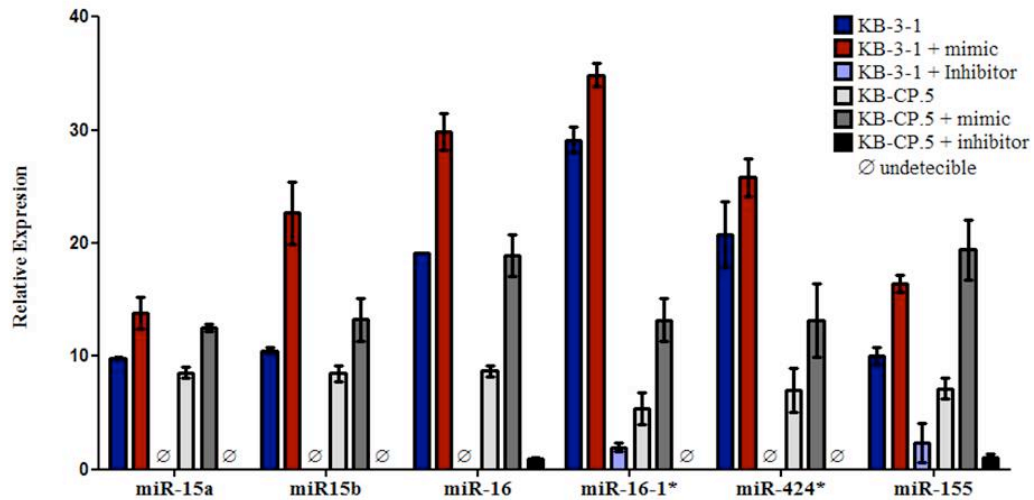


Figure S2, related to Figures 2B-E and 3. Expression of miR family members in KB-3-1 and KB-CP.5 cells. Demonstration of increased expression of each miRNA family member following transfection with miRNA mimic, and loss of expression of each miRNA family member following transfection with siRNA directed against the family member. RNA extraction was performed 72 h after transfection of cells. In most cases, miRNA could not be detected in miRNA inhibitor (siRNA) treated cells (signified by Ø).

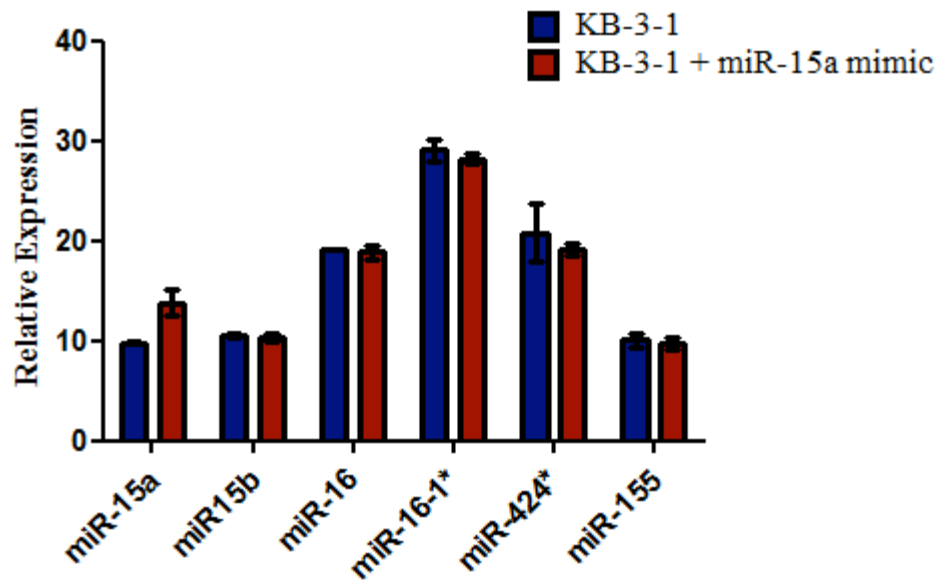


Figure S3, related to Figures 2B-E and 3. Expression of miR family members in KB-3-1 transfected with miR-15a mimic. RT-PCR performed to determine if miR-15a mimic gave off target effects to other family members. miR-15a mimic increases miR-15a levels but has no statistical effect on expression of other family members. Other miRNA mimics examined showed a similar lack of off-target effects (not shown).

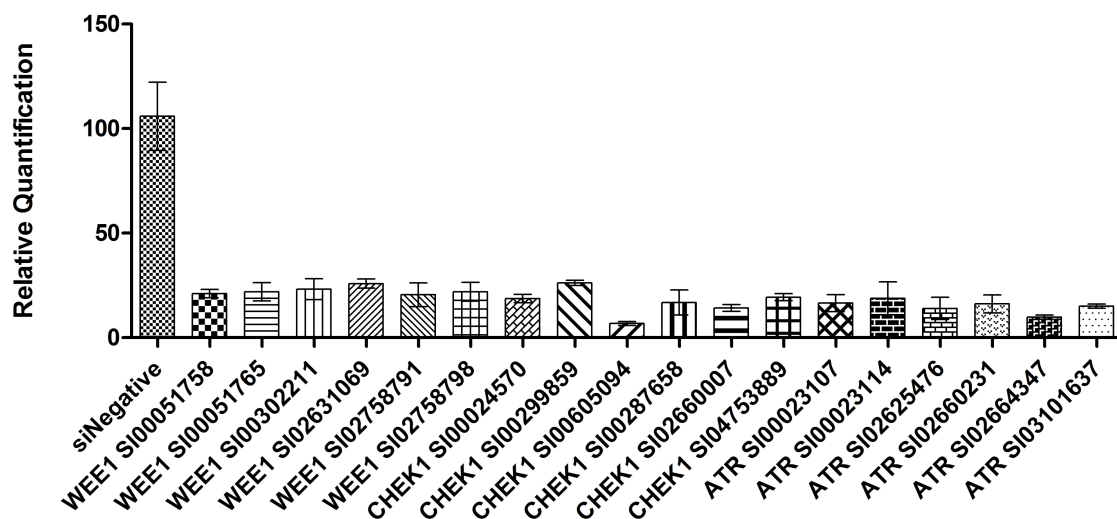


Figure S4, related to Figure 1A. Expression of WEE1, CHEK1 and ATR in KB-CP.5 cells transfected with one of six siRNA against each gene. RT-PCR performed to measure expression, normalized against expression of that gene in siNegative-transfect cells. All data is in triplicate and presented as a percent mean +/- SD.

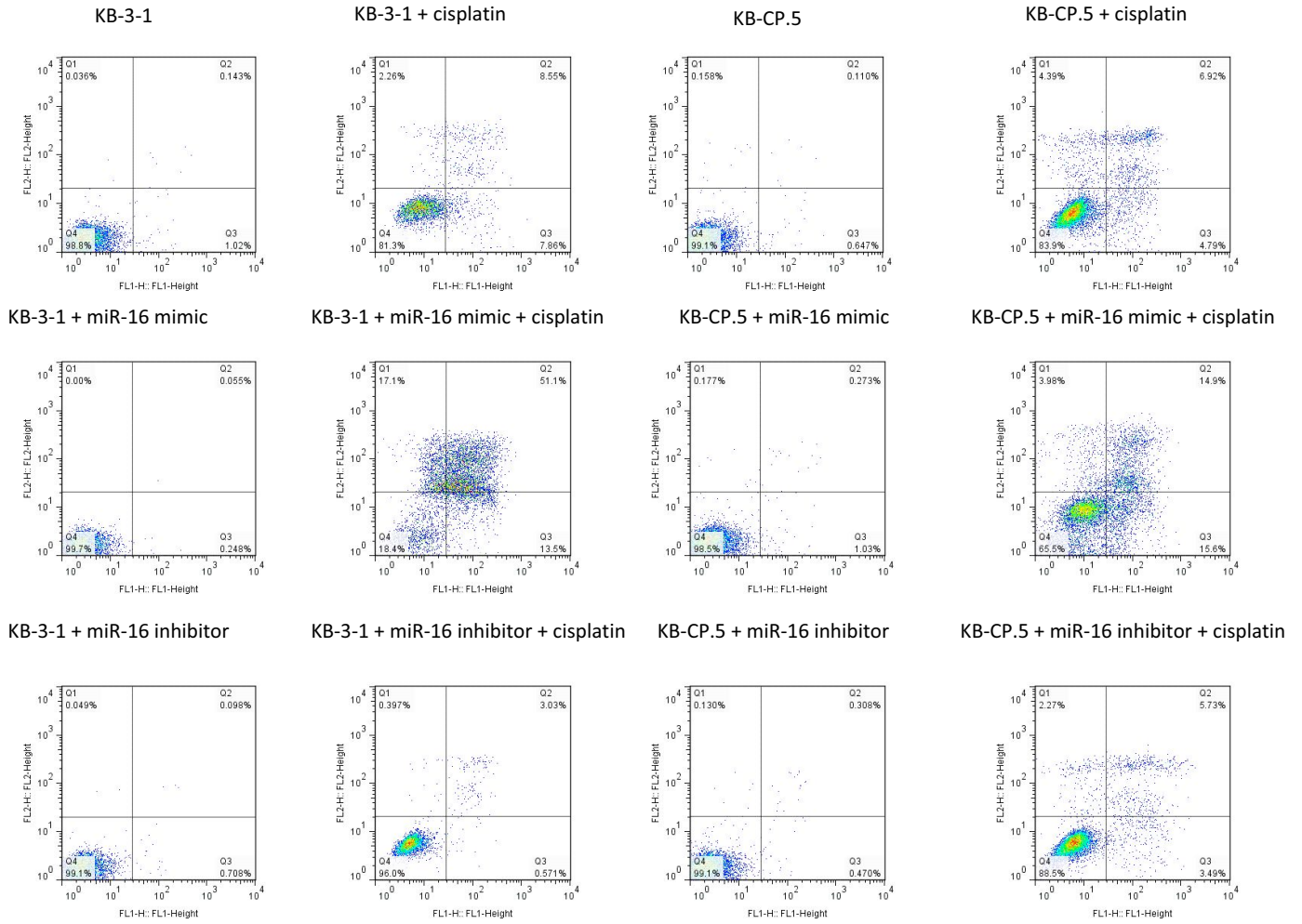


Figure S5, related to Table 4. Representative cell viability analysis dot blots of KB-3-1 and KB-CP.5 cells transfected with either siNeg, miR-16 mimic or miR-16 inhibitor alone or in the presence of cisplatin (5 mM for KB-3-1 or 25 mM for KB-CP.5).

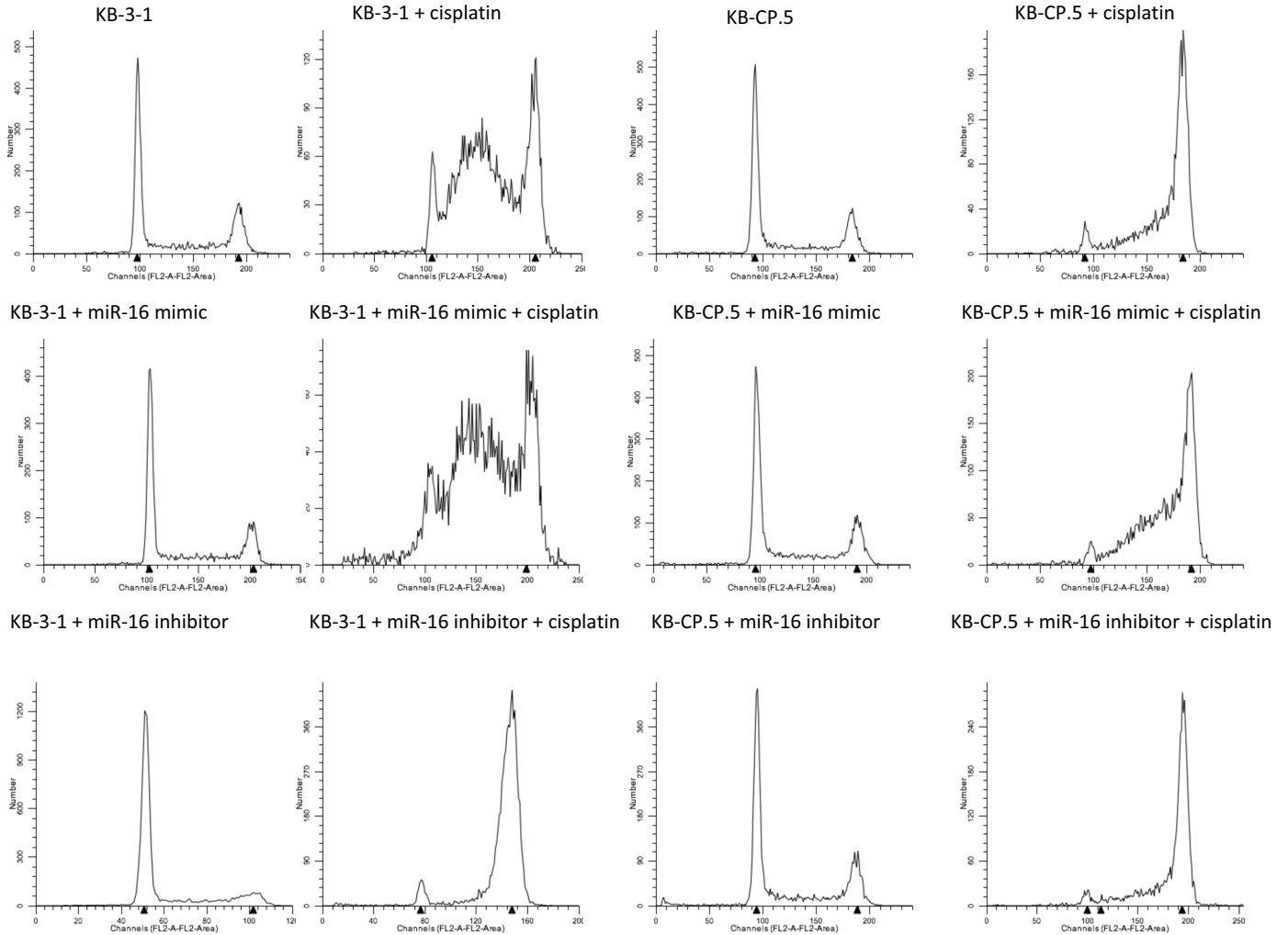


Figure S6, related to Table 4. Representative cell cycle analysis histograms of KB-3-1 and KB-CP.5 cells transfected with either siNeg, miR-16 mimic or miR-16 inhibitor alone or in the presence of cisplatin (5 mM for KB-3-1 or 25 mM for KB-CP.5).

Table S1. Protocol for the high-throughput screens

Sequence	Parameter	Value	Description
1	Reagent	40 μ L	KB-CP.5 lines plated at 1200 cells/well
2	Reagent	0.1 μ L RNAi Max	RNAi
3	Time	24 h	37 °C, 5% CO ₂
4	Reagent	10 μ L	Cells dosed with cisplatin
5	Time	72 h	37 °C, 5% CO ₂
6	Reagent	As per assay instructions	Luminescent viability assay
7	Detection		Read Plate

Table S2. Cytotoxicity (IC₅₀) values for KB-3-1 and KB-CP.5 lines with and without miRNA mimics or miRNA siRNA (inhibitors), and challenged with cisplatin

Cell Type	microRNA	IC ₅₀ (μM) cisplatin	IC ₅₀ (μM) +miRNA mimic	IC ₅₀ (μM) +miRNA inhibitor
KB-3-1	Hsa-miR-15a	3.7 ± 0.4	3.2 ± 0.5	6.1 ± 0.4
KB-3-1	Hsa-miR-15b	3.7 ± 0.4	2.8 ± 0.1	7.0 ± 0.9
KB-3-1	Hsa-miR-16	3.7 ± 0.4	3.0 ± 0.1	5.7 ± 0.3
KB-3-1	Hsa-miR-16-1*	3.7 ± 0.4	2.7 ± 0.1	6.01 ± 0.3
KB-3-1	Hsa-miR-424*	3.7 ± 0.4	2.7 ± 0.1	6.78 ± 0.6
KB-3-1	Hsa-miR-155	3.7 ± 0.4	2.3 ± 0.2	6.6 ± 0.7
KB-CP.5	Hsa-miR-15a	11.5 ± 1.6	8.4 ± 0.2	22.4 ± 1.0
KB-CP.5	Hsa-miR-15b	11.5 ± 1.6	9.4 ± 1.3	20.2 ± 1.9
KB-CP.5	Hsa-miR-16	11.5 ± 1.6	10.8 ± 1.2	20.1 ± 1.2
KB-CP.5	Hsa-miR-16-1*	11.5 ± 1.6	9.6 ± 0.5	20.4 ± 0.0
KB-CP.5	Hsa-miR-424*	11.5 ± 1.6	9.2 ± 0.7	22.2 ± 1.0
KB-CP.5	Hsa-miR-155	11.5 ± 1.6	8.9 ± 0.4	22.2 ± 1.4

Cytotoxicity (IC₅₀) values for KB-3-1 and KB-CP.5 lines with and without miRNA mimics or miRNA siRNA (inhibitors), and challenged with cisplatin. KB-3-1 and KB-CP.5 cells were transfected with a miRNA mimic or an miRNA inhibitor on day one. After 24 h cells were treated with cisplatin (50 μL to 0 μL) in order to determine a dose shown to kill 50% of the cell population (IC₅₀) .

Table S3, related to Table 1 (Excel spreadsheet). Results from miRNA mimic screen.

Cell growth measured as percentage growth after transfection with miRNA mimic treated with vehicle only, and percentage growth after transfection with miRNA treated with cisplatin at EC₅ or EC₃₀ concentrations.

Table S4, related to Table 1 (Excel spreadsheet). Results from kinome siRNA screen.

For each siRNA treatment (gene symbol, gene ID and Ambion siRNA ID), cell growth measured as percentage growth after transfection with siRNA treated with vehicle only, and percentage growth after transfection with miRNA treated with cisplatin at EC₅ or EC₃₀ concentrations.