Evaluation of CHK1 and WEE1 as Candidate Sensitizers to Cisplatin and Paclitaxel in Ovarian Cancer Cells

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Bryan Huber
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Co-Mentors: Dr. Heather Cunliffe, PhD
Dr. David Azorsa, PhD

I want to dedicate this project to my patient, Mrs. K, who was a source of strength and inspiration to me and to those around her. Also, to my mother and sister, who became an example of resilience and resoluteness through their own struggles with ovarian cancer.

Acknowledgements

I wish to thank my mentors Dr. Heather Cunliffe and Dr. David Azorsa. I also wish to thank the lab in which I trained, including Dr. Meredith Henderson, Irma Gonzales, Dr. Daniel Von Hoff, Dr. Haiyong Han, Dr. Shilpi Arora, and the Helios Education Foundation including Dr. Candice Nulsen, Brandy Wells and Kari Trent.

Abstract

Ovarian cancer is the foremost cause of death from gynecologic malignancies in the developed world. The American Cancer Society estimated 22,280 new cases in 2012 and 15,500 deaths. The majority of patients with advanced ovarian cancer relapse from primary treatment and develop drug-resistant disease. The mechanisms underlying drug-resistance are poorly understood. Inhibition of CHK1, a cell cycle G2/M checkpoint kinase has previously been shown to have a synergistic effect with cisplatin in reducing ovarian cancer cell viability. Additional mediators of the G2/M checkpoint have also been found to potentiate the effect of cisplatin and paclitaxel. We chose to evaluate the role of G2/M checkpoint kinases Chk1 and Wee1 and hypothesized that blockade of these kinases would increase the efficacy of cisplatin and paclitaxel either synergistically or additively in the A2780 ovarian cancer cell line model. We determined whether inhibition of CHK1 or WEE1 resulted in an additive or synergistic cytotoxicity in A2780 cells using siRNA technology and specific inhibition using pharmacologic agents. siRNA silencing of CHK1 or WEE1 resulted in an additive effect with Cisplatin and a synergistic effect with Paclitaxel. The response of A2780 cells to Paclitaxel was potentiated in the presence of Chk1 inhibitor PD407824, but not by Wee1 inhibitor MK1775. Our data demonstrates both CHK1 and WEE1 play a role in mediating resistance of A2780 cells to cisplatin and paclitaxel and suggests inclusion of targeted agents against Chk1 or Wee1 may be effective in the treatment of drug-resistant ovarian cancer.

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Introduction

Cancer, in general, accounts for 12.5% of deaths worldwide (1). Among the gynecological cancers, ovarian cancer has the highest mortality rate due to its advanced stage at diagnosis and resistance to therapy (2). The majority of ovarian cancers present at an advanced stage because of non-specific symptoms and inadequate screening tests (3). Due to lack of early detection and the vagueness of the symptoms such as bloating, abdominal pain, urinary symptoms and difficulty eating/feeling full quickly, ovarian cancer is known as a "silent killer" (3). In 2012, the American Cancer Society reported that 22,280 new cases of ovarian cancer were expected that year along with an estimated 15,500 deaths from the disease (4). Although ovarian cancer only accounts for 3% of malignancies in women, the 5 year survival for these patients is below 30% (4,5).

Treatment for ovarian cancer patients includes debulking surgery followed by combination chemotherapy that consists of a taxane plus a platinum drug. This is commonly paclitaxel and carboplatin, which is the current standard of care (6). Initial response rates to this therapy are high, but most patients will develop a recurrence as high as 70% and require retreatment (3). Patients who recur within 6-12 months typically have drug-resistant disease with limited to no effective treatment options (7). To date, addition of other chemotherapeutic agents has not significantly altered survival rates (6). It has been proposed that emergence of drug-resistant ovarian cancer is due to a heterogeneous primary tumor (3), as well as acquired resistance to mechanisms associated with cellular maintenance. Resistance to cisplatin occurs primarily through changes in drug uptake by cancer cells, increase in drug efflux through P-glycoproteins, increase in drug deactivation, decrease in the ability of the cell to undergo apoptosis and increase in the cell's ability to undergo DNA mismatch repair (8). Structurally cisplatin is a platinum-based molecule, which contains the atomic metal platinum with two amine and chloride ligands. Ovarian cancer cells also frequently become resistant to paclitaxel, (a taxane). Paclitaxel acts by inhibiting microtuble depolymerization during anaphase of mitosis, and commonly causes increased drug efflux through P-glycoprotein export.

Currently, newer approaches to target chemoresistance does not focus on the causes of resistance, but rather on pathways or molecules that can be targeted to help potentiate the effects of current cytotoxic chemotherapy (9).

Since molecular signatures of drug resistance are not consistent across primary ovarian tumors or commonly used ovarian cancer cell lines, a dynamic and very specific modality such as RNA interference (RNAi) was previously employed by our laboratory to identify and validate specific genetic targets that allowed the potentiation of chemotherapuetics to resistant cells (10). RNAi, is a targeted loss-of-function genomics modality used to knock down transcripts and thus their corresponding protein products that may be critical for cell function. More specifically, a selection of small interfering RNA (siRNA), which are small synthetic RNA species were utilized to this end in this study (9). RNAi-based screening approaches which target the 'kinome',(families of known kinases), has proved to be an effective method in identifying specific kinase genes whose expression plays a role in mediating drug-resistance in ovarian cancer cells (2). Our laboratory has previously shown that inhibition of Chk1, a kinase at the G2/M checkpoint of the cell cycle (Figure 1) potentiates the effect of cisplatin in an ovarian cell line model (2). The data provided a rationale to extend this finding to functionally evaluate Chk1 and Wee1, both essential kinases which mediate regulation through the G2/M checkpoint. These 2 candidates were selected as they gave the most promising preliminary data in the previous RNAi screen of A2780 ovarian cancer cells with Cisplatin (2)..

Despite differences in mechanism of action of cisplatin and paclitaxel, both agents are known to impact the G2/M phase of the cell cycle. The goal of this study was to interrogate this point of convergence, and evaluate whether inhibition of Chk1 and Wee1 kinases of the G2/M checkpoint will potentiate the effects of cisplatin and paclitaxel either synergistically or additively in A2780 ovarian cancer cells. By accomplishing the goal and specific aims of the project, we anticipate these data will provide insight toward novel combination treatment approaches that will improve therapy by reducing toxicity and survivability for such a lethal disease. Currently, the physiological concentrations used in treatment regimen for cisplatin is 75 mg/m^2 and paclitaxel 135 mg/m^2 every three weeks for six cycles (11). Ultimately, this will accelerate drug discovery and greatly impact future treatment.

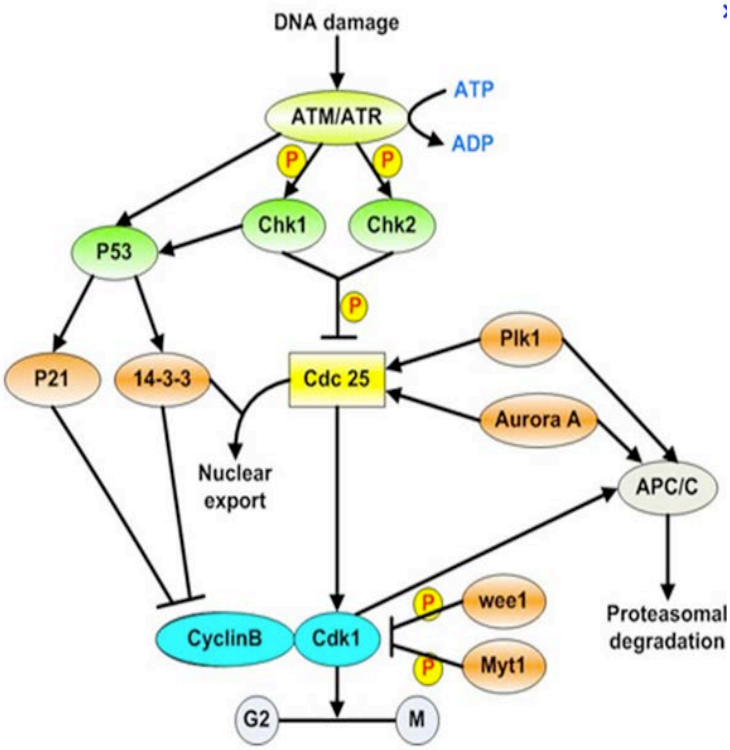


Figure 1. The G2/M checkpoint of the Cell Cycle.

Molecules regulating G2/M are an important site of action for cisplatin and paclitaxel. This is an important regulatory checkpoint for DNA damage. Diagram from Wang et. al. 2009. *Molecular Cancer*

The hypothesis for the project is that blockade of selected kinases, Chk1 and Wee1, in the G2/M checkpoint of the cell cycle will increase the efficacy of cisplatin and paclitaxel either synergistically or additively in the A2780 ovarian cancer cell line model in vitro. Preliminary evidence suggests that regulation of the G2/M checkpoint plays an important role in this process. We addressed our hypothesis with the following set of specific aims.

Specific Aim 1. Define the optimal conditions for RNAi-mediated targeted inhibition of G2/M kinases in A2780 cells treated with cisplatin and paclitaxel.

Sub aim 1.1. Optimize the conditions for transfection of siRNAs into A2780 ovarian cancer cells. This requires definition of the optimal lipid transfection reagent from a panel of four commercially available reagents. The candidate lipid with optimal performance (resulting in the most siRNA being transfected with minimal lipid-based cytotoxicity) will be selected. Also it is important to optimize the vehicle siRNA (negative control) that will produce the least lethality in A2780 cells when transfected. This will control for the reactions of siRNAs with targets.

Sub aim 1.2. Define the IC50 for cisplatin and paclitaxel in A2780 cells. This was accomplished by performing drug-dose response (DDR) assays to measure the viability of A2780 cells against increasing concentrations of each cytotoxic drug. Defining the appropriate IC50 for each drug will facilitate an accurate measurement of the functional effect of siRNA-mediated gene silencing without concerns of drug-induced lethality.

Specific Aim 2. Define whether targeted gene silencing results in an additive, synergistic or antagonistic effect with cytotoxic drugs in A2780 cells. We previously identified targets in the G2/M checkpoint to modulate the effects of either cisplatin or paclitaxel in the treatment of A2780 cells. Guided by the inhibitory concentration 50 (IC50) values for cisplatin and paclitaxel (aim 1), we will test the drugs at several dilutions with siRNAs, of interest.

Specific Aim 3. Validate candidate targets resulting in a synergistic response to paclitaxel or cisplatin in A2780 cells. An available commercial molecular inhibitor to the targets identified by siRNA studies in aim 2 will be used with either cisplatin or paclitaxel to validate the synergy and support the hypothesis that inhibiting Chk1 and Wee1 in the G2/M checkpoint will potentiate the effects of cisplatin and paclitaxel in A2780 ovarian cancer cells.

Research Materials and Methods

Adapted from Aurora et al. (2) and Asorza et al. (11), two recent publications from our laboratory with relevance to this study.

Cell culture:

The A2780 ovarian cancer cells and the A2780cis ovarian cancer were obtained from ECACC (Salisbury, UK). The A2780cis130 and A2780pac cells were selective for in lab for resistance to 130μM of cisplatin and 250nM of paclitaxel respectively. The cells were grown and maintained in RPMI-1640 medium with 10% FBS and penicillin and streptomycin (Invitrogen, Carlsbad, CA) at 37º C in a humidified 5% CO_2 atmosphere. Cisplatin resistant cell lines were grown in 1 μM cisplatin and paclitaxel resistant cell lines were grown in 250 nM paclitaxel.

Reagents:

Cisplatin and paclitaxel were purchased from EMD Chemicals (Gibbstown, NJ) and siRNA library targeting kinases in the G2/M checkpoint as well as non-silencing control were obtained from Qiagen (Valencia, CA). All inhibitors including Chk1 inhibitor PD407824 and Weel inhibitor MK1775 were obtained from Tocris (Ellisville, MI).

Transfection reagent test (TRT):

Four different lipid reagents, SilentFect (Biorad, Hercules, CA), Lipofectamine 2000 (Invitrogen), DharmaFect 3 (Dharmacon, Lafayatte, CO), and RNAiMax (Invitrogen) were tested to determine the optimization for reverse transfection of A2780 cells. Reverse transfection is a method where viable cells are added to wells of tissue culture plates containing immobilized nucleic acid/lipid mixtures, rather than forward transfection, where lipid/nucleic acid complexes are added to cells in culture. Reagents were diluted from 2:1 gradually to 10:1 with OptiMEM (Invitrogen). Transfection reagent and siRNA were allowed to complex for 30 minutes in white Corning 384 plates (Fisher Scientific, Pittsburgh, PA). 1000 A2780 cells were added per well and incubated for 96 hours. Following incubation, a 1:1 dilution of transfection reagent and 50% Cell Titer Blue (Promega, Madison, WI) was formulated per well and the red fluorescence was read with an Envision plate reader (ThermoScientific, Hudson, NH) to measure cell viability.

Drug-dose response (DDR) assays for cisplatin and paclitaxel:

A2780 cells, A2780cis cells, A2780cis130 cells and A2780pac cells (1000 cells/well) were added to white Corning 384 plates (Fisher Scientific, Pittsburgh, PA) and incubated for 72 hours with a range of cisplatin and paclitaxel for respective lines. After incubation, Cell Titer Glo (CTG, Promega) was added to measure relative luminescence units (RLU). IC50 values were calculated using GraphPad Prism software (GraphPad Prism, San Diego, CA).

Functional validation for cisplatin and paclitaxel sensitization in G2/M checkpoint using siRNA drug-dose response assays (siDDR):

Selected siRNAs targeting CHK1 (siRNA target sequences) and WEE1 kinases in the G2/M checkpoint were used. Validated siRNAs of proprietary sequence were obtained from Qiagen, Valencia, CA (product ID SI00024570 for Chk1 and SI00302211 for Wee1) Four different siRNAs were used for each gene to reduce risk of measuring off-target effects. 9.3 ng of siRNA diluted in buffer was printed into each well of a white Corning 384 well plate from a stock solution. Reverse transfection of the cells took place with diluted (6:1) Lipofectamine 2000 (Invitrogen) and OptiMEM (Invitrogen) as well as the appropriate siRNA. The Lipofectamine 2000 6:1 was used for all siDDR (determined by TRT), except for vehicle optimization Lipofectamine 2000 10:1 was used. A2780 cells were resuspended in medium to a final concentration of 1000 cells per well, with 32 replicate wells for each dilution. Plates were incubated at 37º C and 5% CO_2. After 24 hours, 12 point dilutional concentrations of either cisplatin (0 nM, 5.03 nM, 15.1 nM, 45.3 nM, 136 nM, 410 nM, 1.23 uM, 3.7 uM, 11.1 uM, 33.3 uM, 100 uM, and 300 uM) or paclitaxel (0 nM, 0.16 nM, 0.5 nM, 1.5 nM, 4.5 nM, 13.5 nM, 41 nM, 122 nM, 366 nM, 1.1 uM, 3.3 uM, and 10 uM) was added and plates were incubated for an additional 72 hours. The final siRNA concentration was 13 nM. Cell titer glow, an assay designed to measure ATP in a cell, was used to measure cell viability. Viability is directly related to amount of ATP of a cell. Viability was calculated by dividing the average of the relative luminescence units (RLU) values for the drug treated wells by the average of the RLU for the vehicle (serum free media) wells. The IC50 values were determined using GraphPad Prism (11). See Figure 2 for a schematic representation of this experimental design.

Functional validation of cisplatin and paclitaxel modulators with molecular inhibitors (double drug-dose response assays):

For Chk1 and Wee1 inhibitors, A2780 cells were treated with PD407824 or MK1775 respectively in 384-well plates as describe previously (2). After 24 hours, cisplatin or paclitaxel was added to wells at concentrations described in the previous section. After 72 hours, cell viability was measured using Cell Titer Glo and IC50 was calculated using Graphpad Prism as described previously (2).

Results

Optimization of siRNA Transfection in A2780 cells:

A diagram to show our experimental approach to identify potential sensitizers to cisplatin and paclitaxel is shown in Figure 2.

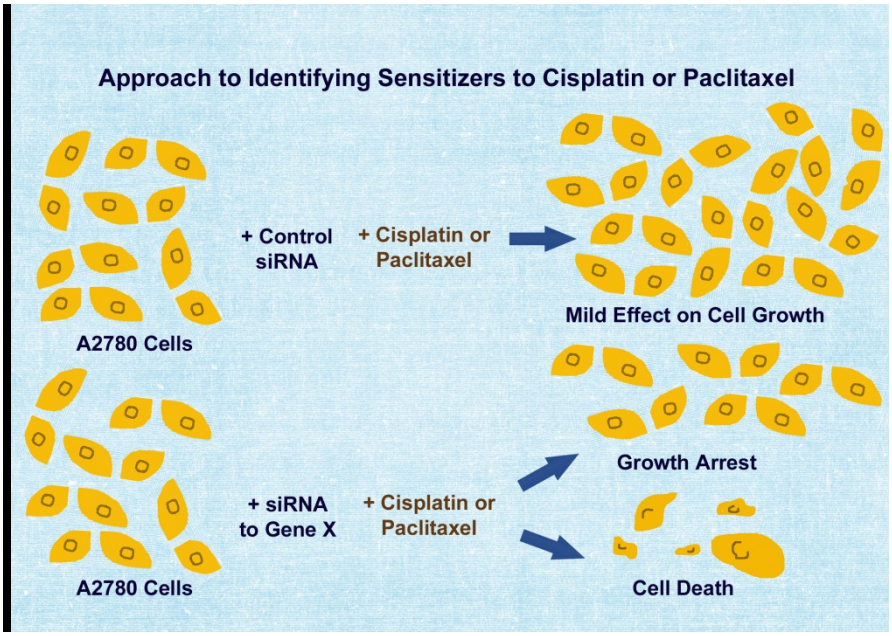


Figure 2. Experimental Setup. Approach to identifying potential sensitizers to cisplatin and paclitaxel. A scrambled siRNA sequence was used as a control and siRNA to candidate genes involved in the G2/M checkpoint were used.

Six different non-silencing siRNAs were tested for their performance in their ability to perform optimally as a negative control in two cell lines (A2780 wild type, and A2780 cisplatin-resistant). Non-silencing 2/3 (NS 2/3), showed the least cell toxicity in the A2780 cells (Figure 3a). NS 2/3 siRNA is a validated negative control siRNA commercially available from Qiagen, with a scrambled sequence that does not target any specific RNA species vital for cell function, thus allowing the cell to survive. We chose to use the NS 2/3 siRNA as the optimal control for subsequent experiments. In contrast, the positive control siRNA ubiquitin B (UBBs1) was lethal to both cell lines in this experiment, indicating an optimal level of transfection efficiency for this assay. Ubiquitin B is essential for cell survival, thus siRNA UBBs1 which silences ubiquitin B results in cell lethality.

Among the four lipid reagents, Lipofectamine 2000 at a ratio of 6:1 Lipid:siRNA had the best performance transfecting siRNA with the least cell lethality (figure 3b and c). Lipofectamine 2000 reagent was therefore selected as the optimal lipid for further experimentation using the A2780 cell line.

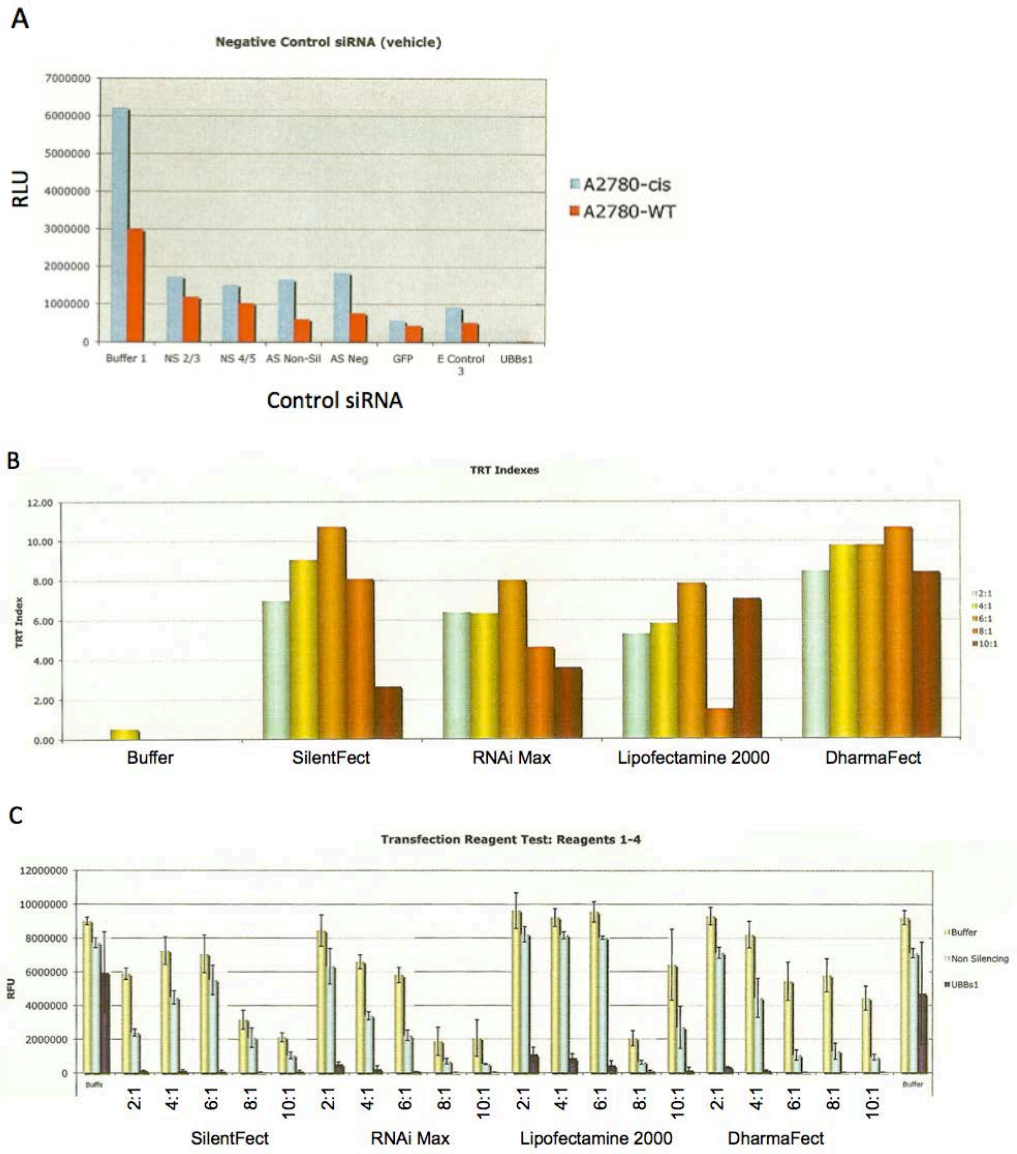


Figure 3. Optimization of experiments with vehicle and Transfection Reagent Test (TRT). A. A Non-silencing 2/3 (NS 2/3), was found to have less toxicity among different vehicles in A2780 cells. **B.** Lipofectamine 2000 at a 6:1 Lipid:siRNA ratio showed lowest toxicity for A2780 cells. **C**. Lipofectamine 2000 and control siRNA NS2/3 showed optimal viability at a 6:1 ratio. RLU, Relative Luminescence Units measured by the Cell Titer Glo (CTG) assay.

To determine the optimum level of cisplatin and paclitaxel to use in combination with siRNAs of interest, it was first necessary to determine the IC50 (the concentration at which 50% of the cells lose viability) for each drug in the A2780 cell line. We included use of drug-resistant derivatives of A2780 cells (A2780-cis and A2780-pacli as qualitative controls. The drug-dose response (DDR) assays for A2780 cells showed an IC50 of 1.8µM for cisplatin and an IC50 of 3.0 nM for paclitaxel (figure 4).

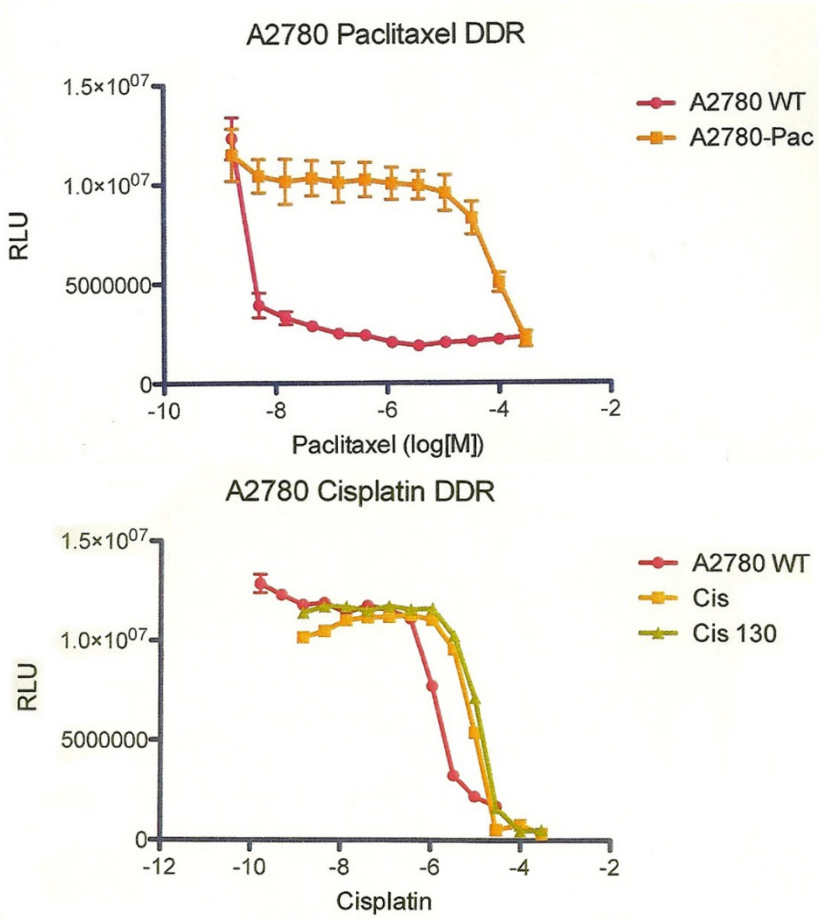


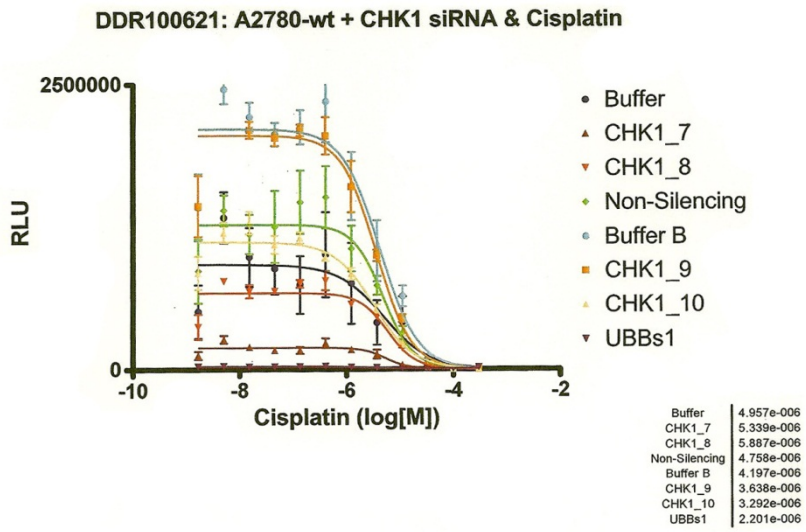
Figure 4. Drug-dose response assays for cisplatin and paclitaxel.

Cisplatin IC50 = 1.8 uM and Paclitaxel IC50 = 3.0 nM. A2780 cells were incubated for 72 hours with a range of cisplatin or paclitaxel concentrations as detailed in the materials and methods. RLU (relative luminescence units) indicates cell viability. IC50 values were calculated using GraphPad Prism.

Functional validation of candidate genes using siRNA drug-dose response (siDDR):

SiRNAs to target and inhibit expression of CHK1 and WEEl were next transfected into A2780 cells using the conditions previously optimized (Figures 3). Cisplatin was used at the IC50 we had previously determined (figure 4). The CHK1 and WEE1 siRNAs did reduce the IC50 for cisplatin (figure 5,) indicating CHK1 and WEE1 silencing produces an additive effect. We noted that not all 4 gene-specific siRNAs resulted in this effect, suggesting not all siRNAs were effective in gene silencing.

The CHK1 siRNA and paclitaxel showed a decrease in the IC50 in 2 of the 4 siRNAs (figure 6). The WEE1 siRNA and paclitaxel showed a decrease in the IC50 in 3 of the 4 siRNAs (figure 6). We did note a significant fold difference between vehicle treatment and siRNA treatment (figure 6), although this was seem previously during optimization experiments, suggesting a general degree of siRNA-mediated toxicity in the A2780 cell line.



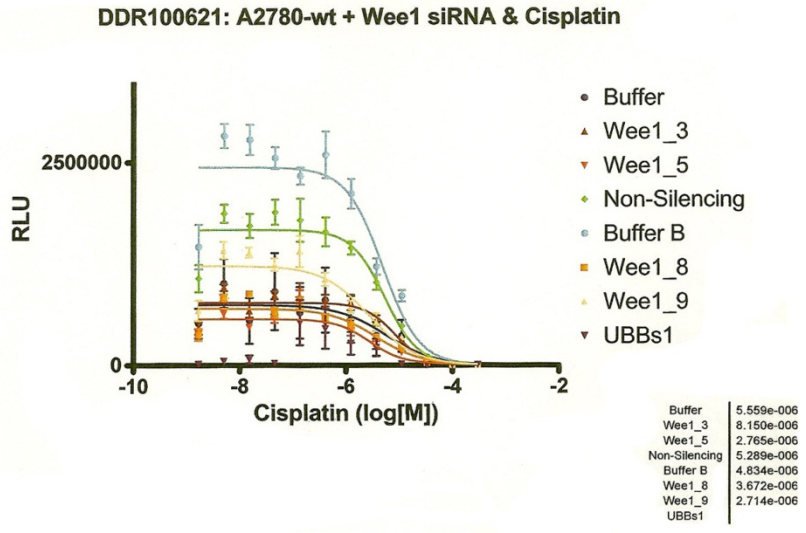


Figure 5. siRNA silencing of CHK1 or WEE1 in A2780 cells results in an additive effect with Cisplatin. Upper graph, CHK1 or control siRNAs + cisplatin. Lower graph, WEE1 and control siRNAs + cisplatin. IC50 values shown bottom right. Lipofectamine 2000 was used to reverse transfect A2780 cells with CHK1 siRNA and WEE1 siRNA in a 384-well plate. After 24 hours, cisplatin was added at concentrations indicated on the x axis and incubated for 72 hours. RLU measured by the CTG assay to measure viability.

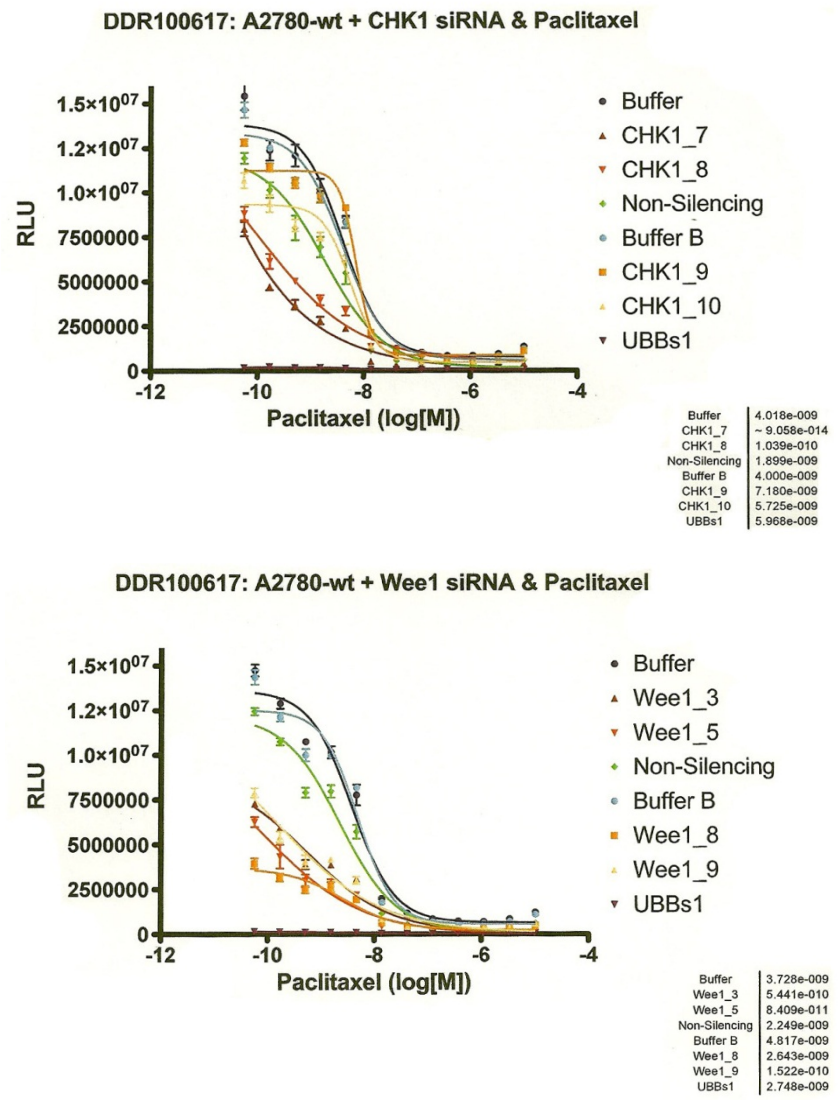


Figure 6.

siRNA silencing of CHK1 or WEE1 in A2780 cells results in a synergistic effect with Paclitaxel. Upper graph, CHK1 or control siRNAs + paclitaxel. Lower graph, WEE1 and control siRNAs + paclitaxel. IC50 values shown bottom right. Lipofectamine 2000 was used to reverse transfect A2780 cells with CHK1 siRNA and WEE1 siRNA in a 384-well plate. After 24 hours, paclitaxel was added at concentrations indicated on the x axis and incubated for 72 hours. RLU measured by the CTG assay to determine viability.

Functional validation of candidate genes using small molecular inhibitors:

To validate our findings in Figures 5 and 6 that silencing of either WEE1 or CHK1 appear to enhance sensitivity of A2780 cells to cisplatin and paclitaxel, we repeated our drug-dose toxicity profiles in the presence and absence of commercially available specific inhibitors to WEE1 and CHK1. Treatment of A2780 cells with PD407824 (Chk1 molecular inhibitor) and paclitaxel showed a decrease in the IC50 between inhibitor compared to no inhibitor (figure 7). Treatment of A2780 cells with MK1775 (Wee1 inhibitor) and paclitaxel showed a slight decrease in the IC50 between no inhibitor and inhibitor (figure 7). These data are consistent with our siRNA findings and supports the hypothesis that inhibition of WEE1 and CHK1, both key mediators of the G2/M cell cycle checkpoint, increases the efficacy of cisplatin and paclitaxel in ovarian cancer cells in vitro.

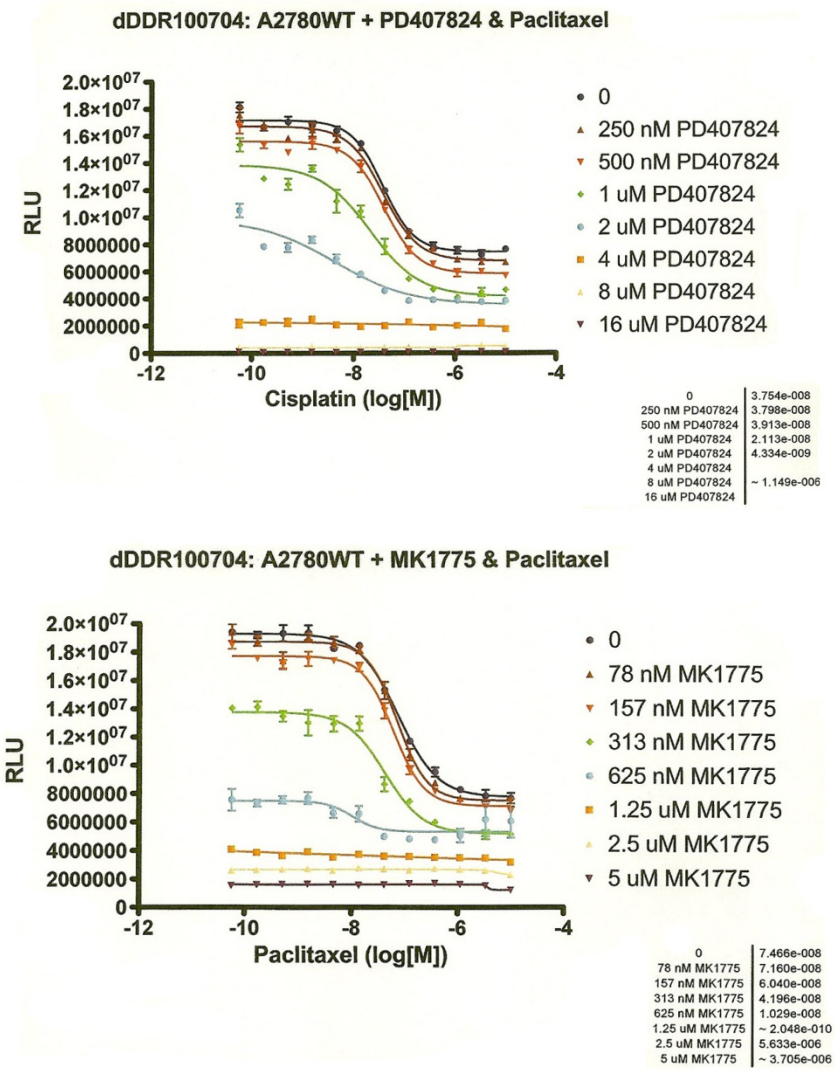


Figure 7. Response of A2780 cells to Paclitaxel is potentiated in the presence of an inhibitor to Chk1 but not by an inhibitor to Wee1. Upper graph, Chk1 inhibitor (PD407824) + paclitaxel. Lower graph, Wee1 inhibitor (MK1775) + paclitaxel. IC50 values shown bottom right. A2780 cells were seeded onto a 384-well plate. At 24 hours, inhibitor was added at the concentrations shown. At 48 hours paclitaxel was added at concentrations indicated on the x axis. A2780 cells were incubated for an additional 72 hours and RLU measured by the CTG assay to determine viability .

Discussion

In this project, we successfully used siRNA drug-dose response assays (siDDR) in the A2780 ovarian cancer cell line to validate WEE1 and CHK1 as sensitizers to cisplatin and paclitaxel in the G2/M checkpoint of the cell cycle. Furthermore, specific inhibition of these same two genes increased the efficacy of each cytotoxic agent. Importantly, our data demonstrated WEE1 and CHK1-mediated sensitization to paclitaxel in the A2780 model is a novel translational finding that has not been previously reported to our knowledge.

Currently the standard chemotherapy for ovarian cancer is a combination of cisplatin (or carboplatin) and paclitaxel. Previous studies have shown that a functional genomics platform, high-throughput RNAi (HT-RNAi), is a reasonable approach in identifying targets that would potential the effects of cisplatin, gemcitabine and cytarabine therapy (2, 11, 12). Although other studies have not focused on identifying targets such as kinases, it is known that kinases are involved in numerous pathways and are also deregulated in malignant cells (11). Therefore, HT-RNAi of the kinome (a family of 572 kinases) has been utilized in past studies and has identified kinases that are found in the G2/M checkpoint (2, 11). Here we validated a selective group of kinases, finding CHK1 and WEE1 being synergistic in combination with paclitaxel. It is interesting to note that other studies have found that *CHK1* and *WEE1* have been potential targets to current therapy in other cell lines (2, 9, 11, 12). A prior study showed that *CHK1* was a sensitizer in SKOV3 ovarian cancer cells with cisplatin (2). These variations may be cell specific and further validation studies would need to occur in other cell lines to continue to make broader claims concerning sensitizers and current therapy when treating ovarian cancer.

Prior studies have noted that Chk1 is a protein kinase that plays an important role in regulating the cell cycle when DNA is damaged (2). More specifically, Chk1 is activated by phosphorylation by ATR which then inactivates Cdc25 via phosphorylation to halt the progression of the cell cycle by not activating Cdc2. When DNA is damaged Chk1 is upregulated in response. In nonmalignant cells, Chk1 will halt the cell cycle, before the cell divides, so that the cell can undergo DNA repair. Inhibition of an inhibitor of the cell cycle (with a siRNA or molecular inhibitor) leads to the growth arrest of A2780 ovarian cells. It has been suggested that promoting the cell cycle through the arrest of CHK1 activity allows for the cell to continue

through mitosis with damaged DNA, which will induce the apoptotic pathways (2). Therefore when the A2780 ovarian cancer cell lines are inhibited with the proper functioning of CHK1 and then are treated with paclitaxel and cisplatin, cell death occurs. It is known that paclitaxel functions by inhibiting microtuble depolymerization during anaphase of mitosis. It is unknown why *CHK1* acts as great sensitizer to paclitaxel. Cisplatin directly crosslinks DNA thus directly inhibiting DNA synthesis. Further studies will need to be done to further elucidate these findings. There was a clear decrease in the IC50 curve showing a clear synergistic response with CHK1 inhibition and paclitaxel in A2780 cells. The same was observed when the molecular Chk1 inhibitor PD407824 was combined with paclitaxel in A2780 cells. There was an additive response with CHK1 inhibition and cisplatin in A2780 cells.

Synergy was also observed between WEE1 inhibition and paclitaxel in A2780 cells. Just as Chk1 inhibits cell cycle progression into mitosis when DNA is damaged, Wee1 also participates in regulating the G2/M checkpoint by inhibiting progression into mitosis when DNA is damage. More specifically, Wee1 inactivates cdc2 by phosphorylation. WEE1 inhibition was only found to have an additive response with cisplatin. WEE1 inhibition did not produce any results when a validated molecular inhibitor MK1775 was added in combination with paclitaxel in A2780 ovarian cells. This may due to the off-targets effects of the siRNA for WEE1. It would be necessary to validate the gene silencing using Western Blot or quantitative real-time PCR (qRT-PCR) to quantify if the transcript for WEE1 is being knocked down.

Although CHK1 and WEE1 were validated genetic sensitizers to paclitaxel in A2780 cells and showed additive effects in cisplatin, more internal optimization was needed. The non-silencing RNAi (NS 2/3), which was the internal negative control for the siDDR validation studies, was unfortunately toxic to the A2780 cells, a cell line-specific phenomenon that is a known technical challenge in RNAi technology approaches. NS 2/3 is a scrambled sequence that was not specific to any of the sequences associated with any of the genetic targets that were being validated. When the NS 2/3 is producing lethality in cells when none should exist this is known as off-target effects.

Future Directions

In this study, we were successful in validating potential sensitizers, CHK1 and WEE1, to chemotherapeutic agents in the A2780 ovarian cancer cell line. While the siRNAs used in this study were selected based on their prior validation by the vendor to show high sensitivity and specificity to inhibit expression of WEE1 and CHK1, it will be important to validate knockdown of these genes, and their protein products using Western blotting. It will also be important to evaluate whether our results for CHK1 and WEE1 in A2780 cells show the same or similar pattern of sensitization to cisplatin and paclitaxel in other ovarian cancer cell lines..

Moving forward, HT-RNAi should be used to identify potential targets in the A2780-cisplatin and A2780-paclitaxel resistant cell lines (derivatives of the A2780 cell lines used in this study) as an important goal for overcoming inherent or acquired resistance to therapy in late stage ovarian cancer. After this step, targets would be validated using siDDR and commercially available molecular inhibitors. The commercially available siRNAs would be validated using quantitative real-time PCR (qRT-PCR) to measure the amount of transcript present after silencing. To make certain that the silencing of targets is producing apoptosis in the A2780 cells, a caspase assay would be performed to show that potential sensitizers are potentiating the cisplatin or paclitaxel response. A more thorough investigation will likely discover sensitizers that could potentiate current therapy.

Conclusion

Over the last several years, RNAi has been a robust way of not only understanding protein function, but has also been vitally important in drug development (16). Late stage ovarian cancer has a very low 5 year survival, even with current therapy. This study validated potential genetic targets that would potentiate the effects of cisplatin and paclitaxel. Applying siDDR, we determined that a few sensitizers in the G2/M checkpoint, CHK1 and WEE1, acted synergistically with paclitaxel in A2780 wild type cells, while acting additively with cisplatin. The molecular inhibitor of Chk1, PD407824, appeared to potentiate the response of A2780 ovarian cancer cells to paclitaxel while the Wee1 inhibitor, MK1775 did not. There were no synergistic affects with cisplatin in the A280 ovarian cancer cells.

Using RNAi is a promising strategy to identify and validate potential genetic sensitizers to current therapy. As noted earlier, using this strategy will allow for a smaller dose of drug to be administered while increasing the cancer cell lethality. This will allow for less toxic side effects and ultimately increase the 5 year survivability for patients.

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