Validation of Candidate Sensitizers to Cisplatin and Paclitaxel in Ovarian Cancer Cells

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Introduction

Ovarian cancer has the highest mortality rate of all gynecological cancers due to its advanced stage at diagnosis and resistance to therapy. Vague symptoms (typically bloating, abdominal pain, urinary symptoms and difficulty eating/feeling full quickly) and inadequate screening tests contribute to diagnosis at advanced stages. 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. Although ovarian cancer only accounts for 3% of malignancies in women, the 5 year survival for these patients is below 30%.

Treatment for primary ovarian cancer includes debulking surgery followed by combination chemotherapy that consists of a taxane plus a platinum drug (commonly paclitaxel and carboplatin). Initial response rates are high, but most patients will develop a recurrence (as high as 70%) and require retreatment (1). Patients who recur within 6-12 months typically have drug-resistant disease with limited to no effective treatment options. To date, addition of other chemotherapeutic agents has not significantly altered survival rates. Ovarian tumors are among the most highly heterogeneous of all the solid tumors, and this heterogeneity is proposed to be a key factor in emergence of drug-resistant disease. Novel treatment approaches to target chemoresistance do 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.

Rationale and Hypothesis

RNAi-based screening approaches which target the 'kinome' (families of known kinases), has proved to be an effective method in identifying mediators of drug-resistance in ovarian cancer cells. Our laboratory previously shown that inhibition of Chk1, a kinase at the G2/M checkpoint of the cell cycle potentiates the effect of cisplatin in the A2780 ovarian cancer cell line model (2). This data provided a rationale for this study, to evaluate the functional role of additional candidate kinases in the G2/M checkpoint in mediating drug sensitivity.

We hypothesized that that blockade of selected kinases Chk1 and Wee1 in the G2/M cell cycle checkpoint will increase the efficacy of cisplatin and paclitaxel either synergistically or additively in the A2780 ovarian cancer cell line model. **Our objectives were to** 1) Define the optimal conditions for RNAi-mediated targeted gene inhibition. 2) Determine whether RNAi silencing results in an additive or synergistic cytoxicity. 3) Determine whether specific inhibitors to Wee1 and Chk1 yield findings consistent with the RNAi data in A2780 cells.

Methods

- Functional validation for cisplatin and paclitaxel sensitization in G2/M checkpoint using siRNA drug-dose response assays: Lipofectamine 2000 was used to reverse transfect wild type A2780 cells with siRNA and control 384-well plate. After 24 h, a concentration titration of cisplatin or paclitaxel were added to the A2780 cells and incubated for 72 h. Cell Titer Glo reagent was then added to the cells and and Relative Light Units (RLU) recorded as a measure of cell viability. Data were plotted as dose-response curves. Method as previously described (2).
- Functional validation of cisplatin and paclitaxel modulators with molecular inhibitors: At 24 h, inhibitor was added at different concentrations. At 48 h, a concentration titration of cisplatin or paclitaxel was added. A2780 cells were incubated for an additional 72 h and cell viability measured again using the cell titer glo assay.

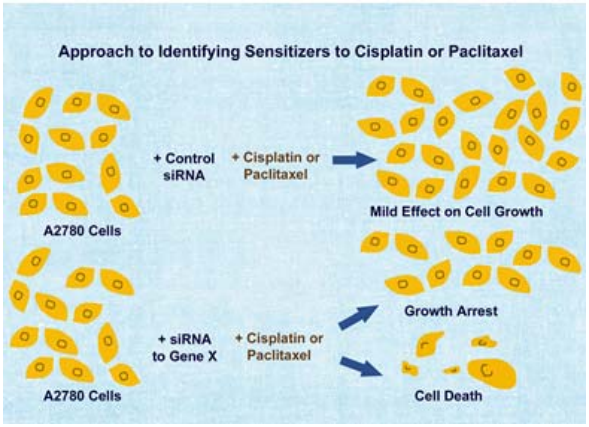

Figure 1. RNA interference strategy to identify potential sensitizers to cisplatin and paclitaxel. A scrambled siRNA sequence was used as a control and siRNA to candidate genes involved in the G2/M checkpoint were used. Method as previously described (2).

References

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(2). Arora, S., Bisanz, KM, Peralta, LA, Basu, GD, Choudhary, A, Tibes, R, Azorsa, DO. RNAi screening of the kinome identifies modulators of cisplatin response in ovarian cancer cells. *Gynecologic Oncology* 2010, 118:220-227.

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Results

RNAi optimization results. 1) We tested 6 negative control siRNAs and determined Non-Silencing to be optimal (data not shown). The positive control lethal siRNA UBBs1 was also validated as optimal in A2780 cells (data not shown). 2) We tested 4 lipid reagents and identified Lipofectamine 2000 reagent to perform optimally according to positive and negative siRNAs in A2870 cells (data not shown).

IC50 determination for Cisplatin and Paclitaxel. Drug-dose response curves were performed in A2780 cells as previously described (2). We determined the IC50 for Cisplatin as 1.8uM and the IC50 for Paclitaxel as 3nM. Data shown in figures 2-3 below were evaluated relative to IC50 values for each drug.

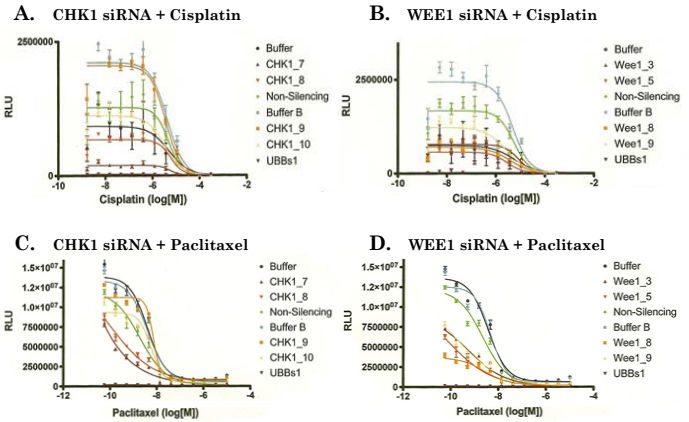

Figure 2. siRNA silencing of CHK1 or WEE1 in A2780 cells results in an additive effect with Cisplatin (A & B) and a synergistic effect with Paclitaxel (C & D).

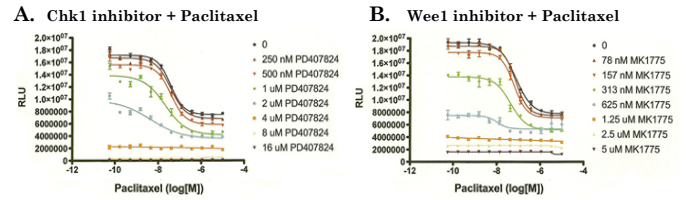

Figure 3. Response of A2780 cells to Paclitaxel is potentiated in the presence of an inhibitor to Chk1 (PD407824) but not by an inhibitor to Wee1 (MK1775).

Conclusions and Future Directions

- RNAi technology was an effective approach to identify and validate sensitizers to standard of care chemotherapy drugs in an in vitro cell line model of ovarian cancer.
- Both CHK1 and WEE1 G2/M checkpoint Kinases appear to play a role in mediating resistance of A2780 cells to cisplatin and paclitaxel.
- Inhibition of CHK1 resulted in consistent potentiation of the paclitaxel response both by RNAi and targeted inhibition, highlighting CHK1 as a candidate translational target that warrants further investigation. Our findings also suggest an investigation of the effect of CHK1 inhibition with a combination of both Cisplatin and Paclitaxel are also warranted.