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Genes Involved in the Cisplatin Response of BRCA2 Cancers

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By

Juliana M Rose

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APPROVED:

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Joseph B. Duffy, Assoc. Prof., Dept. Head, *ad interim*

Department of Biology and Biotechnology

Assoc. Prof., Department of Chemistry and Biochemistry

WPI Project Advisor

Abstract

Breast and ovarian cancers are two of the most prevalent types of cancer in women and kill over 55,000 people annually in the US alone. A number of these cases are deficient in BRCA1 or 2, tumor suppressor genes involved in DNA repair. Although they originally respond to typical chemotherapy treatments, over 40% of BRCA patients develop resistance. Through an unbiased RNA interference screen, we have identified a number of genes which synergize with BRCA2 deficiency to cause chemotherapy resistance. In order to validate these candidates, the genes have been knocked down in BRCA2 cell lines through RNAi and treated with increasing doses of cisplatin to confirm resistance. Common mutations in these genes have also been identified through published literature. One gene specifically has been selected for further study and has shown a marked under expression in chemotherapy resistant cell lines.

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Introduction

There are few words in the English language that invoke a similar response to the word "cancer." It is the second leading cause of death in the United States [24], and people have even gone so far as to call it "The Emperor of All Maladies." (Mukherjee) Cancer will affect an estimated 41% of all people in their lifetime, and this number is only expected to rise as our population ages. [10] Science, however, has made extraordinary strides towards the eradication of cancer, and what used to be a death sentence is now a treatment regime with very hopeful results. Yet we still have a long way to go. In 2007, cancer killed over 562,875 individuals in the US alone, while hundreds of thousands of patients had their lives interrupted by tedious and harsh treatments. [10] Research needs to move towards finding more efficient methods of treatment, both to improve patient care and to save lives.

Although the majority of cancers are sporadic, there are a large number that are hereditary, and more genetic connections are being found daily. The most common form of hereditary cancer is breast cancer, claiming over 10% of all cases. These hereditary mutations tend to affect younger patients more frequently than sporadic cases, contributing to the fact that breast cancer is the second leading cause of cancer death in women. Approximately 40% of hereditary breast cancers are mutated in the Breast Cancer tumor suppressor gene family, commonly known as the BRCA genes. These genes are involved in DNA repair and their mechanisms help ensure sequence integrity. BRCA mutations are also highly prevalent in ovarian cancers, causing 13% of hereditary cases. Treatments are available for these patients, but over 25% of individuals stop responding to treatment over time due to chemoresistance.

With the advent of more precise screening technologies and readily accessible sequencing techniques, the medical field is moving away from broad spectra diagnosis and towards individualized patient care. Molecular biomarkers are being used as precise indicators of disease, and cancer biologists are beginning to discover how to use this technology to treat cancer patients. In the case of chemoresistant BRCA cancers, the question is why are these cancers becoming resistant? Is it because of a genomic mutation? And if so, how can gene expression be utilized in order to predict treatment resistance? This project hopes to chip away at some of these questions.

Background

Cancer Statistics

Breast and ovarian cancers are the second and fifth leading cause of cancer deaths in women, respectively. It is estimated that by 2012, there will be 226,870 new cases of breast cancer and 22,280 new ovarian cancer cases, with a total of 55110 deaths in 2012 alone between the two [2]. A number of these cases are hereditary, and specific genetic mutations have been identified that correlate with cancer occurrence. Mutations in the BRCA gene family have been discovered as a cause of nearly half of all hereditary breast cancers. It has also been witnessed in approximately 13% of hereditary ovarian cancers. This is a staggering statistic, and targeted treatment of BRCA cancers could have a marked clinical significance.

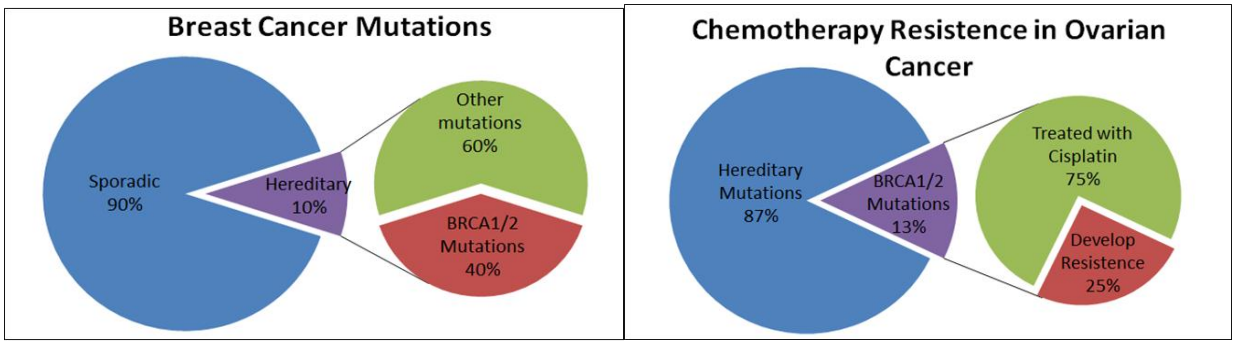


Figure 1: Mutations in Breast and Ovarian Cancers

10% of breast cancers are hereditary, with an impressive 40% of these cancers being deficient in the BRCA family of genes. About 13% of hereditary ovarian cancers also show defects in the RBCA family, with 25% of the BRCA cases becoming resistant to treatment.

		Breast Cancer	Ovarian Cancer	Total
Cases Per Year	New Cases	226,870	22,280	249,150
	Hereditary cases	22,687	1,782	24,469
	BRCA Cancers	9,075	232	**9,307**
	Deaths due to BRCA Mutation	1,597	161	**1,758**

Table 1: Anticipated Cases of Breast and Ovarian Cancers in 2012

In total, the year 2012 is expected to see 249,150 new cases of breast and ovarian cancers in the United States alone. Almost 10,000 of these cases will be due to BRCA mutations [2].

The BRCA family consists of the BRCA1 and BRCA2 genes. Both genes are involved in DNA repair in humans and are classified as tumor suppressor genes. Specifically, the BRCA2 gene is involved in homologous repair, a highly effective method of DNA double strand break repair. Loss

of BRCA2 results in sensitivity to DNA cross-linking agents, a defect in direct repair of double strand breaks through homologous recombination, and issues with cell cycle check point control.

Homologous Recombination

Homologous recombination (HR) is mainly utilized by the cell as a DNA double strand break repair mechanism. This method is highly effective as it maintains the integrity of the genomic sequence. When a double strand break occurs through some form of DNA damage, the 5' end of each strand is resected at the site of the break. This leaves single stranded DNA overhang on each strand.[15] BRCA2 then recruits the protein Rad51 to the site of the break. Rad51 coats the single strand overhang, forming a DNA/Rad51 filament which is seen as foci in damaged DNA. This filament then conducts a homology search, scanning the genome for the appropriate matching sequence on the complementary or sister chromosome.[43] Once the correct sequence is located, the filaments perform a strand invasion, using the alternate chromosome as a template to synthesize the sequence at the break point. The process resolves when the newly synthesized DNA is joined to the 3' end of the original break site on each strand. A simple schematic of this process can be seen in Figure 1.

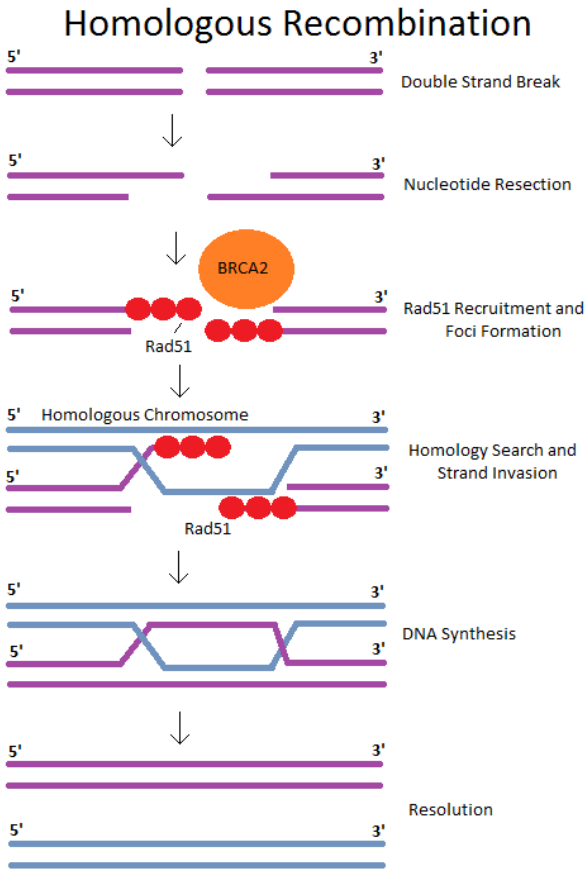


Figure 2: Homologous Recombination

When a double strand break occurs, BRCA2 recruits Rad51 to the break site to initiate homologous recombination. HR is a highly efficient, sequence-conservative method of DNA repair.

Rad51 Foci Formation

The presence of BRCA2 is essential for Rad51 foci formation, which in turn drives the homologous repair mechanism. It has been shown that cells deficient in BRCA2 cannot form Rad51 foci, and therefore do not perform HR. In the lab, it can be seen that cells damaged with cisplatin show significant DNA damage, but only those with WT BRCA2 recruit Rad51.

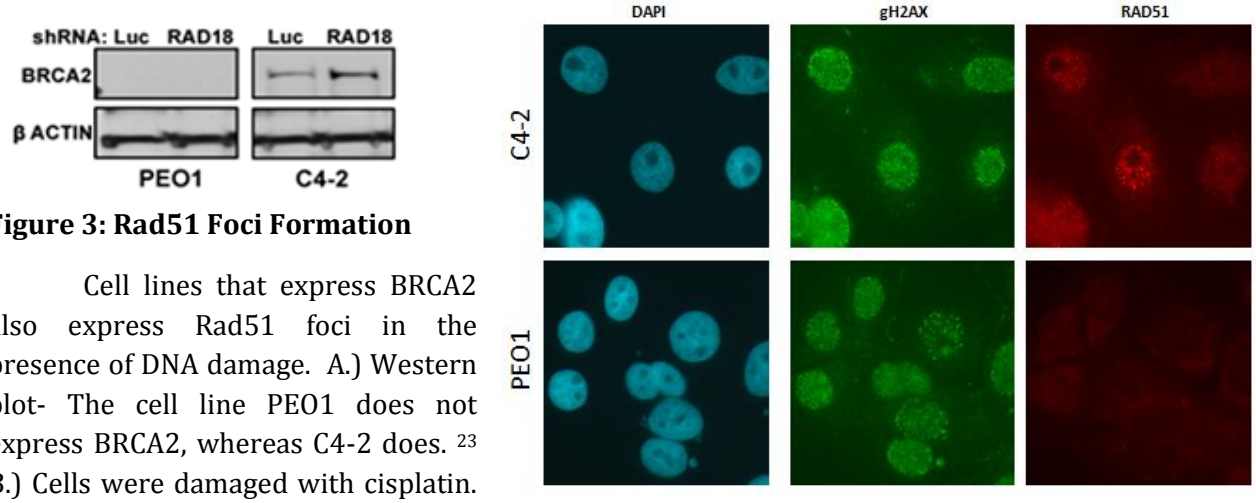


Figure 3: Rad51 Foci Formation

Cell lines that express BRCA2 also express Rad51 foci in the presence of DNA damage. A.) Western blot- The cell line PEO1 does not express BRCA2, whereas C4-2 does. [23] B.) Cells were damaged with cisplatin. Nuclei were visualized using DAPI, a nuclear staining dye. DNA damage can be seen through expression of gH2AX, an indicator of damage. As can be seen, both cells lines express damage. Rad51 foci were visualized. C4-2, the RBCA2 positive cell line, shows Rad51 foci formation at the site of damage, whereas the RBCA2-null PEO1 cell line does not.

A cell deficient in BRCA2 clearly cannot perform homologous recombination. The cell does, however, have other mechanisms to regulate DNA repair. Non-homologous end joining is a process by which two blunt end double stranded DNA molecules are joined together. This can repair double strand breaks, but the integrity of the sequence is severely compromised as there is no way to regulate that the strands being joined are in fact a correct match. There is also a DNA damage tolerance pathway, which allows cells to replicate their DNA and proliferate even in the presence of major DNA damage. When these error-prone mechanisms are utilized by cells to fix DNA, the number of mutations in the genome increases exponentially. [18]

Treatment for BRCA2 Cancers

Many cancers are treated with the chemotherapy cisplatin, including ovarian and breast cancers. Cisplatin is a platinum based therapy and is administered as a drip infusion. Treatment with cisplatin involves several sessions (around 8 hours each) over the course of three to eight months, and may be used alongside other chemotherapy drugs as part of a combination therapy. Direct side effects of cisplatin vary per individual, but have included nausea, vomiting, numbness in the extremities, change in taste and hearing, and kidney damage.[52] Other less common reactions include risk of infection, bruising and bleeding, and anemia. On the molecular level, cisplatin causes inter-strand crosslinks in DNA. In wild type cells, crosslinks are fixed through the homologous

recombination pathway. However in cells lacking imperative tumor suppressor genes involved in HR, such as BRCA2, the crosslinks cannot be resolved and the cell will trigger apoptosis. [52]

Although treatment with cisplatin was originally seen as a novel approach for cancers, the majority of advanced-stage ovarian cancers develop chemoresistance, with a low patient survival rate. Within six months following treatment, ~25% of patients develop platinum-resistant cancer and the overall five-year survival probability is 31% [11]. Defining mechanisms of resistance and developing tools to anticipate when resistance to chemotherapy will be key in fighting this disease on a more targeted level. This knowledge will be critical to guide therapy choice and will provide insight into new therapies targeting the mechanisms of resistance.

A likely explanation for the development of drug resistance is that disease progression is accompanied by genetic or epigenetic alterations in other genes, which confer drug resistance. Consistent with this idea, BRCA-deficiency and associated defects in HR induces genomic instability, which can lead to additional genetic alterations. These mutations could be essential initiate increased growth of BRCA mutant cells, which initially have proliferation defects. For example, mouse models that delete Brca2 in mammary epithelium induce mammary tumors in a manner dependent on the loss of p53 [12]. Functional analysis of the transition from growth defect to proliferation will benefit our understanding of the mechanisms underlying BRCA-FA associated tumorogenesis, as well as the development of therapeutic approaches for patients.

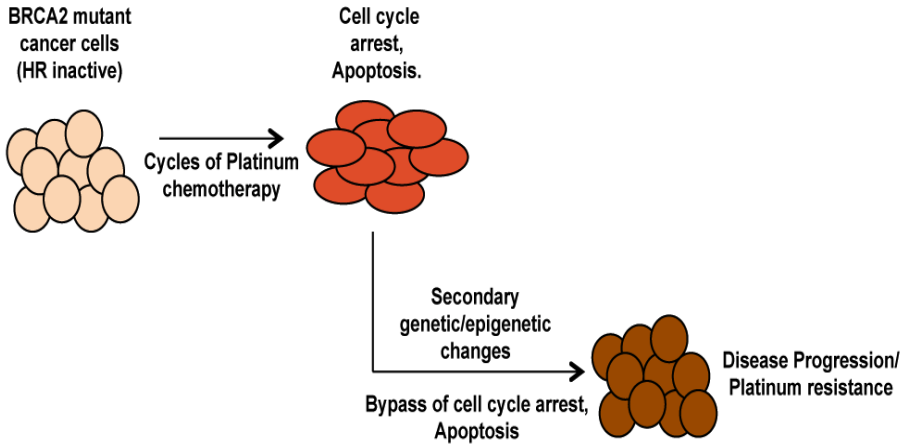


Figure 4: Resistance in BRCA2 Cancers

BRCA2 mutant cancers will originally experience cell cycle arrest and apoptosis under cisplatin treatment. However many tumors will then bypass cell arrest and will continue to proliferate, even in the presence of chemotherapies. We believe that this is due to a secondary genetic or epigenetic change.

Objective

The major objectives of this project are to identify genes that normally suppress ovarian tumorogenesis, and whose loss-of-function cooperates with BRCA deficiency to induce and/or accelerate cancer progression and resistance to toxic chemotherapies. We will refer to such genes as BRCA-suppressor genes (BRCA-SGs). To test the hypothesis that BRCA-SGs normally inhibit error-prone pathways, we will determine how loss of BRCA-SGs leads to the development of drug resistance. The proposed experimental strategy is based upon the fact that BRCA-loss confers cisplatin sensitivity that can be overcome by known (re-instated HR) and unknown mechanisms [19,6,20]. Finally, our goal is to determine whether BRCA-SGs are under-expressed or mutated in tumors from BRCA-patients with cisplatin resistant disease.

We have carried out a genome wide interference (RNAi) screen to identify genes (potential BRCA-SGs) whose knockdown enables BRCA2-mutant cells to gain resistance to cisplatin. With our validated set of genes the goal is to determine the mechanism by which their loss promotes resistance to cisplatin and to identify the regulatory pathways in which they function. We hypothesize that these genes represent candidate suppressors of cancers caused by BRCA-mutations. In this project, a series of experiments involving ovarian cancer cell lines were performed to determine the role of these candidates as suppressors of BRCA-associated cancer. In the future, validated genes whose loss confers cisplatin resistance will be tested for a role in the development of resistance in patient tumors.

The results of these experiments are expected to be significant for several reasons. First, the identification of new genes and regulatory pathways involved in the development of BRCA mutant cancers will substantially enhance our understanding of how BRCA deficiency transforms cells and causes disease. Second, these studies could identify genes that regulate mechanisms of lesion processing. For example, genes whose loss promotes cisplatin resistance (i.e. through BRCA2 reversion mutations) could normally restrict mutagenic pathways, such as non-homologous end joining (NHEJ). Third, this new information may reveal additional targets for therapeutic intervention that can be used instead of, or in conjunction with, conventional therapies.

And lastly, the identification of genes that are required to induce cisplatin sensitivity in HR-defective tumors is likely to have a significant impact on understanding of chemoresistance mechanisms in one of the most challenging diseases, ovarian cancer. Advanced-stage, high-grade serous (HGS) ovarian cancer accounts for 70% of ovarian cancer deaths [13,21]. Of these, about 50% have been linked to BRCA pathway disruption and HR deficiency [11,15], which has resulted in the exploration of BRCA-therapies for HGS ovarian cancer. To exploit these therapies effectively it is crucial that we develop biomarkers to anticipate tumor responses and define resistance pathways. A significant clinical endpoint of studies in this proposal is the discovery of biomarkers for cisplatin response. Genes identified in our screen are likely to be useful as biomarkers because patient response to cisplatin uniquely requires the expression of our candidate genes. Therefore, low expression of these genes in tumors should predict poor response to cisplatin. Thus, it will be essential not only to determine whether these genes are tumor suppressors disrupted in BRCA-cancers, but also whether these genes provide a signature predicting cisplatin response in patients.

Mechanisms by which HR-defective cells overcome sensitivity to DNA damaging agents have recently been identified. Notably, BRCA2-mutant cells can undergo BRCA2 reversion mutations to restore BRCA2 function in HR and resistance to cisplatin [6;20]. These BRCA2-restored cells are also cross resistant to poly(ADP-ribose) polymerase (PARP) inhibitors, a new generation drug that selectively sensitizes BRCA-tumors [5]. Reversion mutations in both BRCA2 and BRCA1 occur in patient tumors when treated with platinum agents [19,22]. Recently, it was determined that ~28% of recurrent ovarian carcinomas undergo reversion mutations and this outcome correlates significantly with poor response to platinum therapies [16]. The likelihood of reversion mutations was increased if a patient had undergone prior chemotherapy [16], supporting the idea that additional gene mutations synergize with BRCA-mutations. Together, these studies demonstrate that BRCA restoration is an important mechanism of drug resistance that also predicts poor response to platinum agents.

Materials and Methods

Cell Lines

The cell lines used in this project were mainly derivatives of the PEO1 cell line. PEO1 is an ovarian cancer cell line that is BRCA2 null, originally sensitive to cisplatin, and can become resistant to cisplatin. It also forms colonies. PEO1 has been widely used throughout literature to study ovarian cancer and cisplatin treatment [23]. Also utilized were derivatives of the cell line, including C4-2, C4-4, C4-11 and C4-13. These four cell lines are resistant to cisplatin. C4-2 is resistant through a spontaneous BRCA2 reversion, but all others are resistant through unknown mechanisms.

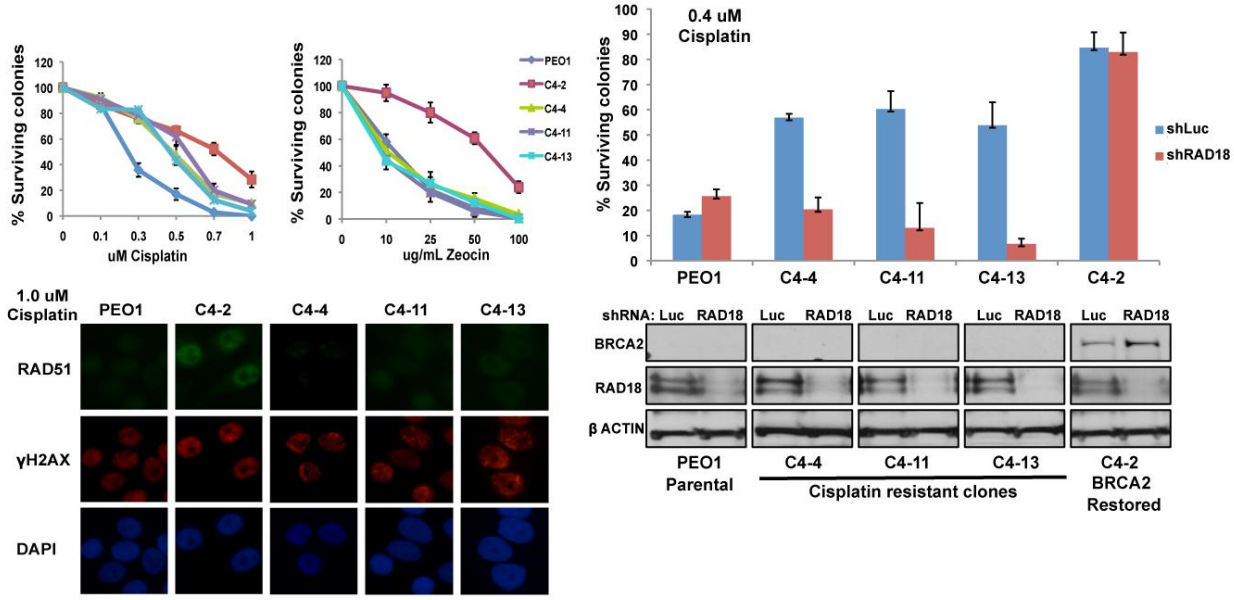


Figure 5: PEO1 Derivative Cell Lines

PEO1 ovarian cancer clones show significant cisplatin resistance: A) Colony survival assays show the differential sensitivity of PEO1 parental and derived clones to cisplatin and DNA double strand breaking agent zeocin. B) Cisplatin induced RAD51 and yH2AX foci were assessed by immunofluorescence. C) Cisplatin sensitivity is shown in PEO1 cell clones that were treated with siRNAs targeting control luc or Rad18. Equal cell numbers were plated and giemsa stained colonies were counted 10-12 days later. Results represent three independent experiments. D.) Western blot shows expression of proteins indicated. Data from the Taniguchi lab. [23]

Also used is an FA-D1 cell line. This cell line is derived from a Fanconi Anemia patient. The cell line is deficient in BRCA2, yet it is not cancerous.

Cell lines were grown in DMEM with 10% FBS and 1% Glutmax in a humidified 5% CO2-containing incubator at 37°C.

Lentiviral Knockdown

Knockout cell lines were created using RNAi technology. Genetic sequences were obtained through BLAST and their complementary sequence was coded into plasmids along with puromycin resistance and GFP. Plasmids were inserted into lentiviruses, and cell cultures were then infected with the viral particles. The complementary strand encoded in the plasmid paired with the target wild type mRNA in the cell line and silence expression. Knockdown colonies were then selected through puromycin treatment.

Bioinformatics

A number of well-documented tumor databases were utilized to gather quantitative information about genes discovered in the RNAi screen. Specifically used were *Tumorscape, COSMIC,* and the *Cancer Genome Atlas. Tumorscape* (*www.broadinstitute.org/**tumorscape)*** was used to gather GISTIC-Q values for genes, as well as to determine is deletions were seen in peak regions of BRCA2 related cancers. *COSMIC* (*www.sanger.ac.uk*- Catalogue of Somatic Mutations in Cancer) and *The Cancer Genome Atlas* (*cancergenome.nih.gov*) gave information on specific mutations seen in patient tumor samples.

Western Blot

Western blots used a 10% Tris Acetate SDS-PAGE gel with Tris-Acetate buffer. The electrophoresis separation ran at 250V, and the western transfer ran at 35V.

Results

Screen Results

To identify genes whose loss confers resistance to cisplatin and synergizes with BRCA2-deficiency, a large scale, unbiased RNAi screen was performed. The screen was performed in the PEO1 cell line, for the reasons described in the Materials and Methods section. The screen used the human pGIZ library pool, and knockdowns were selected through puromycin treatment. It can be reasoned that genes essential for cisplatin resistant mechanisms in BRCA2 cancers would present themselves when the knockdown cell lines were challenged with cisplatin.

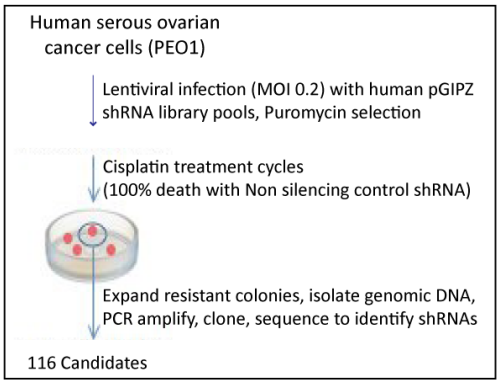


Figure 6: RNAi Screen Schematic

Basic methodology of screen. PEO1 cells were treated with a lentiviral infection of each gene in the human pGIZ gene at a low concentration to ensure single knockdowns. Knockdown colonies were then treated with cycles of cisplatin to determine resistant colonies. The non-silencing control shRNA (infection with lentivirus but no RNA knockdown) showed 100% death with cisplatin treatment. Resistant colonies were then expanded, and genomic DNA was isolated, amplified with PCR and cloned to identify the shRNA in each particular knockdown.

Important to the success of this approach, the screen was performed in collaboration with Dr. Michael Green, an expert in RNAi screen technology [7], [19], [25], [8]. Support for this approach was the UMASS RNAi Core Facility, which provided us with a complete genome-wide human library comprising ~90,000 shRNAs directed against ~27,000 genes that was divided into 15 pools, each containing approximately 6000 clones. High titer viral supernatant pools were obtained, which were used to stably transduce the parental cisplatin sensitive PEO1 cells. Cells infected with shRNA pools were selected with puromycin for 5 days and screened for resistance to cisplatin. Cells were treated with a dose of cisplatin that kills 99%-100% cells transduced with a non-silencing control (NSC) shRNA. In this way, cells harboring an shRNA that conferred resistance were identified by colony formation. To identify gene candidates acquired from the screen, cisplatin-resistant clones were expanded and collected to isolate genomic DNA, and the shRNA region was PCR amplified, cloned and sequenced.

116 genes were identified through this approach. Because the data is currently unpublished, a full list of candidates will not be included in this report, but genes were identified in

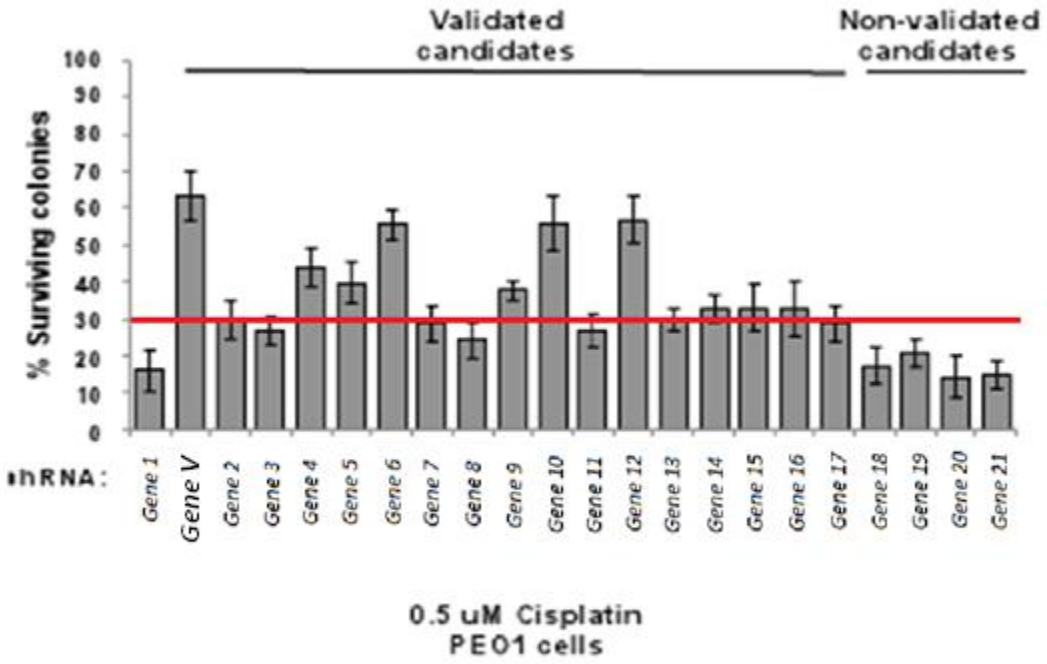
all of the following functions:

Table 2*: *Functions of Screen Candidates

Signal transduction	Intracellular protein transport	Channel pathways
Cell cycle regulation	Vesicle trafficking	Cell motility
Transcription	Cell metabolism	Immunity
DNA repair	Protein metabolism	Unknown
Chromatin remodeling	Lipid metabolism	Translational regulation

Validation of Gene Candidates

As an initial assessment of the primary screen, we selected candidates and tested whether single shRNAs against each gene conferred cisplatin resistance in the PEO1 as well as in the BRCA2 mutant FA patient fibroblast cell line, FA-D1. In brief, cells were stably transduced with a single shRNA against each of the 21 candidates or a non-silencing control (NSC) shRNA. Cells were then treated with cisplatin and growth was assessed by cell colony survival assay. The results below indicate that ~80% of the candidates validated, which suggests, by extrapolation, that ~92 of the 116 candidates from the primary screen will validate. Some genes, whose loss confers robust cisplatin resistance in PEO1, but not FA-D1 cells, are ovarian cancer suppressor genes or cell line specific. Instead, genes, such as ***Gene V*** (Figure 7) whose loss confers robust cisplatin resistance in both lines, could be BRCA2 tumor suppressor genes.



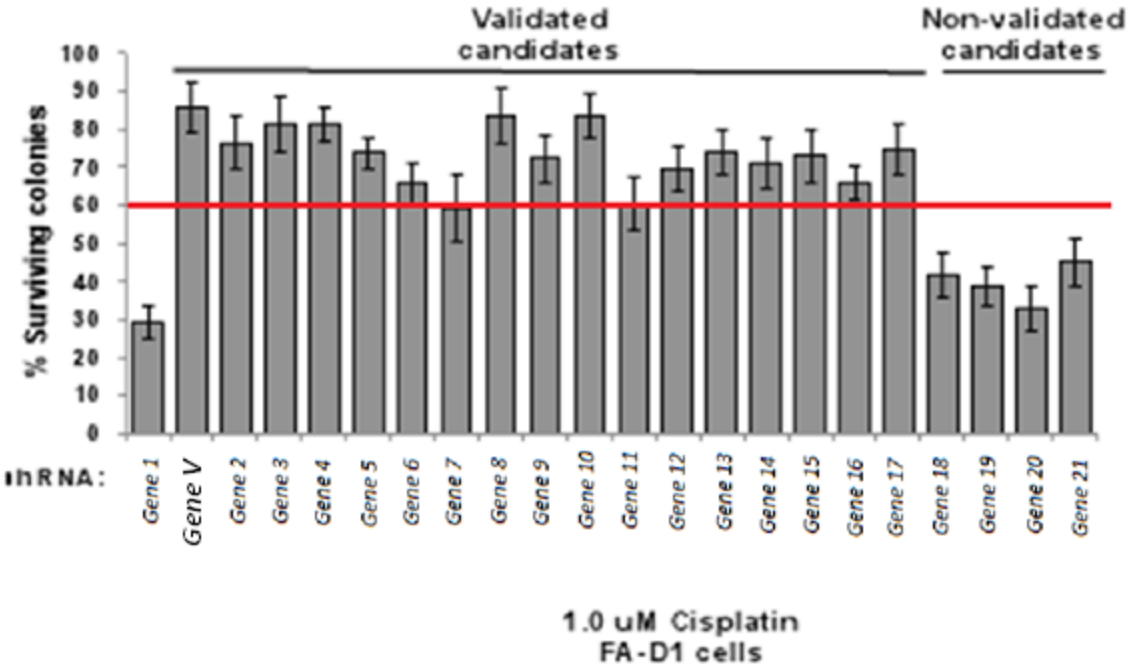


Figure 7: Validation of a subset of randomly selected candidates

Colony formation assays in PEO1 (A) or (B) FA-D1 cells. The red cut-off line indicates a 2-fold increase over the non-silencing control (NSC). Error bars indicate SEM. Gene V (identity has been omitted until publication) shows the most robust validation in both the PEO1 and FA-D1 cell lines.

Genes Commonly Mutated in Ovarian Cancers

The hypothesis of this project predicts that loss-of-function of a gene which confers cisplatin resistance will cooperate with BRCA2-deficiency to induce cancer and/or accelerate disease progression. To determine whether any of the candidate genes we identified in the primary screen are deleted in human cancer, we performed bioinformatic analysis using several publically available databases. In particular, *Tumorscape* was used to identify recurrent copy number variations (a GISTIC Q-value), which point to intervals with oncogenes and tumor suppressors. For a more detailed description of the methods used to analyze these data, see [1]. Using this analysis tool, we identified 7 genes with significantly decreased copy number ($Q<0.25$) in cancer samples compared with reference samples (Table 3). In addition, the Cancer Geonome Atlas revealed that many of our candidate genes are mutated in cancer, especially ovarian cancer (Table 4). In summary, these results establish the biological and clinical relevance of the genes found in our screen.

Table 3: GISTIC-Q Values

Values were derived from the *Tumorscape* cancer databae. These genes surfaced in our screen, and were shown to be significantly deleted in ovarian cancer in the database. A low GISTIC Q-value denotes a likelihood that the gene is deleted across the entire genome , and that the deletion at this locus is enhanced by a selective pressure. These genes' validation data is currently unavailable.

Gene	Chromosomal locus	GISTIC Q-value
DDX1	2p24	0.0694
POLK	5q13	0.0798
COMMD10	5q23.1	0.232
CDO1	5q23.2	0.0776
COL28A1	7p21.3	0.0789
PTGES	9q34.3	0.00556
PRMT7	16q22.1	0.192

Table 4: Mutations in Screen Genes

Using the Cancer Genome Atlas, a number of genes were identified with known mutations in ovarian cancer. This table displays the genomic and amino acid mutations that are observed in these genes. The data confirms that the gene screen is producing valid results, as the genes that have been found are validating in other contexts. As the data for the screen is unpublished at this time, the names of specific genes have been omitted.

Gene	Genomic DNA Mutation	Amino Acid Mutation
GENE A	1060C>T	R354W
	2553A>T	E851D
GENE B	1258G>A	E420K
GENE C	30C>G	I10M
GENE D	633A>C	K211N
GENE F	470G>T	C157F
GENE G	4964C>T	T1655I
	6803C>A	T2268K
	8866C>G	L2956V
GENE H	262C>G	L88V
	299G>T	G100V
	1321A>G	N441D
	5322G>C	M1774I
GENE I	132G>T	E44D
	514A>C	T172P
GENE J	1508A>G	K503R
GENE K	1571C>A	P524H
GENE L	143G>T	R48I
GENE M	1006C>G	Q336E
GENE N	743A>T	D248V
GENE O	1094T>G	V365G
	1281C>G	E427D
	2445C>A	N815K
GENE P	865G>A	A289T
	2015A>C	K672T
GENE Q	220G>A	V74I
GENE R	241_243delAGA	R81del
GENE S	554T>C	M185T
	661T>A	S221T
GENE T	1274G>A	R425Q

Cell Line Testing

After validation, cisplatin resistant ovarian cell lines were blotted for expression of robustly validated gene candidates. The gene which showed the highest level of resistance in both the PEO1 and FA-D1 cell lines was **Gene V** (Figure 7). This gene codes for chromodomain helicase which

helps regulate transcription. Using a purchased antibody, the parental PEO1 and resistant C4-2, C4-4, C4-11 and C4-13 cell lines were blotted for expression (see Figure 5 for cell line information).

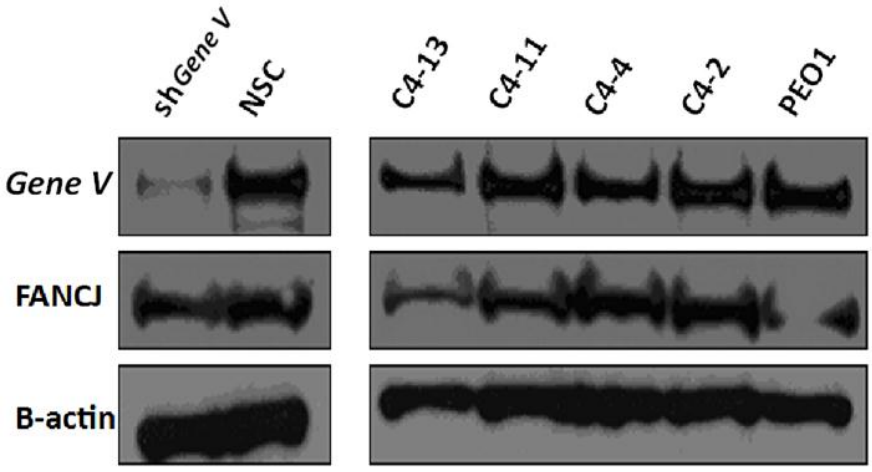


Figure 8: Western Blot for Validated Gene

Resistant cell lines were blotted for protein expression of Gene V. FancJ and B-actin were used as loading controls. Under expression of Gene V can be seen in both the C4-13 and C4-4 cell lines. A bubble in the western blot caused a disruption in the PEO1-FancJ band.

Discussion

Currently, the 116 candidates identified through the genetic screen are being rigorously tested through the validation process. This requires confirmation that the proper gene is being knocked down using two different hairpin sequences, three rounds of cisplatin treatment for each hairpin, and potentially validation through western blotting. It is very promising; however, that many of the genes identified in the screen already have a documented relationship with cancer cases (Tables 3 and 4.) This not only confirms the integrity of the screen, but may also point to genes which are clinically significant and should be further studied in BRCA2 cancers. Once validated, it will be imperative to study these genes in clinical applications- results in the lab may have little significance in actual patient cases.

Validation of the genes has been a long processes, but in the coming months the research will be moving into BRCA2 null cell lines for further testing. It is hypothesized that genes which show a robust resistance to cisplatin (Figure 7) will be under expressed or severely mutated in BRCA2 cell lines. The cell lines from the Taniguichi lab are a good place to start, but other resistant cell lines can be derived simply by treating PEO1 cells with cisplatin and rescuing those colonies which are not sensitive. This may also be done with cell lines other than PEO1, and the results would be more convincing if these procedures were performed in a BRCA2 breast cancer cell line as well.

After validation in cell lines, the next step will be to enter patient tumor samples. Access to the 200 ovarian cancer samples from the UMASS Cancer Center Tissue Bank (a core facility of our Cancer Center) directed by Dr. Stephen Lyle will be very helpful in this endeavor. Ovarian cancer samples corresponding to various histological subtypes and matched normal tissue controls can be retrieved from archival collections of formalin-fixed, paraffin embedded tissue samples (with associated clinical follow-up data). Collectively, through the Dana Farber, University of Washington, and UMASS Medical School repositories we will have access to ovarian cancer samples and expertise to directly test the hypothesis that genes identified in our screen are lost in tumors with poor response to platinum-therapy.

As can be seen in Table 2, the functions of the genes discovered in the screen vary widely, from metabolism to transport to chromosome binding. This implies that there are many different pathways that might be involved in BRCA2 cancer cisplatin resistance. The pathways might be impacted directly by a loss of BRCA2, or they might be defective in other mechanisms, such as shuttling the drug into the cell. This opens up a wide variety of possibilities for clinicians. Individual cases may need to be treated using vastly different methods in order to target the specific genetic inconsistency a patient has. With the advent of faster, cheaper, and more portable sequencing techniques, clinicians will be able to peer directly into a tumor's genome before they decide the best course of action for treatment. The genes identified in this screen could be used as important biomarkers in future cancer patients.

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