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Article

DNA repair protein mutations alter blood cancer sensitivity to cisplatin or gemcitabine *in vitro*

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SUMMARY

Chemotherapy is the most widely used cancer treatment, yet patient responses vary greatly. Thus, identification of cancer genomic biomarkers for heightened sensitivity to conventional chemotherapy is a promising strategy to improve patient outcomes. In our study, we used a publicly accessible database, OncoExpress, to investigate whether chemotherapeutic drugs cisplatin (CDDP: platinum-based DNA crosslinker) and gemcitabine (GEM: DNA-synthesis inhibitor) show enhanced or reduced activity against cancer cell lines originating from human blood cancers carrying different types of DNA-repair mutations. A total of 9 DNA-repair protein genes (BRCA1, PAXIP1, WRN1, PARP1, PARP2, NEIL1, MLH1, PMS2, MSH2) were analysed for their sensitivity to cisplatin and gemcitabine using the independent samples t-test and Glass's delta. We hypothesized that mutations in genes involved in DNA repair could sensitize cancer cells to cytotoxic drugs that induce DNA damage. Our results showed that at a significance level of $p<0.10$, BRCA1 mutations led to significant sensitization to cisplatin ($p=0.057$) or gemcitabine ($p=0.029$). Additionally, WRN ($p=0.099$) or PARP2 ($p=0.045$) mutations led to sensitization to gemcitabine.

INTRODUCTION

The current mainstay treatment for blood cancers is still systemic chemotherapy, but the results are often underwhelming with low patient response rates and high incidence of relapse (1). To address this problem, recent studies have explored cancer genomic biomarkers for sensitivities to chemotherapeutic agents. A Phase III clinical trial found that in breast cancer patients with germline-mutated DNA-repair protein genes *BRCA1* or *BRCA2*, the DNA-damaging agent carboplatin conferred a twice-higher objective response rate in the patients than the anti-microtubule agent docetaxel (2). This finding supported the potential utility of chemotherapeutic agents based on a cancer's genomic profile as biomarker-based precision therapies.

For a chemotherapeutic agent to be regarded as a precision therapy, a clear functional relationship between the target biomarker and the pharmacological action of the agent must be established. Cisplatin and gemcitabine are common DNA-damaging chemotherapeutic drugs used in the treatment of multiple cancers such as breast, lung and ovarian (3). Cisplatin forms inter and intra-strand crosslinks, altering the structure of DNA. This disrupts DNA replication and transcription. Similarly, gemcitabine inhibits DNA synthesis and contributes to DNA replication stress. However, the efficacy of cisplatin and gemcitabine depends not only on the drugs' abilities to induce DNA damage, but also on the cellular response to the DNA damage. The DNA repair protein mutations in blood cancer cell lines may thus confer increased sensitivity or resistance towards cisplatin or gemcitabine.

There are numerous mechanisms of DNA repair in humans composed of countless enzymes. Among them, the homologous recombination repair (HRR), base excision repair (BER), and mismatch repair (MMR) enzyme groups are of special interests in modern oncology. HRR enzymes are important in double-stranded DNA damage repairs — the primary mode of DNA damage repair. Mutations in genes such as *BRCA1, BRCA2, WRN, PAXIP1*, and others have been reported in high frequency in many cancer types including those of breast and prostate among others (4, 5). BER enzymes' roles are primarily in single-stranded DNA repair processes, and they are considered the secondary line of defence against DNA damage involving the enzymes PARP1, PARP2, NEIL1, NEIL2, etc. Hence, mutations in these genes may alter cancer response to DNA-damaging agents. MMR is a highly important system for detecting and repairing base-pair errors, and includes enzymes such as MSH2, MLH1, and PMS2. Tumors with MMR deficiencies have thus been shown to have mutations much more frequently than those without MMR deficiencies (6). Given the importance of these three enzyme groups in DNA repair, we decided to study how mutations in these enzymes affect chemosensitivity of human blood cancer cell lines to cisplatin or gemcitabine *in vitro.*

RESULTS

Data on the average IC_{50} values of the 20 blood cancer cell lines containing mutations in 14 initially selected DNA repair-related genes for cisplatin and gemcitabine were collected from OncoExpress, a publicly available database **(Table 1 and 2)** (7). We observed that the IC_{50} values for gemcitabine are, on average, smaller than the IC_{50} values for cisplatin across the 20 cell lines. This indicated that gemcitabine is generally more cytotoxic than cisplatin in inhibiting the growth and proliferation of these blood cancer cell lines.

Out of the 14 initial genes selected, 4 genes (*ATM, ATR, PARG,* and *TP53*) were found to be mutated in 19 of the 20 blood cancer cell lines and hence were removed from further statistical analysis. One of the genes, *BRCA2*, was wild type in 19 of the 20 blood cancer cell lines, and hence was

No.	Cell Line Name	Tissue	Cancer Type	Cisplatin IC_{50} (μM/mL)	Gemcitabine IC_{50} (μM/mL)
1	Daudi	Blood	Burkitt lymphoma	0.5421	0.0046
2	HL-60	Blood	Acute promyelocytic leukemia	1.7106	0.0056
3	JVM-3	Blood	Chronic B-cell leukemia	0.8980	0.0046
4	K-562	Blood	Chronic myeloid leukemia	7.8955	30.0000
5	ML-2	Blood	Acute myeloid leukemia	1.2802	0.0049
6	MOLM-13	Blood	Acute myeloid leukemia	0.9735	0.0046
7	MOLM-16	Blood	Acute myeloid leukemia	1.7953	0.0046
8	MOLP8	Blood	Multiple myeloma	0.9290	0.0046
9	Molt-4	Blood	Acute lymphoblastic leukemia	0.7159	0.0150
10	MV-4-11	Blood	Acute myeloid leukemia	0.6071	0.0046
11	NALM-6	Blood	B-cell precursor leukemia	1.1796	0.1510
12	NAMALWA	Blood	Burkitt lymphoma	0.2857	0.0076
13	NCI-H929	Blood	Multiple myeloma	2.8741	0.0046
14	Pfeiffer	Blood	Acute T cell leukemia	4.4097	2.3813
15	Raji	Blood	Burkitt lymphoma	2.6230	0.0048
16	Ramos	Blood	Burkitt lymphoma	2.8426	0.0046
17	SU-DHL-6	Blood	B-cell non-Hodgkin's lymphoma	7.1067	0.0064
18	THP-1	Blood	Acute monocytic leukemia	1.7252	0.0074
19	U-937	Blood	Histiocytic lymphoma	2.5016	0.0182
20	WSU-NHL	Blood	Acute lymphoblastic leukemia	0.3129	0.0046

Table 1: Sample of 20 blood cancer cell lines studied. Cancer types, cisplatin and gemcitabine IC_{50} values are shown (Crownbio, OncoExpress).

likewise removed from statistical analysis. The remaining 9 genes were then subjected to independent samples *t*-test and Glass's *delta* statistical test for analysing the effect of each mutation on the efficacy of cisplatin and gemcitabine **(Figure 2A-B)**.

Statistically significant mutation-conferred sensitization to cisplatin versus *BRCA1* (p=0.057), gemcitabine versus *BRCA1* (p=0.029) and *WRN* (p=0.099), as well as to gemcitabine versus *PARP2* (p=0.045) were demonstrated **(Figure 3).** The effect sizes on each of the four were all greater than 0.4, indicative of statistically significant chemosensitization effects upon mutations in the subject genes. Synonymous mutations were not discarded in the process to permit the possibility of RNA-folding anomalies that might impact overall cellular responses.

Interestingly, although above the set significance level of 10%, *PMS2* and *MSH2* mutations resulted in large negative Glass's *delta* values for both cisplatin and gemcitabine, indicating that cell lines with mutations in *PMS2* and *MSH2* may exhibit an increased resistance to the drugs. This observation is consistent with the previous report of cisplatin resistance found in *PMS2* or *MSH2* mutated cell lines (8).

DISCUSSION

Owing to the etiology of the cancers, cisplatin and gemcitabine IC_{50} values may differ significantly between blood cancers with different cellular origins **(Table 1)**. An analysis of diffuse large B-cell lymphomas found that there were two subtypes of the cancer with different cellular origins (9). This resulted in drastically different responses to standard chemotherapeutic treatments. Germinal center B-like diffuse large B-cell lymphoma responded favourably to chemotherapy while chemotherapy had poor efficacy on activated B-like diffuse large B-cell lymphoma. The difference in cellular origins of blood cancers in our study may likewise account for the large error bars seen in **Figure 2.**

The IC_{50} for gemcitabine in the cell line K-562 was 30.000 μM/mL, implying that gemcitabine is ineffective in inhibiting K-562 activity (Table 1). K-562 originates from the bone marrow of chronic myeloid leukemia (CML) patients, characterized by the expression of the tyrosine kinase oncoprotein BRC-ABL1. Hence, as opposed to chemotherapy, CML is most effectively treated using tyrosine kinase inhibitors such as Imatinib mesylate, which blocks the ATP-binding site of BRC-ABL1 and the platelet-derived growth factor receptor (10).

Our study used a publicly available *in vitro* databank to demonstrate that blood cancer cells with mutations in BRCA1 are more sensitive to both cisplatin and gemcitabine (p<0.10, Glass's delta, Figure 3). The formation of cisplatin-DNA adducts is attributed to the disruption of DNA structure by inter-strand and intra-strand crosslinks, therefore hindering DNA replication and transcription (11). Similarly, DNA double-strand breaks are also caused by gemcitabine (12). It is widely accepted that cancer sensitivity to chemotherapeutic drugs not only depends on the drugs' ability to cause DNA damage, but also on the cancer's capacity for repairing the induced DNA damages. BRCA1 mutations resulting in blood cancers are often loss-of-function, evident by how BRCA1 is greatly downregulated and almost undetectable in leukemia cells (13). In this light, it can be argued that mutations that decrease the amount of BRCA1 present — the major DNA repair protein — would hinder cancer's ability to repair damaged DNA, thereby sensitizing it to DNA-damaging drugs such as cisplatin and gemcitabine (12). In further support of this idea, Alli et al. demonstrated BRCA1 mutations causing breast cancer led to greater cancer chemosensitivity to DNA-

DNA repair-related gene	No. of mutated cell lines	Mutated cell lines	No. of wild type cell lines	Wild type cell lines
ATM	20	1 - 20	0	-
ATR	20	1 - 20	0	-
BRCA1	15	1-3, 5, 7-10, 12, 13, 15, 17-20	5	4, 6, 11, 14, 16
BRCA2	20	1 - 20	0	-
MLH1	8	1, 5, 6, 8, 9, 15, 17, 19	12	2-4, 7, 10-14, 16, 18, 20
MSH2	11	1, 4- 8, 12-13, 15, 17, 19	9	2, 3, 9-11, 14, 16, 18, 20
PAXIP1	11	1, 3, 5, 8, 10, 15-20	9	2, 4, 6, 7, 9, 11-14
PMS2	17	1, 2, 4-10, 12, 14-20	3	3, 11, 13
NEIL1	10	1, 8-10, 13-16, 18, 20	10	2-7, 11-12, 17, 19
PARG	1	1	19	2-20
PARP1	11	2-5, 9, 11-13, 15, 17, 19	9	1, 6-8, 10, 14, 16, 18, 20
PARP2	5	4, 5, 9, 11, 19	15	1-3, 6-8, 10, 12-18, 20
TP53	19	1, 3-20	1	2
WRN	13	1, 2, 5, 6, 8-10, 14, 16-10	7	3, 4, 7, 11, 12, 13, 15

Table 2: DNA repair-related genes and the cell lines in which the genes are mutated or wild type. Mutant and wild type cell line numbers correspond to those indicated in Table 1. Rows that have been highlighted in grey indicate genes lacking either mutant or wild type cell lines for comparison.

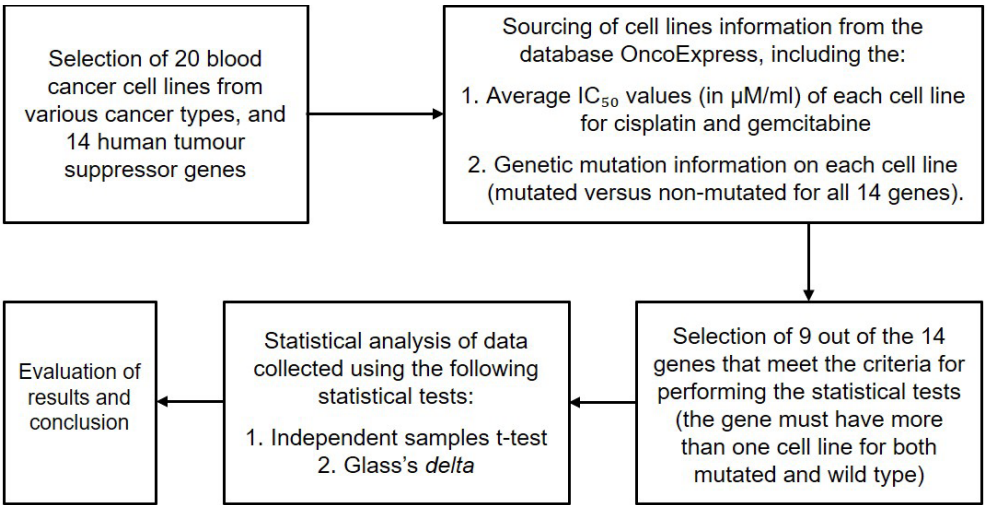

Figure 1: Flowchart of study methodology. Blood cancer cell lines and DNA repair-related genes were selected, evaluated, and compared using statistical analysis.

damaging chemotherapeutics than non-DNA-damaging types (14). Together, this literature supports our findings of cisplatin and gemcitabine sensitization in blood cancers with BRCA1 mutations.

We also observed that cell lines with *WRN* mutations showed increased sensitivity to gemcitabine ($p<0.10$, Glass's *delta*, **Figure 3**). WRN belongs to the group of RecQ helicases (15). These proteins play important roles in the repair of DNA double-strand breaks which occur during meiosis and DNA replication (16). Specifically, WRN is involved in the repair of DNA double-strand breaks by homologous recombination, non-homologous recombination, and base excision repair replication (16). Sallmyr et al. report that mutations in *WRN* results in the up-regulation of WRN production, thus leading to the increased activity of an alternative nonhomologous end-joining repair pathway in leukemia cells (17). This results in an increase in unrepaired double-strand breaks (17). Hence, we speculate that the increased activity of *WRN* arising from its gain-of-function mutation could therefore result in a cell's inability to repair DNA double-strand breaks induced by gemcitabine.

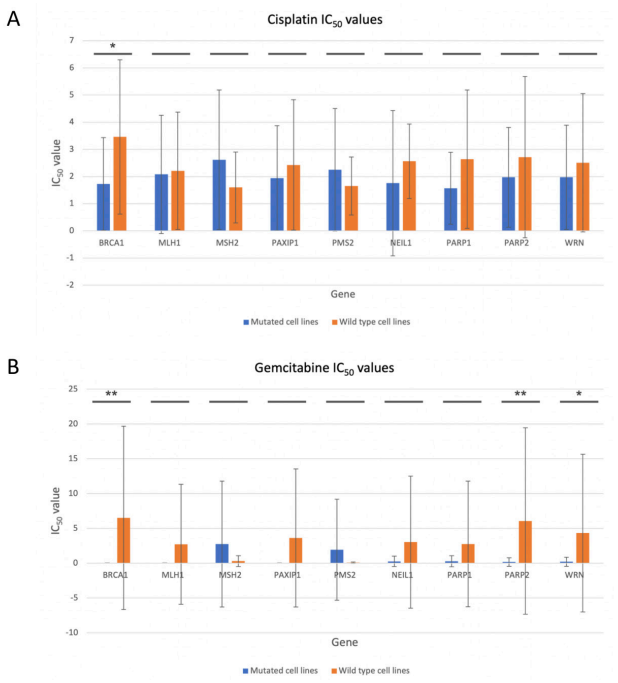

Figure 2: Effects of cisplatin and gemcitabine on blood cancer cell lines. Mean IC_{50} values of (A) cisplatin and (B) gemcitabine for mutated and wild type cell lines of the 9 selected genes (N = 20). Error bars represent standard deviation. One-tailed independent t-test conducted on data. *$p < 0.10$ and **$p < 0.05$. For cisplatin, mutated BRCA1 is significant compared to wild type. For gemcitabine, mutated BRCA1, PARP2 and WRN are significant compared to wild type.

Gemcitabine was also shown to have preferential efficacy towards cells with mutations in *PARP2* ($p<0.10$, Glass's *delta*, **Figure 3**). PARP2 is an enzyme belonging to the family of poly(ADP-ribose) polymerases (PARPS) (18). PARP2 is a DNA repair protein that maintains the cell's genomic stability by participating in pathways in base excision repair, cell-cycle regulation, telomere maintenance, and DNA replication stress reduction (18). As gemcitabine's mechanism of action is masked chain termination that inhibits DNA synthesis and contributes to DNA replication stress (19), this pharmacology may explain the observed sensitization effect in the blood cancer cell lines with *PARP2* mutations.

Some study results were contrary to our original hypothesis. Although statistically insignificant, we noted a trend between the mutations in *PMS2* and *MSH2*, both of which cause mismatch repair (MMR) deficiency, with possible resistance to both cisplatin and gemcitabine **(Figure 3).** This observation is noteworthy as MMR deficiencies were previously reported to cause tumor resistance to cisplatin (20) and gemcitabine (21). However, it is important to note that several studies on the role of MMR mutations in resistance to anticancer treatments have also shown inconclusive or contradictory results. For example, Cooper et al. found that there was no correlation between MMR proficiency and patient survival rate, while Fedier et al. showed that the lack of *PMS2* expression resulted in an increased sensitivity to drugs such as cisplatin and gemcitabine (22, 23). The contradictory literature on MMR deficiency's effects on chemosensitivity may help explain the weak statistical association between the chemosensitivity and

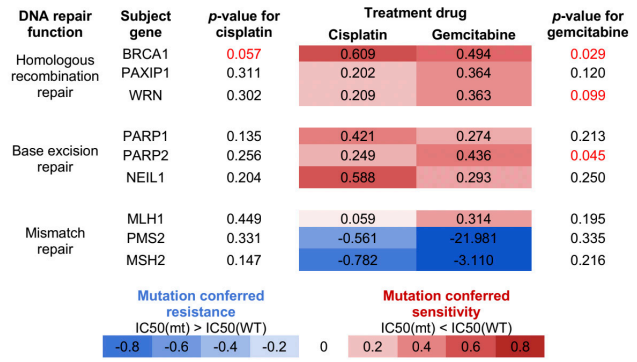

Figure 3: Glass's delta value analysis on mutation effect towards IC_{50} value distribution across the screening panel. p-values were calculated using the one-tailed student's t-test at $p < 0.10$ (significant values shown in red text). For cisplatin, BRCA1 displays a significant difference between the IC_{50} values for mutated and wild type cell lines, as well as the largest Glass's delta value. For gemcitabine, BRCA1, WRN and PARP2 show a significant difference.

PMS2/MSH2 mutations in our study.

Repeated observations of a link between DNA-repair gene deficiencies and preferential chemosensitivity in cancer cells implicates potential pharmacological synergism between DNA damaging drugs and DNA repair pathway inhibitors. Interestingly, the synergistic effects of PARP inhibitors and chemotherapeutic drugs such as gemcitabine and cisplatin are currently under investigation for the treatment of various aggressive cancers such as locally advanced pancreatic cancers and triple-negative breast cancers (24). In this regard, and in extension of our study, combining inhibitors of WRN or BRCA with gemcitabine and or cisplatin may represent new opportunities for cancer treatment.

Our research has several limitations. Firstly, only one database, the CrownBio Oncology database, OncoExpress (7), was used during the study. Relying on a single dataset could have resulted in biased results, thus our findings may require further validation against larger and independent datasets. Additionally, wild type blood cancer cell lines with no mutations in each of the 9 investigated genes were used as a control in our study. Further studies may be improved by using non-cancer, healthy lymphoid cell lines with no mutations in any given gene of interest as a control. Finally, since the correlations between DNA repair mutations and the efficacy of cisplatin and gemcitabine in the current study were assessed *in vitro*, their clinical relevance is yet to be determined.

Withstanding the limitations, our study expands upon previously reported experimental results to highlight the promise of cisplatin and gemcitabine, which are highly affordable, in precision oncology. Future work in this area may enable patients with cancer cell mutations in either *BRCA1, WRN,* or *PARP2* to be treated more effectively and economically. Our findings also help corroborate the utility of an informatic approach using a public oncology database to identify the investigative opportunities of current therapeutics in precision oncology.

MATERIALS AND METHODS

Our investigation included data mining, selection of relevant cancer cell lines and tumor suppressor genes, identification of IC_{50} values, and statistical analysis **(Figure 1)**. Our data was sourced from the CrownBio Oncology database, OncoExpress (7), which collates datasets from various worldwide online databases. OncoExpress provides model information on various genetic drivers behind tumorigenesis, including growth curves, mutations and copy number analysis. Microsoft Excel was used to compile the data. For statistical analysis, online statistics calculators: *t*-Test for 2 independent means and effect size calculators, from Social Science Statistics (25) were used to conduct the independent samples *t*-test and Glass's *delta*.

Initially, a total of 20 cancer cell lines **(Table 1)** from different types of blood cancers, and 14 tumor suppressor genes **(Table 2)** were chosen to be studied in our research investigation. The 20 cancer cell lines and 14 genes were selected if they satisfied the following two conditions: firstly, that they had originated from blood cancers, and secondly, that they had been previously treated *in vitro* with cisplatin and gemcitabine and thus had data available. The average IC_{50} values (in μM/mL) of each human cell line for the chemotherapy drugs cisplatin and gemcitabine were extracted from the database's dose-response curve.

The half maximal inhibitory concentration (IC_{50}) is a commonly used measure of the potency of a substance, such as a drug, in inhibiting a specific biological process by half (26). Thus, the IC_{50} values in **Table 1** indicate the molar concentrations of cisplatin and gemcitabine needed to inhibit, *in vitro*, the respective human cell lines by 50%. The lower the IC_{50} value, the more effective the drug is in inhibiting that particular cell line.

We then obtained genetic mutation information on the cell lines to differentiate between the mutated and wild type cell lines for each of the 14 human DNA repair-related genes. The mutant and wild type cell lines for each mutation are shown in **Table 2**. *ATM, ATR, BRCA2* and *TP53* had an insufficient number of wild type cell lines, while *PARG* had an insufficient number of mutated cell lines to be used in the analysis. Thus, statistical tests could not be performed and these genes were excluded from our analysis.

For our statistical analysis, the independent samples *t*-test was used to determine if differences in the IC_{50} effect of chemotherapy drugs on mutant and wild type cell lines were statistically significant. A one-tailed *t*-Test was conducted, since we hypothesized that mutations in each gene would case increased sensitivity to cisplatin or gemcitabine (IC_{50} (mt) < IC_{50} (WT)). Additionally, Glass's *delta* was used to measure the effect size of the chemotherapy drugs on the mutated cell lines versus the wild type cell lines for each of the genes studied. Glass's *delta* was used over other effect size measures due to large differences in standard deviations of IC_{50} values between mutated and wild type cell lines **(Figure 2).**

The formula for Glass's *delta* is shown below:

$$\text{Glass's } delta = \frac{M_1 - M_2}{S_{control}}$$

We defined M1 and M2 as the following – M1: wild type; M2: mutated; and where wild type is the control group.

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