Molecular Networks of Platinum Drugs and Arsenic Trioxide and Its Interaction With microRNAs in Cancer

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Posted Date: 18 May 2023

doi: 10.20944/preprints202305.1304.v1

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Article

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Simple Summary: The precise mechanism of resistance to platinum drugs or arsenic trioxide is not fully revealed. To reveal the relationships among the drug resistance, the molecular networks of cisplatin, carboplatin, oxaliplatin, and arsenic trioxide were analyzed in several types of cancers. Since diffuse-type gastric cancer (GC), which has epithelial-mesenchymal transition (EMT)-like characteristics, is more malignant compared to intestinal-type GC, the molecular networks in the diffuse- and intestinal-type GC have been analyzed as well. The upstream regulators of cisplatin-treated lung adenocarcinoma included trichostatin A (TSA), a histone deacetylase inhibitor (HDI). Treatment with arsenic trioxide was related to the tumor microenvironment.

Abstract: To reveal the relationship between metallodrugs and cancer malignancy, molecular networks of anti-cancer drugs were analyzed. Molecular networks in several types of cancers were analyzed in Ingenuity Pathway Analysis (IPA). Analysis of carboplatin revealed the causal network in diffuse large B-cell lymphoma. Analysis of 12 analyses of cisplatin treatment identified causal networks including camptothecin and NUPR1. The causal network of camptothecin, which includes PTEN, FAS, and IRF1, was inactivated in diffuse-type GC and activated in intestinal-type GC. Upstream regulator analysis of cisplatin revealed an increase in FAS, BTG2, SESN1 and CDKN1A, and the involvement of the tumor microenvironment pathway. Upstream regulators of cisplatin-treated lung adenocarcinoma included a histone deacetylase inhibitor, trichostatin A (TSA). Causal network of arsenic was inactivated in diffuse-type GC and activated in intestinal-type GC, and included ERK, EGFR, SRC, IKK and TP53. Prediction of RNA-RNA interactions with the causal network of arsenic identified 10 microRNAs including mir-101, mir-103, and mir-22. The results revealed the involvement of EMT in arsenic treatment. Analysis of oxaliplatin, a platinum drug, revealed that the SPINK1 pancreatic cancer pathway is inactivated in ischemic cardiomyopathy. The study showed the importance of the relationship between platinum drugs or arsenic trioxide and the tumor microenvironment in the treatment of resistant cancer in humans.

 doi:10.20944/preprints202305.1304.v1

Keywords: drug resistance; cisplatin; arsenic; tumor microenvironment; microRNA; molecular pathway network

1. Introduction

Metallodrugs, including platinum drugs and arsenic, are widely used as cancer therapeutics [1–5]. Platinum drugs, such as cisplatin and carboplatin, are commonly used for treatment of a variety of cancers including bladder, head and neck, lung, gastrointestinal, ovarian, and testicular cancer [6,7]. Arsenic trioxide is also the front-line drug for the treatment of acute promyelocytic leukemia [8,9]. However, the inherent and acquired drug resistance frequently prevents their widespread use in cancer treatment. Although numerous research had examined the mechanism of chemoresistance, the extent to which metallodrugs produce drug resistance has not yet been fully revealed [10–13]. A study in oral squamous cell carcinoma suggested that microRNA (miRNA)-485-5p targets keratin 17 to regulate oral cancer stemness and chemoresistance through integrin, FAK, Src, ERK, β-catenin pathway [10]. N^6-methyladenosine RNA methylation of centromere protein K in cervical cancer promoted stemness and chemoresistance. The activation of Wnt/β-catenin signaling leads to the enhancement of DNA damage repair pathways that are necessary for cisplatin/carboplatin resistance and epithelial-mesenchymal transition (EMT) involved in metastasis [11]. In non-small cell lung cancer, miRNAs play roles in the resistance to platinum-based chemotherapy such as adjuvant cisplatin (CDDP, *cis*-diammine-dichloro-platinum II) [12]. It has been suggested that miR-129-1-3p, miR-155, miR-200c, miR-17 family (-17, 20a, 20b), miR-15b, miR-27 and miR-181a are involved in CDDP resistance and EMT in non-small cell lung cancer [12]. EMT is one of the features of cancer malignancy and treatment resistance [14–18]. Previous studies demonstrate that EMT is implicated with cisplatin resistance [19–21]; however, precise mechanisms and the underlaid networks are not fully understood. In this context, the present study investigates the relationship between metallodrugs and EMT and tumor microenvironment pathways. Additionally, the molecular networks of carboplatin, cisplatin, and arsenic were investigated in the study.

2. Materials and Methods

2.1. RNA sequencing data collection

The RNA sequencing data of diffuse- and intestinal-type GC are publicly available in The Cancer Genome Atlas (TCGA) of the cBioPortal for Cancer Genomics database at the National Cancer Institute (NCI) Genomic Data Commons (GDC) data portal [22–25]. Publicly available data on stomach adenocarcinoma in the TCGA [24] were compared between diffuse-type GC, which is genomically stable (n = 50), and intestinal-GC, which has a feature of chromosomal instability (n = 223), in TCGA Research Network publications, as previously described [22,26,27].

2.2. Network Pathway Analysis

Data on intestinal- and diffuse-type GC from the TCGA cBioPortal for Cancer Genomics were uploaded and analyzed through the use of Ingenuity Pathway Analysis (IPA) (QIAGEN Inc., Hilden, Germany) [28]. In the IPA database, causal networks were analyzed and bioprofiler analysis was performed on carboplatin. Causal networks, canonical pathways and regulatory networks were analyzed in gastric adenocarcinoma data, lung adenocarcinoma data, diffuse large B-cell lymphoma data, and arsenic treatment data in the IPA database.

2.3. Cell culture of gastric cancer cells

OCUM-2MLN, a scirrhous gastric cancer model cell line, was maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin-streptomycin and 10% Fetal Bovine Serum (FBS) in a humidified incubator at 5% CO_2.

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2.4. Characterization of EMT markers in scirrhous gastric cancer cells

Expression profiles of genes associated with EMT were characterized by reverse transcription (RT)-qPCR in OCUM-2MLN. Cells were seeded in 6-well plates at 0.5 x 10^6 viable cells per well. After 24 hours, the culture medium was replaced with medium containing 0 µM, 1 µM, or 10 µM cisplatin (Sigma Aldrich). Total cell RNA was isolated using an RNeasy mini kit (QIAGEN) after 24 hours. Reverse transcription was carried out with a ReverTraAce qPCR RT Master Mix kit (TOYOBO). Expression of VIM, CDH1, CDH2 and ACTB genes was quantified with qPCR with a FastStart Universal SYBR Green Master (Rox) kit (Roche) using a 7500 Fast Real-Time PCR System (Applied Biosystems). Expression levels compared to untreated control were calculated using the ddCt method with ACTN as an internal reference. The primers used are listed in Table 1.

2.5. Statistic Analysis

Statistical significance was calculated using a Student's t-test on the log-transformed FC data. The data were log-transformed to ensure that the data is normally distributed.

Table 1. The sequences of the primers used in RT-PCR.

Primer name	Primer sequence
CDH1 FWD (forward)	GGGGTAGTGAGGATCTTGAT
CDH1 REV (reverse)	TCCTTTTCCACCCCCAAAGA
CDH2 FWD (forward)	GGCATAGTCTATGGAGAAGT
CDH2 REV (reverse)	GCTGTTGTCAGAAGTCTCTC
VIM FWD (forward)	GCTTTCAAGTGCCTTTCTGC
VIM REV (reverse)	GTTGGTTGGATACTTGCTGG
ACTB FWD (forward)	CCCAAAGTTCACAATGTGG
ACTB REV (reverse)	AAGGGACTTCCTGTAACAAC

3. Results

3.1. Causal networks of carboplatin activity plot in cancer treatment

Platinum drugs were searched with the term "platinum" in Ingenuity Pathway Analysis, which included carboplatin and cisplatin (Table 2). To reveal the causal network of carboplatin treatment, master regulators in depth 3 were investigated in the carboplatin activity plot in IPA analysis. The analysis in the top z-score of 790 analyses for carboplatin in depth 3 of the master regulators was in diffuse large B-cell lymphoma (DLBC) (Figure 1A). In this analysis, EED226 (5 µM, 24 hrs), a potent and selective inhibitor of polycomb repressive complex 2 (PRC2), that directly binds to the histone H3 lysine 27 binding pocket of EED, a subunit of PRC2, was treated to DLBC [29]. Bioprofiler analysis on carboplatin identified the relationship between ALK mutation negative CD274 positive non-small cell lung cancer, ALK mutation negative EGFR sensitizing mutation negative non-squamous non-small cell lung cancer, CD274 low expression positive non-squamous non-small cell lung cancer, EGFR mutation negative CD274 positive non-small cell lung cancer, and locally advanced non-squamous non-small cell lung cancer (Figure 1B, C, Supplementary Table S1). Canonical pathways Regulation of the EMT by growth factors pathway, Tumor microenvironment pathway, NRF2-mediated oxidative stress response, Regulation of the EMT in development pathway and Regulation of the EMT pathway were related to the carboplatin network (Figure 1B, C). Drugs were identified to have direct relationship between the network, which included clazakizumab, anti-interleukin (IL)-6 monoclonal antibody (Figure 1B, C). IL-6 pathway was one of the components signaling pathways in the Regulation of the EMT by growth factors pathway (Figure 1D). IL-6 activates IL6 receptor (IL6R), leading to activation of JAK, STAT3 and TWIST1, which subsequently induces cell migration, cell invasion and metastasis (Figure 1D). Molecules in the Regulation of the EMT by growth factors pathway is listed in Table 3.

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Table 2. Platinum drugs searched with a term "platinum" in Ingenuity Pathway Analysis.

#	Symbol
1	platinum
2	Pt^{2+}
3	carboplatin
4	dicycloplatin
5	enloplatin
6	eptaplatin
7	iproplatin
8	nedaplatin
9	oxaliplatin
10	picoplatin
11	satraplatin
12	sebriplatin
13	zeniplatin
14	platinum agent
15	platinum chemotherapy regimen
16	platinum-based doublet chemotherapy
17	E platinum
18	platinum(II) chloride
19	platinum agent/trastuzumab
20	cisplatin
21	platinum chemotherapy regimen/radiotherapy
22	platinum-based doublet chemotherapy/taxane
23	platinum chemotherapy regimen/vinorelbine
24	platinum chemotherapy/taxoid derivative
25	platinum-based doublet chemotherapy/vinorelbine
26	platinum-based triplet chemotherapy
27	adjuvant chemotherapy/platinum agent
28	cetuximab/platinum chemotherapy regimen
29	gemcitabine/platinum chemotherapy
30	paclitaxel/platinum chemotherapy regimen
31	platinum-based neoadjuvant chemotherapy
32	bevacizumab/platinum chemotherapy/taxoid derivative
33	platinum-norsperimidine complex Pt3NSpd2
34	capecitabine/platinum chemotherapy regimen
35	bevacizumab/gemcitabine/platinum chemotherapy regimen
36	platinum chemotherapy regimen/thoracic radiotherapy
37	nonplatinum-based doublet chemotherapy
38	platinum acetylacetonate-titanium dioxide nanoparticles
39	BP-Cx1-platinum complex BP-C1
40	cetuximab/5-fluorouracil/platinum chemotherapy
41	etoposide/platinum chemotherapy regimen
42	ormaplatin
43	Bamet-UD2
44	NC-4016
45	fluoropyrimidine/platinum-based triplet chemotherapy
46	(diaminocyclohexane)(diacetato)(dichloro)platinum
47	lobaplatin
48	non-pemetrexed containing platinum chemotherapy regimen
49	triplatin tetranitrate
50	dacplatinum
51	cisplatin/etoposide
52	cisplatin/docetaxel
53	cisplatin/paclitaxel
54	carboplatin/paclitaxel
55	cisplatin/epirubicin/5-fluorouracil

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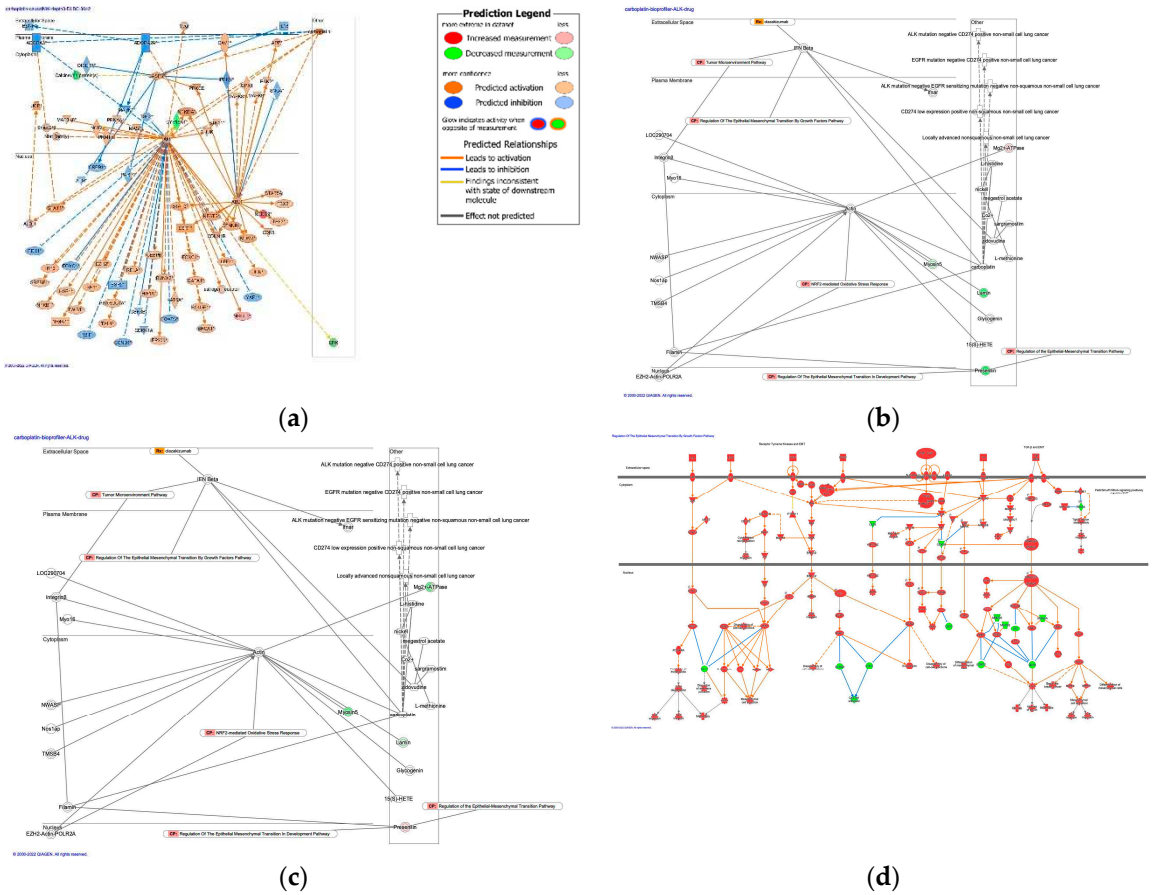

Figure 1. Carboplatin molecular network in cancer. (**a**) Causal network of carboplatin in EED226-treated diffuse large B-cell lymphoma. (**b**) Carboplatin bioprofiler network in diffuse-type gastric cancer (GC). (**c**) Carboplatin bioprofiler network in intestinal-type GC. (**d**) Regulation of EMT by growth factors pathway colored with expected activation states. Red color indicates activation, while green color indicates inactivation.

Table 3. Molecules involved in the EMT by growth factors pathway (Ingenuity Pathway Analysis).

Location	Symbol
Cytoplasm	AKT
	CDC42
	DOCK10
	ERK1/2
	FRS2-GRB2-SHP2
	GAB1
	GRB2
	Grb2-Shc1-Sos
	Ikk
	JAK
	Jnk
	MAP2K4/7
	MAP3K1
	MAP3K7
	MAPK1
	MEK
	MEST
	MIR155
	MIR192
	MIR34A

	MKK3/6
	p38MAPK
	PI3K
	PTPN11
	RAF
	Ras
	RHOA
	SHC1
	SMAD2/3
	SMURF1
	SOS
	TAB1
	VIM
	EGF
	FGF
	FGF dimer
	HGF
	IL6
Extracellular Space	MMP1
	MMP2
	MMP9
	PDGF
	Tgfbeta
	TGFB2
	AP1
	EGR1
	ESRP2
	ETS1
	FOS
	FOXC2
	FOXO1
	GSC
	GSK3B
	HMGA2
	HSF1
Nucleus	ID2
	MTOR
	NFkB
	SMAD2/3/4
	SMAD4
	SNAI1
	SNAI2
	STAT3
	TCF3
	TWIST1
	ZEB1
	ZEB2
	CDH1
	CDH2
	CLDN3
	EGFR
	ERBB2
Plasma Membrane	FGFR
	FRS2
	IL6R
	MET
	OCLN
	Pdgfr

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Other	PDGFRA RAC1 TGFBR1 TGFBR2 Tnfreceptor LATS MIR200 N-Cadherin PAR6 Tnf YAP/TAZ

3.2. Causal networks of cisplatin-treated lung adenocarcinoma

Causal networks of cisplatin treatment were investigated in IPA. In the IPA database, 169 analyses and 171 datasets were found to be related to cisplatin, among which 12 analyses were identified as having treatment of cisplatin (Table 4). Causal network analysis of the cisplatin-treated samples revealed camptothecin as a master regulator of the causal network (Figure 2A). The causal network of camptothecin in cisplatin-treated lung adenocarcinoma included Fas cell surface death receptor (FAS) in the Tumor microenvironment pathway, phosphatase and tensin homolog (PTEN) and MDM2 proto-oncogene (MDM2) in the Cancer drug resistance by drug efflux and interferon regulatory factor 1 (IRF1) in the Production of nitric oxide and reactive oxygen species in macrophages (Figure 2B). Upstream regulator analysis of cisplatin-treated lung adenocarcinoma revealed the up-regulation in FAS, protein kinase C alpha (PRKCA), and cyclin-dependent kinase inhibitor 1A (CDKN1A) in the Molecular mechanisms of cancer, and the involvement of the Tumor microenvironment pathway in the causal network of cisplatin (Figure 3).

Table 4. Analyses of cisplatin treatment.

Analysis Name	Comparison contrast	Upregulated log2 cutoff	Project name	Organism
1- ovarian cancer [ovary] *cisplatin* 1667	ExperimentGroup => cisplatin 1 day recovery 2 weeks vs monolayer culture	0.6374	GSE144232	human
1- breast carcinoma [breast] *cisplatin* 7438	SamplingTime => 10 to 11 hours after treatment vs NA	0.344	GSE28274	human
5- malignant pleural mesothelioma [mesothelium] *cisplatin*;piroxicam 6825	Treatment:TreatTime[hours] => 8 -> cisplatin;piroxicam vs none	0.1488	GSE22445	human
1- lung adenocarcinoma (LUAD) [lung] *cisplatin* 9800	Treatment => cisplatin vs none	0.1224	GSE6410	human
1- malignant pleural mesothelioma [mesothelium] *cisplatin* 6821	Treatment:TreatTime[hours] => 24 -> cisplatin vs none	0.1633	GSE22445	human
140- normal control [liver] *cisplatin* 2334	TreatTime[days]:Treatment => 1 -> cisplatin vs DMSO	1.1422	GSE57805	rat
2- breast carcinoma [breast] *cisplatin* 7439	SamplingTime => 8 to 9 hours after treatment vs NA	0.0815	GSE28274	human
2- malignant pleural mesothelioma [mesothelium] *cisplatin*;piroxicam 6822	Treatment:TreatTime[hours] => 24 -> cisplatin;piroxicam vs none	0.539	GSE22445	human
24- normal control [liver] *cisplatin* 2200	TreatTime[days]:Treatment => 0.67 -> cisplatin vs DMSO	1.0049	GSE57805	rat
256- normal control [liver] *cisplatin* 2324	Treatment:TreatTime[days] => cisplatin -> 1 vs 0.67	0.2943	GSE57805	rat
37- glioblastoma (GBM) [brain] *cisplatin* 4128	Treatment => cisplatin vs DMSO	0.4672	GSE97460	human

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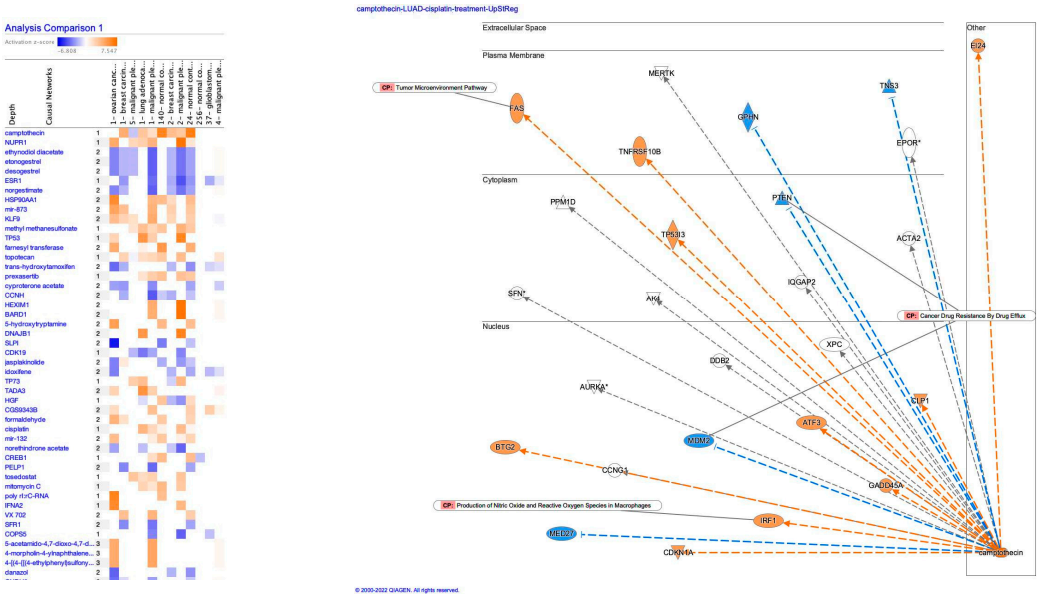
4- malignant pleural mesothelioma [mesothelium] *cisplatin* 6824	Treatment:TreatTime[hours] => 8 -> cisplatin vs none	0.1641	GSE22445	human



(**a**) (**b**)

Figure 2. Causal networks in cisplatin treatment and involvement of tumor microenvironment pathway. (**a**) Camptothecin was identified as a master regulator in cisplatin-treated samples. (**b**) Tumor microenvironment pathway-related FAS was located in plasma membrane in the network of camptothecin in cisplatin-treated lung adenocarcinoma.

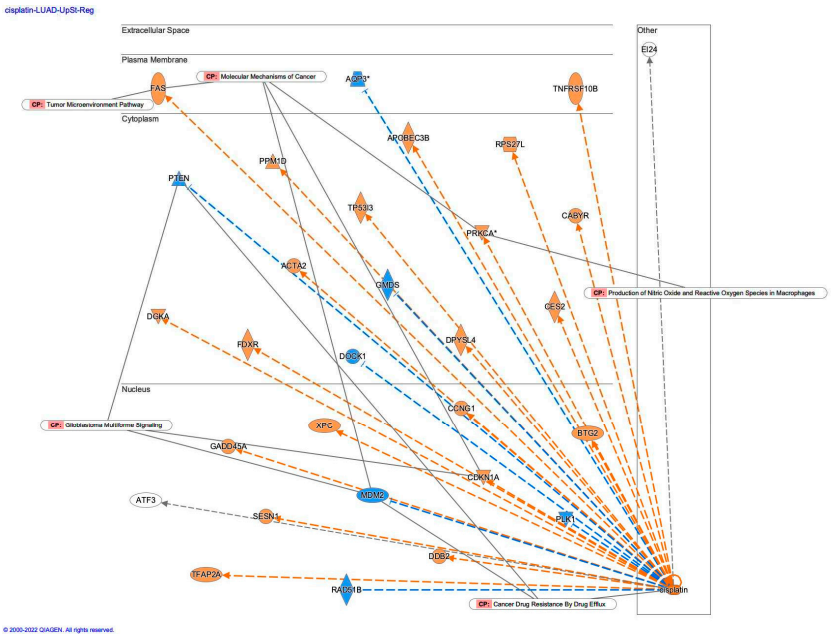


Figure 3. Upstream regulator analysis of cisplatin revealed the up-regulation in FAS, PRKCA and CDKN1A, and involvement of tumor microenvironment pathway. (lung adenocarcinoma (LUAD) [lung] cisplatin 9800).

3.3. Cisplatin as an upstream regulator of gastric adenocarcinoma

The upstream regulator analysis of cisplatin in the activity plot revealed 64880 analyses for cisplatin (Figure 4A). Cisplatin was identified as an activated upstream regulator in pembrolizumab,

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a humanized IgG4 monoclonal antibody against programmed cell death-1 (PD-1), -treated gastric adenocarcinoma in progressive disease (Figure 4B) [30]. The mechanistic network of cisplatin included epidermal growth factor receptor (EGFR) and SMAD family member 3 (SMAD3) in the Regulation of the EMT by growth factors pathway, and Akt, activator protein 1 (Ap1), early growth response 1 (EGR1), Fos proto-oncogene (FOS), Jun proto-oncogene (JUN), NFκB (complex), RELA proto-oncogene (RELA), and signal transducer and activator of transcription 3 (STAT3) in the Regulation of the EMT by growth factors pathway, and Tumor microenvironment pathway (Figure 4B). miR-1195 (miRNAs w/seed GAGUUCG), mir-124, mir-338, mir-379, mir-434, mir-515, mir-622, MIR124, MIR499B, and CpG ODN/STAT3 siRNA CAS3/SS3 (CAS3/SS3) were identified as microRNAs and siRNA to have direct interactions with the network of cisplatin (Figure 4B, Table 5). HIF1A, STAT3, and RELA were the target molecules of the miRNAs. The tumor microenvironment pathway was activated in the pembrolizumab-treated gastric adenocarcinoma (Figure 4C). IL-6 and SPP1, which are regulated by M2 tumor-associated macrophages, were up-regulated in the network (Figure 4C). Since the causal networks of camptothecin were activated in cisplatin-treated lung adenocarcinoma, the gene expression profile of diffuse- and intestinal-type gastric cancer was investigated in the causal network of camptothecin. Camptothecin was activated in intestinal-type GC and inactivated in diffuse-type GC (Figure 5).

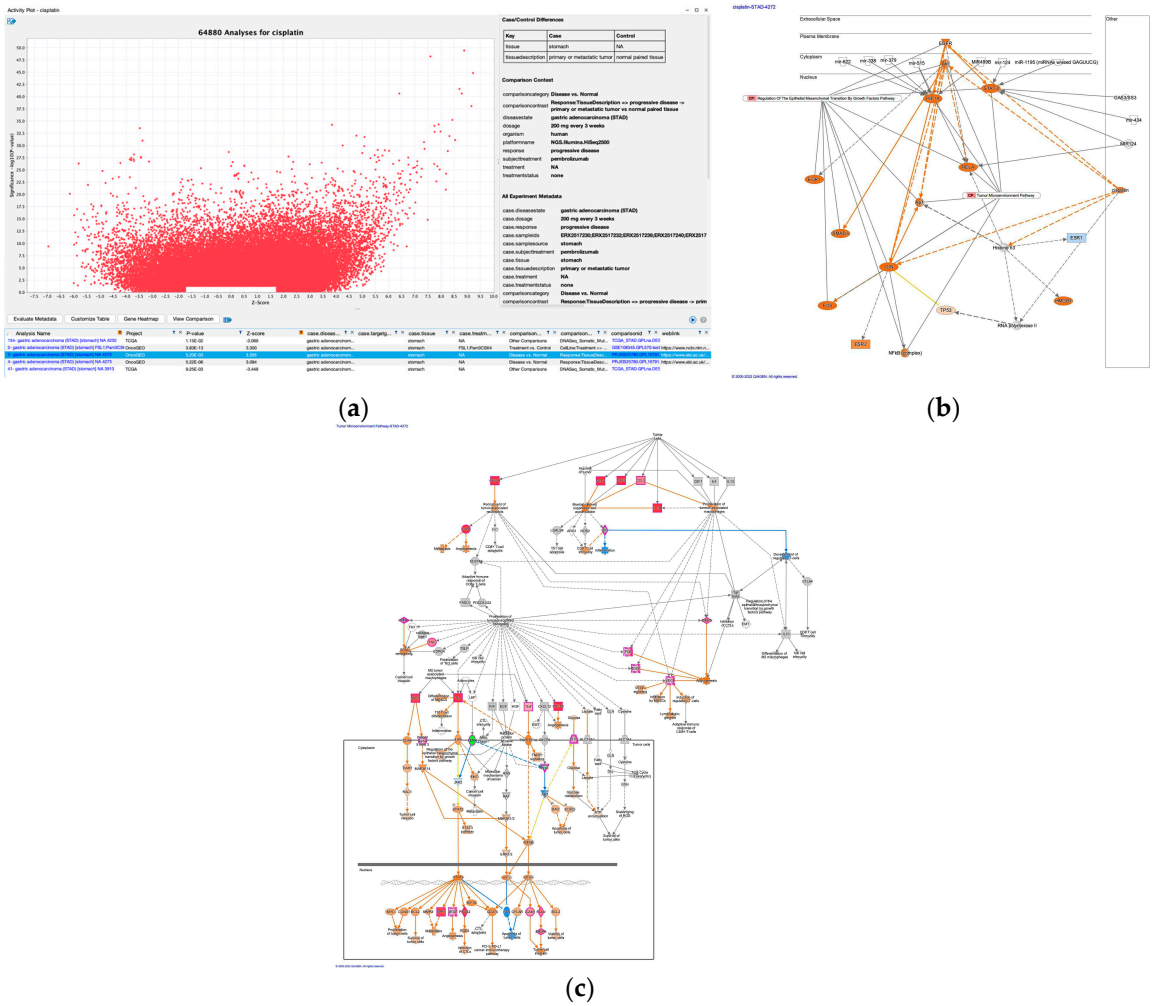

Figure 4. Cisplatin as an upstream regulator in gastric adenocarcinoma. (**a**) The upstream regulator analysis of cisplatin in the activity plot revealed 64880 analyses for cisplatin. (**b**) Cisplatin was identified as an activated upstream regulator in pembrolizumab, a humanized IgG4 monoclonal antibody against programmed cell death-1 (PD-1), -treated gastric adenocarcinoma in progressive disease. (**c**) The tumor microenvironment pathway was activated in the pembrolizumab-treated gastric adenocarcinoma.

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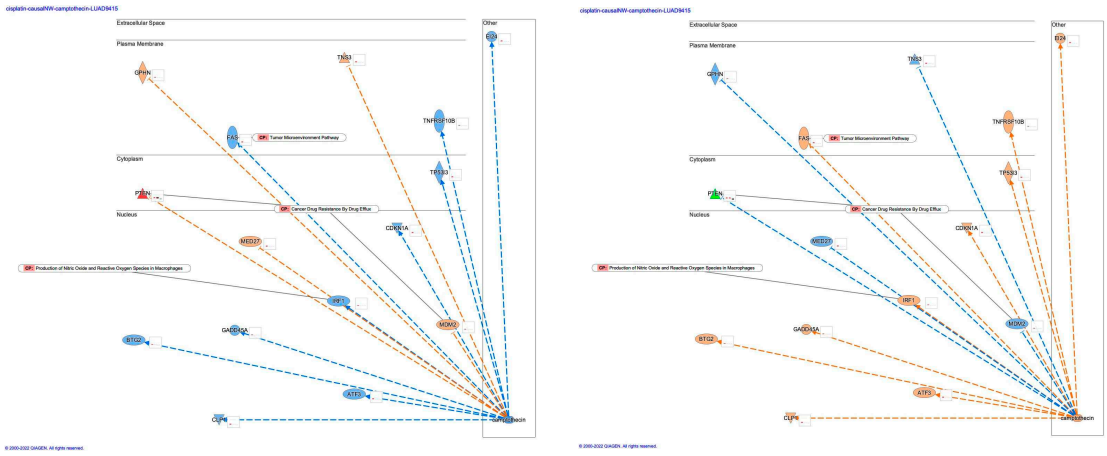
Table 5. microRNAs and a siRNA interacting the cisplatin network in gastric adenocarcinoma.

Symbol	Family
CAS3/SS3	biologic drug
miR-1195 (miRNAs w/seed GAGUUCG)	mature microRNA
mir-124	microRNA
mir-338	microRNA
mir-379	microRNA
mir-434	microRNA
mir-515	microRNA
mir-622	microRNA
MIR124	group
MIR499B	microRNA



(**a**) (**b**)

Figure 5. Causal network of camptothecin in diffuse- and intestinal-type gastric cancer (GC). (**a**) The network of camptothecin in diffuse-type GC. (**b**) The network of camptothecin in intestinal-type GC.

3.4. Arsenic treatment in the EMT by growth factors pathway

3.4.1. Regulatory networks in arsenic-treated liver carcinoma

The network of arsenic was investigated in the EMT by growth factors pathway, which identified regulatory networks of arsenic (III) oxide-treated liver carcinoma (Table 6) [31]. The top regulatory network was involved in cell death of carcinoma cell lines, Cell proliferation of adenocarcinoma cell lines. Regulators of the network were amphiregulin (AREG), cytoskeleton-associated protein 2 like (CKAP2L), H2A.Z variant histone 1 (H2AZ1), HNF1A antisense RNA 1 (HNF1A-AS1), immunity-related GTPase family M member 1 (Irgm1), lin-9 DREAM MuvB core complex component (LIN9), MYB proto-oncogene like 2 (MYBL2), PCNA clamp associated factor (PCLAF), and S100 calcium binding protein A6 (S100A6). AREG is an epidermal growth factor receptor (EGFR) ligand located in extracellular space.

3.4.2. Causal networks of arsenic and direct interaction with microRNAs

A causal network of arsenic was activated in intestinal-type GC and inactivated in diffuse-type GC (Figure 6). The causal network of arsenic had direct RNA-RNA interactions with mir-101, mir-103, miR-125b-2-3pp (and other miRNAs w/seed CAAGUCA), mir-145, mir-22, mir-338, mir-379, mir-515, mir-622, and MIR499B (Table 7). mir-101 targets MTOR and mir-22 targets SP1, mir-103, mir-145, and mir125b-2-3pp (and other miRNAs w/seed CAAGUCA) target TP53, which are inhibited by arsenic (Figure 6). mir-370, mir-515, mir-338, MIR499B and mir-622 targets HIF1A, which is activated by arsenic (Figure 6).

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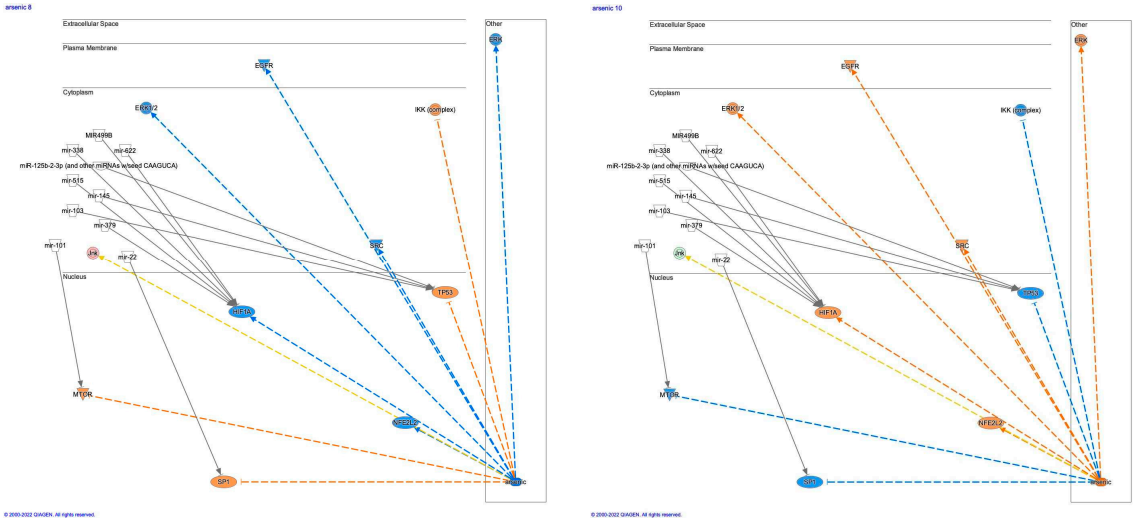
Table 6. Regulatory networks of arsenic (III) oxide-treated liver carcinoma.

ID	Diseases & Functions	Known Regulator-Disease/Function Relationship
1	Cell death of carcinoma cell lines,Cell proliferation of adenocarcinoma cell lines	11% (2/18)
2	Death of embryo,Homologous recombination of DNA	0% (0/18)
3	Cell cycle progression,Cell proliferation of tumor cell lines	65% (13/20)
4	Cell death of breast cancer cell lines,Cell survival	0% (0/6)
5	DNA recombination,Formation of gamma H2AX nuclear focus,Incidence of lymphoma	0% (0/15)
6	Chromosomal aberration,DNA damage,Homologous recombination of DNA,T-cell non-Hodgkin lymphoma	13% (1/8)
7	Formation of gamma H2AX nuclear focus	13% (1/8)
8	Chromosomal aberration,DNA damage,T-cell non-Hodgkin lymphoma	11% (1/9)
9	Death of embryo	0% (0/5)
10	Chromosomal aberration,DNA damage	13% (1/8)



(**a**) (**b**)

Figure 6. Causal network of arsenic in diffuse- and intestinal-type gastric cancer (GC). (**a**) The network of arsenic in diffuse-type GC. (**b**) The network of arsenic in intestinal-type GC.

Table 7. microRNAs which have direct RNA-RNA interactions with causal network of arsenic.

microRNAs
mir-101
mir-103
miR-125b-2-3p (and other miRNAs w/seed CAAGUCA)
mir-145
mir-22
mir-338
mir-379
mir-515
mir-622
MIR499B

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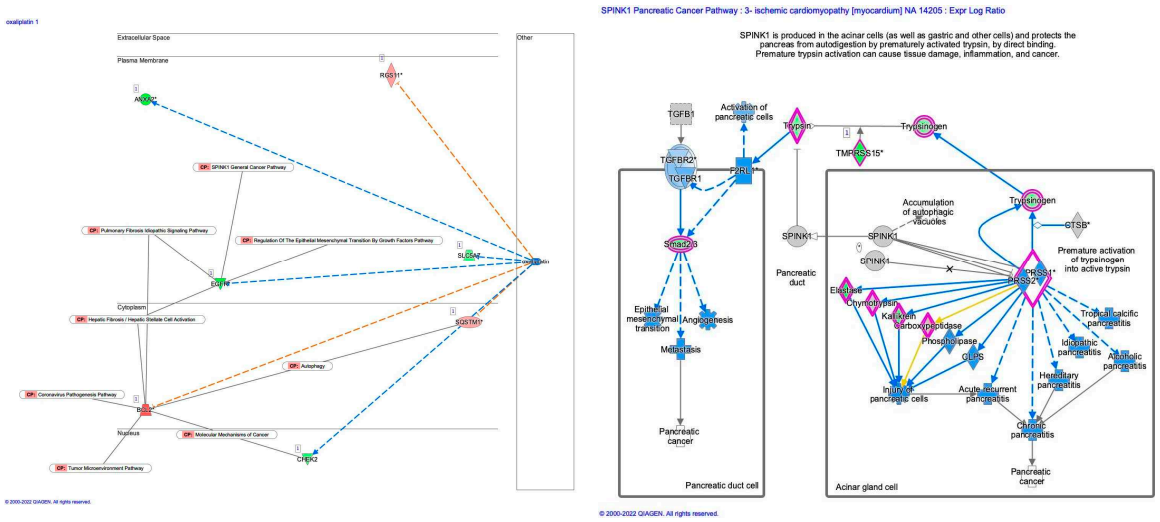
3.5. Analysis of oxaliplatin and SPINK1 pancreatic cancer pathway

Network analysis of oxaliplatin revealed that oxaliplatin as a master regulator was predicted to be inhibited, and SPINK1 pancreatic cancer pathway was inactivated in ischemic cardiomyopathy (Figure 7).



(**a**) (**b**)

Figure 7. Network analysis of oxaliplatin revealed that oxaliplatin as a master regulator was predicted to be inhibited, and SPINK1 pancreatic cancer pathway was inactivated in ischemic cardiomyopathy. (**a**) The network of oxaliplatin. Oxaliplatin was predicted to be inhibited as a master regulator in ischemic cardiomyopathy (**b**) SPINK1 pancreatic cancer pathway was inactivated in ischemic cardiomyopathy.

3.6. Cisplatin treatment increased vimentin expression in scirrhous gastric cancer

To confirm whether the EMT is affected by cisplatin treatment, EMT markers, vimentin and CDH2 expression was examined. Cisplatin treatment increased vimentin expression in scirrhous gastric cancer (Figure 8).

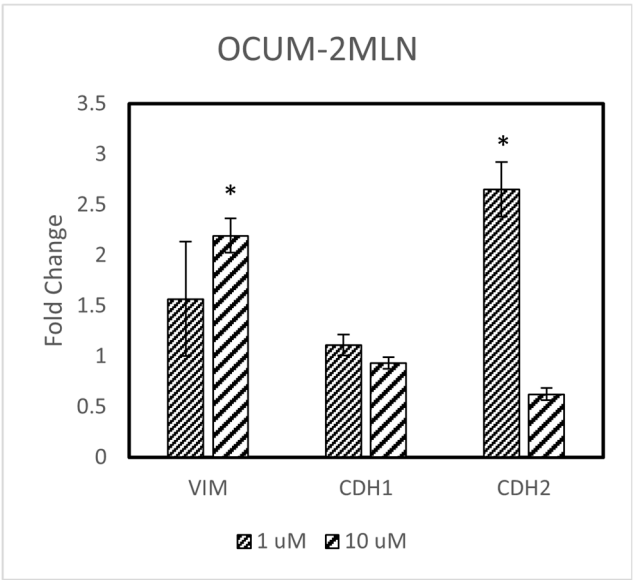


Figure 8. The cisplatin treatment increased vimentin expression in scirrhous gastric cancer. * $p < 0.05$ compared to non-treatment (N= 3).

 doi:10.20944/preprints202305.1304.v1

4. Discussion

Causal network analysis was used to investigate the molecular networks in treatment-resistant cancer, and the results revealed that EED226, a potent inhibitor of PRC2, is involved in DLBC [29]. EED226 inhibits PRC2 that is resistant to an inhibitor of EZH2, a subunit of PRC2 [29]. Since EED226 targets the trimethylated H3K27 binding pocket of EED, histone modification may be involved in cancer treatment resistance.

Several miRNAs, MIR155, MIR192, MIR34A and MIR200, are components of the Regulation of EMT by growth factors pathway. mir-155_5p was up-regulated at lung metastasis and peritoneal metastasis of colorectal cancer compared to primary colorectal cancer [32]. Prior investigation of relationships between platinum drugs such as cisplatin and carboplatin and non-coding RNAs demonstrated that miRNAs are correlated with cisplatin activity [33]. Additionally, recent studies have revealed the association between EMT and miRNAs and their roles in drug resistance [34–37]. Down-regulation of miRNA-214 induces EMT and migration and invasion of gastric cancer [38].

Pembrolizumab, a humanized IgG4 monoclonal antibody against PD-1, is an immune checkpoint inhibitor and can be used for microsatellite instability-high or tumor mutational burden-high advanced gastric cancer [39]. The overall response rate of pembrolizumab in advanced PD-L1-positive gastric cancer was 22.2 % [40]. The patients in IL-1R1^{high} subgroup demonstrated a significantly lower response to pembrolizumab than those in the IL-1R1^{low} subgroup [41]. Sundar R. et al. found a potential role of alternative promoter utilization as a predictive biomarker for resistance to immune checkpoint inhibition [42]. Interferon (IFN)-γ-related gene expression profile predicted response to pembrolizumab [43]. Since the tumor microenvironment pathway was activated in pembrolizumab-treated gastric adenocarcinoma, the treatment resistance is involved in the tumor microenvironment pathway.

In the current study, HIF1A was identified as a target of several miRNAs in the causal network of arsenic. HIF1A and CDKN1A, together with miR-3607-3p, miR-301a-3p, and miR-93-5p, are associated with prolonged survival in glioblastoma patients treated with regorafenib [44]. miR-101 induces HIF1A-mediated apoptosis and cell cycle arrest [45]. HIF1A may be regulated by miRNAs in the causal network of arsenic.

5. Conclusions

In conclusion, platinum drug treatment is related to the tumor microenvironment pathway in cancer. Cisplatin was identified as the upstream regulator of pembrolizumab-treated gastric adenocarcinoma. Several microRNAs were identified to interact with cisplatin network. Carboplatin network was related to regulation of EMT pathway and tumor microenvironment pathway. A close correlation between anti-cancer drug resistance and tumor microenvironment pathway needs to be revealed in future investigations.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Bioprofiler analysis on carboplatin.

Author Contributions: Conceptualization, S.T. and H.C.; formal analysis, S.T.; investigation, S.T., E.B., T.H., H.C.; writing—original draft preparation, S.T. and H.C.; writing—review and editing, S.T., S.Q., R.O., H.C., K.A., H.Y., H.S.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Japan Agency for Medical Research and Development (AMED) grant numbers JP21mk0101216 (ST), JP22mk0101216 (ST) and JP23mk0101216 (ST), JSPS KAKENHI Grant Number 21K12133 (ST and RO).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets analyzed for this study can be found in the Gene Expression Omnibus (GEO) annotation GSE81267 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81267), GSE6970 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6907), and The Cancer Genome Atlas (TCGA) of the

cBioPortal for Cancer Genomics database at the National Cancer Institute (NCI) Genomic Data Commons (GDC) data portal (https://portal.gdc.cancer.gov/).

Acknowledgments: The authors would like to acknowledge members of the National Institute of Health Sciences (NIHS), Japan. This research was supported by the Ministry of Health, Labour, and Welfare (MHLW), Japan.

Conflicts of Interest: The authors declare no conflict of interest.

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