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RNA Sequencing of Carboplatin- and Paclitaxel-Resistant Endometrial Cancer Cells Reveals New Stratification Markers and Molecular Targets for Cancer Treatment

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Abstract
Despite advances in surgical technique and adjuvant treatment, endometrial cancer has recently seen an increase in incidence and mortality in the USA. The majority of endometrial cancers can be cured by surgery alone or in combination with adjuvant chemo- or radiotherapy; however, a subset of patients experience recurrence for reasons that remain unclear. Recurrence is associated with chemoresistance to carboplatin and paclitaxel and consequentially, high mortality. Understanding the pathways involved in endometrial cancer chemoresistance is paramount for the identification of biomarkers and novel molecular targets for this disease. Here, we generated the first matched pairs of carboplatin-sensitive/carboplatin-resistant and paclitaxel-sensitive/paclitaxel-resistant endometrial cancer cells and subjected them to bulk RNA sequencing analysis. We found that 45 genes are commonly upregulated in carboplatin- and paclitaxel-resistant cells as compared to controls. Of these, the leukemia inhibitory factor, (LIF), the protein tyrosine phosphatase type IVA, member 3 (PTP4A3), and the transforming growth factor beta 1 (TGFB1) showed a highly significant correlation between expression level and endometrial cancer overall survival (OS) and can stratify the 545 endometrial cancer patients in the TCGA cohort into a high-risk and low-risk-cohorts. Additionally, four genes within the 45 upregulated chemoresistance-associated genes are ADAMTS5, MICAL2, STAT5A, and PTP4A3 codes for proteins for which small-molecule inhibitors already exist. We identified these proteins as molecular targets for chemoresistant endometrial cancer and showed that treatment with their correspondent inhibitors effectively killed otherwise chemoresistant cells. Collectively, these findings underline the utility of matched pair of chemosensitive and chemoresistant cancer cells to identify markers for endometrial cancer risk stratification and to serve as a pharmacogenomics model for identification of alternative chemotherapy approaches for treatment of patients with recurrent disease.

Keywords Endometrial cancer · Recurrence · Gene expression · Chemoresistance

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Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy diagnosed in the Western world. The majority of patients are diagnosed at an early stage with an overall favorable prognosis. Unfortunately, approximately 20% of patients, despite early stage and seemingly excellent prognosis, will recur [1–3]. Recurrent endometrial cancer is generally treated with a combination of carboplatin and paclitaxel, and while the initial response rate is around 45%, the vast majority of patients relapses and develops a chemoresistant disease, leading to a low survival rate [4]. A number of signaling pathways have been associated with endometrial cancer chemoresistance, including repair mechanisms and pro-survival pathways as well as the upregulation of efflux pumps

which have been specifically associated with chemoresistance to paclitaxel. Tumor biomarkers, including USP14 and L1CAM, have also been associated with poor patient outcome and have predictive value of endometrial cancer recurrence [5–8]. Despite this knowledge, a deep understanding of the cellular mechanisms leading to endometrial cancer chemoresistance is still lacking, and the development of tools for stratifying patients based on their likelihood to respond to chemotherapy and for identification of novel treatments for patients with recurrent disease is still urgently needed. We believe that the paucity of studies focusing on the comprehension of endometrial cancer chemoresistance at the molecular level is in part due to the difficulties of establishing chemoresistant endometrial cancer cells in vitro and has greatly limited our understanding of this disease.

In this study, we established what to our knowledge is the first matched pair of carboplatin-sensitive/carboplatin-resistant and paclitaxel-sensitive/paclitaxel-resistant endometrial cancer cell lines. The obtained matched pairs of cells were subjected to bulk RNA sequencing and their expression profile was compared to the one of their chemosensitive counterpart. We found that 45 genes were commonly upregulated in both paclitaxel- and carboplatin-resistant cells that are compared to the corresponding sensitive cells. Of these, we found that LIF, PTP4A3, and TGFB1 are strong predictors of poor overall survival in the endometrial cancer cohort from The Cancer Genome Atlas (TCGA) and that PTP4A3, ADAMTS5, STAT5A, and MICAL2 are potential molecular targets for chemoresistant endometrial cancer. We believe that our characterized chemoresistant endometrial cancer cells could be a useful platform for further investigating the mechanisms governing endometrial cancer chemoresistance and for preclinical models to test and develop novel chemotherapy agents for chemoresistant endometrial cancer.

Results

Establishment and Characterization of Matching Pairs of Carboplatin- and Paclitaxel-Resistant Human Endometrial Cancer Cells

To gain a better understanding of the pathways that are responsible for chemoresistance, we generated matched pairs of carboplatin-sensitive/carboplatin-resistant and paclitaxel-sensitive/paclitaxel-resistant endometrial cancer cells. To do this, ECC-1 endometrial cancer cells were either mock treated (vehicle) or treated with increasing concentrations of carboplatin (carbo) or paclitaxel (pacli) over a period of several weeks as shown in Scheme 1. At the end of each 6-week period, clones were isolated, allowed to grow, and then retreated with increasing concentrations of the drugs for a total of four cycles (representative pictures are shown in Fig. 1a, b).

This choice of intermittent scheduling of the drugs was made so to mimic the intermittent chemotherapy treatment endometrial cancer patients undergo. Two weeks after the end of the last treatment, carboplatin-resistant clones were approximatively eightfold less sensitive to the drug as compared to vehicle-treated cells (Fig. 2a) and paclitaxel-resistant cells were approximatively tenfold less sensitive to the drug as compared to vehicle cells (Fig. 2b). No cross-resistance was observed. To exclude that the difference in drug sensitivity was due to a difference in proliferation rate, we measured the total cell number of chemosensitive and chemoresistant cells over a period of 4 days. As shown in Fig. 2c, sensitive and resistant cells grew at a comparable rate, suggesting that the difference in drug sensitivity is not due to differences in their proliferation rate.

RNA-seq Analysis in Chemosensitive Versus Chemoresistant Cancer Cells

In order to identify genes that are potentially relevant to chemoresistance in endometrial cancer, we performed gene expression analysis on ECC-1-sensitive (vehicle treated), carboplatin-resistant (ECC-1C), and paclitaxel-resistant (ECC-1P) cell lines via RNA sequencing. Per each cell line, three independent clones were submitted to RNA sequencing. As shown in Fig. 3a, comparison of the gene expression profile between chemosensitive and chemoresistant cells revealed that 691 genes were differentially expressed in paclitaxel-resistant cells versus vehicle-treated cells, and 731 genes were differentially expressed in carboplatin-resistant cells versus vehicle-treated cells (Supplementary Table 1). Of these genes, 175 were commonly altered in both carboplatin- and paclitaxel-resistant cells as compared to vehicle cells. Specifically, 45 of the 175 genes were upregulated in both cases, 89 were downregulated, and 41 genes showed a combination of up-or downregulation. Importantly, comparison of the gene expression of all submitted samples via principal component analysis (PCA) showed reproducible gene expression profile between biological triplicates and very different profiles between the three different conditions (Fig. 3b). Differentially expressed genes whose absolute fold change between conditions was less than twofold and did not show any significant difference after Bonferroni correction ($p = 0.05$) were not considered to be significant and therefore excluded from further analysis. A complete list of all genes is given in Supplementary Table 1.

Upregulation of Genes in Resistant Cells Correlates with Poor Outcome in Endometrial Cancer Patients

Next, we evaluated the prognostic power of the 45 commonly upregulated genes in ECC-1C and ECC-1P cell lines compared with the parental ECC-1 cell line in the
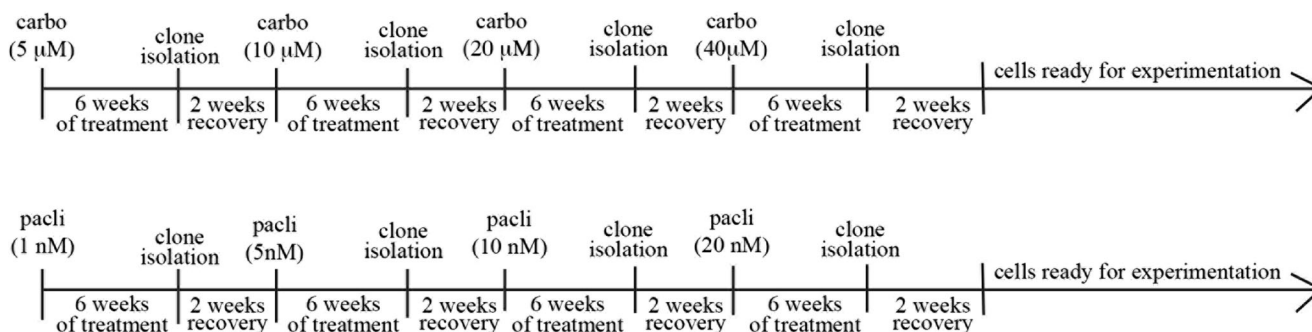
Scheme 1 *Exposure of endometrial cancer cells to increasing concentrations of drugs.* Cells were initially exposed to their IC_{20-30} concentration of carboplatin (carbo) or paclitaxel (pacli) over a period of 6 weeks with the drug being added twice a week. At the end of each cycle, clones were isolated, allowed to grown in absence of drug over a 2-week recovery period prior being re-exposed to increasing concentrations of drugs

endometrial cancer cohort from The Cancer Genome Atlas (TCGA). Specifically, the database was interrogated for correlation between mRNA upregulation and/or DNA amplification and patients' overall survival. Shown in Table 1 is a list of the genes that, out of the 45 upregulated ones, had the highest correlation between gene expression and overall survival (OS). Of those, the leukemia inhibitory factor (LIF), the protein tyrosine phosphatase type IVA, member 3 (PTP4A3), and the transforming growth factor beta 1 (TGFB1) genes showed a highly significant correlation between expression level and OS. Importantly, each of these three genes was able to stratify the 545 endometrial cancer patients into a high-risk and low-risk-cohorts (Fig. 4). Patients with high LIF expression had a statistically significant lower median survival (63.86 months) as compared with patients low LIF expression (> 225.33-month median survival) and a hazard ratio (HR) for death of 3.43 (95% CI, 0.9739 to 12.13; $p = 0.0004$). Patients with high PTP4A3 expression had a statistically significant lower median survival (102.23 months) as compared with patients low PTP4A3 expression (> 225.33-month median survival) and a hazard ratio (HR) for death of 2.59 (95% CI, 0.9739 to 7.09; $p = 0.0009$). Patients with high TGFB1 expression had a statistically significant lower median survival (31.11 months) as compared to patients with low TGFB1 expression (> 225.33-month median survival) and a HR for death of 4.2 (95% CI, 0.9739 to 17.23; $p = 0.0001$).

Fig. 1 *Multi-staged process during the development of chemoresistant cells.* Representative pictures are shown depicting the different stages during the process leading to **a** carboplatin- or **b** paclitaxel-resistant cells. First windows (**a**, **b**) are vehicle-treated parental cells at the beginning of the treatment. Second windows (**a**, **b**) are treated cells after two rounds of chemotherapy which killed about 99% of cells (inserts are the magnification of the surviving cells). Third windows (**a**, **b**) are chemoresistant cells growing in presence of the relative drugs (insert are magnifications of the growing chemoresistant clones). Fourth windows (**a**, **b**) are expansion of chemoresistant cells
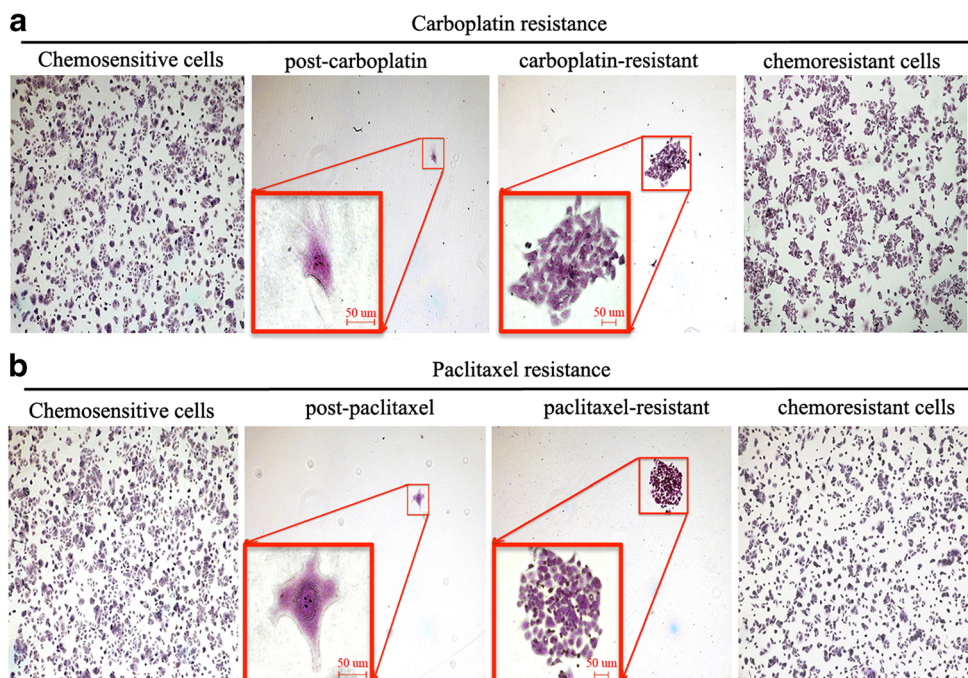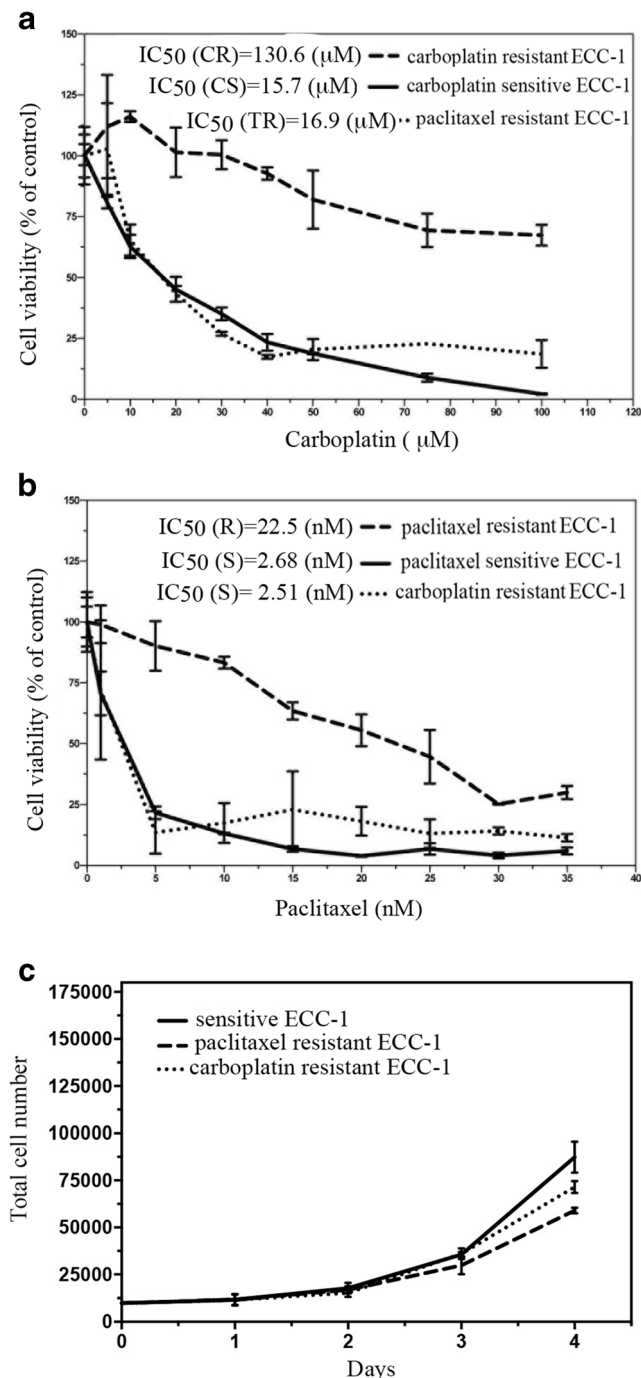
Fig. 2 *Residual cell viability in sensitive and resistant cells exposed to carboplatin and paclitaxel.* Dose-dependent inhibition of cell viability of ECC-1-sensitive and -resistant cells exposed to the indicated concentrations of carboplatin (**a**) or paclitaxel (**b**) over a period of 48 and 96 h, respectively. Percentage of viable cells is relative to mock-treated controls. **c** Proliferation rate of sensitive and resistant endometrial cancer cells as measured by manually counting cells over a period of 4 days using an inverted microscope. Results are expressed as total cell number

We further classified the 175 commonly altered genes by using ingenuity pathway analysis (IPA) and integrated them into canonical pathways (CP). As shown in Supplementary Table 2, ECC-1C cells showed engagement of the tight junction,

osteoarthritis, gamma-glutamyl cycle, epithelial-mesenchymal transition, and folate glutamylation pathways. The ECC-1P cells on the other hand showed activation of CP such as the hepatic fibrosis/hepatic stellate cell activation, osteoarthritis, axonal guidance, atherosclerosis, and netrin signaling pathways. When looking at CP that are significantly modified among the 175 commonly altered genes in both the ECC-1C and ECC-1P cells, the gamma-glutamyl cycle, atherosclerosis, histidine degradation IV, pregnenolone biosynthesis, and ubiquinol-10 biosynthesis signaling pathways appeared to have primarily changed. Importantly, the abovementioned signaling pathways, with the exception of the osteoarthritis pathway, have been previously shown to be associated with human cancers' chemoresistance, progression, and/or poor outcome [9–13]. Taken together, this suggests that our model of chemoresistance can recapitulate some of the clinical features of endometrial cancer patients with respect to transcript levels of the commonly upregulated genes and patients' overall survival. This also suggests that chemoresistant endometrial cancer cells share some of the same activated pathways as other human cancer cells.

Validation of RNA-seq Results via qRT-PCR Analysis and of Protein Expression via Western Blot

Next, we validated our RNA-seq data via quantitative real-time PCR (qRT-PCR). In doing this, we have chosen to focus on validation of genes for which a small-molecule inhibitor is available. This restricted the field to four genes: ADAMTS5, PTP4A3, STAT5A, and MICAL2. As shown in Fig. 5, and consistent with our findings in the gene expression analysis, qRT-PCR showed an upregulation for ADAMTS5, MICAL2, STAT5A, and PTP4A3 in the chemoresistant versus the chemosensitive endometrial cancer cells. Next, we determined whether gene upregulation corresponds to protein upregulation. As shown in Fig. 6, Western blot analysis confirmed that the upregulation of the mRNA corresponded well with an overexpression of each associated protein in the chemoresistant cell lines as compared to the chemosensitive. Taken together, this suggests that ADAMTS5, MICAL2, STAT5A, and PTP4A3 are potential molecular targets for treatment of chemoresistant endometrial cancer using small-molecule inhibitor approach.

Small-Molecule Inhibitors of PTP4A3, ADAMTS5, STAT5A, or MICAL2 Kill Chemoresistant Endometrial Cancer Cells

Next, we tested the feasibility of targeting PTP4A3, ADAMTS5, STAT5A, or MICAL2 for treatment of chemoresistant endometrial cancer cells. To this end, carboplatin-resistant ECC-1 cells were treated with either carboplatin or the FDA-approved STAT5A-inhibitor pimozide [14–19], the FDA-approved PTP4A3 inhibitor pentamidine [20–22], the ADAMTS5A-inhibitor 5-((4-
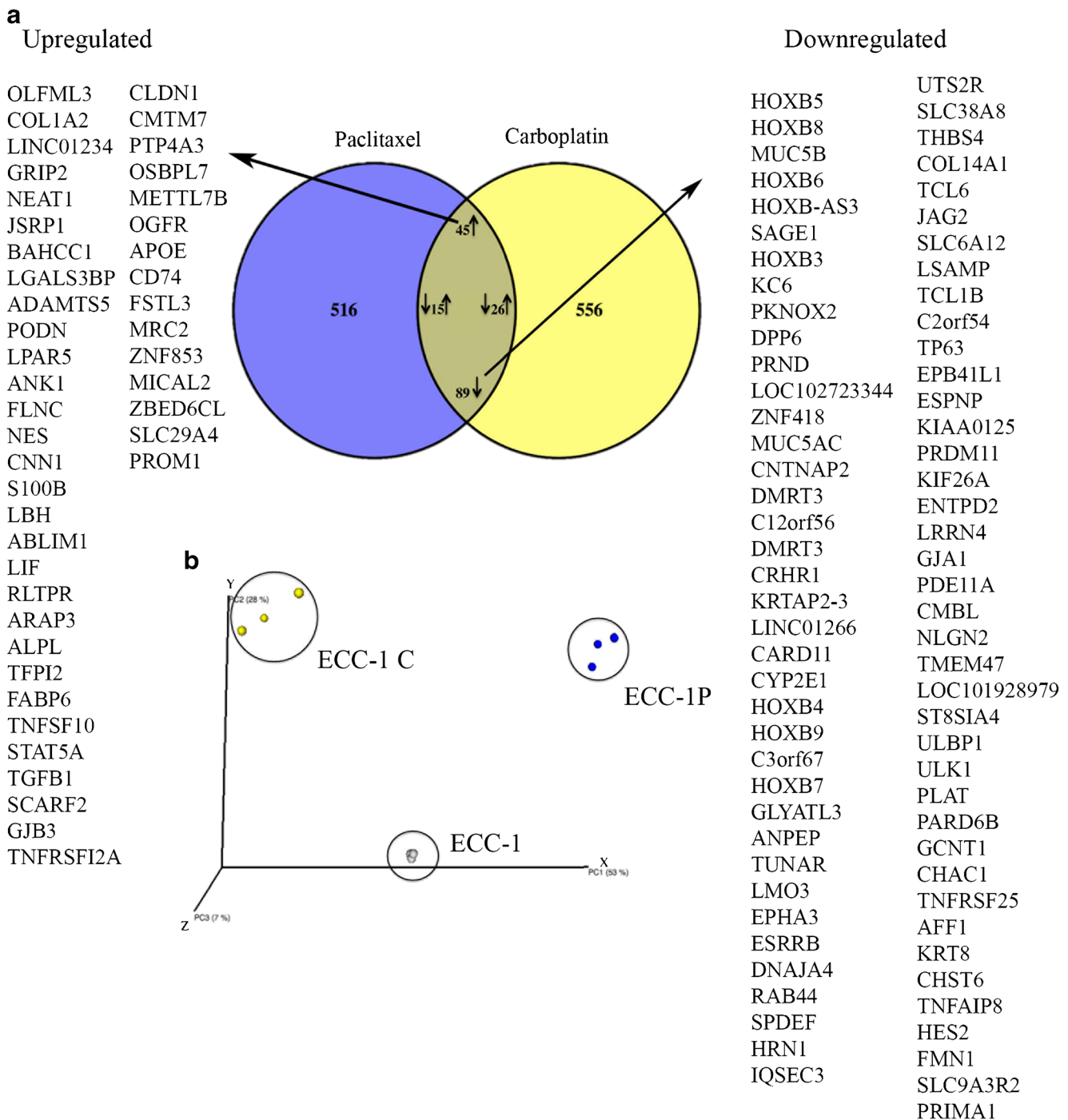
a

Upregulated

OLFML3	CLDN1
COL1A2	CMTM7
LINC01234	PTP4A3
GRIP2	OSBPL7
NEAT1	METTL7B
JSRP1	OGFR
BAHCC1	APOE
LGALS3BP	CD74
ADAMTS5	FSTL3
PODN	MRC2
LPAR5	ZNF853
ANK1	MICAL2
FLNC	ZBED6CL
NES	SLC29A4
CNN1	PROM1
S100B	
LBH	
ABLIM1	
LIF	
RLTPR	
ARAP3	
ALPL	
TFPI2	
FABP6	
TNFSF10	
STAT5A	
TGFB1	
SCARF2	
GJB3	
TNFRSFI2A	

Downregulated

HOXB5	UTS2R
HOXB8	SLC38A8
MUC5B	THBS4
HOXB6	COL14A1
HOXB-AS3	TCL6
SAGE1	JAG2
HOXB3	SLC6A12
KC6	LSAMP
PKNOX2	TCL1B
DPP6	C2orf54
PRND	TP63
LOC102723344	EPB41L1
ZNF418	ESPNP
MUC5AC	KIAA0125
CNTNAP2	PRDM11
DMRT3	KIF26A
C12orf56	ENTPD2
DMRT3	LRRN4
CRHR1	GJA1
KRTAP2-3	PDE11A
LINC01266	CMBL
CARD11	NLGN2
CYP2E1	TMEM47
HOXB4	LOC101928979
HOXB9	ST8SIA4
C3orf67	ULBP1
HOXB7	ULK1
GLYATL3	PLAT
ANPEP	PARD6B
TUNAR	GCNT1
LMO3	CHAC1
EPHA3	TNFRSF25
ESRRB	AFF1
DNAJA4	KRT8
RAB44	CHST6
SPDEF	TNFAIP8
HRN1	HES2
IQSEC3	FMN1
	SLC9A3R2
	PRIMA1

Fig. 3 *Gene expression profiling of carboplatin- and paclitaxel-resistant endometrial cancer cells.* Venn diagram representing the number of genes significantly altered (upregulated or downregulated) in carboplatin- or paclitaxel-resistant cells as compared to vehicle-treated cells. A total of 45 genes were found to be upregulated for both drugs. **b** Principal component analysis (PCA) of RNA-seq data based on normalized read counts. Sample to sample distances after treatment are illustrated for each cell line

chlorobenzylthio-3-trifluoromethyl-N-methyl-1H-pyrazol-4-yl)methylene)-2 thioxothiazolidin-4-one [23, 24], or the MICAL2-inhibitor N-(4-chlorophenyl)-1-(3-(furan-2-yl)benzoyl)piperidine-3-carboxamide [25, 26].

Specifically, cells were treated with increasing concentration of the drugs over a period of 72 h and the residual cell viability was measured by WST1 assay as previously described [27]. As shown in Fig. 7, treatment with pentamidine, pimozide, ADAMTS5, or MICAL2 inhibitors resulted in a dose-dependent inhibition of cell viability of otherwise carboplatin-resistant cells. Specifically, the IC$_{50}$ were 3.35, 12.7, 14.9, and 43.70 μM for pentamidine, pimozide,

Table 1 *Correlation between commonly upregulated genes in carboplatin- and paclitaxel-resistant cells and poor outcome in endometrial cancer patients.* Upregulation of genes for which a correlation between gene expression and endometrial cancer overall survival (OS) was found based on the TCGA database. *Those in italic are the genes for which the correlation was highly significant*

Gene	Prediction of OS
ABLIM1	$p = 2.1375$
ANK 1	$p = 0.5085$
APOE	$p = 1.224$
FABP6	$p = 1.2825$
FSTL3	$p = 1.1025$
LBH	$p = 0.6043$
LGALS3BP	$p = 0.2101$
LIF	$p = 0.0182$
NEAT1	$p = 0.666$
OSBPL7	$p = 0.6795$
PODN	$p = 0.3676$
PTP4A3	$p = 0.035$
SLC9A4	$p = 1.5255$
TGFB1	$p = 0.0054$
TNFRSF12A	$p = 0.0882$
TNFSF10	$p = 1.7415$
ZBED6CL	$p = 1.071$
ZNF853	$p = 0.5895$

Fig. 4 *LIF, PTP4A3, and TGFB1 expression stratified endometrial cancer patients in low- and high-risk groups.* Kaplan–Meier showing stratification of the TCGA endometrial cancer cohort into high- and low-risk groups based on the indicated gene expression

ADAMTS5, or MICAL2 inhibitors, respectively. Similar results were obtained when we tested the paclitaxel-resistant cancer cells (Supplementary Table 3). IC_{50} of the parental (sensitive) ECC-1 cells lines to pentamidine, pimozide, ADAMTS5, or MICAL2 inhibitors are shown in Supplementary Table 4. This suggests that inhibition of PTP4A3, ADAMTS5, STAT5A, or MICAL2 could represent a novel chemotherapy approach for the treatment of chemoresistant endometrial cancer.

Discussion

The use of matched pairs of chemosensitive and chemoresistant cancer cells has greatly improved our understanding of the molecular mechanisms regulating human cancers' chemoresistance in ovarian, lung, prostate, and brain cancers among others [28–32]. Unfortunately, the paucity of matched pairs of chemosensitive and chemoresistant endometrial cancer cells has greatly limited our understanding of this disease whose poor prognosis has been rising over the years mainly due to recurrence and chemoresistance.

Here, we developed and characterized matched pairs of carboplatin-sensitive/carboplatin-resistant and paclitaxel-sensitive/paclitaxel-resistant endometrial cancer cells. This was achieved via cyclic and incremental drug exposure so as to simulate the in vivo development of resistance through multiple cycles of chemotherapy. The resulting chemoresistant cells are approximatively tenfold less sensitive to the drug as compared to their sensitive counterparts. While others have
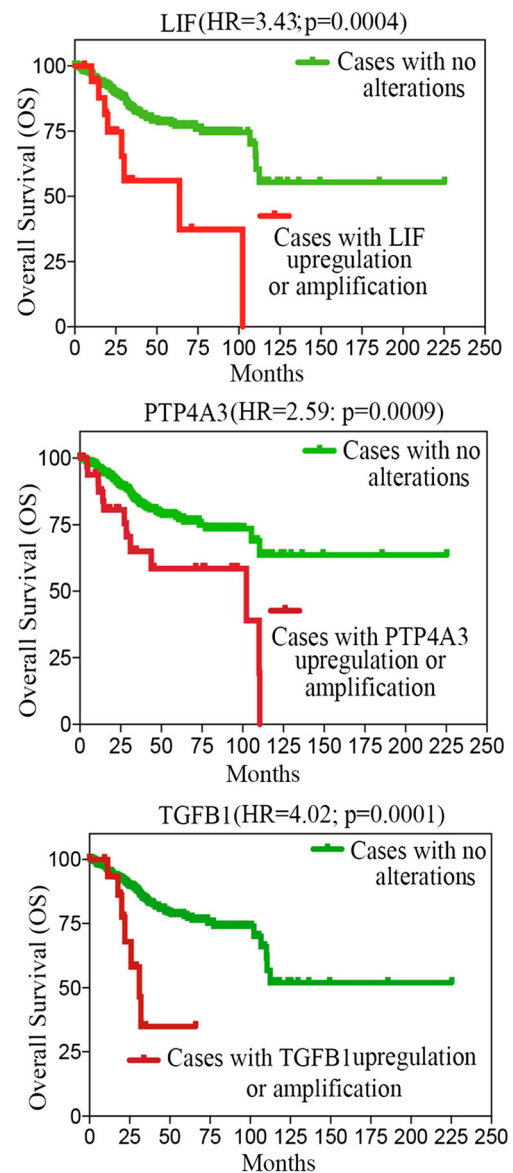
reported the generation of chemoresistant cancer cells that are hundred folds less sensitive to chemotherapy than their sensitive counterpart [29], we think our model is still relevant to the disease. This is because in patients increases in drug dosage are not common practice due to limiting side effects. Importantly, we did not observe cross-resistance, suggesting that different molecular pathways are involved in the development of chemoresistance to carboplatin and paclitaxel.

For the past 20 years, microarray analysis of gene expression has been a widely used technique and precious source of information for understanding the complexities regulating cancer progression, including onset of chemoresistance in both cell lines and primary tumor specimens [33–38].
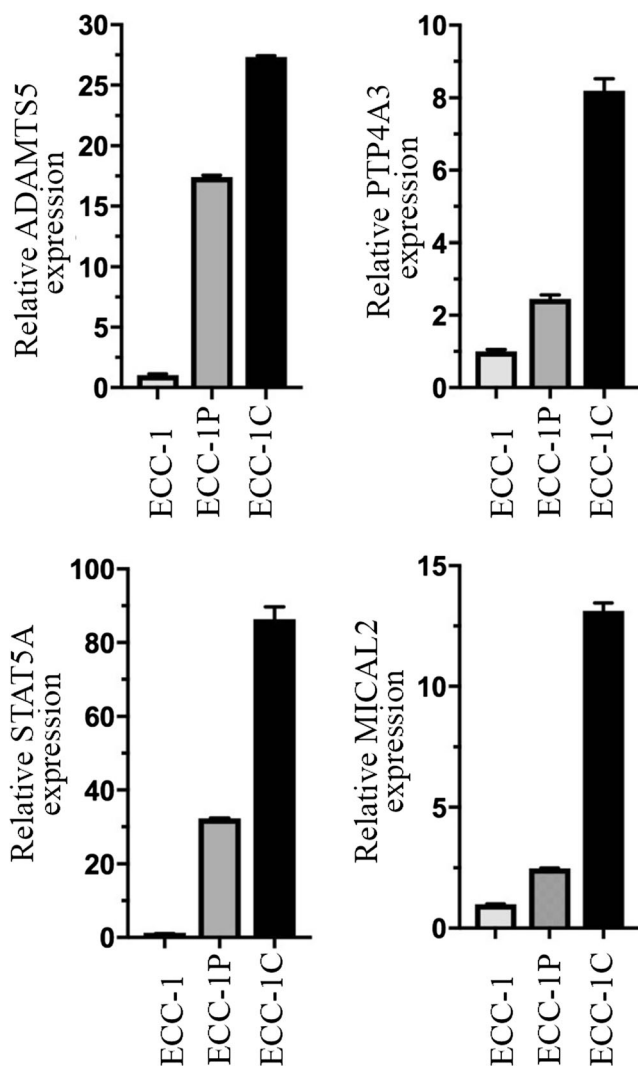
Fig. 5 *Validation of gene expression.* qRT-PCR analysis of ADAMTS5, PTP4A3, STAT5A, and MICAL2 in vehicle-treated (ECC-1) versus chemoresistant (ECC-1P and ECC-1C) endometrial cancer cells. Three independent experiments with means ± standard deviations are presented for each protein

Despite its many advantages, including the possibility to measure the expression levels of thousands of genes simultaneously, microarray analysis suffers a major drawback in that it can only provide information about the genes that are included in a specific array. The recent development of the high-throughput RNA sequencing (RNA-seq) has provided a novel and powerful tool for understanding cancer cells' transcriptome [39, 40]. While still relatively expensive, this technique allows for detection of both known and yet to be discovered transcripts even when their expression is relatively low.

Here, the matched pairs were subjected to bulk RNA-seq and screened for differentially expressed genes (DEGs) commonly found in both carboplatin- and paclitaxel-resistant endometrial cancer cells. These commonly upregulated genes were of particular interest given that endometrial cancer is typically resistant to both drugs. Our analysis of The Cancer Genome Atlas

(TCGA) database revealed a strong correlation between the leukemia inhibitory factor (LIF), the protein tyrosine phosphatase type IVA, member 3 (PTP4A3), and the transforming growth factor beta 1 (TGFB1) gene expression and OS in endometrial cancer patients with each of these genes being able to stratify the 545 endometrial cancer patients in the TGCA into high-risk and low-risk cohorts. This is consistent with previous studies showing a correlation between LIF expression and poor outcome in additional human cancers including melanoma and oral squamous cell carcinoma [41, 42]. Furthermore, PTP4A3 has been shown to be overexpressed in a number of human cancers and its levels to be associated with the severity of the disease and patients' poor survival. This is the case for ovarian cancer [43], lymphoblastic leukemia [44], and breast [45, 46] and gastric [47] cancers. Lastly, aberrant regulation of the TGFB1 pathway has been associated with stemness and metastasis in human cancer, including endometrial cancer [48], via promoting epithelial to mesenchymal transition (EMT) [49].

With the goal of identifying novel therapeutic targets for chemoresistant endometrial cancer, we interrogated our list of 45 commonly upregulated genes to identify the ones for whose products are small-molecule inhibitors available either FDA approved or used in vitro and preclinically. This restricted the field to the previously identified protein tyrosine phosphatase type IVA, member 3 (PTP4A3) and its FDA-approved inhibitor pentamidine, the disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) and its FDA-approved inhibitor pimozide, the signal transducer and activator of transcription 5A (STAT5A) and its inhibitor 5-((4-chlorobenzylthio-3-trifluoromethyl-N-methyl-1H-pyrazol-4-yl)methylene)-2 thioxothiazolidin-4-one, and the microtubule associated monooxygenase, calponin and LIM domain containing 2 (MICAL2) and its inhibitor N-(4-chlorophenyl)-1-(3-(furan-2-yl)benzoyl)piperidine-3-carboxamide.

The PTP4A3 inhibitor pentamidine is an FDA-approved antifungal and antiprotozoal agent. Importantly, pentamidine and its derivatives, as well as a novel class of PTP4A3 allosteric inhibitors, have been shown to have anticancer activity in human cancer cell lines and in preclinical models of human cancers [50, 51]. This includes chemoresistant ovarian cancer [43, 52], renal cancer [20], melanoma [22], and leukemia [21]. The mechanism/s through which small-molecule anti-PTP4A3 exert anticancer activity is/are still poorly understood, but several studies suggest they might do so via inhibiting angiogenesis and the Rho signaling pathway [53, 54]. The STAT5 inhibitor pimozide is an FDA-approved antipsychotic agent which has been recently shown to have antineoplastic effects in human cancers including prostate cancer [14, 15], lung cancer [55], and leukemia [19] via inhibition of STAT5 and wnt/β-catenin signaling pathways. ADAMTS5 has been shown to promote cancer cell migration and invasion and to be a promising molecular target for colorectal cancer [56] and melanoma [5] via a mechanism consistent with inhibition of pro-

Fig. 6 *Validation of protein expression.* Western blot analysis for ADAMTS5 (panel ***a***), PTP4A3 (panel ***b***), STAT5A (panel ***c***), and MICAL2 (panel ***d***) in vehicle-treated (ECC-1) versus chemoresistant (ECC-1P and ECC-1C) endometrial cancer cells. Equal protein loading in each line was verified using amido blots. Quantification of protein expression is given at the bottom of each panel. Three independent experiments with means ± standard deviations are presented for each protein
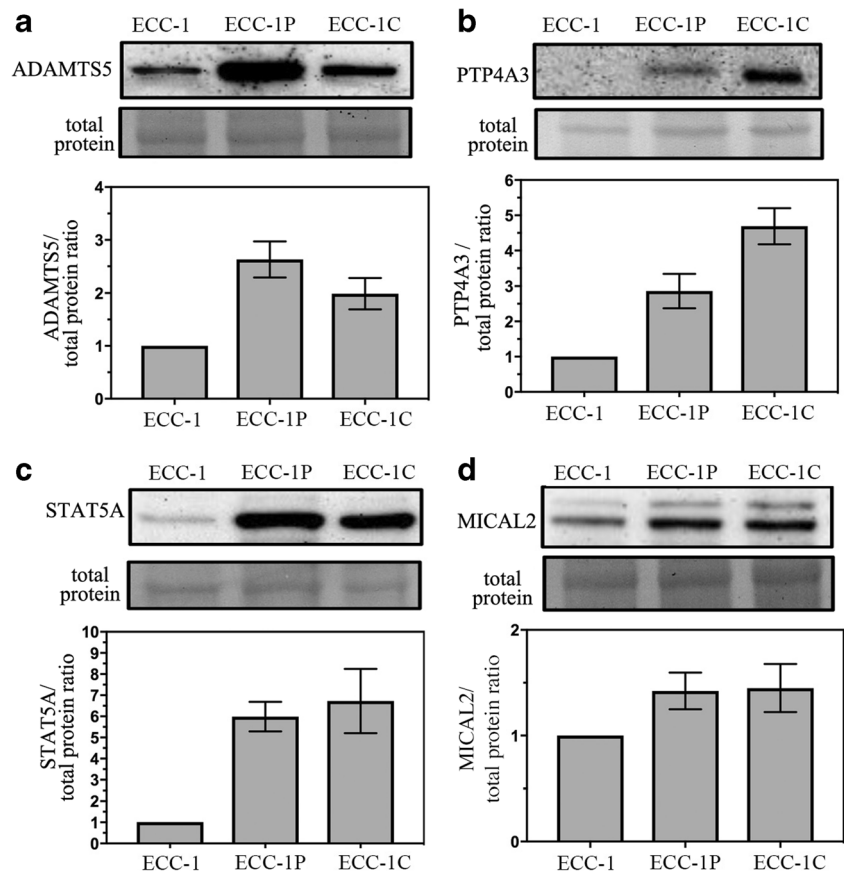
angiogenic factors [6]. Lastly, MICAL2 has been recently shown to play a role in controlling EMT transition via regulating cytoskeletal dynamics [7, 8]. Interestingly, MICAL2 overexpression has been associated with a subpopulation of cells within a tumor with highly migratory behavior, suggesting that its selective targeting could prevent cancer metastasis [57].

In conclusion, we found that the matched pair of chemosensitive and chemoresistant endometrial cancer cells recapitulates the features of human cancers, including endometrial cancer, and can be used to discover biomarkers for risk stratification and as a pharmacogenomics model for identification of alternative cancer treatments. This is particularly true for PTP4A3, which we propose as a novel biomarker that alone or in combination with other markers could be used to stratify endometrial cancer patients based on the likelihood to recur as well as a molecular target for the treatment of chemoresistant disease.

Methods and Material

ECC-1 Cell Line

The endometrial cancer cell line ECC-1 was provided by Dr. Gottfried Konecny (UCLA Medical Center). Cells were cultured in DMEM supplemented with 10% fetal bovine serum at 5% CO_2.

Establishment of Carboplatin- and Paclitaxel-Chemoresistant ECC-1 Cells

Chemoresistant ECC-1 sub-lines were generated by treatment with increasing doses of carboplatin or paclitaxel over a 6-week period followed by a 2-week recovery for a total of four cycles. Chemosensitive, original ECC-1 cells were treated with vehicle (saline).

Crystal Violet Staining and Phase Contrast Microscopy

Cells were stained with crystal violet and images were taken with a Nikon Eclipse T200 microscope and acquired using a NIS-Element F 3.3 camera and software. Magnification was set at ×20 and the scale bar is set at 50 μm.

Cell Viability Assay

Cell viability was determined by (4-[3-(4-iodophenyl)-2-(4-nitro-phenyl)-2H-5-tetrazolio] 1,3-benzene disulfonate) (WST-1) as previously described [58–60]. Briefly, cells were seeded at the concentration of 2000 cells per well in 100 μl medium in a 96-well plate and treated with the indicated concentrations of drugs. At the indicated time points (48, 72, 96 h), cells were incubated according to the manufacturer's protocol with the WST-1 labeling mixture for 4 h. Formazan dye was quantified
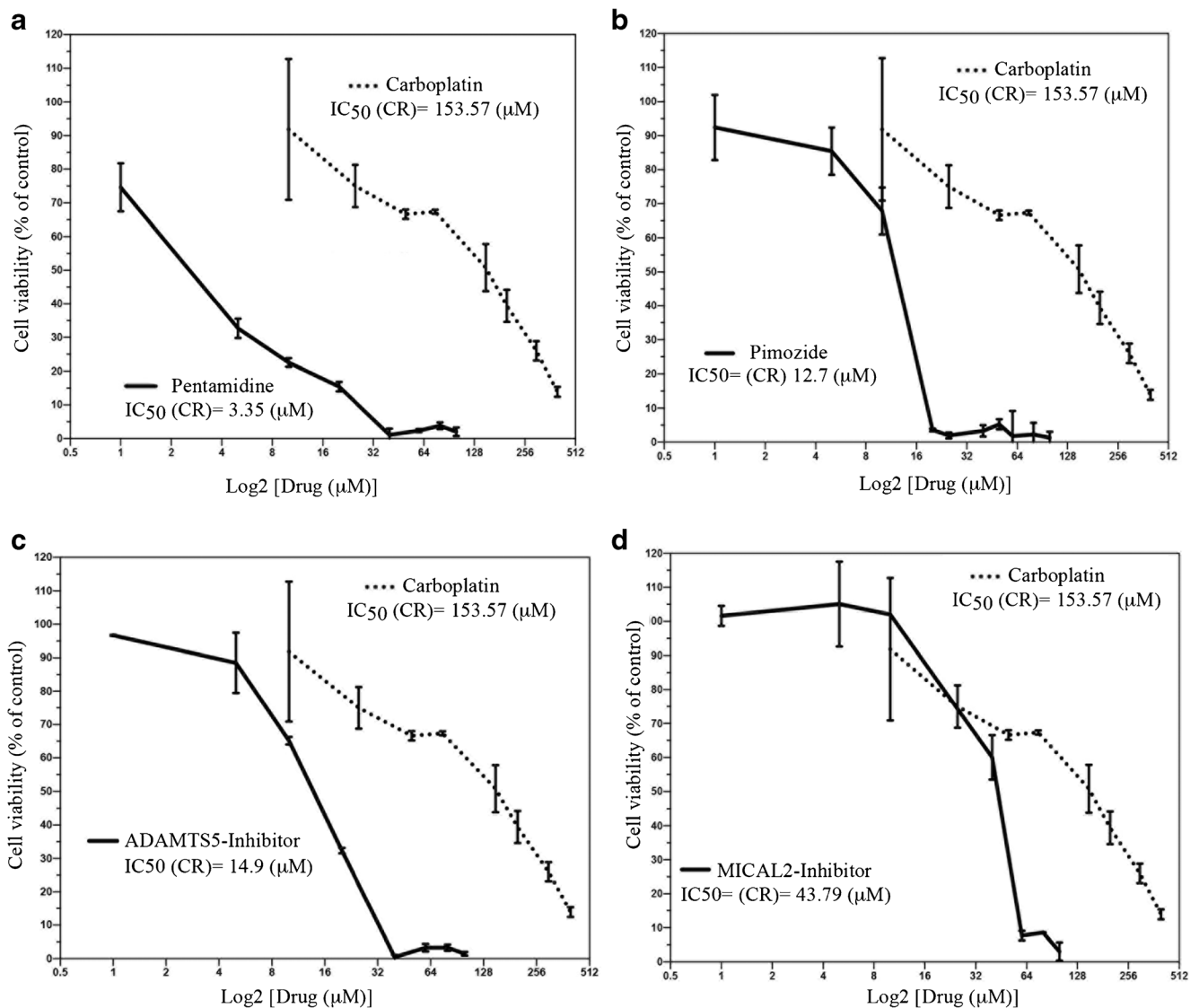
Fig. 7 *PTP4A3, ADAMTS5, STAT5A, and MICAL2 are molecular targets for carboplatin-resistant endometrial cancer cells.* Dose-dependent inhibition of cell viability of carboplatin-resistant ECC-1 cells exposed to increasing concentrations of pentamidine (**a**), pimozide (**b**), ADAMTS-5 inhibitor (**c**), or MICAL-2-inhibitor (**d**) over a period of 72 h. Percentage of viable cells is relative to mock-treated controls. Three independent experiments performed in triplicate; means ± standard deviations are presented

using a spectrophotometric plate reader to measure the absorbance at 480 nm (ELISA reader 190; Molecular Devices). Each experiment was performed in triplicate.

Proliferation Assay

For the cell proliferation assay, 10,000 cells/well were seeded in 24-well plates in triplicate and allowed to grow over a period up to 4 days. At each time point, cells were trypsinized, stained with trypan blue, and counted using a hemocytometer.

Chemicals

Carboplatin and paclitaxel were purchased from Sagent Pharmaceuticals (Schaumburg, IL) and Pharmachemie B.V

(Haarlem, Netherlands) respectively. The MICAL2 inhibitor CCG-203971 and the ADAMTS5 inhibitor CAS# 929634-33-3 were purchased by Calbiochem and resuspended in DMSO. The PTP4A3 inhibitor pentamidine and the STAT5A inhibitor pimozide were purchased from Sigma-Aldrich and resuspended in DMSO. The (4-[3-(4-iodophenyl)-2-(4-nitro- phenyl)-2H-5-tetrazolio] 1,3-benzene disulfonate) (WST-1) was purchased from Cayman Chemicals.

Western Blot Analysis

Total cellular protein (10–20 µg) from each sample was separated by SDS-PAGE, transferred to PVDF membranes, and subjected to Western blot analysis. Antibodies used for

Western blot analysis were obtained from the following commercial sources: rabbit polyclonal anti-ADAMTS5 antibody (abcam), mouse monoclonal anti STAT5A antibody (Thermo Fisher Scientific), rabbit polyclonal anti-MICAL2 antibody (proteintech), and mouse monoclonal PTP4A3 (PRL-3) antibody (Santa Cruz Biotechnology). Peroxidase-linked anti-mouse immunoglobulin G and peroxidase-linked anti-rabbit immunoglobulin G were from Amersham. Each experiment was performed in triplicates.

Statistical Analysis

Per gene, data including mRNA upregulation and/or DNA amplification and patients' overall survival were obtained from the TGCA project via the data portal on 02/13/2018. Kaplan–Meier survival curves were created using the log-rank test for TCGA data to compare high-risk and low-risk groups. Hazard ratio was calculated using GraphPad and statistical significance was set at $p = 0.05$.

RNA-seq Data Analysis

50-bp FastQ paired-end reads ($n = 12.3$ million per sample) were trimmed using Trimmomatic (v 0.33) enabled with the optional "-q" option; 3-bp sliding-window trimming from 3′ end requiring minimum Q30. Quality control on raw sequence data for each sample was performed with FastQC. Read mapping was performed via Hisat2 (v2.0.2) using the UCSC human genome (hg38) as reference. Gene quantification was done via Cuffquant for FPKM values and Feature Counts for raw read counts. Differentially expressed genes were identified using the edgeR (negative binomial) feature in CLCGWB (Qiagen, Valencia, CA) using raw read counts. We filtered the generated list based on a minimum 2× absolute fold change and Bonferroni corrected $p < 0.05$. Principal component analysis (PCA) was performed in CLCGWB to identify and quantify variability in the data. Volcano plots and Venn diagrams were used to visualize the expression data and the results of significance testing. Pair-wise comparisons between treatment groups were made in the Bioconductor (3.2) R package following the standard workflow.

Quantitative Real-Time PCR

Total RNA was extracted using the RNeasy plus kit (Qiagen) according to the manufacturer's instructions. Total RNA concentration and quality were measured using the Epoch microplate spectrophotometer (Biotek). One microgram of total RNA from each cell line was used to generate cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). SYBR green-based RT-qPCR was performed in triplicate using diluted cDNA. Samples were run in the LightCycler 96 (Roche). The comparative Ct method was used to determine the relative expression in each sample using TBP as normalization control. Primers were as follows: STAT5A, forward: 5′- ACATGTACCCACAGAACCCTGACC-3′, reverse: 5′- CACAACACGACCGCTTCACATTGC-3′; PTP4A3, forward: 5′-GGGACTTCTCAGGTCGTGTC-3′, reverse: 5′-AGCCCCGTACTTCTTCAGGT-3′; ADAMTS5, forward: 5′CAC TGT GGC TCA CGA AAT CG 3′, reverse: 5′CGC TTA TCT TCT GTG GAA CCA AA 3′; and MICAL2, forward: 5′-CTCACACGACACCTGGACCTA-3′, reverse: 5′-CCACGCTTATCCAATTTGTACCA-3′.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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