SUPPLEMENTARY DATA

Orthoxenografts of testicular germ cell tumors uncover genomic changes associated to cisplatin resistance and identify PDMP as a re-sensitizing agent

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SUPPLEMENTARY METHODS

Generation process of orthoxenografts of testicular germ cell tumors in nude mice

Primary tumor samples were obtained after surgical resection at Hospital Universitari de Bellvitge, Barcelona (Spain) and placed at room temperature in DMEM medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Fresh surgical specimens of human GCTs were implanted in nude mice. Animals were housed in a

sterile environment, cages and water were autoclaved and bedding and food was γ-ray sterilized. Tumors were implanted in the testis of five-week old male *nu/nu Swiss mice* (Charles River, France) weighting 18-22 g. After anesthesia by isofluorane inhalation, a median laparatomy was performed and the testes were mobilized. Tumor pieces were anchored to the testis surface with prolene 7.0 sutures. After implantation, mice were inspected twice a week, and if no tumor growth was apparent, mice were sacrificed six months after implantation. Serial tumor passaging was performed in two to five animals. Time lags varied for each tumor, depending upon their growth kinetics (**Table S1**). Four orthoxenografts were derived from patients previously treated with cisplatin-based chemotherapy.

Immunohistochemistry characterization of xenografted tumors

Tissues taken for histological studies were fixed in 10% buffered formalin and 3-µm slices of paraffin-embedded tissues were used for immunohistochemistry (IHQ) studies. Monoclonal primary antibody for OCT3/4 was diluted 1:300 (Santa Cruz) Reactions were visualized using the EnVision anti-mouse antibody system, and developed using the DAB-Plus Kit (Dako, Copenhagen, Denmark). Slides were counterstained with Harry's modified hematoxylin.

Determination of mouse serum levels of tumor markers

Serum concentrations of alpha-fetoprotein (AFP) and the β-subunit of human chorionic gonadotropin (β-hCG) concentrations were measured as subrogate tumor growth markers in the serum of nude mice using commercially available two-site enzyme chemiluminometric assays automated on the Immulite® 2000 analyzer (1,2).

Primary response of engrafted NSEs to cisplatin treatments

Small fragments of engrafted tumors were reimplanted in the testicles of 30 nude mice, as described above. When palpable intra-abdominal masses and increased levels of serum tumor marks had both been detected, usually 7–30 days after implantation, mice were randomized into three groups: (i) control group (n = 10), treated with vehicle; (ii) low-dose treatment group (n = 20) (2 mg/kg of cisplatin); and (iii) high-dose treatment group (n = 20) (5 mg/kg of cisplatin). Each treatment group was randomly divided into a *short-term response group* (n = 10), defined by tumor weight at the time of sacrifice of the control group, and a *long-term response group* (n = 10), defined by recurrent tumor mass regrowth post-chemotherapy. Cisplatin was intravenously administered (i.v.) once a week for three consecutive weeks (days 0, 7 and 14). Animals were sacrificed seven days after the final dose (day 21) to examine their short-term response.

Genetic characterization of engrafted NSE tumors

DNA was extracted following standard phenol-chloroform protocols, while total RNA was extracted using TRIZOL reagent following the manufacturers' instructions (Invitrogen). Nude mouse tissues were included in all PCR experiments to avoid mouse DNA and RNA contamination.

Mutations in *TP53* (exons 4-10); K-*ras* (codon 12 and 13), *b-raf* (exons 11 and 15), *EGFR* (exons 18, 19, 20 and 21), c-*Kit* (exons 9, 11, 13 and 17), *PDGFRα* (exons 12 and 14), *PDGFRβ* (exon 12) and *PI3KCA* (exons 9 and 20) were analyzed. All exons were amplified in independent PCR reactions using human intronic primers to avoid amplification of mouse DNA. PCR reactions were carried out using 100-200 ng of genomic DNA in a mixture containing PCR buffer, 100 mM deoxynucleotide

triphosphates, 0.5 µM of each primer and 1 unit of Taq DNA polymerase (Invitrogen). RNA was reverse-transcribed to cDNA using $pd(N)_6$ and the M-MLV retrotranscriptase kit (Invitrogen) and the entire coding *Smad*4 region was analyzed in five overlapping reactions. Primer sequences and PCR conditions are available on request. The presence of gene mutations was detected by direct sequence and/or single-strand chain polymorphism (SSCP). Homozygous deletions or microdeletions in *p15*, *p16* and *Smad4* were evaluated in agarose gels and were defined by the absence of PCR product in three independent experiments.

Genetic instability (MSI) was analyzed using Bethesda's set of five microsatellite markers (D2S123, BAT25, BAT26, D5S346 and BAT40).

FISH analysis

FISH was done by standard methods. We used the UCSC genome browser to select three bacterial artificial chromosomes (BACs) from the K32 BAC library (kindly provided by Dr L. Pérez-Jurado). BAC RP11-582I20 is contained in the amplified 9q32-9q33.1 region while RP11-616C16 flanks it at its distal end. FISH results were analyzed under an Olympus BX60 microscope and images were captured with a Cytovision (Applied Imaging) workstation. One hundred non-overlapping nuclei were scored for each sample.

Quantification of gene and miRNA expression

Total RNA was extracted using Trizol (Invitrogen, San Diego, CA), following the manufacturer's instructions, and reverse-transcribed to cDNA. Quantitative RNA and miRNA analyses was performed as described. Quantitative real-time RT-PCR analyses

were performed using the Light-Cycler 2.0 Roche System and LightCycler FastStart DNA Master SyBR Green I kit (Roche). All the primers were designed specifically to amplify human RNA. Primer sequences and PCR conditions are available on request. Experiments were performed in triplicate using three independent RT reactions. Gene expression was normalized with respect to β-actin.

For miRNA, RNA samples were DNase-treated with Turbo DNA-free (Ambion, Austin, TX), and determined as described (3). Reactions were performed in triplicate and incubated in an Applied Biosystems 7900HT Fast Real-Time PCR system in 384-well plates. All data were normalized with endogenous controls: PPIA, HPRT1 and RPLPO. The relative miRNA levels were calculated using the formula $2^{-\Delta\Delta Ct}$ (4).

C. elegans RNAi and cisplatin-response assays

The *C. elegans* N2 strain (wild type), and the *rrf-3 (pk1436)* and *cgt-1 (ok1045)* mutant strains were provided by the Caenorhabditis Genetic Centre (CGC). The bacterial RNAi clones used were obtained from the ORFeome-based RNAi library (5) or the JA library (6). For adult survival assays, RNAi by feeding was performed on synchronized worms (7) that were cultured in NGM plates containing 50 µg/ml ampicillin, 12.5 µg/ml Tetracycline and 3 mM IPTG. Then, at the young-adult stage, animals were transferred to a 96-well plate. Each well contained S-medium with the corresponding RNAi clone plus 50µg/ml ampicillin, 12.5 µg/ml Tetracycline 3 mM IPTG. Cisplatin (Sigma 15663-27-1) dissolved in water was added to each well to reach the indicated concentration. Lethality was scored as absence of movement that was recorded by an automated tracking system (wmicrotracker) that registers numbers of bins per hour (8). Cisplatin-induced toxicity was evaluated by measuring worm locomotor activity over a 24-h

period. All assays were performed at 20ºC, tracking 20 worms per well each time in each of four replicates.

SUPPLEMENTARY BIBLIOGRAPHY

1. Mora J, Gascón N, Tabernero JM, Germà JR, González F. Alpha-fetoprotein-concanavalin A binding as a marker to discriminate between germ cell tumours and liver diseases. Eur J Cancer. 1995;31A:2239–42.
2. Mora J, Gascón N, Tabernero JM, Rodríguez-Espinosa J, González-Sastre F. Different hCG assays to measure ectopic hCG secretion in bladder carcinoma patients. Br J Cancer. 1996;74:1081–4.
3. Davalos V, Moutinho C, Villanueva A, Boque R, Silva P, Carneiro F, et al. Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. Oncogene. 2012;31:2062–74.
4. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402–8.
5. Rual J-F, Ceron J, Koreth J, Hao T, Nicot A-S, Hirozane-Kishikawa T, et al. Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. Genome Res. 2004;14:2162–8.
6. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, et al. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature. 2003;421:231–7.
7. Porta-de-la-Riva M, Fontrodona L, Villanueva A, Cerón J. Basic Caenorhabditis elegans methods: synchronization and observation. J Vis Exp [Internet]. 2012 [cited 2017 Nov 15];e4019. Available from: http://www.jove.com/video/4019/

8. Simonetta SH, Golombek DA. An automated tracking system for Caenorhabditis elegans locomotor behavior and circadian studies application. J Neurosci Methods. 2007;161:273–80.
9. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45:D362–9.

Supplementary Table S1 Summary of characteristics of primary nonseminoma (NSE) tumors growing as xenografts in nude mice.

Human Primary Tumor					**Orthoxenografts**						
Primary tumor location	**Tumor**	**Histology** [a]	**Stage** [b]	**Patient's response to CDDP**	**Patient's CDDP treatment previous to biopsy**	**Histology**	**Xenograft perpetuated** [c]		**Time-lag between passages (days)** [d]	**Mouse serum markers** [e]	**Pattern of distal dissemination** [f]
							Orthotopic growth	**Subcutaneous growth**			
Testicle	**TGT1X** [J]	YS	Stage I	Not treated	No	YS	Yes	No	69 ± 17	AFP	Lung [g, h1]
	TGT11X	YS, EC, CH, TE	Stage I	Not treated	No	YS, EC, CH	Yes	No	ND	ND	ND
	TGT12X	EC	Good risk	Sensitive	No	EC	Yes	No	97 ± 25	β-hCG, AFP	None
	TGT14X	EC SE	Stage I	Not treated	No	EC	Yes	No	56 ± 17	β-hCG	Peritoneal implants [j, h2] Lymph node affection [j, h4]
	TGT21AX	YS, EC, CH, TE, SE	Stage I	Not treated	No	YS, EC, CH	Yes	Yes	49 ± 11	β-hCG, AFP	None
	TGT21BX	YS, EC, CH, TE, SE	Stage I	Not treated	No	YS, EC, CH	Yes	Yes	64 ± 14	β-hCG, AFP	None
	TGT34X	EC	Poor risk	Sensitive	No	EC	Yes	No	51 ± 8	β-hCG, AFP	Lymph node affection [j,h4]
	TGT38X [k]	CH	Poor risk	Sensitive	No	CH	Yes	No	20 ± 6	β-hCG	Lung [g, h1]
	TGT40X	YS, TE	Stage I	Not treated	No	YS	Yes	No	42 ± 8	AFP	None
Lymph node	TGT39X	YS, EC, TE	Poor risk	Sensitive	No	YS, EC	Yes	No	59 ± 13	β-hCG, AFP	Liver [j, h3] Peritoneal implants [g,h2]
	TGT41X	CH	Poor risk	Refractory	Yes	CH	Yes	No	18 ± 4	β-hCG	ND
	TGT44X [k]	YS, TE	Poor risk	Refractory	Yes	YS	Yes	No	50 ± 8	AFP	ND
Lung metastasis	TGT17X [k]	CH	Poor risk	Refractory	Yes	CH	Yes	No	24 ± 5	β-hCG	Lung [g ,h1]
Brain metastasis	TGT42X	EC	Poor risk	Refractory	Yes	EC	Yes	No	62 ± 10	β-hCG, AFP	ND

[a] Tumor histology: YS, yolk sac; EC, embryonal carcinoma; CH, choriocarcinoma; TE, teratoma; SE, seminoma.
[b] Stage at first diagnosis (Tumor localized in testicle referred as stage I; Stage II to IV classified following the International Germ Cell Cancer Collaborative Group (IGCCCG) classification).
[c] Primary tumor was simultaneously implanted in the testicles and subcutaneous tissues of nude mice. The tumor was considered perpetuated after at least six consecutive passages in nude mice.
[d] Time-lag between passages was calculated on the basis of the first six passages, for a median of 15 mice implanted with each tumor.
[e] Levels of alpha-fetoprotein (AFP) and/or β-subunit of human chorionic gonadotropin (β-hCG) were analyzed as tumor growth markers in the nude mouse serum.
[f] Presence of peritoneal implants has not been described in patients with TGCTs and probably their presence in some animals is a consequence of the methodology used in the implantation of tumors in mouse.
[g] Synchronous lung micrometastases were detected when nude mice were sacrificed.
[j] Liver macrometastases, peritoneal implants and lymph node affection were observed when nude mice were sacrificed.
[h] Orchiectomy was performed to confirm the dissemination patterns when palpable intra-abdominal masses were detected in 5 to 10 mice for each tumor. Animals were sacrificed 6-8 months after surgery, or when they lost weight: [h1], metachronic lung metastasis; [h2], metachronic peritoneal implants; [h3], metachronic liver metastases; [h4], metachronic lymph node.
[k] Xenografted tumors TGT38X and TGT44X characteristics were previously described [20,21,32].
None, absence of metastasis; ND, not determined.
Tumors used in this study are labeled in bold

Supplementary Table S2. Clinicopathological characteristics of patients, by response to cisplatin.

Characteristic	Sensitive (N = 51)		Resistant (N = 24)	
	No.	%	No.	%
Age, years				
Median	27.9		28.1	
Range	(16 – 56)		(15 - 53)	
Histology				
Seminoma	10	19.6	2	8.3
Nonseminoma	41	80.4	22	91.7
Localization				
Testis	51	100	20	83.3
Mediastinum	0	0	4	16.7
IGCCCG stage at diagnosis of metastasis [a]				
Good	38	74.5	7	29.2
Intermediate	7	13.7	7	29.2
Poor	6	11.8	10	41.7
First line of chemotherapy treatment [b]				
EP	9	17.6	2	8.3
BEP	33	64.7	12	50.0
Taxol-BEP	2	3.9	1	4.2
BOMP/EPI	7	13.7	9	37.5
Response to first line of chemotherapy treatment [c]				
Good response (CR, PR-)	51	100	16	66.7
Poor response (PR+, SD, PD)	0	0	8	33.3
Late relapse[d]				
Non	0		22	91.7
Yes	0		2	8.3

[a] IGCCCG International Germ Cell Cancer Collaborative Group.

[b] EP, etoposide/cisplatin; BEP, bleomycin/etoposide/cisplatin; BOMP/EPI, bleomycin/vincristine/methotrexate/ cisplatin-etoposide/cisplatin/ifosfamide.

[c] CR, complete remission characterized by tumor mass reduction by CT scan and negative valor of serum tumor marks; PR-, partial remission characterized by normalization of CT scan and negative valor of serum tumor markers; PR+, partial remission characterized by reduction of tumor mass by CT scan and positive valor of serum tumor markers; SD, stable disease; PD, progressive disease).

[d] Relapse >24 months after first diagnosis.

Supplementary Table S3. Tumors with amplification at 9q32-q33.1 in metastatic GCTs.

Patient	Histology [a]	Status of 9q32-q33.1 [b]	Cisplatin response
# 1	CE	High amplification	Resistant
#2	CE	High amplification	Sensitive
	CE	Low amplification	
	CE	NA	
#3	SE	NA	Resistant
	CE	High amplification	
#4	YS	High amplification	Resistant
	CE	High amplification	
	CH	High amplification	
	SE	High amplification	
#5	SE	High amplification	Sensitive
	CH	High amplification	
	TE	Low amplification	
#6	CH	Low amplification	Resistant
#7	CH	High amplification	Resistant
	CH	Low amplification	
#8	CE	Low amplification	Resistant
	YS	NA	
	TE	High amplification	
#9	SE	High amplification	Sensitive
#10	CE	High amplification	Sensitive
#11	YS	Low amplification	Resistant
#12	SE	Low amplification	
#13	TE	Low amplification	Resistant
#14	TE	Low amplification	Sensitive
#15	CE	High amplification	Sensitive
	CE	NA	
#16	CE	Low amplification	Sensitive
#17	CE	High amplification	Sensitive
	CE	NA	
	TE/CE	NA	
	CH	NA	
#18	CE	Low amplification	Resistant
	TE	Low amplification	

[a]Tumor histology: YS, yolk sac; EC, embryonal carcinoma; CH, choriocarcinoma; TE, teratoma; SE, seminoma.

[b] Low (4 or 5 signals) and high (>5 signals). NA, none amplified.

Supplementary Table S4. Patients classified with respect to 9q32-q33.1 amplification status.

	9q32-q33.1 status [e]				
	Non-amplification (N = 57)		Amplification (N = 18)		
	Number	%	Number	%	*P*
Age, years					0.25
Median	27.6		29.1		
Range	(15 - 56)		(16 - 53)		
Histology					0.52
Seminoma	10	17.5	2	11.1	
Nonseminoma	47	82.5	16	88.9	
Localization					0.96
Testis	54	94.7	17	94.4	
Mediastinum	3	5.3	1	5.6	
IGCCCG stage at diagnosis of metastasis [a]					0.26
Good	37	64.9	8	44.4	
Intermediate	10	17.5	4	22.2	
Poor	10	17.5	6	33.3	
First line of chemotherapy treatment [b]					0.83
EP	9	15.8	2	11.1	
BEP	35	61.4	10	55.6	
Taxol-BEP	2	3.5	1	5.6	
BOMP/EPI	11	19.3	5	27.8	
Response to first line of chemotherapy treatment [c]					0.007
Good response (CR, PR-)	54	94.7	13	72.2	
Poor response (PR+, SD, PD)	3	5.3	5	27.8	
Sensitivity to cisplatin [d]					0.060
Sensitive	42	73.7	9	50.0	
Resistant	15	26.3	9	50.0	

[a] IGCCCG International Germ Cell Cancer Collaborative Group.

[b] EP, etoposide/cisplatin; BEP, bleomycin/etoposide/cisplatin; BOMP/EPI, bleomycin/vincristine/methotrexate/ cisplatin-etoposide/cisplatin/ifosfamide

[c] CR, complete remission characterized by tumor mass reduction by CT scan and negative value of serum tumor marks; PR-, partial remission characterized by normalization of CT scan and negative value of serum tumor markers; PR+, partial remission characterized by reduction of tumor mass by CT scan and positive value of serum tumor markers; SD, stable disease; PD, progressive disease.

[d] Patients who achieved durable complete response with first-line cisplatin-based chemotherapy were considered sensitive. Patients who had either a poor response or relapsed after first-line chemotherapy were considered resistant to cisplatin.

[e] Amplification at 9q determined by FISH using two different probes (see *Material and Methods*).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. Short- and long-term CDDP responses at low (2 mg/kg) and high (5 mg/kg) doses of CDDP were characterized in xenografted tumors. (A) TGT1X, a pure YS; TGT12X and TGT14X, two pure ECs, and TG39X, a mixed tumor with EC and YS components. Dynamic curves of CDDP treatments show a sustained response, characterized by levels of AFP that were maintained for 90 days (TGT39X, right panel). Dynamic CDDP response curves were generated by serial AFP and β-hCG determinations in the serum of control (saline-treated) and drug-treated mice in both types of responses and for 2 and 5 mg/kg doses of CDDP. (B) and (C) Characterization of differential CDDP responses of TGT21AX and TGT21BX, two tumors obtained from the same patient. A complete short- and long-term response was obtained at high doses for both tumors. (C) H&E analyses of relapsed masses in long-term response (2 mg/kg) of TGT21AX, showed the presence of a teratoma (TE), characterized by the absence of tumor serum markers.

Supplementary Fig. 2. Comparative histological analysis of original non-treated orthoxenografts and their pairs with acquired resistance to CDDP after five cycles of treatment in mice. All tumors maintained the same histological appearance, and unlike untreated tumors, higher levels of fibrosis and necrosis were observed. **TGT21AX differentiated to a growing teratoma after the first cycle of CDDP treatment, and did not regrow after mouse reimplantation.

Supplementary Fig. 3. Representative recurrent imbalanced regions in different orthoxenografts with acquired CDDP resistance. (A) Gains at 15q23-q24.1 and 15q26.3 were identified in TGT12XR and TGT21BXR. (B) Loss of the Xp22.33 region happens in TGT12XR, TGT21XR and TGT38XR. Whole-genome mapping was performed by oligonucleotide array CGH analysis (60 kbp window averaging) visually depicted with the SignalMap graphical interface tool from Nimblegen Systems. Arrows indicate regions of new gain/loss in resistant tumors.

Supplementary Fig. 4. Physical and Functional interactions of proteins encoded by 9q32-q33.1 genes expressed in PDOXs of this study. UniProtKB/Swiss-Prot ID names

were loaded to the web-based tool STRING 10.5 (9). The resulting network contains 33 nodes and 4 edges, with a Protein-Protein Interaction (PPI) p-value of 0.221, which means that the network have significantly less interaction than expected. Blue line, Known interaction from curated databases; Pink line, Known interaction from curated databases; Green line, Text mining; and Black line, Co-expres “

Supplementary Fig 5. Impact of some RNAi clones on cisplatin toxicity in *Caenorhabditis elegans* adults

(A) Cisplatin induces a dose-dependent descrease in *C. elegans* adult locomotor activity. Locomotor activity is represented as percentage of variation respect to initial activity of a worm population growing in liquid media. Since cisplatin cause sterility, animals that were not expose to cisplatin show an increase in the movement due to the presence of newborn larvae. (B) To score the effect of RNAi clones in cisplatin toxicity, we plotted the percentage of locomotor activity after 24 hours of exposure to cisplatin. Animals exposed at 500µg/µl keep about 30-35% of their initial locomotor activity. (C) Different response of worms fed with some RNAi clones exposed to 500 µg/µl of cisplatin. Inactivation of *vha-10(RNAi)* and *F27C1.2(RNAi)* cause sensitivity and resistance to cisplatin respectively. *cgt-1* and *cgt-3* are functionally redundant and double inactivation (*cgt-1* mutation plus *cgt-3*(RNAi)) is required to induce sensitivity to cisplatin. RNAi against F27C1.2 was started at L1 stage whereas *vha-10(RNAi) and cgt-3(RNAi)* was initiated at L3 stage. RNAi against the Copper-transporter encoding gene F27C1.2 was performed on the RNAi sensitive background *rrf-3(pk1426).* ***$p<0,001$, **$p<0,01$, *$p<0,05$ compared to control, $^{++}$ $p<0,01$ compared to *cgt-1(ok1045)* and *cgt-3(RNAi)* ^^^ $p<0,001$ compared to *rrf-3(pk1426)* by Student’s t-test.