**ERK regulates HIF-1α-mediated platinum resistance by directly**

**targeting PHD2 in ovarian cancer**

Zhuqing Li1,2,7, Wei Zhou1,2,3,7, Yi Zhang1,2,7, Wei Sun4, Mingo MH Yung5, Jing Sun1,2, Jing Li1,2, Chi-Wei Chen1,2, Zongzhu Li1,2, Yunxiao Meng1,2, Jie Chai 1,2, Yuan Zhou 1,2, Stephanie S Liu6, Annie NY Cheung6, Hextan YS Ngan5, David W. Chan5,\*, Wei Zheng4,\*, and Wenge Zhu1,2,\*

**This file includes Supplementary Fig.S1-S6 and Table S1.**

**Figure S1. Establishment of platinum-resistant ovarian cancer cell lines. Related to Figure 1.**

(**A**) Viability of IGROV1 and IGROV1 CR cells (top), SKOV3 and SKOV3 CR (bottom) treated with increasing concentrations of cisplatin for 5 days. The IC50 values represent the mean of two independent experiments performed in triplicate. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by one-way ANOVA analysis. Data are the mean ± the standard deviation (SD) from three independent experiments performed in triplicate for all following figures, unless otherwise noted. (**B**) Representative flow cytometry dot plots (left) and quantification (right) of IGROV1/IGROV1 CR (top) and SKOV3/SKOV3 CR (bottom) cells treated with cisplatin for 48 hr and analyzed for annexin V and propidium iodide staining by flow cytometry. (**C**) The effect of cisplatin on the proteolytic activation of BCL-XL and cleavage of PARP in the IGROV1/IGROV1 CR (left panel) and SKOV3/SKOV3 CR (right panel) cells. (**D**) Representative images (left) and quantification (right) of TUNEL staining of IGROV1 and IGROV1 CR tumor xenografts after 4 days of treatment with vehicle or cisplatin (2mg/kg twice per week for two weeks), n = 4. Scale bar, 100 μm. (**E**) Relative cell viability of IGROV1/IGROV1 CR (left), and SKOV3/SKOV3 CR (middle) cells treated with increasing concentrations of carboplatin and relative cell viability of PEO1/4 (right) cells treated with increasing concentrations of cisplatin for 5 days. PEO1/4 cells were from the same patient before and after development of chemoresistant to platinum treatment. (**F**) Representative colony formation and quantification of IGROV1/IGROV1CR cells (left panels) and SKOV3/SKOV3 CR cells (right panels) 14 days after treated with cisplatin (0.5 μM for IGROV1/IGROV1 CR cells and 1 μM for SKOV3/SKOV3 CR cells). Colonies were stained with crystal violet.

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**Figure S2. YC-1 targets HIF-1α signaling in PROC cells *in vitro* and *in vivo*. Related to Figure 2.**

(A) Relative cell viability of IGROV1 CR cells treated with YC-1 combined with different doses of cisplatin. (**B**) qPCR analysis of HIF-1α mRNA expression in parental and platinum-resistant ovarian cancer cells. (**C**) Immunoblot analysis of HIF-1α and BCL-XL in IGROV1 CR cells with HIF-1α knockdown by shRNA. (**D**) Viability of HIF-1α knockdown IGROV1 CR cells treated with increasing concentrations of cisplatin for 5 days compared to control non-specific shRNA. (**E**) Immunoblot analysis of of HIF-1α and BCL-XL in IGROV1 CR cells transfected with HIF-1α siRNA. (**F**) Relative cell viability of HIF-1α knockdown IGROV1 CR cells treated with 1 μM cisplatin for 5 days compared to control siRNA (siGL2). (**G**) Activated HIF-1α signaling in IGROV1 cells treated with the indicated concentrations of CoCl2 for 2 hr. (**H**) Relative cell viability of IGROV1 cells treated with increasing concentrations of cisplatin for 5 days after HIF-1α signaling upregulated by CoCl2. (**I**) Representative flow cytometry plots (left) and quantification (right) of viability of IGROV1 CR cells treated with vehicle, 5 μM cisplatin, 1.5 μM YC-1 or the combination (combo) for 48 hr. Annexin V and Propidium Iodide staining by flow cytometry was used for cell viability analysis. (**J**) Immunoblot analysis of HIF-1α and BCL-XL in IGROV1 CR cells treated with vehicle, 5 μM cisplatin, 1.5 μM YC-1 or combo for 48 hr. (**K**) Body weights of IGROV1 CR tumor-bearing mice during treatment as in Figure 2F. (**L**) Representative histopathology of heart, liver, kidney, and lung collected from mice treated as in Figure 2G. Scale bar, 100 μm

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**Figure S3. ERK1/2 regulates HIF-1α activity in ovarian cancer cells. Related to Figure 3.**

(**A**) ERK/HIF-1α signaling in PROC cells treated with selumetinib (MEK inhibitor) of various dosages for 48 hr. (**B**) ERK/HIF-1α signaling in PROC cells treated with cisplatin (5µM), selumetinib (10 µM) or a combination of both (combo) for 48 hr. (**C and D**) Synergetic effect of selumetinib (5 µM) and cisplatin (2 to 32 µM) in PEO4 cells (**C**) and SKOV3 CR cells (**D**) after 72 hr treatment. The combination index (CI) values are presented below the bars. (**E**) Body weights of IGROV1 CR tumor-bearing mice during treatment in Figure 3G. (**F**) Representative histopathology of heart, liver, kidney and lung collected from mice treated in Figure 3H. Scale bar, 100 μm. (**G**) qPCR analysis of HIF-1α mRNA expression in IGROV1 CR cells treated with cisplatin (5 µM), selumetinib (10 µM), or both (combo) for 48 hr.

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**Figure S4. ERK1/2 regulate HIF-1α signaling by directly interacting with and phosphorylating PHD2. Related to Figure 4.**

(**A**) Immunoblot analysis of PHD2 in parental and platinum resistant ovarian cancer cells. (**B**) Immunoprecipitation (IP) analysis revealed elavated phosphorylation of PHD2 (Ser residues) limited binding between PHD2 and HIF-1α, as well as increased HIF-1α protein levels in PROC cells. (**C**) Co-IP assays to detect the direct interaction between endogenous PHD2 and ERK in PEO1 cells. (**D**) Co-IP assays to detect the interaction between PHD2 and ERK in parental and platinum resistant ovarian cancer cells. (**E and F**) Examination of phosphorylated-PHD2 (serine residues) protein levels and the interaction between PHD2 and HIF-1α by immunoprecipitation (IP) (**E**), and immunoblot analysis of hydroxylated-HIF-1α (OH-HIF-1α) and HIF-1α (**F**) in PROC cells treated with 10 µM selumetinib for 48 hours.

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**Figure S5. TGF-β1 receptor inhibitor SB431542 suppresses ERK/HIF-1α signaling. Related to Figure 5.**

(**A**) Relative cell viability of platinum sensitive cells when treated with conditioned medium from platinum sensitive or resistant cells for 72 hr. (**B**) Relative cell viability of PEO1 and SKOV3 cells when treated with different doses of cisplatin (2 to 32 µM) or cisplatin plus 10 μg/mL TGF-β1 for 72 hr. (**C**) Immunoblot analysis of TGF-β1/ERK/HIF-1α signaling in platinum-sensitive cells (PEO1) treated with 10 μg/mL TGF-β, 10 µM selumetinib, or both for 48 hr. (**D and E**) TGF-β1/ERK/HIF-1α signaling (**D**) and IP analysis of phosphorylated-PHD2 (serine residues) protein levels and the PHD2-HIF-1α interaction (**E**) in PROC cells treated with SB431542 (TGF-β1 inhibitor) at 10 µM for 48 hr. (**F**) Synergetic effect of TGF-β1 receptor inhibitor SB431542 (5 µM) and cisplatin (2 to 32 µM) in PEO4 and SKOV3 CR cells after 72 hr treatment. The combination index (CI) is presented below the bars.



**Figure S6. TGF-β1/ERK/HIF-1α pathway activation in platinum sensitive cell lines when treated with different dosages of cisplatin.**

**(A**) TGF-β1/ERK/HIF-1α signaling in platinum sensitive cells treated with cisplatin of various dosages for 4 days. (**B**) Activation of TGF-β1/ERK/HIF-1α signaling in IGROV1 cells treated with 1 μM cisplatin for various time as indicated. (**C**) Immunoprecipitation (IP) analysis of phosphorylated-PHD2 (serine residues) expression and the PHD2-HIF-1α interaction in platinum sensitive cells treated with different doses of cisplatin for 48 hr. (**D and E**) Representative H&E and IHC images (**D**) and quantification of IHC staining (**E**) of tumors in mice bearing IGROV1 xenograft tumors and treated with vehicle or cisplatin (0.5-2 mg/kg intraperitoneally for 2 weeks, twice a week). Scale bar, 100 μm for hematoxylin and eosin (H&E), p-ERK, HIF-1α, and BCL-XL.

**Supplementary Information Table S1**

**Table S1. qHTS data for IGROV CR cells**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample Name** | **Alias** | **IC50 (µM)**  **Vehicle** | **IC50 (µM)**  **with Cisplatin** | **Fold Change** |
| SB 205384 | null | 3.591297333 | 0.0058335 | 615.63 |
| zinc pyrithione | null | 3.639034 | 0.20687533 | 17.59 |
| SU-5416 | null | 1.7640455 | 0.1360355 | 12.97 |
| Cantharidin | null | 3.332017333 | 0.67149367 | 4.96 |
| Lificiguat | YC-1 | 1.091703333 | 0.28741433 | 3.80 |
| Romidepsin | null | 0.077983667 | 0.020544 | 3.80 |
| GW2974 | null | 1.133834 | 0.302515 | 3.75 |
| Suberoylanilide hydroxamic acid | MK-0683;SAHA; SAHA cpd; Vorinostat;Vorinostat; M344 compound;Zolinza | 4.447803 | 1.197932 | 3.71 |
| Suberoylanilide hydroxamic acid | MK-0683;SAHA; SAHA cpd; Vorinostat;Vorinostat; M344 compound;Zolinza | 3.197091333 | 0.98957833 | 3.23 |
| Cantharidin | Cantharidin; Cantharidine; | 1.978521333 | 0.63544833 | 3.11 |
| Phenylmercuric borate | null | 0.84205 | 0.322484 | 2.61 |
| Cytarabine | Aracytidine; Cytidine-5-3H; Aracytine;Cytosine-1-beta-D-arabinofuranoside hydrochloride; ; 6 Cytarabine; Ribonucleoside, Cytosin; Cytosar-U; Riboside, Cytosin; xyloC; Cytosar; Cytosine beta-D-riboside; Arabinocytidine; Cytarabine; Arabinosylcytosine; Cytidine-2-14C; Ara-C hydrochloride; Hydrochloride, Cytarabin;Cytarabine hydrochloride;Cytarabine; Cytosine-1-beta-D-arabinofuranoside hydrochloride; Arabinoside, Cytosin; beta-Ara C; Cytonal; Depocyt; Citarabina | 5.350966 | 2.059601 | 2.60 |
| Plicamycin | null | 0.453674 | 0.17978567 | 2.52 |
| Clofarabine | Clofarabine; ; Clolar;Clofarabine | 0.244087 | 0.097173 | 2.51 |
| Aclarubicin hydrochloride | Aclarubicin; Aclarubicino; Aclaplastin; Aclacinon; Aclacin; | 0.579004 | 0.23225667 | 2.49 |
| Auranofin | Ridaura; ; Auranofin;Auranofin; Auranofina | 1.541314 | 0.61944733 | 2.49 |
| Daunorubicin | WP900 hydrochloride; Cerubidine;Daunorubicin hydrochloride; ;; Daunoblastine;DAUNORUBICIN; Daunorubicin; Daunomycin HCL; Dauno-Rubidomycine; Daunomycin, hydrochloride; Daunomycin hydrochloride; Daunoblastin; Dauno Rubidomycin;Daunomycin hydrochloride; (-)-Daunorubicin | 7.558440333 | 3.089916 | 2.45 |
| Plicamycin | Plicamycin; Mithracin; Mithramycine; Mitramycin; Plicamicina; Mithramycinum; Mitramicina; Acid, Aureoli; | 0.506705 | 0.219788 | 2.31 |
| o-(Chloromercuri)phenol | Phenol-d6; Phenol-2,4,6-d3,OD; Phenol-2,3,4,5,6-d5; Phenol-2,4,6-d3; Hexadeuterophenol; | 2.248817 | 0.98175733 | 2.29 |
| Idarubicin hydrochloride |  | 0.358719333 | 0.160234 | 2.24 |
| Carminomycin | null | 0.547148333 | 0.24660633 | 2.22 |
| PD 0325901 | PD-325901;PD-0325901 | 0.370637333 | 0.18785333 | 1.97 |
| Fludarabine | ; Fludarabine des-phosphate; Fludarabine;fludarabine; F-ara-A;2-Fluoroadenosine; Fludara; Fludarabine Base | 2.577744333 | 1.38021467 | 1.87 |
| Cantharidic Acid | null | 2.101406333 | 1.192091 | 1.76 |
| Nocodazole | Nocodazole;nocodazole;(6-(THIOPHENE-2-CARBONYL)-1H-BENZOIMIDAZOL-2-YL)-CARBAMIC ACID METHYL ESTER | 0.189859333 | 0.11010733 | 1.72 |
| Bardoxolone methyl | Bardoxolone methyl; Bardoxolone methyl | 0.779837667 | 0.455521 | 1.71 |
| Amlenanox | 3 Amlexanox; ; Solfa;Amlexanox; Aphthasol; Amlenanox | 2.161559 | 1.306075 | 1.66 |
| Triclocarban | Triclocarban; Trichlorocarbon; Solubacter; Septivon-Lavril; Septivon; | 3.160458333 | 1.97446867 | 1.60 |
| Suloctdil | null | 4.144732333 | 2.73883467 | 1.51 |
| 4'-Demethylepipodophyllotoxin | 4'-Demethylepipodophyllotoxin;4'-DEMETHYLEPIPODOPHYLLOTOXIN | 0.47066 | 0.342508 | 1.37 |
| Sanguinarine chloride | null | 2.283013333 | 1.662611 | 1.37 |
| Cambendazole | Cambendazole; Cambendazol; Noviben; Camdan; | 3.603659 | 2.64671533 | 1.36 |
| 4-Chloromercuriphenol | Phenol-d6; Phenol-2,4,6-d3,OD; Phenol-2,3,4,5,6-d5; Phenol-2,4,6-d3; Hexadeuterophenol; | 2.208581667 | 1.63599933 | 1.35 |
| Podofilox | podophyllotoxin 4-O-Glcp;AXL-1717/NSC-36407; ;Podophyllotoxin; Picropodophyllin;Picropodophyllin | 0.047066 | 0.03616433 | 1.30 |
| Rubitecan | RUBITECAN; Rubetican; | 0.154862667 | 0.12155333 | 1.27 |
| alpha-Tomatine | null | 2.178235333 | 1.71698733 | 1.27 |
| Idarubicin hydrochloride | ; Idarubicina; Idamycin; Idarubicin hydrochloride;Idarubicin HCl; Idarubicin Hcl; 4 Desmethoxydaunorubici; IDARUBICIN HCl; 4 Demethoxydaunorubici; 4-Desmethoxydaunorubicin;Idarubicin; Idarubicin; Hydrochloride, Idarubici;Idarubicin?HCl | 0.345226667 | 0.278901 | 1.24 |
| Phenylmercuric acetate | Benzene-1,2,3,5-d4; Hexadeuterobenzene; Benzene-d6; Benzene-d5; | 0.303561667 | 0.24544067 | 1.24 |
| MNS | null | 4.555211 | 3.738585 | 1.22 |
| Carminomycin | null | 0.284941333 | 0.23588867 | 1.21 |
| Ethoxazene | null | 3.157481333 | 2.64530533 | 1.19 |
| Bortezomib | Bortezomib; Velcade; PS341 cpd; | 0.012995333 | 0.01101567 | 1.18 |
| CHM-1 hydrate |  | 0.482202333 | 0.42504233 | 1.13 |
| Oxyphenisatin acetate |  | 0.711715 | 0.642862 | 1.11 |
| lestaurtinib | null | 3.047497 | 2.826069 | 1.08 |
| Topotecan HCl | ; Hycamtamine; Topotecane; Nogitecan hydrochloride;Nogitecan hydrochloride/NSC-609699;Topotecan hydrochloride; Topotecan hydrochloride;TOPOTECAN HYDROCHLORIDE; Nogitecan Hydrochloride; Hycamtin | 0.287414333 | 0.26714467 | 1.08 |
| Amsacrine hydrochloride | null | 3.047497 | 2.94947333 | 1.03 |
| Podophyllotoxin | null | 0.061944667 | 0.064344 | 0.96 |
| Fludarabine |  | 0.857969333 | 0.905198 | 0.95 |
| Oxyphenisatin acetate | null | 1.017713667 | 1.157541 | 0.88 |
| Methyl Violet | CIPLUS\_774185; Methyl violet; | 2.283013333 | 2.60596133 | 0.88 |
| Canertinib |  | 1.24309 | 1.42808833 | 0.87 |
| Gemcitabine hydrochloride | Gemcitabine; ; Gemcitabina;Gemzar;Gemcitabine;Inno-D07001 | 0.93909 | 1.09170333 | 0.86 |
| Camptothecin | ;Camptothecin; Campotothecine;Camptothecine (S,+); Camptothecine (S,+); Camptothecine; Sodium Camptothecin; (S)-(+)-Camptothecin;(S)-(+)-Camptothecin; "Camptothecine (S,+)"; Na-CPT;CAMPTOTHECIN | 0.052809 | 0.06282667 | 0.84 |
| PTC-124 | Ataluren;PTC124 | 2.054383667 | 2.48570867 | 0.83 |
| Gentian Violet | ; Triple Dye; Basic Violet 3; ; Methyl violet 2B; Hexamethyl-p-rosaniline chloride (Gentian violet); Crystal Violet;GENTIAN VIOLET; Genapax; Methyl Violet 10B; Gentian violet; Crystal violet solution;Gentian violet | 0.890738 | 1.10155 | 0.81 |
| Carfilzomib | Carfilzomib;ONO-7057;PR-171; Carfilzomib;Carfilzomib (PR-171) | 0.358549667 | 0.44642667 | 0.80 |
| Emetine | ; ; Emetine;Emetine dihydrochloride; Emetine dihydrochloride; Emetine dihydrochloride hydrate;Emetine..2HCl; Hydrochloride, Emetin; Dihydrochloride, Emetin; Emetine Dihydrochloride;Emetine dihydrochloride hydrate; Amebicide;EMETINE | 1.434643 | 1.787748 | 0.80 |
| Amsacrine hydrochloride |  | 3.076838667 | 3.873511 | 0.79 |
| Vinblastine sulfate salt | null | 0.103745333 | 0.13124 | 0.79 |
| Carboquone | Carboquone; Esquinon; Carbazilquinone; Carboquona; | 0.263506 | 0.34834067 | 0.76 |
| Mycophenolic acid | ; ; Acido micofenolico; Acid, Mycophenoli; Mycophenolic acid;Mycophenolic acid;MYCOPHENOLIC ACID | 0.938376333 | 1.27254533 | 0.74 |
| 3,7-Bis(3,4-dimethoxyphenyl)tetrahydro-1H,5H-[1,2,4]triazolo[1,2-a][1,2,4]triazole-1,5-dithione | null | 0.011640333 | 0.015846 | 0.73 |
| Calcimycin | null | 0.487646 | 0.681509 | 0.72 |
| 1,10-Phenanthroline monohydrate | null | 2.178235333 | 3.224842 | 0.68 |
| Vandetanib | ;AZD-6474;Caprelsa;ZD-6474;Zactima;Vandetanib; Vandetanib;CH-331 | 2.3485 | 3.48340633 | 0.67 |
| (-)-Gossypol |  | 1.283832667 | 2.102361 | 0.61 |
| Artesunate | Artesunate (AS);Artesunate;ARTEMISININUM; Artesunate (AS) | 0.73446 | 1.306075 | 0.56 |
| Pralatrexate |  | 0.487406 | 0.929108 | 0.52 |
| Lovastatin | ; ; Lovastatin (Mevinolin); Monacolin K; Mevacor;LOVASTATIN; Mevinolin (lovastatin); 6-Methylcompactin; 6 Methylcompacti; Lovastatin;Lovastatin; 6-alpha-Methylcompactin; 6alpha-Methylcompactin | 0.637903667 | 1.22430933 | 0.52 |
| Raltitrexed | Raltitrexed | 0.619447333 | 1.21081167 | 0.51 |
| Niclosamide | Yomesan;Niclosamide; Niclosamida;NICOLSAMIDE; ; Bayluscide;NICLOSAMIDE; Niclocide; Phenasal; Fenasal; Niclosamid; Radewerm; Bayer 73; Bayer 2353; Niclosamide; Clonitralide; Niclosamidel; Devermine; Niclosamide-olamine; Tr??d??mine | 0.337623 | 0.70753867 | 0.48 |
| Omacetaxine mepesuccinate | Ceflatonin; ; Omacetaxine mepesuccinate;Homoharringtonine | 0.444780333 | 0.98175733 | 0.45 |
| chromomycin | null | 0.032809 | 0.07260833 | 0.45 |
| Niclosamide |  | 1.042268667 | 2.51873667 | 0.41 |
| Parbendazole | Parbendazole | 0.222593 | 0.61017033 | 0.36 |
| Mevastatin | null | 0.938611667 | 2.74223533 | 0.34 |
| THIRAM |  | 0.050903333 | 0.15081067 | 0.34 |
| Artemisinin | null | 1.155264667 | 3.603659 | 0.32 |
| Cerivastatin sodium | Cerivastatin sodium; CERIVASTATIN Na; Baycol; | 0.037697333 | 0.11822467 | 0.32 |
| G-Strophanthin | ;Strophantine octahydrate; Acocantherine;OUABAIN; Strophantine octahydrate; Ouabain octahydrate;Ouabain; Ouabain Octa; K, Acolongiflorosid; G Strophanthi; Acolongifloroside K | 0.103745333 | 0.34834067 | 0.30 |
| Pitavastatin calcium | ; ; Livalo;Itavastatin Ca; ITAVASTATIN Ca;NK-104; Pitavastatin calcium | 0.210236 | 0.71573967 | 0.29 |
| Tyrphostin A9 | null | 0.2644 | 0.908884 | 0.29 |
| AC-93253 iodide | null | 1.096343667 | 4.555211 | 0.24 |
| Docetaxel | Docetaxolum; Docetaxel Hydrate; ; AE-docetaxel;DOCETAXEL; 5 Docetaxel; Docetaxel hydrate; Docetaxel Anhydrous; Docetaxel Trihydrate; Docetaxel;Docetaxel; Taxoltere metro | 0.026339333 | 0.14818 | 0.18 |
| Ammonium pyrrolidinedithiocarbamate | null | 0.260596 | 1.50081267 | 0.17 |
| Rotenone | ;ROTENONE;Rotenone | 0.024857333 | 0.26129933 | 0.10 |
| Rotenone | ;ROTENONE;Rotenone | 0.016312 | 0.20535333 | 0.08 |
| Disulfiram | ;Tetraethylthiuram disulfide; Disulfiram; Tetraethylthiuram disulfide; Disulfiramo; Teturam; Antabuse;Disulfiram;DISULFIRAM | 0.067036 | 0.99372033 | 0.07 |
| Diphenyleneiodonium chloride | null | 0.064344 | 1.37437367 | 0.05 |
| Docetaxel | Docetaxolum; Docetaxel Hydrate; ; AE-docetaxel;DOCETAXEL; 5 Docetaxel; Docetaxel hydrate; Docetaxel Anhydrous; Docetaxel Trihydrate; Docetaxel;Docetaxel; Taxoltere metro | 0.004385333 | 0.159089 | 0.03 |