THE FASEB JOURNAL • RESEARCH • www.fasebj.org

IDH1-mutant cancer cells are sensitive to cisplatin and an IDH1-mutant inhibitor counteracts this sensitivity

Mohammed Khurshed,[*,†] Niels Aarnoudse,[*] Renske Hulsbos,[*] Vashendriya V. V. Hira,[*] Hanneke W. M. van Laarhoven,[*,†] Johanna W. Wilmink,[*,†] Remco J. Molenaar,[*,†] and Cornelis J. F. van Noorden[*,‡,1]

[*]Department of Medical Biology and [†]Department of Medical Oncology, Cancer Center Amsterdam, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and [‡]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia

ABSTRACT: Isocitrate dehydrogenase (*IDH1*)-1 is mutated in various types of human cancer, and the presence of this mutation is associated with improved responses to irradiation and chemotherapy in solid tumor cells. Mutated IDH1 ($IDH1^{MUT}$) enzymes consume NADPH to produce D-2-hydroxyglutarate (D-2HG) resulting in the decreased reducing power needed for detoxification of reactive oxygen species (ROS), for example. The objective of the current study was to investigate the mechanism behind the chemosensitivity of the widely used anticancer agent cisplatin in $IDH1^{MUT}$ cancer cells. Oxidative stress, DNA damage, and mitochondrial dysfunction caused by cisplatin treatment were monitored in $IDH1^{MUT}$ HCT116 colorectal cancer cells and U251 glioma cells. We found that exposure to cisplatin induced higher levels of ROS, DNA double-strand breaks (DSBs), and cell death in $IDH1^{MUT}$ cancer cells, as compared with *IDH1* wild-type ($IDH1^{WT}$) cells. Mechanistic investigations revealed that cisplatin treatment dose dependently reduced oxidative respiration in $IDH1^{MUT}$ cells, which was accompanied by disturbed mitochondrial proteostasis, indicative of impaired mitochondrial activity. These effects were abolished by the $IDH1^{MUT}$ inhibitor AGI-5198 and were restored by treatment with D-2HG. Thus, our study shows that altered oxidative stress responses and a vulnerable oxidative metabolism underlie the sensitivity of $IDH1^{MUT}$ cancer cells to cisplatin.—Khurshed, M., Aarnoudse, N., Hulsbos, R., Hira, V. V. V., van Laarhoven, H. W. M., Wilmink, J. W., Molenaar, R. J., van Noorden, C. J. F. IDH1-mutant cancer cells are sensitive to cisplatin and an IDH1-mutant inhibitor counteracts this sensitivity. FASEB J. 32, 6344–6352 (2018). www.fasebj.org

KEY WORDS: isocitrate dehydrogenase · chemosensitivity · *cis*-diamminedichloroplatinum · 2-hydroxyglutarate

Isocitrate dehydrogenases (IDHs) are homodimeric enzymes that catalyze the conversion of isocitrate to α-ketoglutarate (α-KG) with concomitant reduction of $NAD(P)^+$ to NAD(P)H in the cytoplasm and mitochondria (1). Recurring mutations in the $NADP^+$-dependent *IDH1/2* genes have been observed in substantial percentages of various cancer types, such as glioma (80%), acute myeloid leukemia (20%), cholangiocarcinoma (20%), melanomas (5–10%), and chondrosarcoma (60%) (1–3).

$IDH1^{MUT}$ leads to reprogramming of cellular metabolism, which is one of the hallmarks of cancer (4, 5). The hotspot mutations in *IDH1*, of which $IDH1^{R132H}$ is the most prevalent, cause loss of enzymatic wild-type IDH1 ($IDH1^{WT}$) function and lead to a neomorphic IDH1 activity that converts α-KG into the oncometabolite D-2-hydroxyglutarate (D-2HG) (6). D-2HG exerts its oncogenic effects *via* competitive inhibition of α-KG-dependent dioxygenases (7, 8), which are essential for epigenetic regulation of gene expression, including that of metabolic genes (9).

$IDH1^{WT}$ plays a significant role in the cellular control of oxidative damage through the production of NADPH, which is the most important substrate for generating reducing power such as reduced glutathione for detoxification of oxidants (10, 11). $IDH1^{MUT}$ is associated with a 38% lower total NADPH production capacity in clinical glioblastoma specimens (12). The altered redox responses result in improved responses to therapy in $IDH1^{MUT}$ cancers (1, 12–14). Patients with $IDH1^{MUT}$ tumors in particular respond

ABBREVIATIONS: α-KG, α-ketoglutarate; D-2HG, D-2-hydroxyglutarate; DSB, double-strand break; ETC, electron transport chain; γ-H2AX, γ-histone 2-AX; HCT, human colorectal carcinoma; IDH, isocitrate dehydrogenase; IR, irradiation; MTCO, mitochondrial cytochrome *c* oxidase; MUT, mutation; NAC, *N*-acetyl cysteine; OCR, oxygen consumption rate; oxphos, oxidative phosphorylation; ROS, reactive oxygen species; SDH, succinate dehydrogenase; WT, wild type

[1] Correspondence: Department of Medical Biology, Cancer Center Amsterdam, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. E-mail: c.j.vannoorden@amc.uva.nl

doi: 10.1096/fj.201800547R

0892-6638/18/0032-6344 © The Author(s)

favorably to conventional radiotherapy. We have shown that this response is mediated by D-2HG accumulation, which sensitizes cells to irradiation (IR), whereas inhibition of IDH1^{MUT} reduces oxidative stress in *IDH1*^{MUT} cells and thus protects cells against IR (15). Similarly, patients with *IDH1*^{MUT} cancers may respond well to cytotoxic treatment (6, 10, 16–18), including cisplatin [*cis*-diamminedichloroplatinum(II)] (13, 19). However, the mechanism that induces cisplatin sensitivity in *IDH1*^{MUT} cancers is still unknown.

Cisplatin is one of the most widely used chemotherapeutic agents; it induces intra- and interstrand crosslinks, leading to single-strand breaks and double-strand breaks (DSBs) in the DNA of replicating cells (20). Accumulation of unrepaired DNA lesions, particularly DSBs, can induce cell death. A major limitation of cisplatin is its cytotoxicity. Cisplatin exposure induces production of intracellular reactive oxygen species (ROS). Treatment with antioxidants ameliorates the cytotoxic effects of cisplatin, suggesting involvement of oxidative stress in its toxicity (21–23). ROS cause a massive oxidation of redox-sensitive proteins and lipids, leading to, in particular, mitochondrial damage and cell death through various signaling pathways (24). Mitochondria produce ATP by oxidative phosphorylation (oxphos) and are an important endogenous source of ROS. The formation of mitochondrial ROS is independent of cisplatin-induced DNA damage and is a consequence of impaired mitochondrial protein synthesis (25). The contribution of cisplatin-induced mitochondrial dysfunction to the overall cytotoxic effects of cisplatin varies among cell types and depends on redox status, mitochondrial DNA integrity, and bioenergetic function (25, 26).

We and others showed that *IDH1*^{MUT} cells are more dependent on mitochondrial oxphos, as compared to *IDH1*^{WT} cells (4, 27). In addition, we reported that cells of the patient-derived *IDH1*^{MUT} oligodendroglioma xenograft model E478 are packed with mitochondria, suggesting that *IDH1*^{MUT} gliomas revert to mitochondrial metabolism (28). Therefore, we hypothesized that *IDH1*^{MUT} tumors are more sensitive to cisplatin treatment than *IDH1*^{WT} tumors as a result of its role in the oxidative stress response. The first goal of the present study was to provide *in vitro* evidence that the increased sensitivity to therapy of *IDH1*^{MUT} cancer cells is related to increased oxidative stress after exposure to cisplatin. The second goal was to demonstrate that the metabolic phenotype of *IDH1*^{MUT} cells is a key modulator of sensitivity to cisplatin. Finally, we hypothesize that IDH1^{MUT} inhibitors reverse this metabolic stress response to cisplatin exposure, thus interfering with the survival-prolonging sensitivity of *IDH1*^{MUT} cancer cells.

MATERIALS AND METHODS

Cell culture

HCT116 *IDH1*^{MUT} knockin colon carcinoma cells (*IDH1*^{MUT} HCT116 cells), generated by the adeno-associated virus-targeting technology GENESIS (29), were kindly provided by Horizon Discovery (Cambridge, United Kingdom). U251 glioblastoma cells were stably transduced with lentiviral constructs encoding for IDH1^{WT} or IDH1^{R132H}, as described by Esmaeili *et al.* (30). HCT116 cells were cultured in McCoy's 5A medium (Thermo Fisher Scientific, Waltham, MA, USA) in 5% CO_2 at 37°C. U251 cells were cultured in 5% CO_2 at 37°C in complete DMEM (Thermo Fisher Scientific). All media were supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 100 U/ml penicillin, and 100 mg/ml streptomycin (both Thermo Fisher Scientific).

Reagents

The IDH1^{MUT} inhibitor AGI-5198 was purchased from Medchem Express (Monmouth Junction, NJ, USA), D-2HG, *N*-acetyl cysteine (NAC), carboplatin, oxaliplatin, oligomycin, antimycin A, rotenone, carbonyl-cyanide-(trifluoromethoxy) phenylhydrazone, L-glutamine, and sodium pyruvate were purchased from MilliporeSigma (Burlington, MA, USA); and cisplatin (Platosin) was purchased from Pharmachemie B.V. (Haarlem, The Netherlands).

Colony-forming assays after cisplatin treatment

Colony-forming assays were performed and analyzed after cisplatin treatment (31). Cells were treated before cisplatin exposure for 72 h with D-2HG or the ROS scavenger NAC, or 14 d with AGI-5198, or solvent only (DMSO, 0.5%). Cells were treated with cisplatin at 4 h after plating in the presence or absence of 0–1 μM AGI-5198, 0–10 mM D-2HG, or 0–5 μM NAC. Data are expressed as clonogenic fraction, which is the number of colonies divided by the number of cells plated, corrected for plating efficiency.

Measurement of oxygen consumption rate

HCT116 cells, with and without pretreatment with 1 μM AGI-5198, were grown in 5% CO_2 at 37°C, and the oxygen consumption rate (OCR) was measured with a Seahorse XFe96 analyzer (Agilent Technologies, Santa Clara, CA, USA) (4).

Cellular NADP$^+$, NADPH, and ROS measurements

Cells were incubated for 72 h in the presence or absence of 1 μM AGI-5198 and treated for 24 h with cisplatin. Cells were harvested, prepared, and analyzed for NADP$^+$:NADPH ratios and ROS levels with a colorimetric NADP$^+$:NADPH Ratio Detection Assay Kit (Abcam, Cambridge, MA, USA), and a fluorometric CellRox Deep Red ROS Detection Assay Kit (Thermo Fisher Scientific), respectively, with a POLARStar Galaxy microplate reader (BMG Labtech, Ortenberg, Germany), according to the manufacturers' protocols.

Western blot analysis

Cells were exposed to cisplatin (10 μM) or carboplatin (40 μM) for 24 h and for lysis, the mitochondrial enriched cell lysate RIPA was used (Thermo Fisher Scientific). Western blot analysis was performed with primary anti-succinate dehydrogenase (SDH)-A antibody (0.1 μg/ml; Thermo Fisher Scientific) or with primary anti-mitochondrial cytochrome *c* oxidase (MTCO)-1 antibody (1 μg/ml; Thermo Fisher Scientific) diluted in blocking solution (5% milk in Tris-buffered saline-Tween). For SDS-PAGE and Western blot analysis, 25 μg protein was boiled (for anti-MTCO-1, it was not boiled) with 6× SDS-PAGE loading buffer, and samples were separated on precast NU-PAGE 10% Bis-Tris minigels (Novex Innovations, Winston-Salem, NC, USA). IR anti-mouse (1:5000; Li-cor Biosciences, Lincoln, NE, USA) was used as the secondary antibody. Chemiluminescence was used to detect immunoreactive proteins, and protein abundance was quantified based on band intensities by Odyssey software (Li-Cor Biosciences).

Enzyme activity measurements

Cells were cultured and exposed to 10 μM cisplatin for 24 h and subsequently trypsinized and centrifuged onto microscopy slides (Shandon Cytospin 4 Cytocentrifuge; Thermo Fisher Scientific) at 20 relative centrifugal force for 5 min at room temperature. Cytospins were air-dried for 1 d and subsequently stained with metabolic mapping to visualize activity of SDH. Enzyme activity experiments were conducted and analyzed, as described previously (32, 33). We used nitrotetrazolium blue chloride (NBT; MilliporeSigma) in the enzyme reaction medium, and incubation with substrate and cofactors was performed at 37°C for 60 min of SDH activity. Control reactions were performed in the absence of substrate, but in the presence of cofactors, to assess nonspecific enzyme activity.

γ-Histone 2-AX immunofluorescence staining and quantifications

DNA DSBs were determined with immunofluorescence staining of γ-histone 2-AX(γH2AX) (1:100; MilliporeSigma). Cells were incubated in the presence or absence of 1 μM AGI-5198 and treated with cisplatin. The number of $\gamma H2AX^+$ foci per cell was quantified from deconvoluted stacks of photomicrographs using custom-made software, as previously described (34).

Statistical analysis

Data were processed and analyzed with Excel (Microsoft, Redmond, WA, USA) and visualized using Prism (GraphPad, La Jolla, CA, USA). Two-side Student's *t* tests were used with a significance level cutoff of $\alpha = 0.05$.

RESULTS

Oxidative stress mediates IDH1^{MUT} sensitivity to cisplatin

The effects of cisplatin on *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells were investigated by performing proliferation and colony-forming assays. Relative to *IDH1*^{WT} HCT116 cells, cisplatin caused a significant dose-dependent reduction of the surviving fraction of *IDH1*^{MUT} cells (**Fig. 1*A–D***), suggesting that *IDH1*^{MUT} sensitizes HCT116 cells to cisplatin. The reduced survival of *IDH1*^{MUT} cells was confirmed in a proliferation assay with a U251 glioblastoma cell line that stably overexpressed IDH1^{WT} or IDH1^{MUT} (Fig. 1*B*). We also evaluated the effects of carboplatin and oxaliplatin, both platinum-based cytotoxic agents that form types of DNA lesions similar to those formed by cisplatin, but have a different normal tissue toxicity profile (35, 36). The surviving fractions of *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells after 72 h exposure to carboplatin or oxaliplatin were similar, suggesting that *IDH1*^{MUT} sensitizes HCT116 cells specifically to cisplatin, but not to carboplatin or oxaliplatin (Fig. 1*E–G*).

Furthermore, we investigated whether chemosensitivity of *IDH1*^{MUT} cells is caused by increased vulnerability to oxidative stress, and we treated *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells and IDH1^{R132H}- and IDH1^{WT}-overexpressing U251 cells with the NADPH surrogate and ROS scavenger NAC. NAC equalized the surviving fractions of *IDH1*^{MUT} and *IDH1*^{WT} cells after treatment with cisplatin (Fig. 1*H, I*). This finding suggests that oxidative stress mediates the higher chemosensitivity to cisplatin of *IDH1*^{MUT} cells, as compared to *IDH1*^{WT} cells.

Cisplatin treatment decreases NADPH levels and increases ROS levels in *IDH1*^{MUT} cells, and IDH1^{MUT} inhibitor AGI-5198 attenuates these effects

We investigated the effects of *IDH1*^{MUT} on cellular NADPH and ROS levels, with or without pretreatment with cisplatin. *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells were continuously exposed to cisplatin at a half-maximum inhibitory dose of 25 μM, as determined during 24 h exposure, and ROS levels were measured after 1, 6, 12, and 24 h of exposure. Under steady-state conditions, *IDH1*^{MUT} HCT116 cells had $NADP^+$:NADPH ratios and ROS levels similar to those of *IDH1*^{WT} HCT116 cells, as determined by colorimetric and fluorometric assays and flow cytometry (**Fig. 2**). Across all cell lines, we observed a significantly increased $NADP^+$:NADPH ratio after 72 h of exposure to 25 μM cisplatin (Fig. 2*A*), and ROS levels were significantly increased after 12 and 24 h of cisplatin exposure (Fig. 2*B*). Notably, the increased $NADP^+$:NADPH ratio and ROS levels were higher in *IDH1*^{MUT} than in *IDH1*^{WT} HCT116 cells, and AGI-5198 attenuated this effect in *IDH1*^{MUT} HCT116 cells (Fig. 2*B*). These findings suggest that higher ROS levels in *IDH1*^{MUT} HCT116 cells after cisplatin treatment results in increased NADPH consumption, compared with that in *IDH1*^{WT} HCT116 cells.

IDH1^{MUT} increases the number of DNA DSBs, and the IDH1^{MUT} inhibitor AGI-5198 reverses the effect

The accumulation of DNA strand breaks, particularly DSBs, is an important mediator of cisplatin-induced cell death in replicating cells (20). Therefore, we investigated whether *IDH1*^{MUT} cells are sensitive to cisplatin because of their elevated ROS production and increased $NADP^+$:NADPH ratio, leading to an increased number of DNA DSBs after treatment. We observed more γ-H2AX foci in *IDH1*^{MUT} cells than in *IDH1*^{WT} HCT116 cells in steady-state conditions (**Fig. 3**). Furthermore, the number of γ-H2AX foci was higher in *IDH1*^{MUT} cells than in *IDH1*^{WT} HCT116 cells after treatment for 1 h with 5 and 10 μM cisplatin. To confirm the causal relationship between *IDH1*^{MUT} and increased levels of DNA damage, we pretreated *IDH1*^{MUT} cells with AGI-5198 before treatment with cisplatin, which reversed the number of γ-H2AX foci in *IDH1*^{MUT} cells to levels observed in *IDH1*^{WT} HCT116 cells (Fig. 3).

IDH1^{MUT} sensitizes cells to cisplatin causing increased levels of oxidative respiration

Besides increased $NADP^+$:NADPH levels upon treatment with cisplatin, the distinct metabolic phenotype of *IDH1*^{MUT} cancer cells may sensitize the cells to cisplatin-induced toxic effects (25, 26). We previously determined that

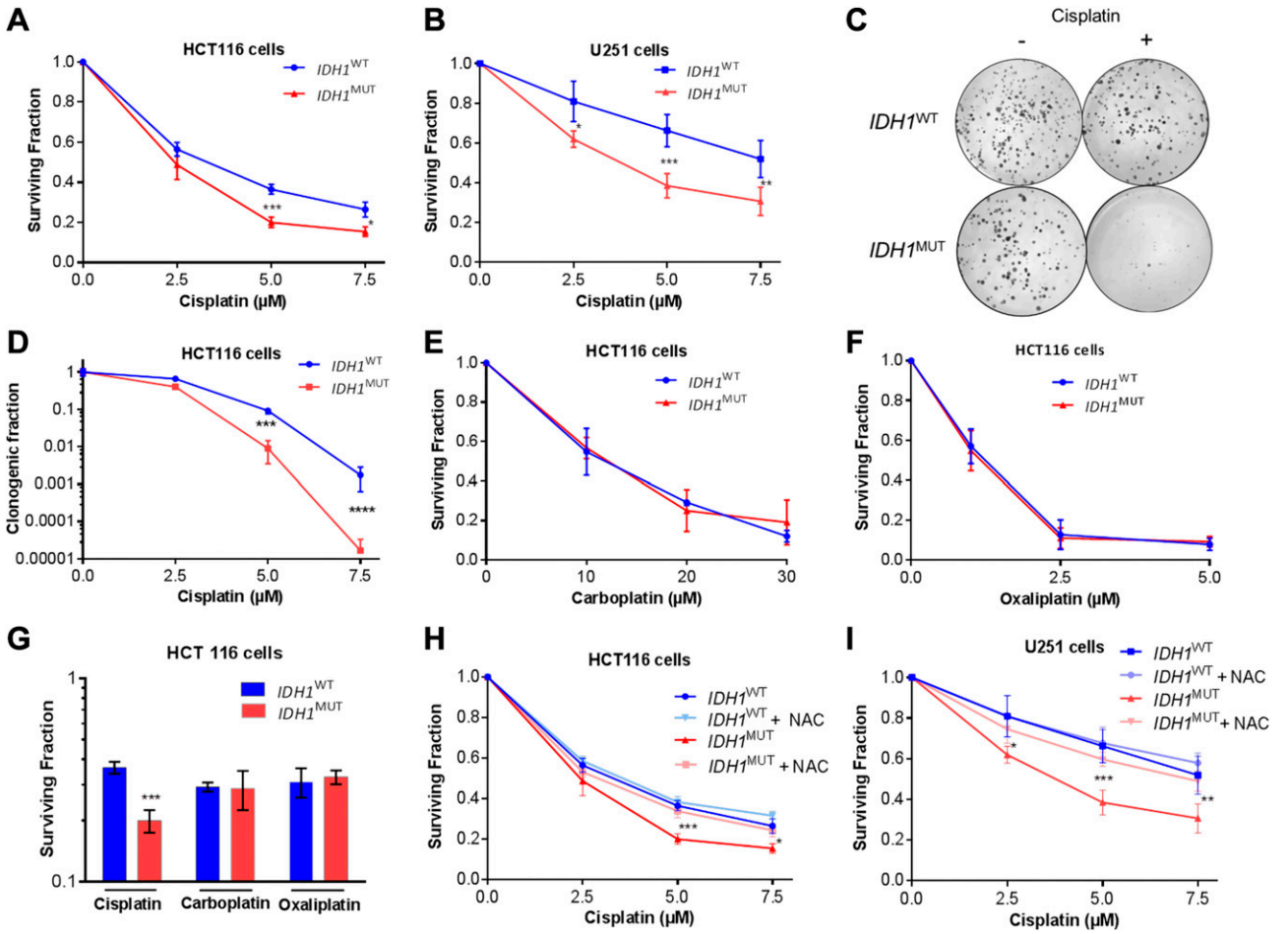

Figure 1. *IDH1*^{MUT} sensitizes cells to cisplatin. *A*) Proliferation assay using *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells. *B*) U251 cell lines transduced with lentiviral vectors harboring the *IDH1*^{WT} and *IDH1*^{MUT} genes. Cells were counted after 72 h of cisplatin exposure, and the number of cells was normalized to the number of cells without treatment. *C*, *D*) Colony-forming assay after 72 h of cisplatin exposure of *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells. The clonogenic fraction is the number of colonies divided by the number of cells plated, corrected for the plating efficiency. The scale on the *y* axis is logarithmic. *E*–*G*) As in *A*, but after 72 h exposure of *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells to carboplatin and oxaliplatin. *H*, *I*) As in *A* and *B*, but in the presence or absence of 5 μM of ROS-scavenging NAC. Significance values were obtained using 1-way ANOVA with the Tukey correction for multiple comparisons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

IDH1^{MUT} HCT116 cells are more dependent on oxphos than are *IDH1*^{WT} HCT116 cells (4). Because cisplatin, but not carboplatin, strongly impairs oxphos and mitochondrial function (37), we investigated whether, besides the reduced NADPH production capacity, the increased sensitivity of *IDH1*^{MUT} cells to cisplatin is caused by this metabolic phenotype. To investigate metabolic vulnerability, OCRs were determined using the Seahorse XFe96 respirometer in *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells in the presence or absence of cisplatin. In the absence of cisplatin, the OCR of *IDH1*^{MUT} cells was ~2-fold higher than that of *IDH1*^{WT} HCT116 cells. Exposure to cisplatin led to a dose-dependent reduction of oxygen consumption in *IDH1*^{MUT} cells, whereas *IDH1*^{WT} cells were not affected (**Fig. 4*A***). Our findings indicate that cisplatin-induced increases in ROS levels are caused by mitochondrial DNA damage. The cisplatin-induced mitochondrial DNA adducts may interfere with mitochondrial DNA transcription, resulting in reduced mitochondrial protein synthesis. Reduced expression of mitochondrial DNA–encoded components of the electron transport chain (ETC) impairs respiration and subsequently leads to ROS generation. The time course of the increase in ROS levels is consistent with such a mechanism. We next determined mitochondrial proteostasis or mitonuclear protein imbalance (38), by analyzing the ratio between a mitochondrial DNA-encoded oxphos subunit (cytochrome *c* oxidase subunit I or MTCO1) and a nuclear DNA-encoded oxphos subunit (SDH-A). Indeed, 24 h treatment with 10 μM cisplatin induced a significant mitonuclear protein imbalance in *IDH1*^{MUT} HCT116 cells (Fig. 4*B*, *C*). Treatment with another platinum-based drug, carboplatin, did not induce mitonuclear protein imbalance. The mitonuclear protein imbalance after treatment with cisplatin was accompanied by a decreased cellular respiration of *IDH1*^{MUT} HCT116 cells, indicative of impaired mitochondrial activity. In an attempt to demonstrate the induced mitonuclear protein imbalance in a functional assay, we performed a histochemical enzyme activity assay to interrogate the 0-order activity of SDH. This

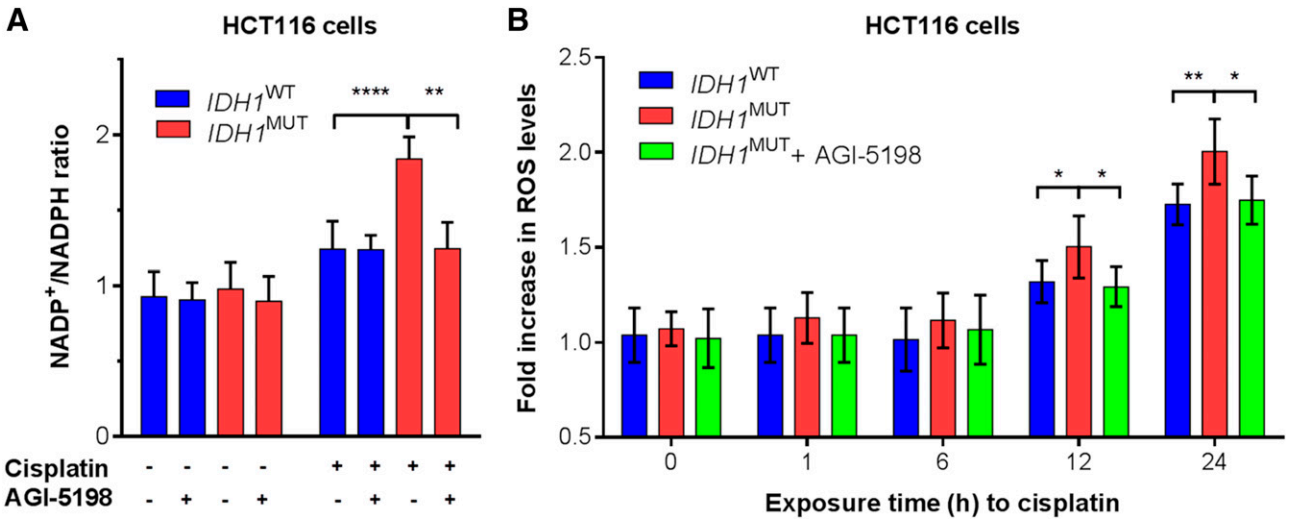

Figure 2. Cisplatin exposure of *IDH1*^{MUT} HCT116 cells decrease NADPH levels and increase ROS levels, and AGI-5198 attenuates these effects. *A*) Cells were incubated in the presence or absence of 1 μM AGI-5198, treated with cisplatin (5 μM), and harvested, prepared, and colorimetrically analyzed for NADP$^+$:NADPH ratios after 72 h. *B*) As in *A*, but cells were treated with cisplatin (25 μM) and analyzed with a fluorometric assay for ROS levels at different time points. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

reaction measures and translates the activity of SDH to reduction of flavin adenine dinucleotide (FAD) to FADH (dark *vs.* light or no staining) and is (partly) dependent on the inhibition of cytochrome oxidase by sodium azide to obtain a maximum dark *vs.* light staining. We performed the assay in the presence or absence of sodium azide on *IDH1*^{MUT} HCT116 cells that were treated or untreated with 10 μM cisplatin to demonstrate cisplatin-induced inactivation of ECT. The assay showed, in the absence of sodium azide, decreased formazan production by SDH. The leakage of electrons *via* the ETC was inhibited by sodium azide (Fig. 4*D*). Treatment with cisplatin increased the FADH production capacity of SDH, suggesting that cisplatin treatment is responsible for inhibition of cytochrome *c* oxidase (Fig. 4*D, E*). These findings suggest that the metabolic phenotype of *IDH1*^{MUT} HCT116 cells (*i.e.*, high oxidative respiration), makes the cells more sensitive to cisplatin treatment *via* inhibition of mitochondrial DNA transcription, which leads to a subsequent reduction in protein synthesis and impaired ETC function.

IDH1^{MUT} inhibitor protects and D-2HG sensitizes *IDH1*^{MUT} cells to cisplatin

The decreased NADPH production capacity and increased metabolic vulnerability of *IDH1*^{MUT} cells is associated with sensitization to cisplatin. Therefore, we investigated whether the lDH1^{MUT} inhibitor AGI-5198 protects *IDH1*^{MUT} cells by restoring NADPH production capacity and altering the metabolic phenotype of *IDH1*^{MUT} cells. We exposed *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells to 1 μM AGI-5198 for 14 d before cisplatin exposure. AGI-5198 did not affect sensitivity of *IDH1*^{WT} HCT116 cells, but reduced sensitivity of *IDH1*^{MUT} HCT116 cells to cisplatin in a manner comparable to that of *IDH1*^{WT} HCT116 cells (**Fig. 5*A–C***). These data show that AGI-5198 blocks IDH1^{MUT}-induced sensitivity to cisplatin. D-2HG is also known to induce oxidative stress in glia and neurons (39). Therefore, we argued that D-2HG is partly responsible for the sensitization of *IDH1*^{MUT} HCT116 cells to cisplatin. We exposed *IDH1*^{MUT} and *IDH1*^{WT} HCT116 cells to D-2HG before cisplatin treatment.

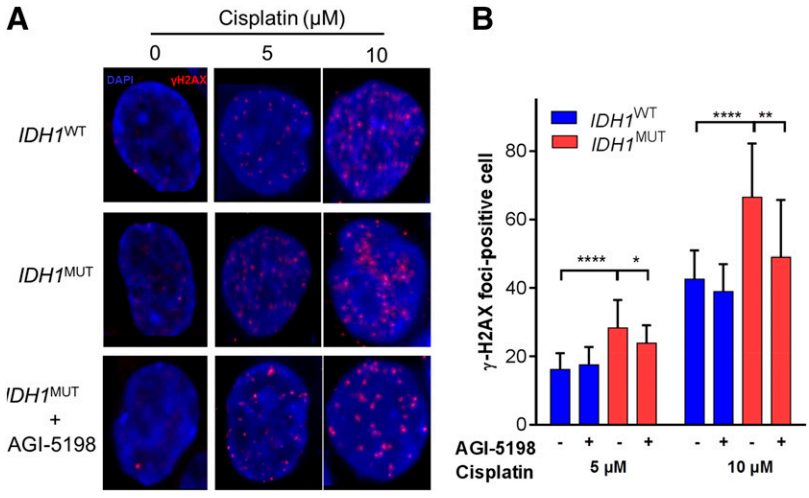

Figure 3. Cisplatin exposure of *IDH1*^{MUT} HCT116 cells increases numbers of DNA DSBs and AGI-5198 reverses this effect. *A*) Representative photomicrographs of cells that were plated on glass coverslips in the presence or absence of 1 μM AGI-5198, treated with 5 or 10 μM cisplatin for 1 h, and fixed after 30 min. γ-H2AX was stained immunocytochemically (red) to demonstrate DNA DSBs and with DAPI (blue) to demonstrate DNA nucleus content. *B*) Plots of γ-H2AX$^+$ foci per cell after cisplatin (5 or 10 μM for 1 h) exposure of *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells after long-term (14 d) incubation in the presence or absence of 1 μM AGI-5198. Plots are visualized with 95% confidence intervals. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

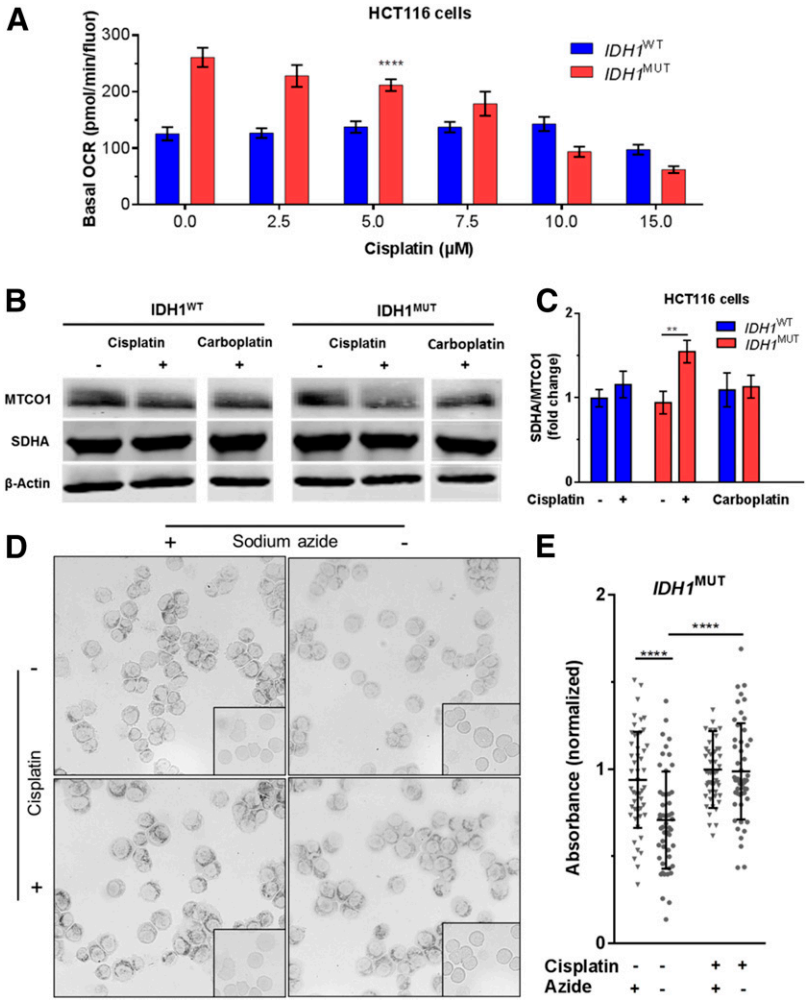

Figure 4. *IDH1*^{MUT} HCT116 cells with high mitochondrial respiration are more vulnerable to cisplatin. *A*) Cisplatin treatment in *IDH1*^{MUT} HCT116 cells dose dependently decreased the OCR, as determined by respirometry. The basal OCR response of HCT116 cells is shown in the presence or absence of cisplatin (24 h). *B*) Western blot of MTCO1 and nuclear DNA–encoded SDH-A expression in HCT116 cells exposed to cisplatin or carboplatin (10 and 40 μM, respectively) for 24 h. *C*) Cisplatin treatment induces mitonuclear imbalance in *IDH1*^{MUT} cells, as shown by the increased ratio of SDH-A and MTCO1. *D*) Representative monochromatic light photomicrographs of *IDH1*^{MUT} HCT116 cells treated with cisplatin (10 μM for 24 h) or left untreated after staining for SDH activity against 50 mM succinate and in the presence or absence of 5 mM sodium azide. This reaction measures and translates the activity of SDH to reduction of FAD to FADH (dark *vs.* light or no staining) and is dependent on the inhibition of cytochrome oxidase by sodium azide to obtain a maximum dark *vs.* light staining. Insets: control metabolic mapping staining patterns in the absence of substrate. *E*) Quantification of the absorbance of blue formazan produced by SDH activity per cell using monochromatic light and image analysis. One representative experiment of 3 is shown, each data point represents means ± SEM. $^{**}P < 0.01$, $^{****}P < 0.0001$.

D-2HG significantly decreased the clonogenic fractions of *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells after cisplatin treatment (Fig. 5C; *i.e.*, D-2HG sensitized HCT116 cells to cisplatin). Of note, AGI-5198 was unable to protect *IDH1*^{MUT} or *IDH1*^{WT} HCT116 cells against cisplatin in the presence of D-2HG; the protective mechanism of AGI-5198 on *IDH1*^{MUT} HCT116 cells therefore depends predominantly on the inhibition of IDH1^{MUT}-mediated D-2HG production.

DISCUSSION

Since the discovery of *IDH1*^{MUT}, it has been known that patients with glioma carrying the *IDH1*^{MUT} have prolonged overall survival compared with their *IDH1*^{WT} counterparts (12, 18, 40). Since then, whether the prolonged survival is merely an association or is attributable to a causative mechanism (1) has been debated. Prospective clinical trials showed that *IDH1*^{MUT} tumors are more sensitive to chemotherapy and radiotherapy than *IDH1*^{WT} tumors (16, 17) and this suggests that *IDH1*^{MUT} predicts the chemo-irradiation response in glioma. For intrahepatic cholangiocarcinoma, there are conflicting data with respect to the prognostic impact of *IDH1*^{MUT} (18, 41). Therefore, the discovery that *IDH1*^{MUT} also sensitizes cancer cells to cisplatin may address this conundrum. On the basis of cisplatin sensitivity, *IDH1*^{MUT} may contribute to a possible prolonged survival of patients with intrahepatic cholangiocarcinoma bearing *IDH1*^{MUT}. *Post hoc* molecular analyses of *IDH1*^{MUT} status in randomized clinical trials of cisplatin treatment in patients with cholangiocarcinoma (42) may provide helpful insights.

We showed that, besides the reduced NADPH production capacity in *IDH1*^{MUT} cancer cells, the oxidative metabolic phenotype is involved in cisplatin sensitivity. We demonstrated with the use of NAC and the AGI-5198 inhibitor that cisplatin's effects were abolished. Increased sensitivity of *IDH1*^{MUT} cells to various cytotoxic agents have been described, including 5-fluorouracil, busulfan, carmustine, daunorubicin, lomustine, temozolomide, and cisplatin (13–15). Whether *IDH1*^{MUT} sensitizes cancer cells to all cytotoxic agents by the same mechanism remains to be established. Cisplatin binds mitochondrial DNA as efficiently as nuclear DNA and accumulates in mitochondria forming adducts with mitochondrial DNA and proteins (43, 44). A difference between cisplatin-induced mitochondrial DNA damage and nuclear DNA damage is that mitochondrial DNA damage is unlikely to be repaired, as mitochondria lack nucleotide excision repair (43).

Cisplatin-induced mitochondrial injury and energy imbalance have been reported (45), and targeted delivery of antioxidants to mitochondria has been shown to reduce

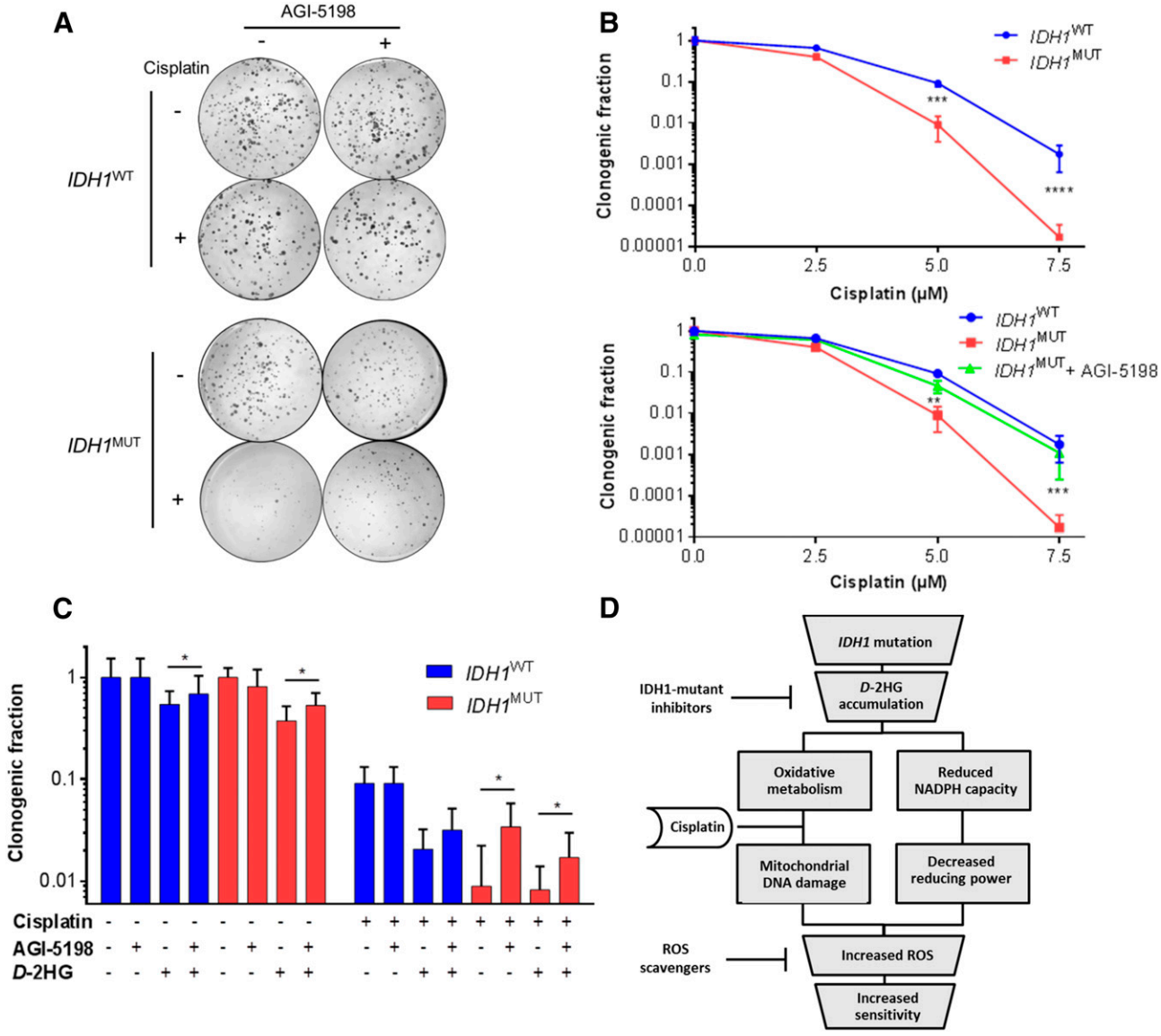

Figure 5. *IDH1*^{MUT} inhibitor AGI-5198 dose dependently protects *IDH1*^{MUT} HCT116 cells against cisplatin exposure. *A*) Colony-forming assay after cisplatin (5 μM) exposure of *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells after long-term (14 d) incubation in the presence or absence of 1 μM AGI-5198. Cells treated with cisplatin were plated in 5-fold higher numbers than untreated cells. *B*) As in *A*, normalized to the clonogenic fraction of untreated *IDH1*^{WT} HCT116 cells. *C*) Colony-forming assay after 72 h of cisplatin exposure with *IDH1*^{WT} and *IDH1*^{MUT} HCT116 cells after long-term (14 d) incubation in the presence or absence of 1 μM AGI-5198 or 4 h incubation in the presence or absence of 10 mM D-2HG, or incubation with both. The scales of the *y* axes in *B* and *C* are logarithmic. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. *D*) *IDH1*^{MUT} sensitized cancer cells against cisplatin by 2 major components: *1*) the reduced NADPH production capacity in *IDH1*^{MUT} cancer cells resulting in decreased reducing power; and *2*) the metabolic phenotype of *IDH1*^{MUT} cancer cells. Cellular exposure to cisplatin causes direct damage to mitochondrial DNA resulting in impairment of the mitochondrial function and subsequently increases intracellular ROS levels in *IDH1*^{MUT} cells. Inhibition of *IDH1*^{MUT} by AGI-5198 disrupts this sensitivity process, which enhances the capacity of *IDH1*^{MUT} cells to reduce oxidative stress and protects them against cisplatin.

the onset of cisplatin-induced renal cell damage (46). The ETC is a significant contributor of cellular oxidative stress, and it has been demonstrated that when cells are more dependent on respiration, cisplatin cytotoxicity is increased (47). Suppression of oxygen consumption by cisplatin has been reported in eukaryotic cells and alterations in oxidative metabolism are a result of uncoupling of respiration (37). This is in agreement with the fact that mitochondrial inhibitors such as metformin depend on oxidative stress to induce cell death (48). We previously showed that *IDH*^{MUT} cancer cells are vulnerable to inhibition of the oxidative metabolism with inhibitors of the ETC, such as the biguanides metformin and phenformin (15). In line with our observations, another study has shown that among the platinum-based cytotoxic agents, specifically cisplatin reduces the expression of mitochondrial DNA–encoded genes and protein levels (25). The difference in potential to induce mitochondrial damage and to generate ROS may be an explanation for the clinical activity and toxicity of carboplatin compared with that of cisplatin.

The findings reported here have therapeutic implications, but there are limitations of our work that warrant

further study. For example, we did not study cisplatin sensitivity of IDH1^{MUT} *in vivo*. Shi *et al*. (13) demonstrated that IDH1^{R132H} overexpression in glioma cell lines treated with cisplatin affected the potential to initiate tumors in nude mice, and this effect was rescued by antioxidant treatment. Demonstration of the *in vivo* sensitivity of *IDH*^{MUT} cancer cells to cisplatin is promising for the translation of our findings into the clinic, in particular in patients with intrahepatic cholangiocarcinoma who receive palliative treatment with the chemotherapeutic combination of cisplatin and gemcitabine as a standard of care. Therefore, we envisage a future study of mouse models of intrahepatic cholangiocarcinoma for *in vivo* validation.

We used HCT116 colorectal carcinoma cells as an *in vitro* model. Although *IDH1*^{MUT} is not as prevalent in colorectal carcinoma as in glioma or cholangiocarcinoma, it occurs in 0.5% of patients (49). The production capacity of D-2HG is 100-fold higher in *IDH1*^{MUT} cells than in *IDH1*^{WT} HCT116 cells (19, 27) and because *IDH1*^{MUT} functions as a heterodimer with *IDH1*^{WT}, *IDH1*^{MUT} HCT116 cells are a more relevant model than the IDH1^{MUT} overexpression models that are frequently used.

In summary, cisplatin-induced sensitivity in *IDH1*^{MUT} cancer cells is mediated by at least 2 major components causing cell death: the redox status and the metabolic activity of *IDH1*^{MUT} cancer cells. Altered oxidative stress responses due to vulnerable metabolism are a plausible mechanism for understanding the sensitivity of *IDH1*^{MUT} cancer cells to cisplatin exposure. Our study may have clinical implications and our results imply that administration of IDH1^{MUT} inhibitors to patients with *IDH1*^{MUT} cancer abolishes the therapeutic effect of cisplatin. Our *in vitro* results suggest that concomitant administration of IDH1^{MUT} inhibitors and cisplatin may result in an unfavorable clinical outcome. FJ

ACKNOWLEDGMENTS

This work was supported by Dutch Cancer Society Grants KWF-UVA 2014-6839 (to M.K., V.V.V.H., R.J.M., and C.J.F.V.N.) and AMC2016.1-10460 (to M.K., R.J.M., J.W.W., and C.J.F.V.N.). The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

M. Khurshed, R. J. Molenaar, and C. J. F. Van Noorden designed the research; M. Khurshed, N. Aarnoudse, R. Hulsbos, and V. V. V. Hira performed the research; M. Khurshed and N. Aarnoudse analyzed the data; H. W. M. van Laarhoven, J. W. Wilmink, R. J. Molenaar, and C. J. F. Van Noorden supervised the research; and M. Khurshed and C. J. F. Van Noorden wrote the paper, all authors read and approved the paper.

REFERENCES

1. Molenaar, R. J., Radivoyevitch, T., Maciejewski, J. P., van Noorden, C. J., and Bleeker, F. E. (2014) The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation. *Biochim. Biophys. Acta* **1846**, 326–341
2. Jiao, Y., Pawlik, T. M., Anders, R. A., Selaru, F. M., Streppel, M. M., Lucas, D. J., Niknafs, N., Guthrie, V. B., Maitra, A., Argani, P., Offerhaus, G. J. A., Roa, J. C., Roberts, L. R., Gores, G. J., Popescu, I., Alexandrescu, S. T., Dima, S., Fassan, M., Simbolo, M., Mafficini, A., Capelli, P., Lawlor, R. T., Ruzzenente, A., Guglielmi, A., Tortora, G., de Braud, F., Scarpa, A., Jarnagin, W., Klimstra, D., Karchin, R., Velculescu, V. E., Hruban, R. H., Vogelstein, B., Kinzler, K. W., Papadopoulos, N., and Wood, L. D. (2013) Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. *Nat. Genet.* **45**, 1470–1473
3. Amary, M. F., Bacsi, K., Maggiani, F., Damato, S., Halai, D., Berisha, F., Pollock, R., O'Donnell, P., Grigoriadis, A., Diss, T., Eskandarpour, M., Presneau, N., Hogendoorn, P. C., Futreal, A., Tirabosco, R., and Flanagan, A. M. (2011) IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. *J. Pathol.* **224**, 334–343
4. Khurshed, M., Molenaar, R. J., Lenting, K., Leenders, W. P., and van Noorden, C. J. F. (2017) In silico gene expression analysis reveals glycolysis and acetate anaplerosis in IDH1 wild-type glioma and lactate and glutamate anaplerosis in IDH1-mutated glioma. *Oncotarget* **8**, 49165–49177
5. Van Lith, S. A., Navis, A. C., Verrijp, K., Niclou, S. P., Bjerkvig, R., Wesseling, P., Tops, B., Molenaar, R., van Noorden, C. J., and Leenders, W. P. (2014) Glutamate as chemotactic fuel for diffuse glioma cells: are they glutamate suckers? *Biochim. Biophys. Acta* **1846**, 66–74
6. Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B. A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G. J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler, K. W., Velculescu, V. E., Vogelstein, B., and Bigner, D. D. (2009) IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* **360**, 765–773
7. Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S. H., Ito, S., Yang, C., Wang, P., Xiao, M. T., Liu, L. X., Jiang, W. Q., Liu, J., Zhang, J. Y., Wang, B., Frye, S., Zhang, Y., Xu, Y. H., Lei, Q. Y., Guan, K. L., Zhao, S. M., and Xiong, Y. (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17–30
8. Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Ward, P. S., Yen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., and Su, S. M. (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**, 739–744
9. Chowdhury, R., Yeoh, K. K., Tian, Y. M., Hillringhaus, L., Bagg, E. A., Rose, N. R., Leung, I. K., Li, X. S., Woon, E. C., Yang, M., McDonough, M. A., King, O. N., Clifton, I. J., Klose, R. J., Claridge, T. D., Ratcliffe, P. J., Schofield, C. J., and Kawamura, A. (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep.* **12**, 463–469
10. Tran, A. N., Lai, A., Li, S., Pope, W. B., Teixeira, S., Harris, R. J., Woodworth, D. C., Nghiemphu, P. L., Cloughesy, T. F., and Ellingson, B. M. (2014) Increased sensitivity to radiochemotherapy in IDH1 mutant glioblastoma as demonstrated by serial quantitative MR volumetry. *Neuro-oncol.* **16**, 414–420
11. Bleeker, F. E., Molenaar, R. J., and Leenstra, S. (2012) Recent advances in the molecular understanding of glioblastoma. *J. Neurooncol.* **108**, 11–27
12. Bleeker, F. E., Atai, N. A., Lamba, S., Jonker, A., Rijkeboer, D., Bosch, K. S., Tigchelaar, W., Troost, D., Vandertop, W. P., Bardelli, A., and Van Noorden, C. J. (2010) The prognostic IDH1(R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. *Acta Neuropathol.* **119**, 487–494
13. Shi, J., Sun, B., Shi, W., Zuo, H., Cui, D., Ni, L., and Chen, J. (2015) Decreasing GSH and increasing ROS in chemosensitivity gliomas with IDH1 mutation. *Tumour Biol.* **36**, 655–662
14. Mohrenz, I. V., Antonietti, P., Pusch, S., Capper, D., Balss, J., Voigt, S., Weissert, S., Mukrowsky, A., Frank, J., Senft, C., Seifert, V., von Deimling, A., and Kögel, D. (2013) Isocitrate dehydrogenase 1 mutant R132H sensitizes glioma cells to BCNU-induced oxidative stress and cell death. *Apoptosis* **18**, 1416–1425
15. Molenaar, R. J., Botman, D., Smits, M. A., Hira, V. V., van Lith, S. A., Stap, J., Henneman, P., Khurshed, M., Lenting, K., Mul, A. N., Dimitrakopoulou, D., van Drunen, C. M., Hoebe, R. A., Radivoyevitch, T., Wilmink, J. W., Maciejewski, J. P., Vandertop, W. P., Leenders, W. P., Bleeker, F. E., and van Noorden, C. J. (2015) Radioprotection of IDH1-mutated cancer cells by the IDH1-mutant inhibitor AGI-5198. *Cancer Res.* **75**, 4790–4802
16. Cairncross, J. G., Wang, M., Jenkins, R. B., Shaw, E. G., Giannini, C., Brachman, D. G., Buckner, J. C., Fink, K. L., Souhami, L., Laperriere,

N. J., Huse, J. T., Mehta, M. P., and Curran, W. J., Jr. (2014) Benefit from procarbazine, lomustine, and vincristine in oligodendroglial tumors is associated with mutation of IDH. *J. Clin. Oncol.* **32**, 783–790
17. Buckner, J. C., Shaw, E. G., Pugh, S. L., Chakravarti, A., Gilbert, M. R., Barger, G. R., Coons, S., Ricci, P., Bullard, D., Brown, P. D., Stelzer, K., Brachman, D., Suh, J. H., Schultz, C. J., Bahary, J. P., Fisher, B. J., Kim, H., Murtha, A. D., Bell, E. H., Won, M., Mehta, M. P., and Curran, W. J., Jr. (2016) Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. *N. Engl. J. Med.* **374**, 1344–1355
18. Wang, P., Dong, Q., Zhang, C., Kuan, P. F., Liu, Y., Jeck, W. R., Andersen, J. B., Jiang, W., Savich, G. L., Tan, T. X., Auman, J. T., Hoskins, J. M., Misher, A. D., Moser, C. D., Yourstone, S. M., Kim, J. W., Cibulskis, K., Getz, G., Hunt, H. V., Thorgeirsson, S. S., Roberts, L. R., Ye, D., Guan, K. L., Xiong, Y., Qin, L. X., and Chiang, D. Y. (2013) Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* **32**, 3091–3100
19. Sulkowski, P. L., Corso, C. D., Robinson, N. D., Scanlon, S. E., Purshouse, K. R., Bai, H., Liu, Y., Sundaram, R. K., Hegan, D. C., Fons, N. R., Breuer, G. A., Song, Y., Mishra-Gorur, K., De Feyter, H. M., de Graaf, R. A., Surovtseva, Y. V., Kachman, M., Halene, S., Günel, M., Glazer, P. M., and Bindra, R. S. (2017) 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. *Sci. Transl. Med.* **9**, aal2463
20. Poklar, N., Pilch, D. S., Lippard, S. J., Redding, E. A., Dunham, S. U., and Breslauer, K. J. (1996) Influence of cisplatin intrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex. *Proc. Natl. Acad. Sci. USA* **93**, 7606–7611
21. Santos, N. A., Catão, C. S., Martins, N. M., Curti, C., Bianchi, M. L., and Santos, A. C. (2007) Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Arch. Toxicol.* **81**, 495–504
22. Dehne, N., Lautermann, J., Petrat, F., Rauen, U., and de Groot, H. (2001) Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. *Toxicol. Appl. Pharmacol.* **174**, 27–34
23. Jiang, Y., Guo, C., Vasko, M. R., and Kelley, M. R. (2008) Implications of apurinic/apyrimidinic endonuclease in reactive oxygen signaling response after cisplatin treatment of dorsal root ganglion neurons. *Cancer Res.* **68**, 6425–6434
24. Juan, M. E., Wenzel, U., Daniel, H., and Planas, J. M. (2008) Resveratrol induces apoptosis through ROS-dependent mitochondria pathway in HT-29 human colorectal carcinoma cells. *J. Agric. Food Chem.* **56**, 4813–4818
25. Marullo, R., Werner, E., Degtyareva, N., Moore, B., Altavilla, G., Ramalingam, S. S., and Doetsch, P. W. (2013) Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. *PLoS One* **8**, e81162
26. Inapurapu, S. P., Kudle, K. R., Bodiga, S., and Bodiga, V. L. (2017) Cisplatin cytotoxicity is dependent on mitochondrial respiration in *Saccharomyces cerevisiae*. *Iran. J. Basic Med. Sci.* **20**, 83–89
27. Grassian, A. R., Parker, S. J., Davidson, S. M., Divakaruni, A. S., Green, C. R., Zhang, X., Slocum, K. L., Pu, M., Lin, F., Vickers, C., Joud-Caldwell, C., Chung, F., Yin, H., Handly, E. D., Straub, C., Growney, J. D., Vander Heiden, M. G., Murphy, A. N., Pagliarini, R., and Metallo, C. M. (2014) IDH1 mutations alter citric acid cycle metabolism and increase dependence on oxidative mitochondrial metabolism. *Cancer Res.* **74**, 3317–3331
28. Navis, A. C., Niclou, S. P., Fack, F., Stieber, D., van Lith, S., Verrijp, K., Wright, A., Stauber, J., Tops, B., Otte-Holler, I., Wevers, R. A., van Rooij, A., Pusch, S., von Deimling, A., Tigchelaar, W., van Noorden, C. J., Wesseling, P., and Leenders, W. P. (2013) Increased mitochondrial activity in a novel IDH1-R132H mutant human oligodendroglioma xenograft model: in situ detection of 2-HG and α-KG. *Acta Neuropathol. Commun.* **1**, 18
29. Fernandez, S. L., Russell, D. W., and Hurlin, P. J. (2007) Development of human gene reporter cell lines using rAAV mediated homologous recombination. *Biol. Proced. Online* **9**, 84–90
30. Esmaeili, M., Hamans, B. C., Navis, A. C., van Horssen, R., Bathen, T. F., Gribbestad, I. S., Leenders, W. P., and Heerschap, A. (2014) IDH1 R132H mutation generates a distinct phospholipid metabolite profile in glioma. *Cancer Res.* **74**, 4898–4907
31. Franken, N. A., Rodermond, H. M., Stap, J., Haveman, J., and van Bree, C. (2006) Clonogenic assay of cells in vitro. *Nat. Protoc.* **1**, 2315–2319
32. Chieco, P., Jonker, A., De Boer, B. A., Ruijter, J. M., and Van Noorden, C. J. (2013) Image cytometry: protocols for 2D and 3D quantification in microscopic images. *Prog. Histochem. Cytochem.* **47**, 211–333
33. Molenaar, R. J., Khurshed, M., Hira, V. V., and Van Noorden, C. J. (2017) Metabolic mapping: quantitative enzyme cytochemistry and histochemistry to determine the activity of dehydrogenases in cells and tissues. *J. Vis. Exp.* 56843
34. Aten, J. A., Stap, J., Krawczyk, P. M., van Oven, C. H., Hoebe, R. A., Essers, J., and Kanaar, R. (2004) Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* **303**, 92–95
35. Go, R. S., and Adjei, A. A. (1999) Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J. Clin. Oncol.* **17**, 409–422
36. Ndagi, U., Mhlongo, N., and Soliman, M. E. (2017) Metal complexes in cancer therapy - an update from drug design perspective. *Drug Des. Devel. Ther.* **11**, 599–616
37. Garrido, N., Pérez-Martos, A., Faro, M., Lou-Bonafonte, J. M., Fernández-Silva, P., López-Pérez, M. J., Montoya, J., and Enríquez, J. A. (2008) Cisplatin-mediated impairment of mitochondrial DNA metabolism inversely correlates with glutathione levels. *Biochem. J.* **414**, 93–102
38. Houtkooper, R. H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., Williams, R. W., and Auwerx, J. (2013) Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* **497**, 451–457
39. Gilbert, M. R., Liu, Y., Neltner, J., Pu, H., Morris, A., Sunkara, M., Pittman, T., Kyprianou, N., and Horbinski, C. (2014) Autophagy and oxidative stress in gliomas with IDH1 mutations. *Acta Neuropathol.* **127**, 221–233
40. Parsons, D. W., Jones, S., Zhang, X., Lin, J. C., Leary, R. J., Angenendt, P., Mankoo, P., Carter, H., Siu, I. M., Gallia, G. L., Olivi, A., McLendon, R., Rasheed, B. A., Keir, S., Nikolskaya, T., Nikolsky, Y., Busam, D. A., Tekleab, H., Diaz, L. A., Jr., Hartigan, J., Smith, D. R., Strausberg, R. L., Marie, S. K., Shinjo, S. M., Yan, H., Riggins, G. J., Bigner, D. D., Karchin, R., Papadopoulos, N., Parmigiani, G., Vogelstein, B., Velculescu, V. E., and Kinzler, K. W. (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807–1812
41. Goyal, L., Govindan, A., Sheth, R. A., Nardi, V., Blaszkowsky, L. S., Faris, J. E., Clark, J. W., Ryan, D. P., Kwak, E. L., Allen, J. N., Murphy, J. E., Saha, S. K., Hong, T. S., Wo, J. Y., Ferrone, C. R., Tanabe, K. K., Chong, D. Q., Deshpande, V., Borger, D. R., Iafrate, A. J., Bardeesy, N., Zheng, H., and Zhu, A. X. (2015) Prognosis and clinicopathologic features of patients with advanced stage Isocitrate Dehydrogenase (IDH) mutant and IDH wild-type intrahepatic cholangiocarcinoma. *Oncologist* **20**, 1019–1027
42. Valle, J., Wasan, H., Palmer, D. H., Cunningham, D., Anthoney, A., Maraveyas, A., Madhusudan, S., Iveson, T., Hughes, S., Pereira, S. P., Roughton, M., and Bridgewater, J.; ABC-02 Trial Investigators. (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N. Engl. J. Med.* **362**, 1273–1281
43. Olivero, O. A., Chang, P. K., Lopez-Larraza, D. M., Semino-Mora, M. C., and Poirier, M. C. (1997) Preferential formation and decreased removal of cisplatin-DNA adducts in Chinese hamster ovary cell mitochondrial DNA as compared to nuclear DNA. *Mutat. Res.* **391**, 79–86
44. Yang, Z., Schumaker, L. M., Egorin, M. J., Zuhowski, E. G., Guo, Z., and Cullen, K. J. (2006) Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. *Clin. Cancer Res.* **12**, 5817–5825
45. Martins, N. M., Santos, N. A., Curti, C., Bianchi, M. L., and Santos, A. C. (2008) Cisplatin induces mitochondrial oxidative stress with resultant energetic metabolism impairment, membrane rigidification and apoptosis in rat liver. *J. Appl. Toxicol.* **28**, 337–344
46. Mukhopadhyay, P., Horváth, B., Zsengellér, Z., Zielonka, J., Tanchian, G., Holovac, E., Kechrid, M., Patel, V., Stillman, I. E., Parikh, S. M., Joseph, J., Kalyanaraman, B., and Pacher, P. (2012) Mitochondrial-targeted antioxidants represent a promising approach for prevention of cisplatin-induced nephropathy. *Free Radic. Biol. Med.* **52**, 497–506
47. Poyton, R. O., Ball, K. A., and Castello, P. R. (2009) Mitochondrial generation of free radicals and hypoxic signaling. *Trends Endocrinol. Metab.* **20**, 332–340
48. Wolf, D. A. (2014) Is reliance on mitochondrial respiration a "chink in the armor" of therapy-resistant cancer? *Cancer Cell* **26**, 788–795
49. Cancer Genome Atlas Network. (2012) Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337

Received for publication March 20, 2018.
Accepted for publication May 14, 2018.