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

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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 (IDH1MUT) enzymes consume NADPH to produce D-2-hydroxyglutarate (D-2HG) resulting in the decreased reductase activity of NADP+ dependent enzymes. The objective of the current study was to investigate the mechanism behind the chemosensitivity of the widely used anticancer agent cisplatin in IDH1MUT cancer cells. Oxidative stress, DNA damage, and mitochondrial dysfunction caused by cisplatin treatment were monitored in IDH1MUT-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 IDH1MUT cancer cells, as compared with IDH1 wild-type (IDH1WT) cells. Mechanistic investigations revealed that cisplatin treatment dose-dependently reduced oxidative respiration in IDH1MUT cells, which was accompanied by disturbed mitochondrial proteostasis, indicative of impaired mitochondrial activity. These effects were abolished by the IDH1MUT 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 IDH1MUT 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.

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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).

IDH1MUT leads to reprogramming of cellular metabolism, which is one of the hallmarks of cancer (4, 5). The hotspot mutations in IDH1, of which IDH1R132H is the most prevalent, cause loss of enzymatic wild-type IDH1 (IDH1WT) 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).

IDH1WT 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). IDH1MUT 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 IDH1MUT cancers (1, 12–14). Patients with IDH1MUT tumors in particular respond...
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\textsuperscript{MUT} reduces oxidative stress in IDH1\textsuperscript{MUT} cells and thus protects cells against IR (15). Similarly, patients with IDH1\textsuperscript{MUT} cancers may respond well to cytotoxic treatment (6, 10, 16–18), including cisplatin \textit{[cis-diaminedichloroplatinum(II)]} (13, 19). However, the mechanism that induces cisplatin sensitivity in IDH1\textsuperscript{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\textsuperscript{MUT} cells are more dependent on mitochondrial oxphos, as compared to IDH1\textsuperscript{WT} cells (4, 27). In addition, we reported that cells of the patient-derived IDH1\textsuperscript{MUT} oligodendroglioma xenograft model E478 are packed with mitochondria, suggesting that IDH1\textsuperscript{MUT} gliomas revert to mitochondrial metabolism (28). Therefore, we hypothesized that IDH1\textsuperscript{MUT} tumors are more sensitive to cisplatin treatment than IDH1\textsuperscript{WT} tumors as a result of its role in the oxidative stress response. The first goal of the present study was to provide \textit{in vitro} evidence that the increased sensitivity to therapy of IDH1\textsuperscript{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\textsuperscript{MUT} cells is a key modulator of sensitivity to cisplatin. Finally, we hypothesize that IDH1\textsuperscript{MUT} inhibitors reverse this metabolic stress response to cisplatin exposure, thus interfering with the survival-prolonging sensitivity of IDH1\textsuperscript{MUT} cancer cells.

**MATERIALS AND METHODS**

**Cell culture**

HCT116 IDH1\textsuperscript{MUT} knockin colon carcinoma cells (IDH1\textsuperscript{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\textsuperscript{WT} or IDH1\textsuperscript{R132H} \textsuperscript{*}, as described by Esmaeili \textit{et al.} (30). HCT116 cells were cultured in McCoy’s 5A medium (Thermo Fisher Scientific, Waltham, MA, USA) in 5% CO\textsubscript{2} at 37°C. U251 cells were cultured in 5% CO\textsubscript{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\textsuperscript{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) phenyldihydrazone, l-glutamine, and sodium pyruvate were purchased from MilliporeSigma (Burlington, MA, USA); and cisplatin (Platinol) was purchased from Pharmachemie B.V. (Haarlem, The Netherlands).

**Colonies-forming assays after cisplatin treatment**

Colonies-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 \mu M AGI-5198, 0–10 mM D-2HG, or 0–5 \mu M 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 \mu M AGI-5198, were grown in 5% CO\textsubscript{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\textsuperscript{+}, NADPH, and ROS measurements**

Cells were incubated for 72 h in the presence or absence of 1 \mu M AGI-5198 and treated for 24 h with cisplatin. Cells were harvested, prepared, and analyzed for NADP\textsuperscript{+}/NADPH ratios and ROS levels with a colorimetric NADP\textsuperscript{+}/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 \mu M) or carboplatin (40 \mu M) 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 \mu g/ml; Thermo Fisher Scientific) or with primary anti-mitochondrial cytochrome c oxidase (MTCO)-1 antibody (1 \mu g/ml; Thermo Fisher Scientific) diluted in blocking solution (5% milk in Tris-buffered saline-Tween). For SDS-PAGE and Western blot analysis, 25 \mu g protein was boiled (for anti-MTCO-1, it was not boiled) with 6 \times SDS-PAGE loading buffer, and samples were separated on precast NU-PAGE 10% Bis-Tris gels (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 γH2AX 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 α = 0.05.

RESULTS

Oxidative stress mediates IDH1MUT sensitivity to cisplatin

The effects of cisplatin on IDH1MUT and IDH1WT HCT116 cells were investigated by performing proliferation and colony-forming assays. Relative to IDH1WT HCT116 cells, cisplatin caused a significant dose-dependent reduction of the surviving fraction of IDH1MUT cells (Fig. 1A–D), suggesting that IDH1MUT sensitizes HCT116 cells to cisplatin. The reduced survival of IDH1MUT cells was confirmed in a proliferation assay with a U251 glioblastoma cell line that stably overexpressed IDH1WT or IDH1MUT (Fig. 1B). We also evaluated the effects of carboxplatin and oxalaplatin, 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 IDH1MUT and IDH1WT HCT116 cells after 72 h exposure to carboxplatin or oxalaplatin were similar, suggesting that IDH1MUT sensitizes HCT116 cells specifically to cisplatin, but not to carboxplatin or oxalaplatin (Fig. 1E–G).

Furthermore, we investigated whether chemosensitivity of IDH1MUT cells is caused by increased vulnerability to oxidative stress, and we treated IDH1MUT and IDH1WT HCT116 cells and IDH1R132H- and IDH1WT-overexpressing U251 cells with the NADPH surrogate and ROS scavenger NAC. NAC equalized the surviving fractions of IDH1MUT and IDH1WT cells after treatment with cisplatin (Fig. 1H, I).

This finding suggests that oxidative stress mediates the higher chemosensitivity to cisplatin of IDH1MUT cells, as compared to IDH1WT cells.

Cisplatin treatment decreases NADPH levels and increases ROS levels in IDH1MUT cells, and IDH1MUT inhibitor AGI-5198 attenuates these effects

We investigated the effects of IDH1MUT on cellular NADPH and ROS levels, with or without pretreatment with cisplatin. IDH1MUT and IDH1WT 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, IDH1MUT HCT116 cells had NADP+ : NADPH ratios and ROS levels similar to those of IDH1WT 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. 2A), and ROS levels were significantly increased after 12 and 24 h of cisplatin exposure (Fig. 2B). Notably, the increased NADP+ : NADPH ratio and ROS levels were higher in IDH1MUT than in IDH1WT HCT116 cells, and AGI-5198 attenuated this effect in IDH1MUT HCT116 cells (Fig. 2B). These findings suggest that higher ROS levels in IDH1MUT HCT116 cells after cisplatin treatment results in increased NADPH consumption, compared with that in IDH1WT HCT116 cells.

IDH1MUT increases the number of DNA DSBs, and the IDH1MUT 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 IDH1MUT 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 IDH1MUT cells than in IDH1WT HCT116 cells in steady-state conditions (Fig. 3). Furthermore, the number of γ-H2AX foci was higher in IDH1MUT cells than in IDH1WT HCT116 cells after treatment for 1 h with 5 and 10 μM cisplatin. To confirm the causal relationship between IDH1MUT and increased levels of DNA damage, we pretreated IDH1MUT cells with AGI-5198 before treatment with cisplatin, which reversed the number of γ-H2AX foci in IDH1MUT cells to levels observed in IDH1WT HCT116 cells (Fig. 3).

IDH1MUT sensitizes cells to cisplatin causing increased levels of oxidative respiration

Besides increased NADP+ : NADPH levels upon treatment with cisplatin, the distinct metabolic phenotype of IDH1MUT cancer cells may sensitize the cells to cisplatin-induced toxic effects (25, 26). We previously determined that...
IDH1\textsuperscript{MUT} HCT116 cells are more dependent on oxphos than are IDH1\textsuperscript{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\textsuperscript{MUT} cells to cisplatin is caused by this metabolic phenotype. To investigate metabolic vulnerability, OCRs were determined using the Seahorse XFe96 respirometer in IDH1\textsuperscript{MUT} and IDH1\textsuperscript{WT} HCT116 cells in the presence or absence of cisplatin. In the absence of cisplatin, the OCR of IDH1\textsuperscript{MUT} cells was 2-fold higher than that of IDH1\textsuperscript{WT} HCT116 cells. Exposure to cisplatin led to a dose-dependent reduction of oxygen consumption in IDH1\textsuperscript{MUT} cells, whereas IDH1\textsuperscript{WT} cells were not affected (Fig. 4A). 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 \(\mu\)M cisplatin induced a significant mitonuclear protein imbalance in IDH1\textsuperscript{MUT} HCT116 cells (Fig. 4B, 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\textsuperscript{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...
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 IDH1MUT 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. 4D). Treatment with cisplatin increased the FADH production capacity of SDH, suggesting that cisplatin treatment is responsible for inhibition of mitochondrial DNA transcription, which leads to a subsequent reduction in protein synthesis and impaired ETC function.

Figure 3. Cisplatin exposure of IDH1MUT 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 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.

**IDH1MUT inhibitor protects and d-2HG sensitizes IDH1MUT cells to cisplatin**

The decreased NADPH production capacity and increased metabolic vulnerability of IDH1MUT cells is associated with sensitization to cisplatin. Therefore, we investigated whether the IDH1MUT inhibitor AGI-5198 protects IDH1MUT cells by restoring NADPH production capacity and altering the metabolic phenotype of IDH1MUT cells. We exposed IDH1MUT and IDH1WT HCT116 cells to 1 μM AGI-5198 for 14 d before cisplatin exposure. AGI-5198 did not affect sensitivity of IDH1WT HCT116 cells, but reduced sensitivity of IDH1MUT HCT116 cells to cisplatin in a manner comparable to that of IDH1WT HCT116 cells (Fig. 5A–C). These data show that AGI-5198 blocks IDH1MUT-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 IDH1MUT HCT116 cells to cisplatin. We exposed IDH1MUT and IDH1WT HCT116 cells to d-2HG before cisplatin treatment.

Figure 2. Cisplatin exposure of IDH1MUT 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.
D-2HG significantly decreased the clonogenic fractions of IDH1<sup>WT</sup> and IDH1<sup>MUT</sup> 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<sup>MUT</sup> or IDH1<sup>WT</sup> HCT116 cells against cisplatin in the presence of D-2HG; the protective mechanism of AGI-5198 on IDH1<sup>MUT</sup> HCT116 cells therefore depends predominantly on the inhibition of IDH1<sup>MUT</sup>-mediated D-2HG production.

**DISCUSSION**

Since the discovery of IDH1<sup>MUT</sup>, it has been known that patients with glioma carrying the IDH1<sup>MUT</sup> have prolonged overall survival compared with their IDH1<sup>WT</sup> 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<sup>MUT</sup> tumors are more sensitive to chemotherapy and radiotherapy than IDH1<sup>WT</sup> tumors (16, 17) and this suggests that IDH1<sup>MUT</sup> predicts the chemo-irradiation response in glioma. For intrahepatic cholangiocarcinoma, there are conflicting data with respect to the prognostic impact of IDH1<sup>MUT</sup> (18, 41). Therefore, the discovery that IDH1<sup>MUT</sup> also sensitizes cancer cells to cisplatin may address this conundrum. On the basis of cisplatin sensitivity, IDH1<sup>MUT</sup> may contribute to a possible prolonged survival of patients with intrahepatic cholangiocarcinoma bearing IDH1<sup>MUT</sup>. Post hoc molecular analyses of IDH1<sup>MUT</sup> 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<sup>MUT</sup> 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<sup>MUT</sup> cells to various cytotoxic agents have been described, including 5-fluorouracil, busulfan, carmustine, daunorubicin, lomustine, temozolomide, and cisplatin (13–15). Whether IDH1<sup>MUT</sup> 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...
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$^{\text{MUT}}$ 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.

Figure 5. IDH$^{\text{MUT}}$ inhibitor AGI-5198 dose dependently protects IDH$^{\text{MUT}}$ HCT116 cells against cisplatin exposure. A) Colony-forming assay after cisplatin (5 $\mu$M) exposure of IDH$^{\text{WT}}$ and IDH$^{\text{MUT}}$ HCT116 cells after long-term (14 d) incubation in the presence or absence of 1 $\mu$M 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 IDH$^{\text{WT}}$ HCT116 cells. C) Colony-forming assay after 72 h of cisplatin exposure with IDH$^{\text{WT}}$ and IDH$^{\text{MUT}}$ HCT116 cells after long-term (14 d) incubation in the presence or absence of 1 $\mu$M 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) IDH$^{\text{MUT}}$ sensitized cancer cells against cisplatin by 2 major components: 1) the reduced NADPH production capacity in IDH$^{\text{MUT}}$ cancer cells resulting in decreased reducing power; and 2) the metabolic phenotype of IDH$^{\text{MUT}}$ 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 IDH$^{\text{MUT}}$ cells. Inhibition of IDH$^{\text{MUT}}$ by AGI-5198 disrupts this sensitivity process, which enhances the capacity of IDH$^{\text{MUT}}$ cells to reduce oxidative stress and protects them against cisplatin.
further study. For example, we did not study cisplatin sensitivity of IDH1MUT in vitro. Shi et al. (13) demonstrated that IDH1R132H 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 vitro sensitivity of IDH1MUT 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 IDH1MUT 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 IDH1MUT cells than in IDH1WT HCT116 cells (19, 27) and because IDH1MUT functions as a heterodimer with IDH1WT, IDH1MUT HCT116 cells are a more relevant model than the IDH1WT HCT116 expression models that are frequently used.

In summary, cisplatin-induced sensitivity in IDH1MUT cancer cells is mediated by at least 2 major components causing cell death: the redox status and the metabolic activity of IDH1MUT cancer cells. Altered oxidative stress responses due to vulnerable metabolism are a plausible mechanism for understanding the sensitivity of IDH1MUT cancer cells to cisplatin exposure. Our study may have clinical implications and our results imply that administration of IDH1MUT inhibitors to patients with IDH1MUT cancer abolishes the therapeutic effect of cisplatin. Our in vitro results suggest that concomitant administration of IDH1MUT inhibitors and cisplatin may result in an unfavorable clinical outcome.

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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.

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