Exploiting the acquired vulnerability of cisplatin-resistant tumors with a hypoxia-amplifying DNA repair–inhibiting (HYDRI) nanomedicine

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Various cancers treated with cisplatin almost invariably develop drug resistance that is frequently caused by substantial DNA repair. We searched for acquired vulnerabilities of cisplatin-resistant cancers to identify undiscovered therapy. We herein found that cisplatin resistance of cancer cells comes at a fitness cost of increased intracellular hypoxia. Then, we conceived an inspired strategy to combat the tumor drug resistance by exploiting the increased intracellular hypoxia that occurs as the cells develop drug resistance. Here, we constructed a hypoxia-amplifying DNA repair–inhibiting liposomal nanomedicine (denoted as HYDRI NM), which is formulated from a platinum(IV) prodrug as a building block and payloads of glucose oxidase (GOx) and hypoxia-activatable tirapazamine (TPZ). In studies on clinically relevant models, including patient-derived organoids and patient-derived xenograft tumors, the HYDRI NM is able to effectively suppress the growth of cisplatin-resistant tumors. Thus, this study provides clinical proof of concept for the therapy identified here.

INTRODUCTION

Platinum anticancer drugs (cisplatin, carboplatin, and oxaliplatin) are widely applied in standard-of-care chemotherapy for many types of cancer and provide substantial benefits to patients. The initial therapeutic successes of platinum-based medications are based on their induction of intrantradna DNA cross-links; however, most patients ultimately relapse with resistant lesions (1–3). It has been well documented that spontaneous nucleotide excision repair in cancer cells is the major pathway for developing resistance to platinum drugs (4). Inhibition of DNA repair by targeted drugs is a pivotal approach to potentiating the therapeutic efficacy of cisplatin and achieving more sustained clinical benefits (5), but adaptive drug resistance is still mostly inevitable. Following treatment with DNA repair inhibitors, adaptive drug resistance is frequently caused by activation of other alternative pathways, including decreased drug transport, increased cellular detoxification, increased tolerance of DNA adducts, and defects in the apoptotic cell death pathway (6, 7). Given these problems of platinum drugs, we were inspired to explore an effective strategy to combat tumor resistance by finding and exploiting the inevitable weakness in drug-resistant cancer cells.

It has been demonstrated that drug resistance of cancer cells comes at a fitness cost of some collateral physical hallmarks, which might result in an acquired vulnerability and elicit inspirations to identify new therapeutics (8, 9). Numerous studies have unveiled that hypoxia in tumor microenvironment has a crucial role as orchestrators of clinical resistance to platinum-based chemotherapy through a variety of signaling pathways in DNA damage, mitochondrial activity, apoptosis, autophagy, and drug efflux (10–13). It is common sense that hypoxia in solid tumors is a result of inadequate oxygen being consumed by rapidly proliferating tumor cells far from the capillaries that provide the immediate diffusion of oxygen (14–16). However, some histopathologic examinations in the clinic call this long-held classic view into question, because tumor hypoxia can be observed at the early stage of tumor progression or even at the area adjacent to the capillary vessels in patients after chemotherapy. Puzzled by the questions from clinicians, we are curious of the intracellular hypoxic state of platinum drug–resistant cancer cells, which has seldom been investigated to date.

In our study, we compared three pairs of cancer cells and their drug-resistant derivatives, and we unveiled that the level of intracellular oxygen in cisplatin-resistant cells is much lower than that in cisplatin-sensitive cancer cells. This elevated intracellular hypoxia may expose cisplatin-resistant cancer cells to an acquired weakness. Inspired by this finding and hypothesis, we constructed a hypoxia-amplifying DNA repair–inhibiting nanomedicine (HYDRI NM), with the aim of realizing synergistic therapy by exploiting the acquired weakness of cisplatin-resistant tumors via increasing intracellular hypoxia. The HYDRI NM (also denoted as GOx/TPZ@Lipo-Pt) was formulated from a platinum(IV) prodrug as a building block and encapsulated payloads of glucose oxidase (GOx) and the hypoxia-activatable tirapazamine (TPZ). The loaded GOx was competent to consume oxygen by catalyzing the

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oxidation of the abundant glucose in the tumor region, which amplified the intracellular hypoxia within the cisplatin-resistant cancer cells. In response to the aggravated hypoxia, the initially inactive TPZ was successively activated to induce cytotoxicity and significant down-regulation of xeroderma pigmentosum group F (XPF), an overexpressed DNA self-repairing protein that causes acquired resistance of cancer cells to cisplatin. Thus, the as-designed HYDRI NM was able to efficiently suppress the growth of cisplatin-resistant tumors, providing proof of concept for this identified therapeutic approach, which takes advantage of the increased levels of intracellular hypoxia in drug-resistant cells.

RESULTS

Cisplatin-resistant cancer cells show increased intracellular hypoxia

To identify alternative therapeutic strategies for cisplatin-resistant tumors, we generated cisplatin-resistant cell line derivatives (BEL7404DDP, A549DDP, and KB-3-1DDP) by culturing their parent cell lines (BEL7404, A549, and KB-3-1) with stepwise increases in cisplatin for several months (17). Quantifications of cell viability by cell counting kit 8 (CCK-8) assay confirmed the more sustained viability of the cisplatin-resistant derivatives than the parent cell lines in the presence of cisplatin (fig. S1, A to D). The IC_{50} (half-maximal inhibitory concentration) values of cisplatin in the cisplatin-resistant derivatives were 10.34-, 5.22-, and 6.12-fold higher than those in the cisplatin-sensitive parent cells. Similarly, apoptosis assays showed that incubation with cisplatin caused more apoptosis of the parent cell lines than the drug-resistant derivatives (fig. S1, E to G). These results underscored the successful generation of the cisplatin-resistant cancer cell derivatives after long-term exposure to cisplatin-containing medium, which recapitulated the occurrence of acquired cisplatin resistance in clinical chemotherapy.

There is a fact that the cisplatin-resistant derivative cells exhibited much lower proliferation rate than the cisplatin-sensitive parental cells at the same culture conditions. It is suspected that the slow growth of the derivatives may have some associations with unknown changes in levels of oxygen consumption in cells. Thus, we attempted to unveil potential alterations of intracellular hypoxia in the cisplatin-resistant cell line derivatives. The intracellular hypoxia was analyzed by confocal laser scanning microscopy (CLSM) using a ROS-ID® hypoxia/ oxidative stress detection kit (Fig. 1A and fig. S2) (18). The red fluorescent signals in the derived cells were significantly stronger than those in the cisplatin-sensitive parental cells, which indicates that the intracellular environment is more hypoxic in the cisplatin-resistant cells (Fig. 1, A and B). This is an interesting hint, because most of the previous studies focused on the hypoxic microenvironment within tumor tissue rather than individual cells (19). Considering that hypoxia-inducible factor-1α (HIF-1α) is the pivotal transcriptional regulator of the cellular response to hypoxia, we measured HIF-1α levels by Western blotting and immunofluorescence staining (20). As shown in Fig. 1 (C and D), the drug-resistant derivatives expressed significantly higher levels of HIF-1α protein than their parental cells. The spatial distribution, as observed by CLSM, revealed that HIF-1α levels were high in the nuclei of the drug-resistant cells (Fig. 1F). Moreover, it is believed that the intracellular hypoxia can result in a transcellular oxygen concentration gradient and is positively correlated with the oxygen diffusion fluxes around the cells (21, 22). A self-referencing oxygen electrode was manipulated around the cells to determine the oxygen diffusion fluxes by a noninvasive microtest technology (fig. S3A). The concentration gradient of O₂ was determined by selective microsensors repeatedly “vibrating” between two points in a defined manner, and the O₂ flux was calculated according to Fick’s law of diffusion (23). The absolute value of the O₂ diffusion flux around BEL7404DDP cells was almost threefold higher than that around BEL7404 cells, indicative of the more intense intracellular hypoxia of BEL7404DDP cells (fig. S3, B and C). Collectively, these lines of evidence documented that the three cisplatin-resistant cancer cells showed increased intracellular hypoxia. To unveil the underlying mechanism of the increased intracellular hypoxia, we tested the glucose uptake rate of both BEL7404 and BEL7404DDP cancer cells. As shown in fig. S3D, the drug-resistant cells exhibited lower glucose uptake rate than the sensitive cells, which was consistent with the observations in the previous study (24, 25). The alteration of glucose uptake implies potential changes of glucose metabolic process in drug-resistant derivatives with respect to the sensitive parental cells. RNA-sequencing technology was used to profile the global transcriptome of BEL7404 and BEL7404DDP cells. More than 5000 genes showed significant alterations in the mRNA level in BEL7404DDP cells (fig. S3E). Specifically, the obvious changes of the mRNA level were found in numerous glucose metabolism-related genes that were associated with glucose transmembrane transport, insulin secretion, glucose starvation, and glucose homeostasis (fig. S3, F and G). Therefore, we speculated that the increased intracellular hypoxia of resistant cells was closely related to their reshaped landscape in glucose uptake and metabolism behavior. However, more fundamental investigations are requested before making a clear statement on the mechanism of the acquired intracellular hypoxia. Here, we just provided some primary discussions on the mechanism and left it as an open issue for further studies. In addition, we also characterized the cellular expression of XPF protein, which serves as a master regulator of DNA repair and causes compromised efficacy of platinum drugs (26, 27). Western blot and immunofluorescence analyses both revealed that the expression of XPF protein was markedly up-regulated in the cisplatin-resistant derivatives compared with their parental cells (Fig. 1, C, E, and G).

Preparation and characterization of the HYDRI NMs (GOx/TPZ@Lipo-Pt)

We hypothesized that the increased intracellular hypoxia levels may be an acquired weakness in the sense that a further enhancement of hypoxia could be exploited to kill the drug-resistant cells. Therefore, we prepared a versatile cisplatin prodrug—containing liposomal NM (denoted as GOx/TPZ@Lipo-Pt NM or HYDRI NM) aimed at efficiently inhibiting the growth of cisplatin-resistant cancer by taking advantage of the acquired intracellular hypoxia, as illustrated in Fig. 2. The cisplatin prodrug was first prepared by conjugating cis,trans,cis-[Pt(NH₃)₂(OH)₂Cl₂] with excess octane isocyanate (28), as depicted in fig. S4. The results of nuclear magnetic resonance [¹H NMR (nuclear magnetic resonance), ¹³C NMR, and ¹⁹⁵Pt NMR] validated the molecular structure of the obtained platinum(IV) complex. The GOx/TPZ@Lipo-Pt NM was prepared through supramolecular self-assembly of the cisplatin prodrug conjugate together with dipalmitylophosphatidylcholine, cholesteral, and 1,2-Distearylsn-Glycero-3-Phosphoethanolamine with conjugated methoxyl poly(ethylene glycol) (DSPE-mPEG₂₀) at the molar ratio of 4:12:4:1 (12, 29–31). The hypoxia-activatable drug TPZ and the oxygen consumer GOx were spontaneously encapsulated into the hydrophilic cavity of the liposomes.
As controls, Lipo-Pt, TPZ@Lipo-Pt, GOx/TPZ@Lipo, TPZ@Lipo, and GOx@Lipo-Pt liposomes were prepared in a similar way. The morphology and size distributions of the different liposomal NMs were evaluated by transmission electron microscopy (TEM) imaging and dynamic light scattering (DLS). The TEM images revealed that all the liposomal NMs have similar spherical morphology with average sizes of approximately 60 nm (Fig. 3A). The TEM measurements are slightly smaller than the hydrodynamic diameters measured by DLS due to the shrinkage in the dry state (Fig. 3B and fig. S5A). The presence of TPZ and GOx in the GOx/TPZ@Lipo-Pt NM was validated by the characteristic absorption peaks at 475 and 275 nm, respectively, in the ultraviolet-visible (UV-vis) absorbance spectrum (Fig. 3C). As displayed in fig. S5B, the TPZ loading capacity (2.01%) and GOx loading capacity (3.1%) of the GOx/TPZ@Lipo-Pt NM were quantified by UV-vis absorption spectroscopy and GOx activity assay kit, respectively. The Pt content measured by inductively coupled plasma mass spectrometry (ICP-MS) was 7.8 weight % (wt %).

We expected that the GOx/TPZ@Lipo-Pt NM would further positively augment the intracellular hypoxia via GOx-mediated oxygen consumption. GOx catalyzes the oxidation of glucose, thus inducing a synergistic effect between TPZ and the cisplatin prodrug on drug-resistant cancer cells. To assess the oxygen-consuming capacity of the GOx/TPZ@Lipo-Pt NM, we prepared suspensions of GOx/TPZ@Lipo-Pt and control formulations in glucose solution and detected the oxygen concentration using an oxygen-sensitive phosphorescent molecular probe (32) and a portable dissolved oxygen meter (18, 33). As shown in Fig. 3 (D and E), in the presence of GOx-containing liposomes (GOx/TPZ@Lipo and GOx/TPZ@Lipo-Pt NMs), marked decreases in the dissolved oxygen concentration in the suspensions were continuously recorded. The GOx encapsulated in the liposomes showed no obvious loss in oxygen-consuming ability compared with the free GOx. Notably, a faster oxygen consumption rate was achieved with increasing concentrations of GOx/TPZ@Lipo-Pt (Fig. 3F). In addition, the platinum drug and TPZ release profiles of the GOx/TPZ@Lipo-Pt NM were evaluated in phosphate-buffered saline (PBS) with or without 10 mM dl-dithiothreitol (DTT) (18). As presented in Fig. 3G and fig. S5C, the GOx/TPZ@Lipo-Pt NM showed good stability without obvious leakage of platinum in the absence of DTT. In contrast, in the presence of DTT, the platinum(IV) complexes were readily reduced to platinum(II), and up to 60% of the
In vitro inhibition of tumor cell growth by GOx/TPZ@Lipo-Pt HYDRI NM

One of the fundamental arguments in this study is that GOx catalyzes oxygen consumption to aggravate hypoxia in drug-resistant cancer cells, thus eliciting the synergistic effect between TPZ and the cisplatin complex. A ROS-1D® hypoxia/oxygen stress detection kit was used to visualize the intracellular hypoxia level within BEL7404DDP cells subjected to different treatments. CLSM imaging showed that the cells incubated with GOx-free liposomal NMs (Lipo-Pt and TPZ@Lipo-Pt) displayed moderate red signals (Fig. 4A), which stems from the intrinsic intracellular hypoxia within the cisplatin-resistant BEL7404DDP cells as demonstrated above. In contrast, treatment with GOx-containing liposomal NMs (GOx/TPZ@Lipo and GOx/TPZ@Lipo-Pt) led to enhanced red fluorescence in the cells, which indicates that the intracellular hypoxia was amplified by GOx-mediated oxygen consumption. The dynamic evolution of the intracellular hypoxia was also continuously monitored using the MitoXpressXtra-Oxygen assay in BEL7404DDP cells treated with various liposomes. Upon the addition of glucose into the suspensions, the cells treated with GOx/TPZ@Lipo and GOx/TPZ@Lipo-Pt showed a much higher oxygen consumption rate than those treated with Lipo-Pt or TPZ@Lipo-Pt (Fig. 4B).

Next, we carefully assessed the in vitro synergistic effects of the GOx/TPZ@Lipo-Pt NM on the cisplatin-resistant BEL7404DDP cell line (Fig. 4C). The CCK-8 assay revealed that the presence of TPZ or GOx in liposomes did not cause increased cytotoxicity compared with Lipo-Pt (fig. S6, A and B). This suggests that GOx or TPZ causes negligible toxicity in a normoxic environment. Next, BEL7404DDP cells were incubated with TPZ@Lipo-Pt at various fixed oxygen concentrations. The viability of the BEL7404DDP cells and the IC50 values of TPZ@Lipo-Pt were markedly reduced as the oxygen levels decreased from 21 to 1% (fig. S6, D and E), which confirms that the hypoxia-activated cytotoxicity of TPZ is related to the hypoxic stress. It is noteworthy that GOx/TPZ@Lipo-Pt in 21% oxygen induced a similar cell growth inhibition pattern to TPZ@Lipo-Pt in 1% oxygen (fig. S6C). This demonstrates that the GOx-catalyzed oxygen consumption is able to create sufficient hypoxia to readily elicit the toxicity of TPZ. The cell-killing efficacy of the liposomal NMs (at an equivalent Pt concentration of 1 µM) was also assessed on the drug-resistant cell lines A549DDP and KB-3-1DDP. As displayed in fig. S6 (G to I), GOx/TPZ@Lipo-Pt was the most cytotoxic. In agreement with the above quantitative CCK-8 assay results, flow cytometry measurements of BEL7404DDP cells treated with various liposome formulations showed that GOx/TPZ@Lipo-Pt resulted in the highest percentage of late apoptotic cells (up to 57.5%; Fig. 4E). This confirmed the efficacy of the GOx/TPZ@Lipo-Pt NM. The synergistic effect between TPZ and the platinum(IV) complex of GOx/TPZ@Lipo-Pt was quantitatively determined by calculating the combination index (CI). According to the CI theorem, CI values lower than 1 indicate synergism, CI values higher than 1 indicate antagonism, and CI values equal to 1 indicate an additive effect (34–36). The inhibitory concentrations at various effect levels of Lipo-Pt, GOx/TPZ@Lipo, and GOx/TPZ@Lipo-Pt NMs were derived from the above CCK-8 assays and displayed in fig. S6F. A Chou-Talalay plot of CI values versus effect levels is presented in Fig. 4D. All the CI values were much lower than 1, indicating that a strong synergistic effect was achieved by the GOx/TPZ@Lipo-Pt NM.

We further searched for a more fundamental understanding of the mechanism underlying the synergistic efficacy of GOx/TPZ@Lipo-Pt against the drug-resistant cells. A general consensus is that the cisplatin resistance of cells mainly arises from a DNA repair response. XPF protein plays a prominent role in this process and is up-regulated in drug-resistant cells (37). Thus, we used Western blotting to investigate the expression levels of XPF protein in the three drug-resistant cell lines subjected to various treatments. As shown in fig. S7, all the untreated cell lines expressed high levels of XPF protein, which were significantly down-regulated after incubation with TPZ under hypoxic conditions (1% oxygen). In contrast, treatments with TPZ under normoxic conditions caused a moderate reduction in XPF levels. These results suggest that TPZ displayed potent down-regulation of XPF expression at hypoxia in addition to the activated cytotoxicity. BEL7404DDP cells treated with GOx/TPZ@Lipo-Pt or GOx/TPZ@Lipo showed statistically significant down-regulation of XPF expression compared with cells
treated with other liposome formulations (Fig. 4, F and G). Then, ICP-MS was used to quantify the formation of intracellular platinum (Pt)–DNA adducts, which is thought to be crucial to the antitumor potency of cisplatin. A marked increase in Pt-DNA adducts was detected in cells after treatment with GOx/TPZ@Lipo-Pt for 7 hours (Fig. 4H) (38). Collectively, these results indicate that the synergistic therapeutic efficacy of GOx/TPZ@Lipo-Pt in cisplatin-resistant cancer cells can be attributed to the aggravation of intracellular hypoxia by GOx-mediated oxygen consumption, which increases cytotoxicity and reinforces the ability of TPZ to down-regulate XPF and thus results in collateral drug sensitivity of cisplatin-resistant cancer cells.

Response of patient-derived tumor organoids to GOx/TPZ@Lipo-Pt HYDRI NM

Patient-derived tumor organoids (PDOs) are able to recapitulate the genotypic and phenotypic landscape of the original solid tumors. PDOs have thus been exploited as clinically relevant in vitro models to imitate patient-specific pathology and to evaluate the efficacy of chemodrugs for each patient. Therefore, the intracellular hypoxia status was also characterized using PDOs. The used PDOs were derived from colon tumor sample of one patient (denoted as PDO_0) and metastatic liver tumor tissue of primary colon cancer of two patients (denoted as PDO_1 and PDO_2) who were receiving clinical cisplatin chemotherapy. After incubating PDOs with Lipo-Pt at fixed concentrations for 2 days, the viabilities were quantified by CCK-8 assay to assess the drug resistance of the PDOs. The proliferation inhibition results in Fig. 5A suggested that PDO_0 showed sensitive responses to platinum drug, PDO_2 showed moderate resistance, and PDO_1 showed strong resistance to platinum drug. The intracellular hypoxia level of the PDOs was then determined by the expression of HIF-1α via immunofluorescence assay. As displayed in Fig. 5B, the highest expression of HIF-1α on PDO_0 showed sensitive responses to platinum drug, PDO_2 showed moderate resistance, and PDO_1 showed strong resistance to platinum drug. The intracellular hypoxia level of the PDOs was then determined by the expression of HIF-1α via immunofluorescence assay. As displayed in Fig. 5B, the highest expression of HIF-1α on PDO_0 showed sensitive responses to platinum drug, PDO_2 showed moderate resistance, and PDO_1 showed strong resistance to platinum drug.
derived from the PDO analysis implied a positive correlation between the hypoxia degree and the drug resistance extent, which verified the relevance of the observations from comparing parental tumor cell lines with their cisplatin-resistant derivatives. To evaluate the therapeutic effects of GOx/TPZ@Lipo-Pt, the established PDOs were incubated with various liposomal NMs at different Pt-equivalent concentrations (2, 5, and 10 μM) for 48 hours. The survival rate of the PDOs after 2 days of incubation and the recurrence rate 10 days after primary treatment were analyzed. The results for PDO_1 that have the most resistant response to cisplatin are shown in Fig. 5 (C to F). Morphological changes were observed under the microscope, and the size changes of the PDO_1 were calculated to evaluate the proliferation inhibition ability of the liposomal NMs (Fig. 5, C and D).
In addition, the CCK-8 assay was used to quantify PDO_1 viability (Fig. 5, E and F). The results showed that both GOx/TPZ@Lipo and GOx/TPZ@Lipo-Pt treatments significantly inhibited the proliferation of PDO_1 compared with the saline control (Fig. 5E). Although GOx/TPZ@Lipo-Pt was slightly (and nonsignificantly) more effective than GOx/TPZ@Lipo at inhibiting proliferation, the relapse rate of PDO_1 10 days after treatment was significantly decreased by GOx/TPZ@Lipo-Pt (Fig. 5F). This indicates that GOx/TPZ@Lipo-Pt may have potent efficacy with good prognosis against cisplatin-resistant tumors. To investigate the mechanism underlying the therapeutic effect, we used immunofluorescence to analyze the expression levels of HIF-1α and XPF in PDO_1 after the various treatments (Fig. 5G). Compared with the untreated group, the slices of PDO_1 subjected to GOx/TPZ@Lipo-Pt or GOx/TPZ@Lipo treatment displayed enhanced green fluorescence and decreased red signals, which indicates elevated expression of HIF-1α and down-regulation of XPF levels. These changes in HIF-α and XPF in PDOs treated with GOx/TPZ@Lipo-Pt verified the reinforced hypoxia and the subsequent activation of TPZ, which boosts the potency of the platinum(IV) complex. The therapeutic efficacy of the liposomal NMs was also evaluated on PDO_0 and PDO_2. The NMs tested on PDO_2 that has moderate cisplatin resistance acted in a similar fashion of therapy to those tested on PDO_1 (fig. S8, B to E). As shown in fig. S8A, all the liposomal NMs realized considerable proliferation inhibition on PDO_0. PDO_0 was almost completely killed by GOx/TPZ@Lipo-Pt even at the Pt concentration of 2 μM. It is worth noting that the better killing potency of the HYDRI NMs on PDO_0 than that on PDO_1 or PDO_2 is attributed to the inherent sensitivity of PDO_0 to cisplatin. In parallel with pursuing efficient drug-resistant cancer killing, it is not exclusive to realize better inhibition potency on drug-sensitive cells than on drug-resistant cells. Therefore, it is reasonable that the as-prepared HYDRI NMs resulted in higher killing efficiency on the cisplatin-sensitive cells (PDO_0) than on the cisplatin-resistant cells (PDO_1).

**In vivo anticancer efficacy of GOx/TPZ@Lipo-Pt in cell line–derived xenograft models**

Next, we evaluated the anticancer effectiveness of GOx/TPZ@Lipo-Pt on cell line–derived xenograft (CDX) models in vivo. We subcutaneously inoculated immunodeficient nude mice (NOD)/severe combined immunodeficient (SCID) mice with BEL7404 cells and BEL7404DDP cells to establish cisplatin-sensitive and cisplatin-resistant tumors, respectively. When the tumor size reached 50 mm³, we characterized the hypoxia landscape within the tumors before treatments using photoacoustic (PA) imaging. PA is a powerful technology for the visualization of physiological and pathological processes at the molecular level with deep tissue penetration and good spatial resolution. The PA signals of deoxygenated hemoglobin at 750 nm were recorded under the Oxy-Hemo mode in a PA imaging system (39). As displayed in fig. S9A, stronger PA signals were detected from the BEL7404DDP tumor than from the BEL7404 tumor, suggestive of the lower blood oxygen level in cisplatin-resistant tumors than in cisplatin-sensitive tumors. Then, the mice were euthanized, and the expression levels of HIF-1α in tumor slices were determined by immunofluorescence staining to further characterize the cellular hypoxia within the tumor tissue (fig. S9B). The up-regulated HIF-1α expression in the BEL7404DDP tumor implies a more hypoxic cellular microenvironment in cisplatin-resistant tumors. Overexpression of XPF protein was also verified in the tumor tissue, which was in agreement with the findings from the experiments on cultured cells. These data confirmed the characteristics of elevated hypoxia and up-regulated XPF expression in the cisplatin-resistant tumors, which may cause acquired vulnerability to the GOx/TPZ@Lipo-Pt NM. Afterward, the BEL7404DDP tumor–bearing mice were randomly divided into four groups (four mice per group) and subjected to intravenous administrations of different liposomal NMs at equivalent platinum(IV) or TPZ doses every 3 days. As presented in fig. S9 (C and D), the tumor-bearing mice showed a weak response to Lipo-Pt because of the severe drug resistance. Formulations containing TPZ were more effective, and GOx/TPZ@Lipo-Pt induced the most potent inhibition of tumor growth. This suggests that GOx/TPZ@Lipo-Pt has a synergistic effect on drug-resistant tumors, as we showed above for cultured cells. No appreciable body weight loss was observed in the mice throughout the treatments (fig. S9E).

**GOx/TPZ@Lipo-Pt enhances cancer therapy in a patient-derived xenograft model**

Next, we carried out a more clinically relevant proof of concept for the therapy proposed here using patient-derived xenograft (PDX) models. PDX tumors can inherit the original features of the primary patient tumors, and this approach has thus emerged as a powerful tool for predicting drug response in the clinic. In our work, the PDX models of liver cancer were passaged from highly immunodeficient mice that received subcutaneous transplants of fresh liver cancer tissue from patients. When the average tumor size reached about 100 mm³, the mice received intravenous administration of different liposomal NMs. Levels of deoxygenated hemoglobin, determined by PA imaging at 4 hours after injection of GOx/TPZ@Lipo or GOx/TPZ@Lipo-Pt, were significantly enhanced compared to pre-injection values (Fig. 6, A and B). In contrast, the injection of saline, Lipo-Pt, or TPZ@Lipo-Pt did not significantly change the PA signals at the tumor site. This confirmed that GOx-catalyzed oxygen consumption amplified the intratumor hypoxia. Then, the therapeutic efficacy of the different liposome formulations was systematically assessed in the PDX tumor–bearing mice. Mice received five injections of saline or the indicated liposomal NMs every 3 days. As displayed in Fig. 6 (C to E), the mice injected with Lipo-Pt exhibited rapid tumor growth with no statistical difference from the mice injected with saline. This hints at the failure of chemotherapy with platinum drugs on drug-resistant liver cancer. Weak tumor growth suppression was observed in mice with injections of TPZ@Lipo-Pt, which ought to stem from the incomplete activation of TPZ under the mildly hypoxic conditions within the drug-resistant tumor. The multiple injections of GOx/TPZ@Lipo led to a moderate inhibition of tumor growth (61.72% with respect to control), which was evidently associated with increased cytotoxicity of TPZ following GOx-induced amplification of hypoxia. The tumors of mice injected with GOx/TPZ@Lipo-Pt were almost completely inhibited (82.08% growth suppression relative to control). All the tumors were harvested at the end of the therapy (fig. S9F), which validated the synergistic effect of GOx/TPZ@Lipo-Pt. Furthermore, at the end of the therapy, livers were harvested to analyze the level of metastasis from the primary subcutaneous PDX tumor. Mice treated with multiple injections of GOx/TPZ@Lipo-Pt showed a markedly reduced level of liver metastasis, as evidenced by the photos in Fig. 6G. With the synergistic therapy, the average number of liver metastasis sites was markedly decreased from ~100 per mouse.
It is of great importance and significance to evaluate the side effect of the HYDRI NMs. Fluorescence imaging in vivo was used to spatiotemporally track the whole-body biodistribution of the HYDRI NMs loaded with IR780 dyes in the PDX-bearing mice. As shown in fig. S10 (A to C), increased distinct fluorescence signals in the tumor area were observed after intravenous injection, suggestive of the tumor site–specific accumulation of HYDRI NMs due to enhanced permeability and retention effect. The semiquantitative analysis of ex vivo fluorescence imaging of the major organs indicated the appreciable uptake efficiency (7.5 ± 2.2% injected dose) of the (saline control) to ~2 per mouse (GOx/TPZ@Lipo-Pt therapy) (Fig. 6H).

Fig. 6. In vivo antitumor effects of GOx/TPZ@Lipo-Pt on PDX tumors. (A) PA images of blood oxygen saturation levels in PDX tumors before and 2 hours after the indicated treatments. (B) Quantification of PA signals preinjection and 4 hours after injection at $\lambda = 750$ nm. (C) PDX tumor growth kinetics in BALB/c nude mice treated five times every other day with saline, Lipo-Pt, TPZ@Lipo-Pt, GOx/TPZ@Lipo, or GOx/TPZ@Lipo-Pt. Plots show tumor growth curves of each individual mouse from each group. (D) Combined tumor volume data from the mice shown in (C). (E) Inhibition of tumor growth at the end of treatment with different formulations at equivalent Pt and TPZ doses. (F) Average body weight of mice in each treatment group versus time. (G) Livers of mice were collected on day 25 after the final injection and examined for metastases (white arrows). (H) Quantitative evaluation of liver metastatic nodules. Data are expressed as means ± SD ($n = 6$ mice per group), *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (two-tailed unpaired Student’s t test). Photo credit: Jing Chen, National Center for Nanoscience and Technology.
HYDRI NMs (fig. S10, D and E). To evaluate the potential in vivo adverse systemic effects of the HYDRI-based nanochemotherapy, the body weights of the mice were recorded throughout the therapeutic period and were not significantly affected by the various treatments compared with the control group (Fig. 6F and fig. S10A). The major organs and blood of the mice receiving various treatments were collected at the end of therapy for hematoxylin and eosin (H&E) staining and blood biochemistry analysis. As displayed in fig. S10F, no obvious hydropic damage or necrotic lesions were found on the H&E-stained slides of all the liposomal NM–treated groups compared with the saline-treated control group. Multiple injections of the liposomal NMs did not cause significant alterations on the level of biochemical blood biomarkers compared with injections of saline (fig. S10G). Together, the HYDRI NMs showed favorable tumor-targeting ability and thus were able to induce efficient tumor-specific killing without severe adverse effects.

DISCUSSION
Platinum anticancer drugs provide substantial benefits to patients as the most widely used medications in various cancer chemotherapy in clinic. Along with the long-term chemotherapy, most patients have less efficient response and ultimately suffer from severe resistance to the platinum-based medications, which is mainly attributed to the spontaneous nucleotide excision repair of cancer cells as the major pathway. It is common sense to inhibit the DNA repair occurrence with some targeting drugs, which is a pivotal strategy to synergize the therapy of platinum drugs and give rise to more sustained clinical benefit. However, new drug resistance issues will occur to the DNA repair inhibitors or platinum drugs because of activation of alternative pathways in addition to nucleotide excision repair. It is believed that drug resistance occurrence of cancer cells will come at a fitness cost that in turn can cause some collateral physical hallmarks, which might result in an acquired vulnerability of drug-resistant cancer cells. Thus, with a converse thinking different from inhibiting the pathways associated with drug resistance, we are inspired to explore newly emerged characteristics of cancer as the inevitable drug resistance develops and identify undiscovered therapeutic strategy by leveraging acquired vulnerability of the cancer cells.

In our work, we compared three pairs of cancer cells and their cisplatin-resistant derivatives and found an intriguing finding of elevated intracellular hypoxia of cisplatin-resistant derivatives in comparison with the cisplatin-sensitive parental cells. Accordingly, the cisplatin-resistant derivatives showed up-regulated expression of HIF-1α, which is the pivotal transcriptional regulator of the cellular response to hypoxia. It has been demonstrated that the degradation resistance of HIF-1α under cisplatin treatment is intensely associated with cisplatin-resistance level in cancer cells, and interference of HIF-1α can induce re-regulation of aerobic glycolysis in drug-resistant cancer cells (40). We found that the cisplatin-resistant cells exhibited lower glucose uptake rate than the sensitive cells, and obvious changes of mRNA level in cisplatin-resistant cells were found in numerous glucose metabolism–related genes. Collectively, the increased intracellular hypoxia might be an apparent feature of cisplatin resistance that might essentially arise from alterations of glucose metabolism. Therefore, the finding of increased intracellular hypoxia in cisplatin-resistant cells might elicit inspirations on underlying mechanism discovery and better understanding of the drug resistance issue.

On the basis of the finding, we devised a scheme to combat the tumor cisplatin resistance by leveraging rather than inhibiting the intracellular hypoxia that emerges along with the inevitable development of drug resistance. We successfully engineered the GOx/TPZ@Lipo-Pt HYDRI NM, which carries a platinum(IV) prodrug in the outer hydrophobic layer and has payloads of TPZ and GOx in the hydrophilic cavity. When cisplatin-resistant cancer cells were treated with the HYDRI NM, the intracellular hypoxia was further markedly amplified by the GOx-catalyzed oxidation of glucose. The in vitro cell experiments revealed that the enhanced intracellular hypoxia levels were able to boost the cytotoxicity and bioactivity of TPZ. The activated TPZ displayed increased antitumor activity and inhibited the overexpressed XPF protein, which is associated with DNA repair in drug-resistant cells. The integration of GOx and TPZ within the GOx/TPZ@Lipo-Pt NM led to a synergistic effect with the platinum(IV) complex against drug-resistant tumors. As a clinical proof of concept, the synergistic therapeutic efficacy of the GOx/TPZ@Lipo-Pt NM was further verified on PDO, CDX, and PDX tumor models in vitro and in vivo. The results confirmed that the identified novel therapeutic approach has substantial benefits to overcome the major issue associated with chemotherapy of cisplatin-resistant cancers.

MATERIALS AND METHODS
Materials
CCK-8, cisplatin (DDP, 98%), and TPZ (≥98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further purification. GOx (110 U/mg) was purchased from Coolaber (Beijing, China). ROS-ID® hypoxia/oxidative stress detection kit (Enzo Life Sciences), HIF-1α antibody (Cell Signaling Technology, 36169s), and anti-XPF antibody (Abcam, ab76948) were used as received. GOx activity detection kit and RPMI Medium 1640 without phenol red were purchased from Solarbio Life Sciences (Beijing, China). Annexin V–FITC (fluorescein isothiocyanate) apoptosis detection kit was purchased from Keygen Biotech (Nanjing, China). Alexa Fluor 488–labeled goat anti-rabbit immunoglobulin G (IgG)/(H+L) was obtained from Beyotime Institute of Biotechnology (Shanghai, China). RPMI-1640 medium, Dulbecco’s modified Eagle’s medium (DMEM), PBS, fetal bovine serum (FBS), and RPMI-1640 medium were purchased from WISENT (Beijing, China). All other reagents were provided by Beijing Chemical Reagents Institute (Beijing, China), and all of the reagents were used without further purification unless otherwise noted. Deionized water for all tests was obtained by a Milli-Q purification system (Millipore, Millford, MA, USA).

Preparation of GOx/TPZ@Lipo-Pt HYDRI NMs
Briefly, platinum(IV) complexes (6.0 mg) in N,N-dimethylformamide, DPPC (17.6 mg), cholesterol (3.1 mg), and DSPE-mPEG2k (5.5 mg) were mixed and dissolved in 10 ml of chloroform at the molar ratio of 4:12:4:1 in a round-bottom flask. A phospholipid thin film was formed through a rotary evaporator under reduced pressure. Afterward, 2 ml of deionized water containing TPZ (1 mg/ml) and GOx (1 mg/ml) was added into the round-bottom flask for hydration under bath sonication for 30 min. Then, the obtained liposome solution was continuously subjected to 21 times extrusion through a 200-nm polycarbonate filter using an extruder. The as-prepared GOx/TPZ@Lipo-Pt was purified by centrifugation (20,000g, 30 min) three times to remove free TPZ and GOx. The obtained GOx/TPZ@Lipo-Pt
HYDRI NMs were dispersed in 1 ml of PBS (1×, pH 7.4) and stored at 4°C for further usage. The other formulations of Pt@Lipo, TPZ@Lipo-Pt, and GOx/TPZ@Lipo were prepared following the same process except for the addition of TPZ, GOx, or platinum(IV) complex. The drug (Pt, TPZ, and GOx) loading capacity of liposomes was quantified as the weight percentage of drug loaded in the whole nanostructure, indicating the drug content in liposomes.

**Cell lines and cell culture conditions**

BEL7404, BEL7404DDP, KB-3-1, and KB-3-1DDP cell lines were gifts from M. Gottesman’s laboratory at the National Cancer Institute (USA). The A549 cell line was purchased from Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences/Peking Union Medical College (Beijing, China). Following the protocol developed by Gottesman (17), the resistant derivatives A549DDP were selected by culturing parental A549 cells with stepwise increases in cisplatin for several months. The BEL7404 and BEL7404DDP cell lines were cultured in RPMI-1640 medium. The A549, A549DDP, KB-3-1, and KB-3-1DDP cell lines were cultured in DMEM. All the cancer cells were supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin. Cisplatin (5 μM) was added to the BEL7404DDP, A549DDP, and KB-3-1DDP culture medium to maintain cisplatin resistance, and the cells were passaged twice to avoid residual cisplatin bound to the cells before use in experiments. The cancer cells were cultured in a three-gas incubator (Biospherix C21) with 5% CO₂ at 37°C.

**Intracellular hypoxia detection**

A hypoxia/oxidative stress detection kit (Enzo Life Sciences) was used to detect the level of hypoxia generated inside cells. Briefly, cancer cells were seeded into an eight-well glass bottom plate with a density of 5 × 10⁴ cells per well. At 80% confluency, the culture medium was removed, and the cancer cells were left untreated or incubated with different liposome formulations at a Pt-equivalent concentration of 10 μM for 2 hours. Then, the cells were washed in PBS twice, and the hypoxia/oxidative stress detection mix was added to the cells (according to the manufacturer’s instruction) for 30 min of incubation. The nuclei were stained by Hoechst 33342. Last, the cells were washed in PBS twice and observed through a CLSM. Positive controls, i.e., cells treated with the hypoxia inducer from the kit, were also included in the experiment according to the manufacturer’s instruction. The presence of hypoxia and Hoechst 33342 was detected by CLSM with excitation/emission wavelengths at 543/595 ± 20 nm and 405/455 ± 20 nm, respectively.

**Immunofluorescence of cells and organoids**

The medium was replaced with fresh medium, and cells were fixed by 95% ethanol at 4°C for 15 min. The cells were blocked with 5% milk for 2 hours and incubated overnight with primary antibodies against HIF-1α or XPF. The samples were washed five times with PBS and then incubated with secondary antibodies at room temperature for 1 hour. Actin was stained by ActinRed 555 Ready Probes (Thermo Fisher Scientific, R37112) and observed by CLSM. Organoids were fixed with 4% paraformaldehyde at room temperature for 20 min and blocked in Dulbecco’s PBS ( Gibco, 14190-250) with 0.3% Triton X-100 and 5% horse serum at room temperature for 1 hour. Samples were then incubated with primary antibody at 37°C for 2 hours and secondary antibodies at 37°C for 1 hour. The primary antibodies were HIF-1α rabbit antibody (1:200 dilution) and XPF rabbit antibody (1:500 dilution). The secondary antibody was Alexa Fluor 488–labeled goat anti-rabbit IgG(H+L) (1:200 dilution). Nuclei were counterstained by 4’,6-diamidino-2-phenylindole (1:100, Beyotime, C1002) for 30 min at room temperature.

**Western blotting**

Western blotting was used to investigate protein expression levels. After different treatments, cells were washed twice with cold PBS and incubated with radioimmunoprecipitation assay (RIPA) lysis buffer containing protease inhibitors in an ice-cold bath for 30 min to release the proteins. The lysed cells were collected and then subjected to ultrasonic pulverization in an ice-cold bath for 20 s. The lysate was centrifuged at 12,000g at 4°C for 15 min, and the supernatant was quantified and mixed with loading buffer. Samples containing equivalent amounts of protein were separated by SDS–polyacrylamide gel electrophoresis, and the bands were transferred to cellulose nitrate membrane and blocked with 5% nonfat milk in TBST (25 mM tris, 137 mM NaCl, 3 mM KCl, and 0.1% Tween 20, pH 7.4) for 2 hours. Membranes were incubated with primary antibodies against HIF-1α, XPF, and β-actin overnight. After washing five times with TBST (5 min per wash), the membranes were incubated with secondary antibody for 1 hour at room temperature and then imaged with a Bio-Red ChemiDoc Touch Imaging System.

**MitoXpress Kit**

The MitoXpress Kit (Cayman Chemical) was used to investigate the oxygen consumption rate of liposomes according to the manufacturer’s protocol. Briefly, 150 μl of glucose solution (1 mg/ml) and 15 μl of MitoXpress probe were placed in a 96-well plate. Then, the indicated formulations were added and covered with oil. The MitoXpress probe generates a fluorescent signal as oxygen is consumed. The fluorescence in each well was detected by a microplate reader using time-resolved fluorescence mode with excitation/emission wavelengths of 380/650 nm every 30 s.

**Portable dissolved oxygen meter**

A portable dissolved oxygen meter was also used to investigate the oxygen consumption capacity of liposomes according to the manufacturer’s protocol. First, a 50-ml sample tube was prepared with 4 ml of glucose solution (1 mg/ml), and the indicated formulations and free GOx were added at an equivalent enzyme activity of 125 U/ml. An HI-2400 dissolved oxygen meter was used to measure the O₂ consumption in real time, and the oxygen concentration was recorded every 30 s for about 900 s in total. The O₂ consumption of different concentrations of GOx/TPZ@Lipo-Pt (enzyme activity: 0, 50, 125, 250, and 500 mU/ml) was also investigated.

**Drug release**

The drug release assay was conducted by dialyzing (molecular weight cutoff = 3500) 500 μl of GOx/TPZ@Lipo-Pt (5 mg/ml) in 13.5 ml of buffered solution (PBS or PBS + 10 mM DTT) and gently shaken at 100 rpm in a shaker at 37°C. At predetermined time intervals, 300 μl of external buffer solution was taken out and replaced with fresh buffer solution. The UV absorption intensity of the released TPZ was measured by a microplate reader at the absorption wavelength of 475 nm. The concentration of Pt released was analyzed by ICP-MS.

**CCK-8 assay**

Cisplatin-sensitive tumor cells (BEL7404, A549, and KB-3-1) were grown in 96-well plates at a density of 7 × 10³ cells per well, and
cisplatin-resistant tumor cells (BEL7404DDP, A549DDP, and KB-3-1DDP) were seeded in 96-well plates at a density of 1 × 10^4 cells per well in 100 μl of medium. After incubation overnight, the medium was replaced with fresh medium containing different liposomes at certain concentrations. Another 24-hour incubation was performed, and then 10 μl of CCK-8 was added to every well and incubated for 2 hours. Next, the plate was shaken for 15 s at 37°C, and the absorbance at 450 nm was recorded by microplate reader.

Aptosis analysis
Cancer cells were seeded in six-well plates and cultivated with various liposomes or cisplatin at a certain concentration for 24 hours. Then, cells were harvested from the plates, washed with PBS, and collected by centrifugation at 1000 rpm for 3 min. Subsequently, 5 μl of FITC-annexin V, 5 μl of propidium iodide (PI), and 500 μl of binding buffer were mixed with each cell suspension for 15 min in the dark. Last, flow cytometry was used to quantitatively determine the apoptotic stages.

Animals and tumor models
Female NOD/SCID mice and BALB/c nude mice (~18 g, 5 weeks) were bought from Beijing Vital River Laboratories. The care and treatment of animals was conducted with the approval of the Animal Ethics Committee of Guangzhou Medical University (Guangzhou, China).

For drug-resistant cell–derived xenograft tumors, cisplatin-resistant BEL7404DDP liver cancer cells were suspended in 100 μl of PBS and Matrigel (Corning, USA) at a 1:1 ratio (v/v), and then 10^5 cells were subcutaneously injected into the right flanks of NOD/SCID mice.

Patient-derived tumor of hepatocellular carcinoma was freshly planted into the subcutaneous of BALB/c nude mice. Three generations were transplanted, and the fourth was used for experiments.

In vivo combination therapy
A subcutaneous liver cancer model was established in mice to evaluate the synergistic effect of therapy. A total of 10^5 cisplatin-resistant BEL7404DDP liver cells mixed with Matrigel were injected into the right flanks of NOD/SCID mice. When the tumor volume reached ~30 mm^3, 16 mice were randomly divided into four groups. Lipo-Pt, TPZ@Lipo-Pt, GOx/TPZ@Lipo, and GOx/TPZ@Lipo-Pt (equivalent to 1.03 mg/kg Pt) were intravenously injected through the tail vein every 3 days for a total of three times. Mouse weight and tumor volume were measured twice every week. The tumor volume was measured by Vernier caliper and calculated using the following formula: V = (a × b^2)/2, where a refers to the tumor length and b refers to the tumor width. The mice were euthanized at the end of therapy, and the tumors were collected.

BALB/c nude mice bearing subcutaneous PDX tumor were randomly divided into five groups (five mice per group) when the tumor volume reached ~100 mm^3: (i) intravenously injected with PBS, (ii) intravenously injected with Lipo-Pt, (iii) intravenously injected with TPZ@Lipo-Pt, (iv) intravenously injected with GOx/TPZ@Lipo, and (v) intravenously injected with GOx/TPZ@Lipo-Pt. The dose of Pt was kept at 1.03 mg/kg body weight. In vivo real-time PA imaging was performed before and 2 hours after the first injection to detect the hypoxia level in the tumor. Mice were intravenously injected every 2 days, and the tumor volume and body weight were measured every 2 days.

Statistical analysis
GraphPad Prism 7.0 software (GraphPad Software) was used for statistical analysis. All the results are expressed as means ± SD. Statistical differences were determined by two-tailed unpaired Student’s t test: *P < 0.05, **P < 0.01, and ***P < 0.001.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/13/eabc5267/DC1

View/request a protocol for this paper from Bio-protocol.

REFERENCES AND NOTES
1. D. Chen, Y. Zhou, C. H. Chui, K. H. Lam, S. Law, A. S. Chan, X. Li, A. K. Lam, J. C. O. Tang. Expression of insulin-like growth factor binding protein-5 (IGF5BP5) reverses cisplatin-resistance in esophageal carcinoma. Cell 7, 143 (2018).
2. Z. C. Liu, K. Cao, Z. H. Xiao, L. Qiao, X. Q. Wang, B. Shang, Y. Jia, Z. Wang, VRK1 promotes cisplatin resistance by up-regulating c-MYC via c-Jun activation and serves as a therapeutic target in esophageal squamous cell carcinoma. Oncotarget 8, 65654–65658 (2017).
3. R. Argawal, S. B. Kaye. Ovarian cancer: Strategies for overcoming resistance to chemotherapy. Nat. Rev. Cancer 3, 502–516 (2003).
4. L. P. Martin, T. C. Hamilton, R. J. Schilder. Platinum resistance: The role of DNA repair pathways. Clin. Cancer Res. 14, 1291–1295 (2008).
5. H. Farmer, N. McCabe, C. J. Lord, A. N. Tutt, D. A. Johnson, T. B. Richardson, M. Santarosa, K. J. Dillion, J. Hickson, C. Knights, N. M. Martin, S. P. Jackson, G. C. Smith, A. Ashworth, Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921 (2005).
6. D. J. Hutchison. Cross resistance and collateral sensitivity studies in cancer chemotherapy. Adv. Cancer Res. 7, 235–250 (1963).
7. L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castero, G. Kroemer. Molecular mechanisms of cisplatin resistance. Oncogene 31, 1869–1883 (2012).
8. R. G. Bristow, R. P. Hill. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. Nat. Rev. Cancer 8, 180–192 (2008).
9. J. M. Brown, W. R. Wilson. Exploiting tumour hypoxia in cancer treatment. Nat. Rev. Cancer 4, 437–447 (2004).
10. J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad. Cancer nanomedicine: Progress, challenges and opportunities. Nat. Rev. Cancer 17, 20–37 (2017).
11. B. Thienpont, J. Steinbacher, H. Zhao, F. D’Anna, A. Kuchnio, A. Ploumakis, B. Ghesquiere, L. Van Dyck, B. Boeckx, L. Schoonjans, E. Hermans, F. Amant, V. N. Kristensen, K. Peng Koh, M. Mazzone, M. Coleman, T. Carell, P. Carmeliet, D. Lambrechts. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. Nature 537, 63–68 (2016).
12. D. Clever, R. Roychoudhuri, M. G. Constantiniades, M. H. Askene, M. Sukumar, C. A. Klebanoff, R. Eil, H. D. Hickman, Z. Yu, J. H. Pan, D. C. Palmer, A. T. Phan, J. Goulding, L. Gattinoni, A. W. Goldrath, Y. Belkaid, N. P. Restifo. Oxygen sensing by T cells establishes an immunologically tolerant metastatic niche. Cell 166, 1117–1131.e14 (2016).
13. D. M. Lewis, K. M. Park, V. Tang, Y. Xu, K. Pak, T. S. Eisinger-Mathason, M. C. Simon, S. Gerecht, Intratumoral oxygen gradients mediate sarcoma cell invasion. Proc. Natl. Acad. Sci. U.S.A. 113, 9292–9297 (2016).
14. Y. Liu, Y. Jiang, M. Zhang, Z. Tang, M. He, W. Bu, Modulating hypoxia via nanomaterials chemistry for efficient treatment of solid tumors. Acc. Chem. Res. 51, 2502–2511 (2018).
15. D.-w, Shen, S.-i, Akiyama, P. Schoelen, I. Pasant, M. M. Gottesman, Characterization of high-level cisplatin-resistant cell lines established from a human hepatoma cell line and human KB adenocarcinoma cells: Cross-resistance and protein changes. Br. J. Cancer 71, 676–683 (1995).
16. S. Xu, X. Zhu, C. Zhang, W. Huang, Y. Zhou, D. Yan, Oxygen and Pt(II) self-generating conjugate for synergistic photo-chemo therapy of hypoxic tumor. Nat. Commun. 9, 2053 (2018).
19. P. H. Maxwell, G. U. Dachs, J. M. Gleadle, L. G. Nicholls, A. L. Harris, I. J. Stratford, D. Hankinson, C. W. Pugh, P. J. Ratcliffe, Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc. Natl. Acad. Sci. U.S.A. 94, 8104–8109 (1997).

20. W. Wang, Y. Cheng, P. Yu, H. Wang, Y. Zhang, H. Xu, Q. Ye, A. Yuan, Y. Hu, J. Wu, Perfluorocarbon regulates the intratumoral environment to enhance hypoxia-based agent efficacy. Nat. Commun. 10, 1580 (2019).

21. L. R. Wang, X. Xue, X. M. Hu, M. Y. Wei, C. Q. Zhang, G. L. Ge, X. J. Liang, Structure-dependent mitochondrial dysfunction and hypoxia induced with single-walled carbon nanotubes. Small 10, 2859–2869 (2014).

22. X. Ma, L. H. Zhang, L. R. Wang, X. Xue, J. H. Sun, Y. Wu, G. Zou, X. Wu, P. C. Wang, W. G. Wang, J. J. Yin, K. Zheng, X. J. Liang, Single-walled carbon nanotubes alter cytochrome c electron transfer and modulate mitochondrial function. ACS Nano 6, 10486–10496 (2012).

23. G. H. Henriksen, D. R. Raman, L. P. Walker, R. M. Spanwick, Measurement of net fluxes of ammonium and nitrate at the surface of barley roots using ion-selective microelectrodes: II. Patterns of uptake along the root axis and evaluation of the microelectrode flux estimation technique. Plant Physiol 99, 734–747 (1992).

24. X. J. Liang, T. Finkel, D. W. Shen, J. J. Yin, A. Aszalos, M. M. Gottesman, SIRT1 contributes in part to cisplatin resistance in cancer cells by altering mitochondrial metabolism. Mol. Cancer Res. 6, 1499–1506 (2008).

25. N. Vasan, J. Baselga, D. M. Hyman, A view on drug resistance in cancer. Nature 575, 299–309 (2019).

26. Z. Wang, Z. Xu, G. Zhu, A platinum(ii) anticancer prodrug targeting nucleotide excision repair to overcome cisplatin resistance. Angew. Chem. Int. Ed. Engl. 55, 15564–15568 (2016).

27. M. Dabholkar, J. Vionnet, F. Bostick-Bruton, J. J. Yu, E. Reed, Messenger RNA levels of cytochrome c electron transfer and modulate mitochondrial function. Cancer Res. 59, 734–747 (1999).

28. X. Xu, L. Chen, J. Cui, J. Shi, J. Wu, P. W. Kantoff, J. Shen, H. C. Kim, J. Wolfram, C. Mu, W. Zhang, H. Liu, Y. Xie, A. Yuan, H. Chen, J. Wu, Y. Hu, Perfluorocarbon nanoparticles enhance reactive oxygen levels and tumour growth inhibition in photodynamic therapy. Nat. Commun. 6, 8785 (2015).

29. J. Chen, L. Liu, M. S. Motevali, X. Wu, H.-Y. Yang, X. Li, L. Han, A. Magrini, W. Guo, J. Chang, B. Massimo, X.-J. Liang, Light-triggered retention and cascaded therapy of albumin-based theranostic nanomedicines to alleviate tumor adaptive treatment tolerance. Adv. Funct. Mater. 28, 1707291 (2018).

30. X. Song, L. Feng, C. Liang, K. Yang, Z. Liu, Ultrasound triggered tumor oxygenation with oxygen-shuttle nanoperfluorocarbon to overcome hypoxia-associated resistance in cancer therapies. Nano Lett. 16, 6145–6153 (2016).