Targeting glutamine metabolism enhances responses to platinum-based chemotherapy in triple-negative breast cancers (TNBC)

Reprogramming of metabolic pathways, a hallmark of human cancer, results from a process in which cancer cells become dependent on specific metabolic pathways such as glutamine catabolism or glutaminolysis for growth and survival. Previous studies have demonstrated that triple-negative breast cancers (TNBC) may use glutamine as an extracellular nutrient source to generate lipids, proteins, and nucleic acids. Glutamine catabolism in cancer cells also contributes to the production of the antioxidant, glutathione (GSH), which is critical for redox homeostasis and for protection of cells from oxidative stress elicited by reactive oxygen species (ROS). Considering TNBC cell lines are often dependent on glutamine for growth and survival, we sought to determine whether targeting glutaminolysis with a small molecule inhibitor, CB-839, in combination with platinum-based chemotherapy drug, would elicit significant anti-tumor activity.

We first evaluated mRNA expression of glutaminase 1 (GLS1), a rate-limiting enzyme in glutaminolysis, in breast cancer tissues using the TCGA breast cancer cohort (n = 1005). We also evaluated GLS1 protein expression by immunohistochemistry in an independent breast cancer tissue cohort. Analyses indicated that higher GLS1 expression in breast carcinomas correlated with worse overall survival in both the TCGA cohort (P = 0.0375) (Fig. 1A) and the independent cohort (P = 0.005) (Fig. S1A, B). GLS1 protein expression in the independent cohort was positively associated with tumor grade (Fig. S1C) and was significantly higher in TNBC than in the other breast cancer subtypes (Fig. S1D). Elevated GLS1 protein expression in TNBCs (n = 71) showed a trend toward correlation with worse overall survival (HR = 2.258, P = 0.055, log-rank test) (Fig. S1E).

We next evaluated GLS1 expression by Western blot in a panel of breast cancer cell lines derived from different subtypes (Fig. S2A, B). The TNBC cell lines, except for MDA-MB-468, exhibited elevated GLS1 expression, and most of them were sensitive to glutamine depletion from the culture medium. CB-839 targets GLS1 in an allosteric and reversible fashion. We tested potency of CB-839 to kill three GLS1-high TNBC lines and a GLS1-low TNBC line, MDA-MB-468. Survival of the GLS1-high lines was profoundly inhibited by CB-839 (Fig. S2C). Conversely, replenishing culture medium with an antioxidant, N-acetyl cysteine (NAC), or a tricarboxylic acid (TCA) cycle intermediate, α-ketoglutarate (α-KG), abrogated CB-839-induced cytotoxicity (Fig. S2C).

Platinum-based chemotherapy is a current option for treating TNBC patients because many TNBCs harbor BRCA1/BRCA2 mutations, causing functional deficiency in DNA homologous recombination repair and rendering tumor cells susceptible to DNA chelating drugs such as carboplatin and cisplatin. However, with continued platinum chemotherapy, many TNBCs develop chemoresistance. Moreover, some TNBCs are intrinsically refractory to platinum drugs. Therefore, developing an effective and safe strategies to promote platinum responsiveness and to overcome platinum resistance are urgently needed.

Glutaminolysis not only replenishes intermediates in the TCA cycle and reductive carboxylation but also produces antioxidant GSH necessary for redox homeostasis. Through its highly reactive thiol groups, GSH detoxifies drugs by binding to and neutralizing xenobiotic agents, including platinum drugs. GSH can also attenuate drug-induced oxidative stress by scavenging excessive ROS induced by anti-cancer drugs. To test potential involvement of GSH in redox homeostasis in TNBC, we treated TNBC cells with a combination of carboplatin and CB-839 or with each individual drug. Effective reduction of intracellular GSH redox status, defined by the GSH/GSSG ratio, was identified in...
Figure 1  Therapeutic efficacy of the GLS inhibitor CB-839 in combination with carboplatin. (A) Association of GLS1 mRNA levels in primary breast cancers and probability of overall survival. Patient and tumor data were obtained from The Cancer Genome Atlas (TCGA) breast cancer database. Patients were stratified according to tumor GLS expression: GLS^{low} (n = 251) or GLS^{high} (n = 754),
TNBC cells treated with CB-839/carboplatin drug combination (Fig. 1B). Surviving cell colonies were evaluated concurrently, and GLS\textsuperscript{high} cancer cells were more sensitive to both CB-839 single agent and CB-839/carboplatin combination treatments than GLS\textsuperscript{low} cancer cells (Fig. 1C, S3A). The drug combination index (CI) evaluated by incubating breast cancer cells with various ratios of carboplatin/CB-839 indicated a synergistic drug combinatorial effect in GLS\textsuperscript{high} cells (Fig. S3B). Collectively, the data suggest that GLS inhibition may interfere with the capacity of tumor cells to generate antioxidant glutathione (GSH) which detoxifies platinum drugs and balances cellular redox state. Consequently, GLS inhibition enhances cytotoxic potency of platinum drugs.

We previously reported that CB-839 potently induced replication stress in ovarian tumors. Here, we assessed the effect of GLS1 inhibition on cell cycle progression and found that CB-839 induced a G1 phase arrest in GLS\textsuperscript{high} cells, likely preventing premature S phase entry. On the other hand, carboplatin single agent treatment caused an increase in the percentage of cells in S phase (Fig. 1D, S4A). These effects were accompanied by a concomitant increase in replication stress, reflected by increased γH2AX levels (Fig. 1E). To evaluate whether CB-839 impacted DNA synthesis during replication, we used a DNA fiber assay to quantify the length of pulse-labeled nucleoside fibers. CB-839 substantially decreased the velocity of DNA replication progression (Fig. 1F). We previously demonstrated that GLS inhibition by CB-839 reduced nucleotide pools. Based on the data in aggregates, it is plausible that the restrained nucleotides may delay replication fork progression and ultimately result in replication stress.

The in vivo efficacy of CB-839/carboplatin combination treatment was evaluated in two xenograft models of TNBC: HCC1806-GLS\textsuperscript{high} and MDA-MB-468-GLS\textsuperscript{low}. Mice with xenograft tumors were treated with CB-839 and carboplatin singly or in combination according to the treatment schedule illustrated in Figure 5A. Combination treatment with CB-839 and carboplatin markedly delayed tumor growth and enhanced survival in mice bearing HCC1806-GLS\textsuperscript{high} xenografts (Fig. 1G). However, MDA-MB-468-GLS\textsuperscript{low} xenograft tumors were sensitive to carboplatin single-agent treatment, and the addition of CB-839 yielded only a modestly increased therapeutic benefit (Fig. 1G).

To determine the effect of CB-839 on glutamine metabolic flux in TNBC xenograft tumors, we performed MS-based stable isotope-resolved metabolomics with [U-\textsuperscript{13}C\textsubscript{5}] glutamine to track glutamine metabolites in mice treated with CB-839 or vehicle control. Significant increases in m5 glutamine (denotes that all five carbons in the glutamine backbone were labeled by \textsuperscript{13}C) and decreases in m5 glutamate, m5 α-ketoglutarate, m4 fumarate, and m4 malate were observed in the CB-839-treated group (Fig. S5). Notably, CB-839 treatment also inhibited the production of m5 citrate and m3 malate, which are often generated through reductive carboxylation of glutamate, a metabolic pathway activated in hypoxic conditions or when tumor cell mitochondria are impaired.

Similar to breast TNBCs, ovarian high-grade serous carcinoma cells often develop resistance to platinum-based chemotherapy after initial treatment. Several teams, including our own, have reported chemoresistant ovarian cancers which are glutamine-dependent and become sensitive to glutamine-targeted therapy. Cancer genome sequencing studies have revealed that high-grade ovarian serous carcinoma and breast TNBC share common molecular features, including a high prevalence of TP53 mutations and increased chromosomal instability associated with aggressive clinical behaviors. Thus, both TNBC and advanced ovarian cancers can develop glutamine reliance, and targeting this metabolic vulnerability may impede tumor progression. CB-839 has been tested in multiple clinical studies, in combination with paclitaxel for advanced TNBC (NCT03057600) and in combination with anti-PD1 plus carboplatin or pemetrexed for non-small cell lung cancer (NCT04265534). Our current study supports the promise of glutamine catabolism inhibitor and platinum combination therapy, and these findings warrant further evaluation in clinical studies.

...
Author contributions

JH, YAS, CCC, AT, and CYH conducted experiments and analyzed data. JH, YAS, IMS, SG, and TLW provided critical insights, and conceived and designed the experiments. JH, YAS, CCC, and TLW wrote the manuscript. CYH provided biospecimens and pathology diagnoses. Lastly, TLW, IMS, and SG supervised and coordinated the work. All authors reviewed the manuscript, agreed with the results, and provided comments.

Conflict of interests

All authors declare that there are no potential conflicts of interest.

Funding

The study was supported by Ovarian Cancer Research Alliance and the Johns Hopkins-Allegheny Health Network Cancer Research Fund, United States.

Acknowledgements

We thank Calithera Biosciences (South San Francisco, CA) for providing CB-839 for in vitro and in vivo studies reported here. We thank Marina Gelman and Tianhe Li for critically reading the manuscript and giving valuable comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.02.009.

References

1. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16(10): 619–634.

2. DeBerardinis RJ, Cheng T. Q’s next: the diverse functions of glutamine in metabolism, cell biology and cancer. Oncogene. 2010;29(3):313–324.

3. Tutt A, Tovey H, Cheang MCU, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med. 2018;24(5):628–637.

4. Galuzzi L, Senovilla L, Vitale I, et al. Molecular mechanisms of cisplatin resistance. Oncogene. 2012;31(15):1869–1883.

5. Shen YA, Hong J, Asaka R, et al. Inhibition of the MYC-regulated glutaminase metabolic axis is an effective synthetic lethal approach for treating chemoresistant ovarian cancers. Cancer Res. 2020;80(20):4514–4526.

Jiaxin Honga,b,c, Yao-An Shenb,b,**, Chih-Yi Hsuh, Peng Huanga, Alicja Tomaszewskia,d, Edward Gabrielsona,b, Ie-Ming Shihb,d, Stephanie Gaillarda,d, Tian-Li Wanga,b,d,*

a Sidney Kimmel Comprehensive Cancer Center and Department of Oncology, Baltimore, MD 21231, USA
b Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
c Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, PR China
d Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

*Corresponding author. Department of Pathology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Room 306, CRB2, 1550 Orleans Street, Baltimore, MD 21231, USA.

**Corresponding author. Department of Pathology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Room 376, CRB2, 1550 Orleans Street, Baltimore, MD 21231, USA.

E-mail addresses: yaoan.shen@gmail.com (Y.-A. Shen), tlw@jhmi.edu (T.-L. Wang)

3 December 2021
Available online 21 March 2022