BMB
Reports

BMB Rep. 2022; 55(12): 645-650
www.bmbreports.org

Selective cytotoxicity of a novel mitochondrial complex I inhibitor, YK-135, against EMT-subtype gastric cancer cell lines due to impaired glycolytic capacity

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Epithelial-to-mesenchymal transition (EMT)-subtype gastric cancers have the worst prognosis due to their higher recurrence rate, higher probability of developing metastases and higher chemoresistance compared to those of other molecular subtypes. Pharmacologically actionable somatic mutations are rarely found in EMT-subtype gastric cancers, limiting the utility of targeted therapies. Here, we conducted a high-throughput chemical screen using 37 gastric cancer cell lines and 48,467 synthetic small-molecule compounds. We identified YK-135, a small-molecule compound that showed higher cytotoxicity toward EMT-subtype gastric cancer cell lines than toward non-EMT-subtype gastric cancer cell lines. YK-135 exerts its cytotoxic effects by inhibiting mitochondrial complex I activity and inducing AMP-activated protein kinase (AMPK)-mediated apoptosis. We found that the lower glycolytic capacity of the EMT-subtype gastric cancer cells confers synthetic lethality to the inhibition of mitochondrial complex I, possibly by failing to maintain energy homeostasis. Other well-known mitochondrial complex I inhibitors (e.g., rotenone and phenformin) mimic the efficacy of YK-135, supporting our results. These findings highlight mitochondrial complex I inhibitors as promising therapeutic agents for EMT-subtype gastric cancers and YK-135 as a novel chemical scaffold for further drug development. [BMB Reports 2022; 55(12): 645-650]

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https://doi.org/10.5483/BMBRep.2022.55.12.150

Received 26 September 2022, Revised 20 October 2022, Accepted 14 November 2022

Keywords: EMT, Gastric cancer, Glycolytic capacity, Mitochondrial complex I, OXPHOS

INTRODUCTION

Gastric cancer is the fifth most diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide (1). Advances in genomic technologies have enabled a better understanding of cancers by uncovering genetic heterogeneity that enabled molecular classifications (2-5) which can affect prognosis and therapeutic outcomes in gastric cancer. The Cancer Genome Atlas (TCGA) project classified gastric cancers into four molecular subtypes: tumors positive for Epstein-Barr virus (EBV), chromosomal instable tumors, tumors with microsatellite instability (MSI), and genomically stable (GS) tumors. Of these, the GS subtype is associated with epithelial-to-mesenchymal transition (EMT) features (2). Later, the EMT-activated gastric cancer subtype was consistently found in diverse cohorts of gastric cancer (4-6). The EMT-subtype gastric cancers are also chemoresistant due to their low proliferation rate (6). In addition, the rare druggable oncogenic mutations and low mutation burden of this subtype render targeted therapies and immunotherapies ineffective, respectively (7, 8). Consequently, there are vital unmet needs that demand the development of new therapeutic interventions targeting the EMT-subtype gastric cancer to improve the survival outcomes of these patients.

In the present study, we conducted a large-scale chemical screen using 37 gastric cancer cell lines and 48,467 synthetic small-molecule chemical compounds to identify novel drug candidates with selective cytotoxicity against EMT-subtype gastric cancer cell lines. We further investigated the molecular targets and the underlying selectivity mechanisms of one of the hit compounds, YK-135. This study highlights the altered central energy metabolism as a synthetic lethal target pathway for the selective intervention of EMT-subtype gastric cancers. Hence, the findings from this study provide a novel precision drug candidate for further drug development.

ISSN: 1976-670X (electronic edition)

RESULTS

Large-scale chemical screening identified small-molecule compounds selectively targeting EMT-subtype gastric cancer cell lines

Previously, we classified a panel of 29 gastric cancer cell lines into intrinsic molecular subtypes by analyzing whole exome sequencing and RNA sequencing data (9). Here, we have expanded this panel by adding eight additional gastric cancer cell lines, SNU5, SK-GT-2, IM95, MKN7, NUGC3, NUGC4, RERF-GC-1B, and SCH (Supplementary Table 1). We performed an unsupervised hierarchical clustering analysis based on expression levels of EMT signature genes (5, 9) and found that seven of the 37 gastric cancer cell lines exhibit robust EMT features (Fig. 1A). We then performed a high-throughput drug screen using 37 gastric cancer cell lines and 48,467 small-molecule compounds to identify compounds with selective cytotoxicity against EMT-subtype gastric cancer cell lines (Fig. 1B). The readout for our screening process was the cytotoxicity of each compound using the CellTiter-Go assay, which measures ATP concentration in each well of the screening plates. The measurements from this assay were highly correlated with the live cell counts measured by combined staining of nuclei with Hoechst and propidium iodide (PI) (Supplementary Fig. 1). A series of screening identified seven hit compounds (Fig. 1B, Supplementary Data 1) that had a wider selective margin between the EMT cancer cell lines and the non-EMT lines in a cumulative distribution function (AUC KS test $P \leq 0.05$ and KS distance (D) > 0.6) (Fig. 1C). Molecular structures of the final seven hit compounds are shown in Fig. 1D. Next, to prioritize hit compounds targeting the distinct molecular features of EMT-subtype gastric tumors, we conducted gene set enrichment analysis (GSEA) of the EMT-subtype gastric tumors vs. other gastric tumors using the TCGA dataset. We observed that mitochondrial respiration-related gene sets (especially mitochondrial respiratory chain complex assembly) were most substantially reduced in EMT-subtype gastric cancer patients compared to non-EMT-subtype (Fig. 1E and Supplementary Fig. 2). This observation suggested that reduced mitochondrial mass or activity may be a therapeutic vulnerability in the EMT-subtype gastric cancer. Thus, we evaluated the effects of the seven hit compounds on mitochondrial oxygen consumption rate (OCR) in SNU484, an EMT-subtype cell line. We observed that only YK-135 showed significant inhibition of mitochondrial respiration, which was similar to other known mitochondrial inhibitors (phenformin and rotenone) (Fig. 1F). We further validated the selective toxicity of YK-135 in 4 EMT (SNU484, MKN1, SNU668 and HGC27), 3 non-EMT (NCI-N87, MKN45 and SNU719) gastric cancer cell lines and 3 non-cancerous cell lines (THLE-2, CCD18CO and HK-2). YK-135 was confirmed to selectively exert cytotoxic effects on EMT-subtype gastric cancer cell lines (Fig. 1G).

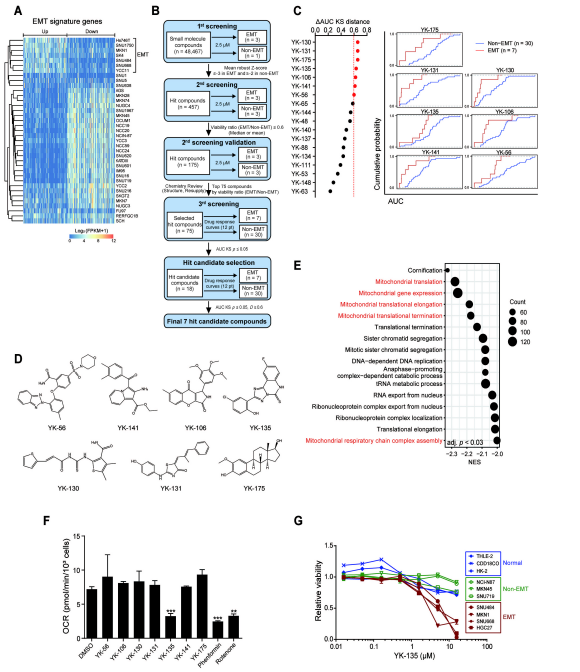

Fig. 1. A large-scale chemical screen identifies YK-135 as a selective cytotoxic agent against EMT-subtype gastric cancer cell lines. (A) Classification of the 37 gastric cancer cell lines according to their EMT signatures. Columns represent EMT signature genes (149 upregulated genes on the left, 161 downregulated genes on the right). (B) Screening workflow. All 48,467 chemicals in the library were screened once at a concentration of 2.5 μM in three EMT (Hs746T, SNU484 and SNU668) and one non-EMT (SNU719) cell lines. Four hundred fifty-seven molecules with selective response profiles were re-screened at 2.5 μM in six cell lines (3 EMT (Hs746T, SNU484 and SNU668) vs. 3 non-EMT (SNU719, MKN45 and NCC59)). One hundred seventy-five chemicals with selective viability were selected and re-assayed in the same conditions, followed by filtering by chemical structures or properties (e.g., availability of resupply and chemical structural similarity) (Supplementary Data 1). Seventy-five chemicals were re-screened in a multi-dose format (12-point dose responses) against a panel of 37 cell lines in singlicate. Nineteen chemicals with EMT-selective toxicity were selected and re-assayed across the 37 cell lines using 12 doses (half-log dilution series). (C) Dot plot of KS test distances of AUC in EMT cell lines compared to non-EMT cell lines against 18 selected chemicals (left). Red dots indicate selected 7 hit candidates (ΔAUC KS distance ≥ 0.6). Cumulative distribution function plots of 7 hit candidates demonstrating enrichment for AUC of EMT (red) and non-EMT (blue) cell lines (right). (D) Structures of the 7 hit candidate chemicals. (E) Dot plot of GSEA results illustrating GO gene sets decreased in EMT-type gastric tumor of TCGA stomach adenocarcinoma cohort. The figure shows the top 16 enriched GO terms with NES < -2.0 and adjusted P-value < 0.03. Red text indicates mitochondria-related gene sets. (F) Basal respiration measurement using a Seahorse bioanalyzer. All values are mean ± SD of triplicated experiments. P-values were determined by one-way ANOVA, followed by a Tukey multiple comparison test (**P < 0.01, ***P < 0.0001). (G) YK-135 dose-response in 4 EMT, 3 non-EMT-subtype gastric cancer cell lines and 3 normal cell lines.

YK-135 blocked mitochondrial respiration by inhibiting mitochondrial complex I activity

Our initial observation that YK-135 inhibited mitochondrial respiration in SNU484 led us to further examine the consequence of YK-135 treatment in an expanded gastric cancer cell line panel including two EMT- (SNU484 and HGC27) and two non-EMT-subtype gastric cancer cell lines (MKN45 and NCI-N87). Measuring the cellular oxygen consumption rate (OCR) using the Seahorse XF analyzer revealed a significant inhibition of OCR by YK-135 in all cell lines at concentrations of 5-10 μM (Fig. 2A), which was more potent than the biguanides (metformin or phenformin), as they usually inhibit OCR at millimolar concentrations (10). Next, to determine which mitochondrial complex YK-135 inhibits, we examined mitochondrial respiration in membrane-permeabilized cells by supplying either complex I or complex II substrate. In the complex I-linked respiration assay supplied with adenosine-5′-diphosphate (ADP) and the complex I substrates pyruvate and malate, YK-135 (10 μM) inhibited oxygen consumption in permeabilized SNU484 cells similar to the complex I inhibitor, rotenone (Fig. 2B, Left). Meanwhile, YK-135 had a minor effect on the oxygen consumption in the cells supplemented with ADP and the complex II substrate succinate, wherein mitochondrial complex II inhibitor, 2-thenoyltrifluoroacetone (TTFA), efficiently inhibited complex II-linked respiration (Fig. 2B, Right). YK-135 also dose-dependently inhibited the mitochondrial complex I enzyme activity in isolated mitochondria of SNU484 cells *in vitro* (Fig. 2C). In line with these observations, YK-135 mediated cytotoxicity was rescued by rosiglitazone, a PPAR-γ agonist and an inducer of mitochondrial biogenesis (Supplementary Fig. 3). Next, we compared YK-135 with other mitochondrial complex I inhibitors, phenformin and rotenone, with respect to the EMT-subtype selective cytotoxicity. This assay revealed that rotenone also showed significantly increased cytotoxicity against the EMT-subtype gastric cancer cell lines, although its therapeutic margin is narrower than YK-135 (Fig. 2D). This observation indicated that YK-135 is a novel mitochondrial complex I inhibitor displaying superior selective cytotoxicity toward EMT-subtype gastric cancer cell lines compared with other mitochondrial complex I inhibitors.

YK-135 selectively induced AMPK-mediated apoptosis in EMT-subtype gastric cancer cell lines

Biguanide-mediated inhibition of mitochondrial respiration activates AMP-activated protein kinase (AMPK), a major energy sensor in the cell, by increasing the intracellular AMP and ADP levels (11). YK-135 treatment activated AMPK, as demonstrated by increased phospho-AMPK (Thr172) levels, only in EMT-subtype gastric cancer cell lines (Fig. 3A), indicating that the ATP depletion in the EMT-subtype gastric cancer cell lines was more robust than that in the non-EMT cell lines. AMPK activation causes a cell cycle arrest and inhibits the synthesis of macromolecules required for the cell growth and proliferation (12), as evidenced by reduced S6K phosphorylation in

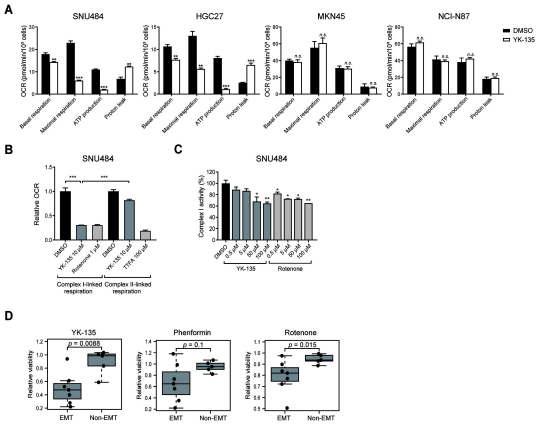

Fig. 2. YK-135 inhibits mitochondrial OXPHOS complex I activity. (A) Assessment of OXPHOS functions for YK-135. Basal, maximal, ATP-linked respiration and proton leak in 2 EMT (SNU484 and HGC27) and 2 non-EMT (MKN45 and NCI-N87) gastric cancer cell lines 24 hours post-YK-135 treatment by a Seahorse bioanalyzer. P-values were determined by Student's *t* test (**P < 0.01, ***P < 0.0001, n.s.: not significant). (B) SNU484 cells were treated with DMSO or YK-135 (10 μM) for 24 h. Cells were permeabilized using plasma membrane permeabilizer (PMP), and OCR was measured with sequential administration of a combination of pyruvate (10 mM), malate (0.5 mM), and ADP (4 mM) for complex I-linked respiration or rotenone (1 μM), succinate (10 mM) and ADP (4 mM) for complex II-linked respiration. (C) Complex I enzymatic activity was measured from mitochondrial fractions of SNU484 cells. Mitochondrial fractions were isolated and then treated with YK-135 or rotenone *in vitro*. (D) Selective sensitivity of OXPHOS inhibitors in 7 EMT (HGC27, SK4, SNU668, MKN1, YCC11, SNU484, and Hs746T) and 5 non-EMT cell lines (NCI-N87, MKN45, SNU719, SNU216, and NUGC4). Boxplots represent mean of relative viability 72 h post-treatment of YK-135 (5 μM), phenformin (0.63 mM) or rotenone (0.16 μM). P-values were determined by Student's *t* test.

EMT-subtype cell lines (Fig. 3A). Therefore, we next examined whether YK-135 selectively exerts anti-proliferative effects on EMT-subtype cancer cell lines. Our investigation revealed that YK-135 treatment induced G2/M cell cycle arrest specifically in EMT-subtype gastric cancer cell lines (HGC27 and SNU484) but not in non-EMT gastric cancer cell lines (MKN45 and NCI-N87) (Fig. 3B). It is known that the intact mitochondrial respiratory electron transport chain (ETC) generates an electrochemical proton gradient that establishes the mitochondrial membrane potential used by mitochondrial complex V to generate ATP. However, impaired mitochondrial ETC leads to a decreased mitochondrial membrane potential and increased harmful reactive oxygen species (ROS) generation, which also contributed to cell cycle arrest and cell proliferation inhibition (13). We, therefore, examined the effect of YK-135 on the mitochondrial membrane potential and mitochondrial ROS production. Upon YK-135 treatment, SNU484 and HGC27 cells, but not MKN45 and NCI-N87 cells, showed a decreased mitochondrial membrane potential (measured by the MitoTracker™ Red CMXRos dye staining) (Fig. 3C) and significantly increased

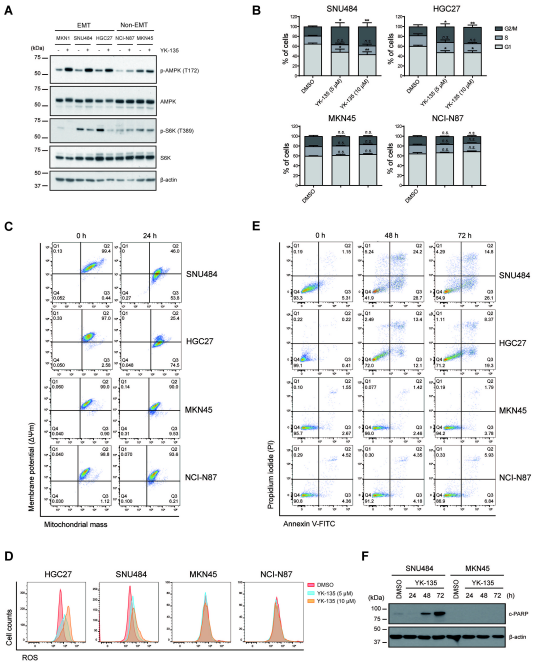

Fig. 3. YK-135 induces AMPK-mediated apoptosis in EMT-subtype gastric cancer cell lines. (A) YK-135-induced AMPK signaling was assessed by western blotting of whole-cell lysates from the indicated EMT and non-EMT cell lines. (B) Evaluation of cell cycle arrest by propidium iodide staining and flow cytometry analysis after YK-135 treatment for 24 h. Ten thousand cells were counted per analysis. Data represent mean ± SD from experiments in triplicate. P-values were determined by two-way ANOVA (*P < 0.05, **P < 0.01, n.s.: not significant). (C) Flow cytometry analysis of MitoTracker™ Red CMXRos and MitoTrackerTM Green FM-stained cells to detect changes in mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial mass induced by YK-135. (D) Representative flow cytometry histogram of MitoSOX staining in DMSO (light red), 5 μM YK-135 (light blue), and 10 μM YK-135 (orange). (E) Representative scatter plots of apoptosis analysis using Annexin V-FITC/PI dual staining and flow cytometry after YK-135 (5 μM) treatment for 48 and 72 h. (F) YK-135 induced apoptosis was assessed by western blotting using anti-cleaved PARP antibody in SNU484 and MKN45 cells post-incubation for indicated time points.

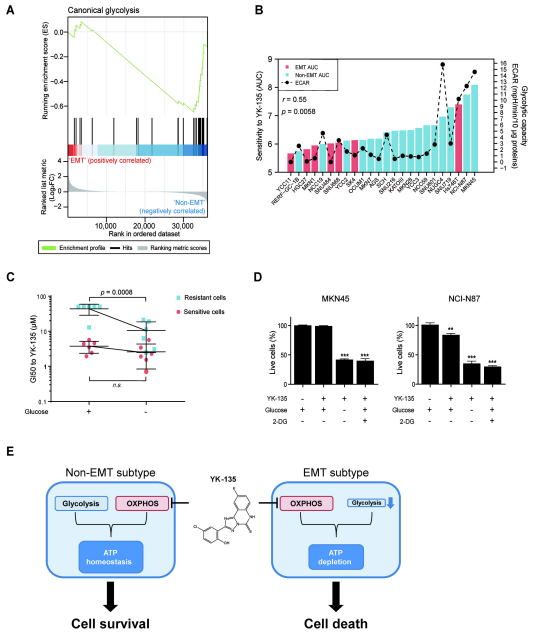

Fig. 4. Low glycolytic capacity confers hypersensitivity to YK-135. (A) Gene set enrichment analysis showed decreased canonical glycolysis gene set expression in the EMT-subtype gastric tumor patient cohort (NES = −1.46, P = 0.0481). (B) The graphs represent the correlation between AUC and glycolytic capacity. A Seahorse bioanalyzer assessed the glycolytic capacity in 24 gastric cancer cell lines. r, Spearman correlation coefficient. Spearman correlation test P-value is indicated. (C) YK-135 sensitivity in gastric cancer cell lines with/without glucose supplement. 50% growth inhibition (GI50) was calculated in sensitive (n = 6) and resistant (n = 6) cell lines 72 h-post YK-135 treatment. Unpaired Student's *t* test P-value is indicated (n.s.: not significant). (D) Inhibition of glycolysis sensitizes the resistant cell line, MKN45 and NCI-N87 against YK-135 (15.8 μM). Cell lines were treated with a non-lethal concentration of the glycolysis inhibitor 2-deoxyglucose (2-DG, 10 mM) with YK-135 for 72 h. P-values were determined by one-way ANOVA, followed by a Tukey multiple comparison test (**P < 0.01, ***P < 0.001). (E) Graphical summary to describe selective toxicity of YK-135 against EMT-subtype gastric cancer cell lines. The low glycolytic capacity of EMT-subtype gastric cancer cell lines generates synthetic lethality to OXPHOS inhibition.

ROS production (Fig. 3D). To further understand the consequence of mitochondrial dysfunction caused by YK-135 treatment in EMT-subtype gastric cancer cells, we examined its effect on cell death. Flow cytometry analysis using PI and Annexin V staining (Annexin V-FITC Apoptosis Kit) showed significant increases in cellular apoptosis 72 h post-YK-135 treatment, as compared with the vehicle control in SNU484 and HGC27 cells but not in MKN45 and NCI-N87 cells (Fig. 3E). We further examined the expression of cleaved PARP-1 (c-PARP), a hallmark of cellular apoptosis. We observed that YK-135 treatment-induced PARP-1 cleavage only in the EMT-subtype gastric cancer cells in a time-dependent manner (Fig. 3F). These data collectively indicated that YK-135 induced robust ATP depletion and consequently led to selective apoptotic cell death in EMT-subtype gastric cancer cell lines.

Low glycolytic capacity of EMT-subtype gastric cancer cells conferred hypersensitivity to YK-135

Since YK-135 treatment activates AMPK only in the EMT-subtype gastric cancer cells (Fig. 3A), we hypothesized that EMT-subtype cancer cells might have a defect in compensatory mechanisms for dealing with mitochondrial ATP depletion. In this regard, GSEA analysis revealed that the glycolysis-related gene set was significantly depleted in the EMT-subtype gastric tumors in the TCGA dataset (Fig. 4A). Since glycolysis is critical

for the survival of cancer cells in the presence of mitochondrial complex I inhibitor (14), we measured extracellular acidification rate (ECAR), an indicator of glycolysis, in 24 gastric cancer cell lines, and found that there was a positive correlation between glycolytic capacity and resistance to YK-135 (r = 0.55, P = 0.0058 by Spearman's rank correlation) (Fig. 4B). This result demonstrated that the gastric cancer cell lines that were sensitive to YK-135, including the six EMT-subtype gastric cancer cell lines, showed lower glycolytic capacity than that of the resistant cell lines. Notably, one outlier EMT-subtype gastric cancer cell line, Hs746T was relatively resistant to YK-135 but showed higher glycolytic capacity than other EMT-subtype gastric cancer cell lines (Fig. 4B). To determine whether defects in glycolysis caused the sensitivity to YK-135, we investigated the effect of glucose removal from the culture medium on YK-135-mediated cytotoxicity. A significant decrease in viability (50% growth inhibition, GI50) was observed only in YK-135-resistant gastric cancer cell lines by glucose removal (Fig. 4C). We further found that a glycolysis inhibitor, 2-deoxyglucose, significantly sensitized MKN45 and NCI-N87 cell lines to YK-135 treatment (Fig. 4D). These data indicated that the YK-135-dependent inhibition of mitochondrial complex I activity decreases cell proliferation and induces cell death in EMT-subtype gastric cancer cell lines by leveraging their low glycolytic capacity (Fig. 4E). These findings collectively highlight how mitochondrial complex I inhibitors can be promising targeted therapeutic agents for treating EMT-subtype gastric cancer patients and emphasize the role of YK-135 as a novel chemical scaffold for further therapy development.

DISCUSSION

The oxidative phosphorylation (OXPHOS) pathway plays a crucial role in cancer cells by providing ATP and metabolites to support rapid proliferation and migration. Thus, OXPHOS inhibition holds excellent potential for an anticancer strategy with many therapeutic opportunities. To date, the biguanide metformin which can act as a putative mitochondrial complex I inhibitor has been tried in multiple clinical trials as an anticancer agent in breast and colorectal cancers (14, 15). Beyond metformin, the more potent biguanide phenformin (for hepatocellular carcinoma (16)) and mitochondrial complex I inhibitors such as IACS-010759 (for Glioblastoma and AML (17)) have shown efficacy in clinical trials. In addition, other mitochondrial OXPHOS inhibitors, such as Gracillin (complex II inhibitor for non-small cell lung carcinoma (18)) and gboxin (complex V inhibitor for glioblastoma (19)), have shown anticancer efficacy in pre-clinical studies. Despite these successes, the use of OXPHOS inhibitors has some limitations. First, in the absence of any precise biomarker, these drugs are likely to exhibit some side effects toward non-cancerous cells with high-energy demands. Mitochondrial dysfunction or fragmentation by OXPHOS inhibitors induces a compensatory shift to glycolysis and pyruvate fermentation, resulting in secretion and accumulation of lactate leading to lactic acidosis (16, 20). Second, cancer cells exhibit flexibility in switching energy metabolism between glycolysis and OXPHOS to adapt to the tumor microenvironment, which causes resistance to OXPHOS inhibitor treatment. In the case of malignant lymphocytes, metabolic adaptation to biguanides is acquired by transcriptionally reprogramming glucose metabolism (21). Thus, identifying tumor subtypes that may benefit from OXPHOS inhibition is necessary to overcome the limitation of mitochondrial inhibitors in clinical applications.

Thus, our discovery that EMT-subtype gastric tumors and gastric cancer cell lines exhibit significantly reduced expression of the glycolysis-related genes and low glycolytic capacity, respectively, provides evidence supporting the utility of mitochondrial complex I inhibitors against EMT-subtype gastric cancers. However, of the various mitochondrial complex I inhibitors, YK-135 showed the highest selectivity against EMT-subtype gastric cancer cell lines, indicating that YK-135 has a unique pharmacological property differing from other mitochondrial complex I inhibitors. One of the possible modes of action of YK-135 explaining the higher selectivity to the EMT-subtype gastric cancer cell lines might be its partial inhibitory effect on mitochondrial complex II, as shown in Fig. 2B and Supplementary Fig. 4, since mitochondrial complex II can serve as a bypass when complex I is blocked. Despite the potent efficacy of YK-135 in gastric cancer cell lines, it has limited bioavailability and stability *in vivo*, preventing its immediate application in a mouse model (data not shown). Therefore, further chemical optimization of its pharmacokinetic properties is needed for this compound's further pre-clinical and clinical development.

In conclusion, the present study demonstrates that a novel mitochondrial complex I inhibitor, YK-135, exhibits specific cytotoxicity toward EMT-subtype gastric cancer cell lines exerted due to their impaired glycolytic capacity. This finding holds tremendous translational implications for synthetic lethal strategies against treatment-refractory EMT-subtype gastric cancers. Further studies are needed to improve the poor pharmacokinetic properties of YK-135 and to determine if YK-135 has similar effects in in other cancer types.

MATERIALS AND METHODS

Materials and methods are available in the Supplemental Information.

ACKNOWLEDGEMENTS

The chemical library used in this study was kindly provided by Korea Chemical Bank (http://www.chembank.org/) of Korea Research Institute of Chemical Technology (Daejeon, Korea).

This study was supported by grants from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (HI14C1324), the National Research Foundation of Korea (NRF) (2020R1A2C3007792 and 2022

R1A2B5B03001199), and the "Team Science Award" of Yonsei University College of Medicine (6-2021-0194). SBK was supported by the Brain Pool Program funded by the Ministry of Science and ICT through the NRF (2019H1D3A2A01050712). HK was supported by the Global Ph.D. Fellowship Program funded by the NRF (2019H1A2A1075632).

CONFLICTS OF INTEREST

HSK is a founder, chief scientific officer, and shareholder of Checkmate Therapeutics Inc. The authors have no other conflicting interests.

REFERENCES

1. Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71, 209-249
2. Cancer Genome Atlas Research Network (2014) Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513, 202-209
3. Chen EI, Hewel J, Krueger JS et al (2007) Adaptation of energy metabolism in breast cancer brain metastases. Cancer Res 67, 1472-1486
4. Cheong JH, Yang HK, Kim H et al (2018) Predictive test for chemotherapy response in resectable gastric cancer: a multi-cohort, retrospective analysis. Lancet Oncol 19, 629-638
5. Cristescu R, Lee J, Nebozhyn M et al (2015) Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 21, 449-456
6. Cho J, Lee HJ, Hwang SJ et al (2020) The interplay between slow-cycling, chemoresistant cancer cells and fibroblasts creates a proinflammatory niche for tumor progression. Cancer Res 80, 2257-2272
7. Sholl LM, Hirsch FR, Hwang D et al (2020) The promises and challenges of tumor mutation burden as an immunotherapy biomarker: a perspective from the international association for the study of lung cancer pathology committee. J Thorac Oncol 15, 1409-1424
8. Lin C, Shi X, Zhao J et al (2020) Tumor mutation burden correlates with efficacy of chemotherapy/targeted therapy in advanced non-small cell lung cancer. Front Oncol 10, 480
9. Lee J, Kim H, Lee JE et al (2018) Selective cytotoxicity of the NAMPT inhibitor FK866 toward gastric cancer cells with markers of the epithelial-mesenchymal transition, due to loss of NAPRT. Gastroenterology 155, 799-814 e713
10. Bridges HR, Jones AJ, Pollak MN and Hirst J (2014) Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. Biochem J 462, 475-487
11. Hardie DG (2013) AMPK: a target for drugs and natural products with effects on both diabetes and cancer. Diabetes 62, 2164-2172
12. Motoshima H, Goldstein BJ, Igata M and Araki E (2006) AMPK and cell proliferation–AMPK as a therapeutic target for atherosclerosis and cancer. J Physiol 574, 63-71
13. Maharjan S, Oku M, Tsuda M, Hoseki J and Sakai Y (2014) Mitochondrial impairment triggers cytosolic oxidative stress and cell death following proteasome inhibition. Sci Rep 4, 5896
14. Wheaton WW, Weinberg SE, Hamanaka RB et al (2014) Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. eLife 3, e02242
15. Andrzejewski S, Gravel SP, Pollak M and St-Pierre J (2014) Metformin directly acts on mitochondria to alter cellular bioenergetics. Cancer Metab 2, 12
16. Veiga SR, Ge X, Mercer CA et al (2018) Phenformin-induced mitochondrial dysfunction sensitizes hepatocellular carcinoma for dual inhibition of mTOR. Clin Cancer Res 24, 3767-3780
17. Molina JR, Sun Y, Protopopova M et al (2018) An inhibitor of oxidative phosphorylation exploits cancer vulnerability. Nat Med 24, 1036-1046
18. Min HY, Jang HJ, Park KH et al (2019) The natural compound gracillin exerts potent antitumor activity by targeting mitochondrial complex II. Cell Death Dis 10, 810
19. Shi Y, Lim SK, Liang Q et al (2019) Gboxin is an oxidative phosphorylation inhibitor that targets glioblastoma. Nature 567, 341-346
20. Ball S, Woods HF and Alberti KG (1974) Lacticacidosis, ketoacidosis, and hyperalaninaemia in a phenformintreated diabetic patient. Br Med J 4, 699-700
21. Khan H, Anshu A, Prasad A et al (2019) Metabolic rewiring in response to biguanides is mediated by mROS/HIF-1a in malignant lymphocytes. Cell Rep 29, 3009-3018. e4