A Small-Molecule Inhibitor of Glucose Transporter 1 Downregulates Glycolysis, Induces Cell-Cycle Arrest, and Inhibits Cancer Cell Growth *In Vitro* and *In Vivo*

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Abstract

The functional and therapeutic importance of the Warburg effect is increasingly recognized, and glycolysis has become a target of anticancer strategies. We recently reported the identification of a group of novel small compounds that inhibit basal glucose transport and reduce cancer cell growth by a glucose deprivation–like mechanism. We hypothesized that the compounds target Glut1 and are efficacious *in vivo* as anticancer agents. Here, we report that a novel representative compound WZB117 not only inhibited cell growth in cancer cell lines but also inhibited cancer growth in a nude mouse model. Daily intraperitoneal injection of WZB117 at 10 mg/kg resulted in a more than 70% reduction in the size of human lung cancer of A549 cell origin. Mechanism studies showed that WZB117 inhibited glucose transport in human red blood cells (RBC), which express Glut1 as their sole glucose transporter. Cancer cell treatment with WZB117 led to decreases in levels of Glut1 protein, intracellular ATP, and glycolytic enzymes. All these changes were followed by increase in ATP-sensing enzyme AMP-activated protein kinase (AMPK) and declines in cyclin E2 as well as phosphorylated retinoblastoma, resulting in cell-cycle arrest, senescence, and necrosis. Addition of extracellular ATP rescued compound-treated cancer cells, suggesting that the reduction of intracellular ATP plays an important role in the anticancer mechanism of the molecule. Senescence induction and the essential role of ATP were reported for the first time in Glut1 inhibitor–treated cancer cells. Thus, WZB117 is a prototype for further development of anticancer therapeutics targeting Glut1-mediated glucose transport and glucose metabolism.

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Introduction

The Warburg effect (1–3), or upregulated glycolysis, recently has been intensively studied and recognized as one of the critical missing pieces of the puzzle for understanding cancer and formulating more effective anticancer strategies (4–6). Almost all cancers analyzed upregulated glucose transport and aerobic glycolysis regardless of their oxygen status (7, 8). Furthermore, cancer cells were found to be addicted to glucose and very sensitive to glucose concentration changes (7, 9). Glucose deprivation is sufficient to induce growth inhibition and cell death in cancer cells (10–12). The increased glucose transport in cancer cells has been attributed primarily to the upregulation of glucose transporter 1 (Glut1), 1 of the more than 10 glucose transporters that are responsible for basal glucose transport in almost all cell types (13, 14). Glut1 has not been targeted until very recently due to the lack of potent and selective inhibitors. First, Glut1 antibodies were shown to inhibit cancer cell growth (15). Other Glut1 inhibitors and glucose transport inhibitors, such as fasentin (16) and phloretin (17), were also shown to be effective in reducing cancer cell growth. A group of inhibitors of glucose transporters has been recently identified with IC50 values lower than 20 μmol/L for inhibiting cancer cell growth (18). However, no animal or detailed mechanism studies have been reported with these inhibitors.

Recently, a small molecule named STF-31 was identified that selectively targets the von Hippel-Lindau (VHL)-deficient kidney cancer cells (19). STF-31 inhibits VHL-deficient cancer cells by inhibiting Glut1. It was further shown that daily intraperitoneal injection of a soluble analogue of STF-31 effectively reduced the growth of tumors of VHL-deficient cancer cells grafted on nude mice (19). On the other hand, STF-31 appears to be an inhibitor with a narrow cell target spectrum.

We recently reported the identification of a novel group of small compounds that inhibit glucose transport and cell...
growth in several cancer cell lines and these 2 inhibitory activities were correlated (20). We further showed that these compounds inhibit cancer cell growth using a glucose deprivation–like mechanism (20). The compounds’ inhibitory activities were greater than most of the other Glut1-inhibitory agents reported. On the basis of these activities and the universal presence of Glut1 in cancer cells, we hypothesized that these novel compounds downregulate glucose transport and glycolysis by inhibiting Glut1 and that they should be effective in inhibiting cancer growth in vivo. To test these hypotheses, WZB117, a structural and functional analogue derived from the compounds used in our previous studies (21) with higher potencies, was used to treat multiple cancer cell lines and tumor-bearing nude mice to determine its in vivo anticancer efficacy and identify its inhibition target and anticancer mechanism.

Materials and Methods

Compound inhibitors and other chemicals

Compound WZB117 was synthesized as previously reported (20, 21). Compound solutions were freshly prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) before each experiment. Chemicals oligomycin, cisplatin, paclitaxel, and ATP were from Sigma-Aldrich.

Cell line, cell culture, and experimental controls

Human non–small cell lung cancer (NSCLC) cell lines H1299 and A549, human breast ductal carcinoma MCF7, as well as human nontumorigenic NL20 lung and MCF12A breast cells were purchased from American Type Culture Collection and were not authenticated. All these cells were maintained in American Type Culture Collection recommended cell culture media and conditions.

Cells were treated with compound WZB117 for 24 or 48 hours. WZB117 (10 μmol/L) was used in the experiments unless otherwise noted. Mock-treated and glucose deprivation samples served as negative and positive controls, respectively. In glucose deprivation, Dulbecco’s Modified Eagle’s Media (DMEM) with reduced glucose concentration (2 mmol/L or 8% of glucose concentration in the regular cell culture medium) was prepared by mixing glucose-free DMEM with regular DMEM.

Glucose uptake assay in cancer cells and in human red blood cells

The inhibitory activity of compounds on glucose transport was analyzed by measuring the cell uptake of 2-deoxy-d-18F-1H glucose as previously described (20, 21).

Similar procedure was used for glucose uptake assay in human red blood cells (RBC), except that RBCs were washed and collected by centrifugation at 2,000 × g for 5 minutes as they are suspension cells, and the treated RBCs were solubilized in 0.1% SDS before radioactivity was measured.

Cell proliferation (MTT) and clonogenic assays

Cell proliferation and viability rates were measured using the MTT Proliferation Assay Kit (Cayman) or clonogenic assays (21).

Hypoxia studies

Cancer cell study in hypoxia was conducted using the Anaerobe Gas Generating Pouch System with indicator (BD GasPak EZ). The pouch formed an oxygen-free environment in which the compound-treated cells were incubated for 24 hours. After the hypoxic incubation, the treated cells were measured for their viability by the MTT assay.

Animal study

Male NU/J nude mice of 6 to 8 weeks of age were purchased from The Jackson Laboratory and were fed with the Irradiated Teklad Global 19% protein rodent diet from Harlan Laboratories. To determine the in vivo anticancer efficacy of compound WZB117 on human tumor xenograft growth, NSCLC A549 cells in exponential growth phase were harvested, washed, precipitated, and resuspended in PBS. Each mouse was injected subcutaneously with 5 × 10⁶ cancer cells in the flank. Compound treatment started 3 days after the cancer cells injection and when all tumors became palpable. Tumor cell–injected mice were randomly divided into 2 groups: control group (n = 10) treated with PBS/DMSO (1:1, v/v) and WZB117 treatment group (n = 10) treated with WZB117 (10 mg/kg body weight) dissolved in PBS/DMSO solution (1:1, v/v). Mice were given intraperitoneal injection with either PBS/DMSO vehicle or compound WZB117 (10 mg/kg) daily for 10 weeks. Tumor sizes were measured every 7 days with calipers, and tumor volume (L × W²/2) was calculated and presented as means ± SEM. All of the procedures involved in animal study were conducted in conformation with the guidelines of both Ohio University (Athens, OH) and NIH.

Protein target studies I: RBC membrane vesicle preparation and glucose uptake assay

RBC and RBC-derived vesicles were prepared using published protocols (22) with minor modifications. The glucose uptake assay using sealed vesicles was similar to that in RBCs, except that the centrifugation was at 18,000 × g for 20 minutes to precipitate the vesicles after each washing step.

Protein target studies II: docking studies

A molecular model of WZB117 was constructed using Spartan 10 (Wavefunction Inc.; ref. 23). Following molecular mechanics energy minimization with the Merck molecular force field, the compound structures were exported to Macromodel (Schrodinger) and docked to the Glut1 homology modeled PDB structure 1SUK (24) Protein and grid preparations were conducted using the Glide module of FirstDiscovery 2.7 (Schrodinger) with default protocols (25) and centered in the middle of the
transport channel with the bounding box encompassing the entire channel. WZB117 was then docked using Glide, and the best docked structure for the compound was selected on the basis of the Glide-calculated E
\textsubscript{mode} value.

**Western blot analyses and RNA isolation and real-time PCR**

Western blot analyses were conducted using the standard protocol. Antibodies for Glut1 (H-43), eIF2α, and cyclophosphamide–Adriamycin–vincristine–prednisone (CHOP) were from Santa Cruz; PGAM1 antibody was from Novus Biologicals. Antibody for p-eIF2α was from Invitrogen. All other antibodies were from Cell Signaling.

RNA from treated A549 cells was isolated using RNeasy total RNA extraction kit (Qiagen), and cDNA was synthesized with the Bio-Rad iScript Select cDNA Synthesis Kit (Bio-Rad). The produced cDNA was used to specifically quantify the transcript of SLC2A1 (Glut1) using the Bio-Rad iCycler with the Bio-Rad iQ Sybr Green Supermix. The RT–PCR primer sets for human SLC2A1 and β-actin were from SuperArray. For quantifying transcript levels, 6C\textsubscript{m} method was used. β-actin mRNA was used as an internal control for normalizing Glut1 mRNA.

**Lactate and ATP measurements and ATP rescue study**

Extracellular lactate concentration was measured using the Lactate Assay Kit II (BioVision). Intracellular ATP concentration was measured using ATPlite luminescence ATP detection assay system from Perkin-Elmer. Briefly, cells were seeded at a density of 50,000 cells in each well of a 96-well plate. ATP levels were measured after 6, 12, and 24 hours of treatment. Protein concentration of cells in each well was determined for both lactate and ATP measurements for signal normalization.

In the cell rescue study, ATP of various concentrations were added in cell culture medium of cancer cells in 96-well plates with or without 30 μmol/L WZB117. Intracellular ATP levels and cell viability were measured by an MTT assay 24 hours after the treatment.

**Cell-cycle analysis and detection of apoptotic and necrotic cells**

Cell cycle was analyzed as previously described (20). For identification of apoptotic and necrotic cells among WZB117-treated A549 cells, the treated cells were stained with propidium iodide and Annexin V–FITC according to the manufacturer’s instructions (BD Pharmingen) and then subjected to flow cytometric analysis.

**Senescence study**

Senescence was examined by a senescence associate β-galactosidase (β-gal) assay kit (Cell Signaling) under microscope for both β-gal expression and enlarged cell morphology compared with untreated cells. Cells were photographed using a microscope (ECLIPSE E600; Nikon).

**Statistical analysis**

Samples were in triplicate or hexad in cell studies. Each experiment was repeated at least twice with the exception of the animal study. Data are reported as mean ± SD. Data were analyzed using one-way ANOVA. P ≤ 0.05 was considered statistically significant.

**Results and Discussion**

We previously showed that the human dietary compound α-PGG mimics insulin action by binding and activating the insulin receptor (IR), resulting in glucose uptake in target cells (26). We recently reported that α-PGG inhibits cancer cells through IR-mediated apoptosis in human colon cancer RKO cells (27). In the process of structural and function optimization for the anti-diabetes activity of PGG-derived compounds, numerous glucose transport inhibitory compounds were synthesized and tested in cancer cells (21, 28). These compounds inhibited cancer cell growth in a glucose deprivation–like manner (20). This study was done to determine the anticancer efficacy in vivo and identify the anticancer mechanism of the inhibitors using the compound WZB117.

Glucose uptake assays showed that WZB117 (Fig. 1A) inhibits glucose transport in cancer cells in a dose-dependent manner (Fig. 1B). It also revealed that the inhibition of glucose transport induced by WZB117 occurred within 1 minute after the assay started (Fig. 1C, second time point partially overlapped with the time point at 0), suggesting that the inhibitory activity is likely to be via a direct and fast mechanism. Cell viability assay showed that WZB117 inhibited cancer cell proliferation with an IC\textsubscript{50} of approximately 10 μmol/L (Supplementary Fig. S1A). The inhibitory activity of WZB117 on cancer cell growth was also confirmed with a clonogenic assay (Fig. 1D), which also indicates that the inhibition is irreversible in nature. WZB117 treatment resulted in significantly more cell growth inhibition in lung cancer A549 cells than in non-tumorigenic lung NL20 cells (Fig. 1E). Similar results were also observed in breast cancer MCF7 cells and their non-tumorigenic MCF12A cells (Supplementary Fig. S1B). When WZB117 was added to cancer cells grown under hypoxic conditions, more cell growth inhibition was observed than under normoxic conditions (Fig. 1F). These results suggested that cancer cells are very sensitive and vulnerable to biologic changes under hypoxic conditions, which further sensitized cancer cells to the glucose transport inhibitor WZB117. Synergistic anticancer effects between WZB117 and anticancer drug cisplatin or paclitaxel were also observed (Supplementary Fig. S1C).

After showing the anticancer activity of WZB117 in cultured cancer cells, we went on to address the question whether WZB117 inhibits cancer growth in animal tumor models. The animal study showed that after daily intraperitoneal injection of WZB117 at 10 mg/kg body weight, the sizes of the compound-treated tumors were on average more than 70% smaller than those of the mock (PBS/DMSO)-treated tumors (Fig. 2A and B).
Notably, 2 of the 10 compound-treated tumors disappeared during the treatment and never grew back even at the end of the study (Fig. 2B). Body weight measurement and analysis revealed that the mice treated with WZB117 lost about 1 to 2 grams of body weight compared with the mock-treated mice (Supplementary Fig. S2A). With most of the weight loss in the fat tissue (Supplementary Table S1). Blood counts and analysis of mice at the end of the study showed that lymphocytes and platelets were changed in the compound-treated mice compared with the vehicle-treated mice, but the cell counts remained in the normal ranges (Supplementary Table S2). One of the concerns for using glucose transport inhibitors was that the inhibitor might produce hyperglycemia in the treated mice. It was found that a single injection of WZB117 produced only mild and temporary hyperglycemia that disappeared 1 to 2 hours after the compound injection without generating persistent hyperglycemia (Supplementary Fig. S2B and unpublished observations). The relatively high anticancer efficacy and relatively low toxicity of WZB117 observed in animals may be partially explained by cancer cells’ higher sensitivity and vulnerability to glucose concentration changes induced by WZB117 than normal cells (Fig. 1E) and by cancer cells’ sensitivity to glucose transport inhibition under hypoxia conditions (Fig. 1F), in which a majority of cancer cells were growing in animals.

We previously found that our small-molecule inhibitors of glucose transport inhibited glucose transport in all the cancer cell lines tested internally. With additional data (Fig. 1C), we speculated that the target of these inhibitors is Glut1, as Glut1 is responsible for basal glucose transport in almost all cell types (13), and Glut1 was upregulated in many cancer cells tested (8, 20). To test this hypothesis, RBCs were chosen as a cell model for determining whether Glut1 is the target of compound WZB117 because RBCs express Glut1 as their sole glucose transporter (29) and are an established model for...
As expected, WZB117 indeed inhibited glucose transport across the cell membrane, Glut1 located on either IOV or ROV should transport glucose in both directions across the cell membrane, Glut1 located on either IOV or ROV should be able to transport glucose down the glucose gradient. As expected, WZB117 indeed inhibited glucose transport in both IOV and ROV (Fig. 3B and C), strongly supporting the hypothesis that Glut1 is the target of WZB117.

To find additional evidence for direct WZB117-Glut1 interactions, ligand docking studies were conducted using the Glide module of FirstDiscovery 2.7 (Schrödinger). Glide conducts flexible ligand docking to a rigid receptor using a grid-based docking method and scoring function (23). The docking study revealed that the binding of WZB117 to Glut1 involved 3 hydrogen bonds, one each with Asn34, Arg126, and Trp412 (Fig. 3D). These amino acid residues are located in the central channel region of Glut1.

After showing that Glut1 was very likely to be the target of WZB117, we went on to determine the sequence of molecular events of WZB117 treatment on glycolysis. Real-time quantitative reverse transcription PCR (RT-qPCR) and Western blot analysis of Glut1 revealed that similar to the glucose deprivation control, the level of Glut1 mRNA was upregulated 24 hours after the treatment (Fig. 4A), whereas Glut1 protein level was decreased by the WZB117 treatment as early as 12 hours (Fig. 4B). These apparently inconsistent results could be explained thus: Inhibition of glucose transport by WZB117 decreased glucose supply to cancer cells, resulting in an urgent requirement for increasing glucose import and the upregulation of Glut1 mRNA level. However, because of the limited supply of glucose required for the processing of glycogen and membrane-bound proteins including Glut1, Glut1 protein levels were not increased. The possibility of the involvement of other mechanisms such as AMP-activated protein kinase (AMPK)/mTOR signaling-mediated arrest of protein synthesis cannot be ruled out.

The addition of WZB117 to cancer cells led to reduction of extracellular lactate levels (Fig. 4C) and intracellular ATP (Fig. 4D) as early as 6 to 12 hours after the WZB117 treatment with a further decline at 24 hours. Importantly, addition of extracellular ATP to the cell culture media at the time of compound addition significantly increased intracellular ATP levels (Supplementary Fig. S3A) and rescued the compound-treated cancer cells 24 hours after the treatment (Fig. 4E). If the addition of extracellular ATP was delayed for 12 hours or longer, the ATP started losing its rescue ability (Supplementary Fig. S3B). These results suggest that the reduced ATP level is largely responsible for the cancer cell inhibitory activity of the compound, at least for the first 24 hours of the compound treatment. The same ATP addition was ineffective in rescuing cancer cells treated by paclitaxel (Fig. 4E), a drug that inhibits cancer cells using a mechanism not directly involving ATP. Although ATP has been known to cross cell plasma membrane (33, 34), the mechanism by which ATP enters cells is not presently known. This is the first time that extracellular ATP is shown to be important in rescuing cancer cells with deprived glucose transport and glucose metabolism. Extracellular ATP may contribute to oncogenesis and cancer metabolism significantly more than previously thought.
Autophagy occurred as early as 6 hours after the compound treatment (Supplementary Fig. S3C), suggesting that autophagy and extracellular ATP might work together to rescue compound-treated cancer cells by providing needed biomaterial and energy, respectively.

Oligomycin, a specific mitochondrial inhibitor, did not reduce cell proliferation rate at a concentration of 50 nmol/L (data not shown). However, when WZB117 was used to treat A549 cells together with 50 nmol/L oligomycin, the presence of oligomycin further reduced the proliferation rate of A549 cells compared with the samples treated with WZB117 alone (Fig. 4F). This result indicates that at 50 nmol/L, an effective mitochondria-inhibitory dose (35), oligomycin alone did not change cell proliferation rate but sensitized cancer cells to the inhibitory activity of WZB117 (Fig. 4F). Mitochondria inhibition is known to upregulate glycolysis (35). These data suggest that when a Glut1/glycolysis inhibitor, WZB117 in this case, was added to oligomycin-treated cells, these cells were unable to compensate for oligomycin-induced inhibition of mitochondria with upregulating glycolysis. The double inhibitions further reduced the proliferation rate of the treated cancer cells. This result also shows that WZB117 is more effective in inhibiting cell proliferation in cells that have some degree of mitochondrial dysfunction and dependence on glycolysis such as cancer cells. This finding can also partially explain why WZB117 was more effective in inhibiting cancer cells than their noncancerous cell counterparts (Fig. 1E and Supplementary Fig. S1B).

Other key glycolytic enzymes, first rate-limiting hexokinase II (36), and cancer cell–specific PKM2 (37), were reduced at 6 and/or 12 hours but upregulated at 24 hours, whereas PGAM1 (38), an enzyme catalyzes an alternative step for pyruvate synthesis, was not affected by WZB117 treatment (Fig. 4G).

After identifying and characterizing some of the biologic and biochemical changes in cancer cells related to glucose transport and glucose metabolism, other cell growth, survival, and cell death processes were examined in WZB117-treated cancer cells to identify molecular participants and consequences of the treatment. Western blot analyses showed that key cell growth signaling proteins
such as Akt (39), mTOR (40), and AMPK (41) were affected by the compound treatment in ways similar to the changes found in glucose deprivation controls (Fig. 5A). Phosphorylated Akt and mTOR were found to decrease 6 and 12 hours after the compound treatment. Notably, the upregulation of phosphorylation of the energy (ATP) sensor AMPK coincided with the start of the decline in ATP levels (Fig. 4D) and with the time when extracellular ATP started to lose its rescue ability (Supplementary Fig. S3B). All these changes suggest that the treated cancer
cells responded to changes in glycolysis and energy status by downregulating phosphorylation levels of enzymes involved in cell growth signaling pathway and energy homeostasis. AMPK is very likely to act as the key link between the ATP reduction and the subsequent cancer cell inhibition.

An endoplasmic reticulum stress study showed that the protein level of GRP78/Bip, an endoplasmic reticulum stress marker (42, 43), steadily increased from 6 to 48 hours after WZB117 treatment. The onset of endoplasmic reticulum stress, as indicated by the start of the GRP78/Bip phosphorylation was accompanied by an elevation of GRP78/Bip, but CHOP expression was not increased to a detectable level (Fig. 5B). These results indicate that WZB117 induces endoplasmic reticulum stress, which likely leads to PKR-like endoplasmic reticulum kinase (PERK) activation and eIF2α phosphorylation (44). However, the stress level is not severe enough to induce CHOP expression and significant apoptosis as indicated by the very low level of PARP cleavage (Fig. 5C) and very small changes in the numbers of apoptotic cells in the treated cells (Fig. 6A, right). The role of endoplasmic reticulum stress played in the inhibition of WZB117-treated cancer cells is presently unclear.

WZB117 treatment led to approximately 30% and 50% reductions in cell proliferation rate 24 and 48 hours after compound treatment, respectively (Fig. 6A, left). Flow cytometric study, which used 10,000 cells regardless of the treatments, showed that WZB117 treatment resulted in 8% increase in necrosis at 48 hours [Fig. 6A, top right quadrant of (iv)] with only about 2% apoptosis [Fig. 6A, bottom right quadrant of (iv)]. Flow cytometric analysis revealed that WZB117 treatment led to cell-cycle arrest. WZB117 treatment resulted in approximately 23% and 4% more cells in G0–G1 and G2–M phases, respectively and approximately 30% less S-phase cells (Fig. 6B). This number, 30%, also matched with the MTT assay result at 24 hours [Fig. 6A, (ii) of left], indicating that at 24 hours, almost all the reduction in cancer cell proliferation was due to the cell-cycle arrest.

G0 arrest is known to be regulated by phosphorylated retinoblastoma (pRb), whose activity is regulated by its phosphorylation. The phosphorylation of Rb is regulated by cyclin-dependent kinase (CDK)2/cyclin E2 complex (45, 46). As expected, levels of CDK2 and cyclin E2 as well as pRb were decreased at the same time point (Fig. 6C). These changes were likely to be responsible for, at least in
part, the cell-cycle arrest. p16 is known to be involved in pRb and cell-cycle regulations. However, p16 gene is homozygously deleted in A549 cells and therefore plays no role in pRb and cell-cycle regulation of A549 cells (47). Additional but presently unknown mechanism may be involved in the regulation of pRb and cell-cycle arrest in A549 cells treated with WZB117.

Cell staining and observation revealed enlarged cell morphology and significant expression of β-gal, a widely used marker of cancer cell senescence (48), in A549 cells 24 hours after WZB117 treatment (Fig. 6D). Combining the β-gal expression and enlarged cell morphology with irreversible cell inhibition (Fig. 1D) and changes in phosphorylated Rb, common and important features of senescence (49, 50), it was concluded that WZB117-treated A549 cells became senescent concomitant to or following cell-cycle arrest. This is the first time that senescence was reported in cancer cells treated by a Glut1 inhibitor. One possible explanation for WZB117-treated cancer cells undergoing senescence and necrosis, rather than apoptosis, is that apoptosis is an ATP-utilizing process whereas necrosis and senescence are not. The compound treatment might deplete intracellular ATP so much that the cancer cells were unable to carry out apoptosis, forcing the cells to undergo senescence and necrosis (51).

Taken together, data reported here show that after the exposure to WZB117, cancer cells experienced an immediate reduction in glucose transport. Consequently, some
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Figure 7. A proposed mechanism for the anticancer activity of WZB117. Detailed description of the mechanism can be found in Results and Discussion. Solid arrows indicate cause-effect relationships supported by the experimental evidence of this study whereas the dotted arrows indicate speculative relationships. ER, endoplasmic reticulum.

key glycolytic enzymes and metabolites (ATP and lactate) were decreased in the first few hours. These led to changes in key enzymes, particularly AMPK in ATP sensing and energy homeostasis. All these changes culminated in cell-cycle arrest, accompanied by senescence and upregulation of some glycolytic enzymes, which is likely to be a response to senescence (52). Prolonged inhibition of glucose transport and reduction of glycolysis induced necrosis (19, 53), further inhibiting cancer cell growth. Cell-cycle G1 arrest, mediated by downregulation of cyclin E2 and phosphorylation of Rb, and subsequent senescence and necrosis were the major mechanisms underlying the inhibitory action of WZB117 on cancer cell growth. Reduced ATP levels appear to play an essential role in the WZB117-induced cancer cell inhibition in the first 24 hours of the compound treatment. Figure 7 graphically depicts the hypothetical mechanism and time sequence of molecular and cellular events described above. Both ATP reduction and senescence were described for the first time as potential anticancer mechanisms of Glut1 inhibitors.

All these data indicate that WZB117, a novel Glut1 inhibitor, is effective both in vitro and in vivo in inhibiting cancer cell growth and can serve as a prototypical compound for the further development of Glut1 and glucose transport inhibitors as a new group of anticancer therapeutics.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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