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Dietary D-mannose from fruits as a natural metabolic modulator against breast cancer: Targeting the Warburg effect for precision nutrition intervention

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ABSTRACT: Cancer cells' preferential reliance on glucose metabolism creates therapeutic opportunities for food-derived metabolic modulators. D-mannose, a glucose analog naturally abundant in cranberries, apples, and other common fruits, represents a promising nutritional intervention that can selectively target cancer metabolism while preserving normal cellular function. This study investigated the anticancer efficacy of fruit-derived D-mannose against breast cancer and elucidated its metabolic regulatory mechanisms using integrative approaches. Four molecularly distinct breast cancer cell lines were treated with D-mannose to evaluate cytotoxicity and apoptosis induction. Comprehensive metabolomics, ^{13}C -glucose flux analysis, and xenograft studies assessed central carbon metabolism reprogramming and therapeutic efficacy. D-mannose demonstrated subtype-specific anticancer activity while sparing normal breast epithelial cells. Metabolomic analysis revealed sophisticated multi-pathway disruption, including upstream glycolytic intermediate accumulation with dramatic lactate reduction, tricarboxylic acid cycle reprogramming, and pentose phosphate pathway bottlenecks that deprive cancer cells of biosynthetic precursors. ^{13}C -flux analysis confirmed impaired glucose utilization across metabolic networks, with cancer cells unable to compensate due to metabolic inflexibility. *In vivo* validation demonstrated significant tumor growth inhibition with excellent safety profiles. Plasma analysis confirmed systemic metabolic normalization and significant inhibition of key glycolytic enzymes. These findings establish D-mannose as a sophisticated food-derived metabolic modulator that exploits cancer's glucose dependency through competitive interference, demonstrating how naturally occurring fruit sugars can serve as precision nutrition tools for breast cancer intervention. This research advances evidence-based functional food development and validates the "food as medicine" paradigm for safer, accessible cancer prevention and complementary therapy strategies.

Keywords: D-mannose; precision nutrition; breast cancer; Warburg effect; metabolomics; functional foods

1. Introduction

Breast cancer continues to pose a formidable global health challenge, affecting over 2.3 million women annually and representing the leading cause of cancer-related mortality in women worldwide [1]. Despite substantial progress in molecular-targeted therapies and precision oncology, persistent issues including treatment resistance, severe systemic toxicity, and limited accessibility of advanced therapeutics underscore the urgent need for innovative therapeutic strategies [2]. These clinical limitations have driven increasing

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interest toward food-derived bioactive compounds as complementary therapeutic approaches. Unlike synthetic drugs that often cause significant side effects such as immunosuppression, cardiotoxicity, and gastrointestinal complications, natural compounds from fruits and vegetables can provide therapeutic benefits while maintaining biocompatibility with normal physiological processes [3-7]. Several prospective studies have demonstrated that dietary patterns rich in fruits, vegetables, and plant-based compounds correlate with improved breast cancer outcomes and reduced recurrence rates [8, 9]. The Women's Healthy Eating and Living (WHEL) randomized trial, involving over 3,000 breast cancer survivors, showed that increased consumption of vegetables and fruits was associated with reduced mortality risk, particularly in specific patient subgroups [10]. More specifically, dietary interventions targeting cancer metabolism have shown promising results in clinical settings. A clinical trial investigating ketogenic diets in breast cancer patients demonstrated metabolic changes consistent with reduced tumor glucose utilization and improved treatment tolerance [11]. These clinical observations, combined with mechanistic studies demonstrating metabolic vulnerabilities in cancer cells, provide compelling rationale for investigating specific dietary compounds as precision nutrition tools targeting cancer metabolism.

Cancer cells exhibit fundamentally different energy production patterns compared to normal healthy cells, creating unique vulnerabilities that can be therapeutically exploited [12]. While normal cells efficiently generate energy through oxygen-dependent processes when oxygen is available, cancer cells preferentially rely on a less efficient but faster energy production method called glycolysis, even in oxygen-rich environments. This metabolic shift, known as the "Warburg effect", allows cancer cells to rapidly generate the energy and building blocks needed for their aggressive growth and division [13]. However, this metabolic preference creates a critical dependency: cancer cells become heavily reliant on glucose as their primary fuel source and lose the metabolic flexibility that normal cells possess to switch between different energy sources when needed [13, 14]. This glucose dependency represents a therapeutic opportunity that can potentially be targeted using natural dietary compounds. Food-derived molecules can compete with glucose for cellular uptake and processing, disrupting key metabolic pathways including glycolysis, the TCA cycle, and the pentose phosphate pathway. Since cancer cells have limited ability to switch to alternative fuel sources, they are more vulnerable to glucose competition strategies than normal cells, which retain metabolic flexibility [15]. This provides a selective targeting approach that can potentially harm cancer cells while preserving normal cellular functions.

Among food-derived simple carbohydrates, D-mannose emerges as a particularly compelling therapeutic candidate due to its unique metabolic properties and extensive presence in the human diet. This naturally occurring monosaccharide, abundant in cranberries, apples, peaches, and other commonly consumed fruits, exists as the C-2 epimer of glucose and shares structural similarity [16]. Originally recognized for its clinical efficacy in preventing and treating urinary tract infections through competitive inhibition of bacterial adhesion, D-mannose has recently attracted attention for broader biological activities including anti-inflammatory, immunomodulatory, and metabolic regulatory effects that extend its

therapeutic potential beyond infectious diseases [17–19]. Studies have shown that D-mannose can compete for cellular uptake through shared glucose transporters (GLUTs) and potentially interfere with glycolytic enzymes positions it as a selective metabolic intervention tool that can preferentially target cancer cell metabolism while minimizing impact on normal cellular processes [20]. This metabolic selectivity, combined with the established safety profile of food-derived compounds, suggests that D-mannose could serve as an effective anticancer agent with minimal systemic toxicity.

Given D-mannose's demonstrated ability to selectively target cancer cell metabolism, it emerges as a potential therapeutic strategy for breast cancer due to the profound energy metabolism reprogramming observed across molecular subtypes. However, previous investigations have primarily focused on single cancer cell models or endpoint metabolite measurements, limiting understanding of subtype-specific efficacy patterns and dynamic metabolic mechanisms. The molecular classification of breast cancer into distinct subtypes (Luminal A, Luminal B, HER2-enriched, and triple-negative) exhibits fundamentally different metabolic characteristics [21]. Estrogen receptor-positive tumors display enhanced glycolytic activity, HER2-positive subtypes demonstrate increased oxidative phosphorylation, while triple-negative breast cancers exhibit aggressive metabolic reprogramming with heightened glucose dependency [22–26]. This metabolic diversity necessitates systematic evaluation across all major breast cancer classifications to understand therapeutic potential and enable personalized nutritional interventions.

Therefore, this study aimed to comprehensively investigate the anticancer activity of D-mannose against breast cancer through three distinct innovations: (1) systematic evaluation of subtype-specific efficacy across four molecularly distinct breast cancer classifications; (2) integrative mechanistic elucidation combining targeted metabolomics with stable isotope flux analysis to distinguish metabolic flux alterations from endpoint metabolite changes; and (3) comprehensive assessment of three-pathway metabolic network disruption associated with the Warburg effect (glycolysis, TCA cycle, and pentose phosphate pathway) with *in vivo* validation through plasma metabolomics and enzymatic analysis. These findings are expected to advance the “food as medicine” paradigm by establishing how naturally occurring dietary compounds can serve as precision tools for metabolism-targeted cancer therapy, with mechanistic validation enabling development of evidence-based nutritional interventions.

2. Materials and Methods

2.1 Chemicals and reagents

D-mannose ($\geq 98\%$ purity) were procured from Sigma-Aldrich (St. Louis, MO, USA), while uniformly labeled ^{13}C -glucose ($\geq 99\%$ ^{13}C enrichment) was obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Cell culture reagents including Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640 medium, fetal bovine serum (FBS), and antibiotic solutions were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). CCK-8 cell viability kit and the Annexin V-FITC/PI apoptosis detection kit were purchased from Dojindo Laboratories (Kumamoto, Japan) and BD Biosciences (Franklin Lakes, NJ, USA), respectively. Caspase-3 and caspase-9 activities were measured using

colorimetric assay kits (Beyotime, Shanghai, China). All chromatographic solvents were of LC-MS grade and obtained from Fisher Scientific (Hampton, NH, USA).

For targeted metabolomics analysis, authentic standards for central carbon metabolism were acquired from Sigma-Aldrich, including D-glucose-6-phosphate disodium salt (G6P), D-fructose-6-phosphate disodium salt dihydrate (F6P), D-3-phosphoglycerate disodium salt (3PG), phosphoenolpyruvate monopotassium salt (PEP), pyruvate (PYR), citrate (CIT), α -ketoglutarate (α -KG), malate (MAL), cis-aconitate (Cis-AA), succinate (SUC), fumarate (FUM), 6-phosphogluconate trisodium salt (6PG), and D-ribose-5-phosphate disodium dihydrate (R5P). Methylsuccinate (Me-SUC) was used as the internal standard. Chemical derivatization reagents including 3-nitrophenylhydrazine hydrochloride (3-NPH) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich to enhance detection sensitivity and chromatographic separation of the target metabolites.

2.2 Cell culture

Four well-characterized breast cancer cell lines representing distinct molecular subtypes were employed in this study: MCF-7 (Luminal A subtype), BT474 (Luminal B subtype), MDA-MB-453 (HER2-positive subtype), and MDA-MB-231 (triple-negative subtype). Normal breast epithelial MCF-10A cells served as the non-malignant control. All cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in their respective optimal growth media supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin solution under standard culture conditions (37°C, 5% CO₂, humidified atmosphere).

2.3 Cell viability and proliferation assays

Cell viability was quantitatively assessed using the CCK-8 colorimetric assay. Specifically, exponentially growing cells were seeded at a density of 1×10^4 cells per well in 96-well microplates and allowed to adhere overnight. Cells were then exposed to varying concentrations of D-mannose (0, 5, 10, 25, 50, and 75 mM) dissolved in complete growth medium for 24 hours. Following treatment, CCK-8 reagent was added according to the manufacturer's protocol, and absorbance was measured at 450 nm using a microplate spectrophotometer.

Long-term proliferative capacity was evaluated through clonogenic survival assays. Specifically, cells were seeded at low density (500 cells per well) in 6-well plates and treated with D-mannose at concentrations of 0, 5, 25, and 50 mM for 24 hours. After treatment removal, cells were cultured in fresh complete medium for 7-10 days to allow colony formation. Resulting colonies were fixed with 4% paraformaldehyde solution, stained with 0.5% crystal violet, and manually counted. Only colonies containing more than 50 cells were considered viable, and colony formation efficiency was expressed as a percentage of untreated controls.

2.4 Apoptosis analysis

Programmed cell death was quantitatively assessed using dual-parameter flow cytometry with Annexin V-FITC and propidium iodide staining. Cells were seeded at 1×10^5 cells per well in 6-well plates and treated with D-mannose at concentrations of 5, 25, and 50 mM for 24 hours. Following treatment, both adherent and floating cells were collected, washed twice with ice-cold phosphate-buffered saline, and resuspended in binding buffer. Annexin V-FITC and propidium iodide were added according to the manufacturer's specifications, and samples were incubated in darkness for 15 minutes at room temperature. Flow cytometric analysis was performed using a BD FACSCalibur system with at least 10,000 events recorded per sample. Data acquisition and analysis were conducted using CellQuest Pro software, with early apoptotic cells identified as Annexin V-positive/PI-negative and late apoptotic cells as double-positive for both markers.

For Caspase-3 and Caspase-3 activity assays, cells were lysed and protein concentrations determined by Bradford assay. Caspase-3 activity was measured by monitoring Ac-DEVD-pNA substrate cleavage at 405 nm, while caspase-9 activity was assessed through Ac-LEHD-pNA cleavage. Reactions were conducted at 37°C for 2 hours, and specific activities calculated as optical density change per microgram protein per minute.

2.5 Targeted metabolomics analysis

Comprehensive metabolomic profiling of central carbon metabolism was performed using targeted liquid chromatography-tandem mass spectrometry. Cells were cultured to 70-80% confluence in 10-cm dishes and treated with 25 mM D-mannose for 24 hours. At the end of treatment, culture medium was rapidly aspirated, and cells were washed twice with ice-cold saline to remove extracellular metabolites. Intracellular metabolites were extracted using ice-cold 80% methanol solution, and samples were immediately stored at -80°C until analysis.

Metabolite extracts were subjected to chemical derivatization using 3-nitrophenylhydrazine (3-NPH) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to enhance detection sensitivity and chromatographic separation^[27,28]. The derivatized samples were analyzed using a high-performance liquid chromatography-triple quadrupole mass spectrometry system (LC-20A/4000 QTrap, Shimadzu/Sciex). Target metabolites encompassed key intermediates of glycolysis, tricarboxylic acid cycle, and pentose phosphate pathway, including G6P, F6P, 3PG, PEP, PYR, CIT, α -KG, MAL, Cis-AA, SUC, FUM, 6PG, and R5P. Quantification was achieved using multiple reaction monitoring mode with authentic standards and Me-SUC as internal standards for normalization. Supplementary materials offer comprehensive details regarding the sample preparation, chromatographic settings, mass spectrometry parameters and methodology validation results.

2.6 Stable isotope tracing and metabolic flux analysis

Dynamic metabolic flux analysis was conducted using uniformly labeled ^{13}C -glucose to trace carbon atom fate through central metabolic pathways. Cells were first acclimated in glucose-free medium for 6 hours to deplete endogenous glucose stores, then incubated with 25 mM U- ^{13}C -glucose in the presence or absence of 25 mM D-mannose for 24 hours.

Following isotope incubation, cells were rapidly quenched using liquid nitrogen, and metabolites were extracted as described above. Mass isotopomer distributions of target metabolites were determined using the same LC-MS/MS system with modified acquisition parameters to monitor all possible mass isotopomers. Natural isotope abundance was corrected using IsoCor software to obtain true ^{13}C -enrichment values. Metabolic flux ratios and pathway activities were calculated based on isotopomer distribution patterns and steady-state assumptions, providing quantitative assessment of metabolic pathway utilization under different treatment conditions.

2.7 *In vivo efficacy studies*

A group of female BALB/c mice aged 5-6 weeks were obtained from Shenyang Pharmaceutical University (Quality Certification No. 110322211102996085) and housed in a specific pathogen-free environment. All experimental procedures were formally approved by the Institutional Animal Care and Use Committee of Shenyang Pharmaceutical University (Approval No. SYPUIACUC-S2021-12-1-101).

After one week acclimatization, MCF-7 cells (1×10^7 cells in 100 μL PBS) were subcutaneously injected into the right axillary region of mice. When tumors reached $\sim 100 \text{ mm}^3$, mice were randomly allocated into four groups ($n=8$): vehicle control, tumor-bearing control, D-mannose treatment (245 mg/kg body weight daily via oral gavage), and positive control doxorubicin (2 $\mu\text{g/g}$ body weight twice weekly via intraperitoneal injection). Tumor volumes were measured every other day using digital calipers and calculated as $V = L \times W^2 / 2$ (L = longest diameter, W = perpendicular width). After 40 days treatment, the mice were euthanized. Plasma samples were collected, and major organs (tumors, heart, liver, kidneys, lungs) were harvested for histopathological examination and biochemical analyses. Tissues were either fixed in 10% neutral buffered formalin for histology or snap-frozen in liquid nitrogen for molecular analyses.

2.8 *Plasma metabolomics and enzymatic activity assessment*

Systemic metabolic changes were evaluated through plasma targeted metabolomics analysis using the same LC-MS/MS approach described for cellular samples. The extraction and derivatization methods of metabolites can be found in the supplementary materials.

The activities of key glycolytic enzymes in plasma samples were determined using commercially available colorimetric assay kits (Solarbio, Beijing, China). Hexokinase activity was measured by monitoring glucose-6-phosphate formation coupled to NADPH generation, lactate dehydrogenase activity was assessed through pyruvate-to-lactate conversion monitoring NADH oxidation, and pyruvate kinase activity was evaluated by measuring ATP formation coupled to NADH generation. All enzymatic assays were performed according to manufacturer's specifications using a microplate spectrophotometer, with results expressed as units per milligram of total protein determined by Bradford assay.

2.9 *Molecular docking*

The protein structures of hexokinase (HK, PDB: 2NZT), lactate dehydrogenase (LDH, PDB: 1II0), and pyruvate kinase (PK, PDB: 4B2D) were retrieved from the RCSB PDB protein Crystal Structure Database

(<https://www.pdbus.org/>). PyMOL 2.4 (Schrodinger, New York, NY, USA) was applied to remove water molecules and ligands from the structures. AutoDock 4.2.6 (Scripps Research, La Jolla, CA, USA) was employed for hydrogenation and charge assignment. The Mol2 structure of D-mannose was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and semiflexible molecular docking between the receptors and D-mannose was performed using AutoDock 4.2.6. Finally, PyMOL 2.4 was used to visualize and analyze the molecular docking results.

2.10 Statistical analysis

In our study, all experimental data are presented as mean $\pm$ standard deviation (SD) from at least three independent biological replicates. Statistical significance was assessed using appropriate parametric tests after confirming data normality through Shapiro-Wilk tests and homogeneity of variance through Levene's test. For two-group comparisons, unpaired Student's *t*-tests were performed. For multiple group comparisons, one-way analysis of variance (ANOVA) was conducted, followed by appropriate post-hoc tests with multiple comparison corrections. *p*-values less than 0.05 (after appropriate corrections) were considered statistically significant, with specific significance levels indicated as **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 D-mannose inhibits breast cancer cell proliferation

To investigate the potential anticancer effects of D-mannose, we first evaluated its impact on cell viability across four molecularly distinct breast cancer subtypes representing the major clinical classifications. The D-mannose concentration range was selected based on literature precedent in cancer metabolism research and the requirement for effective competitive inhibition against the 25 mM glucose present in culture medium [17, 18, 29]. CCK-8 viability assays revealed dose-dependent growth inhibition following 24-hour treatment, with IC₅₀ values of 62.38 $\pm$ 0.91 mM for MCF-7 (Luminal A), 55.59 $\pm$ 2.33 mM for BT474 (Luminal B), 72.72 $\pm$ 2.09 mM for MDA-MB-453 (HER2-positive), and 56.77 $\pm$ 2.61 mM for MDA-MB-231 (triple-negative) cells (Figure 1A). Notably, the HER2-positive subtype (MDA-MB-453) demonstrated the lowest sensitivity to D-mannose treatment, with an IC₅₀ value approximately 1.3-fold higher than the average of other subtypes. The Luminal B subtype (BT474) showed the highest sensitivity, followed closely by the triple-negative subtype (MDA-MB-231), while the Luminal A subtype (MCF-7) exhibited intermediate sensitivity. This sensitivity pattern suggests that D-mannose may target distinct metabolic vulnerabilities that vary among breast cancer molecular classifications, with metabolic dependencies rather than hormone receptor status being the primary determinant of therapeutic response. Importantly, normal breast epithelial MCF-10A cells showed minimal cytotoxicity even at the highest tested concentration (75 mM, Figure 1B), indicating selective targeting of malignant cells while preserving normal cellular function—a critical advantage for food-derived therapeutic compounds.

The antiproliferative effects of D-mannose were further validated through clonogenic survival assays, which assess the long-term reproductive capacity of individual cells under low-density conditions. Treatment with D-mannose significantly suppressed colony formation across all breast cancer subtypes in a concentration-dependent manner (Figure 1C-D). At 25 mM, colony numbers were reduced by 20%, 26%, 13%, and 21% in MCF-7, BT474, MDA-MB-453, and MDA-MB-231 cells, respectively, with higher concentrations (50 mM) achieving >90% reduction across all cell lines. This profound inhibition of clonogenic potential indicates that D-mannose not only affects immediate cell viability but also compromises the self-renewal capacity essential for tumor progression and metastatic colonization.

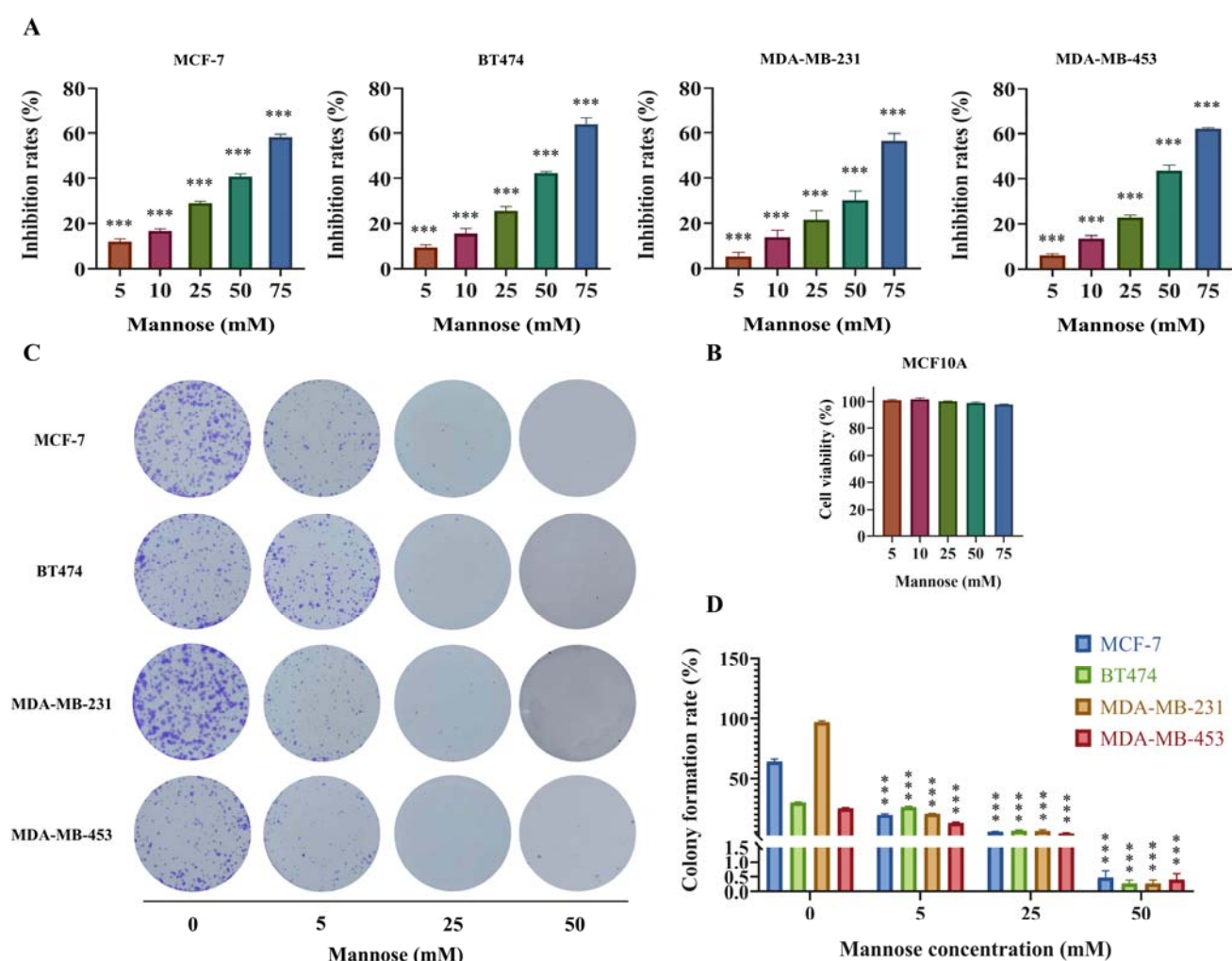
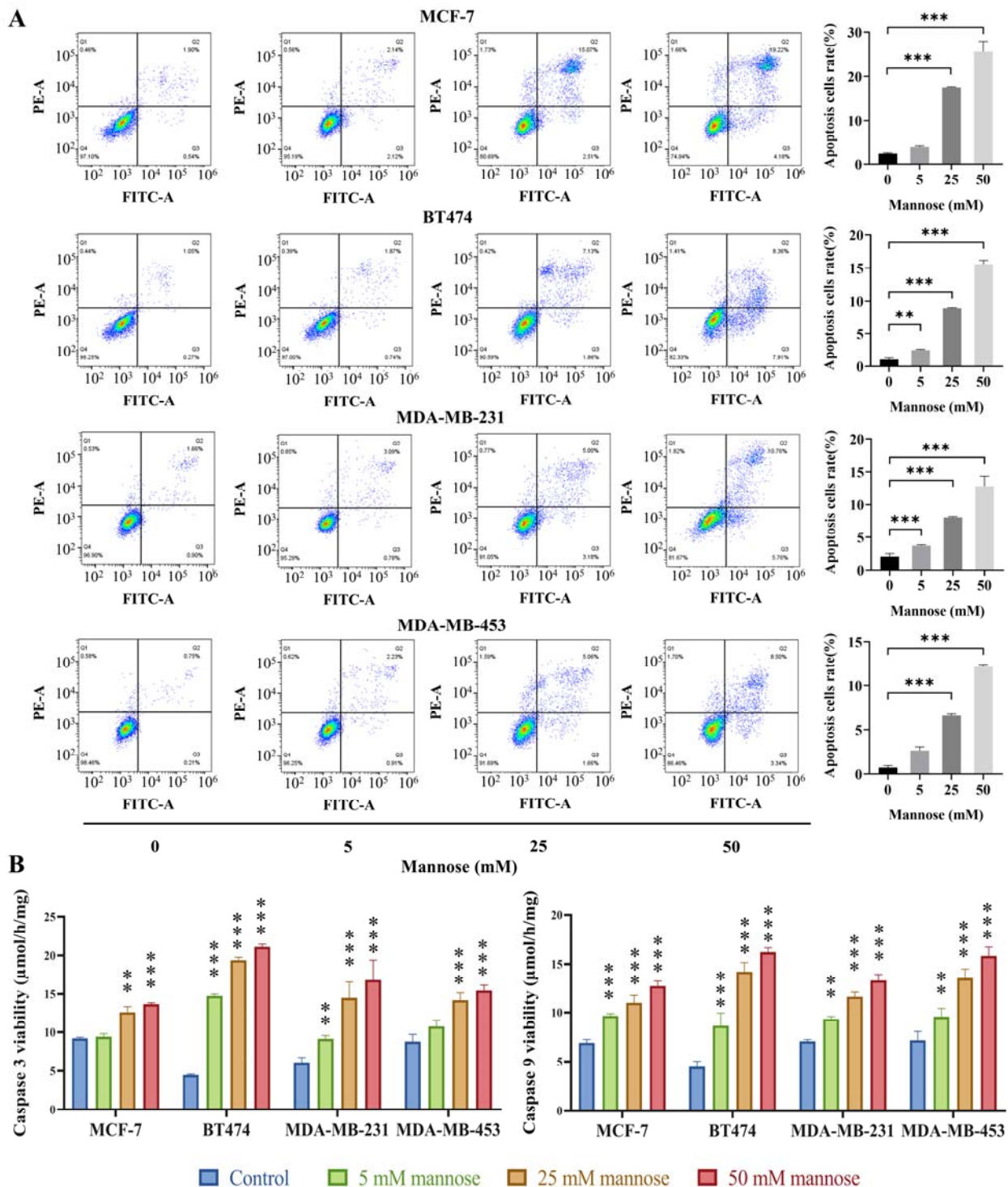


Figure 1. Fruit-derived D-mannose selectively inhibits breast cancer cell proliferation while preserving normal cell viability. (A) Dose-response analysis of breast cancer cell viability following D-mannose treatment. Four molecularly distinct breast cancer cell lines representing major clinical subtypes were treated with D-mannose (0–75 mM) for 24 h. (B) Selective cytotoxicity assessment in normal breast epithelial cells. (C–D) Long-term antiproliferative effects evaluated by clonogenic survival assay. Representative images (C) and quantitative analysis (D) showing dose-dependent suppression of colony formation capacity across all breast cancer subtypes. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.2 D-mannose induces apoptosis in breast cancer cells

To elucidate whether the observed growth inhibition was mediated through programmed cell death, we performed dual-parameter flow cytometry and caspase activity analysis. D-mannose treatment induced dose-dependent apoptosis across all breast cancer subtypes, with early apoptotic populations increasing from baseline levels of 1.0–4.9% to 6.7–17.6% following 25 mM treatment for 24 hours (Figure 2A). Total

apoptotic cell populations (early plus late apoptotic) reached 5.1–15.1% at 25 mM concentration compared to 0.8–3.1% in untreated controls. Estrogen receptor-positive subtypes (MCF-7 and BT474) demonstrated higher apoptotic sensitivity compared to HER2-positive and triple-negative variants. At 25 mM treatment, MCF-7 cells showed the highest total apoptosis (17.6 ± 0.7%), followed BT474 by (9.0 ± 0.2%), MDA-MB-231 (8.2 ± 0.1%), and MDA-MB-453 (6.7 ± 0.1%) cells. This differential sensitivity correlated with the metabolic vulnerability patterns observed in viability assays.



Caspase activity analysis revealed significant activation of both executioner and initiator caspases (Figure 2B). Caspase-3 activity increased 1.4-fold, 4.3-fold, 1.6-fold, and 2.4-fold in MCF-7, BT474, MDA-MB-453, and MDA-MB-231 cells, respectively, following 25 mM D-mannose treatment. Caspase-9 activity similarly increased 1.6-fold, 3.2-fold, 1.9-fold, and 1.7-fold across the respective cell lines. The pronounced activation of caspase-9 preceding caspase-3 activation confirms that D-mannose triggers apoptosis through the intrinsic mitochondrial pathway, likely resulting from metabolic stress and energy depletion caused by disrupted glucose utilization.

3.3 D-mannose disrupts central carbon metabolism in breast cancer cells

To investigate the metabolic basis underlying the subtype-specific anticancer effects of D-mannose, we performed comprehensive targeted metabolomics analysis of central carbon metabolism across four molecularly distinct breast cancer subtypes. Treatment with D-mannose (25 mM, 24 h) revealed profound metabolic reprogramming that exhibited both common patterns and subtype-specific responses across the interconnected networks of glycolysis, tricarboxylic acid (TCA) cycle, and pentose phosphate pathway (Figure 3).

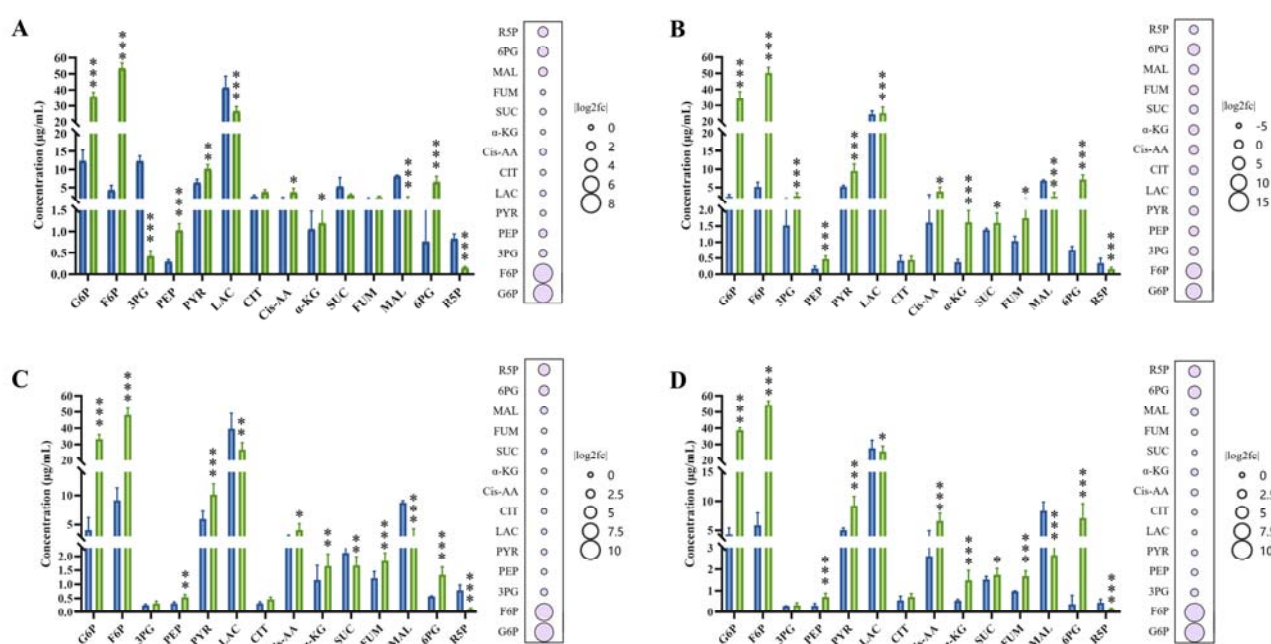


Figure 3. Targeted metabolomics analysis revealing D-mannose-induced perturbations in glycolysis, tricarboxylic acid (TCA) cycle, and pentose phosphate pathway (PPP) metabolites across breast cancer subtypes. (A) MCF-7; (B) BT474; (C) MDA-MB-231; (D) MDA-MB-453 cells. Metabolite abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; LAC, lactate; CIT, citrate; Cis-AA, cis-aconitate; α -KG, α -ketoglutarate; SUC, succinate; FUM, fumarate; MAL, malate; 6PG, 6-phosphogluconate; R5P, ribose-5-phosphate. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

D-mannose treatment profoundly disrupted the glycolysis pathway across all breast cancer subtypes, creating a characteristic metabolite accumulation pattern that revealed systematic interference with glucose utilization. The upstream glycolytic intermediates G6P and F6P showed significant elevation across MCF-7, BT474, MDA-MB-231, and MDA-MB-453 cells, likely resulting from D-mannose cellular uptake and phosphorylation to M6P, which undergoes enzymatic interconversion with F6P through mannose-6-phosphate isomerase activity [29]. This metabolite accumulation indicates competitive

interference at the hexokinase and phosphofructokinase regulatory checkpoints, disrupting the normal glucose flux through the energy investment phase of glycolysis. Downstream metabolites exhibited subtype-specific perturbations, with 3PG significantly increasing in MCF-7 cells, PEP accumulating in MCF-7, MDA-MB-231, and MDA-MB-453 cells, and PYR elevation observed in BT474, MDA-MB-231, and MDA-MB-453 cells. Most critically, LAC levels were dramatically reduced across all cell lines, indicating impaired pyruvate-to-lactate conversion that represents the hallmark terminal step of the Warburg effect. This metabolic signature demonstrates that D-mannose effectively disrupts the rapid ATP generation pathway that cancer cells depend upon for sustained proliferation, while simultaneously increasing ATP consumption in the early glycolytic steps, creating an energetic crisis that selectively targets malignant metabolism.

The tricarboxylic acid cycle demonstrated significant metabolic reprogramming following D-mannose treatment, with key intermediates *cis* AA and α -KG showing marked elevation across all four breast cancer subtypes. This upregulation of TCA cycle intermediates represents a reversal of the typical Warburg effect pattern, where cancer cells characteristically suppress oxidative metabolism in favor of glycolytic energy production [30]. The substantial increase in α -KG levels holds particular therapeutic significance, as this metabolite serves as a critical cofactor for α -ketoglutarate-dependent dioxygenases that regulate chromatin modifications and gene expression. In many cancers, isocitrate dehydrogenase (IDH) mutations reduce α -KG availability while promoting the aberrant production of the oncometabolite D-2-hydroxyglutarate (D-2-HG), which drives tumorigenesis through epigenetic dysregulation and metabolic reprogramming [31]. The D-mannose-induced restoration of α -KG levels suggests active inhibition of oncometabolite formation and potential reversal of cancer-associated epigenetic alterations. Furthermore, the accumulation of TCA cycle intermediates indicates that D-mannose treatment forces cancer cells toward oxidative metabolism, increasing mitochondrial activity and reactive oxygen species production that can overwhelm the antioxidant defense systems that cancer cells rely upon for survival under metabolic stress.

The pentose phosphate pathway exhibited distinctive disruption characterized by significant elevation of 6-phosphogluconate (6PG) across all breast cancer cell lines, coupled with substantial depletion of ribose-5-phosphate (R5P) in MCF-7, BT474, and MDA-MB-231 cells. This metabolic bottleneck represents a strategic disruption of cancer cell biosynthetic capacity, as the pentose phosphate pathway serves dual critical functions in supporting malignant proliferation. The pathway's oxidative branch generates NADPH essential for reductive biosynthesis of fatty acids and cholesterol, while simultaneously maintaining glutathione in its reduced state for antioxidant defense against oxidative stress [32-34]. Concurrently, the non-oxidative branch produces ribose-5-phosphate as the essential precursor for nucleotide synthesis required for DNA replication and RNA transcription during rapid cell division [35]. The observed accumulation of 6PG with concurrent R5P depletion indicates that D-mannose specifically disrupts the flow through the oxidative branch of the pentose phosphate pathway, creating a dual metabolic crisis by limiting both NADPH generation for biosynthetic processes and ribose availability for nucleotide synthesis.

The comprehensive metabolic profiling demonstrates that D-mannose functions as a sophisticated multi-pathway metabolic disruptor that simultaneously targets the three core components of central carbon metabolism through distinct but complementary mechanisms.

3.4 ^{13}C -metabolic flux analysis reveals impaired glucose utilization

While traditional targeted metabolomics provides valuable insights into metabolite concentration changes, it cannot distinguish whether metabolite accumulation results from enhanced synthesis or impaired degradation, particularly at metabolic crossroads where multiple pathways converge. Metabolic flux analysis addresses this limitation by tracking the dynamic transfer of isotope-labeled carbons through metabolic networks, enabling quantitative assessment of pathway activities, carbon flow directions, and real-time metabolic reprogramming responses [36]. To directly assess the dynamic metabolic flux alterations underlying D-mannose's differential anticancer effects across breast cancer molecular subtypes, we employed stable isotope tracing using uniformly labeled ^{13}C -glucose (U- ^{13}C -glucose) as a metabolic tracer.

D-mannose treatment profoundly disrupted glucose utilization across all breast cancer subtypes, demonstrating consistent impairment of central carbon metabolism through distinct isotopomer distribution alterations. In MCF-7 cells, D-mannose treatment significantly increased the [M+0] isotopomer fraction of G6P and F6P while dramatically reducing their [M+6] fractions, indicating decreased glucose entry into glycolysis and reduced phosphorylation of glucose to G6P (Figure 4). This metabolic bottleneck at the hexokinase step represents the initial disruption point where D-mannose competes with glucose for cellular uptake and phosphorylation. The downstream glycolytic intermediate pyruvate showed a significant increase in [M+0] abundance coupled with substantial reduction in [M+3] incorporation, confirming impaired glucose flux through the entire glycolytic pathway. Within the tricarboxylic acid cycle, citrate demonstrated increased [M+0] levels with decreased [M+2] incorporation, while α -ketoglutarate showed elevated [M+2] abundance, and fumarate exhibited increased [M+0] with reduced [M+2] labeling. These isotopomer patterns collectively indicate reduced glucose-derived carbon entry into the TCA cycle, suggesting metabolic reprogramming toward alternative carbon sources and decreased glucose utilization efficiency in MCF-7 cells.

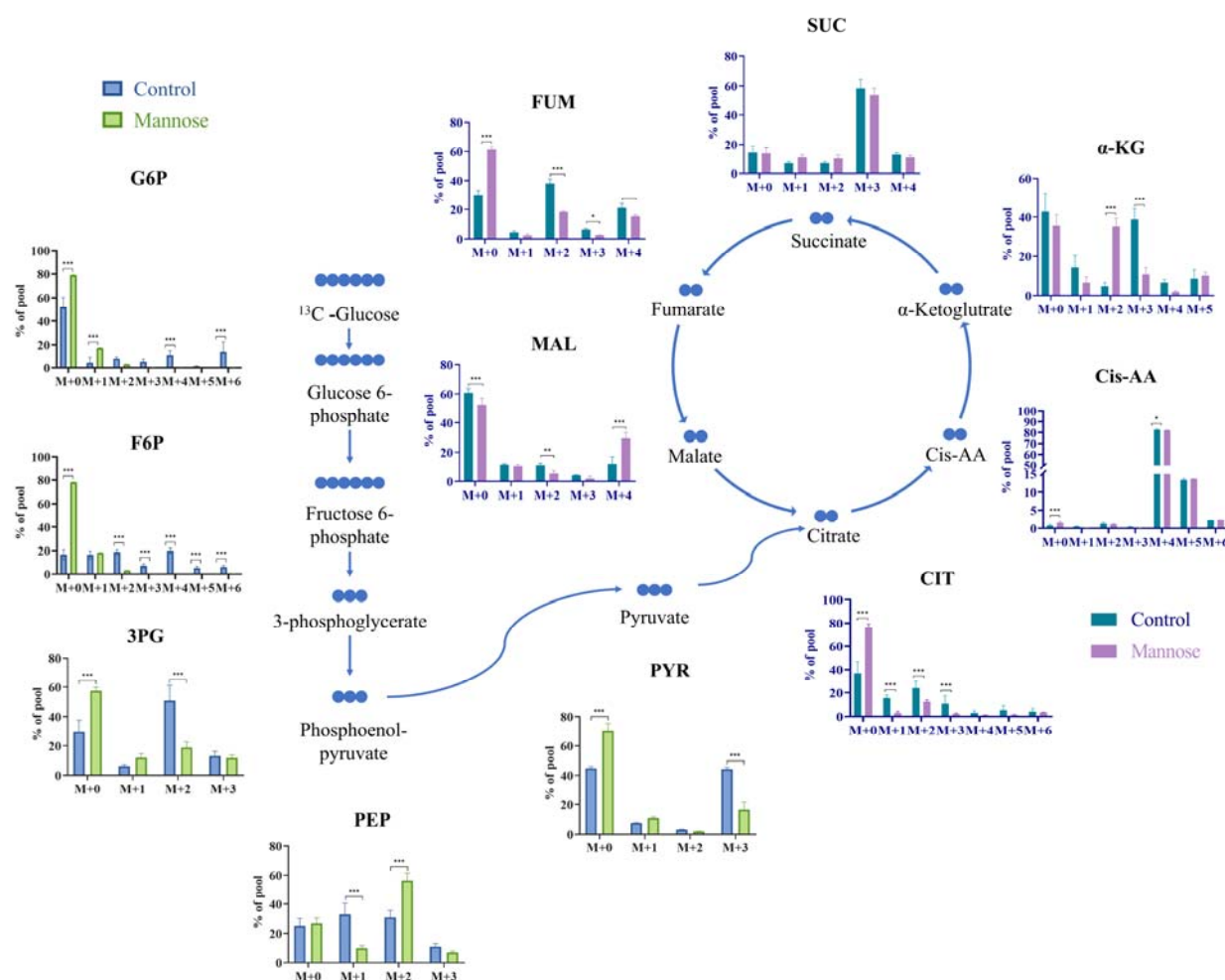


Figure 4. ^{13}C -glucose metabolic flux analysis demonstrates impaired glucose utilization in MCF-7 cells. Metabolite abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; CIT, citrate; Cis-AA, cis-aconitate; α -KG, α -ketoglutarate; SUC, succinate; FUM, fumarate; MAL, malate. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

BT474 cells exhibited similar metabolic flux disruption patterns following D-mannose treatment, with enhanced glycolytic impairment and TCA cycle perturbations (Supplementary Figure S1). The glycolytic pathway showed significant increases in [M+0] fractions of G6P and F6P, accompanied by marked reductions in [M+6] isotopomer abundance, confirming decreased glucose entry into glycolysis. Downstream intermediates 3PG, PEP, and PYR all demonstrated significantly reduced [M+3] incorporation, with PEP and PYR showing concurrent increases in [M+0] abundance. This isotopomer profile indicates comprehensive glycolytic flux reduction throughout the pathway, from the initial glucose phosphorylation through PYR formation. The TCA cycle revealed extensive metabolic reprogramming, with CIT, Cis-AA, SUC, FUM, and MAL all showing elevated [M+0] fractions, while CIT, FUM, and MAL exhibited decreased [M+2] incorporation. Notably, α -KG displayed increased [M+2] labeling, suggesting potential metabolic compensation through alternative carbon sources. These findings demonstrate that D-mannose effectively disrupts central carbon metabolism in Luminal B breast cancer cells, forcing metabolic adaptation away from glucose-dependent energy production.

MDA-MB-231 triple-negative breast cancer cells demonstrated the most pronounced glycolytic inhibition among all subtypes tested, with substantial metabolic flux alterations throughout central carbon metabolism (Supplementary Figure S2). Glycolytic pathway analysis revealed significant increases in [M+0] isotopomer fractions of G6P and F6P, coupled with dramatic reductions in [M+6] labeling, indicating severe impairment of glucose entry into glycolysis. The downstream metabolites 3PG, PEP, and pyruvate all showed markedly reduced [M+3] incorporation with concurrent increases in [M+0] abundance, confirming comprehensive glycolytic flux inhibition. This profound glycolytic disruption aligns with the triple-negative subtype's characteristic dependence on glycolytic metabolism for rapid proliferation. Within the TCA cycle, citrate, fumarate, and malate demonstrated elevated [M+0] fractions with decreased [M+2] incorporation, while α -KG showed increased [M+2] labeling. Notably, the TCA cycle perturbations were less pronounced than glycolytic changes, suggesting potential metabolic compensation through alternative carbon sources such as glutamine or fatty acid oxidation. This metabolic adaptation pattern indicates that while D-mannose effectively targets the primary glycolytic dependency of triple-negative breast cancer, these highly adaptable cells may partially compensate through alternative metabolic pathways.

MDA-MB-453 HER2-positive breast cancer cells exhibited significant central carbon metabolism disruption, though with distinct isotopomer patterns reflecting this subtype's unique metabolic characteristics (Supplementary Figure S3). Glycolytic pathway analysis revealed substantial increases in [M+0] fractions of G6P and F6P with corresponding decreases in [M+6] labeling, confirming impaired glucose entry into glycolysis. The downstream intermediates 3PG, PEP, and pyruvate all demonstrated increased [M+0] abundance, with 3PG and pyruvate showing significantly reduced [M+3] incorporation. This pattern indicates moderate glycolytic flux inhibition, consistent with the HER2-positive subtype's relatively lower sensitivity to D-mannose treatment observed in our viability assays. The tricarboxylic acid cycle showed comprehensive perturbations, with citrate, cis-aconitate, succinate, and fumarate all exhibiting elevated [M+0] fractions, while citrate, cis-aconitate, fumarate, and malate demonstrated decreased [M+2] incorporation. These isotopomer changes suggest that D-mannose effectively disrupts glucose utilization in HER2-positive breast cancer cells, though the metabolic impact may be moderated by this subtype's enhanced oxidative metabolism capacity and metabolic flexibility.

Overall, the comprehensive ^{13}C -metabolic flux analysis establish D-mannose as a food-derived metabolic modulator capable of disrupting cancer cell energy production through targeted interference with glucose utilization, providing mechanistic validation for its therapeutic potential in breast cancer intervention.

3.5 D-mannose suppresses tumor growth in vivo

To validate the therapeutic potential of D-mannose as a food-derived anticancer agent, we evaluated its antitumor efficacy in MCF-7 tumor-bearing nude mice. This xenograft model was selected based on the pronounced sensitivity of ER⁺ breast cancer cells to D-mannose treatment observed in our cellular studies, representing approximately 70% of clinical breast cancer cases [37].

The sustained tumor growth suppression throughout the treatment period indicates that D-mannose maintains its anticancer activity during chronic administration, supporting its potential for long-term therapeutic applications (Figure 5A-B). D-mannose treatment administered orally for 40 days resulted in significant tumor growth inhibition compared to untreated controls. Final tumor volumes were dramatically reduced by 65% in the D-mannose group ($518 \pm 56 \text{ mm}^3$) versus the model group ($951 \pm 116 \text{ mm}^3$, $P < 0.001$). Histopathological examination using H&E staining provided comprehensive evidence of D-mannose's therapeutic effects and excellent safety profile. Tumor tissues from D-mannose-treated mice exhibited increased cell death markers, including nuclear fragmentation and chromatin condensation characteristic of apoptosis, along with markedly reduced proliferation compared to control tumors (Figure 5C). These morphological changes confirmed that the observed tumor volume reduction resulted from active induction of cancer cell death rather than simple growth inhibition. Importantly, systematic examination of major organs including heart, liver, and kidney revealed no pathological changes in D-mannose-treated animals, contrasting sharply with the significant cardiac and hepatic toxicity observed in the doxorubicin-treated positive control group. These results collectively confirm that D-mannose, as a naturally occurring food-derived compound, can effectively inhibit breast cancer growth *in vivo* while maintaining an exemplary safety profile, strongly supporting its potential as a safe and effective anticancer agent suitable for long-term therapeutic applications and preventive interventions.

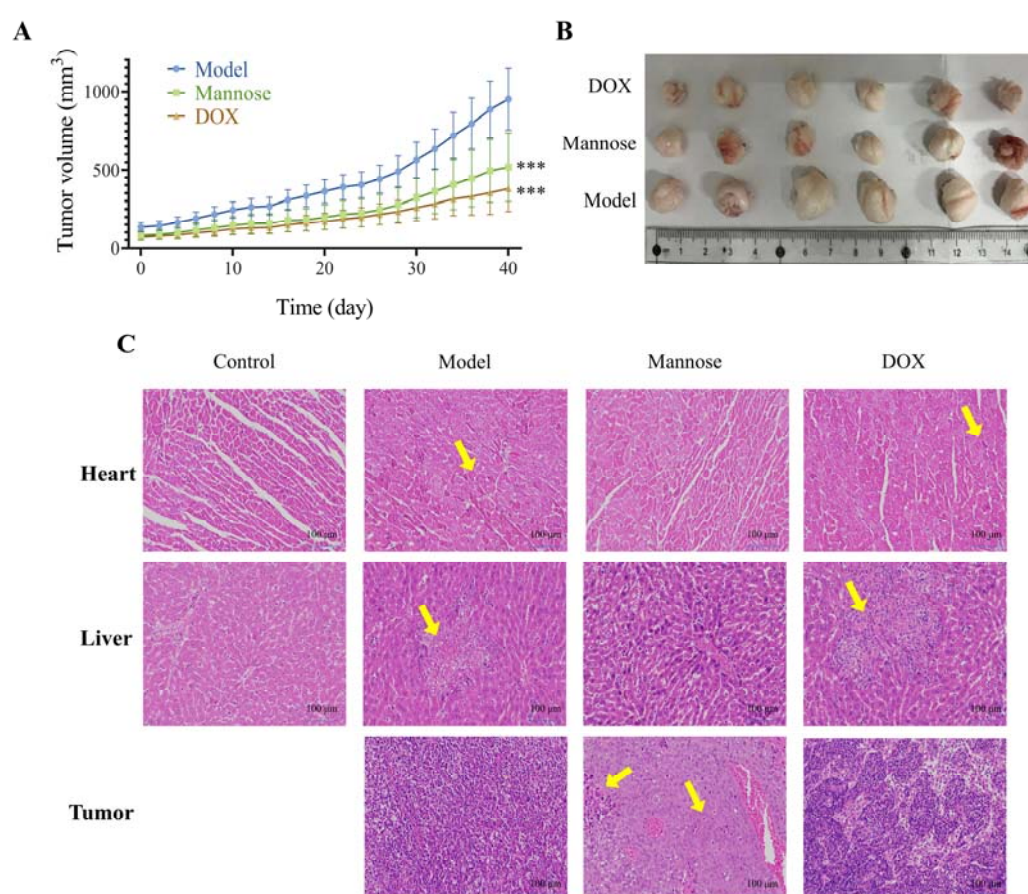


Figure 5. D-mannose demonstrates significant antitumor efficacy with excellent safety profile in MCF-7 xenograft model. (A) Tumor growth kinetics over 40-day treatment period. **(B)** Representative tumor images at study termination demonstrating marked size reduction in D-mannose-treated group. **(C)** Histopathological analysis of heart, liver and tumor tissues by H&E staining.

3.6 Plasma metabolomics confirms systemic metabolic reprogramming

To validate the metabolic reprogramming observed in cellular studies, we performed comprehensive targeted metabolomics analysis of central carbon metabolites in plasma samples from MCF-7 tumor-bearing nude mice. The systemic metabolic analysis revealed profound alterations in central carbon metabolism that mirrored and extended our cellular findings. Tumor-bearing mice exhibited significant elevations in key metabolites across all three major pathways of central carbon metabolism (Figure 6A). In the glycolytic pathway, G6P, F6P, and 3PG levels were markedly increased, indicating enhanced glucose utilization and metabolic flux through glycolysis. The tricarboxylic acid cycle showed comprehensive upregulation with significant increases in CIT, α -KG, SUC, FUM, MAL, and Cis-AA, reflecting the heightened oxidative metabolism required to support tumor growth and energy demands. Similarly, the pentose phosphate pathway demonstrated activation through elevated levels of 6PG and R5P, indicating increased nucleotide synthesis capacity and NADPH production necessary for tumor cell proliferation and antioxidant defense. D-mannose treatment effectively normalized these tumor-induced metabolic perturbations, with all elevated central carbon metabolites showing significant reversal toward control levels. This systemic metabolic normalization provided compelling evidence that D-mannose functions as a comprehensive metabolic modulator capable of reversing cancer-associated metabolic reprogramming at the organismal level. The plasma metabolomics data demonstrated that the anticancer effects of D-mannose observed at the cellular level translate to meaningful therapeutic benefits *in vivo*, supporting its potential as a metabolism-targeted intervention for breast cancer.

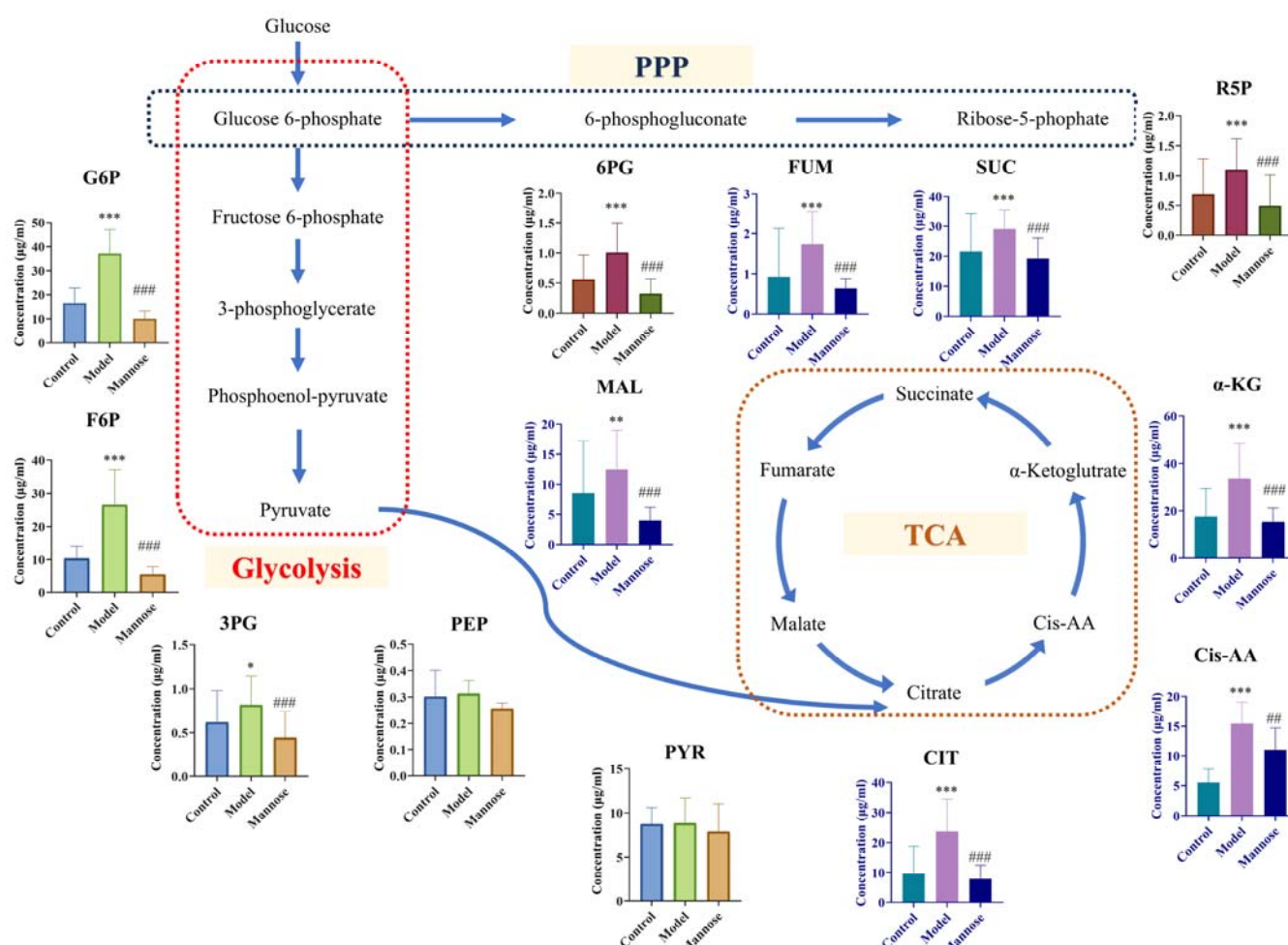


Figure 6. D-mannose normalizes systemic metabolic reprogramming and inhibits key glycolytic enzymes. Plasma metabolomics analysis demonstrating systemic metabolic alterations. Tumor-bearing mice showed elevated central carbon metabolites across glycolysis (G6P, F6P, 3PG), TCA cycle (CIT, α -KG, SUC, FUM, MAL, Cis-AA), and PPP (6PG, R5P). D-mannose treatment effectively normalized these perturbations, restoring metabolite levels toward healthy control values.

Metabolite abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; CIT, citrate; Cis-AA, cis-aconitate; α -KG, α -ketoglutarate; SUC, succinate; FUM, fumarate; MAL, malate. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control group. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. Model group.

3.7 Key glycolytic enzymes are inhibited by D-mannose treatment

To further elucidate the molecular mechanisms underlying the metabolic disruption observed in D-mannose-treated mice, we examined the activities of key glycolytic enzymes in plasma samples. Hexokinase (HK), lactate dehydrogenase (LDH), and pyruvate kinase (PK) represent critical rate-limiting enzymes in glycolysis and serve as reliable indicators of glycolytic flux [38-43]. These enzymes are frequently overexpressed in cancer tissues and correlate with tumor aggressiveness and poor prognosis in breast cancer patients. Our analysis revealed that tumor-bearing mice exhibited significantly elevated activities of all three glycolytic enzymes compared to healthy controls, confirming the systemic activation of glycolytic pathways characteristic of cancer metabolism. D-mannose treatment effectively countered this metabolic reprogramming, significantly reducing the activities of all three enzymes (Figure 7). Specifically, HK activity decreased by 71% ($P < 0.01$), LDH by 29% ($P < 0.01$), and PK by 60% ($P < 0.05$) compared to untreated tumor mice. These findings demonstrate that D-mannose functions as a natural glycolytic

inhibitor, targeting multiple enzymatic checkpoints to effectively disrupt cancer cell energy metabolism and restore systemic metabolic homeostasis.

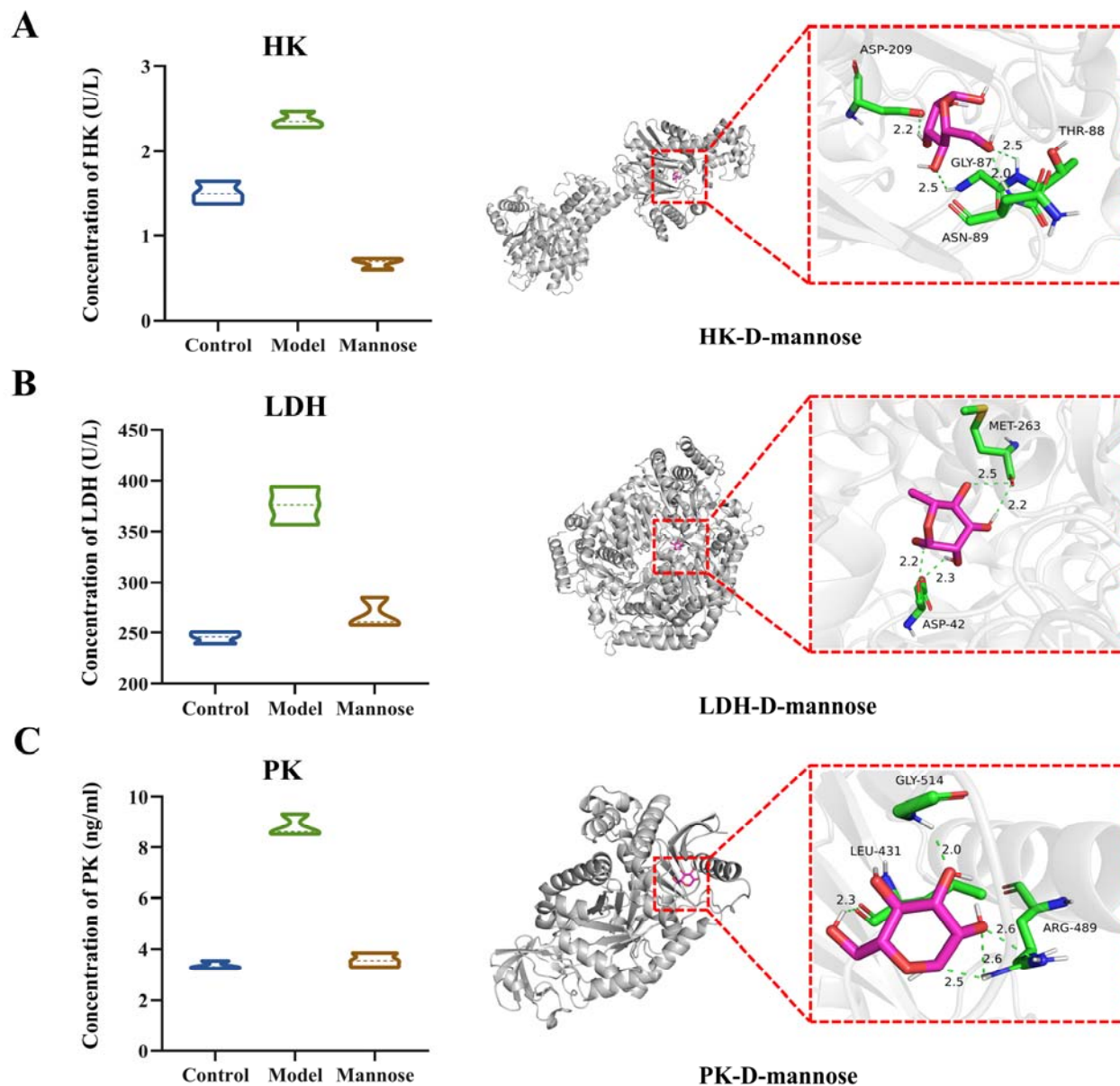


Figure 7. Effects of D-mannose on glycolytic enzyme activities in plasma of tumor-bearing mice and molecular docking analysis. (A) Hexokinase (HK) activity, (B) Lactate dehydrogenase (LDH) activity, (C) Pyruvate kinase (PK) activity and its molecular docking results.

Molecular docking revealed that D-mannose binds to the active site of HK with a binding energy of -5.8 kcal/mol, forming hydrogen bonds with key residues Asp-209, Glu-87, Thr-88 and Asn-89 in the glucose-binding pocket (Figure 7A). This competitive binding at the active site likely provides evidence for the glucose-competitive inhibition mechanism observed in our metabolic flux analysis. Molecular docking also demonstrated that D-mannose can interact with the NADH-binding pocket of LDH (binding energy: -5.9 kcal/mol), potentially competing with pyruvate binding and explaining the dramatic lactate reduction observed across all breast cancer subtypes (Figure 7B). Meanwhile, D-mannose showed favorable binding interactions with PK (binding energy: -6.4 kcal/mol), particularly at the substrate-binding domain, forming

hydrogen bonds with Gly-514, Leu-431 and Arg-489 (Figure 7C). This interaction likely interferes with PEP-to-pyruvate conversion, consistent with our observed PEP accumulation in metabolomic profiling.

4. Discussion

This study provides evidence that D-mannose, a naturally occurring monosaccharide found in fruits such as cranberries, apples, and peaches, exhibits anticancer activity against breast cancer through metabolic intervention. Our investigation indicates that D-mannose may represent a nutraceutical compound with potential therapeutic properties, contributing to understanding how food-derived bioactive molecules can influence metabolic processes in cancer cells. The observed selective cytotoxicity against malignant cells while sparing normal breast epithelial cells represents a characteristic of food-based compounds compared to conventional chemotherapy, supporting the concept of nutritional interventions as potential complementary approaches in cancer management.

The mechanistic foundation underlying D-mannose's anticancer efficacy centers on its sophisticated ability to disrupt cancer cell energy metabolism through competitive interference with glucose utilization pathways (Figure 8). Our integrative metabolomics analysis revealed that D-mannose induces coordinated metabolic perturbations across the three interconnected networks of central carbon metabolism—glycolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway. This multi-pathway disruption exploits the fundamental metabolic reprogramming characteristic of cancer cells, particularly their dependence on glycolytic metabolism known as the Warburg effect^[44]. The observed accumulation of upstream glycolytic intermediates coupled with dramatic lactate reduction demonstrates effective blockade of the rapid ATP generation pathway that cancer cells require for sustained proliferation^[45, 46]. This metabolic signature is particularly significant because it capitalizes on cancer cells' limited metabolic flexibility compared to normal cells, which retain the capacity to utilize alternative energy sources when glucose metabolism is compromised. The competitive relationship between D-mannose and glucose, mediated through shared glucose transporters (GLUTs) and enzymatic processing pathways, creates a selective metabolic stress that preferentially affects malignant cells while minimizing impact on normal cellular functions^[29].

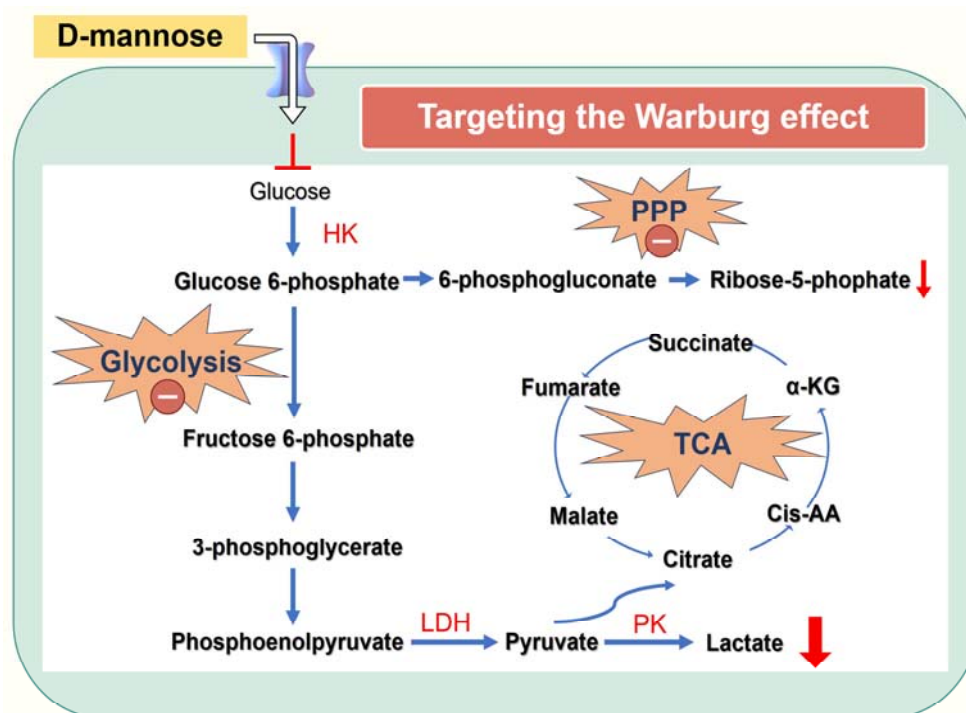


Figure 8. Mechanistic illustration of D-mannose-mediated metabolic disruption in breast cancer cells.

The application of ^{13}C -glucose metabolic flux analysis provided crucial mechanistic validation that extends beyond conventional metabolomics approaches by enabling real-time tracking of carbon atom movement through interconnected metabolic networks [47]. The systematic reduction in ^{13}C -glucose incorporation across glycolytic intermediates—evidenced by decreased [M+6] labeling in G6P and F6P, and reduced [M+3] incorporation in downstream metabolites—demonstrated comprehensive blockade of glucose entry and flux disruption throughout the entire glycolytic pathway. Within the TCA cycle, altered isotopomer patterns characterized by increased [M+0] fractions and decreased [M+2] incorporation provided direct evidence that D-mannose forces cancer cells to reduce glucose-derived carbon utilization for oxidative metabolism. The concurrent elevation of α -ketoglutarate abundance holds particular therapeutic significance, as this metabolite serves as a critical cofactor for epigenetic regulatory enzymes that are frequently dysregulated in cancer. These dynamic flux alterations establish D-mannose as a sophisticated metabolic modulator rather than a simple glucose competitor, providing mechanistic foundation for its therapeutic potential in precision nutrition applications.

The demonstration of subtype-specific anticancer efficacy patterns across molecularly distinct breast cancer classifications provides crucial insights for developing personalized nutritional interventions in oncology [48]. Our findings revealed differential sensitivity with important metabolic implications: Luminal B (BT474) and triple-negative subtypes (MDA-MB-231) exhibited the highest sensitivity to D-mannose treatment, Luminal A showed intermediate sensitivity (MCF-7), while HER2-positive cells (MDA-MB-453) demonstrated relative resistance. The pronounced resistance observed in HER2-positive breast cancer can be mechanistically attributed to this subtype's distinctive metabolic architecture characterized by elevated oxidative phosphorylation (OXPHOS) capacity and enhanced metabolic plasticity [49]. Unlike other breast

cancer subtypes that exhibit heavy glycolytic dependence (the Warburg effect), HER2-amplified tumors maintain robust mitochondrial function with significantly higher OXPHOS activity driven by HER2-mediated PI3K/AKT/mTOR signaling cascade activation^[50]. This metabolic phenotype confers several resistance mechanisms. First, HER2⁺ cells possess superior mitochondrial reserve capacity that enables efficient ATP production through oxidative metabolism even when glycolytic flux is compromised^[51]. Our ¹³C-metabolic flux analysis supports this mechanism, showing that MDA-MB-453 cells maintained relatively higher glucose-derived carbon incorporation into TCA cycle intermediates compared to other subtypes following D-mannose treatment. Second, HER2-positive tumors exhibit enhanced metabolic flexibility with greater capacity to utilize alternative fuel sources beyond glucose—including glutamine oxidation, fatty acid β -oxidation, and amino acid catabolism^[52-54]—when D-mannose competitively inhibits glucose utilization. In contrast, the high sensitivity of Luminal B tumors reflects their characteristic metabolic phenotype combining estrogen receptor-driven proliferative signaling with enhanced glycolytic dependency, creating vulnerabilities that D-mannose effectively exploits^[55]. Similarly, triple-negative breast cancer's aggressive phenotype relies on extreme glycolytic reprogramming with minimal oxidative metabolism and limited metabolic flexibility, rendering these cells exquisitely sensitive to glycolytic disruption^[26]. The intermediate sensitivity of Luminal A subtype reflects its more balanced metabolic profile with moderate glycolytic activity^[56]. These mechanistic insights have important implications for precision nutrition applications.

The successful translation of cellular findings to *in vivo* efficacy demonstrates the therapeutic potential of D-mannose as a food-derived anticancer agent suitable for clinical development. The significant tumor growth inhibition achieved with oral D-mannose administration, coupled with excellent tolerability and absence of systemic toxicity, contrasts sharply with conventional chemotherapeutic approaches that often cause severe adverse effects limiting patient compliance and quality of life^[57-59]. The plasma metabolomics analysis confirming systemic metabolic reprogramming provides evidence that the anticancer effects observed at the cellular level translate to meaningful therapeutic benefits *in vivo*. The inhibition of key glycolytic enzymes—hexokinase, lactate dehydrogenase, and pyruvate kinase—further validates D-mannose's mechanism as a comprehensive metabolic modulator capable of reversing cancer-associated metabolic reprogramming at the organismal level.

While positioning D-mannose within the broader functional carbohydrate landscape reveals complementary intervention strategies. Unlike complex polysaccharides from *Poria cocos* and *Ganoderma* species, which exhibit high molecular weight (10-1000 kDa) and structural heterogeneity, D-mannose offers rapid systemic absorption and predictable pharmacokinetics^[60]. These polysaccharides primarily exert immunomodulatory effects through pattern recognition receptor activation and gut-mediated mechanisms, including intestinal mucus enhancement and microbiome modulation^[61]. This mechanistic diversity suggests synergistic potential. Specifically, D-mannose provides direct cellular metabolic intervention, while polysaccharides establish systemic benefits through gut barrier strengthening and immune enhancement.

Such complementarity aligns with precision nutrition principles, where individual variability—including gut integrity, microbiome composition, and metabolic status—determines optimal carbohydrate selection. Future research should investigate combined formulations to individual biological profiles for personalized cancer prevention strategies.

From a functional food development perspective, our findings establish a scientific foundation for creating targeted food products and dietary interventions that leverage specific metabolic vulnerabilities in cancer cells. The natural occurrence of D-mannose in commonly consumed fruits provides a practical advantage for developing accessible nutritional interventions that can be readily incorporated into existing dietary patterns. For breast cancer patients, dietary supplementation with D-mannose-rich fruits or standardized D-mannose formulations could provide a safe and accessible complementary therapeutic approach that enhances conventional treatment efficacy. Nevertheless, several limitations of this study should be acknowledged. The first concerns the translational relevance of our *in vitro* findings. A notable discrepancy exists between the effective IC₅₀ concentrations observed *in vitro* (55–73 mM) and the physiologically achievable plasma levels *in vivo* (5–15 mM). Nonetheless, several factors may mitigate this concern. D-mannose is already widely used clinically for urinary tract infection (UTI) prevention with excellent safety profiles. Standard clinical dosage: 2–3 grams daily for UTI prevention/treatment (equivalent to ~ 30–45 mg/kg for a 70 kg adult) [62]. Furthermore, tumors may accumulate D-mannose due to enhanced GLUT transporter expression in cancer cells [63]. Consequently, while comprehensive pharmacokinetic-pharmacodynamic studies are needed to optimize clinical translation, the achievable intratumorally concentrations may be sufficient to exert anti-tumor effects. The second limitation lies in the use of established cell lines, which cannot fully recapitulate the complex heterogeneity of clinical breast tumors. This inter- and intratumorally heterogeneity gives rise to metabolically diverse subpopulations with varying drug sensitivities. Future studies employing patient-derived xenografts and three-dimensional organoids will be essential to comprehensively evaluate the efficacy of D-mannose across this spectrum of heterogeneity and to identify predictive biomarkers for personalized nutritional intervention.

5. Conclusions

This study establishes D-mannose, a naturally occurring monosaccharide abundant in fruits, as an effective anticancer agent against breast cancer through targeted metabolic intervention. Comprehensive metabolomics and ¹³C-flux analysis revealed that D-mannose disrupts the Warburg effect by blocking glucose utilization across glycolysis, TCA cycle, and pentose phosphate pathways, while demonstrating subtype-specific efficacy patterns. *In vivo* validation confirmed significant tumor growth inhibition with excellent safety profiles, contrasting favorably with conventional chemotherapy toxicity. These findings advance the “food as medicine” paradigm by providing mechanistic foundation for developing accessible, metabolism-targeted nutritional interventions for breast cancer prevention and treatment through precision nutrition applications.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All the data used to support the findings of this study are available from the corresponding authors upon reasonable request.

Reference

- [1] X. Xiong, L.W. Zheng, Y. Ding, et al., Breast cancer: pathogenesis and treatments, *Signal Transduct. Target. Ther.* 10 (2025) 33–49. <https://doi.org/10.1038/s41392-024-02108-4>.
- [2] G. Curigliano, H.J. Burstein, M. Gnant, et al., Understanding breast cancer complexity to improve patient outcomes: The St Gallen International Consensus Conference for the Primary Therapy of Individuals with Early Breast Cancer 2023, *Ann. Oncol.* 34 (2023) 970–986. <https://doi.org/10.1016/j.annonc.2023.08.017>.
- [3] F. Kroschinsky, F. Stölzel, S. von Bonin, et al., New drugs, new toxicities: severe side effects of modern targeted and immunotherapy of cancer and their management, *Crit. Care.* 21 (2017) 11–89. <https://doi.org/10.1186/s13054-017-1678-1>.
- [4] M. Samtiya, R.E. Aluko, T. Dhewa, et al., Potential Health Benefits of Plant Food-Derived Bioactive Components: An Overview, *Foods.* 10 (2021) 25–839. <https://doi.org/10.3390/foods10040839>.
- [5] B. Cong, Perspectives in Food & Medicine Homology, *Food & Medicine Homology.* 1 (2024) 9420018. <https://doi.org/10.26599/FMH.2024.9420018>.
- [6] H. Niu, Aruhan, S. Surenjidiin, et al., Yinshan Zhengyao: exploring the power of food and inheriting healthy thoughts, *Food & Medicine Homology.* 1 (2024) 9420006. <https://doi.org/10.26599/FMH.2024.9420006>.
- [7] J.-T. Li, A. Gu, N.-N. Tang, et al., Exploring anti-tumor potential of food and medicine homology substances: An in-silico evaluation of Citri Grandis Exocarpium against gallbladder cancer, *Food & Medicine Homology.* (2025) <https://doi.org/10.26599/FMH.2026.9420084>.
- [8] S.E. Stanton, S. Adams, M.L. Disis, Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review, *JAMA Oncol.* 2 (2016) 1354–1360. <https://doi.org/10.1001/jamaoncol.2016.1061>.
- [9] R.L. Prentice, B. Caan, R.T. Chlebowski, et al., Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial, *Jama.* 295 (2006) 629–642. <https://doi.org/10.1001/jama.295.6.629>.
- [10] J.P. Pierce, L. Natarajan, B.J. Caan, et al., Influence of a Diet Very High in Vegetables, Fruit, and Fiber and Low in Fat on Prognosis Following Treatment for Breast CancerThe Women's Healthy Eating and Living (WHEL) Randomized Trial, *JAMA.* 298 (2007) 289–298. <https://doi.org/10.1001/jama.298.3.289>.

- [11] R.J. Klement, C.E. Champ, U. Kämmerer, et al., Impact of a ketogenic diet intervention during radiotherapy on body composition: III—final results of the KETOCOMP study for breast cancer patients, *Breast Cancer Research*. 22 (2020) 94. <https://doi.org/10.1186/s13058-020-01331-5>.
- [12] Z.E. Stine, Z.T. Schug, J.M. Salvino, et al., Targeting cancer metabolism in the era of precision oncology, *Nat. Rev. Drug Discov.* 21 (2022) 141-162. <https://doi.org/10.1038/s41573-021-00339-6>.
- [13] M. Liao, D. Yao, L. Wu, et al., Targeting the Warburg effect: A revisited perspective from molecular mechanisms to traditional and innovative therapeutic strategies in cancer, *Acta Pharmaceutica Sinica B*. 14 (2024) 953-1008. <https://doi.org/10.1016/j.apsb.2023.12.003>.
- [14] M. Tufail, C.H. Jiang, N. Li, Altered metabolism in cancer: insights into energy pathways and therapeutic targets, *Mol. Cancer*. 23 (2024) 40. 203. <https://doi.org/10.1186/s12943-024-02119-3>.
- [15] S. Bose, C. Zhang, A. Le, Glucose Metabolism in Cancer: The Warburg Effect and Beyond, *Adv Exp Med Biol*. 1311 (2021) 3-15. https://doi.org/10.1007/978-3-030-65768-0_1.
- [16] J. Luo, T. Wu, J. Zhang, et al., D-mannose promotes diabetic wound healing through inhibiting advanced glycation end products formation in keratinocytes, *Molecular Medicine*. 31 (2025) <https://doi.org/10.1186/s10020-025-01070-3>.
- [17] S. Torretta, A. Scagliola, L. Ricci, et al., D-mannose suppresses macrophage IL-1 β production, *Nature Communications*. 11 (2020) <https://doi.org/10.1038/s41467-020-20164-6>.
- [18] Y.-L. Deng, R. Liu, Z.-D. Cai, et al., Mannose inhibits the growth of prostate cancer through a mitochondrial mechanism, *Asian Journal of Andrology*. 24 (2022) 540-548. <https://doi.org/10.4103/aja2021104>.
- [19] S. Al Hadeethi, C. El-Baba, K. Araji, et al., Mannose Inhibits the Pentose Phosphate Pathway in Colorectal Cancer and Enhances Sensitivity to 5-Fluorouracil Therapy, *Cancers*. 15 (2023) <https://doi.org/10.3390/cancers15082268>.
- [20] Y. Saito, M. Kinoshita, A. Yamada, et al., Mannose and phosphomannose isomerase regulate energy metabolism under glucose starvation in leukemia, *Cancer Science*. 112 (2021) 4944-4956. <https://doi.org/10.1111/cas.15138>.
- [21] A. Rios-Hoyo, N.-L. Shan, P.L. Karn, et al., Clinical Implications of Breast Cancer Intrinsic Subtypes, *Advances in experimental medicine and biology*. 1464 (2025) 435-448. https://doi.org/10.1007/978-3-031-70875-6_21.
- [22] Y.M. Liu, J.Y. Ge, Y.F. Chen, et al., Combined Single-Cell and Spatial Transcriptomics Reveal the Metabolic Evolvment of Breast Cancer during Early Dissemination, *Advanced Science*. 10 (2023) <https://doi.org/10.1002/advs.202205395>.
- [23] N. Lorito, M. Bacci, A. Smiriglia, et al., Glucose Metabolic Reprogramming of ER Breast Cancer in Acquired Resistance to the CDK4/6 Inhibitor Palbociclib(), *Cells*. 9 (2020) <https://doi.org/10.3390/cells9030668>.
- [24] H.M. Brechbuhl, A. Han, K.V. Paul, et al., Metabolic Switch in Endocrine Resistant Estrogen Receptor Positive Breast Cancer, *bioRxiv : the preprint server for biology*. (2024) <https://doi.org/10.1101/2024.12.28.630631>.
- [25] Y. Zhang, M.J. Wu, W.C. Lu, et al., Metabolic switch regulates lineage plasticity and induces synthetic lethality in triple-negative breast cancer, *Cell Metab*. 36 (2024) 193-208.e198. <https://doi.org/10.1016/j.cmet.2023.12.003>.
- [26] Y. Gong, P. Ji, Y.S. Yang, et al., Metabolic-Pathway-Based Subtyping of Triple-Negative Breast Cancer Reveals Potential Therapeutic Targets, *Cell Metab*. 33 (2021) 51-64.e59. <https://doi.org/10.1016/j.cmet.2020.10.012>.
- [27] X. Meng, H. Pang, F. Sun, et al., Simultaneous 3-Nitrophenylhydrazine Derivatization Strategy of Carbonyl, Carboxyl and Phosphoryl Submetabolome for LC-MS/MS-Based Targeted Metabolomics with Improved Sensitivity and Coverage, *Anal Chem*. 93 (2021) 10075-10083. <https://doi.org/10.1021/acs.analchem.1c00767>.
- [28] D. Niether, S. Wiegand, Thermodiffusion and hydrolysis of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *Eur Phys J E Soft Matter*. 42 (2019) 117. <https://doi.org/10.1140/epje/i2019-11880-1>.
- [29] P.S. Gonzalez, J. O'Prey, S. Cardaci, et al., Mannose impairs tumour growth and enhances chemotherapy, *Nature*. 563 (2018) 719-723. <https://doi.org/10.1038/s41586-018-0729-3>.
- [30] A. Samad, R. Samant, K. Venkateshwara Rao, et al., Oxaloacetate as a Holy Grail Adjunctive Treatment in Gliomas: A Revisit to Metabolic Pathway, *Cureus*. 15 (2023) e48821. <https://doi.org/10.7759/cureus.48821>.
- [31] M. Khurshed, R.J. Molenaar, K. Lenting, et al., In silico gene expression analysis reveals glycolysis and acetate anaplerosis in IDH1 wild-type glioma and lactate and glutamate anaplerosis in IDH1-mutated glioma, *Oncotarget*. 8 (2017) 49165-49177. <https://doi.org/10.18632/oncotarget.17106>.

- [32] M.M. Adeva-Andany, N. Pérez-Felpete, C. Fernández-Fernández, et al., Liver glucose metabolism in humans, *Biosci Rep.* 36 (2016) <https://doi.org/10.1042/bsr20160385>.
- [33] T. TeSlaa, M. Ralser, J. Fan, et al., The pentose phosphate pathway in health and disease, *Nat Metab.* 5 (2023) 1275-1289. <https://doi.org/10.1038/s42255-023-00863-2>.
- [34] J. Hu, N. Liu, D. Song, et al., A positive feedback between cholesterol synthesis and the pentose phosphate pathway rather than glycolysis promotes hepatocellular carcinoma, *Oncogene.* 42 (2023) 2892-2904. <https://doi.org/10.1038/s41388-023-02757-9>.
- [35] A. Ahamed, R. Hosea, S. Wu, et al., The Emerging Roles of the Metabolic Regulator G6PD in Human Cancers, *Int J Mol Sci.* 24 (2023) <https://doi.org/10.3390/ijms242417238>.
- [36] L. Liang, F. Sun, H. Wang, et al., Metabolomics, metabolic flux analysis and cancer pharmacology, *Pharmacology & Therapeutics.* 224 (2021) <https://doi.org/10.1016/j.pharmthera.2021.107827>.
- [37] L. Ma, Z. Chen, M. Feng, et al., A diverse treatment with the extract of *Euphorbia fischeriana* Steud. and *Ziziphus jujuba* Mill. for breast cancer nude mice of MCF-7 (ER+) cells or MDA-MB-453 (ER-) cells via modulation of the PI3k/Akt signalling pathway, *Pharmacological Research - Modern Chinese Medicine.* 5 (2022) 100198. <https://doi.org/10.1016/j.prmcm.2022.100198>.
- [38] D. Guo, Y. Tong, X. Jiang, et al., Aerobic glycolysis promotes tumor immune evasion by hexokinase2-mediated phosphorylation of I κ B α , *Cell Metab.* 34 (2022) 1312-1324.e1316. <https://doi.org/10.1016/j.cmet.2022.08.002>.
- [39] N. Fan, X. Zhang, W. Zhao, et al., Covalent Inhibition of Pyruvate Kinase M2 Reprograms Metabolic and Inflammatory Pathways in Hepatic Macrophages against Non-alcoholic Fatty Liver Disease, *Int J Biol Sci.* 18 (2022) 5260-5275. <https://doi.org/10.7150/ijbs.73890>.
- [40] A. De Jesus, F. Keyhani-Nejad, C.M. Pusec, et al., Hexokinase 1 cellular localization regulates the metabolic fate of glucose, *Mol Cell.* 82 (2022) 1261-1277.e1269. <https://doi.org/10.1016/j.molcel.2022.02.028>.
- [41] W. Sun, M. Jia, Y. Feng, et al., Lactate is a bridge linking glycolysis and autophagy through lactylation, *Autophagy.* 19 (2023) 3240-3241. <https://doi.org/10.1080/15548627.2023.2246356>.
- [42] F. Li, W. Si, L. Xia, et al., Positive feedback regulation between glycolysis and histone lactylation drives oncogenesis in pancreatic ductal adenocarcinoma, *Mol Cancer.* 23 (2024) 90. <https://doi.org/10.1186/s12943-024-02008-9>.
- [43] S. Zhu, Y. Guo, X. Zhang, et al., Pyruvate kinase M2 (PKM2) in cancer and cancer therapeutics, *Cancer Lett.* 503 (2021) 240-248. <https://doi.org/10.1016/j.canlet.2020.11.018>.
- [44] M. Rigoulet, C.L. Bouchez, P. Paumard, et al., Cell energy metabolism: An update, *Biochim Biophys Acta Bioenerg.* 1861 (2020) 148276. <https://doi.org/10.1016/j.bbabi.2020.148276>.
- [45] T. Wang, Z. Ye, Z. Li, et al., Lactate-induced protein lactylation: A bridge between epigenetics and metabolic reprogramming in cancer, *Cell Prolif.* 56 (2023) e13478. <https://doi.org/10.1111/cpr.13478>.
- [46] C. Li, F.Y. Liu, Y. Shen, et al., Research progress on the mechanism of glycolysis in ovarian cancer, *Front Immunol.* 14 (2023) 1284853. <https://doi.org/10.3389/fimmu.2023.1284853>.
- [47] C.L. Sake, A.J. Metcalf, M. Meagher, et al., Isotopically nonstationary (13)C metabolic flux analysis in resting and activated human platelets, *Metab Eng.* 69 (2022) 313-322. <https://doi.org/10.1016/j.ymben.2021.12.007>.
- [48] D.M. Wolf, C. Yau, J. Wulfschle, et al., Redefining breast cancer subtypes to guide treatment prioritization and maximize response: Predictive biomarkers across 10 cancer therapies, *Cancer Cell.* 40 (2022) 609-623.e606. <https://doi.org/10.1016/j.ccell.2022.05.005>.
- [49] N. Arai, N. Hattori, S. Yamashita, et al., HSD17B4 methylation enhances glucose dependence of BT-474 breast cancer cells and increases lapatinib sensitivity, *Breast Cancer Res Treat.* 201 (2023) 317-328. <https://doi.org/10.1007/s10549-023-07013-y>.
- [50] L. Boscolo Bielo, D. Trapani, E. Nicolò, et al., The evolving landscape of metastatic HER2-positive, hormone receptor-positive Breast Cancer, *Cancer Treat Rev.* 128 (2024) 102761. <https://doi.org/10.1016/j.ctrv.2024.102761>.
- [51] J. Madoz-Gúrpide, J. Serrano-López, M. Sanz-Álvarez, et al., Adaptive Proteomic Changes in Protein Metabolism and Mitochondrial Alterations Associated with Resistance to Trastuzumab and Pertuzumab Therapy in HER2-Positive Breast Cancer, *Int J Mol Sci.* 26 (2025) <https://doi.org/10.3390/ijms26041559>.
- [52] V.M. Youngblood, L.C. Kim, D.N. Edwards, et al., The Ephrin-A1/EPHA2 Signaling Axis Regulates Glutamine Metabolism in HER2-Positive Breast Cancer, *Cancer Res.* 76 (2016) 1825-1836. <https://doi.org/10.1158/0008-5472.Can-15-0847>.

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- [53] M. Wang, Y. Zhang, Z. Li, et al., Reprogramming of amino acid metabolism in breast cancer, *Biochim Biophys Acta Rev Cancer*. 1880 (2025) 189412. <https://doi.org/10.1016/j.bbcan.2025.189412>.
- [54] M. Xiong, D. Liu, X. Wang, et al., PPAR γ Activation of Fatty Acid Metabolism Drives Resistance to Anti-HER2 Therapies in HER2-Positive Breast Cancer, *Int J Biol Sci*. 21 (2025) 2396-2414. <https://doi.org/10.7150/ijbs.99275>.
- [55] M. Ishfaq, N. Bashir, S.K. Riaz, et al., Expression of HK2, PKM2, and PFKFB3 Is Associated with Metastasis and Late Disease Onset in Breast Cancer Patients, *Genes (Basel)*. 13 (2022) <https://doi.org/10.3390/genes13030549>.
- [56] E. Shin, J.S. Koo, Glucose Metabolism and Glucose Transporters in Breast Cancer, *Front Cell Dev Biol*. 9 (2021) 728759. <https://doi.org/10.3389/fcell.2021.728759>.
- [57] R. Zhang, Y. Yang, W. Dong, et al., D-mannose facilitates immunotherapy and radiotherapy of triple-negative breast cancer via degradation of PD-L1, *Proc Natl Acad Sci U S A*. 119 (2022) <https://doi.org/10.1073/pnas.2114851119>.
- [58] D.B. Abreu, J.R. Cernadas, Management of adverse reactions induced by chemotherapy drugs, *Curr Opin Allergy Clin Immunol*. 22 (2022) 221-225. <https://doi.org/10.1097/aci.0000000000000833>.
- [59] Y.L. Ai, W.J. Wang, F.J. Liu, et al., Mannose antagonizes GSDME-mediated pyroptosis through AMPK activated by metabolite GlcNAc-6P, *Cell Res*. 33 (2023) 904-922. <https://doi.org/10.1038/s41422-023-00848-6>.
- [60] Y.-K. Xie, X.-Y. Pan, X.-R. Liang, et al., Research progress on structural characterization and bioactivities of Poria cocos and Ganoderma polysaccharides, *Food & Medicine Homology*. 2 (2025) 9420040. <https://doi.org/10.26599/FMH.2025.9420040>.
- [61] Y.-Y. Yu, Y.-N. Duan, S. Ma, et al., Research progress on the mechanism of functional activity of edible fungi polysaccharides—focusing intestinal mucus as a key and entry point, *Food & Medicine Homology*. 2 (2025) 9420042. <https://doi.org/10.26599/FMH.2025.9420042>.
- [62] B. Kranjčec, D. Papeš, S. Altarac, d-mannose powder for prophylaxis of recurrent urinary tract infections in women: a randomized clinical trial, *World Journal of Urology*. 32 (2014) 79-84. <https://doi.org/10.1007/s00345-013-1091-6>.
- [63] P.-B. Ancsey, C. Contat, E. Meylan, Glucose transporters in cancer – from tumor cells to the tumor microenvironment, *The FEBS Journal*. 285 (2018) 2926-2943. <https://doi.org/https://doi.org/10.1111/febs.14577>.