Sialic acid-binding lectin-modified fructose-coated nanoparticles: a promising targeted therapeutic synthetic for breast cancers

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Abstract

Background: Sialic acid-binding lectin (cSBL) specifically kills tumor cells rather than healthy cells. Glycopolymer-coated nanoparticles are selectively ingested by tumor cells because they can interact with the enriched carbohydrate receptors located on the surface of tumor cells. In this context, we synthesized cSBL-modified fructose-coated nanoparticles (LMFN) and cSBL-modified glucose-coated nanoparticles (LMGN) to investigate their anticancer activity in various molecular subtypes of breast cancer cell lines.

Methods: The syntheses of fructose-coated nanoparticles and glucose-coated nanoparticles were based on the chemicals of 1,2:4,5-di-*O*-isopropylidene- β -D-fructopyranose and 1,2:4,5-di-*O*-isopropylidene- β -D-glucopyranose, respectively. The carbodiimide-based method was employed to synthesize LMFN and LMGN. The antitumor mechanism was explored by cell cycle analysis with flowcytometry and the antitumor activity was assessed by cytotoxicity assay and multiple drug effects analysis.

Results: The cytotoxicity assay showed that LMFN had robust antitumor activity against all breast cancer phenotype cell lines whereas LMGN was rarely efficacious to against human epidermal growth factor receptor 2-positive/overexpression (HER2+/overexpression) breast cancer cells. The intrinsic reason for these findings was that the overexpression of fructose transporter, GLUT5, was observed in all breast cancer subtype cell lines but only a paucity of glucose transporter, GLUT1, was expressed in HER2+/overexpression breast cancer cell lines that dampened the uptake of LMGN to these cells. The cell cycle analysis indicated that the anticancer activity of LMFN was achieved by arresting cell cycle in S phase. The multiple drug effects analysis suggested the synergistic effect in the combinations of LMFN and tamoxifen to kill estrogen receptor+ breast cancer cells and LMFN and trastuzumab to kill HER2+/overexpressed breast cancer cells.

Conclusion: Our work pinpoints that LMFN may be a new-onset selection for molecularly targeted therapy of breast cancers and paves the way for establishing its clinical application in the future.

Introduction

Carbohydrate is a crucial source of energy for organisms and an indispensable component which participates in the procedures of cell adhesion, cell-cell recognition and cell proliferation. Cancer cells frequently proliferate actively and grow vigorously through abnormal metabolism. Given the Warburg effect, they are apt to implement active glycolysis and produce a large amount of lactic acid even under the conditions of sufficient oxygen and normal mitochondrial function[1]. Their rapid proliferation and the Warburg effect both consume a myriad of glucose, giving rise to a low glucose-microenvironment. Therefore, they have to harness other energy surrogates to complete their growth in this landscape; fructose has the same capability as glucose to promote their colony formation and migration[2] and can functionally replace it to maintain cell proliferation when its provision is insufficient. Fructose is the second largest sugar consumed by human body and accounts for more than 40% of sweetener consumption in Western diet[3]. With the highest sweetness among all natural sugars, it is about 1.8

times as sweet as sucrose[4]. Actually, fructose is more prone to be metabolized than glucose as its metabolism bypasses the rate-limiting enzyme of glycolytic pathway and is not controlled by insulin[5].

Recent epidemiological studies indicate that excessive intake of fructose is related to the occurrence and development of some tumors[6-9]. High intake of fructose increases the incidence rate and accelerates the progression of pancreatic cancer[10, 11]. In the study of human breast cancer cell lines, it was uncovered that fructose prompted the proliferation of MCF7 and MDA-MB-231 in a dose-dependent manner beneath the glucose-efficiency condition, but had no effect on normal cells[2]. Glucose transporter 5 (GLUT5) is exclusively responsible for the fructose absorption by cells[12] and is highly expressed in several breast cancer cell lines and tumor tissues while the normal cell lines are devoid of its presentation[2]. Knockdown of it can obviously inhibit the proliferation and growth of MCF-7 cells in fructose-containing medium but not in glucose-containing medium[2].

The selective expression of GLUT5 in cancer tissues implies that GLUT5 can be used as a potential therapeutic target. An *in vitro* study of Zhao *et al*[13] demonstrated that fructose-coated nanoparticles (FCN) could complete targeted drugs delivery into MDA-MB-231 cells, thanks to the overexpression of GLUT5 found on their plasma membranes. Cancer cells recognize fructose molecules on the surface of FCN and induce GLUT5-mediated endocytosis. The specific affinity between fructose molecules and GLUT5 spurs these micelles preferentially binding to cancer cells rather than normal cells, thus enabling the drastically higher uptake rate of them by tumor cells than that by normal cells. Increasing the amount of fructose molecules on their surface can increase the uptake rate whereas blocking GLUT5 can decrease it as well as impedes the metastasis, proliferation and apoptosis of cancer cells[14, 15].

GLUT1 is a kind of plasma membrane protein that widely exists on the surface of mammalian cell membrane and plays a key role in the process of glucose uptake by tissues and organs[16]. The overexpression of GLUT1 has been identified as an important hypoxia biomarker in malignant tumors and a predictor of tumor angiogenesis. Breast cancer is molecularly classified into five subtypes including Luminal A, Luminal B, Luminal-HER2+, HER2 overexpression and triple-negative breast cancer (TNBC) in terms of the expressed level of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2(HER2) and Ki-67[17]. Hitherto, the comparisons of GLUT1 and GLUT5 expressions across these breast cancer subtype cell lines were unclear, and it was an uncertainty whether these cancer cell lines all proliferate in a dose-dependent manner in glucose-containing medium and fructose-containing medium.

Sialic acid-binding lectin (cSBL) is a protein extracted from the cytoplasm of *Rana catesbeiana* eggs, which can bind to specific carbohydrate structures. With the biological multifunction such as the insecticide activity, the anti-infection and antitumor capabilities[18], cSBL is currently utilized into the research of treating several types of cancers. The proliferation of tumor cells and tumor angiogenesis can be inhibited when lectins bind to carbohydrate-binding sites and the resultant obstructions in migration and invasion of tumor cells occur[19]. Of note, lectin solely has robust binding capability to primary and metastatic cancer tissues but not to normal human tissues[19], consistent with its outstanding cell killing

effects on tumor cells such as breast cancer, cervical cancer, oral cancer, glioblastoma and T cell leukemia but not on healthy cells, for instance, diagonalized cells, fibroblasts and lymphocytes[20-22]. The exclusive binding-tumor phenomenon and the thorough antitumor capability of lectin signify that it may be employed as a latent diagnostic tool and a therapeutic method for cancer.

Lectin suppresses the presentations of ER, PR and HER2 in breast cancer cells[23]; trastuzumab, the first anti-HER2 monoclonal antibody, kills the HER2+/overexpressed breast cancer cells efficiently because it is against the extracellular domain of HER2 protein that reduces the overexpression of HER2[24]; and tamoxifen competitively inhibits the binding of estrogen and ER, resulting in the blockade of ER-mediated signaling that is the backbone for the growth of ER+ breast cancers[25]. However, it was unclear that cSBL acted synergistically or antagonistically with tamoxifen and trastuzumab in repressing the proliferation of ER+ breast cancer cells and HER2+/overexpressed breast cancer cells, respectively.

The purpose of this work was to settle those aforementioned questions. To reinforce the interactive activity between cSBL and tumor cells, the glucose-coated nanoparticles (GCN) and FCN were employed as the nanocarrier to synthesize cSBL-modified GCN (LMGN) and cSBL-modified FCN (LMFN). Both synthetics were respectively applied to treat various breast cancer phenotype cell lines to compare their cell killing effects across these cell lines.

Materials And Methods

Materials. Five molecular subtypes of breast cancer cell lines involving MDA-MB-361 (Luminal A), MCF7 (Luminal B), BT474 (Luminal-HER2+), MDA-MB-231 (TNBC) and SKBR3 (HER2-overexpression) were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China)[26]. The RPMI-1640 cell culture medium, fetal bovine serum (FBS), PBS buffer, agar powder, D-glucose, D-fructose, Cell Counting Kit-8 (CCK8) solution, crystal violet and RIPA lysate were purchased from Solarbio Beijing Science & Technology Co. Ltd (Beijing, China) and the cSBL was originated from Yu Bo Biotech Co. Ltd (Shanghai, China). The chemicals including 4-dimethylaminopyridine (DMAP), propidium iodide (PI), 1,2:4,5-di-*O*-isopropylidene- β -d-fructopyranose, pyridine, methacrylic anhydride, 1,2:4,5-di-*O*-isopropylidene- β -d-glucopyranose, anhydrous dichloromethane, sodium bicarbonate (NaHCO₃), dichloromethane, magnesium sulfate (MgSO₄), ethyl acetate, N-hexane, 1,4-dioxane, 2,2'-azobisbutyronitril (AIBN), 4-cyanopentanoic acid dithiobenzoate (CPADB), diethyl ether, carbodiimide (EDAC), N-hydroxysuccinimide (NHS), tamoxifen and trastuzumab were attained from Zhonghua Science & Technology Co. Ltd (Hangzhou, China).

Soft agar clone formation. The 40°C, 2× RPMI-1640 cell culture mediums (20%FBS) containing different concentrations of fructose were respectively mixed with an equal volume of 40°C, 1.2% soft agar medium. The mixtures were dripped into 6-well plates to prepare the bottom culture mediums followed by cooling and solidifying at room temperature. The 40°C, 0.7% soft agar medium was added into identical volume of these fructose mediums and followed by being mixed with cell suspension. The mixtures were dropped onto the solidified bottom culture medium and the 6-well plates were placed into 5% CO₂, 37°C

cell incubator for 10 days. Counting: The cell culture wells were stained by 0.005% crystal violet more than 1 hour. Then, 10 fields in each well were randomly selected to be observed beneath 100-fold microscope and only the cell colonies harboring cell number more than 50 were counted. The soft agar clone formation experiment with reference to glucose was implemented with the same parameters and procedures.

Western blot analysis. The harvested cells were washed with PBS followed by fully lysis with adding 4°C RIPA lysis buffer and 4 times of vibration by an ultrasonic vibrator. Insoluble substances were removed by a centrifugation at 10,000 rpm for 10 minutes, and protein was quantified by bicinchoninic acid. The obtained protein content was resolved by SDS-PAGE electrophoresis and transferred onto nitrocellulose membrane. The GLUT1 and GLUT5 expressions were respectively detected by correspondent rabbit primary antibody followed by overnight incubation with rabbit secondary antibody and chemiluminescence detection.

Synthesis of FCN. An appropriate proportion of 1,2:4,5-di-*O*-isopropylidene- β -d-fructopyranose, DMAP, pyridine and 0°C methacrylic anhydride were added into anhydrous dichloromethane. After stirring at room temperature for 48 hours, the organic layer was separated by ice-cold saturated NaHCO₃ solution. The aqueous layer was extracted with dichloromethane and the organic layer was dried with MgSO₄ followed by concentrating under reduced pressure. The product was further purified by flash column chromatography by using ethyl acetate: N-hexane (1:1) as the eluent.

In a Schlenk tube, the purified product was dissolved in 1,4-dioxane and presently AIBN and CPADB were added in and mixed up. Placing the tube in a freeze-pump-thaw for 3 cycles made the mixture be degassed, followed by a polymerization at 70°C that would be halted with ice water after 15 hours. The polymerized solution was poured into diethyl ether for precipitation and then the harvest viscous polymer was dried under vacuum for 24 hours.

Synthesis of GCN. Substituting the 1,2:4,5-di-*O*-isopropylidene- β -d-fructopyranose with 1,2:4,5-di-*O*-isopropylidene- β -d-glucopyranose, the applied methods and the rest chemicals were as exactly same as the synthesis of FCN.

Synthesis of LMFN. A specific proportion of EDAC and NHS were concurrently dissolved into PBS as the activating solution. The cSBL (0.6 mg) was dissolved in PBS (5 mL) preparing cSBL/PBS solution (0.12 mg/mL). The FCN (1 g) was dissolved in activating solution (5 mL) that sequently activated at room temperature for four hours followed by a centrifugation at 4000 rpm for 10 minutes. Extracting the supernatant, it was poured into a sterile 15 mL centrifuge tube and the cSBL/PBS solution (5 mL) was added synchronically. The mixture was incubated in the 4°C refrigerator for 14 hours. Fetching out the centrifuge tube and centrifuging it at 4000 rpm for 10 minutes, the harvested supernatant was LMFN.

Synthesis of LMGN. The synthesizing process of LMGN was identical as that of LMFN, with exception of replacing the FCN as the same weight of the GCN.

Cytotoxicity assay. The CCK8 Test was applied for the cytotoxicity assay. The dosing cohort, non-dosing cohort and blank cohort were set up in a 96-well plate. All wells of the first two cohorts were plated 10×10^3 cells accompanied by no cells in the wells of the blank cohort. The dosing cohort was treated with 10% LMFN/RPMI-1640 cell culture medium or 10% LMGN/RPMI-1640 cell culture medium, while the other groups were only treated with RPMI-1640 cell culture medium. After cultivating 24 hours, 10 μ L CCK8 solution was added followed by an incubation away from light for 3 hours. The absorbance at 450 nm of each group was measured by enzyme-labeled instrument, and the cell viability and drug inhibition rate were calculated.

Cell cycle analysis. Cells were cultivated in 3 control wells and 3 opposite experimental wells. The experimental wells were treated with 500ug/ml LMFN and continuously cultured for 48 hours. Cells in both groups were washed and resuspended with PBS, adjusting the cell concentration to 1×10^6 /ml. The cell resuspension was translated into a flow tube followed by centrifuging at 800 rpm for 5 minutes. The supernatant was cast away and the cells were fastened with 4°C, 75% ethanol at 4°C refrigerator for 2 hours. The fastened cells were treated with 100ul RNase A and placed in a 37°C-thermostat water bath for 30 minutes. Cell cycle was detected by flowcytometry after cells fully mixed with PI solution and stored under shade in 4°C refrigerator for 30 minutes.

Multiple drug effects analysis. The LMFN group, the tamoxifen group and the combination group (LMFN plus tamoxifen) were set up in six 96-well plates. Each group contained the dosing subgroup, non-dosing subgroup and blank subgroup. ER+ breast cancer cell lines MCF7 and MDA-MB-361 were placed in the former two subgroups. Similar to above, only the dosing subgroups were treated with agent. After dosing overnight, the inhibition rates of all groups with 6 different concentrations of agents were calculated.

For the multiple agent effects analysis on HER2+ breast cancer cell lines SKBR3 and BT474, the LMFN group, the trastuzumab group and the combination group (LMFN plus trastuzumab) were set up in six 96-well plates.

Combination index (CI) was calculated in terms of the Chou-Talalay method by inputting the agent concentrations coupled with the corresponding inhibition rates into Calcosyn Version 2.0 (Biosoft, Cambridge, UK)[27]. Mean CI (CI_m) value <0.9 indicates a synergistic effect, value between 0.9 to 1.1 implies an additive effect, and value >1.1 represents an antagonistic effect[28].

Statistical analysis. Results of measurement data were expressed as means $\pm$ SD. All experiments were repeated thrice. Using the GraphPad Prism Version 5.0 software, the statistical comparisons between two groups adopted an unpaired t-test and among multiple groups the One-way ANOVA was used followed by a Bonferroni test to compare differences across the interior-group. A P-value of <0.05 was considered to be statistically significant.

Results

All phenotypes of breast cancer cell lines proliferated in a dose-dependent manner in the fructose-containing medium. The five molecular subtypes of breast cancer cell lines were fostered in the RPMI-1640 cell culture mediums containing fructose with 4 gradient concentrations of 2500mg/L, 5000mg/L, 7500mg/L and 10000mg/L, respectively. As shown in **Figure 1A**, the number of cell colony formation increased significantly with the increment of fructose concentration.

The growth of HER2+ cell lines showed difference with that of HER2- cell lines in glucose-containing medium. The proliferation of HER2- breast cancer cell lines MDA-MB-361, MCF7 and MDA-MB-231 in the fructose-containing medium was in the dose-dependent manner. By contrast, the number of colony formation of HER2+ breast cancer cell lines BT474 and SKBR3 gradually decreased when the glucose concentration exceeded 5000 mg/L, i.e. reached 7500mg/L and 10000mg/L (**Figure 1B**). This inconsistent phenomenon of proliferation implied that the absorption and usage of fructose and glucose by these cell lines might be in diversity.

HER2+ breast cancer cell lines underexpressed GLUT1. GLUT1 and GLUT5 are the glucose transporter and the fructose transporter, respectively, which embed in the cell membrane [12, 16]. These 5 breast cancer subtype cell lines all overexpressed GLUT5 with no significance (**Figure 2A-B**) while the GLUT1 was only overexpressed in HER2- breast cancer cell lines MDA-MB-361, MCF7 and MDA-MB-231. The expressions of GLUT1 in HER2+ breast cancer cell lines BT474 and SKBR3 were significantly lower than those in HER2- breast cancer cell lines (**Figure 2B**). These results explained the reason why the proliferation of HER2+ breast cancer cell lines was suppressed in the high concentrations of glucose-containing mediums. The underexpressed GLUT1 limited the glucose uptake by tumor cells, and the high concentration of glucose converted the tumor microenvironment, collectively leading to inhibit the proliferation and growth of HER2+ breast cancer cell lines.

LMFN manifested robust cell killing effects on all breast cancer subtype cell lines. Without significant difference, LMFN showed vigorous inhibition efficacy to all phenotypes of breast cancer cell lines. However, the inhibition efficacy of LMGN on HER2+ breast cancer cell lines SKBR3 and BT474 was lower than that on HER2- breast cancer cell lines (**Figure 3**), which might be related to their underexpression of GLUT1.

LMFN inhibited the proliferation of breast cancer cells by arresting cell cycle. Given that LMGN did not experience strong cell killing effect on HER2+ breast cancer cell lines, we only investigated the mechanism of LMFN to kill tumor cells. For all breast cancer phenotypes cell lines, the cell population arrested in S phase of LMFN groups compared with the blank groups significantly increased (**Figure 4**), indicating that LMFN inhibited cell proliferation via arresting cell cycle.

LMFN synergized with tamoxifen to kill ER+ breast cancer cells and with trastuzumab to kill HER2+ breast cancer cells. Herein, LMFN was continuously applied to combine with other agents for analyzing the multiple drug effects. As outlined in **Figure 5A** and **5B**, the combination of LMFN and tamoxifen synergistically killed MFC7 ($CI_m=0.54$) and MDA-MB-361 ($CI_m=0.37$); additionally, LMFN combined with

trastuzumab had a synergistic effect on killing BT474 and SKBR3, with CI_m value of 0.79 and 0.68, respectively (**Figure 5C** and **5D**).

Discussion

Dietary fructose is closely associated with a variety of metabolic diseases[29, 30] and is functionally alike to glucose in boosting the proliferation and metastasis of tumor cells[2]. Our work demonstrates that fructose universally prompts the clone formation of these five molecular subtypes of breast cancer cell lines in a dose-dependent manner but the high concentrations of glucose inhibit the proliferation of HER2+/overexpressed breast cancer cell lines, because the overexpression of GLUT5 exists in all breast cancer cell lines whereas GLUT1 is underexpressed in HER2+ breast cancer cell lines.

The uptake of carbohydrates by cells is dependent on the members of GLUT family that are located on their plasma membrane and encoded by *SLC2A1*[31, 32]. GLUT1 is the first characterized GLUT glucose transporter, particularly focusing on the constant uptake of glucose into cells through facilitative diffusion[33, 34]. Research findings have shown that the elevated level of GLUT1 can be observed in myriad types of tumorigenesis procedure and has become an important hypoxia biomarker for malignant tumors[35]. With an exception, our work found that GLUT1 was lowly expressed in HER2+/overexpressed breast cancer cell lines, indicating that GLUT1 cannot be employed as an all-around predictive biomarker for these tumors. By now, it needs to further investigate whether it is a coincidence or there has some detailed mechanisms with pertinent to the HER2+ status restricting the expression of GLUT1.

GLUT5 is the only glucose transporter specifically binding to fructose[36]. Knockdown of it markedly inhibits the growth of several types of breast cancer cells in fructose-containing medium but not in glucose-containing medium[2]. In normal tissues, GLUT5 is mainly presented in small intestine but is underexpressed in the brain, adipose tissue, kidney, testis and skeletal muscle. Previous study had demonstrated that a crowd of breast cancer cells, cervical cancer cells and liver cancer cells overexpress GLUT5[2], and our work further confirms the elevated level of it in five molecular phenotypes of breast cancer cell lines, suggesting that GLUT5 overexpression can be used as a potential biomarker for predicting tumorigenesis. The high level of GLUT5 protein increases the fructose intake by tumor cells and accelerates the cancer development, thereby the dietary fructose is supposed to be limited and even abstained in cancer patients.

The antitumor activity of the lectin is principally attained by the following paths. (1) Lectin directly bind to various integrins and epidermal growth factor receptor (EGFR) on cancer cell surface and promote the internalization and autophagic degradation of these molecules, inducing caspase-8 dependent cell apoptosis[37]. (2) Lectin downregulates vascular endothelium integrins to inhibit the neovascularization in a dose-dependent manner[37]. (3) Tumor immunogenicity can be improved by lectin due to the decreased expression of B7-H4 that is a negative regulator of T cell mediated immunity[37]. (4) Lectin incurs the downregulation of *STMN1* and *MCM4* as well as the upregulation of *WEE1*, *RAD1* and *ATR*, giving rise to the cell cycle arrest in S phase[38]. (5) The increase of cytochrome C deposition and *Bax*

expression and the decrease of *Bcl-2* expression simultaneously emerge in breast cancer cells treated with lectin, which clearly suggests a mitochondrial pathway mediating the lectin-induced cell apoptosis[39]. (6) Additionally, the treatment of breast cancer cells with lectin leads to the occurrence of apoptosis and autophagy via inhibiting the EGFR-mediated Ras-Raf-MEK-ERK pathway[39]. (7) Moreover, lectin binding to cancer cells inhibits cell migration, invasion and adhesion, which may be in association with a membrane protein, glycoprotein POTE ankyrin domain family member F[40].

Recently, glycopolymer-coated nanoparticles have attracted great interest due to the specific binding between them and carbohydrate receptors located on the cancer cell surface, suggesting that these synthetics are potential to deliver agents into tumor cells in a targeted way[13]. Several studies have addressed that the intake of FCN can be substantially observed in tumor cells but rarely in healthy cells[13, 41], which is correlated to the overexpressed GLUT5 found on the surface of tumor cells[42, 43]. Of note, nanoparticles that are coated with hydrophilic shell manufactured by long polymer chains with high fructose density have great affinity to tumor cells[14]. Since biocompatibility, relative non-toxic property and the capability to cross the blood-brain barrier, GCNs are widely used as the radiosensitizer in radiotherapy and can carry therapeutic biomolecules into brain[44, 45]. Because of the specific antitumor effect of lectin and the selective interaction between glycopolymer-coated nanoparticles and tumor cells, we synthesized LMFN and LMGN by EDAC-based method and found that LMFN could arrest the cell cycle in S phase and had potent antitumor activity to all molecular subtypes of breast cancer cell lines. Furthermore, the synergistic effect was presented in the combination of LMFN and tamoxifen to killing ER+ breast cancer cell lines and in that of LMFN and trastuzumab to killing HER2+ breast cancer cell lines. Our findings offer a novel-innovative scenario for the targeted treatment in breast cancer. Admittedly, the stride towards establishing the clinical application of LMFN will be laborious and time-consuming.

There were some limitations in our work. Firstly, only a single kind of cell line was chosen for each breast cancer subtype cell population, which might result in selection bias. Second, the healthy cell lines were not involved to verify the selective antitumor activity of LMFN to malignant cells. Third, we only conducted cell cycle analysis to investigate the mechanism of action with respect to the antitumor efficacy of LMFN, more investigations such as an apoptosis assay and an autophagy assay might be also relevant. Lastly, the knockdown of GLUT5 might be needed to further prove the close association between the uptake of LMFN by tumor cells and the elevated expression of GLUT5 embedded in their surface.

Conclusion

Collectively, LMFN exhibits extensively robust antitumor efficacy to various molecular phenotypes of breast cancer cells, implicating that it may become a new choice for targeted therapy in breast tumors. Further studies regarding the molecular mechanism of LMFN-inhibited tumor cell proliferation and the LMFN as a single therapeutic biomolecule or in combination with other agents to explore its antitumor activity *in vivo* will be worthily performed in the future.

Declarations

Ethics Statement

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Lin He: Writing; charting; experiments.

Biyuan Zhang: Statistic analysis; experiments.

Haiji Wang: experiments.

Yuhua Song: Conception/Design; funding; experiments; final approval of manuscript.

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Figures

Figure 1

The soft agar clone formation experiments of fructose medium and glucose medium. (A) The RPMI-1640 cell culture mediums containing the concentrations of fructose are 2500 mg/L, 5000 mg/L, 7500 mg/L and 10000 mg/L, respectively. (B) The identical concentration parameters of glucose are applied.

*** $P < 0.001$. ** $P < 0.01$. # $P > 0.05$. Error bars represent the standard deviation for separate experiments.

A

Western blot analysis showing protein levels of GLUT1 (56kDa), GLUT5 (54kDa), and GADPH (36kDa) in five breast cancer cell lines: SKBR3, BT474, MDA-MB-231, MCF7, and MDA-MB-361. The blots are arranged in four rows, with the first row showing GLUT1, the second row showing GADPH, the third row showing GLUT5, and the fourth row showing GADPH. The lanes are labeled with the cell line names: SKBR3, BT474, MDA-MB-231, MCF7, and MDA-MB-361. The protein levels are indicated by the intensity of the bands.

Protein	SKBR3	BT474	MDA-MB-231	MCF7	MDA-MB-361
GLUT1 (56kDa)	Low	Low	High	High	High
GADPH (36kDa)	High	High	High	High	High
GLUT5 (54kDa)	High	High	High	High	High
GADPH (36kDa)	High	High	High	High	High

Figure 2

The expressions of GLUT1 and GLUT5 in different breast cancer subtype cancer cell lines. (A) Western blot analysis. GADPH is taken as the internal reference. (B) Grayscale analysis of western bolt. Solid line, the comparison between SKBR3 with non-HER2+ breast cancer cell lines; dotted line, the comparison between BT474 with non-HER2+ breast cancer cell lines. *** $P < 0.001$. # $P > 0.05$.

Figure 3

The cytotoxicity assay of LMFN and LMGN by CCK8 test. LMFN, sialic acid-binding lectin-modified fructose-coated nanoparticles; LMGN, sialic acid-binding lectin-modified glucose-coated nanoparticles. The left cohort is the cytotoxicity assay of LMFN and the right cohort is the cytotoxicity assay of LMGN. Both cohorts employ MCF7 as the control. ***P<0.001. #P>0.05. Error bars represent the standard deviation for separate experiments.

Figure 4

The cell cycle analysis of five phenotypes of breast cancer cell lines treated with LMFN. All LMFN cohorts of these cell lines are treated with 500 ug/ml LMFN and continuously cultured for 48 hours. (A) SKBR3 (HER2-overexpression subtype). (B) BT474 (Luminal-HER2+ subtype). (C) MCF7 (Luminal B subtype). (D) MDA-MB-361 (Luminal A subtype). (E) MDA-MB-231 (triple-negative breast cancer). ***P<0.001. **P<0.01. Error bars represent the standard deviation for separate experiments.

Figure 5

The multiple drug effects analysis of LMFN combined with other agents against breast cancer cells. (A) LMFN combines tamoxifen to kill MCF7. (B) LMFN combines with tamoxifen to kill MDA-MB-361. (C) LMFN combines with trastuzumab to kill BT474. (D) LMFN combines with trastuzumab to kill MDA-MB-231. (A, B) Open rounds, tamoxifen alone; the concentrations of tamoxifen in this group are 10000, 5000, 2500, 1250, 625 and 312.5 nmol/L, respectively. Dark squares, LMFN alone; the concentrations of LMFN in this group are 500, 250, 125, 62.5, 31.25 and 15.625 ug/mL, respectively. Grey triangles, tamoxifen plus LMFN, the concentrations of tamoxifen and LMFN are identical to above two groups. (C, D) Open rounds, trastuzumab alone; the concentrations of trastuzumab in this group are 10, 5, 2.5, 1.25, 0.625 and 0.3125 ug/mL, respectively. Dark squares, LMFN alone; the concentrations of LMFN in this group are 500, 250, 125, 62.5, 31.25 and 15.625 ug/mL, respectively. Grey triangles, trastuzumab plus LMFN; the concentrations of trastuzumab and LMFN are identical to above two groups. Mean combination index

(CI_m) <0.9 indicates a synergistic effect for the combinations. Error bars represent the standard deviation for separate experiments.