Original Article
High glucose induced human breast cancer cell MCF-7 viability, migration and invasion via the expression of AKT, MAPKs and STAT3

Qi Wu1, Wenhuan Li2, Shan Zhu1, Juanjuan Li1, Juan Wu1, Xiang Li1, Chuang Chen1, Wen Wei1, Shengrong Sun1, Changhua Wang3

1Department of Breast and Thyroid Gland Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, P. R. China; 2Department of Thyroid and Breast Surgery, The Central Hospital of Wuhan, Wuhan 430071, Hubei Province, P. R. China; 3Department of Pathophysiology, Wuhan University School of Basic Medical Sciences, Wuhan 430060, Hubei Province, P. R. China

Received January 10, 2016; Accepted April 6, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: High glucose induces human breast cancer cell viability, migration and invasion in vitro and in vivo. However, the mechanism of high glucose induces human breast cancer cell viability, migration and invasion remains poorly understood. In our study, we investigate the effects of high glucose induces MCF-7 human breast cancer cell viability, migration and invasion in vitro experiments. High glucose induces activation of cell viability, migration and invasion in a time- and concentration-dependent manner. The mechanism reveals that high glucose significantly increases the phosphorylation of AKT, ERK1/2 and STAT3 and inhibits the phosphorylation of p38 MAPK. These results suggest that high glucose induces human breast cancer cell MCF-7 viability, migration and invasion via the expression of AKT, MAPKs and STAT3.

Keywords: High Glucose, MCF-7, AKT, MAPKs, STAT3

Introduction
Breast cancer is the most commonly occurring tumor among women worldwide. In China, approximately one in every ten women will develop the disease in their lifetime, and it is the second leading cause of cancer-related death in women [1]. Type2 diabetes (T2DM) is characterized by a chronic hyperglycemic state caused by insulin resistance in skeletal muscle, adipose tissue, the liver, and/or impaired insulin secretion [2]. It is now well-established that T2DM is linked to increasing breast cancer incidence and mortality [3-5]. According to recent studies, T2DM conferred as much as 37% increased risk of breast cancer in women [6] and both T2DM and breast cancer incidence are increasing at alarming rates worldwide. Even prediabetes may also increase the risk of breast cancer [7-9]. The detailed mechanisms remains unknown, however, that the hyperglycemia in T2DM patients may promote cancer progression is a possible mechanism.

The central role of AKT in the PI3Ks pathway makes it one of the most activated downstream effectors, the AKT kinase family includes three members AKT1, AKT2, and AKT3. It is becoming increasingly clear that AKT isoforms underline their distinct functional role in cancer development and progression [10-12]. Therefore, hyperglycemia enhances the viability of non-tumorigenic and malignant mammary epithelial cells through increased leptin/IGF1R signaling and activation of AKT/mTOR [13].

The mitogen-activated protein kinase (MAPK) pathway is an important signaling pathway in living beings in response to extracellular stimuli. There are 3 main subgroups manipulating by a set of sequential actions: ERK1/2, JNK/SAPK, p38 MAPK. ERK1/2 can be triggered by growth factors or the transcription factors involved in tumor cell differentiation, proliferation, survival, migration, and angiogenesis, meanwhile glucose uptake and metabolism are required for ERK1/2 activation [14, 15]. p38 MAPK that
High glucose induced MCF-7 viability, migration and invasion

mediates various cellular functions such as apoptosis, cell growth and differentiation are activated by inflammatory cytokines and a variety of environmental stresses. p38 MAPK activity play important roles in tumour progression [16, 17].

Of the seven members of signal transducer and activator of transcription (STAT) protein family, STAT3 has been demonstrated to be the most important for cancer progression [18, 19]. It’s not only crucial for transducing signals from numerous receptor and non-receptor tyrosine kinases that are frequently activated in cancer cells, but they are also transcription factors that regulate the expression of a wide range of genes [20, 21], thereby contributing to tumour progression.

Taken together, this evidence suggests that high glucose induced human breast cancer cell viability, migration and invasion, but its possible mechanism have not been investigated. To our knowledge, this is the study demonstrating that high glucose induced human breast cancer cell MCF-7 viability, migration and invasion via the expression of AKT, MAPKs and STAT3.

Materials and methods

Materials

Tubulin, AKT and the phospho-AKT (Ser473) antibody, STAT3 and the phospho-STAT3 (Ser727), the MAPK family antibody sampler kit, and the phospho-MAPK family antibody sampler kit were purchased from Cell signaling Technology (USA). D-Mannitol were purchased from Sigma (USA).

Cell culture and treatment

Human breast cancer cell line, MCF-7 (ATCC, Manassas, USA) were cultured in RPMI 1640 (Gibco BRL, GrandIsland, NY, USA) containing 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA) and 1% penicillin/streptomycin (Invitrogen, Grand Island, NY) at 37°C in a humidified atmosphere of 5% CO₂. Cells were cultured in normal-glucose (5.5+16.5 mM D-Mannitol for osmotic balance) and high-glucose (11+11 mM D-Mannitol for osmotic balance or 22 mM) conditions. At 80-90% confluence, cells were subjected to serum starvation in serum-free RPMI 1640 overnight and then stimulated with wogonin under normal- and high-glucose conditions for 48 h.

MTT assay

The thiazolyl blue tetrazolium bromide (MTT) (Amresco, Solon, OH, USA) was dissolved in phosphate buffered saline (PBS) at a concentration of 5 mg/ml, filtered, and stored at 4°C. Cells were seeded into a 96-well plate, washed three times with PBS and starvation in serum-free RPMI 1640 overnight. Cells were treated with wogonin under normal- and high-glucose conditions for 24 h, 48 h, 72 h. For the viability assay, 20 μl MTT was added into each well. An ELISA plate reader (Biotek, Winooski, Vermont, USA) was used to measure the optical density at 490 nm. The viability of control cells was 100%.

TUNEL assay

The TUNEL assay was performed using an In Situ Cell Death Detection Kit (Roche, Basel, Switzerland) following the manufacturer’s instructions. Cells that were cultured in serum-free RPMI 1640 containing 5.5 mM glucose were used as a negative control. Images were taken with the Olympus FluoView FV1000 Confocal Microscope.

Wound healing assay

MCF-7 cells were grown to confluent monolayers on 6-well plates and a pipette tip was used to create linear scratch wounds. Mitomycin C (Amresco) was used to inhibit cell viability. 1%
High glucose induced MCF-7 viability, migration and invasion

FBS also was used in the assay. Cells migrated into the wound surface and the relative wound closure were determined under an inverted microscopy at various times, five randomly chosen fields were analyzed for each well. The percentage of inhibition was expressed using control wells at 100%. Wound images were taken with a digital camera mounted on light microscope. The wound gap widths were measured using Image J software.

**Transwell assay**

The upper chamber of each 8.0 μm pore size Transwell apparatus (Corning, NY, USA) was coated with Matrigel (BD Biosciences, San Jose, CA). MCF-7 cells were added to the upper chamber at a density of 2*10⁶ cells/ml (100 μl per chamber) in serum-free RPMI 1640 and incubated for 48 h with 10% FBS and wogonin under normal- and high-glucose conditions in the lower compartment. Cells on the upper surface were removed by a cotton swab. Cells that penetrated to the lower membrane surface were fixed in 4% paraformaldehyde, stained with crystal violet, and quantified by manual counting and ten randomly chosen fields were analyzed for each group.

**Western blots**

Cells were collected with lysis buffer after being washed three times with ice-cold PBS. Lysates were boiled in SDS loading buffer for 10 min

---

Figure 2. Effect of high glucose on MCF-7 cells viability, migration, invasion in vitro. Cells were exposed to different glucose concentrations for 48 h in 5% CO2 incubator at 37 °C. A. MTT assay was used to exam the effects of high glucose on cells viability. B. TUNEL assay was used to exam the effects of high glucose on cells apoptosis. C. Confluent monolayer was scraped with a pipette tip and cells migrated into the wounded monolayer was assessed by microscope equipped with a camera. D. Cells were seeded in the upper chamber of transwell coated with matrigel, after 48 h incubation, invasive cells passed through the membrane and were measured by crystal violet staining. The bars represent the mean values ± SD triplicate (n = 3). *P < 0.05, **P < 0.01 vs. the 5.5 mM group.
then cleared by centrifugation (14,000 rpm, 10 min, 4°C). The proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected with specific antibodies.

Figure 3. Effect of high glucose on MCF-7 cells via the expression of AKT, MAPKs and STAT3. Cells were exposed to different glucose concentrations for 48 h in 5% CO\textsubscript{2} incubator at 37°C. Cell lysates were then examined by Western blot analysis to verify the total and phosphorylation of AKT, MAPKs and STAT3. Band intensities were normalized to Tubulin and presented as a bar graph. The bars represent the mean values ± SD triplicate (n = 3). *P < 0.05, **P < 0.01 vs. the 5.5 mM group.
High glucose induced MCF-7 viability, migration and invasion

Statistical analysis

All experiments were done independently at least three times. Results are represented as the mean ± SD. The quantification of the increase in protein expression and phosphorylation was performed using NIH Scion Image software and was normalized with the control protein expression in each experiment. Significant differences between groups were analyzed by using the paired t-test. A P-value of < 0.05 was considered statistically significant.

Results

Effect of high glucose on MCF-7 cell viability in a concentration-dependent and time-dependent manner

The overall effects of high glucose in MCF-7 cells was assessed using an MTT assay. As shown in Figure 1, high glucose promoted MCF-7 cell viability in a concentration-dependent and time-dependent manner when compared to the control. At glucose 22 mM group, cell viability after 48 h of treatment was promoted 153.2% compared to glucose 5.5 mM group.

Effect of high glucose on MCF-7 cells viability, migration, invasion in vitro

To investigate the effects of high glucose-induced MCF-7 cells growth, MCF-7 cells were incubated under normal- and high-glucose conditions for 48 h. MTT and TUNEL assays were performed to measure cell viability and apoptosis. As demonstrated in Figure 2A, high glucose improved MCF-7 cell viability, accordingly decreased MCF-7 cell apoptosis (Figure 2B). We used wound healing and transwell assays as described in the materials and methods section to test the effect of high glucose-induced MCF-7 cell migration and invasion. As shown in Figure 2C, after 48 h the wound was almost covered due to the influx of highly migratory cells in high glucose groups, whereas low glucose groups cells remained close to the initial state. As shown in Figure 2D, compared with the control, a dose-dependent augment in the number of invasive cells was seen in high glucose groups.

Effect of high glucose on MCF-7 cells via the expression of AKT, MAPKs and STAT3

AKT, MAPKs and STAT3, which are important glucose effectors in tumour progression, are involved in the regulation of MCF-7 growth, migration, invasion. Western blotting assay was also used to find out the mechanism of high glucose-induced MCF-7 cells growth, migration, invasion. We tested the total and phosphorylation of AKT expression in MCF-7 cells exposed under normal- and high-glucose conditions for 48 h. As shown in Figure 3, the phosphorylation of AKT was increased in high glucose groups. The amount of phosphorylation p38 reduced in high glucose groups, while ERK1/2 increased in high glucose groups. JNK1/2 was not significantly influenced under high glucose conditions. Also the phosphorylation of STAT3 was increased in high glucose groups.

Discussion

As cancer cells use glucose as the source of energy for their viability, high glucose provides a favorable environment for the growth and survival of breast cancer cells [22, 23]. Further, it has been reported that high glucose confers resistance to chemotherapy in malignant cancer cells but not in non-malignant cell [23, 24]. We demonstrate that high glucose induce MCF-7 viability, migration and invasion, moreover the mechanism study that the effect is associated with the expression of AKT, MAPKs and STAT3.

Viability, migration and invasion of tumor is the major cause of morbidity and mortality. The mechanism is a multistep and complex process involving the extracellular signalling, tumor microenvironment and stimulating factors. It is reported that activation of AKT plays a critical role in high glucose-induced cancer cell lines viability, migration and invasion [13, 25-29]. It is well-established that hyperglycemia enhances the viability of non-tumorigenic and malignant mammary epithelial cells through increased leptin/IGF1R signaling and activation of AKT/mTOR [13].

MAPKs are involved in a multitude of cellular pathways and functions in response to a variety of ligands and cell stimuli. In many cancer cell types, the ERK1/2 are linked to cell proliferation. ERK1/2 are thought to play a role in some cancers, because mutations in Ras and B-Raf, which can activate the ERK1/2, are found in many human tumors [15]. Reactive oxygen species (ROS) are well known to induce the activation of the Raf/MEK/ERK1/2 signaling pathways in a variety of cell types [30, 31]. In pancreatic cancer ROS intermediates act
High glucose induced MCF-7 viability, migration and invasion
directly on growth receptors, such as the high glucose [32], and that induce the activation of
ERK1/2 and p38 signaling. p38 kinases are members of MAPK family that transduce sig-
als from various environmental stresses, growth factors and steroid hormones. p38 has
recently gained attention as a tumor suppres-
sor. This effect is reported that increased
tumorigenicity of the lack of p38 MAPK cells is
called mainly by a decrease in the apoptosis rate indicating that the lack of the p38 MAPK
causely increase the ERα:ERβ ratio and a reduction in the activity of the p53
tumor suppressor protein [33]. Further, There is
much evidence to support a role for p38α as a
tumour suppressor, and this function of p38α
is mostly mediated by both negative regulation
cell cycle progression and the induction of
apoptosis, although the induction of terminal
differentiation also contributes to tumour sup-
pressive function [34]. These data suggest that
high glucose can activate ERK1/2 and inhibit
p38 that this may promote growth, migration,
invasion and prevent apoptosis in MCF-7.

STATs are latent transcription factors that medi-
ate cers are reported to contain constitutively
activated cytokine- and growth factor-directed
transcription [19, 20, 35]. In many human can-
cers and transformed cell lines, STAT3 is per-
sistentaly activated, and in cell culture, active
STAT3 is either required for transformation,
enhances transformation or blocks apoptosis
[36-38]. There is study provide evidence that
glucose can promote both migration and inva-
sion of CT-26 cells, and that the STAT3-induced
MMP-9 signal pathway is involved in this pro-
cess [39]. It is well-established that high glu-
cose can increase mitochondria-derived ROS
generation and subsequently upregulate VEGF
expression possibly through JAK2/STAT3 acti-
vation [40]. It demonstrates that progestins
(PR) also induce STAT3 phosphorylation at
Ser727 residue, which occurs via activation of
ERK1/2 pathways [41]. These data suggest that
high glucose can activate STAT3 that this
may promote growth, migration, invasion and prevent apoptosis in MCF-7.

In conclusion, this is the major study to show
that high glucose induces human breast cancer
cell MCF-7 viability, migration and invasion via
the expression of AKT, MAPKs and STAT3. Never-
theless, we find that high glucose induces
MCF-7 viability, migration and invasion just by
the experiments in vitro, whether those need to
be further investigated in vivo.

Acknowledgements
This work was partially supported by a NSFC
grant to Prof. Shengrong Sun (Grant NO:
30701032), and a NSFC grant to Prof. Changhua
Wang (Grant NO: 30770758/H0178). This work
was also supported by a NSFC grant to Dr.
Juanjuan Li (Grant NO: 81302314/H1622), the
Fundamental Research Funds of Hubei Pro-
vince Health and Family Planning Commission
to Wen Wei (Grant NO: JS-20110118) and the
Fundamental Research Funds for the Central
Universities of China to Shan Zhu (Grant NO:
2042014kf0189).

Disclosure of conflict of interest
None.

Address corresponson rule to: Shengrong Sun, De-
partment of Breast and Thyroid Surgery, Renmin
Hosptial of Wuhan University, 99 Zhang Zhidong
Road, Wuhan 430060, Hubei Province, P. R. China.
E-mail: sun137@sina.com; Changhua Wang, Depart-
ment of Pathophysiology. Wuhan University School
of Basic Medical Sciences. Wuhan 430060, Hubei
Province, P. R. China. E-mail: chwang0525@whu.
edu.cn

References
[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer statis-
diabetes mellitus. Diabetes Care 2010; 30:
S42-S47.
[3] Boyle P, Boniol M, Koechlin A, Robertson C,
Valentini F, Coppens K, Fairley LL, Boniol M,
Zheng T, Zhang Y, Pasterk M, Smans M, Cur-
do MP, Mullie P, Gandini S, Bota M, Bolli GB,
Rosenstock J, Autier P. Diabetes and breast
107: 1608-1617.
Kaufman B. Diabetes Mellitus and Breast Can-
[5] Lipscombe LL, Goodwin PJ, Zinman B, McLaugh-
lin JR, Hux JE. Diabetes mellitus and breast
cancer: a retrospective population-based co-
hort study. Breast Cancer Res Treat 2006; 98:
349-356.
C, Sun S. Association between diabetes melli-
tus and breast cancer risk: a meta-analysis of
the literature. Asian Pac J Cancer Prev 2011;
12: 1061-1065.
High glucose induced MCF-7 viability, migration and invasion


[29] Yamamoto M, Patel NA, Taggart J, Sridhar R, Cooper DR. A shift from normal to high glucose levels stimulates cell proliferation in drug sensitive MCF-7 human breast cancer cells but
High glucose induced MCF-7 viability, migration and invasion

not in multidrug resistant MCF-7/ADR cells which overproduce PKC-betaII. Int J Cancer 1999; 83: 98-106.


