Glucose promotes cell proliferation, glucose uptake and invasion in endometrial cancer cells via AMPK/mTOR/S6 and MAPK signaling

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Abstract

Objectives—Obesity and diabetes are well-known risk factors for the development of endometrial cancer. A high rate of aerobic glycolysis represents a key mechanism by which endometrial cancer cells consume glucose as its primary energy source. The up-regulated glycolytic pathway is a common therapeutic target whose inhibition has implications for anti-tumor activity in cancer cells. This study aimed to investigate the effect of various concentrations of glucose on cell proliferation in endometrial cancer.

Methods—ECC-1 and Ishikawa cells were treated with low glucose (1mM), normal glucose (5mM) and high glucose (25mM), and cytotoxicity, apoptosis, cell cycle, adhesion/invasion, and changes of AMPK/mTOR/S6 and MAPK pathways were evaluated.

Results—Our results revealed that high glucose increased cell growth and clonogenicity in two endometrial cancer cell lines in a dose dependent manner. Low glucose induced the activity of cleaved caspase 3 and caused cell cycle G1 arrest. High glucose increased the ability of adhesion

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and invasion by decreasing E-Cadherin and increasing Snail expression. In addition, high glucose increased glucose uptake and glycolytic activity through modulating the AMPK/mTOR/S6 and MAPK pathways.

Conclusions—Our findings suggest that glucose stimulated cell proliferation through multiple complex signaling pathways. Targeting glucose metabolism may be a promising therapeutic strategy in the treatment of endometrial cancer.

Keywords

endometrial cancer; glucose; glycolysis; invasion

Introduction

Endometrial cancer is the most common gynecologic malignancy in women in the Western world, and it is the fourth most common cancer among women in the United States. Both the incidence of and mortality due to endometrial cancer are on the rise, and in 2014, 54870 new cases and 10170 deaths are predicted[1]. Women have a 2.6% lifetime risk of developing this malignancy in the US [2]. One potential contributor to the increased incidence of endometrial cancer is the rising rates of obesity and diabetes in the United States. Obesity and diabetes are well known risk factors for the development of endometrial cancer. The risk of developing this malignancy increases by 50% to 60% for every 5-unit increase in body mass index (BMI). Obesity and diabetes may also be associated with worse outcomes for this disease [2, 3]. Women with early stage disease (FIGO stage I and II) and endometrioid histology have a relatively good prognosis with surgery alone or surgery plus radiation. However, patients with advanced stage disease (FIGO stage III and IV) have a 5-year survival of 21–56%. Patients with advanced stage III or IV disease are unlikely to be cured by surgery, conventional chemotherapy, radiation or a combination of these modalities [4]. Given the rising incidence of this disease and the paucity of effective treatments for advanced and recurrent endometrial cancer, understanding the energy metabolism in endometrial cancer cells will help develop new approaches for the effective management of this obesity-driven malignancy.

One of the major hallmarks of cancer is the reprogramming of a cell's energy machinery by oncogenes and tumor suppressor genes to inhibit oxidative phosphorylation and alternatively employ aerobic glycolysis to induce the degradation of glucose into lactate [5]. Most cancer cells use aerobic glycolysis as a means of energy production regardless of whether they are under normoxic or hypoxic condition. Heavy consumption of glucose and increased glycolysis are essential to generate both catabolic and anabolic precursors for the synthesis of DNA, RNA, proteins and lipids for cancer cell growth [5]. Diabetic patients are twice as likely to develop endometrial cancer[6]. Hyperglycemia, which is usually associated with obesity, insulin resistance and hyperinsulinemia, is an independent risk factor for endometrial cancer [5, 7]. Increased blood glucose levels are associated with contributing to the growth or carcinogenesis of endometrial cancer [7]. Recent studies confirmed that glucose transporters, and glycolytic and lipogenic enzymes are upregulated in the malignant endometrium when compared to their nonmalignant counterparts, indicating that metabolic profiling showed higher rates of glycolysis and lower glucose oxidation in endometrial

cancer cells[8, 9]. Moreover, patients with type I endometrial cancer who had the alterations of metabolic profiling (including those with stages II–IV and obese patients) had worse overall survival than those without such changes [8].

Although glucose metabolism has been widely studied in a number of malignant cell types, the mechanistic role of glucose in cell growth of endometrial cancer remains poorly understood. To improve our understanding of the mechanism linking glucose metabolism and endometrial cancer cell growth, we investigated the effect of different glucose concentrations on cell proliferation in endometrial cancer cells in vitro. Our results indicate that targeting glucose metabolism is a promising therapeutic strategy for endometrial cancer.

Materials and Methods

Cell culture and reagents

The human endometrial cancer cell lines, ECC-1 and Ishikawa, were used. ECC-1 cells were maintained in 1640 medium with 5% fetal bovine serum (FBS) and Ishikawa cells were cultured in DMEM medium supplemented with 5% FBS. All media were supplemented with 100 U/ml of penicillin and 100 ug/ml of streptomycin. The cells were cultured in a humidified 5% CO_2 at 37 °C. Glucose solution, MTT and DMSO were purchased from Sigma-Aldrich (St. Louis, MO). 2-NBDG was bought from Life Technologies (Grand Island, NY). Cleaved caspase 3 Activity Assay kit was bought from AAT Bioquest (Sunnyvale, CA). The anti-phospho-AMPK antibody, anti-phospho-pS6 antibody, anti-phospho-p44/42 antibody and others were purchased from Cell Signaling (Danvers, MA). Enhanced chemiluminescence (ECL) detection reagents were purchased from GE Health care (Piscataway, NJ). For glucose studies, the cells were cultured in RPMI-1640 medium or DMEM medium without glucose (Cat #11879-020 and 11966-025, Gibco) containing 5% FBS and supplied with various concentrations of glucose.

Cell proliferation assay

ECC-1 and Ishikawa cells were seeded at 4000 cells/well in 96-well plates in their culture media. After 24 hours, cells were cultured in media with different concentrations of glucose for 48 hours or 72 hours. Cell proliferation was measured by adding 5 ul of MTT solution (5 mg/ml) per well for an additional incubation of 1 hour. The MTT reaction was terminated through the replacement of the media with100 ul DMSO. Viable cell densities were determined by measuring absorbance of metabolic conversion of the colorimetric dye at 570 nm. Each experiment was performed in triplicate and repeated three times to assess for consistency of results.

Colony formation assay

ECC-1 and Ishikawa cells growing in log phase were seeded (400 cells/well in a 6-well plate) in their complete regular growth medium. Cells were allowed to adhere for 24 hours, and medium was replaced with fresh complete regular growth medium containing the indicated concentrations of glucose. Cells were cultured at 37°C for 14 days, with medium changes every third or fourth day. Cells were stained with 0.5% crystal violet, and colonies were counted under the microscope.

Cell cycle analysis

The cells were plated at a density of 1.5×10^5 cells/well in 6-well plates overnight, and then treated with various concentrations of glucose for 48 hours. Cells were collected by 0.05% trypsin, washed with phosphate-buffered saline (PBS) solution, fixed in a 90% methanol solution and then stored at −20 °C until cell cycle analysis was performed. On the day of analysis, the cells were washed with PBS and centrifuged, resuspended in 50 ul RNase A solution (250 ug/ml) with 10 mM EDTA, followed by incubation for 30 min at 37 °C. After incubation, 50 μl of propidium iodide (PI) staining solution was added to each tube and incubated for 10 min in the dark. The cells were assessed by Cellometer (Nexcelom, Lawrence, MA). The results were analyzed by using the FCS4 express software (Molecular Devices, Sunnyvale, CA).

Cleaved caspase 3 assay

Cleaved caspase 3 was assessed with Cleaved caspase 3 Activity Assay kit. After we finished the treatment of the cells in 96-well plate at 6000 cells/well, we added 10ul of caspase 3 assay loading buffer into each well, mixed gently and then incubated the plates for 60 min at 37 °C, 5% CO2. The fluorescence intensity was measured at an excitation wavelength of 350 nm and an emission wavelength of 450 nm using a plate reader from Tecan (Morrisville, NC).

Adhesion assay

Each well in a 96-well plate was coated with 100 ul laminin-1(10ug/ml) and incubated at 37 C for 1 hour. This fluid was then aspirated and 200 ul blocking buffer was added to each well for 45–60 min at 37C. The wells were then washed with PBS and the plate was allowed to chill on ice. To each well, 2.5×10^3 cells were added with their media with varying concentrations of glucose directly. The plate was then allowed to incubate at 37°C for 2 hours. After this period, the medium was aspirated and cells were fixed by directly adding 100 ul of 5% glutaraldehyde and incubating for 30 min at room temperature. Adhered cells were then washed with PBS and stained with 100 ul of 0.1% crystal violet for 30 min. The cells were then washed repeatedly with water, and 100 ul of 10% acetic acid was added to each well to solubilize the dye. After 5 min of shaking, the absorbance was measured at 570 nm using a microplate reader from Tecan.

Invasion assay

Cell invasion assays were performed using 96-well HTS transwells (Corning Life Sciences, Wilmington, NC) coated with 0.5-1X BME (Trevigen, Gaithersburg, Maryland). Starved (serum-free media for 12 hours) ECC-1and Ishikawa cells (50,000 cells/well) were seeded for 12 hours in the upper chambers of the wells in 50 μl FBS-free medium, and the lower chambers were filled with 150 μl regular medium with different concentrations of glucose. The plate was incubated for 12 hours at 37°C to allow the cells to invade into the lower chamber. After washing the upper and lower chambers with PBS once, 100 ul Calcein AM solution was added into the lower 37°C chamber and incubated at for 30–60 min. The lower chamber plate was measured by the plate reader for fluorescence at EX/EM 485/520 nM. All experiments were performed in duplicate to assess for consistency of response.

Glucose uptake assay

The ECC-1 and Ishikawa cells were seeded into 96-well plates at 4000 cells per well overnight and then were treated with 100ul of glucose free culture medium containing 100ug/ml 2-NBDG with varying concentrations of glucose for 20 minutes. The medium was then replaced with 200ul HBSS (Life technologies corporation, Grand Island, NY), and the plate was centrifuged for 5min at 400rpm. The fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a plate reader from Tecan.

Western blot analysis

Total protein was extracted from endometrial cancer cells using RIPA buffer (Boston Bioproducts, Ashland, MA). Protein samples with equal loading (30 ug) were separated by 10–12% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk and then incubated with a 1: 1000 dilution of primary antibodies for overnight at 4°C. The membranes were washed and incubated with a secondary peroxidase-conjugeted antibody for 1 hour at room temperature. The membranes were developed using an enhanced ECL at Alpha Innotech Imaging System (Protein Simple, Santa Clara, CA). After developing, the membranes were re-probed using antibody against α-tubulin to confirm equal loading. The bands' intensity were measured and normalized to α-tubulin. Each experiment was repeated at least twice for consistency of results.

Statistical analysis

Data are expressed as mean ± SEM. Data were compared using two-tailed Student's t-test, and $p < 0.05$ was considered significant.

Results

Glucose promotes cell growth in endometrial cancer cells

Glucose is an essential energy and nutrient source for the growth and survival of cancer cells. To test the effect of glucose on the growth of endometrial cancer cells, two endometrial cancer cell lines, ECC-1 and Ishikawa, were treated in their culture media with three concentrations of glucose (low glucose (LG 1mM), normal glucose (NG, 5mM) and high glucose (HG, 25mM)) for 48 and 72 hours. 5 mM glucose in the media corresponds to normal physiological levels in human blood (100 mg/dl), whereas 25 mM glucose is equivalent to a patient with uncontrolled severe diabetes[10]. Cell proliferation was assessed by MTT assay. The results showed that cell proliferation was increased by 2.8 to 3.3 fold at 48 hours (Figure 1A) and 3.6 to 4.2 fold at 72 hours (Figure 1B) in Ishikawa and ECC-1 cells treated with high glucose compared with those for the control cells without glucose incubation (no glucose (N)). Given that in vitro colony formation assays are excellent indicators of long term tumor cell survival, we then assessed whether glucose had an effect on the colonization ability of ECC-1 and Ishikawa cells. The results showed high glucose significantly increased clonogenicity of ECC-1 and Ishikawa cells by 300% and 400%, respectively, compared with low glucose groups after exposure to glucose for 10 days

(Figure 1C and D). Together, these results confirm that glucose is a critical nutrient for growth of endometrial cancer cells.

Low glucose induces apoptosis in endometrial cancer cells

To elucidate the mechanisms of glucose on cell growth, the effects of glucose on apoptosis were analyzed. ECC-1 and Ishikawa cells were cultured with three concentrations of glucose for 14 hours, and the activity of cleaved caspase3 was analyzed using ELISA assay. Incubation of the cells with low glucose increased cleaved caspase3 activity an approximately 40% in both cell lines compared with normal glucose groups. Western blotting analysis also showed that protein expression of BCL-2 and MCL-1 was markedly decreased in low glucose groups after glucose exposure of 18 hours compared with these for the cells cultured under conditions of normal and high glucose, suggesting that low glucose induced mitochondrial apoptosis in endometrial cancer cells.

Low glucose induces cell cycle arrest in endometrial cancer cells

To further confirm whether the growth inhibition of the cells induced by low glucose was related to cell cycle arrest, the cell cycle profile was analyzed by Cellometer after treating the ECC-1 and Ishikawa cells with three concentrations of glucose for 48 hours. Low glucose increased G1 population from 52.2 % to 67.8 % in ECC-1 cells, and from 50.76 % to 61.44 % in Ishikawa cells, respectively, compared with normal glucose groups. Meanwhile the S phase population increased with increasing concentrations of glucose in both cell lines (Figure 3A and B). To further understand the effect of glucose on cell cycle checkpoints, the expression of CDK4 and CDK6 was measured by western blotting. Low glucose significantly reduced protein expression of CDK4 and CDK6 compared with normal and high glucose groups after 24 hours treatment (Figure 3C). These results suggest that high glucose promotes the passage of cells into S phase from G1.

Low glucose inhibits adhesion and invasion in endometrial cancer cells

The effect of glucose on the migration of endometrial cancer cells was analyzed by a laminin-1 adhesion and transwell invasion assays. The ECC-1 and Ishikawa cells treated with low glucose had a reduced ability to adhere to laminin-1, as compared to normal and high glucose cells (Figure 4A). Similarly, the migratory capacity of the endometrial cancer cells was also reduced after treatment with low glucose, while ability of invasion was significantly increased by around 50 % in high glucose groups in both cell lines after 12 hours of culture (Figure 4B). To further explore the effect of glucose on adhesion and migration of endometrial cancer cells, the expression of E–cadherin, an invasion suppressor, and Snail, a pleiotropic protein that represses transcription of E–cadherin, were analyzed by Western blot (Figure 4C). Treatment with low glucose for 24 hours reduced Snail expression and increased E-cadherin expression in ECC-1 and Ishikawa cells. These findings suggest that glucose is able to affect motility in endometrial cancer cells.

The effect of glucose on glycolytic metabolism in endometrial cancer cells

To better understand the effect of glucose on glycolytic metabolism, the cells were cultured with various concentrations of glucose for 12 hours, and cellular glucose uptake was

detected with 2-NBDG fluorescence assay. Glucose induced an increase in glucose uptake by about 25% in normal glucose groups and 40% in high glucose groups in both cell lines compared with low glucose groups (Figure 5A). ECC-1 cells exhibited more glucose uptake than the Ishikawa cells. We then monitored the effect of glucose on glucose transporter 1 (GLUT1). The levels of GLUT1 were analyzed using western blotting after cells were treated with low glucose, normal glucose and high glucose for 12 hours. High glucose significantly increased the expression of GLUT1 protein in both cells (Figure 5B), suggesting that Glut1 was involved in the mechanism of glucose-induced glucose uptake. To address the alternations of glycolytic pathway, the expression of pyruvate dehydrogenase (PDH) and lactate dehydrogenase A (LDHA) were analyzed after incubation with different concentrations of glucose for 12 hours. We observed a decreased PDH protein expression and an increased phosphorylation of LDHA expression in high glucose groups in both cells, suggesting a direct effect of glucose on glycolytic flux and the increasing activity of glycolysis.

Glucose affects the AMPK and AKT/mTOR/S6 pathways

Given that AMPK and mTOR are master protein kinases regulating glucose metabolism, we investigated the effect of glucose on AMPK and mTOR pathways in endometrial cancer cells. Treating both cells with low glucose increased phosphorylation of AMPK within 12 hours (Figure 6A). We next examined whether different concentrations of glucose would affect the activities of MAPK and AKT/mTOR/S6 pathways. The results showed that high glucose stimulated both p42/44 and AKT phosphorylation and decreased phosphorylation of S6 after incubation with glucose for 3 and 6 hours, whereas high glucose reduced phosphorylation of p42/44, increased phosphorylation of S6 and demonstrated minimal changes in phosphorylation of AKT after glucose treatment for 12 hours in both cell lines (Figure 6B). This indicates that multiple complex signaling pathways impact the regulation of glucose metabolism through different mechanisms in endometrial cancer.

Discussion

In the current study, we used two endometrial cancer cell lines to address the role of different concentrations of glucose in cell growth. We showed that glucose increased cell proliferation through the AMPK/mTOR/S6 and MAPK pathways. To elucidate the underlying mechanism, we demonstrated that high glucose inhibited cell apoptosis and promoted cell cycle progression along with increased CDK4 and CDK6 expression and decreased cleaved caspase 3 activity. Low glucose was able to cause cell cyle G1 arrest and decrease the abilities of adhesion and invasion in endometrial cancer cells, which led to upregulation of E-cadherin and downregulation of Snail expression. In addition, high glycolytic activity was observed in high glucose-cultured cells, resulting in increased glucose uptake and protein expression of Glut1 and LDHA. These results support the notion that glucose is a major source of energy for cell growth and invasiveness, and that targeting glucose metabolism with molecular interventions may be a viable strategy for endometrial cancer therapy [11, 12].

Our results showed that low glucose inhibited cell growth, induced significant apoptosis and caused cell cycle G1 arrest, whereas high glucose increased the ability of adhesion and invasion in endometrial cancer. Moreover, glucose increased glycolytic activity and stimulated cell proliferation by regulating the AMPK, AKT/mTOR/S6 and MAPK pathways. These results indicate that targeting glucose metabolism is a promising therapeutic strategy for endometrial cancer.

Glucose is an essential nutrient that maintains cellular energy homeostasis. There is extensive evidence that cancer cells are more sensitive to different concentrations of glucose than normal cells owing to their higher consumption ratio of energy[13, 14]. The consequence of glucose restriction is an energy crisis that non-specifically or specifically activates numerous death pathways that converge to apoptosis and/or cell cycle arrest [15, 16]. Recent in vitro and in vivo studies have shown that there are differential responses of cancer cells to glucose deprivation under different genetic and epigenetic background, indicating that metabolic profiles of tumors are likely dependent on both the genotype and tissue of origin [17, 18]. The restriction of glucose induced G1 phase arrest and apoptosis in breast, leukemia, prostate and lung cancer cells[15, 18–21], while N-GlcNAc2-modified protein-producing renal carcinoma and bladder cancer cells arrested in G2/M-phase alone, with the changes triggered by glucose deprivation[22, 23]. In this study, we examined the changes in cell cycle and apoptosis in two endometrial cancer cell lines treated with different concentrations of glucose. Our results demonstrated that low glucose inhibited cell proliferation via increased cleaved caspase 3 activity and induced cell cycle G1 arrest. As a result, the expressions of CDK4, CDK6, BCL-2 and Mcl-1 were down-regulated in low glucose cultured cells; thus establishing the conditions that brought cells to a G1 cell cycle arrest and apoptosis. These results indicate that the anti-proliferative effects exerted by glucose deprivation can be attributed to the induction of cell cycle arrest and apoptosis.

The progression and invasion of cancer appears to be secondary to a series of unique biological events within the tumor microenvironment, in part due to the aberrant metabolism of glucose resulting in significantly higher amounts of lactic acid in cancer cells. The acidic microenvironment produced by anaerobic glucose metabolism is beneficial for progression, invasion and metastasis of cancer cells because it increases extracellular matrix degradation by proteolytic enzymes and promotes formation of angiogenesis [24, 25]. Furthermore, the acidification may also lead to increased activation of matrix metalloproteinases (MMPs) activities [24]. High glucose has been previously shown to increase lactate production and decrease E-cadherin protein expression in colon, breast, bladder and pancreatic cancer cells [24, 26]. Cancer cells with low E-cadherin expression are more prone to evade a tumor mass and to invade the surrounding tissue. Treating our endometrial cancer cells with high glucose for 24 hours resulted in increased adhesion and invasion capabilities and was accompanied by decreased expression of E–cadherin and increased expression of Snail. Adhesion and invasion are two important aspects that lead to the ability of cancer cells to metastasize. E-cadherin is a cell-cell adhesion protein that contributes to the maintenance of the epithelial barrier function through homotypic interactions [27]. The loss of function of E-cadherin is mainly due to an overexpression of transcriptional repressors including Snail and Slug. Upreglation of Snail and Slug resulted in the downregulation of E-cadherin, enhanced cell motility, and increased cell invasiveness in ovarian cancer [28]. The

complexity of the mechanisms by which high glucose promoted invasion and affected the expression of Snail and E-cadherin provides opportunities for further investigation.

Glycolysis is a complex biochemical process that is mediated by multiple glycolytic genes and a multitude of pathways that activate glucose metabolism in cancer cells [29]. Glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) are tightly coupled processes. PDH interconnects the processes and, thus, represents a key regulatory step in glucose metabolism. Transport of glucose across the plasma membrane is the first rate-limiting step for glucose metabolism and is mediated by facilitative glucose transporter proteins (GLUTs)[9]. Recent results showed that poorly differentiated endometrial cancer had significantly higher GLUT1 and GLUT3 expression than well-differentiated tumors. The expression of lactate dehydrogenase 5 (LDH5) in tumor tissues is an independent prognostic marker in endometrial cancer and has a strong association with poor prognosis [30]. Targeting LDHA by Galloflavin was found to have promising anti-tumor activity in endometrial cancer cell lines and primary cultures of endometrial cancer[12]. These data suggests that survival of endometrial cancer cells was highly coupled with glycolysis [8]. Culturing endometrial cancer cells with high concentration of glucose for 12 hours increased glucose uptake and expression of GLUT1 in both cell lines. These changes lead to a shift in metabolic fluxes to glycolysis, ultimately causing the reduction of PDH expression and the induction of LDHA activity. Therefore, our data support the concept that targeting glucose-derived metabolism could preferentially be exploited as a therapeutic strategy for endometrial cancer.

AMPK is a pivotal energy sensor governing normal and cancer cell metabolism. The PI3K/Akt and MAPK signal transduction pathways, which are involved in the regulation of cell proliferation, differentiation, cell growth, and apoptosis, are the most frequently altered biochemical pathways in endometrial cancer, [31, 32]. Although the AMPK pathway and the PI3K-AKT cascade converge on mTOR with opposing regulatory effects, frequently functioning in concert in response to distinct extracellular cues or physiological conditions to coordinate bioenergetics and cell viability[33], little is known about the interaction between AMPK and MAPK pathways in cancer cells [34]. Deprivation of glucose induced supra-physiological levels of phospho-tyrosine signaling and a modest increase in phosphorylation of AMPK, AKT, and p42/44 in breast, kidney, lung, colon, prostate and ovarian cancer cells [21, 34–36]. On the other hand, it has recently been reported that high glucose stimulated phosphorylation of AKT, reduced AMPK phosphorylation and activated MAPK pathways in cancer cells [37–40]. These studies suggest these signaling pathways have switch-like properties that produce differential kinetics under different metabolic stresses. Here, we demonstrated that high glucose activated both AKT/mTOR/S6 and MAPK pathways and low glucose induced the expression of phosphorylation of AMPK in Ishikawa and ECC-1 cells, suggesting that AMPK may act as a switch that controls the functions of these signaling networks.

In conclusion, increased glucose metabolism via glycolysis provides endometrial cancer cells with essential substrates for rapid growth. Low glucose activated AMPK signaling, induced cell cycle G1 arrest and caused apoptosis in Ishikawa and ECC-1 endometrial cancer cells. High glucose enhanced the adhesion and invasion activity of endometrial

cancer cells by mediating the upregulation of Snail and downregulaton of E-Cadherin expression. Managing hyperglycemia or targeting glucose metabolism may serve as potential strategies for treatment of endometrial cancer. Our results warrant further investigation of this hypothesis.

Acknowledgments

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Highlights

- High glucose promotes proliferation of endometrial cancer cells through AMPK/mTOR/S6 and MAPK signaling

- High glucose promotes cell cycle progression and induces the ability of adhesion and invasion in endometrial cancer cells

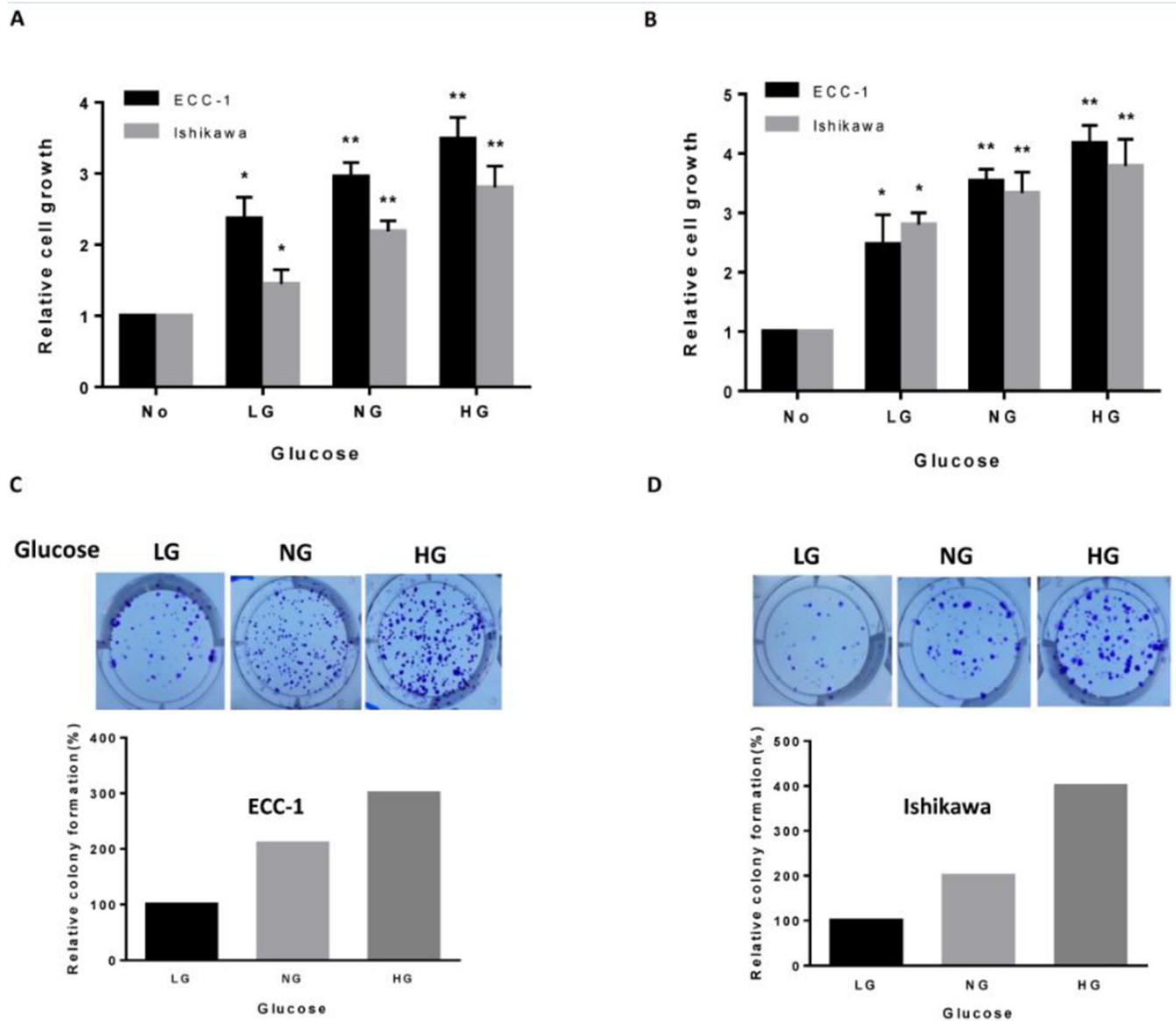- High glucose increases glucose uptake and glycolytic activity in endometrial cancer cells

Figure 1. Glucose promotes cell proliferation in ECC-1 and Ishikawa cells
ECC-1 and Ishikawa cells were cultured for 24 hours and treated with no glucose (NO), low glucose (1 mM, LG), normal glucose (5 mM, NG) and high glucose (25 mM, HG) in 96-well plates for 48 hours (A) and 72 hours (B). Cell proliferation was assessed by MTT assay. The effect of glucose on long term growth in endometrial cancer cells was assessed through colony-forming assay (C and D). *p < 0.05 and **p < 0.01.
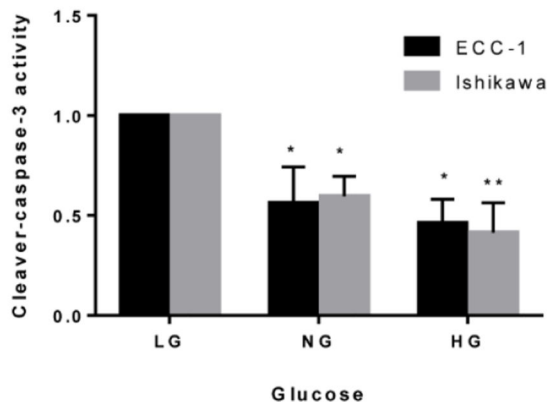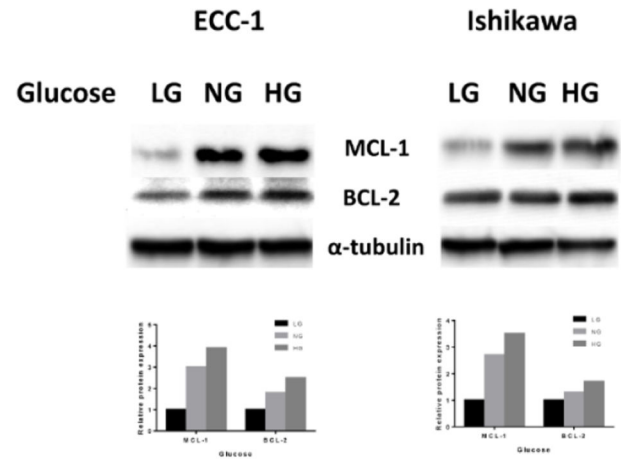
Figure 2. Low glucose induced apoptosis in endometrial cancer cells
The ECC-1 and Ishikawa cells were cultured in the presence of varying concentration of glucose (LG, NG and HG) in regular medium for 14 hours. Cleaved caspase-3 activity was determined by ELISA assay (A). The protein expression of BCL-2 and MCL-1 was detected by Western blotting in the both cells after exposure to glucose for 18 hours (B).
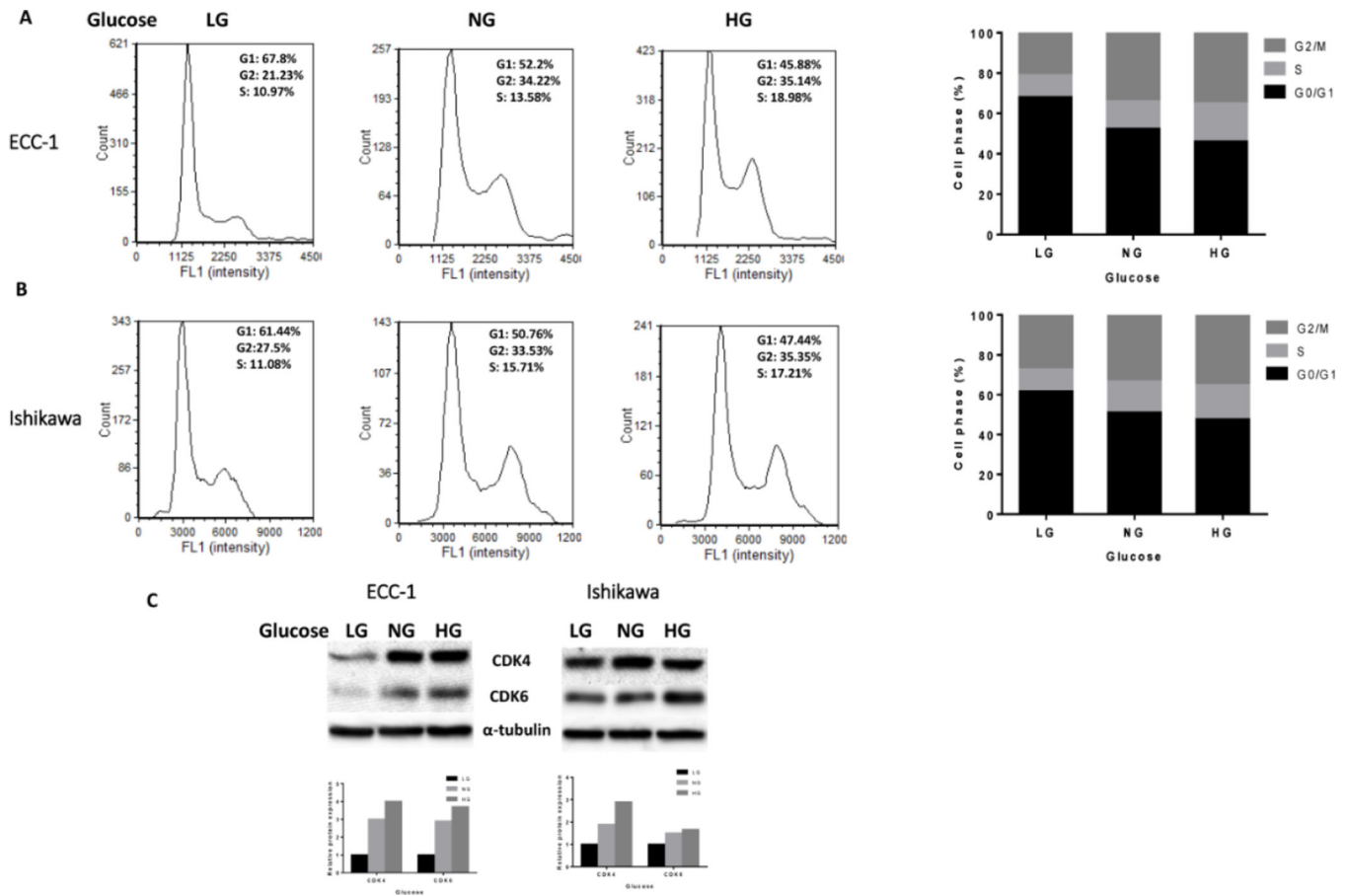
Figure 3. Low Glucose induced cell cycle G0/G1 arrest in endometrial cancer cells
ECC-1 and Ishikawa cells were cultured for 24 hours and then treated with different
concentrations of glucose (LG, NG and HG) for 48 hours. Cell cycle analysis was performed
by Cellomte. Low glucose induced cell cycle G0/G1 phase arrest in the both cells (A and B).
The expression of CDK4 and CDK6 were examined by Western blotting in ECC-1 and
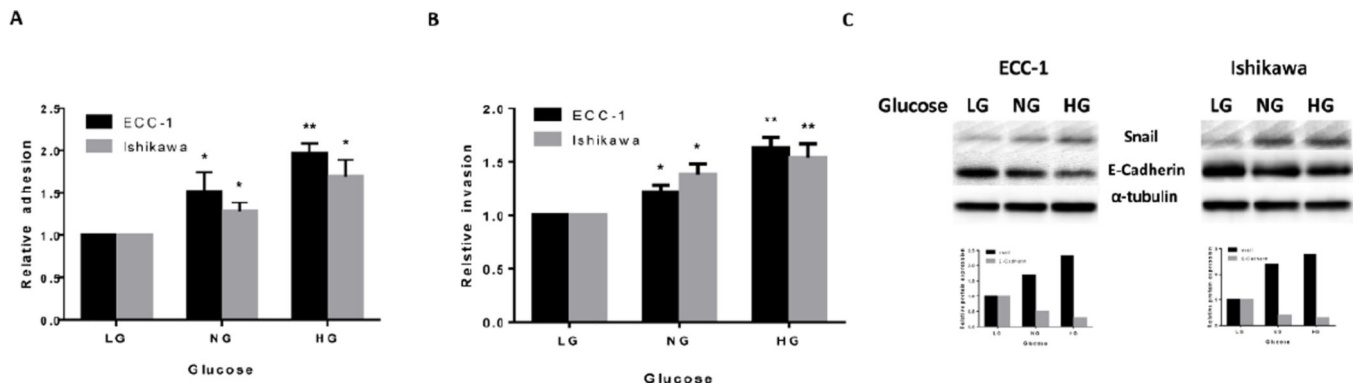Ishikawa cells after exposure to glucose for 24 hours at the indicated concentrations (C).

Figure 4. High glucose induced the ability of adhesion and invasion in endometrial cancer cells
ECC-1 and Ishikawa cells were treated with glucose as indicated in a laminin coated 96 well plates or BME coated 96 transwell plates for 12 hours. Adhesion and invasion were assessed using a plate reader. High glucose significantly increased the ability of adhesion and invasion in the both cells (A and B). (B). The expression of Snail and E-Cadherin were determined by Western blotting in the ECC-1 and Ishikawa cell lines after exposure to glucose for 24 hours (C). *p < 0.05 and **p < 0.01.
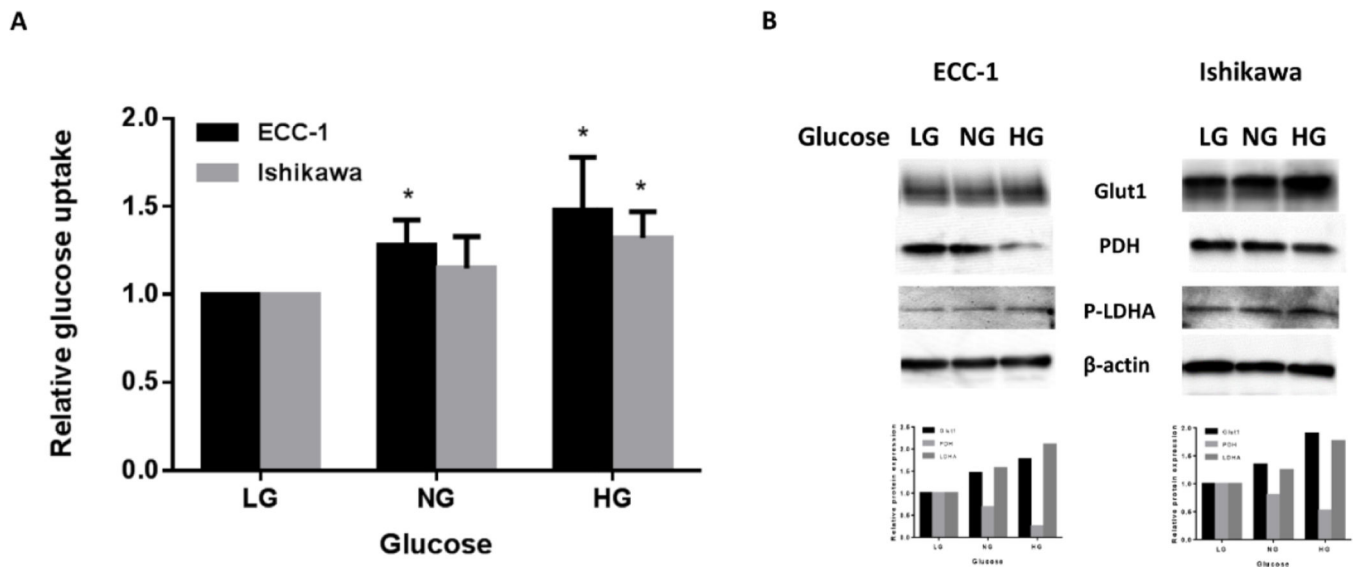
Figure 5. High glucose promoted the activity of glycolysis in endometrial cancer cells
ECC-1 and Ishikawa cells were treated with different concentrations of glucose for 12 hours. The levels of cellular glucose uptake were measured by 2-NBDG assay (A). The expression of GLUT1, phos-LDHA and PDH was analyzed using Western blotting (B). High glucose significantly increased glucose uptake, accompanied by an increase in the expression of GLUT1 and Phos-LDHA and a decrease in PDH.
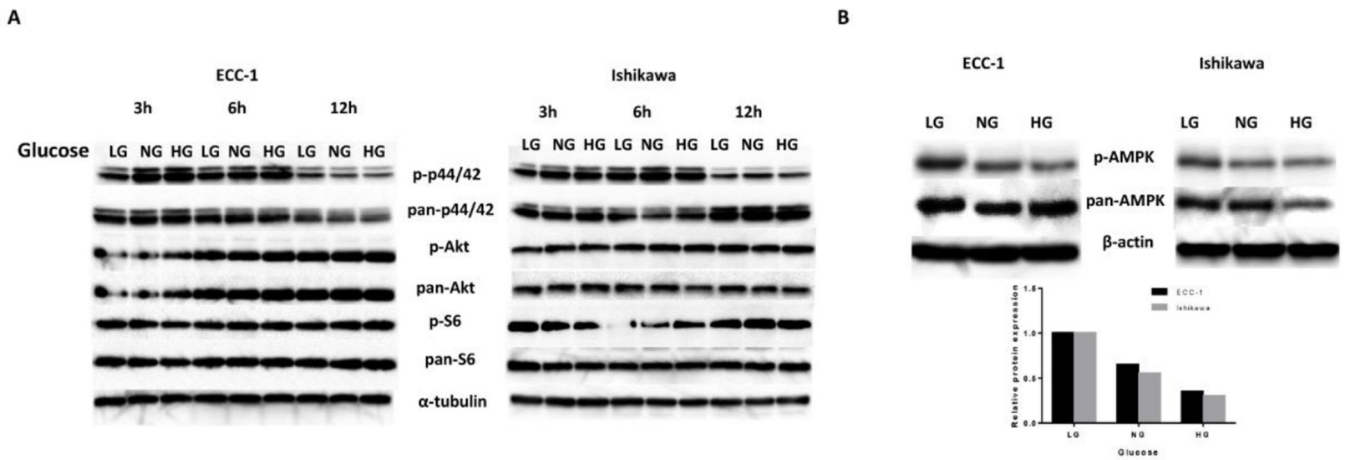
Figure 6. Glucose affects the AMPK and AKT/mTOR/S6 pathways
ECC-1 and Ishikawa cells were treated with glucose as indicated in their regular media for 3, 6 and 12 hours. The expression of phosphorylated pS6, AKT and p42/44 was analyzed using western blotting (A). The phosphorylation of AMPK was detected by Western blotting after incubation of glucose for 12 hours in both cells (B).