

Hyperglycemic Conditions Proliferate Triple Negative Breast Cancer Cells: Role of Ornithine Decarboxylase

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Abstract

Several cancer subtypes (pancreatic, breast, liver, and colorectal) rapidly advance to higher aggressive stages in diabetes. Though hyperglycemia has been considered as a fuel for growth of cancer cells, pathways leading to this condition are still under investigation. Cellular polyamines can modulate normal and cancer cell growth, and inhibitors of polyamine synthesis have been approved for treating colon cancer, however the role of polyamines in diabetes-mediated cancer advancement is unclear as yet. We hypothesized that polyamine metabolic pathway is involved with increased proliferation of breast cancer cells under high glucose(HG) conditions. Studies were performed with varying concentrations of glucose (5mM-25mM) exposure in invasive, triple negative breast cancer cells, MDA-MB-231; non-invasive, estrogen/progesterone receptor positive breast cancer cells, MCF-7; and non-tumorigenic mammary epithelial cells, MCF-10A. There was a significant increase in proliferation with HG (25mM) at 48-72h in both MDA-MB-231 and MCF-10A cells but no such effect was observed in MCF-7 cells. This was correlated to higher activity of ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine synthesis pathway. Inhibitor of polyamine synthesis (difluoromethylornithine, DFMO, 5mM) was quite effective in suppressing HG-mediated cell proliferation and ODC activity in MDA-MB-231 and MCF-10A cells. Polyamine (putrescine) levels were significantly elevated with HG treatment in MDA-MB-231 cells. HG exposure also increased the metastasis of MDA-MB-231 cells. Our findings are the first to indicate that polyamine inhibition can improve prognosis of breast cancer patients with diabetes, and also prevent proliferation of normal breast epithelial cells, which could potentially become tumorigenic.

Introduction

Diabetes and cancer have been postulated to have a correlation for the past eighty years, however, it is only within the past decade that significant epidemiological evidence has been compiled to suggest a causal link [1, 2]. Strong evidence suggests that the risk for several cancer types, including cancers of the liver, pancreas, colorectum, urinary tract, breast, and female reproductive organs, is increased in diabetic patients [1, 3]. Diabetes increases metastasis, recurrence, and mortality of cancer [3, 4]. Currently, diabetes-cancer link has primarily been hypothesized to rely on hormonal (insulin and insulin-like growth factor 1), inflammatory, and metabolic (hyperglycemia) characteristics of diabetes [5]. Hyperglycemia promotes rapid cancer cell proliferation thus increasing cancer progression [6]. Altered glucose metabolism also contributes to rewiring of metabolic pathways for cell growth and survival [3]. Although hyperglycemia has been examined as a contributor to rapidly progressing cancer, pathways leading to this condition remain under investigation.

Studies into the molecular biology underlying breast cancer have found that polyamines, organic molecules that play a large role in eukaryotic cell growth and development, have elevated amounts in breast cancer tissue [7–9]. Polyamines control gene expression at transcriptional, posttranscriptional, and translational levels, modulating functions of DNA, nucleotide triphosphates, RNA, and proteins [10, 11]. Multiple pathways regulate cellular polyamine levels, including synthesis from amino acid precursors, uptake mechanisms that utilize polyamines from diet and intestinal microorganisms, and stepwise

degradation and efflux [12]. A significant body of evidence demonstrates that numerous oncogenic pathways are involved in regulating the transcription and translation of enzymes involved in polyamine metabolism, and that upregulation of polyamine biosynthetic enzymes correlates with both increased cell proliferation and tumorigenesis [13–18]. In fact, increased polyamine levels have been linked to breast, prostate, colon, and skin cancers [7, 19–22]. While normal physiological conditions maintain intracellular polyamine concentrations within narrow limits, dysregulation of polyamine metabolism can lead to various pathological conditions besides cancer, including inflammation, renal failure, stroke, and diabetes [10, 23].

The polyamine pathway involves the conversion of L-ornithine (from dietary sources or conversion from L-arginine) to form putrescine, the first polyamine in the pathway [24]. This reaction is mediated by ornithine decarboxylase (ODC), the rate-limiting enzyme in the polyamine pathway, which has been shown to have increased activity in cancer (Fig. 1) [25]. Putrescine is further converted to other polyamines including spermidine and spermine through spermidine synthase (SpdS) and spermine synthase (SpmS), respectively. Polyamine levels are tightly regulated by catabolism through spermine oxidase (SMOX, converts spermine to spermidine), spermidine spermine N^1 -acetyl transferase (SSAT), and N^1 -acetylpolyamine oxidase (PAOX) [24]. A parallel pathway that feeds into the polyamine synthesis maintains intracellular levels if the primary pathway is not sufficient or is affected. This involves decarboxylation of S-adenosylmethionine (AdoMet), the aminopropyl donor for synthesis of spermidine and spermine.

While inhibition of polyamines through the ornithine decarboxylase enzyme has been shown to provide protection against breast cancer [9, 26], it is not known whether this would be true for diabetes-linked cancer advancement as well. In fact, polyamine pathway can be a potential target to treat chemoresistance in triple negative breast cancer [9, 27]. Thus, we hypothesized that high glucose (HG) conditions advance breast cancer cell proliferation, with a possible role of polyamine pathway. Our results showed that advanced stage breast cancer cells as well as non-tumorigenic mammary epithelial cells proliferate in hyperglycemic states, and polyamine inhibition is protective in these conditions.

Materials And Methods

Reagents and supplies

Dulbecco's Modified Eagle Medium/F12 (DMEM/F12, 1:1), phenol red-free DMEM, horse serum, penicillin/streptomycin, GlutaMAX, PrestoBlue® cell viability reagent, were obtained from Thermo Fisher Scientific (Rockford, IL, USA). All cell lines (MDA-MB-231, and MCF-10A), Eagle's minimum essential medium (EMEM) and fetal bovine serum (FBS) were obtained from American Type Culture Collection (Manassas, VA, USA). Epidermal growth factor (EGF) was obtained from Peprotech (Rocky Hill, NJ, USA). Glucose, insulin, hydrocortisone, and cholera toxin were obtained from Millipore Sigma (Milwaukee, WI, USA). Alpha-difluoromethylornithine (DFMO) was obtained from Bachem Americas Inc (Torrance, CA, USA). One-step cDNA kit, and SYBR Green supermix were obtained from Quantabio (Beverly, MA, USA).

Cell Culture

The cell lines used in this study included two different breast cancer cells: highly invasive, triple negative breast cancer cells (TNBC), MDA-MB-231 and poorly aggressive, non-invasive, estrogen/progesterone receptor positive breast cancer cells, MCF-7 cells. In addition, normal mammary epithelial cells, MCF-10A, were used as a control cell line to mimic effects on healthy tissue surrounding cancer. All cell lines were cultured at 37°C in a humidified atmosphere with 5% CO₂, under following conditions:

Maintenance and subculture- MDA-MB-231 and MCF-7 were grown in DMEM/F12 supplemented with 5% fetal bovine serum, 1% GlutaMax, 100IU/ml penicillin, and 100ug/ml streptomycin. MCF-10A cells were grown in DMEM/F12 supplemented with 5% horse serum, 20ng/ml EGF, 0.5µg/ml hydrocortisone, 100ng/ml cholera toxin, 10µg/ml insulin, 100IU/ml penicillin, and 100µg/ml streptomycin.

Treatment- Cells were treated for 24-72h in glucose-free, phenol red-free DMEM media supplemented with varying concentrations of glucose (5mM-25mM) and/or 5mM of DFMO, 1% FBS/1% horse serum, 1% GlutaMAX, 100IU/ml penicillin, 100µg/ml streptomycin.

Cell proliferation assay

Cells were seeded in 96-well plates at a density of 8000 cells per well and allowed to adhere for 24h. Cells were then starved and treated (90µl per well) with varying concentrations of glucose (5mM-25mM), in the presence or absence of 5mM DFMO. Mannitol (20mM) with glucose (5mM) was used as an osmotic control. After 48-72hh, cells were incubated with PrestoBlue® reagent (10µl per well) for 10min at 37°C. Fluorescence was monitored at 560/590nm (ex/em) using a microplate reader and Gen5 software (BioTek Synergy 2; Winooski, VT, USA). Fluorescence is directly proportional to the number of living cells in each well. Blank wells included media with no cells, and this reading was subtracted from all treated wells. Percent change was calculated relative to the 5mM glucose treatment as control.

Clonogenic assay

Cells were plated at a density of 2000 cells per well, allowed to attach overnight, and then treated with glucose and/or DFMO for 72h. At the end of the treatment period, cells were washed with ice-cold PBS, and further incubated with complete media (no treatment) for another week, with change of media every two days. This is to allow establishment of colony formation from surviving cells. Colonies were washed with cold PBS, fixed with methanol for 15 min, and further stained with 0.1% crystal violet for 30 min. Plates were submerged in tap water to wash off the dye, allowed to dry overnight, and colonies with more than 50 cells were counted using a stereomicroscope. Survival fraction was calculated using the formula:

$$\text{Plating Efficiency (PE)} = (\# \text{ of colonies formed} / \# \text{ of cells seeded}) \times 100$$

$$\text{Survival Fraction (SF)} = (\# \text{ of colonies formed} / \# \text{ of cells seeded}) \times \text{PE}$$

Polyamine Assay

For polyamine analysis, cells were grown and treated in T-175 flasks, trypsinized, pelleted and stored at -80°C until analysis. Samples were shipped on dry ice and sent to the proteomics and metabolomics facility at University of Nebraska at Lincoln for analysis. Polyamines were extracted from the cell pellets using 400 µL of chilled 5% trichloroacetic acid and after incubation on ice for 1 h with frequent vortexing. The samples were then centrifuged at 5000 rpm for 5 min at 4°C. Supernatants were transferred to a new microfuge tube and neutralized by adding 2 M K₂HPO₄. The extracted polyamines were derivatized using AccQ-Tag chemistry as described previously [28]. The derivatized compounds were then analyzed using the Shimadzu Nexera II UPLC coupled to the Sciex QTRAP 6500 + mass spectrometer equipped with a TurbolonSpray (TIS) electrospray ion source. For LC separation, a ACCQ-TAG ULTRA C18 1.7 um (2.1 x 100 mm, Waters) was used flowing at 0.7 mL/min. The gradient of the mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in 100% acetonitrile) was as follow: 5% B for 2 min, to 90% B in 2.5 min, hold at 90% B for 2 min, then back to 5% B in 0.5 min. The QTRAP 6500 + mass spectrometer was tuned and calibrated according to the manufacturer's recommendations. The instrument was set-up to acquire in positive mode. Analyst software (version 1.6.3) was used to control sample acquisition and data analysis. All the metabolites were detected using MRM (Multiple Reaction Monitoring) transitions that were previously optimized using standards. For quantification, an external standard curve was prepared using a series of standard samples containing different concentrations of metabolites and fixed concentration of the internal standard.

Assay for ODC enzyme activity

For preparing samples for ODC activity assay, cells were grown and treated in T-175 flasks, and subsequently harvested in buffer containing 25 mM Tris HCl pH 7.5, 0.1 mM EDTA, and 2.5 mM DTT. Samples were stored at -80°C until analysis. ODC activity was determined by radioactive assay, as described previously[29], to measure the amount of ¹⁴CO₂ liberated from L-[1-¹⁴C]ornithine. Enzymatic activity was expressed as pmol of CO₂/h per mg of protein. This assay was performed at John Hopkins Medical Institute in Dr. Casero's lab.

Statistical analysis

All values obtained were expressed as mean ± SEM. Results shown were analyzed with Graph Pad software (Prism 9.0) and are representative of at least three experiments performed in replicates. Statistical comparison between more than two different groups was performed using one-way ANOVA followed by Tukey's test. Differences were considered to be statistically significant at p < 0.05.

Results

Time and dose response assessment of cell proliferation under hyperglycemia

To assess dose response effects of glucose treatments in these cell lines, proliferation was monitored at 72h after treatment. Both MDA-MB-231 and MCF-10A cells displayed marked increase in proliferation at

10mM and 25mM glucose concentrations compared to 5mM (Fig. 2). Mannitol was used as an osmotic control, and did not cause any measurable change in proliferation compared to 5mM glucose control. This effect was not evident in MCF-7 cells even with the highest glucose concentrations.

With respect to periods of incubation, in both MDA-MB-231 (triple negative breast cancer cell) and MCF-10A (non-tumorigenic mammary epithelial cell), HG resulted in significantly increased proliferation as early as 48h after treatment (Fig. 3A and 3B) and similar trends continued 96h post-treatments.

Effect of polyamine inhibitor DFMO on cell proliferation and colony formation

The increase in proliferation observed with HG in MDA-MB-231 and MCF-10A, was markedly suppressed with the polyamine inhibitor, DFMO, returning growth to that exhibited in LG conditions at 72h (Fig. 4A). Addition of DFMO had negligible effects on the proliferation of both cell lines grown in LG conditions.

Colony formation increased remarkably with HG treatments in MDA-MB-231 and MCF-10A cells (Fig. 4B). Interestingly, DFMO (2mM) was effective in suppressing colonies formed for LG as well as HG treatments in all cells. Lower concentration of DFMO (2mM) was used for these experiments due to less number of cells plated per well; higher concentration (5mM) was cytotoxic upon longer incubation times.

Ornithine decarboxylase (ODC) activity under hyperglycemia with polyamine inhibition

Similar to proliferation and colony forming assays, ODC enzyme activity with HG exposure was elevated about 2–5 fold higher than LG in both MDA-MB-231 and MCF-10A (Fig. 5). As expected, DFMO suppressed ODC activity under basal (LG) and even under induced (HG) conditions. ODC activity with DFMO in HG-treated cells was either similar or lower than LG exposures. Since DFMO acts only by affecting ODC activity, ODC gene and protein levels were not monitored.

Supplementation of Polyamines to Reverse DFMO Effect

Polyamines- 10 μ M putrescine, 1 μ M spermidine, or 1 μ M spermine were supplemented after 8 h treatment with DFMO. Cell proliferation was increased with HG, which was decreased marginally in the presence of DFMO; however, supplementation with polyamines failed to show any reversal of DFMO effect (Fig. 6).

Metastasis of Late Stage Cancer Cells in Diabetes

Scratch wound healing assay was performed in late-stage, metastatic, MDA-MB-231 cells. Migration of cells towards the scratch is indicative of metastasis *in vivo*. While wound healing was increased with incubation times, near complete healing was observed after 48h only in HG-treated wells, indicative of metastasis (Fig. 7, Table 1). Cells treated with LG (5mM) showed only a partial reversal. Polyamine inhibition was ineffective in repairing the scratch under normal or high glucose treatments.

Polyamine levels in cells after glucose treatments

Intracellular polyamine concentrations were assayed at 48h after treatment with glucose (5, 25mM). Putrescine (PUT) levels significantly increased in MDA-MB-231 cells (Table 2A) after treatment with HG but this effect was not evident in MCF-10A cells (Table 2B). Spermidine (SPD) and spermine (SPM) did not change with HG in both these cell types.

Discussion

Current evidence shows a strong link between diabetes and increased risk of several cancer types including breast, endometrial, pancreatic, hepatic, colorectal, and urinary tract [1]. Diabetes also negatively impacts cancer treatment outcomes, increasing metastasis, recurrence, and mortality of cancer [3, 4]. Hyperglycemia has been considered a leading factor in promoting cancer cell proliferation [6], however controlling hyperglycemia has not shown to be effective in reducing the risk of cancer prognosis, hence other strategies to prevent and treat cancer need to be considered [1]. To our knowledge, a comparison of the effects of hyperglycemia on cell proliferation and effect of polyamine inhibition in breast cancer lines has not been performed. Therefore, the present study tested the effects of hyperglycemia on proliferation of MCF-7 and MDA-MB-231 cells (representative of low and highly invasive breast carcinomas respectively), as well as MCF-10A cells (normal mammary epithelial cells). We also sought to elucidate the role of polyamines in diabetic cancer advancement, using a polyamine synthesis inhibitor DFMO.

Here, we have shown that MDA-MB-231 (highly invasive triple negative breast cancer) and MCF-10A (noncancerous) cells proliferate markedly after 48-72h exposure to HG, however such effects were not observed in MCF-7 cells. Similar trends have also been reported earlier when MCF-7 cells were subjected to hyperglycemia and hyperinsulinemia, which support the differential response [30–32]. The varied response we observed could be attributed to the fact that MCF-7 cells are estrogen receptor positive whereas MDA-MB-231 and MCF-10A cells lack this receptor. Future studies will be performed to investigate the role of estrogen receptor in inducing proliferation of breast cancer cells under diabetic conditions. A similar study, mimicking type 2 diabetic condition in MCF-7 cells, emphasized that presence of estrogen in hyperinsulinemic states remarkably affected cell growth as compared to hyperinsulinemia alone [33].

Several cellular pathways have been investigated to understand the prognosis of breast cancer advancement in diabetes. Leptin signaling, including activation of Akt/mTOR pathway, contributes to hyperglycemia mediated increased risk of cancer in normal mammary cells, as well as cancer progression in malignant cells [31]. Proliferation, migration, and invasiveness of breast cancer cells in hyperglycemic-hyperinsulinemic states has also been attributed to oxidative stress which elevates urokinase plasminogen activator [34]. We tested whether polyamines are involved in this pathway, as polyamines are required for cell growth, and elevated in several cancer subtypes including skin, colon, and breast. Inhibition of the polyamine synthesis pathway, using DFMO abrogated proliferation of cells

and colony formation observed with HG treatments. This was observed in both MDA-MB-231 and MCF-10A cells after 72h treatments. Interestingly although HG did not increase proliferation of MCF-7 cells compared to controls, there was marginal increase in colony formation at same concentration, which was inhibited using DFMO. Lower number of cells plated per well and longer incubation time post-treatment are likely the reason for an increase in colonies with HG observed with MCF7 cells in clonogenic assay.

To understand the role of polyamine pathway in regulating HG-mediated cell proliferation, we also measured enzyme activity of ODC, the rate-limiting enzyme involved in the polyamine pathway (Fig. 1). ODC activity was elevated considerably with HG in all cell types, and DFMO treatment was protective in reversing these effects. ODC is directly involved in the production of putrescine, the first product in the polyamine biosynthetic pathway (Fig. 1). Putrescine levels were elevated with HG in MDA-MB-231 cells but did not change in MCF-10A cells. Quite remarkably, spermidine and spermine concentrations did not change with HG treatments in cancer cells and normal cells. Our data provides the first evidence where HG-induced polyamine production could transform normal mammary epithelial cells to a hyperproliferative phenotype.

It has been suggested that DFMO induces cytostasis through depletion of polyamines and through depletion of thymidine, which is involved in supplementing synthesis of polyamine through a parallel pathway involving S-adenosylmethionine (Fig. 1) [35]. In the case of MDA-MB-231 cells, supplementation of polyamines (putrescine, spermine, or spermidine) did not restore the inhibition of proliferation through DFMO action which suggests that DFMO might be acting through additional mechanism than polyamine metabolism alone. While polyamine synthesis enzymes such as ODC, arginine decarboxylase, and agmatinase have been shown to be decreased in diabetic patients, and increased in breast cancer patients, there is not a measurable difference for patients with co-existing diabetes and breast cancer [8]. This is the first study to our knowledge to also report the metastatic potential of triple negative breast cancer cells, MDA-MB-231 is significantly enhanced with HG treatments though DFMO is not protective towards this.

In conclusion, our data suggests that restricting polyamine (putrescine) synthesis can be a plausible therapeutic option in preventing proliferation of breast cancer cells in diabetic states, while also affecting the growth profile of non-tumorigenic mammary epithelial cells (Fig. 7). Future studies would involve treating MDA-MB-231 cells for prolonged periods to assess tumor formation *in vitro* using soft gel assays and *in vivo* using xenograft mouse models. It is suggested that polyamine inhibitors can be combined with common breast cancer therapeutics in diabetic breast cancer patients, which can dramatically improve the prognosis of these patients. A comprehensive comparison of enzymes and metabolites of polyamine pathway in cancer patients with pre-existing diabetes in relation to those with cancer or diabetes alone, will help in customizing effective therapeutic regimen.

Declarations

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Author Contributions: CC, the primary author was involved with project design, experimentation, and writing the manuscript; JO and JM performed cellular assays; MD under the supervision of RAC performed enzyme activity assays at John Hopkins; HT edited the manuscript; and SC supervised the work, helped with project design, and edited the manuscript. We thank Dr Sophie Alvarez at the Proteomics & Metabolomics Facility, Nebraska Center for Biotechnology at the University of Nebraska-Lincoln for the polyamines analysis. The facility and instrumentation are supported by the Nebraska Research Initiative.

Declarations of interest: none

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest/Competing interests: None

Availability of data and material: Upon request from corresponding author

Code availability: Upon request from corresponding author

Authors' contributions: provided earlier

Ethics approval: N/A

Consent to participate: N/A

Consent for publication: This manuscript is being submitted upon consent from all authors listed

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Tables

Table 1: Rate of wound healing compared to scratch area at 0h was calculated for different treatments for 24 and 48h.

Table 1

Treatments	Scratch Wound Healing Rate		
	0h	24h	48h
5mM Glu	0	19.1±4.7	30.2±3.8
5mM Glu+D	0	23.7±2.3	45.7±5.2
25mM Glu	0	65.9±6.1*	85.9±1.5*
<u>25mM Glu+D</u>	<u>0</u>	<u>54.3±1.6*</u>	<u>71.1±2.5*</u>

Table 2: Levels of polyamines (ng/10⁶ cells in pellet)- putrescine (PUT), spermidine (SPD), and spermine (SPM) levels in (A) MDA-MB-231 cells, and (B) MCF-10A cells treated with glucose. Data is representative of 3 experiments performed in replicates; *p<0.05 compared to LG (5mM) control

Table 2

(A)

Treatments	Polyamines (ng/10 ⁶ cells in pellet)		
	Putrescine	Spermidine	Spermine
5mM Glu	45.3±10.5	613.1±191.9	237.8±85.5
<u>25mM Glu</u>	<u>60.5±11.6*</u>	<u>668.9±229.2</u>	<u>205.6±77.1</u>

(B)

Treatments	Polyamines (ng/10 ⁶ cells in pellet)		
	Putrescine	Spermidine	Spermine
5mM Glu	3.9±0.1	522.2±9.7	130.8±7.3
<u>25mM Glu</u>	<u>2.9±1.8</u>	<u>476.5±270.6</u>	<u>149.8±92.3</u>

Figures

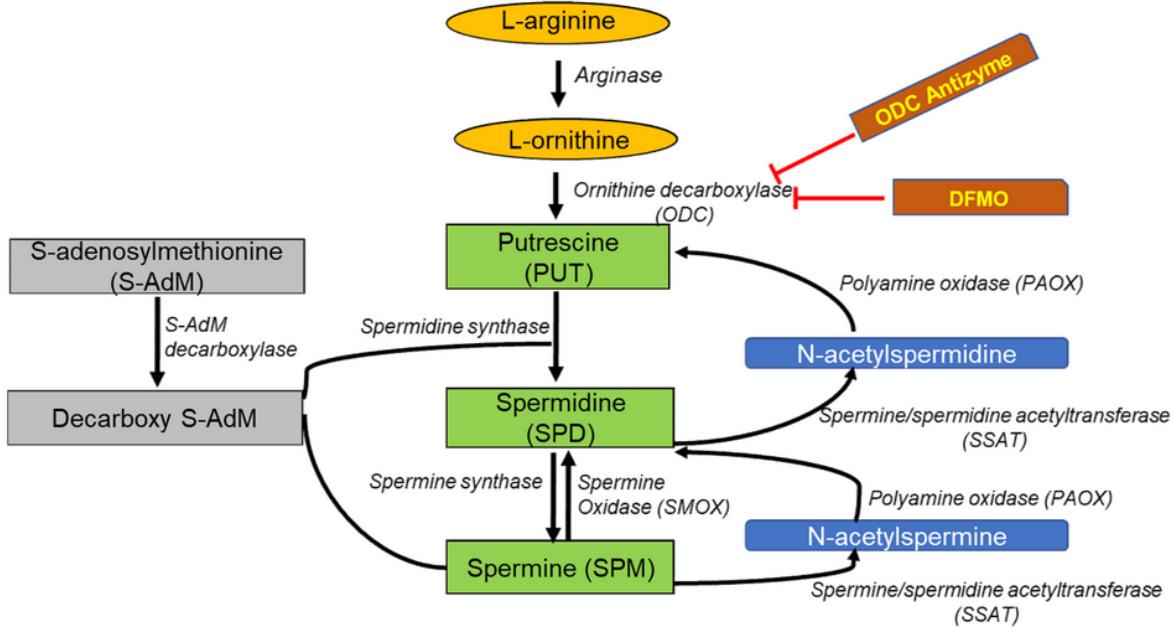


Figure 1

Schematic of polyamine pathway in animal cells. Primary pathway for polyamine synthesis is presented in the middle which involves the action of ornithine decarboxylase to produce the polyamines (putrescine, PUT, spermidine, SPD, and spermine, SPM) from L-ornithine. In addition, low levels of SPD or SPM also trigger input of these metabolites from a parallel alternate pathway through the decarboxylation of S-adenosylmethionine (S-AdM). Catabolism of SPD and SPM is mediated through the enzymes spermine/spermidine acetyltransferase (SSAT), and polyamine oxidase (PAOX). ODC, the first enzyme in the pathway, can be endogenously inhibited by ODC antizyme or exogenously using DFMO.

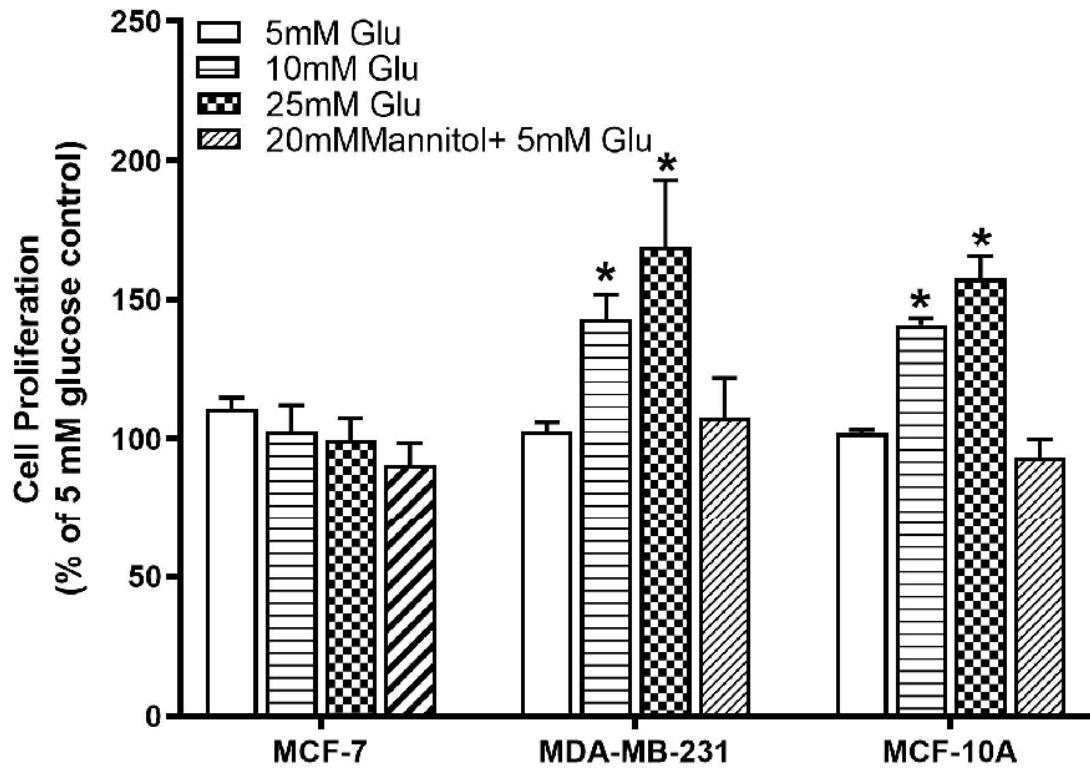


Figure 2

Dose response of proliferation with glucose treatment in MCF-7, MDA-MB-231, and MCF-10A cells treated with 5mM, 10mM, 25mM glucose, or mannitol (20mM with 5mM glucose) for 72h; *p<0.05 vs. respective 5mM glucose). Values are presented as %mean ± SEM compared to respective 5mM Glu treatment at that time point, n=3, each treatment with replicates.

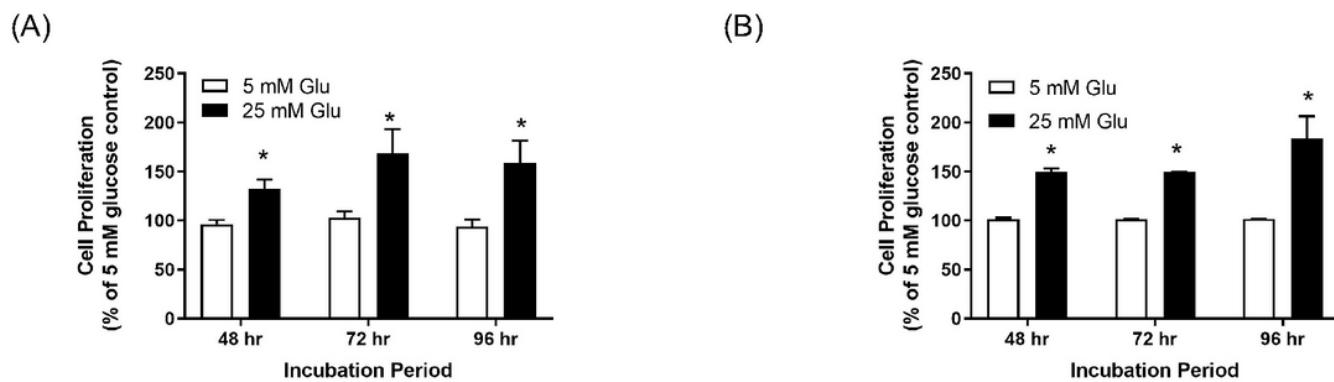
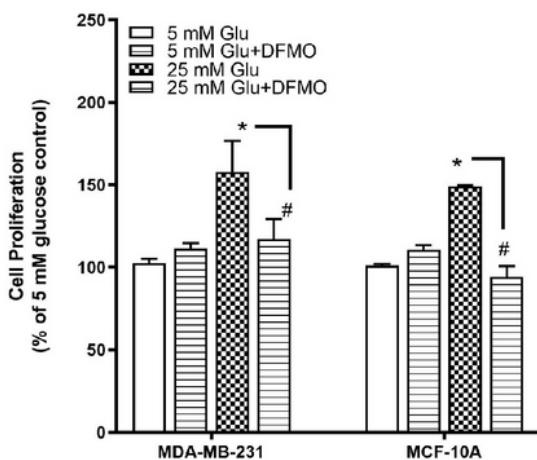


Figure 3

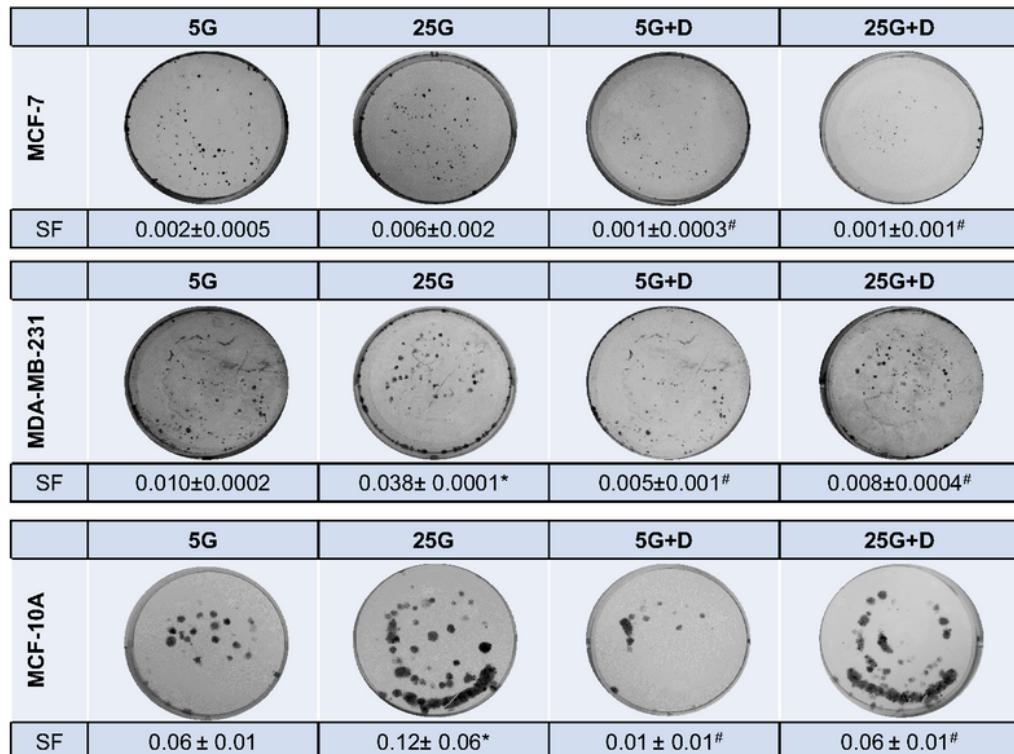
Time response of proliferation with glucose treatment in (A) MDA-MB-231, and (B) MCF-10A cells. LG (5mM Glu) and HG (25mM Glu); *p<0.05 vs. respective 5mM glucose. Values are presented as %mean ±

SEM compared to respective 5mMGlut treatment at that time point, n=3, each treatment with replicates.

(A)



(B)



Survival Fraction (SF) = # of colonies formed after treatments/# of cells plated

Values represent average \pm SD

Figure 4

A) Cell proliferation of MDA-MB-231 and MCF-10A cells in LG (5mM Glu) and HG (25mM Glu), in presence or absence of polyamine inhibitor, DFMO (5mM) for 72h (*p<0.05 vs. 5mM glucose, #p<0.05 vs. 25mM glucose). Values are mean \pm SEM, n=3, each treatment with replicates. (B) Colony forming assay (clonogenic) for MCF-7, MDA-MB-231, and MCF-10A cells in LG (5mM Glu) and HG (25mM Glu), with and

without polyamine inhibitor, DFMO (2mM) for 72h (*p<0.05 vs. 5mM glucose, #p<0.05 vs. 25mM glucose). Survival fraction (SF) represents the number of colonies formed based on number of cells plated and cells survived after treatments. Colonies with \geq 50 cells were counted for calculations. Values are mean \pm SEM, n=3, each treatment with replicates.

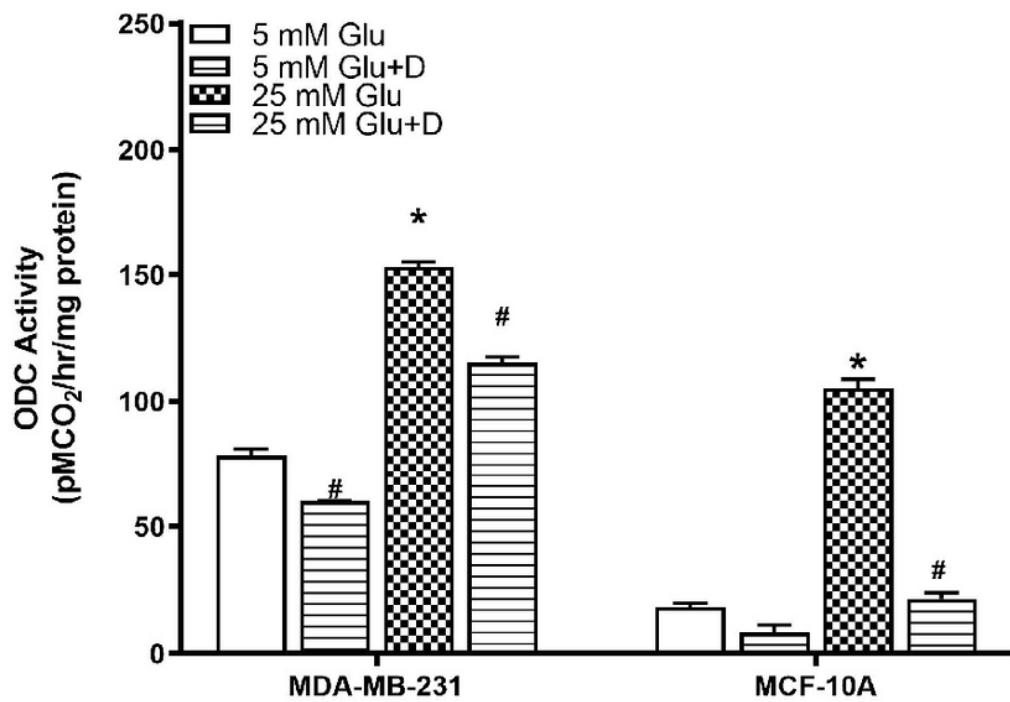


Figure 5

ODC activity assay in cells treated with varying concentrations of glucose (5mM, 25mM) and/or DFMO for 72h. Values are mean \pm SEM, *p<0.05 vs 5mM glucose, #p<0.05 vs. 25mM glucose.

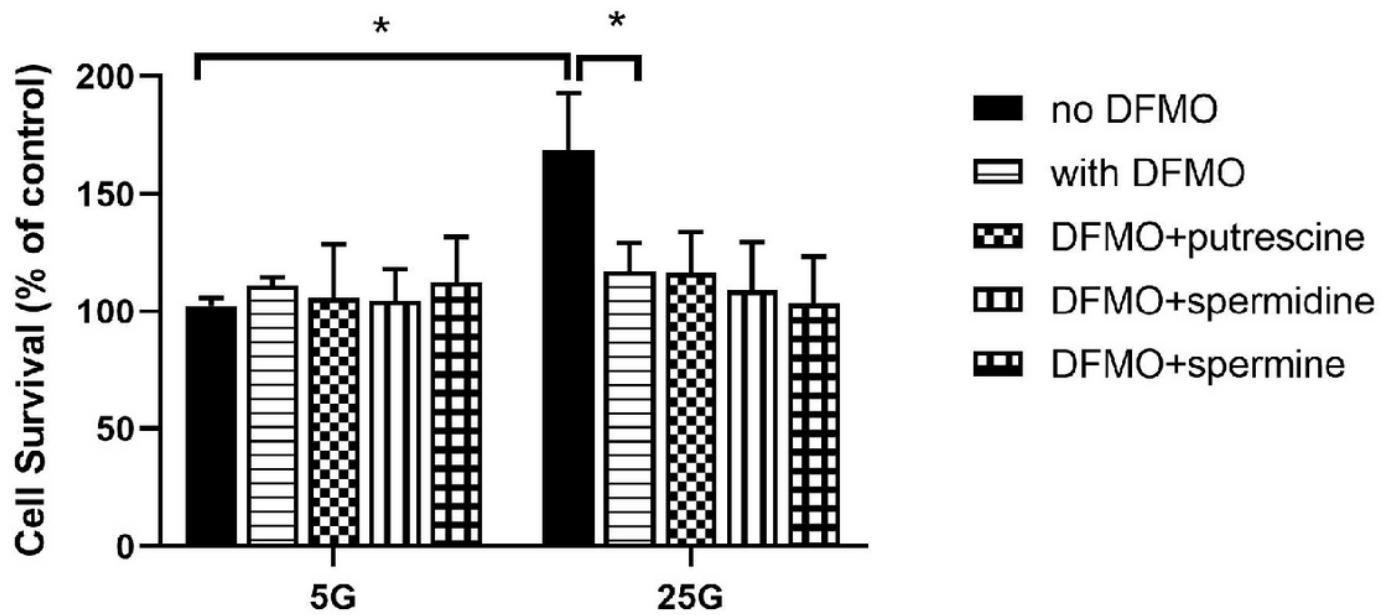


Figure 6

Cell proliferation following 8-hour inhibition with DFMO and 48-hour supplementation of natural polyamines. The concentrations of polyamines used were: 10 μ M putrescine, 1 μ M spermidine, and 1 μ M spermine. (*p<0.05 vs. respective glucose control). Values are mean \pm SEM, n=3.

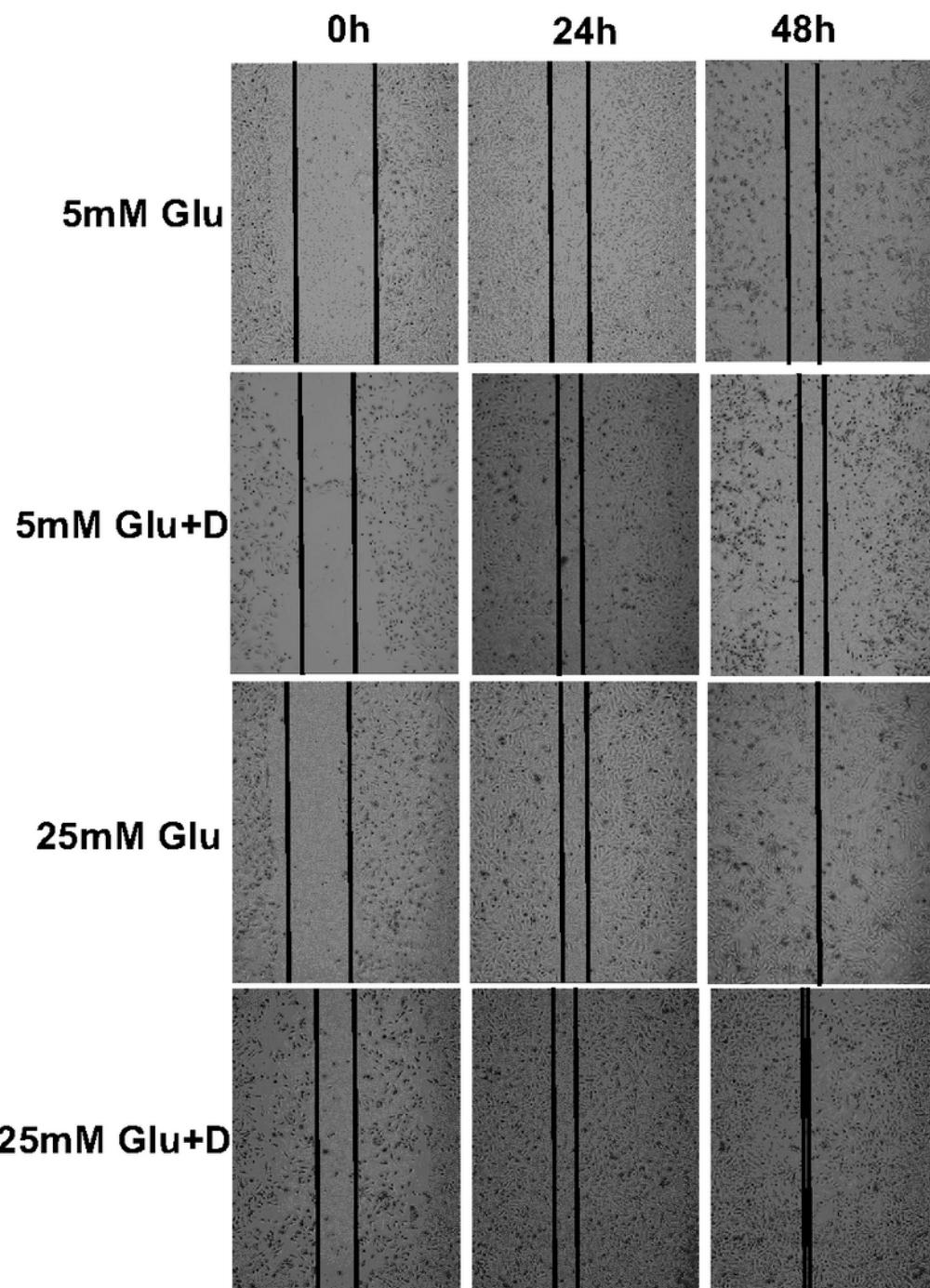


Figure 7

Scratch wound healing assay in MDA-MB-231 cells treated with glucose and/or DFMO for 0, 24, 48h. Images were taken using phase contrast microscopy, and area of wound (% of total area) was calculated using ImageJ (NIH) software. Multiple images were taken for each treatment, and averaged. All treatments were performed in replicates. Values are mean \pm SEM, *p<0.05.

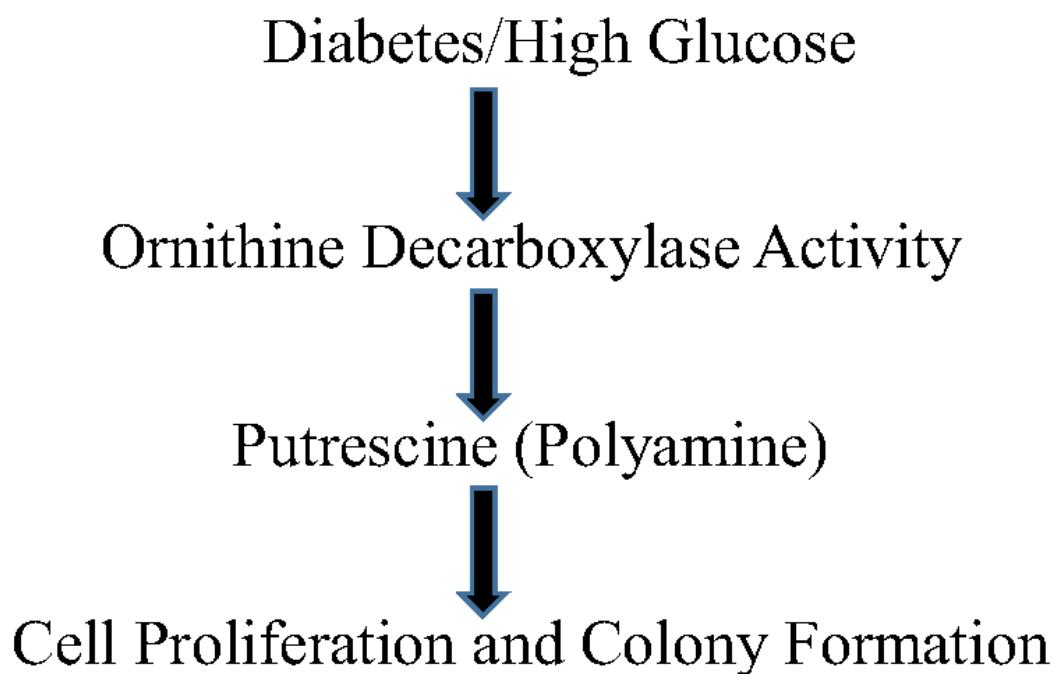


Figure 8

Schematic of proposed model for diabetes-mediated aggression of breast cancer states Table 1: Rate of wound healing compared to scratch area at 0h was calculated for different treatments for 24 and 48h.