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Metabolic Features of Lymphoblastoid and Myeloma Cells: Commonality and Differences

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Abstract: Glucose is the main source of energy for most cells. Glucose transport across the cytoplasmic membrane is known to be mediated by a family of glucose transporters (*GLUT*s). Therefore, studying the regulation of the *GLUT*s gene expression at different glucose levels in human multiple myeloma cells is relevant. The total amount of ATP was determined using a bioluminescent assay. Gene expression was analyzed using qRT-PCR. It has been established that the metabolism of lymphoblastoid cells IM9 ($CD138^+$, $CD38^-$), as well as myeloma lines RPMI8226 ($CD138^+$, $CD38^{++}$) and H929 ($CD138^+$, $CD38^{+++}$), depends on the availability of glucose. These cell lines exhibited glucose-dependent ATP synthesis, which was higher in RPMI8226 and H929 cells than in IM9 cells. *GLUT1* mRNA was found to be overexpressed under glucose deficiency in RPMI8226 and H929 cells, but *GLUT3* mRNA was overexpressed in IM9 cells. It was shown that the *GLUT1* gene is sensitive to glucose levels in RPMI8226 and H929 cells, and the *GLUT3* gene is sensitive to glucose in lymphoblastoid cells. It was found that during glucose deprivation, the *GLUT2* gene is sensitive to glucose and overexpressed in all types of cells, while the *GLUT4* gene is insensitive to glucose.

Keywords: multiple myeloma; ATP synthesis; expression; glucose transporter genes GLUT1-GLUT4.

1. Introduction

Multiple myeloma (MM) is a malignancy of antibody-producing plasma cells that develop from differentiating B cells characterized by infiltration of the bone marrow by plasma cells, the presence of monoclonal immunoglobulin in the blood serum and/or urine, and osteolytic bone lesions [1]. MM is the second-most common hematologic malignancy and is estimated to account for 34,470 new cases (male - 19,100; female - 15,370) and 12,640 (male -7,090; female -5,550) estimated deaths in 2022 in the United States [2].

Despite great advances in therapy, MM is incurable, with an overall survival of approximately 5.2 years after diagnosis, depending on age and therapy. Although the drug store has grown significantly in the last few years, there are no definitive curative actions for MM, and the majority of patients relapse with drug-resistant disease [3-8]. Drug resistance is a

multicausal phenomenon, and the mechanisms behind its occurrence are, in part, associated with metabolic changes [9]. It is thus not surprising that over the last decade, more studies have shown the connections between metabolic state, disease progression, and therapy resistance [1]. However, the contribution of cellular metabolism and intrinsic/extrinsic metabolites to therapy sensitivity and resistance mechanisms is less well understood. Therefore, further research into key metabolic features of MM cells is ongoing to help identify new therapeutic treatment strategies [10-35].

Metabolism of tumor cells needs to evolve throughout cancer progression, and it has to increase compared to normal cells. Cancer cells change cellular metabolism to promote cell growth and proliferation, with specific dependencies dictated by the cell's clonal origin and differentiation variation. Clonality with the subsequent development of subpopulations within a tumor may influence metabolic heterogeneity due to differential glucose uptake and ATP synthesis, an indicator of metabolically active cells [36]. Therefore, elucidating cell-specific metabolic status is critical to understanding the contribution of metabolism to therapeutic efficacy and resistance mechanisms.

It is known that MM is a malignancy characterized by the clonal proliferation of antibody-producing plasma cells that develop from differentiating B cells, which were originally characterized by low metabolic activity. Differentiation from B cells to plasma cells requires increased glucose uptake and ATP synthesis to prepare for antibody production [37]. Metabolic changes are also instrumental in regulating gene and protein expression, which are implicated in bioenergetics and biosynthesis [36]. For instance, a notable upregulation of genes involved in glucose metabolism is seen when comparing plasma cells from newly diagnosed MM patients *vs* normal donors and in relapsed patients *vs* newly diagnosed MM patients [38]. So, the glucose uptake and ATP synthesis can be associated with the differentiation status of MM cells as well as MM tumor progression. This is why elucidating the relationship between MM cell differentiation status and metabolism will allow this phenomenon to be used as a marker of cancer progression and to predict the overall viability of patients with MM disease, as well as sensitivity to therapy.

This study aims to investigate glucose-dependent ATP synthesis and the expression of glucose transporter genes involved in glucose uptake - *GLUT1, GLUT2, GLUT3,* and *GLUT4* in two human myeloma cell lines RPMI8226 and H929, as well as the lymphoblastoid cell line IM9 with clearly established criteria for their differentiation.

2. Materials and Methods

2.1. Materials.

We used the growth medium RPMI1640 (25 mM HEPES, sodium pyruvate, OOO NPP PanEco, Russia), the growth medium DMEM without glucose (25 mM HEPES, sodium pyruvate, OOO NPP PanEco, Russia), Glucose (400 mg/mL, OOO GROTECS, Saint Petersburg, Russia). Fetal Bovine Serum (FBS, Biosera, South America Origin), L-glutamine (OOO NPP PanEco, Russia), Gentamicin (10 mg/ml, OOO NPP PanEco, Russia). Plastic dishes (culture flasks 25 cm^2, disposable pipettes) and 96-well plates for growing the cell culture were purchased from Corning-Costar (USA).

2.2. Cell lines.

Three different types of cell lines were used with clearly establish criteria for differentiating them: two established human multiple myeloma cell line (HMCL) RPMI8226 ($CD138^{+}$, $CD38^{++}$, B-B_4 $^{++}$, $CD28^{++}$, $CD19^{-}$, $CD20^{-}$, $CD11a^{+/-}$, $CD49e^{+}$) and H929 ($CD138^{+}$, CD^{38+++}, B-B_4 $^{+}$, $CD28^{+}$, $CD19^{-}$, $CD20^{-}$, $CD11a^{-}$, $CD49e^{-}$) which express the differentiation markers indicated in parentheses on their surface, and one lymphoblastoid cell line (LCL) IM9 (CD^{138+}, CD^{38-}, B-$B_4^{+/-}$ $CD28^{-}$, $CD19^{++}$, $CD20^{+}$, $CD11a^{+}$, $CD49e^{+}$) which result from the immortalization of nonmalignant B cells by *Epstein-Barr virus* (EBV) [39]. Cell lines H929 and IM9 were purchased from the Russian Collection of Cell Cultures (Institute of Cytology RAN, Saint-Petersburg, Russia). Cell line RPMI8226 (human multiple myeloma) is a kind gift from Dr. Kakpakova E.S. (FSBI «N.N. Blokhin RCRC», Russia). Cells cultured in growth medium RPMI with 10% FBS at 37°C, 5% CO_2, and 95% humidity. The cells were grown to 90% density in culture flasks.

2.3. Colorimetric MTT assay.

The compound MTT was adopted to assess cell proliferation. Briefly, IM9, RPMI8226, and H929cells were seeded into 96-well plates at a density of $20x10^3$ cells per well in triplicate in 150 µl of medium containing serum with 25 мM glucose (control) or without glucose. These conditions were repeated for a serum-free medium. After incubation for 72 h, 20 µl 5 mg/ml MTT was added to each well, and the cells were incubated at 37˚C for another 4 h. Subsequently, the media was discarded, followed by the addition of 150 µl DMSO per well to dissolve the purple precipitate. The absorbance (A) was measured at 570 nm using a microplate reader (Multiskan FC 357-908226, Thermo Scientific, USA). The cell growth (%) was calculated according to the following equation: % viable cells = $(A_{sample} - A_{blank})/(A_{control} - A_{blank})$ x 100%, where $A_{control}$ is the absorbance of the untreated cells, A_{sample} is the absorbance of the cells without glucose, and A_{blank} is the absorbance of the media.

2.4. Analysis of glucose-dependent synthesis of total amount ATP.

Analysis of glucose-dependent synthesis of the total amount of ATP was determined in cells by the ATP-Glo™ Bioluminometric Cell Viability Assay Kit (Biotium, Germany) in accordance with the instructions. This ATP detection kit uses firefly luciferase, which uses ATP to oxidize D-luciferin and subsequently produce light to estimate the amount of ATP available. Since ATP is an indicator of metabolically active cells, the number of viable cells can be estimated based on the amount of ATP present. ATP-Glo™ is a flash-type luminescent assay. The generated luminescence signal is stable for up to 1 minute. Luminescence intensity was measured on a spectrofluorimeter (Cary Eclipse, Varian Medical Systems, USA) at E_{ex}/E_{em} = 546/620 nm.

The effect of glucose on the total amount of ATP was analyzed as follows [40]. Cells IM9, RPMI8226, and H929 were grown in culture flasks to a density of 10^6 cells. MM cells were then washed twice from serum and glucose with serum-free DMEM without glucose. After each wash, the cells were pelleted by centrifugation at 3000 g for 5 min. The cell pellet was resuspended in 5 ml serum-free DMEM containing 5 mM glucose (low glucose) and incubated for 12 h. After 12 h, the cells were pipetted repeatedly, divided equally into 2 parts, and pelleted by centrifugation. IM9, RPMI8226, and H929 cells were seeded at a density of $2x10^4$ cells per well.

Then, serum-free DMEM containing 0 mM glucose was added to one part of the cells, and 100 µL was added to every 5 wells of a 96-well plate. Serum-free DMEM with 25 mM glucose (high glucose level) was added to the second part of the cells, 100 µl in every 5 wells of a 96-well plate.

After this, the cells were incubated at 37°C for 10 and 30 minutes. Then, 5 µl of ATP-Glo cocktail was added to each well, and the luminescence intensity was measured on a spectrofluorimeter as described above. The total amount of ATP was calculated based on the constructed calibration standard curve. Data are presented as the mean of triplicate experiments.

2.5. Analysis of the effect of glucose deprivation on the level of GLUT mRNA expression.

IM9, RPMI8226, and H929 cells were grown in growth medium RPMI1640 to a density of $3x10^6$ cells; then, the cells were washed to remove serum and glucose with serum-free DMEM without glucose. After washing, the cells were pelleted by centrifugation at 3000 g for 5 min. The cell pellet was resuspended in 5 ml of serum-free DMEM containing 0 mM glucose and serum-free DMEM with 25 mM glucose and incubated for 12 h. After this time, the cells were collected by centrifugation at 3000 g for 5 min, and total RNA was extracted.

2.6. RNA extraction, reverse transcription, and quantitative real-time PCR for gene expression analysis.

Total RNA was extracted using a single-step technique using TRI Reagent (Sigma, USA) according to the manufacturer's protocol. For cDNA synthesis, 2 µg of total RNA was reverse-transcribed using oligo dT18 primers and MMLV RT enzyme. Quantitative polymerase chain reaction (qPCR) was performed on a CFX 96 Touch Real-Time PCR (Bio-Rad, USA), using SYBR Green reporter fluorescent dye according to the manufacturer's protocol. The set of reagents for qPCR (Syntol, Russia) was used. The cycling conditions were as follows: 95°C for 5 min and 40 cycles of 95°C for 15 sec followed by 60°C for 25 sec and 72°C for 25 sec. Under these conditions, no amplification was observed in the non-template or RT controls. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The comparative CT method analyzed the data, and the fold change was calculated by the $2-\Delta\Delta CT$ method. Amplification of a housekeeping gene, β-actin, was used to normalize cDNA synthesis efficiency. All samples were assayed in triplicate, and mean expression values were used. The sequences of the oligonucleotide primers and mRNA NCBI reference sequences used are shown in Table 1.

Table 1. Primer pairs for qPCR detection

Primer name	Primer sequence (5′-3′)	NCBI reference sequence
GLUT1	F: CTTCACTGTCGTGTCGCTGT	NM_006516.2
GLUT1	R: TGAAGAGTTCAGCCACGATG	
GLUT2	F: GGTTTGTAACTTATGCCT AAG	NM_000340.1
GLUT2	R: GCCTAGTTATGCATTGCA G	
GLUT3	F: GACCCAGAGATGCTGTAATGGT	NM_006931.2
GLUT3	R: GACCCCAGTGTTGTAGCCAA	
GLUT4	F: GCCATGAGCTACGTCTCCATT	M20747.1
GLUT4	R: GGCCACGATGAACCAAGGAA	
β-Actin	F: AGCCATGTACGTTGCTATCCA	NM_001101.3
β-Actin	R: ACCGGAGTCCATCACGATG	

2.7. Statistical analysis.

All cell experiments were performed in three or more independent repeats. Statistical analysis of the obtained data was performed using the computer program GraphPad Prizm 5.02 (GraphPad Software Inc., USA). Data are expressed as means ± standard error of the mean (SEM). The Student t-test for two-group comparisons was used to compare the differences among gene expression levels of experiments *vs* control. The differences were considered significant at *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3. Results and Discussion

3.1. Glucose-dependent cell viability and ATP synthesis in the presence or absence of FBS.

We examined whether the presence of FBS affects the glucose-dependent viability of IM9, RPMI8226, and H929 cells. MTT assay showed that glucose deprivation in the absence of FBS compared with the presence of FBS resulted in a marked decrease in the viability of all cell types (Figure 1). The average viability of IM9 cells decreased from 57% to 9.5% (6-fold) (Figure 1A and Figure 1D). The average viability of RPMI8226 cells decreased from 42% to 14% (3-fold) (Figure 1B and Figure 1E). And the average viability of H929 cells decreased from 55% to 21% (2.6-fold) (Figure 1C and Figure 1F). Thus, this result showed that cell viability is very sensitive to the presence of serum.

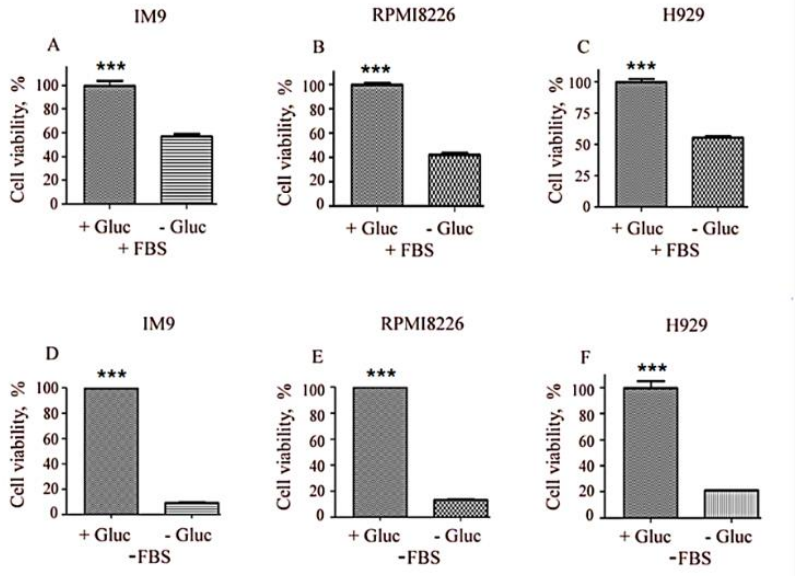

Figure 1. Glucose-dependent viability of **(A, D)** IM9; **(B, E)** RPMI8226; **(C, F)** H929 cells in the presence or absence of FBS. Glucose deprivation in the absence of FBS compared with the presence of FBS showed a more than two-fold decrease in the viability of all cell types. MTT assay, for 72 h. Data are presented as the mean ± standard deviation (n=3). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ *vs.* control group (+Gluc).

Next, we assessed whether the presence of FBS affects the total glucose-dependent amount of ATP in IM9, RPMI8226, and H929 cells. Our data showed that glucose deprivation in the absence of FBS compared with the presence of FBS leads to a marked decrease in the amount of ATP in all cell types (Figure 2). The average amount of ATP in IM9 cells decreased from 54% to 15% (3.6-fold) (Figure 2A and Figure 2D). The average amount of ATP in RPMI8226 cells decreased from 34.9% to 4.4% (8-fold) (Figure 2B and Figure 2E). The average amount of ATP in H929 cells decreased from 65.8% to 44.6% (1.5-fold) (Figure 2C and Figure 2F).

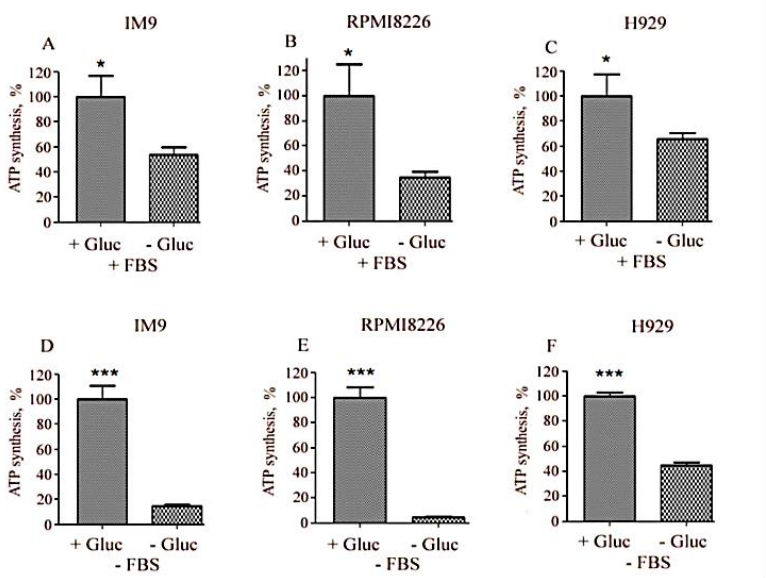

Figure 2. Glucose-dependent amount of ATP in **(A, D)** IM9; **(B, 3)** RPMI8226; **(C, F)** H929 cells in the presence or absence of FBS. Glucose deprivation in the absence of FBS compared with the presence of FBS resulted in a more than 1.5-fold decrease in the amount of ATP for all cell types. ATP synthesis was analyzed after 72 h. Data are presented as the mean ± standard deviation (n=3). *P<0.05 *vs.* control group (+Gluc).

Thus, our experiment showed that for IM9, glucose-dependent cell viability and ATP amount in the absence of FBS compared to the presence of FBS were reduced by 6- and 3.6-fold, accordingly (Table 2). For H929, glucose-dependent cell viability and ATP amount in the absence of FBS were reduced by 2.6- and 1.5-fold accordingly (Table 2). However, for RPMI8226, only a 3-fold decrease in cell viability in the absence of FBS leads to an 8-fold decrease in the amount of ATP (Table 2). Based on these data, we hypothesize that RPMI8226 requires more glucose-dependent ATP per cell than IM9 and H929 cells.

Table 2. Comparison of fold decrease in glucose-dependent cell viability and ATP amount in the absence of FBS compared to the presence of FBS.

Type of cells	Decrease of glucose-dependent cell viability (fold)	Decrease of the glucose-dependent amount of ATP (fold)
IM9	6	3,6
RPMI8226	3	**8**
H929	2,6	1,5

Data are presented as the average fold reduction in cell viability and ATP amount.

Overall, we determined that all cell types used were very sensitive to the presence of glucose in the medium without FBS, so we performed subsequent experiments without FBS. It is important to note here that FBS contains lipids, of which fatty acids are the simplest form of lipids. Along with glucose, fatty acids represent one of the main sources of energy metabolism of myeloma cells [41]. When energy is required, fatty acid degradation is activated to produce ATP molecules for rapid proliferation of tumor cells and growth [42, 43]. Thus, we will completely exclude fatty acids from the metabolism study in our future experiments without FBS.

3.2. Comparing of glucose-dependent synthesis of ATP in cells.

Next, we compared the effect of glucose-dependent total ATP synthesis in IM9, RPMI8226, and H929 cells after 10 and 30 min. As noted above, we performed all experiments

on cells in the absence of FBS to avoid complications associated with the participation of fatty acids in ATP production. The experimental design was modified in accordance with [40].

First, MM cells were washed from serum and glucose with serum-free DMEM without glucose, and to reduce cellular ATP stores, cells were maintained in serum-free DMEM containing low glucose (5 mM) for 12 h. Then, cells were treated with serum-free DMEM containing 0 mM glucose and 25 mM (high) glucose for 10- and 30 min. The total amount of ATP in each cell type was then calculated (Figure 3).

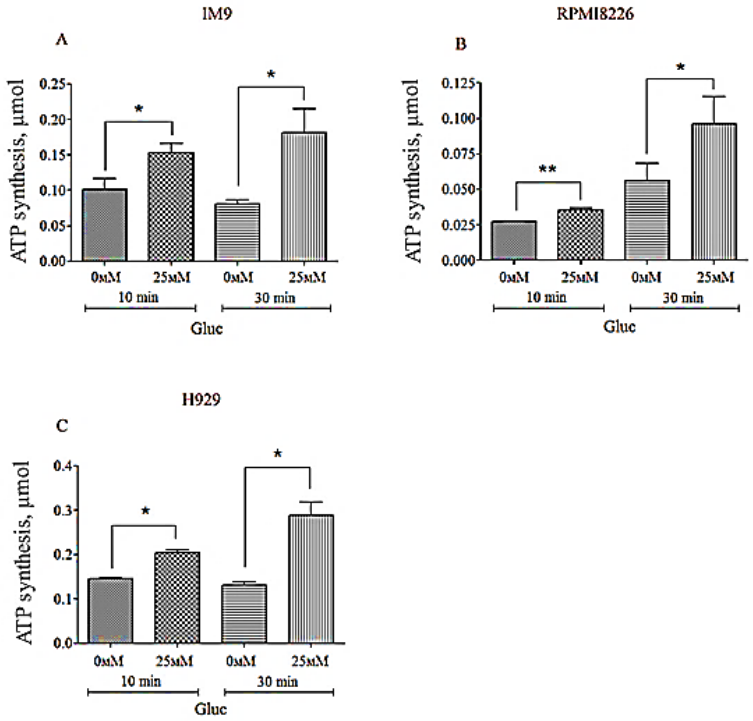

Figure 3. Glucose-dependent ATP synthesis by **(A)** IM9; **(B)** RPMI8226; **(C)** H929 cells in the presence or absence of glucose after 10- and 30 min. Glucose deprivation was carried out in the absence of FBS. ATP synthesis was analyzed after 72 h. Data are presented as the mean ± standard deviation (n=3). *P<0.05 *vs.* control group (+Gluc).

It was shown that at a glucose concentration of 25 mM, the total amount of ATP for all cell types continues to increase for 30 min, which is greater than 10 min (Figure 3). The amount of ATP at a glucose concentration of 25 mM for 10- and 30 min is greater than that at a glucose concentration of 0 mM (control) during this time. Overall, this means all cell types at 5 mM glucose for 12 h depleted ATP. The amount of ATP at a glucose concentration of 0 mM for 10- and 30 min did not change noticeably in IM9 and H929 cells but increased within 30 min in RPMI 8226 cells. Thus, the amount of ATP in RPMI8226 cells could increase without glucose. It is known that along with glucose and fatty acid, glutamine is one of the main sources of myeloma cells' energy metabolism (ATP synthesis) [41, 43]. But at the same time, RPMI8226 cells are also glucose-dependent, and in the absence of glucose (0 mM), the amount of ATP is significantly less than in the presence of 25 mM glucose both after 10 min and after 30 min. To exclude the possible involvement of glutamine and establish only the glucose-dependent amount of ATP at both 10 and 30 min, we subtracted the ATP value at 0 mM glucose from the ATP value at 25 mM glucose and determined Δ_1ATP_{10min} and Δ_2ATP_{30min} for each cell type (Table 3). To compare the rate of ATP synthesis by IM9, RPMI8226, and H929 cells over 20 min (from 10 to 30 min), the ratio $\Delta_2ATP_{30min}/\Delta_1ATP_{10min}$ was calculated. Our data showed that the minimum rate of ATP synthesis is demonstrated by IM9 cells (1.9), and the

maximum rate of ATP synthesis is demonstrated by RPMI8226 cells (5.1). The rate of ATP synthesis by H929 cells is intermediate (2.7) (Table 3).

Thus, we concluded that ATP synthesis in IM9, RPMI8226, and H929 cells depends on glucose metabolism, and the rate of glucose-dependent ATP synthesis is higher in RPMI8226 and H929 myeloma cells compared to IM9 lymphoblastoid cells. This is also consistent with our hypothesis above that RPMI8226 requires more ATP synthesis compared to IM9 and H929 cells (Table 2).

It was previously shown that MM cells in the bone marrow exhibit increased glucose uptake to sustain elevated lactate production, in contrast to normal plasma cells in the BM [36]. Upregulation of glucose metabolism-related genes is revealed while comparing plasma cells from newly diagnosed MM patients *vs* normal donors and in relapsed *vs* newly diagnosed MM patients [36]. MM cells rely heavily on glucose metabolism for aerobic glycolysis and oxidative phosphorylation and, ultimately, for ATP synthesis to maintain elevated levels of protein synthesis and secretion [43].

Table 3. The rate of glucose-dependent ATP synthesis by cells over 20 min.

Type of cells	Δ_1ATP_{10min} (µmol)	Δ_2ATP_{30min} (µmol)	Δ_2ATP_{30min} / Δ_1ATP_{10min}
IM9	0,0522	0,100	1,9
RPMI8226	0,0079	0,040	**5,1**
H929	0,0588	0,158	2,7

Δ1ATP10 min and Δ2ATP30 min – glucose-dependent ATP synthesis for 10 min and 30 min, respectively. The ratio Δ2ATP30 min /Δ1ATP10 min shows the rate of glucose-dependent ATP synthesis by cells over 20 min (from 10 min to 30 min).

3.3. Comparison of basal GLUT1-GLUT4 mRNA expression levels between and within each cell type.

Glucose is the main energy source in cells, generating ATP through glycolysis and oxidative phosphorylation. A key step in glucose consumption is the glucose transport across the plasma membrane into the cytosol, which is mediated by a family of glucose transporters (GLUTs) through a facilitated diffusion mechanism [44,45]. Although patterns of GLUTs expression in cancer have already been identified, extensive research evidence for the role of glucose in regulating individual GLUTs is still needed. Identifying and targeting glucose depending on GLUTs provides a promising approach to blocking glucose-regulated metabolism and signaling in cancer cells.

GLUT1 has a high affinity for glucose and has been found to be overexpressed in various malignancies and in MM cells, and GLUT1 upregulation increases glucose uptake [44, 45]. In MM cells expressing GLUT1, a selective GLUT1 inhibitor completely suppresses glucose uptake activity, induces apoptosis, and sensitizes multiple myeloma cells to conventional chemotherapeutic agents [46]. GLUT2 has been shown to transport glucose and fructose with relatively low affinities [44, 45]. GLUT3 is a high-affinity glucose transporter that has been found to be expressed predominantly in tissues with high levels of glucose needs [44]. GLUT4 has been found in insulin-sensitive tissues and plays a key role in maintaining the viability and proliferation of MM cells [47]. Based on protein sequence and structural similarity, the GLUT1- GLUT4 transporters form class I “glucose transporters” [44, 45]. There are three classes of glucose transporters, but the class I is known as the classical glucose transporters and is the most well-studied [44,45]. Because GLUTs are often overexpressed in

many types of cancer cells, new GLUT1- GLUT4 inhibitors are being developed, used alone or in combination with drugs to block glucose uptake [48-55].

We compared basal *GLUT1-GLUT4* mRNA expression between cell types and found high basal *GLUT1* expression in myeloma cell lines RPMI8226 (17.5 ± 0.55) and H929 (19.9 ± 0.59) compared to lymphoblastoid cells IM9 (1 ± 0.5) (Table 4). *GLUT2*, *GLUT3*, and *GLUT4* are also preferentially expressed in RPMI8226 and H929 cells compared to IM9 cells (Table 4). Thus, we conclude that IM9 lymphoblastoid cells express *GLUT1* - *GLUT4* at a lower level than RPMI8226 and H929 myeloma cells. IM9 is a lymphoblastoid cell line that results from immortalizing nonmalignant B cells by the *Epstein-Barr virus*; thus, IM9 cells are not myeloma cells [39]. The high basal level of all GLUT1, GLUT2, GLUT3, and GLUT4 mRNAs in RPMI8226 and H929 myeloma cells compared to IM9 lymphoblastoid cells may be associated with the acquisition of malignant properties by plasma cells.

Table 4. Comparison of basal *GLUT1-GLUT4* mRNA expression levels between cell types.

Gene name	IM9	RPMI8226	H929
GLUT1	1 ± 0,50	**17,5 ± 0,55**	**19,9 ± 0,59**
GLUT2	1 ± 0,43	8,4 ± 0,48	2,9 ± 0,51
GLUT3	1 ± 0,40	2,1 ±0,40	1,5 ± 0,22
GLUT4	1 ± 0,50	4,0 ± 0,43	2,9 ± 0,15

the mRNA expression values of *GLUT1* - *GLUT4* were normalized to those in IM9 cells. Data are presented as the mean ± standard deviation (n=3).

We then compared basal *GLUT1-GLUT4* mRNA expression in each cell type and found very low *GLUT4* expression in all cell lines (Table 5). In IM9 cells, only *GLUT3* (11.2 ± 0.64) is expressed at high basal levels compared to *GLUT1*, *GLUT2*, and *GLUT4*. Again, *GLUT1* shows high expression in RPMI8226 (17 ± 0.43) and H929 (26.5 ± 0.60) myeloma cells compared to *GLUT2*, *GLUT3* and *GLUT4* (Table 5). Thus, Table 3 and Table 4 conclude that *GLUT1* is the major glucose transporter for RPMI8226 and H929 myeloma cells, whereas *GLUT3* is important only for IM9 lymphoblastoid cells.

Our data are consistent with high levels of *GLUT3* mRNA expression being a feature of normal B cells [47]. MM is a malignancy of antibody-producing plasma cells that develop from differentiating B cells. Perhaps the increase in *GLUT1* expression in RPMI8226 and H929 myeloma cells is associated with acquiring new malignant properties by plasma cells.

Table 5. Comparison of basal *GLUT1- GLUT4* mRNA expression levels within each cell type.

Cell name	*GLUT1*	*GLUT2*	*GLUT3*	*GLUT4*
IM9	3,1 ± 0,72	2,3 ± 0,43	**11,2 ± 0,64**	1 ± 0,50
RPMI8226	**17 ± 0,43**	4,8 ± 0,30	7,1 ± 0,14	1 ± 0,23
H929	**26,5 ± 0,60**	2,3 ± 0,51	7,3 ± 0,23	1 ± 0,13

The mRNA expression values of *GLUT1- GLUT4* were normalized to those in *GLUT4* within each cell type. Data are presented as the mean ± standard deviation (n=3).

3.4. Effects of glucose deprivation on the GLUT1, GLUT2, GLUT3 and GLUT4 mRNA expression levels.

We investigated the effect of glucose deprivation on the *GLUT1*, *GLUT2*, *GLUT3*, and *GLUT4* mRNA expression levels in IM9, RPMI8226, and H929 cells. Our results show that during glucose deprivation, the glucose transporter *GLUT1* is overexpressed in RPMI8226 and H929 myeloma cells (Figure 4B and Figure 4C) and is not altered in IM9 lymphoblastoid cells (Figure 4A). These data also confirmed that *GLUT1* is an important glucose transporter for

RPMI8226 and H929 cells, as its basal high expression level (Table 4 and Table 5) is complemented by increased expression levels in the absence of glucose (Figure 4B and Figure 4C). Our data are consistent with the above quote that *GLUT1* is an important glucose transporter in MM cells [46]. It was previously shown that glucose deprivation increases the expression of *GLUT1* in rat C6 cells [56], in 3T3-L1 adipocytes [57], and in L6 myocytes [58]. *GLUT1* is negatively regulated by glucose in L8 myocytes and NIH 3T3 [59].

MM is a malignancy of antibody-producing plasma cells that develop from differentiating B cells. Differentiation of B cells into plasma cells requires an increase in glucose consumption rate to allow proliferation and to prepare for antibody production. Real-time-PCR analysis of GLUTs family gene expression changes in differentiating B cells revealed a decline of *GLUT1*. An assessment of GLUT1 protein mirrored the trends seen at the transcriptional level [47]. IM9 is a lymphoblastoid cell line (LCL) that results from the immortalization of nonmalignant B cells by *Epstein-Barr virus* [39]. Thus, IM9 cells are not myeloma cells and may retain the properties of differentiating B cells; therefore, they do not show any increase in *GLUT1* expression upon glucose deprivation. Perhaps the increase in *GLUT1* expression in MM cells under glucose deprivation is associated with the acquisition of new malignant properties by plasma cells.

In this part of our work, we concluded that in human myeloma cells RPMI8226 and H929, glucose deprivation increases the expression of the glucose transporter gene *GLUT1*, whereas in lymphoblastoid cells IM9, the expression of *GLUT1* does not change.

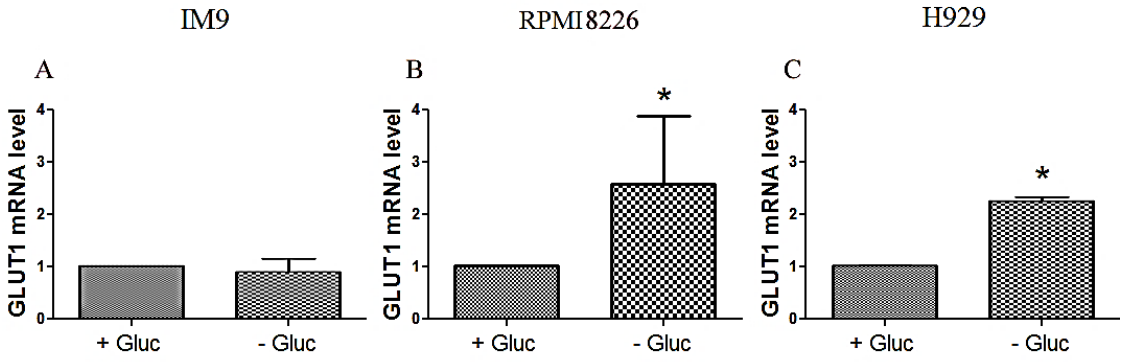

Figure 4. Level of *GLUT1* mRNA expression level in response to glucose deprivation in IM9, RPMI8226, and H929 cells. During the 12 h glucose deprivation, the *GLUT1* gene is overexpressed in **(4B)** RPMI8226; **(4C)** H929 myeloma cells and is statistically different from controls. At the same time, *GLUT1* expression does not change in **(4A)** IM9 lymphoblastoid cells. The mRNA level was measured using qRT-PCR. All experiments were carried out in triplicate. Data are presented as the mean ± standard deviation (n=3). *P<0.05 *vs.* control group (+ Gluc).

Our results show that the glucose transporter gene GLUT2 is overexpressed under glucose deprivation in all three cell lines, IM9, RPMI8226, and H929 (Figure 5A, Figure 5B, and Figure 5C). *GLUT2* is present in pancreatic islet beta cells and is required for glucose-stimulated insulin secretion [60]. *GLUT2* expression is required for physiological control genes sensitive to glucose, and their inactivation in the liver leads to disruption of glucose-stimulated insulin secretion [60]. In the nervous system, *GLUT2*-dependent glucose sensing controls feeding, thermoregulation, pancreatic islet cell mass and function, and sympathetic and parasympathetic activities [60]. Thus, the *GLUT2* transporter is associated with various glucose-sensing cells that are activated either by hypoglycemia or hyperglycemia.

In our study, we showed that the *GLUT2* gene has a moderate basal level of expression in IM9, RPMI8226, and H929 cells (Table 5), but upon glucose deprivation, *GLUT2* expression

is significantly increased in all these cells. We did not find information in the literature on the effect of glucose deprivation on *GLUT2* mRNA expression in MM cells. However, based on our data, we conclude that *GLUT2* is a glucose-sensitive gene in IM9 and in RPMI8226, H929 myeloma cells, and its expression is regulated by glucose.

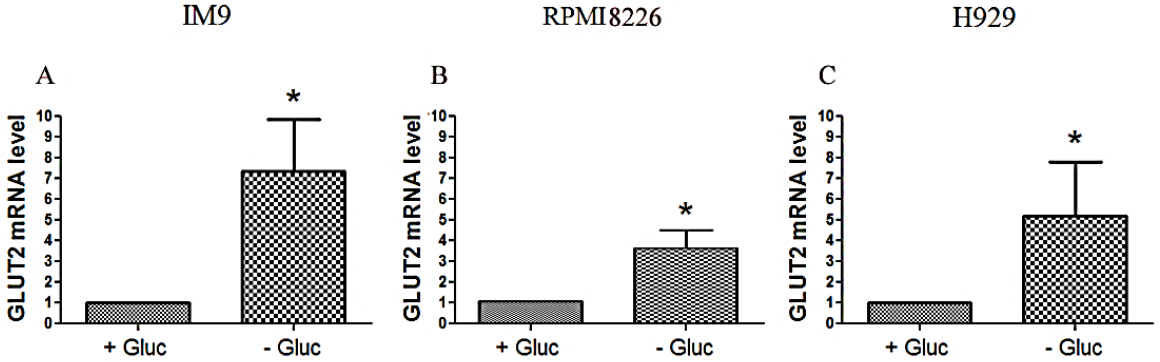

Figure 5. Level of *GLUT2* mRNA expression in response to glucose deprivation in IM9, RPMI8226, and H929 cells. During glucose deprivation, the glucose transporter gene *GLUT2* is overexpressed in **(5A)** IM9 lymphoblastoid cells; **(5B)** RPMI8226; **(5C)** H929 myeloma cells. The mRNA level was measured using qRT-PCR. All experiments were carried out in triplicate. Data are presented as the mean ± standard deviation (n=3). *P<0.05 *vs.* control group (+Gluc).

Our results show that during glucose deprivation, the glucose transporter gene *GLUT3* is overexpressed in IM9 lymphoblastoid cells (Figure 6A) compared with those in RPMI8226 (Figure 6B) and H929 (Figure 6C) myeloma cells. It is shown above that in IM9 cells, the expression of the basal level of *GLUT3* (11.2 ± 0.64) is significantly higher compared to *GLUT1* (3.1 ± 0.72), *GLUT2* (2.3 ± 0.43) and *GLUT4* (1 ± 0.5) (Table 5). Based on this, we hypothesized that high levels of *GLUT3* mRNA expression are a feature of lymphoblastoid IM9 cells. The *GLUT3* gene has previously been shown to be overexpressed in normal B cells compared to that in myeloma cells [47], although our comparative data show that basal *GLUT3* mRNA expression in IM9 cells (1 ± 0.4) is slightly lower compared to RPMI8226 (2.1 ± 0.4) and H929 (1.5 ± 0.22) (Table 4). However, IM9 lymphoblastoid cells may retain some B cell features, indicating that *GLUT3* is important for glucose homeostasis in IM9 lymphoblastoid cells compared to RPMI8226 and H929 myeloma cells, and therefore, its expression is glucose-sensitive.

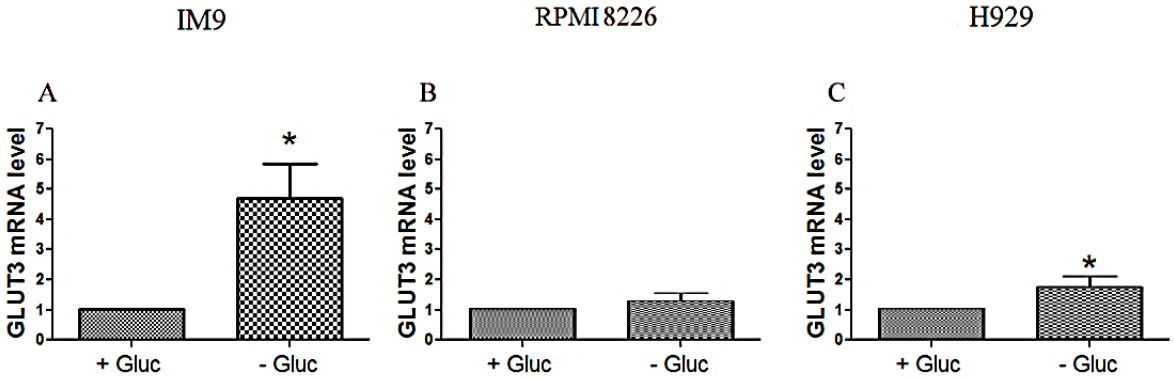

Figure 6. Level of *GLUT3* mRNA expression in response to glucose deprivation in IM9, RPMI8226, and H929 cells. During glucose deprivation, the glucose transporter gene *GLUT3* is overexpressed in **(6A)** IM9 lymphoblastoid cells compared with those in **(6B)** RPMI8226; **(6C)** H929 myeloma cells. The mRNA level was measured using qRT-PCR. All experiments were carried out in triplicate. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. control group (+ Gluc).

Thus, we conclude that upregulation of *GLUT3* mRNA in response to glucose deficiency is predominantly a property of IM9 lymphoblastoid cells as compared with RPMI8226 and H929 myeloma cells. It was previously shown that glucose deprivation upregulated *GLUT3* mRNA and protein in mouse brains and in primary neuronal cultures from rat embryos [61], in chromaffin cells from bovine adrenal medullae [62], in granulocytes and monocytes [63], in rat brain neurons [64].

Our examination of the effect of glucose deprivation on *GLUT4* mRNA expression levels revealed only an increasing trend in IM9 (Figure 7A), RPM82826 (Figure 7B), and H929 (Figure 7C) cells.

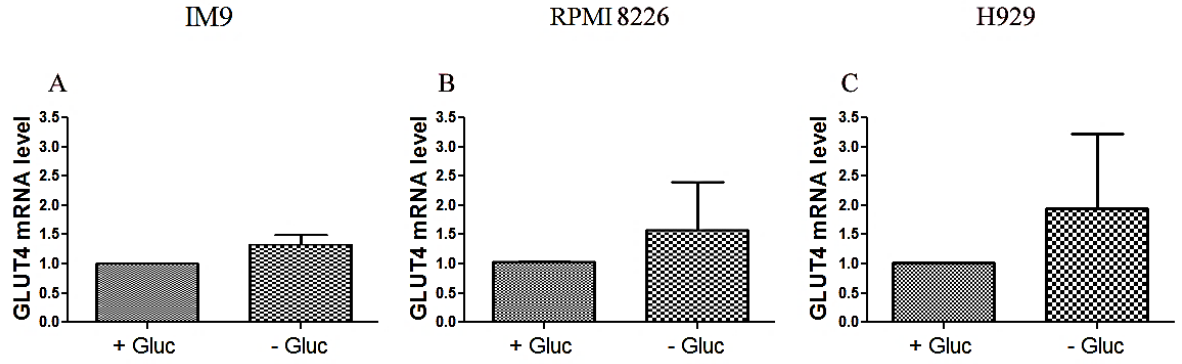

Figure 7. Level of *GLUT4* mRNA expression in response to glucose deprivation in IM9, RPMI8226, and H929 cells. During glucose deprivation, the glucose transporter gene *GLUT4* shows only an increasing trend in **(7A)** IM9 lymphoblastoid cells; **(7B)** RPMI8226; **(7C)** in H929 myeloma cells. The difference in GLUT4 mRNA expression between the control group (+Gluc) and the experimental group (-Gluc) for each cell type was not statistically significant. All experiments were carried out in triplicate. The mRNA level was measured using qRT-PCR.

It is known that activation of B lymphocytes after interaction with the B cell antigen receptor includes a proliferative response and an associated increase in the rate of glucose metabolism. Exposure to anti-IgM caused an increase in proliferation and glucose consumption, as measured by the uptake of the fluorescent glucose analog 2-NBDG. Real-time-PCR analysis of changes in the expression profiles of *GLUT* family genes in differentiating B cells revealed activation of the *GLUT4* gene. And the GLUT4 protein score mirrored the trends observed at the transcriptional level [47]. It has been suggested that myeloma cells may aberrantly use this mechanism to support the malignant phenotype [47].

Moreover, myeloma cells have been shown to exhibit glucose dependence and exhibit overexpression of *GLUT4* mRNA compared to normal B cells [47]. Our data are consistent with those of these authors, and we showed that *GLUT4* gene expression is low in IM9 lymphoblastoid cells (1 ± 0.5) compared to RPMI8226 (4.0 ± 0.43) and H929 (2.9 ± 0.15) myeloma cells (Table 4). Glucose deprivation did not lead to a statistically significant increase in *GLUT4* expression in IM9, RPMI8226, and H929 cells (Figure 7). This is consistent with previous information: no glucose-responsive elements have been found in the *GLUT4* gene, and no information on glucose metabolites mediate the response of *GLUT4* gene expression to glucose availability [65].

It was previously shown that in i3T3-L1 fat cells, glucose deprivation does not affect GLUT4 protein levels [66], and in myocytes in glucose-free or high-glucose medium did not alter GLUT4 protein content [67]. A decrease in plasma glucose may be an important consequence of food restriction. It was shown that the expression level of *GLUT4* mRNA in the heart decreased during fasting and increased during refeeding [68], and food restriction did

not affect *GLUT4* expression in skeletal muscle [69]. A common feature of many tumors is increased glucose catabolism during tumor growth. In Ehrlich ascites tumor cells, *GLUT1* and *GLUT3*, but not *GLUT4*, mRNA levels progressively increased during tumor development [70]. It has been shown that under conditions of hypoglycemia, the level of GLUT4 in human granulocytes increases, while in monocytes, GLUT4 remains unchanged [71]. These studies of *GLUT4* expression during glucose deprivation provide evidence that the *GLUT4* gene is not normally regulated by glucose.

4. Conclusions

The human lymphoblastoid cell line IM9 ($CD138^{+}$, $CD38^{-}$), as well as two human myeloma cell lines RPMI8226 ($CD138^{+}$, $CD38^{++}$) and H929 ($CD138^{+}$, $CD38^{+++}$), were used in the present study. We found that glucose deprivation reduced cell viability and resulted in decreased ATP synthesis in each cell line. Thus, we conclude that lymphoblastoid IM9 and myeloma RPMI8226 and H929 cells depend on glucose availability for energy maintenance.

The rate of glucose-dependent ATP synthesis was higher in RPMI8226 and H929 cells compared to IM9 cells. This may be due to myeloma cells acquiring malignant properties requiring an increased energy supply.

Comparison of basal levels of *GLUT1*, *GLUT2*, *GLUT3*, and *GLUT4* mRNA expression between cell types revealed that IM9 cells express all of these *GLUTs* mRNAs at lower levels compared with those in RPMI8226 and H929 cells. *GLUT1* mRNA shows high levels of expression in RPMI8226 and H929 myeloma cells. This suggests that higher expression of glucose transporters in myeloma cells allows them to increase glucose uptake rate to promote ATP synthesis.

Comparison of basal *GLUT1*, *GLUT2*, *GLUT3*, and *GLUT4* mRNA expression levels within each cell type revealed that *GLUT1* is the major glucose transporter for RPMI8226 and H929 myeloma cells, whereas *GLUT3* is important only for IM9 lymphoblastoid cells. Investigation of the effect of glucose deprivation showed that the glucose transporter *GLUT1* gene is overexpressed in RPMI8226 and H929 cells and is not altered in IM9 cells; the *GLUT3* gene is overexpressed in IM9 cells compared with those in RPMI8226 and H929. This suggests that *GLUT1* is a glucose-sensing gene specific to myeloma cells, and *GLUT3* is a glucose-sensing gene specific to lymphoblastoid cells.

During glucose deprivation, the glucose transporter gene *GLUT2* does not show any cell-specific dependence, is overexpressed in all cell types, and is a glucose-sensing gene. Under these conditions, *GLUT4* gene expression tended to increase, but its change was not statistically significant in all cell types, leading us to conclude that it is not a glucose-sensing gene.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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