Transcription Factor C-Jun Modulates GLUT1 in Glycolysis and Breast Cancer Metastasis

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Abstract

As one of the main isoforms of membranous glucose transporters (GLUT), GLUT1 involves tumorigenesis, metastasis and prognosis in a variety of cancers. However, its role in breast cancer metastasis remains to be elucidated. Here we examined its transcriptional and survival data in patients with breast cancer from several independent databases including the Oncomine, Gene Expression Profiling Interactive Analysis, Gene Expression across Normal and Tumor tissue, UALCAN, cBioPortal, Kaplan-Meier Plotter and PROGgeneV2. We found that its mRNA expression was significantly high in cancer tissues, which was associated with metastasis and poor survival. Transcription factor c-Jun might bind to GLUT1 promoter to downregulate its gene expression or mRNA stability, therefore to suppress glycolysis and metastasis. By qRT-PCR, we verified that GLUT1 was significantly increased in 38 paired human breast cancer samples while JUN was decreased. Furthermore, the protein level of GLUT1 was higher in tumor compared to normal tissues through IHC assay. We also performed GO and KEGG analysis of genes related to GLUT1 and JUN to explore underlying pathways. Our results further support that transcription factor c-Jun regulates GLUT1 to influence glycolysis and breast cancer metastasis.

Background

Breast cancer is the most commonly diagnosed cancer with the highest mortality in females worldwide. Approximately 2.1 million women were diagnosed with breast cancer in 2018, accounting for almost 25% of cancer cases in females [1]. Metastasis is the leading cause of cancer-related death (about 90%) [2, 3]. The prognosis remains poor in spite of multiple advances in surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapies. Thus, it is important to understand the molecular mechanisms underlying the metastatic process.

Altered metabolism increases cell survival and/or proliferation as well as the metastatic potential in various cancers, including the breast cancer. Warburg effect, also known as aerobic glycolysis (though it occurs also under anaerobic conditions in cancers), is a well-known dysregulation of cancer metabolism with increased glucose consumption and lactic acid production [4]. Dysregulated glucose metabolism is a hallmark in human cancer [5–7]. Glucose is the primary energy source for mammalian cells. Cancer cells typically rely on aerobic glycolysis to meet their biosynthetic need for energy, nucleotides, lipids, and amino acids, thus promoting cell survival and proliferation [6]. However, rapidly proliferating cancer cells can maximally elevate glucose uptake via a dramatic increase in expression of membranous glucose transporters (GLUT). There are four main isoforms. GLUT1 overexpresses in most cancers and associates with poor survival [8, 9]. As a primary switch of aerobic glycolysis, GLUT1 is also known as the solute carrier family 2 member 1 protein SLC2A1, involving in tumor survival and metastasis [7, 10–12]. GLUT1 accelerates aerobic glycolysis and provides energy and biomass for the movement of primary tumor cells to distant locations in the body. However, its regulatory mechanism in breast cancer metastasis remains unknown.

In recent years, many mutated or dysregulated transcription factor targets were found in cancers. They are a distinct class of anticancer drug targets that modulate aberrant gene expression, including genes related to carbohydrate metabolism and metastasis [13–15]. Numerous novel approaches could regulate the activity of these important proteins via post-transcriptional modifications (PTMs) of transcription factors, including phosphorylation of serine (Ser) and/or threonine (Thr) or tyrosine (Tyr), and methylation of lysine (Lys) or arginine (Arg). c-Jun encoded by gene JUN is one of the important cancer-related transcription factors and is a key molecule in glucose metabolism and cancer metastasis. However, its relation to GLUT1 in glucose metabolism-mediated metastasis in breast cancer has not been fully investigated. In the current study, we comprehensively analyzed their expression patterns and relation to survival using numerous publicly available databases from various online platforms.

Materials And Methods

Clinical samples

38 paired breast cancer tissue and normal adjacent tissue samples were collected from patients, undergoing surgery in 2018 in the department of breast surgery, Zhongshan Hospital, Fudan University, Shanghai, China. Samples were snap frozen in liquid nitrogen within 60 min and then stored at -80°C until analysis. This study was approved by the Medical Ethics Committee of Fudan University.

Quantitative reverse transcription-polymerase chain reaction analysis

Total RNA was isolated from cell lines and clinical tissues with Trizol reagent (Invitrogen); reverse transcription reactions with ReverTra Ace qPCR RT Kit (TOYOBO), and qRT-PCR with HieffTMqPCR SYBR& Green Master Mix on ABI 7300 Fast Real-Time PCR System (Applied Biosystems), according to the manufacturer's instructions. Relative portions of mRNAs were normalized to β -actin. Fold changes were calculated with the standard $\Delta\Delta\text{Ct}$ method.

Immunohistochemistry

Paraffin-embedded tissue samples were deparaffinized at 65°C for 40 min, cleared in xylene, rehydrated in a graded series of alcohol, and then heated with saline sodium citrate buffer at 95° to 100°C for antigen retrieval. After cooling and two PBS washes, slides were incubated with primary antibody (GLUT1, PA6583, Elabscience) diluted in blocking solution at 4°C overnight, followed by a horseradish peroxidase (HRP)-conjugated secondary antibody incubation at room temperature for 1 h. Slides were then developed with 3,3'-diaminobenzidine (DAB), counterstained with hematoxylin, and dehydrated in graded ethanols. Positive staining density was detected under a light microscope.

Oncomine Analysis

The transcription level of GLUT1 in various types of cancers was analyzed with the Oncomine database (<https://www.oncomine.org/resource/login.html>). The threshold had a p-value less than $1E-4$, with a fold change greater than 2 and gene ranking in the top 10%.

GEPIA Database

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is a newly developed interactive web server for analyzing the RNA sequencing expression data. It includes 9736 tumor and 8587 normal samples from the TCGA and the GTEx projects, with a standard processing pipeline. GEPIA provides customized analysis, such as tumor/normal differential expression, profiling for cancer types or pathological stages, patient survival, similar gene detection, correlation and dimensionality reduction [16].

GENT Database

Tissue-wide gene expression patterns across 72 paired tissues and significant test results by two-sample T test are examined with GENT database (Gene Expression across Normal and Tumor tissue) (<http://gent2.appex.kr/gent2/>). For each tissue, p-value and log2 fold change were calculated.

UALCAN Database

UALCAN (<http://ualcan.path.uab.edu/>) is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data. It is built on PERL-CGI with high quality graphics of javascript and CSS [17].

cBioPortal Web Tool

The breast invasive carcinoma (The Cancer Genome Atlas, Firehose Legacy) dataset was further analyzed by cBioPortal (<https://www.cbioportal.org/>). The genomic profiles of GLUT1 include mutations, putative copy number alterations from genomic identification of significant targets in cancer (GISTIC) and mRNA expression Z scores (microarray).

The Human Protein Atlas

The Human Protein Atlas (<https://www.proteinatlas.org/>) is a Swedish-based program initiated in 2003. It maps all the human proteins in the cell, tissue and organ with integration of various omics technologies,

including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology [18].

Kaplan-Meier Plotter

Kaplan-Meier Plotter contains gene expression and survival data of breast cancer patients (<https://kmplot.com/analysis/index.php?p=service&cancer=breast>) [19]. The best probe set (201249_at) of GLUT1 was used to obtain the plots. Patient samples were split into two groups by auto selection of best cutoff value. The overall survival (OS), recurrence-free survival (RFS), distant metastasis-free survival (DMFS) and post-progression survival (PPS) of patients were analyzed with the hazard ratio (HR) with 95% confidence intervals (CIs) and log rank p value.

PROGgeneV2 Database

PROGgeneV2 database (<http://genomics.jefferson.edu/proggene/>) is for survival analysis on single genes, or on mean expression of a group of user defined genes including 27 types of tissue [20]. The relationship between GLUT1 mRNA expression and survival outcomes (metastasis, relapse and death) was examined.

MethSurv Web Tool

MethSurv web tool (<https://biit.cs.ut.ee/methsurv/>) was used to analyze the relationship between gene methylation and prognosis [21].

bc-GenExMiner Web Tool

Breast Cancer Gene-Expression Miner (bc-GenExMiner) is a new mining module that analyzes breast cancer gene-expression correlation and gene prognosis (<http://bcgenex.centregauducheau.fr/bc-gem/gem-avertissement.php>) [22].

GO and KEGG Analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were explored by R/Bioconductor with a cluster Profiler package.

Results

GLUT1 Transcription Level Increased in Most Types of Cancer

We assessed GLUT1 mRNA expression in cancer and its corresponding normal tissue with four independent cancer data mining databases. 1) we used the Oncomine database. GLUT1 upregulated in almost all cancers, including breast, lung, kidney, pancreatic, bladder, head and neck, colorectal, esophageal, gastric, ovarian cancer and leukemia and lymphoma (Fig 1a). The upregulation in breast cancer was the greatest with 10 up and 1 down datasets. 2) we examined the mRNA expression in 33 types of human cancer with a combined TCGA and GTEx database (Fig 1b). It was significantly high in 16 types and low in 2 types. 3) we used the GPL570 Platform (HG-U133_Plus_2) in the GENT database and found it overexpressed in certain cancers, including breast cancer (Fig 1c). 4) using the UALCAN web tool, we found that the expression was significantly high in most cancers, including the breast (Fig 1d).

GLUT1 mRNA and Protein Increased in Breast Cancer

GLUT1 mRNA level upregulated in 10 out of 11 datasets in breast cancer (Oncomine). In Curtis Breast dataset [23] (Table 1), it increased by 2.317 folds in invasive breast carcinoma, 2.083 folds in invasive ductal breast carcinoma, and 1.615 folds in invasive lobular breast carcinoma (Fig 2a). In The Cancer Genome Atlas dataset (TCGA) (Table 1), it also increased in invasive breast carcinoma (2.251 folds), invasive ductal breast carcinoma (2.557 folds), and invasive lobular breast carcinoma (1.628 folds) compared to their normal counterparts (Fig 2b). Similarly, augmented expression was observed in invasive breast carcinoma compared to normal tissue (1.643 folds) in Gluck Breast dataset [24] (Table 1). Zhao et al. [25] (Table 1) reported an increase in invasive ductal (2.8 folds) and in lobular (2.075 folds) breast carcinomas versus normal samples. The similar results were obtained with GEPIA (Fig 2c) and UALCAN (Fig 2d) web tool. In addition, GLUT1 protein expression upregulated significantly in breast cancer tissues compared to normal tissues (Fig 2e).

Relation of GLUT1 mRNA and Pathological Characteristics

Using the UALCAN web tool (Table 2), we found that GLUT1 significantly upregulated in breast cancer independence of race (Fig S1 a), age (Fig S1 b), nodal metastasis status (Fig S1 d) and menopause status (Figure S1 g). The expression also significantly increased in different stages except stage4 (Fig S1 c), probably because of the small sample size of stage4. GLUT1 increased in almost all major subclasses (with TNBC types) except TNBC-LAR and TNBC-MSL (Fig S1 e). Apart from metaplastic, it increased in all histology subtypes (Fig S1 f).

GLUT1 Associated with Poor Prognosis and Metastasis

GLUT1 expression was negatively correlated with OS (Fig 3a) (GENT database), metastasis free survival and lung metastasis free survival (Fig 3b, Table 3) (PROGgeneV2 database) of patients. High mRNA expression of GLUT1 was predicted to decrease DMFS in all patients (Fig 3c) with different subclasses (Fig 3d). It was also negatively correlated with OS, RFS, DMFS and PPS in lymph node positive breast cancer (Fig 3e). Thus, the Kaplan-Meier curve and log rank test analyses revealed that the increase was significantly associated with recurrence, distant metastasis and poor prognosis.

Mutations, Methylation and Copy Number Alterations Analysis

We further analyzed GLUT1 mutations, methylation and copy number alterations in breast cancer. Mutations were altered in about 2% of sequenced patients in 33 types of cancer (Fig 4a) and about 6% of sequenced patients in breast cancer (Fig 4b) (cBioPortal web tools). Methylation was negatively correlated with mRNA expression with spearman and pearson analyses (Fig 4c). In the CNAs analysis, amplification and gain were predominantly correlated with GLUT1 expression (Fig 4d). These results suggest that the overexpression of GLUT1 could partly result from alterations in methylation and CNAs. Heatmaps of top (1-25) genes with hyper (Fig 4e) and hypo (Fig 4f) methylated promoters were also examined with UALCAN web tools.

Relation of GLUT1 and JUN and their expression in human breast cancer tissues

We investigated the transcription factors that have the capacity to bind to the promoter of GLUT1 in GeneCard and PROMO databases and found that transcription factor c-Jun encoded by gene JUN can bind to the GLUT1 promoter. We predicted the binding site sequences (Table 4). Our results suggest that the binding may regulate the GLUT1 gene expression or mRNA stability.

We analyzed the expression and prognostic value of JUN. The mRNA expression ($p < 1E-12$) and protein phosphorylation level at T239S243 ($P = 0.002$) of JUN significantly decreased in breast cancer compared to normal tissue (UALCAN database) (Fig 5a, Fig 5b). JUN expression varied with tumor stages (Fig 5c), and was positively correlated with OS in all patients (Fig 5d). In addition, the decreased methylation level was associated with increased overall survival ($p = 0.022$, $HR = 1.736$), indicating that the low expression of JUN might be partly due to methylation (Fig 5e). By using the bc-GenExMiner web tool, we found that GLUT1 expression was negatively correlated with JUN ($p = 0.0001$, $r = -0.13$) (Fig 5f).

We further found that GLUT1 was upregulated in 38 paired human breast cancer tissues while JUN was downregulated by qRT-PCR (Fig 5g, Fig 5h). Through IHC, we verified that the protein level of GLUT1 was significantly increased in tumor samples (Fig 5i).

Pathway Analysis of Alterations in GLUT1 and JUN and their Co-expressed Genes

We examined genes that co-expressed with GLUT1 and JUN in breast cancer in Adorno cell line dataset [26] (Fig S2 a, Fig S2 b), and found that the transcription level of GLUT1 and JUN were closely correlated and might contribute to certain signaling pathways. Hence, we performed GO and KEGG analysis of genes associated with GLUT1 and JUN.

With GO analysis, we found both GLUT1 and JUN regulated three aspects, i.e. cellular components [proteinaceous extracellular matrix, extracellular matrix component, basement membrane and collagen trimer (Fig 6a)], biological processes [Extracellular structure organization, extracellular matrix organization and collagen-activated signaling pathway (Fig 6b)], and molecular functions [Transcription coactivator activity, transcription factor activity, RNA polymerase II transcription factor binding and extracellular matrix constituent (Fig 6c)]. These results are known to relate to cancer metastasis.

With KEGG analysis, we found GLUT1 and JUN are associated with seven breast cancer related pathways. Among them, focal adhesion and ECM-receptor interaction were involved in tumorigenesis and metastasis (Fig 6d, Fig S3).

Discussion

GLUT1 plays a role in tumorigenesis, metastasis and prognosis in a variety of cancers [7, 10, 11]. However, its role in breast cancer remains unclear. The present study is the first to use bioinformatics to demonstrate that the increased mRNA expression of GLUT1 was associated with poor prognosis and metastasis of breast cancer, which may be regulated via JUN by binding to GLUT1 promoter.

Dysregulation of carbohydrate metabolism is well-known to be associated with tumorigenesis, metastasis and prognosis [7, 11, 27, 28]. Warburg pathway enzyme (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4) acts as a molecular fulcrum that couples sugar metabolism to transcriptional activation by stimulating SRC-3 to promote tumor metastasis [27]. The inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (a major enzyme in glycolysis) could downregulate cancer cell adhesion molecules in endothelia by suppressing NF- κ B signaling [28]. PFKFB3-blockade can enhance chemotherapy [28]. SMAD4 deficiency increases in matrix metalloprotease 9 enhanced hypoxia-induced GLUT1 expression and aerobic glycolysis, which was responsible for the enhanced migration of colon cancer cells [11]. In oral squamous cell carcinoma, miR-378a-3p represses GLUT1 expression by targeting its 3'UTR and inhibits cell migration and invasion both *in vitro* and *in vivo* [7]. In our study, GLUT1 mRNA expression was much higher in breast cancer than normal tissues. Using UALCAN web tool, we found that the expression of GLUT1 was significantly associated with the clinicopathological characteristics (race, age, metastasis and menopause status). Moreover, in several independent webs, we observed that GLUT1 expression was negatively correlated with OS, metastasis free survival and lung metastasis free survival. In addition, the Kaplan-Meier curve analysis revealed that increased GLUT1 mRNA level was significantly

associated with poor OS, RFS, DMFS and PPS in breast cancer patients with positive lymph node. Thus, inhibition of GLUT1 might suppress breast cancer proliferation and metastasis.

Transcription factor c-Jun is closely associated with metastasis, prognosis and glucose metabolism in many types of cancer. It acts as both tumor suppressor and oncogene. Increased c-Jun expression (by inhibition of ATF2 via RNA interference) leads melanoma to undergo cell apoptosis and inhibits growth and metastasis [29]. Moreover, it binds to the promoter region of human miR-374a and suppress miR-374a in A549 and pc-9 cells, leading to inhibition of cell growth, migration, invasion and metastasis [30]. However, c-Jun can promote tumorigenesis and metastasis in some other types of cancer. It binds and activates human FOXK1 (Forkhead box (FOX) K1) gene promoter, enhancing FOXK1-mediated proliferation and metastasis in gastric cancer [31]. Transcription factors (NFATc, ATF2, and c-Jun) upregulated in lung cancer aggressive cells, increasing angiopoietin-like protein 2 (ANGPTL2) expression, and cell motility and invasion [32]. In gastric cancer, SIRT1 suppresses migration and invasion by downregulating ARHGAP5 through inhibiting c-Jun [33]. Moreover, c-Jun is closely related to glucose metabolism. TGF- β 1b/c-Jun targets G6PC3 of FoxO signaling to promote gluconeogenesis and maintain a high glucose level [34]. cJun also binds to and activates the β 1,3-N-acetyl-glucosaminyltransferase8 (β 3GnT8) promoter and thus upregulates the β 3GnT8 expression, leading to malignancy in gastric cancer [35]. However, mechanisms of controlling the interplay between GLUT1 and c-Jun remain to be elucidated. In our report, the mRNA expression and protein phosphorylation at T239S243 of JUN were significantly downregulated in breast cancer tissues. The low expression of JUN might be partly due to methylation. In addition, the JUN expression and the methylation were correlated with survival. The expressions of GLUT1 and JUN were negatively correlated in the bc-GenExMiner web. The underlying mechanism might be that c-Jun binded to GLUT1 promoter at several sites to suppress its expression.

In GO and KEGG analysis, we found that GLUT1 and JUN regulated proteinaceous extracellular matrix, extracellular structure organization and transcription coactivator activity and modulate focal adhesion and ECM-receptor interaction signaling pathways, which are well-known to be associated with metastasis.

In conclusion, high expression of GLUT1 is significantly associated with poor prognosis and promotes glucose metabolism and tumor metastasis. The GLUT1 effect may be regulated by hypomethylation and JUN expression. Thus, GLUT1 could be a novel target for breast cancer treatment.

Declarations

Funding

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Competing interests

The authors declare no competing interests.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author.

Code availability

Not applicable

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Authors' contributions

Ping Zhou, Yangbai Sun and Ping Zhu designed the study. Ping Zhu performed all analyses with the help from collaborators: Jingjing Lu, Yue Zhou, Yabiao Gao and Shuyi Chen. Jerry Yu helped clarify the language and grammar usage in our manuscript. All authors contributed and were involved in final approval of the paper.

Ethics approval

This study was approved by the Medical Ethics Committee of Fudan University.

Consent to participate

Patients are consent to participate in this study.

Consent for publication

All authors have approved the manuscript and agree with the publication.

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Tables

Due to technical limitations, table 1 to 4 is only available as a download in the Supplemental Files section.

Figures

Fig 1

Figure 1

Transcription Level of GLUT1 in Different Types of Cancer versus their Corresponding Normal Tissue a. The number of datasets with GLUT1 mRNA expression (red: over expression, blue: low expression) from Oncomine database. Parameters: p-value: $1E-4$, fold-change: 2, gene ranking: 10%. Cell color is determined by the best gene rank percentile for the analyses within the cell. b. The mRNA expression from The Cancer Genome Atlas (TCGA) through GEPIA database. Each dot represents a sample. c. Box plot for the mRNA expression pattern in GPL570 Platform (HG-U133_Plus_2) from the GENT database (Gene Expression across Normal and Tumor tissue). Box represents the median, and 25th and 75th percentiles. Dots represent outliers. d. The mRNA expression pattern of GLUT1 in The Cancer Genome Atlas (TCGA) database from UALCAN database. Box represents the median, and 25th and 75th percentiles.

Figure 2

Upregulation of GLUT1 Transcription and Protein Levels in Breast Cancer versus normal tissues a and b. Box plot of the mRNA expression from Curtis Breast dataset and TCGA Breast dataset (Oncomine database). IDBC: Invasive Ductal Breast Carcinoma, ILBC: Invasive Lobular Breast Carcinoma, IBC: Invasive Breast Carcinoma. c. The transcription level in GEPIA database, $p < 0.05$. d. The mRNA expression from UALCAN database, $p < 1E-12$. E. The protein expression from the Human Protein Atlas.

Figure 3

GLUT1 mRNA Level and Survival in Breast Cancer a. Overall Survival (OS) from GENT2 database ($p < 0.001$). b. Metastasis Free Survival (MFS, $p < 0.001$) and Lung Metastasis Free Survival (LMFS, $p = 0.023$) from PROGgeneV2 database. c. and d. Distant Metastasis Free Survival (DMFS) ($p = 0.003$) in all patients and with its subclasses respectively from Kaplan-Meier Plotter. Luminal A: $p = 0.032$, Luminal B: $p = 0.04$, HER2+: $p = 0.018$, Basal-like: $p = 0.013$. e. OS ($p = 0.072$), Recurrence-Free Survival (RFS, $p = 0.016$), DMFS

($p=0.004$) and Post-Progression Survival (PPS, $p=1.4e-05$) in lymph node positive breast cancer from Kaplan-Meier Plotter.

Figure 4

Relation of GLUT1 to Mutations, Methylation and Copy Number Alterations in Breast Cancer a. Mutations in 33 types of cancer. Altered in 195 (2%) of 10967 sequenced patients (10967 total) from TCGA PanCancer Atlas Studies (cBioPortal web tool). b. Mutations in breast cancer. Altered in 65 (6%) of 1108

sequenced patients (1108 total) from TCGA, Firehose Legacy (cBioPortal web tool). c. Methylation level using spearman ($r = -0.46$, $p = 1.21 \times 10^{-12}$) and pearson ($r = -0.43$, $p = 5.53 \times 10^{-11}$) from TCGA, Firehose Legacy (cBioPortal web tool). d. Copy number alterations from TCGA, Firehose Legacy (cBioPortal web tool). SD: Shallow Deletion, D: Diploid, G: Gain, A: Amplification. e. and f. Heatmap of top (1-25) genes with hyper and hypo methylated promoters, respectively (UALCAN database).

Figure 5

Relation of GLUT1 and JUN and their expression in human breast cancer tissues a. and b. The mRNA expression and protein phosphorylation at T239S243 of JUN (UALCAN database). c. Tumor stage, $p=0.000634$ (GEPIA database). d. Overall survival, $p<0.001$ (GENT2 database). e. Relationship between methylation (CpG island at 5'UTR and 1stExon of JUN) and overall survival (MethSurv web tool). f. The correlation of JUN and GLUT1, $p = 0.0001$, $r = -0.13$ (bc-GenExMiner web tool). g. and h. The mRNA expression of GLUT1 and JUN in 38 paired human breast cancer samples by qRT-PCR. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ $****p < 0.0001$. Error bars, mean $\pm$ SD. i. IHC results of GLUT1. Scale bars, 50 μ m.

Fig 6
a

Figure 6

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis (Correlation with GLUT1 and JUN by R/Bioconductor) a. Cellular components. b. Biological processes. c. Molecular functions. d. KEGG pathway.

Supplementary Files

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