



Adipose Tissue-Derived Factors Promote Raji B-Cell Lymphoma Survival during Doxorubicin Treatment

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Obesity is associated with increased risk and poorer outcomes in several cancers, but its effects on lymphoma responses to chemotherapy remain incompletely understood. This study examined whether soluble factors derived from adipose tissue influence the survival of Raji B-cell lymphoma cells during doxorubicin treatment. Raji cells were exposed to adipose tissue-derived medium with or without doxorubicin, and viability was assessed using LIVE/DEAD Fixable Red staining and flow cytometry. Addition of the adipose-derived preparation increased cell viability during doxorubicin exposure, with significant increases observed in the 20-, 30-, and 40- μ L treatment groups relative to doxorubicin alone. Exploratory RT-qPCR analysis suggested treatment-associated changes in TNF- α and GLUT1 expression; however, these measurements were performed in a single experiment and require replication. These findings suggest that soluble adipose-derived factors may partially protect Raji lymphoma cells from doxorubicin-induced cytotoxicity and support further investigation of the relationship between adipose tissue and chemotherapy resistance.

Cancer remains one of the leading causes of mortality worldwide, with chemotherapy serving as a cornerstone of treatment for many malignancies, including non-Hodgkin B cell lymphoma.^{1,2} Among commonly used chemotherapeutic agents, doxorubicin, an anthracycline antibiotic, is widely utilized due to its ability to induce cancer cell death through DNA intercalation, inhibition of topoisomerase II, and generation of reactive oxygen species.^{3,4} Despite its effectiveness, resistance to doxorubicin remains a major clinical challenge, limiting treatment success and contributing to disease relapse. Emerging evidence suggests that the tumor microenvironment plays a critical role in modulating chemotherapy response.⁵ Particularly, adipose tissue has gained attention as an active endocrine organ that can influence cancer progression and therapeutic outcomes. Adipocytes can secrete a wide range of cytokines, chemokines, and adipokines which can impact tumor cell behavior.⁶ These secreted factors are increasingly recognized as key contributors to cancer cell survival, proliferation, and drug resistance.

Adipose tissue is now understood to be metabolically active and highly involved in systemic and local signaling. Adipocytes release signaling molecules such as tumor

necrosis factor- α (TNF- α), which regulate inflammation, metabolism, and cellular survival pathways.^{7,8} These adipocyte-derived factors can alter the tumor microenvironment and promote tumor progression. Adipocytes located near tumors, often referred to as cancer-associated adipocytes, undergo phenotypic changes that enhance their ability to support tumor growth.⁹ These cells exhibit increased secretion of inflammatory mediators and metabolic substrates, such as free fatty acids, which can be utilized by cancer cells for energy production and survival. This metabolic crosstalk is a key mechanism contributing to therapy resistance.

In addition to secreting protective factors, adipocytes can also alter drug availability. Evidence suggests that adipocytes can sequester and metabolize chemotherapeutic agents, effectively reducing the concentration of active drug reaching cancer cells.¹⁰ This phenomenon further contributes to decreased drug efficacy.

Cytokines play a central role in mediating communication between adipocytes and cancer cells. TNF- α , a pro-inflammatory cytokine produced by adipose tissue, has been implicated in cancer progression and treatment resistance.¹¹ While TNF- α can induce apoptosis under certain conditions, it can also activate survival pathways such as NF- κ B signaling, promoting cell proliferation and resistance to chemotherapy.

In addition to inflammatory signaling, metabolic adaptations are critical for can-

cer cell survival. Glucose transporter 1 (GLUT1) is a key regulator of glucose uptake and is often upregulated in cancer cells to support increased metabolic demands.^{12,13} Enhanced GLUT1 expression allows cancer cells to maintain energy production under stress conditions, including exposure to chemotherapy.

While previous studies suggest that adipocytes contribute to chemotherapy resistance, the specific role of adipocyte-derived cytokines in lymphoma cells during doxorubicin treatment remains unclear.¹⁴ In particular, the effects of these cytokines on inflammatory signaling and metabolic adaptation require further investigation. This study aims to examine whether modelled adipocyte-conditioned media influences the survival of lymphoma cells treated with doxorubicin. In addition, the study evaluates changes in gene expression of TNF- α and GLUT1 to assess the impact of adipocyte-derived factors on inflammatory and metabolic pathways.

Understanding how adipocyte-derived cytokines influence cancer cell survival during chemotherapy is important for improving treatment outcomes. As obesity rates continue to rise, the interaction between adipose tissue and cancer cells becomes increasingly relevant in clinical settings. Insights from this study may help explain variability in chemotherapy response and contribute to the development of strategies targeting the tumor microenvironment.

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Materials and Methods

Raji B-Cells Culture

Raji B-cell lymphoma cells were cultured in RPMI medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Cytiva HyClone) and 1% penicillin-streptomycin (Gibco). Cells were maintained in a humidified incubator at 37°C with 5% CO₂. Cell concentration and viability were monitored every two days using a hemocytometer and trypan blue (Gibco) exclusion assay at a 1:1 ratio of cells to trypan blue.

Modelled Adipocyte Conditioned Media Preparation

To model adipocyte conditioned media, approximately 800 mg of porcine adipose tissue was trimmed and homogenized. A total of 3.5 mL of sterile PBS-Tween (0.1%) solution was added to the tissue sample. The mixture was vortexed for 2 minutes and centrifuged at 5000 rpm for 1 minute. Following centrifugation, the upper lipid layer was removed, and the adipocyte-conditioned supernatant was collected. The collected supernatant was filtered twice using a sterile 20 mm filter before use in cell culture experiments.

Cell Seeding Assay

Raji cells were seeded into 24-well plates at different concentrations including 5×10^3 , 1×10^4 , 1×10^5 , 5×10^5 , and 1×10^6 cells/mL. Each condition was prepared

in triplicate. Cells were incubated for 24 hours before viability assessment.

Cell viability was measured using the LIVE/DEAD™ Fixable Red Dye assay (ThermoFisher, L23102). Cells were washed with sterile PBS and stained with 1 µL of LIVE/DEAD™ Fixable Red Dye for 30 minutes at room temperature in the dark. Samples were analyzed using flow cytometry. The optimal seeding density was selected based on the highest percentage of viable cells.

Lethal Dose 50 Determination

Raji cells were seeded at a concentration of 1×10^5 cells/mL in 24-well plates. Cells were treated with different concentrations of doxorubicin hydrochloride (MP Biochemicals, 25316-40-9), including 1, 2, 4, 8, 16, and 20 µg/mL, for 24 hours. Following treatment, cell viability was assessed using the LIVE/DEAD™ Fixable Red Dye assay and flow cytometry. The lethal dose 50 (LD₅₀) concentration was determined from the resulting viability curve.

Treatment with Modelled Adipocyte-Cytokine Conditioned Media and Doxorubicin

Raji cells were cultured with modelled adipocyte-conditioned media at concentrations of 10, 20, 30, and 40 µL. Cells were incubated with modelled adipocyte-conditioned media for 12 hours prior to treatment with the LD₅₀ concentration of doxorubi-

cin. Following doxorubicin addition, cells were incubated for an additional 24 hours. Cell viability was assessed using the LIVE/DEAD™ Fixable Red Dye assay and analyzed by flow cytometry. All treatment conditions were performed in triplicate. Counts of live Raji cells and concentrations of drugs were controlled consistently to Raji cells with doxorubicin in adipocyte-cytokine conditioned media. Two-tailed, unpaired Student's t-tests assuming equal variances were used to determine statistical significance with a threshold of $p \leq 0.05$.

RT-qPCR

Total RNA was extracted from each treatment condition using the Monarch Total RNA Miniprep Kit (New England Biolabs) according to the manufacturer's instructions. RNA concentration and purity were measured using a NanoDrop spectrophotometer. Complementary DNA (cDNA) was synthesized in a 20 µL reaction containing 4 µL of 5X iScript Supermix, 500 ng RNA template, and nuclease-free water. Reverse transcription was performed using the following thermal conditions: priming at 25°C for 5 minutes, reverse transcription at 46°C for 20 minutes, and RT inactivation at 95°C for 1 minute.

Quantitative PCR (qPCR) reactions were prepared in a total volume of 20 µL containing 10 µL SYBR Green qPCR Master Mix, 1 µL forward/reverse primer mixture, 1 µL cDNA template, and 8 µL nu-

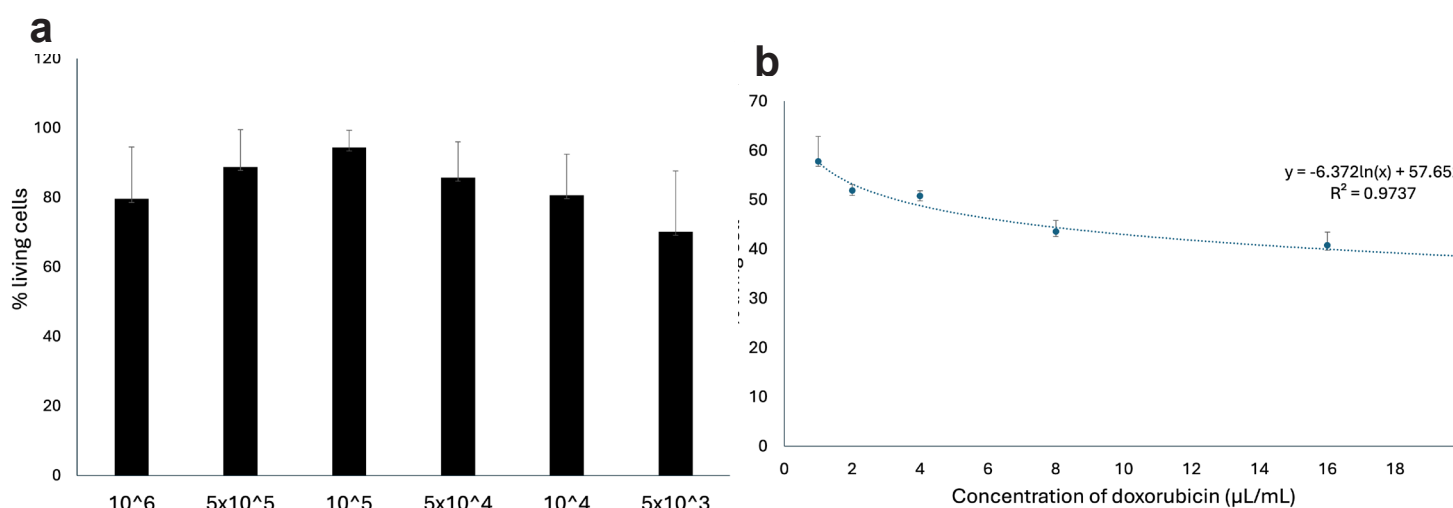


Figure 1 | Optimization of RAJI cell culture conditions and determination of doxorubicin LD₅₀. a, RAJI cell viability at different seeding densities after 24 hours. b, Dose-response curve of RAJI cells treated with increasing concentrations of doxorubicin. Cell viability decreased as doxorubicin concentration increased. The regression curve used to estimate the LD₅₀ concentration is shown. Data are presented as mean ± SD (n=3).

cleave-free water. qPCR amplification was performed on a real-time PCR system under the following cycling conditions: initial denaturation at 95°C for 4 minutes, followed by repeated cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute. Relative gene expression of TNF- α and GLUT1 was analyzed using the $2^{-\Delta\Delta C_t}$ method and normalized to the housekeeping gene, GAPDH.

Results

Cell Seeding Optimization

Different Raji cell densities, including 5×10^3 , 1×10^4 , 1×10^5 , 5×10^5 , and 1×10^6 cells/mL, were evaluated to determine the optimal concentration for viability assays. Among the tested conditions, 1×10^5 cells/mL demonstrated the highest percentage of viable cells after 24 hours and was selected for all subsequent experiments (Fig. 1a).

Determination of Doxorubicin LD₅₀

Treatment with increasing concentrations of doxorubicin resulted in a progressive decrease in Raji cell viability. The dose-response curve demonstrated a nonlinear relationship between drug concentration and survival, where higher concentrations produced greater cytotoxic effects. The fitted dose-response curve yielded an estimated LD₅₀ of approximately 3.3 μ g/mL. (Fig. 1b). This concentration was selected for downstream experiments involving modelled adipocyte-conditioned media because it produces substantial but incomplete cell death, allowing potential protective effects of adipocyte-derived cytokines to be observed.

Effect of Modelled adipocyte-conditioned media on Raji Cell Viability

Raji cells treated with modelled adipocyte-conditioned media alone maintained high viability across all tested concentrations. No substantial decrease in cell survival was observed, indicating that adipocyte-derived cytokines were not directly toxic to the cells (Fig. 2).

In contrast, Raji cells treated with doxorubicin alone demonstrated a marked reduction in viability. However, the addition of modelled adipocyte-conditioned media increased cell survival compared to doxorubicin treatment alone (Fig. 3). This pro-

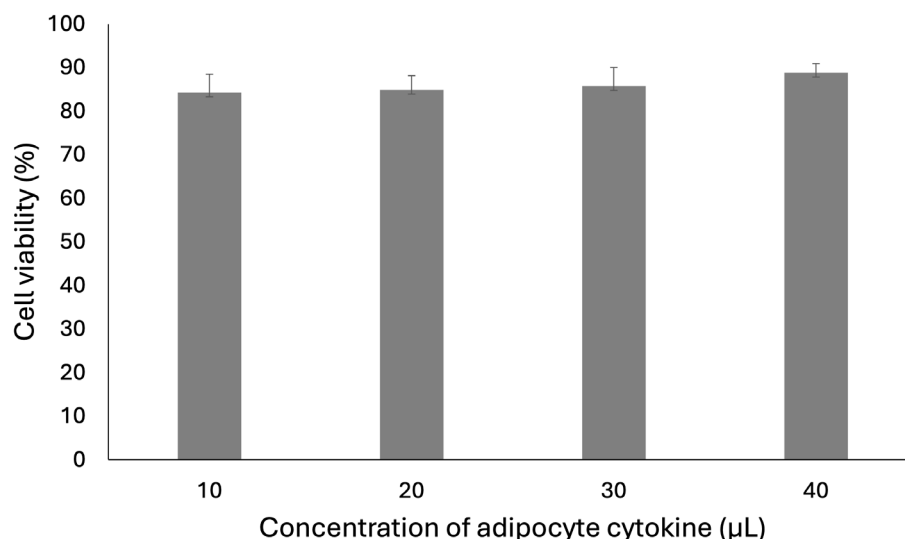


Figure 2 | Effects of adipocyte-conditioned media on Raji cell viability. Raji cells were treated with increasing volumes of adipocyte-conditioned media, and cell viability was measured after 24 hours using the LIVE/DEAD assay. Data are presented as mean $\pm$ SD (n=3).

TECTIVE effect became more pronounced at higher concentrations of modelled adipocyte-conditioned media, suggesting that adipocyte-derived factors may reduce the cytotoxic effects of doxorubicin.

Statistical analysis demonstrated significant increases in viability in several modelled adipocyte-conditioned media treatment groups compared to the doxorubicin-only condition, supporting the observation that adipocyte-derived factors promote lymphoma cell survival during chemotherapy exposure.

Statistical analysis showed significant increases in cell viability in the 20 μ L, 30 μ L, and 40 μ L modelled adipocyte-conditioned media groups compared to doxorubicin treatment alone ($p < 0.05$), while the 10 μ L group was not statistically significant ($p > 0.05$).

TNF- α Gene Expression

Relative TNF- α expression was evaluated using RT-qPCR. Doxorubicin treatment resulted in a strong decrease in TNF- α expression compared to untreated Raji cells. Unexpectedly, Raji cells treated with modelled adipocyte-conditioned media alone also showed reduced TNF- α expression relative to the control condition (Fig. 3a). In the combined modelled adipocyte-conditioned media and doxorubicin treatment group, TNF- α expression remained low. These findings suggest that modelled adipocyte-conditioned media did not increase TNF- α expression in Raji cells under these experimental conditions. However, be-

cause this experiment was performed only once, additional replicates are required to validate these observations.

GLUT1 Gene Expression

GLUT1 expression was analyzed to evaluate potential metabolic changes associated with modelled adipocyte-conditioned media treatment. Raji cells exposed to modelled adipocyte-conditioned media demonstrated increased GLUT1 expression compared to untreated control cells, suggesting enhanced metabolic activity and glucose uptake (Fig. 3b).

Doxorubicin treatment alone caused a substantial reduction in GLUT1 expression. However, cells treated with both modelled adipocyte-conditioned media and doxorubicin exhibited higher GLUT1 expression compared to the doxorubicin-only condition. Although expression levels remained below untreated controls, this partial restoration suggests that adipocyte-derived factors may support metabolic adaptation and survival during chemotherapy stress.

Discussion

This study investigated whether adipocyte-derived factors influence lymphoma cell survival during doxorubicin treatment. The results demonstrated that modelled adipocyte-conditioned media increased Raji cell viability during chemotherapy exposure, supporting the hypothesis that soluble

adipocyte-derived factors may contribute to chemotherapy resistance.

Modelled adipocyte-conditioned media alone did not reduce cell viability, indicating that the adipose-derived preparation was not detectably toxic to Raji cells under the tested conditions. However, when combined with doxorubicin treatment, modelled adipocyte-conditioned media partially protected lymphoma cells from drug-induced cytotoxicity. This protective effect became more pronounced with increasing concentrations of modelled adipocyte-conditioned media, suggesting that the apparent protective effect increased with the volume of preparation added.

The observed increase in GLUT1 expression following modelled adipocyte-conditioned media treatment suggests that adipocyte-derived factors may promote metabolic adaptation in lymphoma cells. GLUT1 is responsible for glucose uptake and is commonly upregulated in cancer cells to support increased metabolic demands. The exploratory GLUT1 result is consistent with a possible effect on metabolic regulation, but replication and functional metabolic assays are needed.

Unexpectedly, TNF- α expression decreased rather than increased following modelled adipocyte-conditioned media treatment. Since adipocytes are known to produce inflammatory cytokines, an increase in TNF- α expression was initially expected.

This result may indicate that adipocyte-derived factors do not directly induce TNF- α transcription in Raji cells, or that Raji cells respond differently to adipocyte signaling under these conditions. Additional experiments are required to further investigate this observation.

Overall, these findings support previous evidence suggesting that adipose tissue contributes to cancer cell survival and chemotherapy resistance through interactions within the tumor microenvironment.

Limitations

Several limitations were present in this study. First, RT-qPCR experiments were performed only once, limiting the ability to interpret these TNF- α and GLUT1 gene expression results as anything more than suggestive and exploratory. Additional biological replicates are required to validate these findings. Second, the sample size for viability experiments was relatively small consisting of three technical wells from one experiment, which may reduce statistical power. Third, modelled adipocyte-conditioned media contains a mixture of cytokines and signaling molecules, making it difficult to identify which specific factors contributed to the observed protective effects. Finally, gene expression was evaluated only at the mRNA level and was not confirmed at the protein level using methods such as Western blotting or ELISA.

Future studies should repeat the RT-qPCR experiments with more biological replicates to confirm the trends observed in TNF- α and GLUT1 expression. Since this study only examined gene expression at the mRNA level, additional experiments such as Western blotting or ELISA could be used to determine whether these changes are also seen at the protein level.

Future Direction

It would also be important to identify which specific adipocyte-derived cytokines are responsible for increasing Raji cell survival during doxorubicin treatment. Testing additional lymphoma cell lines could help determine whether this protective effect is unique to Raji cells or occurs more broadly in lymphoma.

Future work could further investigate survival pathways involved in chemotherapy resistance, such as NF- κ B and PI3K/AKT signaling. In addition, comparing modelled adipocyte-conditioned media from lean and obese adipose tissue may provide more insight into how obesity contributes to lymphoma progression and drug resistance.

Author Biography

Gia Luc Vo is a graduate of the KU Biotechnology program. He completed an internship at the University of Kansas Medical Center, where he conducted research on Autosomal Dominant Polycystic Kidney Disease. Following graduation, he plans to pursue a career in biomedical research and continue studies in cancer biology and biotechnology.

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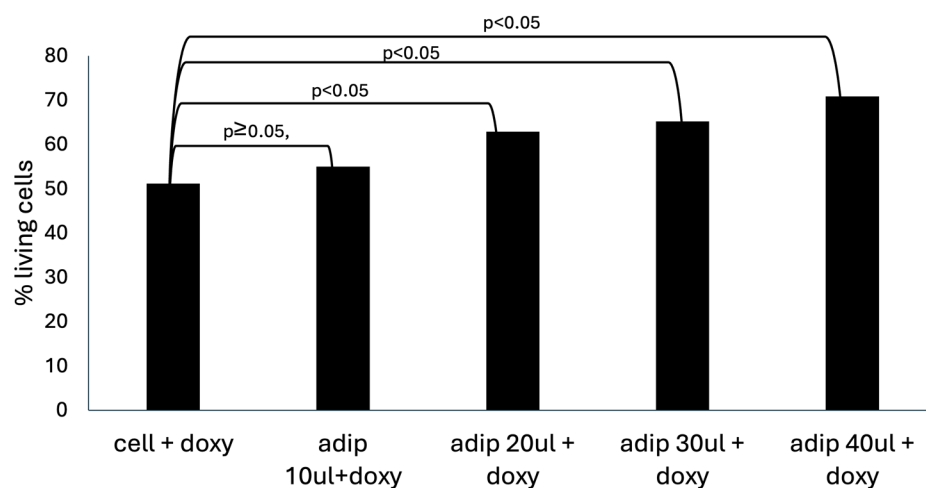


Figure 3 | Effects of adipocyte-conditioned media on RAJI cell viability during doxorubicin treatment. RAJI cells were treated with doxorubicin at the LD50 concentration in the presence of increasing volumes of adipocyte-conditioned media. Cells were cultured with adipocyte-cytokine condition media for 12 hours before add doxorubicin to LD50 concentration. Cell viability was measured after 24 hours using the LIVE/DEAD assay. Statistical significance was determined by Student's t-test compared with the doxorubicin-treated control group. Data are presented as mean $\pm$ SD (n=3).

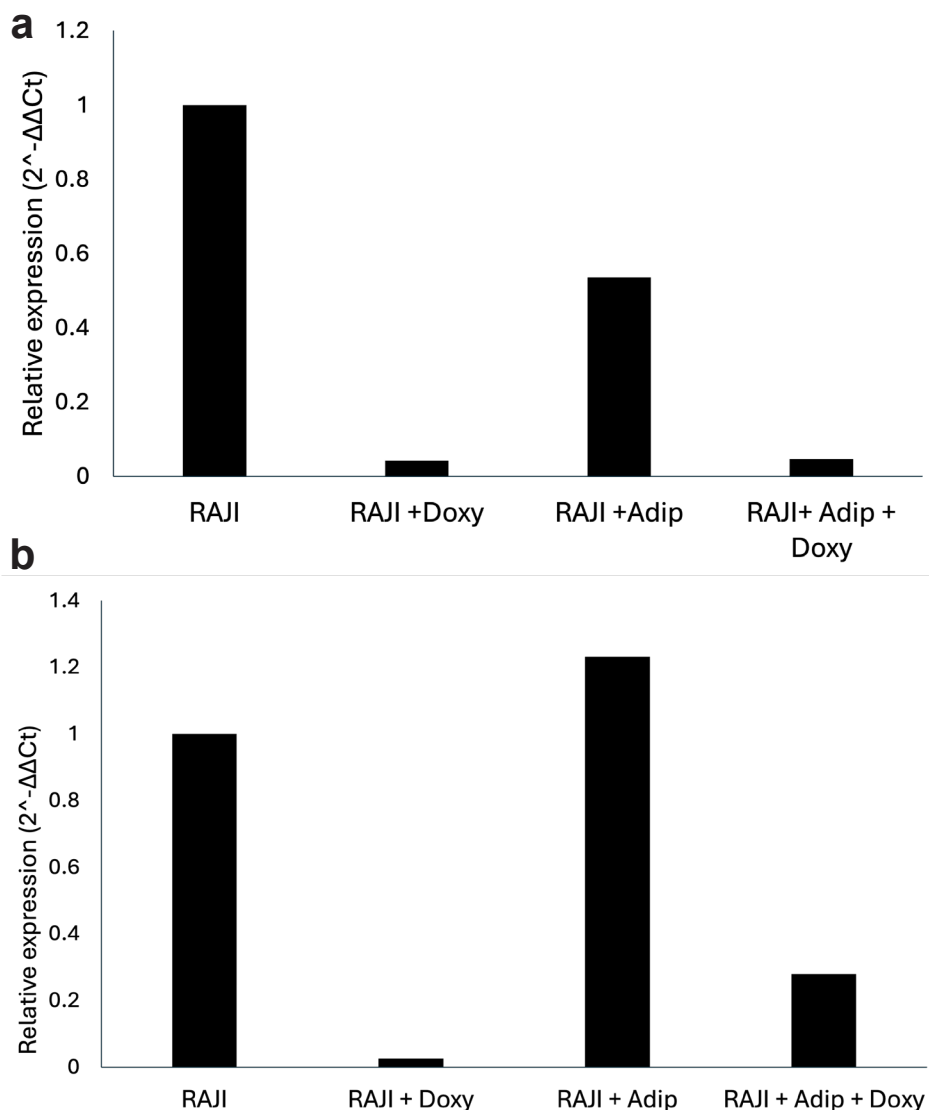


Figure 4. Relative expression of TNF- α and GLUT1 in RAJI cells under different treatment conditions.

a, Relative TNF- α expression in untreated RAJI cells, doxorubicin-treated cells, adipocyte-conditioned media-treated cells, and combined adipocyte-conditioned media plus doxorubicin treatment. **b**, Relative GLUT1 expression under the same treatment conditions. Gene expression levels were normalized to a housekeeping gene and analyzed using the $2^{-\Delta\Delta Ct}$ method.

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