HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

Extracellular matrix detached cancer cells resist oxidative stress by increasing histone demethylase KDM6 activity

Mohamed A. Alfaleh [a,b], Mohammed Razeeth Shait Mohammed [f], Anwar M Hashem [b,c], Turki S Abujamel [b,d], Nabil A Alhakamy [a,e], Mohammad Imran Khan [g,*]

[a] Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[b] Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[c] Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[d] Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[e] Center of Excellence for Drug Research and Pharmaceutical Industries, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[f] Moores Cancer Centre, University of California San Diego, 3855 Health Sciences Dr, La Jolla, CA 92037, USA
[g] Research Center, King Faisal Specialist Hospital and Research Center, P.O. Box 40047, Jeddah 21499, Saudi Arabia

ARTICLE INFO

Keywords:
Anoikis resistant
KDM6A/B
Glycolysis
ROS
Oxidative stress

ABSTRACT

Epithelial cancer cells rely on the extracellular matrix (ECM) attachment in order to spread to other organs. Detachment from the ECM is necessary for these cells to seed in other locations. When the attachment to the ECM is lost, cellular metabolism undergoes a significant shift from oxidative metabolism to glycolysis. Additionally, the cancer cells become more dependent on glutaminolysis to avoid a specific type of cell death known as anoikis, which is associated with ECM detachment.

In our recent study, we observed increased expression of H3K27me3 demethylases, specifically KDM6A/B, in cancer cells that were resistant to anoikis. Since KDM6A/B is known to regulate cellular metabolism, we investigated the effects of suppressing KDM6A/B with GSK-J4 on the metabolic processes in these anoikis-resistant cancer cells.

Our results from untargeted metabolomics revealed a profound impact of KDM6A/B inhibition on various metabolic pathways, including glycolysis, methyl histidine, spermine, and glutamate metabolism. Inhibition of KDM6A/B led to elevated reactive oxygen species (ROS) levels and depolarization of mitochondria, while reducing the levels of glutathione, an important antioxidant, by diminishing the intermediates of the glutamate pathway. Glutamate is crucial for maintaining a pool of reduced glutathione.

Furthermore, we discovered that KDM6A/B regulates the key glycolytic genes expression like hexokinase, lactate dehydrogenase, and GLUT-1, which are essential for sustaining glycolysis in anoikis-resistant cancer cells.

Overall, our findings demonstrated the critical role of KDM6A/B in maintaining glycolysis, glutamate metabolism, and glutathione levels. Inhibition of KDM6A/B disrupts these metabolic processes, leading to increased ROS levels and triggering cell death in anoikis-resistant cancer cells.

1. Introduction

Cancer poses a significant threat to human life globally, with approximately Death from metastasis accounts for 90 % of all cases of cancer. Understanding the mechanisms underlying the establishment of metastasis is of utmost importance (Endo et al., 2020). When cancer cells split off from the primary tumor and start settling in at new locations, this is known as the metastatic stage. However, the metastasis process is hindered by a cell death known as anoikis, which is cause by loss of the extracellular matrix (ECM) (Guadamillas et al., 2011).

The resistance to anoikis involves a complex network of events, including alterations in glucose metabolism, maintenance of redox homeostasis, and production of adenosine triphosphate (ATP), which are critical property of cellular metabolism enabling ECM-independent

Peer review under responsibility of King Saud University.
* Corresponding author at: Research Center, King Faisal Specialist Hospital and Research Center, P.O. Box 40047, Jeddah 21499, Saudi Arabia.
E-mail addresses: maalfaleh@kau.edu.sa (M.A. Alfaleh), mshaitmohammed@ucsd.edu (M. Razeeth Shait Mohammed), amhashem@kau.edu.sa (A.M. Hashem), tabujamel@kau.edu.sa (T.S. Abujamel), nalhakamy@kau.edu.sa (N.A. Alhakamy), mikhan@kfshrc.edu.sa (M. Imran Khan).

https://doi.org/10.1016/j.sjbs.2023.103871
Received 4 August 2023; Received in revised form 16 September 2023; Accepted 6 November 2023
Available online 9 November 2023

cancer cells survival (Hawk and Schafer 2018). As compare to normal cells, cancer cells display a distinct metabolic characteristic known as the Warburg effect, wherein they rely on glycolysis for energy acquisition. Research on the Warburg effect in the context of cancer has been considerable (Ward and Thompson 2012). However, whether or not cancer cells lacking an ECM rely on the Warburg effect for survival is not well understood.

Glutamate, the most abundant amino acid in the blood, plays a crucial function in metabolism as a precursor for metabolic intermediates via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) (Cairns et al., 2011). Recent research has highlighted the significance of glutamine metabolism in the development and cancer cells survival, supporting bioenergetics and redox balance (Zhang et al., 2017). Additionally, matrix-detached cancer cells often experience hypoxia, which promotes glycolysis, facilitating cell proliferation and viability (Shait Mohammed et al., 2021).

It is well established that hypoxia can influence epigenetic modifications. The hypoxia-inducible factor (HIF)-α subunits are influenced not only by oxygen levels but also by reactive oxygen species (ROS). Mitochondria, essential organelles involved in various cellular processes, including ATP production, cell survival, and cell death, are a significant source of ROS (Labuschagne et al., 2019). HIF-1α, a hypoxic transcription factor, modulates chromatin in various ways, mainly by regulating the levels of expression of many JmjC-Jumonji-domain histone demethylases (KDMs). Our recent studies (Shait Mohammed et al., 2022) have demonstrated the epigenetic regulation of HIF-1α by KDM6A/B. For better understanding of the contributions of KDM6A/B to metabolic regulation in ECM-detached cancer cells, we conducted an investigation using two cell lines in an ECM-detached model, focusing on the regulation of glycolysis, mitochondrial membrane potential, ROS production, and expression of glycolytic target genes. Our study provides the first evidence that inhibiting KDM6A/B affects the metabolic profile, transcription of glycolytic genes, ROS levels, and mitochondrial membrane potential in ECM-detached cancer cells.

2. Materials and methods

2.1. Cell cultures and treatment

In this study, we used ATCC (United States) HCT116 and 22Rv1 cells. All cell lines were grown in 10 % foetal bovine serum (FBS; Gibco one-shot, Brazil), penicillin (50 U/mL) and streptomycin (50 mg/mL) supplemented DMEM (Gibco, Invitrogen) at 37 °C and 5 % CO2.. For ECM detachment experiments, Poly-HEMA(P3932-Sigma) 8 mg/mL in 95 % ethanol was used to coat 6 well tissue culture plates, which were subsequently incubated at 37 °C until dry for maximum period of 5 h. Media was changed every two days until the cells reached 70—90 % confluence. The1X10^6 cells were grown in a poly-HEMA coated plate at 5 % CO2 and 37 °C. The ECM detached cells were treated for sixe days with GSK J4 (Abcam-144396, Cambridge, MA USA) at varying doses we used GSK-J4 5 μM for 22Rv1 and 10 μM for HCT116 to treat the ECM detached cells (Shait Mohammed et al., 2022).

2.2. Extraction of metabolites

Metabolites were isolated from ECM detached and attached cells and followed with GSK J4 KDM6A/B inhibitor treatment. ECM cells were lysed within 30 s using a tissue homogenizer with a 2:1:1 v/v of ice-cold methanol, acetonitrile, and water mixture. Incubated at − 20 ∘C for 60 min and for 15 min at 4 degrees Celsius, 13,000 rpm they were spin. Samples were analyzed in LC-MS/MS.. (Alzahrani et al., 2021) (Timmerman et al., 2013).

2.3. Mass spectrometry

The samples have been analysed using a linear ion trap mass spectrometer (LC-MS/MS LTQ XLTM; Thermo Fisher Scientific). Parameters for MSn, with complete scanning mode covering 100–1000 *m/z*. For run 40, arbitrary units were established as flow rate, and Helium was employed as the buffer gas while Nitrogen was used as the sheath gas. We used a spray voltage of 3.0 kV and a capillary voltage of 4.0 V. The capillary temperature was fixed at 270C.

2.4. Data analysis

The raw data is available in the form of a file that was manipulated in the freely available XCMS online database. Peaks have been cross-referenced with the Human Metabolome Database to identify human metabolites. Metaboanalyst was used for statics and pathway analysis.

2.5. Real-time qPCR

A Reverse Transcription kit (applied biosystems) was used to transcribe RNA isolated from all cell lines after diverse experimental settings. Using gene-specific primers (Table-1), cDNA (1–100 ng) was amplified three times. The fold change of each mRNA was determined using CT data from the instrument's software. The difference between the CT value of the housekeeping gene and the mRNA of interest was used to determine CT. The difference between the control and experimental CT values was then used to determine CT for each mRNA. The formula for determining the fold change was $2 - \Delta\Delta CT$ (Livak KJ, Schmittgen TD, et al., 2001).

2.6. Determination of ATP content

To assess the level of ATP intracellularly, the ATP determination kit (Invitrogen-A22066, USA) was used. The equal protein concentration of protein was loaded in 96 well plates and followed as kit protocol. Via microplate luminometer Relative luminescence units have been measured (SpectraMax i3). Protein concentrations were used as a standard to normalize all data..

2.7. ROS assays

The concentration of ROS in living cells was determined using the CELLROX (Invitrogen). The ROS assay was carried out by cells grown as monolayer and ECM detached condition and treated with GSK-J4. In culture medium Cells were grown with 500 nM CELLROX for 60 min plate at 37 °C and 5 % CO2 and Immediately via flow cytometry analyze the samples.

2.8. Mitochondria membrane potential and cell viability assays

Briefly, Adherent or poly-HEMA-coated 6-well plates were used to seed 1×10^6 cells into each well, and after GSK-J4 treatment, Solution Guava Mito Potential Kit was added as per manufacture protocol.After incubation of one hour in a 37 °C and 5 % CO2 and via flow cytometry immediately the samples were analyze.

2.9. Statistical analysis

We used GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA) to analyse the data and find statistically significant differences between the control and GSK-J4 treated groups. $P > 0.05$ was chosen as the significance criterion for the tests.

3. Results

3.1. KDM6A/B inhibition alters global metabolic landscape of anoikis resistant cancer cells

In order to gain insights into the intracellular metabolism of attached

cells, matrix-detached cells (representing anoikis resistance), and matrix-detached cells treated with GSK-J4, we performed LC-MS/MS analysis of intracellular metabolites. We obtained high-quality spectra from three replicates of HCT116 and 22Rv1 cell lines. The spectral profiles within each cell line were consistent, indicating reproducibility of the metabolic profiles of individual replicates. Supplementary Fig. 1A displays the LC-MS/MS spectral separation of the cellular metabolic extracts, and a correlation heat map is also provided.

Metabolomic analysis clearly distinguished the different cell groups, including adherent cells, matrix-detached cells, and GSK-J4 treated matrix-detached cells, as shown by the PLS-DA score plot and VIP scores (Fig. 1A, B). The PLS-DA analysis demonstrated clusters of metabolites with the highest VIP scores (FDR correction $q < 0.05$ and $p < 0.05$) (Table 1; Supplementary Table 1). A metabolite heat map was generated with an FDR-corrected q-value < 0.05, highlighting key differences between adherent cells, matrix-detached cells, and GSK-J4 treated matrix-detached cells, as depicted by Ward clustering (Fig. 1C).

To gain further insights into the metabolic pathways involved, we performed enrichment pathway analysis by mapping the differentially regulated metabolites to the KEGG database using MetaboAnalyst 4.0. The top 25 enriched pathways are displayed in Fig. 1D and E, with a significant p-value < 0.05. These enriched pathways encompass energy metabolism (like glycolysis, gluconeogenesis, and the TCA cycle), amino acid metabolism (including tyrosine metabolism, serine and glycine metabolism), and purine metabolism. These pathways are crucial for cell proliferation, ATP production, and the biosynthesis of nucleotides, fatty acids, and lipids. Perturbations in metabolites implicated in these pathways were observed.

Supplementary Fig. 2 provides the mRNA expression levels of KDM6A/B in HCT116 and 22Rv1 cell lines.

3.2. KDM6A/B inhibition reduces glycolytic metabolites in anoikis resistant cancer cells

To investigate the metabolic adaptations promoting survival of cancer cell during ECM detachment, we examined metabolic changes in cells cultured in different conditions. Specifically, we compared cells grown as monolayers (ECM attached) to cells grown on ultralow attachment plates that prevent attachment to the extracellular matrix, forcing cells to grow in suspension (detached condition). We conducted these experiments using HCT116 and 22Rv1 cancer cell lines and assessed glycolysis and TCA cycle intermediates using LC-MS.

To assess the glycolytic status of ECM-detached cells and ECM-detached cells treated with a KDM6 inhibitor, we examined glucose metabolism by analyzing glycolysis and TCA cycle intermediate metabolites. Our findings revealed an increase in phosphoenol pyruvate levels and LDH activity in detached cells, indicating a more glycolytic phenotype (Fig. 2A, B). However, when KDM6A/B was inhibited, these glycolytic characteristics were reduced, suggesting a role of KDM6A/B in promoting the glycolytic phenotype during ECM detachment.

To validate these observations, we measured intracellular ATP levels. The results showed that detachment from the ECM led to a decrease in ATP levels, further confirming the glycolytic nature of detached cells (Fig. 2C). Conversely, KDM6A/B inhibition resulted in increased intracellular ATP levels, indicating that KDM6B-inhibited detached cells exhibit an oxidative phosphorylation phenotype.

These findings collectively indicate that ECM-detached cells display a glycolytic phenotype, which is attenuated by KDM6A/B inhibition. Furthermore, KDM6A/B inhibition promotes an oxidative phosphorylation phenotype in detached cells, as supported by increased intracellular ATP levels.

3.3. KDM6A/B epigenetically regulate glycolysis in anoikis resistant cancer cells

The observed metabolomic changes prompted us to investigate the transcriptional perturbations associated with KDM6A/B-H3K27me3 demethylase inhibitor treatment and their impact on detached cell glycolytic transcripts. The transcriptional changes aligned with the metabolomic findings, revealing raised glucose transporters (GLUT3 and GLUT1) expression and key enzymes involved in glycolysis, like hexokinase 2 (HK-2), phosphofructokinase (PFKL), phosphoglycerate mutase 1 (PGAM1), lactate dehydrogenase (LDHA) and enolase 2 (ENO-2), indicating an elevation in glycolytic activity. Conversely, elevated levels of pyruvate dehydrogenase kinase 1 (PDK1) suggested a reduction in the conversion of glycolytic metabolic intermediates into the TCA cycle

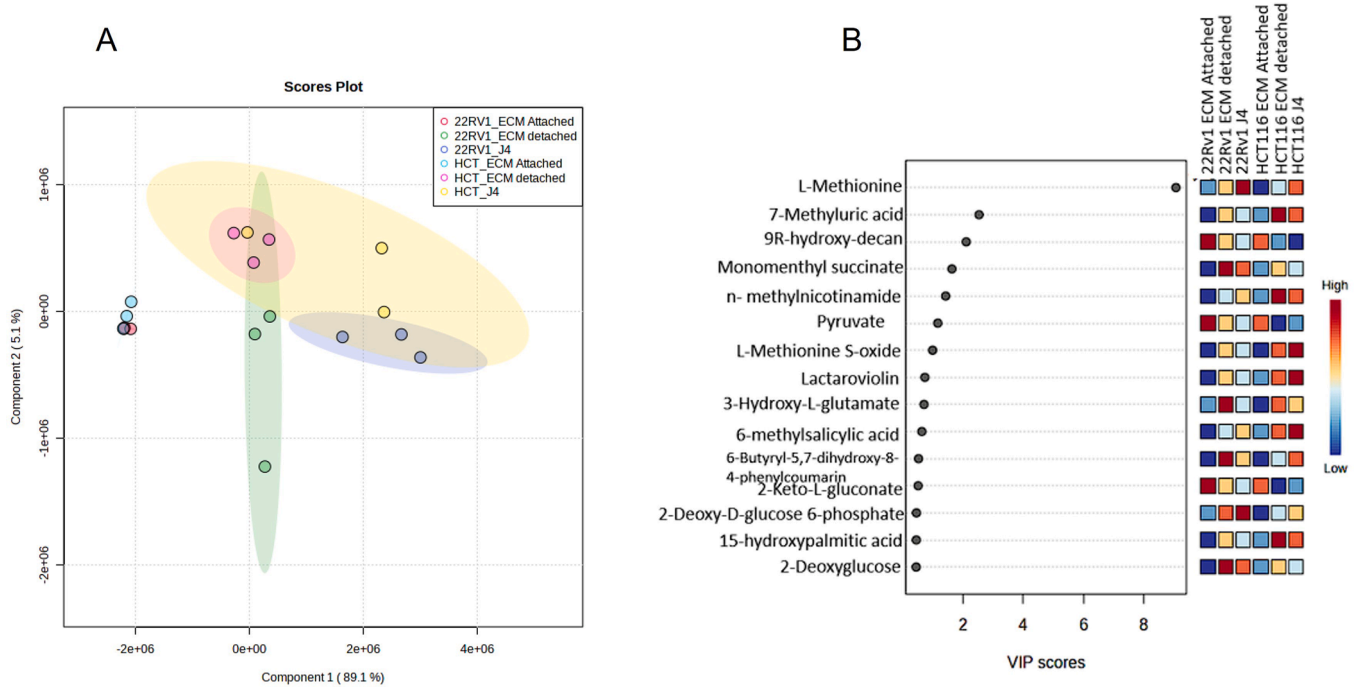

Fig. 1.

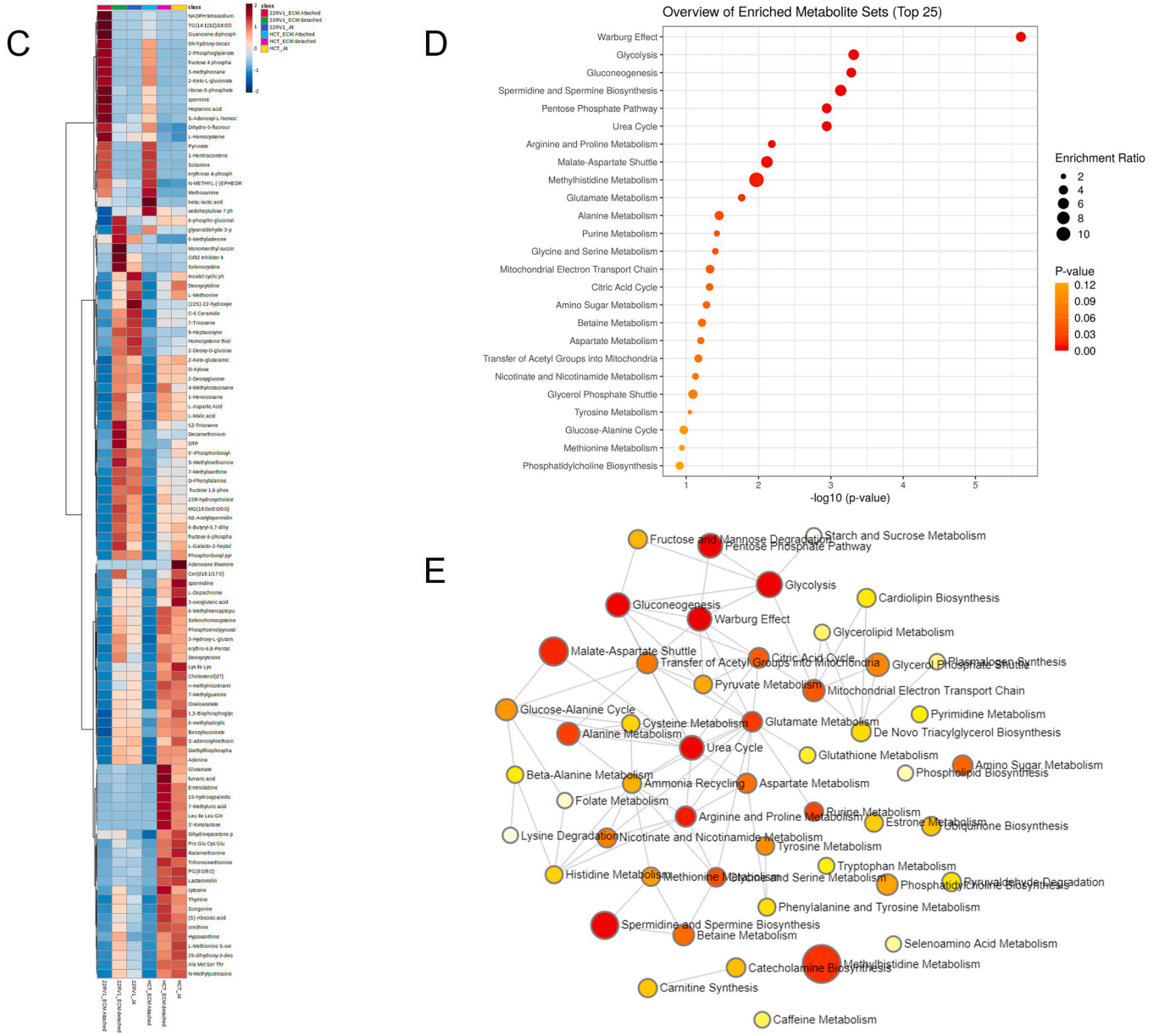

Fig. 1. (*continued*).

Table 1
Primers list.

	Forward Primer	Reverse
GLUT1	CATCCCATGGTTCATCGTGGCTGAACT	GAAGTAGGTGAAGATGAAGAACAGAAC
GLUT4	TTTTGAGATTGGCCCTGGCCCCAT	C TCAGGTACTCTTAAGAAGGTGAAG
HK2	GCCATCCTGCAACACTTAGGGCTTGAG	GTGAGGATGTAGCTTGTAGAGGGTCCC
PFKL	GGAGAAGCTGCGCGAGGTTTAC	ATTGTGCCAGCATCTTCAGCATGAG
ALDOA	AGGCCATGCTTGCACTCAGAAGT	AGGGCCCAGGGCTTCAGCAGG
GAPDH	TTCCGTGTCCCCACTGCCAACGT	CAAAGGTGGAGGAGTGGGTGTCGC
PGK1	ATGTCGCTTTCTAACAAGCTGA	GCGGAGGTTCTCCAGCA
PGAM1	GGAAACGTGTACTGATTGCAGCCC	TTCCATGGCTTTGCGCACCGTCT
ENO1	GACTTGGCTGGCAACTCTG	GGTCATCGGGAGACTTGAA
ENO2	TCATGGTGAGTCATCGCTCAGGAG	ATGTCCGGCAAAGCGAGCTTCATC
PKM2	GCCCGTGAGGCAGAGGCTGC	TGGTGAGGACGATTATGGCCC
LDHA	ATGGCAACTCTAAAGGATCA	GCAACTTGCAGTTCGGGC
GLUT1-Chip	TCATGTCCTTAATGTCCTATCATG	CCCGTTTGTGAGCCAAGCCTTGCT
HK-2-chip	AGGAAGAGCAAAGACCCTTGGGTG	GCCACTCACCCTCACAGCCAGTC
PFKL-1-chip	TGGGATTACAGGCGCACACCACC	AACACAGGCTGGGCGCAGTGGCT
LDHA-1-chip	GATCAGCCTGACCAACATGGTGAAA	TGAAACGGAGTCGCTCTGTCGCC

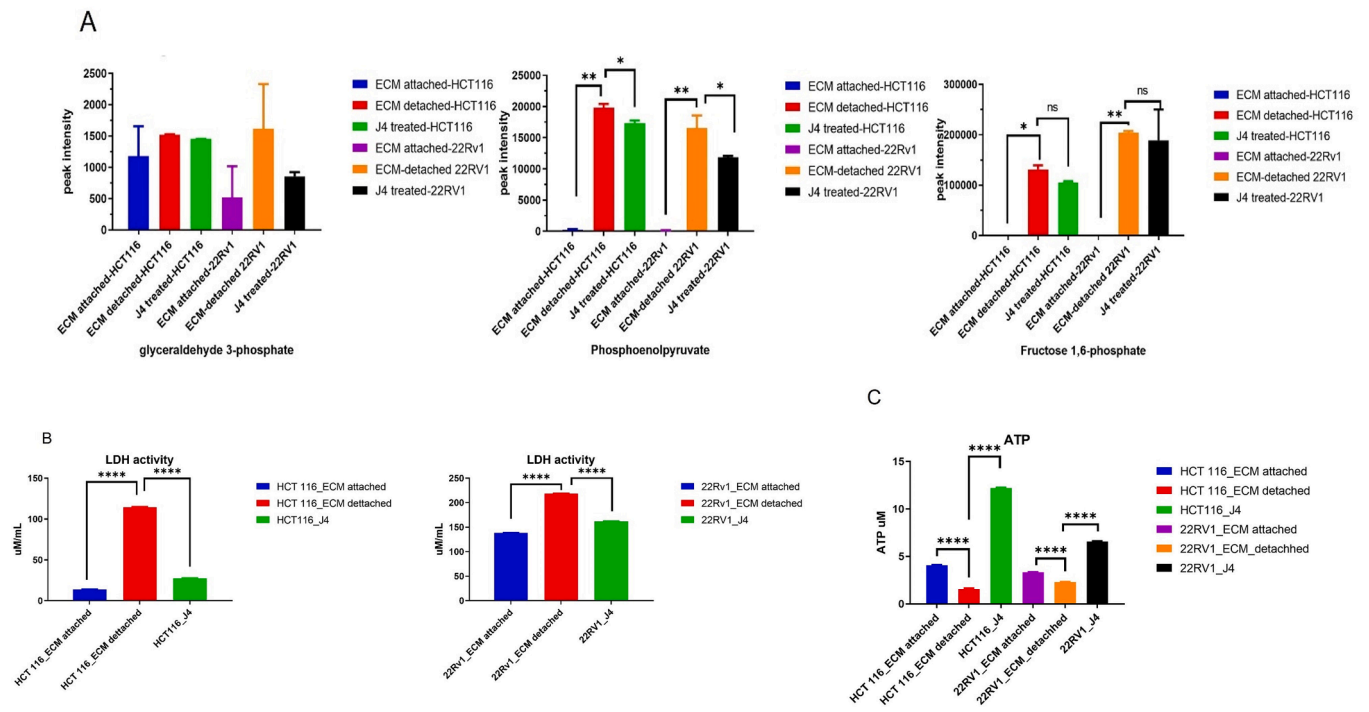

Fig. 2.

(Fig. 3A). Importantly, KDM6A/B inhibition resulted in a significant decrease in glycolytic genes expression.

To investigate whether KDM6A/B transcriptionally regulates the expression of glycolytic genes, we examined putative KDM6A/B binding sites within the promoter regions of these genes, spanning approximately 3 kb (Buchheit et al., 2014). Results of CHIP assay shows that KDM6A/B was attracted to the areas where the binding sites are and enhanced the promoter-reporter activity of HK-2, LDHA, GLUT-1, and PFKL. However, KDM6A/B inhibition with GSK-J4 treatment diminished the binding of these sites and altered the promoter-reporter activity (Fig. 3B). These findings suggest that KDM6A/B promotes the glycolytic genes transcription via promoter sites attachments.

3.4. *KDM6A/B inhibition modifies glutamine metabolism and reduces cellular GSH level in anoikis resistant cancer cells*

Glutamine metabolism intermediates play a crucial role in supporting cellular antioxidant defense mechanisms. Glutamate-glutamine conversion contributes to the biosynthesis of glutathione (GSH) (Li et al., 2018). Cancer cells surviving in ECM detachment exhibit significant alterations in the reductive carboxylation of glutamine metabolism within the mitochondria, which helps limit mitochondrial reactive oxygen species (ROS) production. To assess the impact of KDM6A/B inhibition on glutamine metabolism, we examined the levels of glutamine and other TCA cycle intermediates in ECM detached cells and ECM detached cells treated with a KDM6 inhibitor. The results demonstrated an raise in glutamate, glutamine and α-ketoglutarate in detached cells, which was reduced upon KDM6A/B inhibition (Fig. 4A). This suggests that ECM detached cells experience a decrease in mitochondrial capacity, leading to an increased reliance on glycolysis maintained via carboxylation of glutamine to malate conversion (Fig. 4B). Glutamine metabolism in detached cells plays a significant role in repressing oxidative stress mechanisms through GSH and superoxide dismutase (SOD) (Fig. 4C and D). Supporting this hypothesis, measurements of GSH and SOD activity revealed increased antioxidant levels in detached cells, which were significantly reduced upon KDM6A/B inhibition (Fig. 4C and D).

To further investigate the relationship between KDM6A/B inhibition and the regulation of redox mechanisms, We looked at how redox-maintenance enzymes' genes were expressed. The results showed increased expression of glutaminase (GLS), glutaminase-2 (GLS-2), and glutamine synthetase (GS) in ECM detached cells, indicating an enhancement in antioxidant activity (Fig. 3D). Importantly, KDM6A/B inhibition significantly decreased the genes expression related with the regulation of redox mechanisms. These changes in gene transcript levels correlated with the observed alterations in metabolomics.

To explore whether KDM6B transcriptionally regulates redox gene expression, we conducted CHIP assays using primers targeting the promoter regions of the respective genes. The CHIP assay results revealed that KDM6B was recruited to the regions containing the binding sites and enhanced the promoter-reporter activity of GLS2 and GLS1. However, KDM6B inhibition with GSK-J4 treatment reduced the binding of these sites and altered the promoter-reporter activity (Fig. 4E). These findings suggest that KDM6B promotes gene transcription by binding to the genes promoter sites involved in the glutamine pathway.

3.5. *KDM6A/B inhibition increases intracellular ROS and mitochondrial depolarization in anoikis resistant cancer cells*

Moreover, ECM detached cells treated with a KDM6A/B inhibitor exhibited a notable raise in levels of reactive oxygen species (ROS) (Fig. 5A) and a noticeable decrease in the ROS scavenger superoxide dismutase, as indicated by a reduced glutathione (GSH) ratio (Fig. 4C and D), contrast to untreated detached cells. This elevation in ROS was accompanied by raise in cell death in KDM6A/B inhibited detached cells (Fig. 5B). These findings highlight the involvement of KDM6B histone demethylase in the metabolic alterations associated with oxidative stress in detached cells.

To understand the mechanism underlying the ROS burst in KDM6A/B inhibition, we focused on mitochondria, which are organelles responsible for producing reactive oxygen species (mROS) and significant amounts of ATP. Mitochondria possess a regulatory mechanism that can prevent excessive ROS production, and mitochondrial depolarization triggers a rapid increase in ROS generation. Supporting this

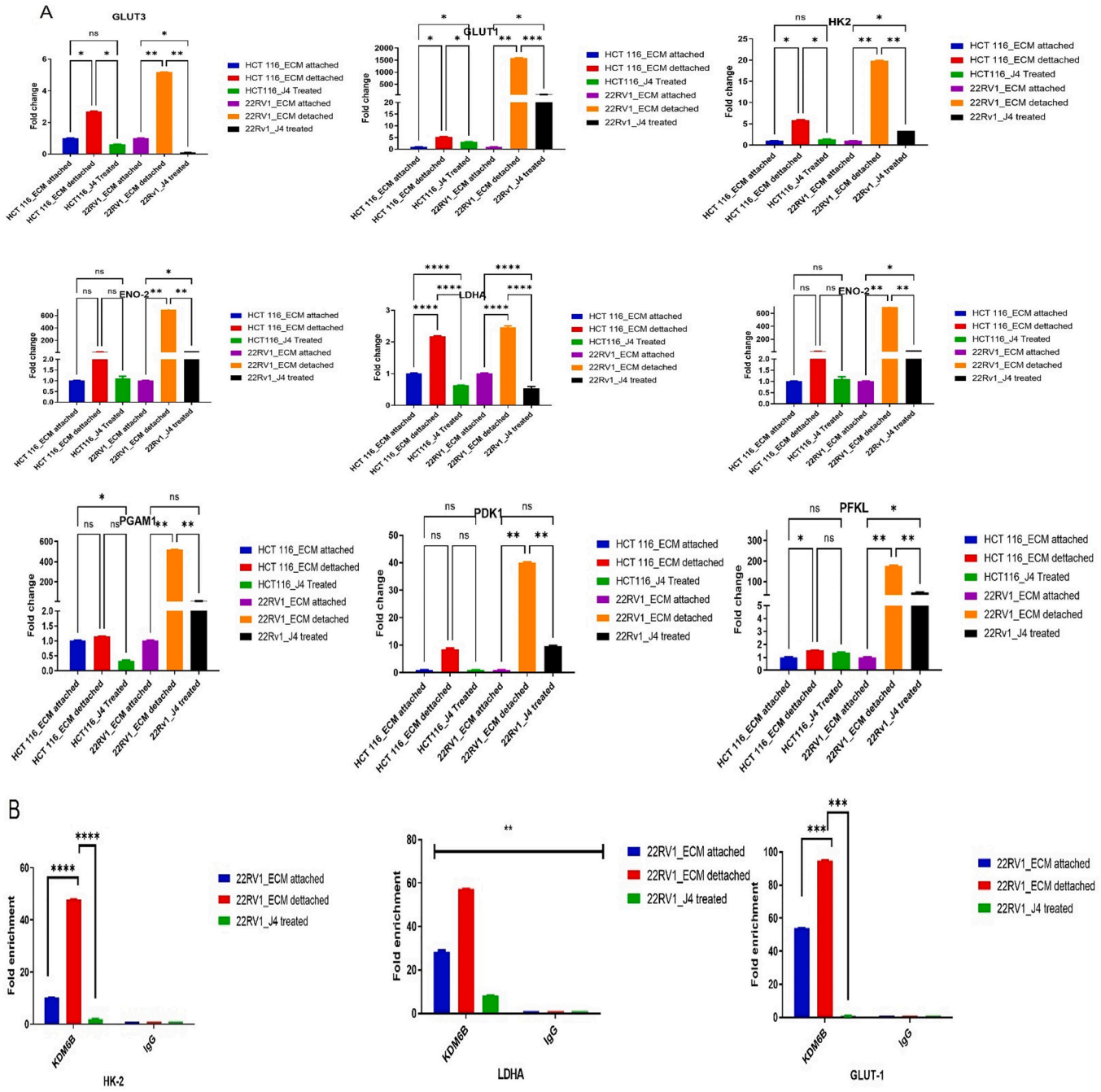

Fig. 3.

hypothesis, we observed a significant increase in mitochondrial depolarization in response to KDM6A/B inhibition, leading to oxidative stress (Fig. 5C). Consequently, KDM6A/B inhibition resulted in enhanced ROS production and induced apoptosis. To further investigate this observation, we treated ECM detached cells with both the KDM6A/B inhibitor and mitoTEMPO, a mitochondria-targeted antioxidant. Remarkably, the ECM detached cells treated with the KDM6A/B inhibitor and mitoTEMPO exhibited reduced ROS levels and an increase in the population of healthy cells (Fig. 5D and E).

4. Discussion

The metastatic stage of cancer involves the detachment of tumor cells from the primary site and their attachment to secondary sites. Successful metastasis requires the ability of cancer cells to adapt to the stress of extracellular matrix (ECM) detachment and overcome the challenges posed by this process (Cantor and Sabatini 2012). It has been reported that regulation of reactive oxygen species (ROS) and cell clustering can promote cancer metastasis. In this study, we provide insights into the epigenetic regulatory mechanisms involved in the metabolic adaptations of detached cells.

Building upon our previous research showing the significance of histone demethylases KDM6A/B in stemness and hypoxia in detached cells (Shait Mohammed et al., 2022), we expand our understanding of KDM6A/B as critical regulators of metabolic phenotypes in ECM detachment cells (Khan et al., 2021). We demonstrated that this epigenetic regulation of metabolic adaptation plays a crucial role in promoting cell survival. We show that inhibition of KDM6A/B in ECM detachment affects mitochondrial metabolism significantly, resulting in more reactive oxygen species being produced. The survival of Cell relies

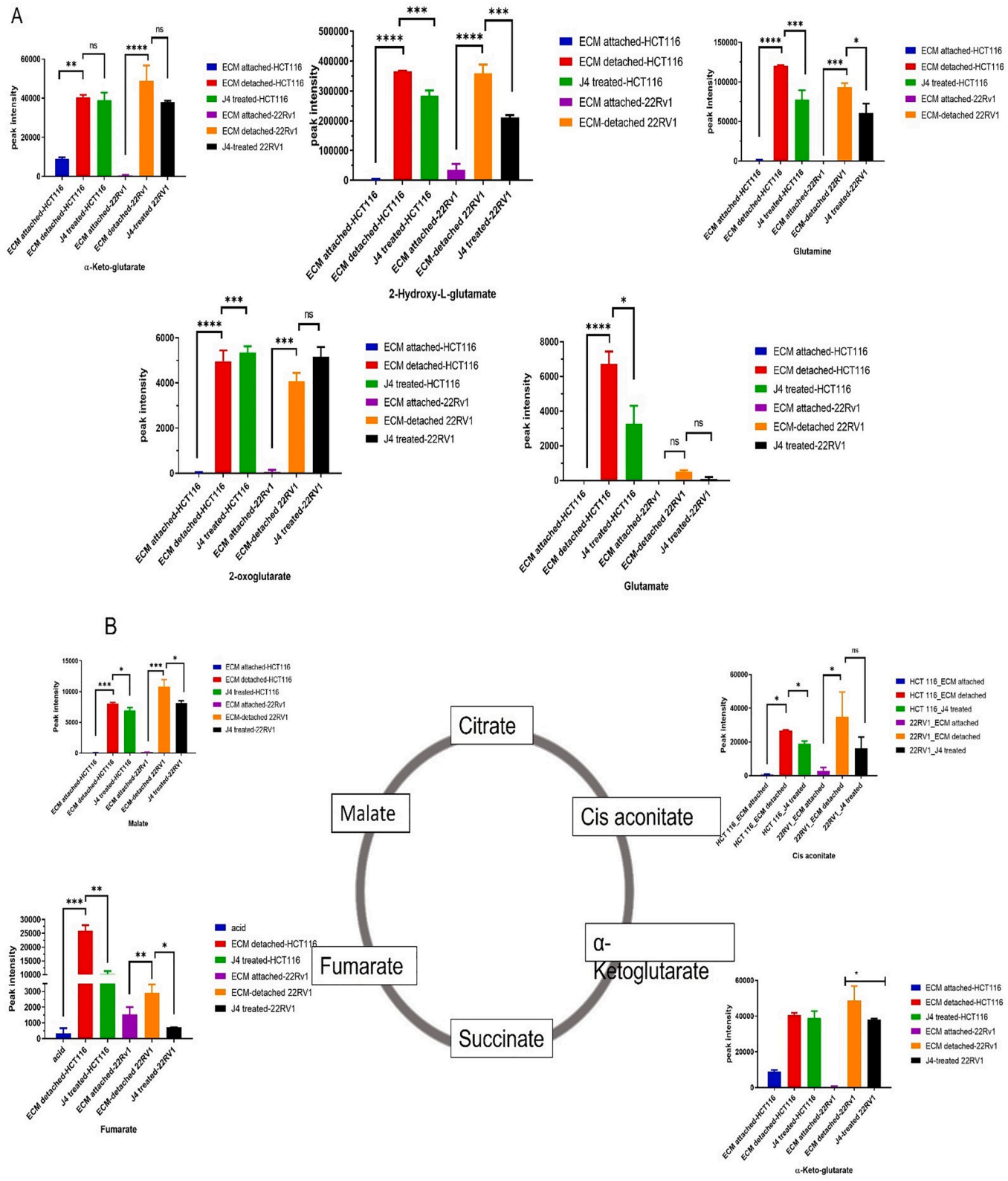

Fig. 4.

on the induction of antioxidant mechanisms following detachment, involving the activation of superoxide dismutase (SOD) and glutathione (GSH) to counteract ROS generation.

Our previous data revealed that ECM detached spheroids create a hypoxic environment, consistent with reports indicating the presence of a hypoxic core in tumor spheroids (Alkhatabi et al., 2022). This hypoxic tumor microenvironment leads to the stabilization of Hif1α and Hif2α, which trigger mitophagy—a process contributed in the removal of damaged mitochondria and mitochondrial ROS production regulation. Our previous study demonstrated that KDM6B transcriptionally

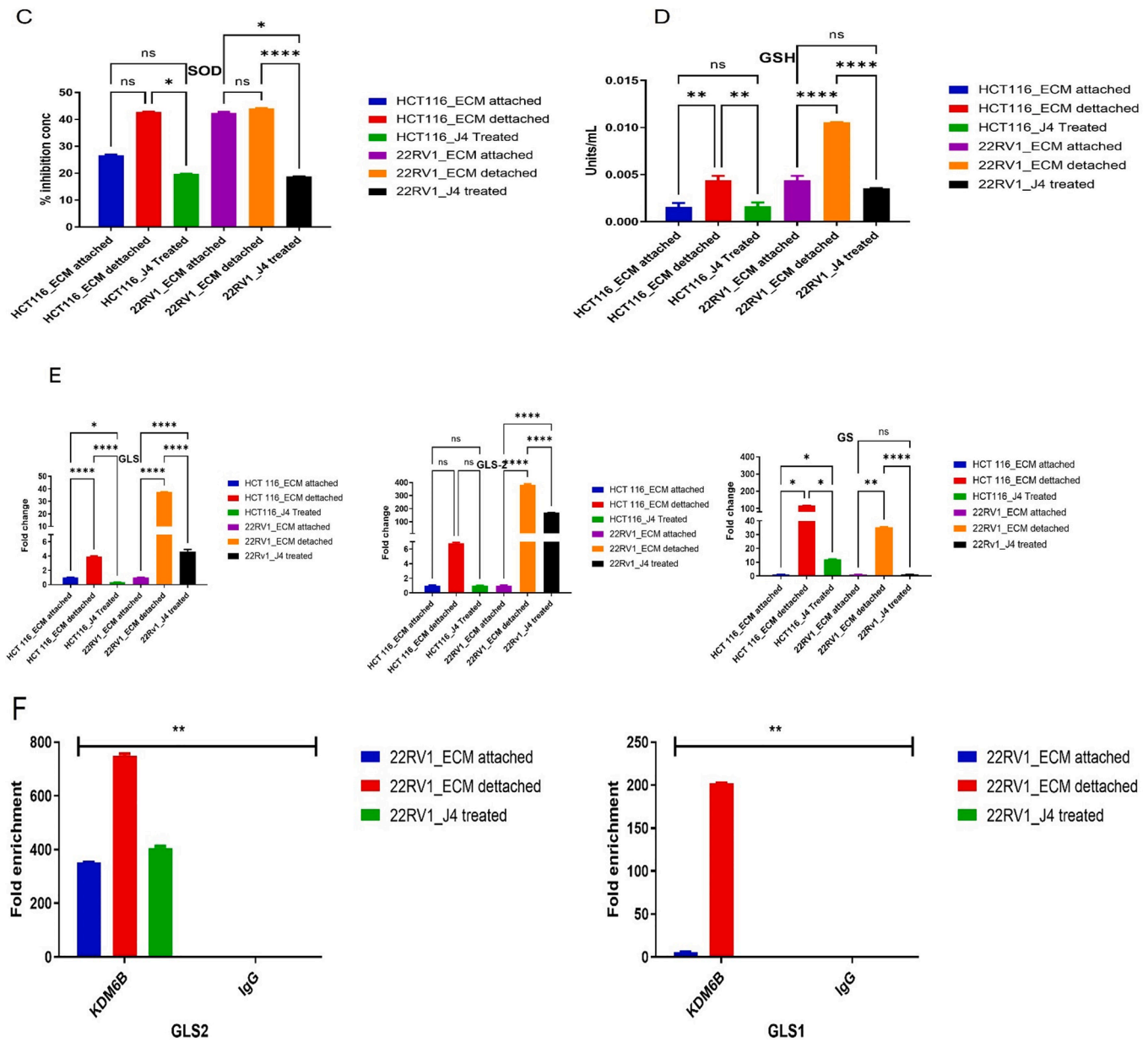

Fig. 4. (*continued*).

regulates the expression of Hif1α. In this work, we show that KDM6A/B inhibition leads to an increase in ROS levels, further supporting the association between KDM6A/B inhibition, mitochondrial depolarization, mitochondrial damage, and mitochondrial ROS production. Importantly, we demonstrated that the addition of MitoTEMPO, a mitochondria-specific ROS scavenger, reduces ROS production and promotes cell survival in detached cells. The hypoxic conditions in detached cells eliminate damaged mitochondria and limit ROS production.

Our findings indicate that KDM6A/B partially contributes to the metabolic switch observed in ECM detached cells. We show that KDM6A/B inhibition in detached cells shifts the metabolic profile towards oxidative phosphorylation (OXPHOS) and raise citrate levels to maintain high ATP levels. This observation aligns with previous studies suggesting that citrate can be utilized for mitochondrial NADPH production. This metabolic reprogramming is crucial to the development and sustenance of malignant characteristics. (Saha et al., 2018).

The Warburg effect postulates that enhanced glucose metabolism is crucial for cell proliferation (Pereira et al., 2017). Our data showed that ECM detached cells rely on glycolysis and glutamine-mediated reductive carboxylation for energy metabolism (Franchi et al., 2017). We propose that epigenetic regulation plays a role in governing these metabolic reprogramming-based survival mechanisms during ECM detachment. A recent study by Adem P. et al. (2020) supports the notion that KDM6A/B play a vital part in effector T cells the metabolic reprogramming.

Our data reveal that KDM6B occupies the promoter regions of genes involved in glycolysis metabolism, of GLUT-1, HK-1, and LDHA, (Fig. 4B) indicating its transcriptional regulation of these genes. Additionally, we provide further support for KDM6B's role in transcriptionally regulating genes take part in metabolism of glutamine, like GLS and GLS-1.

In conclusion, our findings underscore the critical role of KDM6A/B in detached cells, as they control the metabolic switches important for ECM detached cells to adapt to environmental stress (Fig. 6). Our results provide valuable insights into the regulation of glycolysis, reductive carboxylation, and oxidative stress by KDM6A/B in ECM detached cells. Further investigations utilizing metabolic inhibitors will help deepen our understanding of these crucial aspects of metabolic adaptation and pave the way for potential therapeutic approaches.

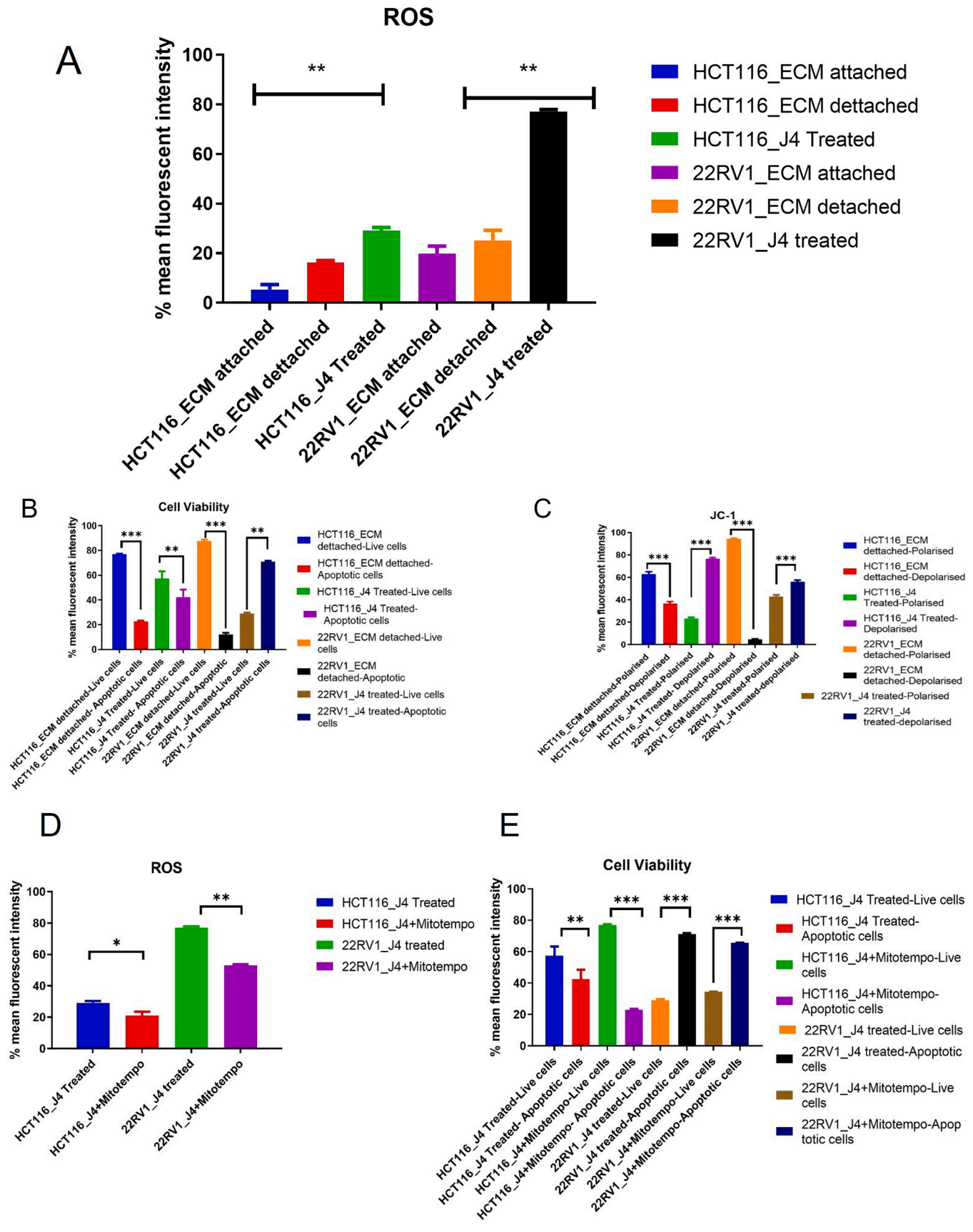
A
ROS
% mean fluorescent intensity
HCT116_ECM attached
HCT116_ECM dettached
HCT116_J4 Treated
22RV1_ECM attached
22RV1_ECM detached
22RV1_J4 treated
B
Cell Viability
% mean fluorescent intensity
HCT116_ECM dettached-Live cells
HCT116_ECM dettached-Apoptotic cells
HCT116_J4 Treated-Live cells
HCT116_J4 Treated-Apoptotic cells
22RV1_ECM detached-Live cells
22RV1_ECM detached-Apoptotic
22RV1_J4 treated-Live cells
22RV1_J4 treated-Apoptotic cells
C
JC-1
% mean fluorescent intensity
HCT116_ECM dettached-Polarised
HCT116_ECM dettached-Depolarised
HCT116_J4 Treated-Polarised
HCT116_J4 Treated-Depolarised
22RV1_ECM detached-Polarised
22RV1_ECM detached-Depolarised
22RV1_J4 treated-Polarised
22RV1_J4 treated-depolarised
D
ROS
% mean fluorescent intensity
HCT116_J4 Treated
HCT116_J4+Mitotempo
22RV1_J4 treated
22RV1_J4+Mitotempo
E
Cell Viability
% mean fluorescent intensity
HCT116_J4 Treated-Live cells
HCT116_J4 Treated-Apoptotic cells
HCT116_J4+Mitotempo-Live cells
HCT116_J4+Mitotempo-Apoptotic cells
22RV1_J4 treated-Live cells
22RV1_J4 treated-Apoptotic cells
22RV1_J4+Mitotempo-Live cells
22RV1_J4+Mitotempo-Apoptotic cells

Fig. 5.

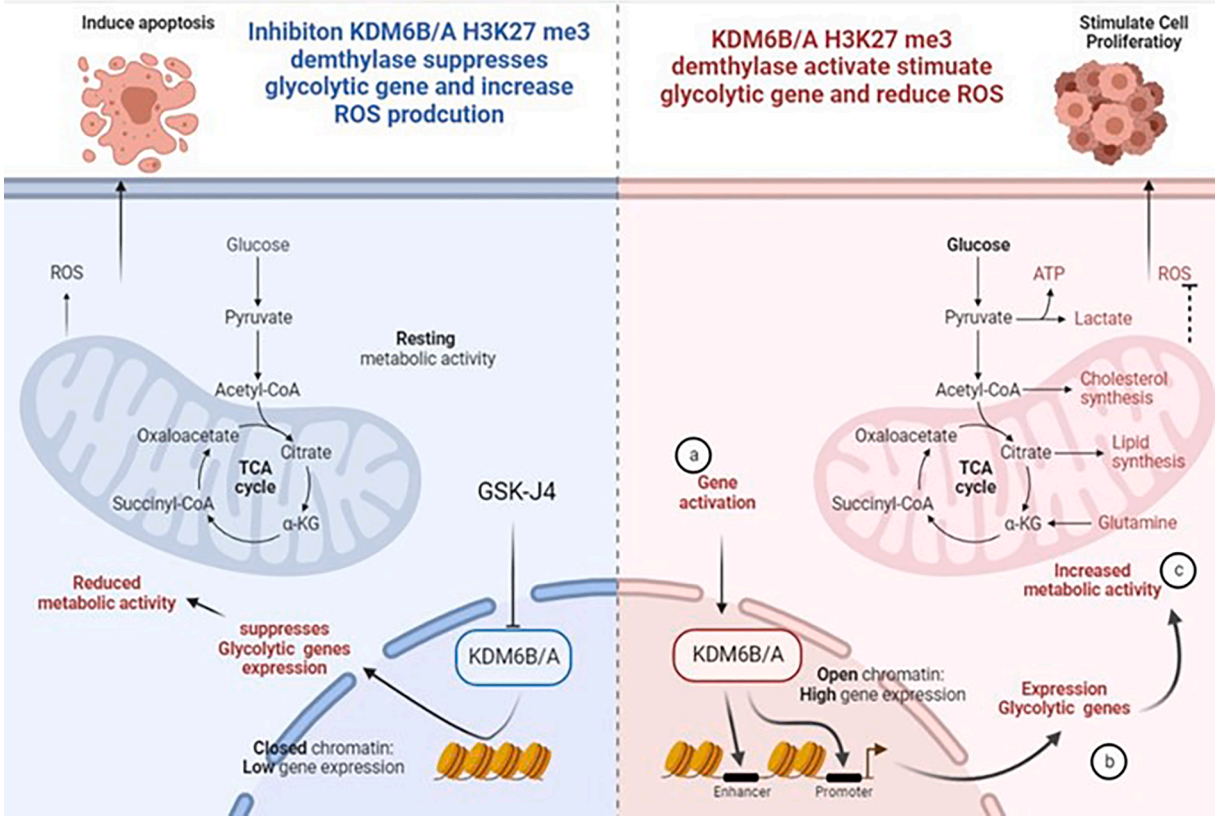

Fig. 6.

Funding

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number IFPRC-189-166-2020 and King Abdulaziz University, DSR, Jeddah, Saudi Arabia.

CRediT authorship contribution statement

Mohamed A. Alfaleh: Funding acquisition, Supervision, Project administration, Investigation. **Mohammed Razeeth Shait Mohammed:** Conceptualization, Methodology, Data curation, Writing – original draft. **Turki S Abujamel:** Funding acquisition, Supervision, Project administration, Investigation. **Nabil A Alhakamy:** Funding acquisition, Supervision, Project administration, Investigation. **Mohammad Imran Khan:** Conceptualization, Methodology, Data curation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank DSR technical and financial support. A work has been as part of thesis title " EXPLORATION OF METABOLOMIC AND EPIGENETIC LANDSCAPE OF CANCER CELLS DURING EXTRACELLULAR MATRIX DETACHMENT (MRS Mohammed – 2021)"

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103871.

References

Alkhatabi, H.A., Zohny, S.F., Shait Mohammed, M.R., Choudhry, H., Rehan, M., Ahmad, A., Ahmed, F., Khan, M.I., 2022. Venetoclax-resistant mv4-11 leukemic cells activate pi3k/akt pathway for metabolic reprogramming and redox adaptation for survival. Antioxidants (Basel). https://doi.org/10.3390/antiox11030461.

Alzahrani, F.A., Shait Mohammed, M.R., Alkarim, S., Azhar, E.I., El-Magd, M.A., Hawsawi, Y., Abdulaal, W.H., Yusuf, A., Alhatmi, A., Albiheyri, R., Fakhurji, B., Kurdi, B., Madani, T.A., Alguridi, H., Alosaimi, R.S., Khan, M.I., 2021. Untargeted metabolic profiling of extracellular vesicles of sars-cov-2-infected patients shows presence of potent anti-inflammatory metabolites. Int. J. Mol. Sci. https://doi.org/10.3390/ijms221910467.

Buchheit, C.L., Weigel, K.J., Schafer, Z.T., 2014. Cancer cell survival during detachment from the ecm: Multiple barriers to tumour progression. Nat. Rev. Cancer. https://doi.org/10.1038/nrc3789.

Cairns, R.A., Harris, I.S., Mak, T.W., 2011. Regulation of cancer cell metabolism. Nat. Rev. Cancer. https://doi.org/10.1038/nrc2981.

Cantor, J.R., Sabatini, D.M., 2012. Cancer cell metabolism: One hallmark, many faces. Cancer Discov. https://doi.org/10.1158/2159-8290.Cd-12-0345.

Endo, H., Owada, S., Inagaki, Y., Shida, Y., Tatemichi, M., 2020. Metabolic reprogramming sustains cancer cell survival following extracellular matrix detachment. Redox Biol.

Franchi, L., Monteleone, I., Hao, L.Y., Spahr, M.A., Zhao, W., Liu, X., Demock, K., Kulkarni, A., Lesch, C.A., Sanchez, B., Carter, L., Marafini, I., Hu, X., Mashadova, O., Yuan, M., Asara, J.M., Singh, H., Lyssiotis, C.A., Monteleone, G., Opipari, A.W., Glick, G.D., 2017. Inhibiting oxidative phosphorylation in vivo restrains th17 effector responses and ameliorates murine colitis. J. Immunol. https://doi.org/10.4049/jimmunol.1600810.

Guadamillas, M.C., Cerezo, A., Del Pozo, M.A., 2011. Overcoming anoikis–pathways to anchorage-independent growth in cancer. J. Cell Sci.

Hawk, M.A., Schafer, Z.T., 2018. Mechanisms of redox metabolism and cancer cell survival during extracellular matrix detachment. J. Biol. Chem.

Khan, M.I., Zamzami, M.A., Ahmad, A., Choudhry, H., 2021. Molecular profiling of epigenetic landscape of cancer cells during extracellular matrix detachment. Sci. Rep. https://doi.org/10.1038/s41598-021-82431-w.

Labuschagne, C.F., Cheung, E.C., Blagih, J., Domart, M.C., Vousden, K.H., 2019. Cell clustering promotes a metabolic switch that supports metastatic colonization. Cell Metab. https://doi.org/10.1016/j.cmet.2019.07.014.

Li, L., Liang, Y., Kang, L., Liu, Y., Gao, S., Chen, S., Li, Y., You, W., Dong, Q., Hong, T., Yan, Z., Jin, S., Wang, T., Zhao, W., Mai, H., Huang, J., Han, X., Ji, Q., Song, Q., Yang, C., Zhao, S., Xu, X., Ye, Q., 2018. Transcriptional regulation of the warburg effect in cancer by six1. Cancer Cell. https://doi.org/10.1016/j.ccell.2018.01.010.

Pereira, P.M.R., Berisha, N., Bhupathiraju, N., Fernandes, R., Tomé, J.P.C., Drain, C.M., 2017. Cancer cell spheroids are a better screen for the photodynamic efficiency of glycosylated photosensitizers. PLoS One. https://doi.org/10.1371/journal.pone.0177737.

Saha, M., Kumar, S., Bukhari, S., Balaji, S.A., Kumar, P., Hindupur, S.K., Rangarajan, A., 2018. Ampk-akt double-negative feedback loop in breast cancer cells regulates their adaptation to matrix deprivation. Cancer Res. https://doi.org/10.1158/0008-5472.Can-17-2090.

Shait Mohammed, M.R., Alghamdi, R.A., Alzahrani, A.M., Zamzami, M.A., Choudhry, H., Khan, M.I., 2021. Compound c, a broad kinase inhibitor alters metabolic fingerprinting of extra cellular matrix detached cancer cells. Front. Oncol. https://doi.org/10.3389/fonc.2021.612778.

Shait Mohammed, M.R., Zamzami, M., Choudhry, H., Ahmed, F., Ateeq, B., Khan, M.I., 2022. The histone h3k27me3 demethylases kdm6a/b resist anoikis and transcriptionally regulate stemness-related genes. Front. Cell Dev. Biol. https://doi.org/10.3389/fcell.2022.780176.

Timmerman, L.A., Holton, T., Yuneva, M., Louie, R.J., Padró, M., Daemen, A., Hu, M., Chan, D.A., Ethier, S.P., van 't Veer, L.J., Polyak, K., McCormick, F., Gray, J.W., 2013. Glutamine sensitivity analysis identifies the xct antiporter as a common triple-negative breast tumor therapeutic target. Cancer Cell. https://doi.org/10.1016/j.ccr.2013.08.020.

Ward, P.S., Thompson, C.B., 2012. Metabolic reprogramming: A cancer hallmark even warburg did not anticipate. Cancer Cell.

Zhang, J., Pavlova, N.N., Thompson, C.B., 2017. Cancer cell metabolism: The essential role of the nonessential amino acid, glutamine. Embo j. https://doi.org/10.15252/embj.201696151.