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RESEARCH

FOXM1 boosts glycolysis by upregulating SQLE to inhibit anoikis in breast cancer cells

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Abstract

Background Resisting anoikis is a prerequisite for cancer to spread and invade and a major cause of cancer-related deaths. Yet, the intricate mechanisms of how cancer cells evade anoikis remain largely unknown. There is a significant need to explore how these mechanisms play out in breast cancer (BC).

Methods Bioinformatics analysis revealed the expression levels of SQLE and FOXM1 in BC tissue, along with their correlation. The enrichment pathways of SQLE were also explored. qPCR detected the expression of SQLE and FOXM1 in BC cells. CCK-8 assessed cell viability, while flow cytometry measured anoikis. Western blot was employed to examine the protein expression of key genes in glycolytic metabolism and apoptosis-related proteins. Extracellular acidification rate was quantified, and corresponding kits evaluated glucose consumption, lactate production, and adenosine triphosphate levels in cells. Dual-luciferase reporter assays and chromatin immunoprecipitation tests unveiled the binding relationship between FOXM1 and SQLE.

Results SQLE was found to be highly expressed in BC and enriched in pathways associated with anoikis and glycolysis. SQLE curbed anoikis in BC via the aerobic glycolysis pathway. There was also a direct binding between FOXM1 and SQLE and a positive correlation between their expression. Recovery experiments substantiated that FOXM1 targeted SQLE to suppress anoikis in BC cells.

Conclusion FOXM1 upregulates SQLE, which in turn mediates glycolysis to suppress anoikis in BC. The FOXM1/SQLE axis is a promising therapeutic target for BC treatment.

Keywords Anoikis · Breast cancer · FOXM1 · Glycolysis · SQLE

Introduction

Cancer remains the leading cause of population death in every country in the world (Sung et al. 2021). According to reports, in 2020, there were approximately 19.3 million new cancer cases worldwide (excluding 18.1 million cases of non-melanoma skin cancer) and about 10 million cancer-related deaths (excluding 9.9 million deaths from non-melanoma skin cancer). Among these, breast cancer (BC) accounted for approximately 2.3 million new cases, surpassing lung cancer as the most common cancer type, with a mortality rate of about 6.9%[1]. Compared to other cancers, BC treatment faces unique challenges, including its high heterogeneity. Different subtypes (e.g., hormone receptor-positive, HER2-positive, triple-negative) exhibit significantly varied responses to treatment, necessitating personalized therapeutic approaches (Burguin et al. 2021). The standard treatment strategies of surgery and

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chemo/radiotherapy, while effective, come with significant drawbacks. Surgery often leads to patient discomfort and diminished quality of life, while chemotherapeutic drugs not only cause severe side effects such as fatigue, hair loss, and neuropathy but also frequently result in drug resistance (Burguin et al. 2021). Therefore, developing more effective alternative treatments is urgently needed to improve patient survival rates and quality of life.

The dysregulation of apoptosis in cancer cells is a key driver of tumor onset and progression. Due to their malignant potential, cancer cells can evade apoptosis to survive (Paoli et al. 2013). Anoikis, a specialized type of programmed cell death, occurs when cells detach from the extracellular matrix or neighboring cells, but numerous mechanisms can cause resistance to anoikis in cancer cells (Grossmann 2002). For example, in pancreatic cancer, the Gli2-YAP1 axis fosters resistance to anoikis, thus promoting cancer metastasis (Yu et al. 2022). Anoikis resistance is a major factor in the survival of BC cells and the development of metastasis (Dai et al. 2023). Similarly, in BC, migrating cancer cells develop resistance to anoikis following their passage through microporous membranes mimicking confined migration (CM), culminating in enhanced metastasis (Fanfone et al. 2022).

Tumors not only display abnormalities in cell growth, metastasis, and invasion but in energy metabolism. Glycolysis, in particular, is a critical part in reshaping the microenvironment of metastatic tumors (Hanahan and Weinberg 2011; Icard et al. 2018; Zhao et al. 2021). BC cells rely heavily on glycolysis to meet their energy demands, and glycolysis plays a crucial role in the progression of BC (Wu et al. 2020). It has been reported that lactate produced by tumor cells through glycolysis competes metabolically with infiltrating immune cells, thereby influencing the tumor immune microenvironment and affecting the response to immune checkpoint inhibitors (Schreier et al. 2022). Research has shown that glycolysis can modulate apoptosis in cancer cells. Cisplatin-induced acetylation of PFKFB3 at lysine 472, for example, can incapacitate the nuclear localization signal, leading to an increase in cytoplasmic PFKFB3 that enhances glycolysis and shields cells from apoptosis (Li et al. 2018). Although previous studies have explored the relationship between glycolysis and anoikis, their specific roles in cancer metastasis remain unclear. This study aimed to delve deeper into this relationship, uncovering its potential implications for metastatic cancer treatment, thereby providing a solid foundation for the development of more effective therapeutic strategies.

SQLE, a rate-limiting enzyme in the cholesterol synthesis pathway, catalyzes the transformation of squalene into 2,3-oxidosqualene (Li et al. 2022). Its expression levels have been linked to the advancement of multiple tumors. SQLE is highly expressed in pancreatic cancer, promoting cell proliferation and invasion through the lncRNA-TTN-AS1/miR-133b/SQLE axis (Wang et al. 2022). In lung squamous cell carcinoma, SQLE boosts cell proliferation and metastasis via the extracellular signal-regulated kinase signaling pathway (Ge et al. 2019). Overexpression of SQLE promotes the proliferation, migration and invasive capacity of BC cells (Tang et al. 2021). Moreover, lncRNA030 sustains the stemness of BC stem cells by stabilizing SQLE mRNA and increasing cholesterol synthesis (Qin et al. 2021). Zhang et al. (Zhang et al. 2020). also demonstrated that SQLE can advance the differentiation and apoptosis of mesenchymal stem cells derived from bovine skeletal muscle. The part SQLE plays in tumor anoikis is still a mystery. Therefore, this study further probed the connection between SQLE and anoikis, potentially unravelling more about BC genesis.

We have uncovered the mechanism by which SQLE affects anoikis in BC cells. It turns out that SQLE, which is highly expressed in BC, inhibits anoikis through glycolysis. There is an upstream regulator of SQLE, FOXM1, which activates SQLE to mediate glycolysis and prevent anoikis in BC. The study hints that SQLE in BC blood samples before treatment might be a reliable predictor for therapy efficacy. Our study not only reveals the important role of SQLE in BC but also provides a theoretical basis for the development of new therapeutic strategies and biomarkers. Through further research, we can better understand the mechanism of SQLE in different cancer types and provide new ideas for personalized treatment.

Materials and methods

Bioinformatics

The mRNA expression data of BC (normal samples: 113, tumor samples: 1113) were downloaded from the TCGA database (http://portal.gdc.cancer.gov). Differential analy sis was performed using the edgeR package ($|logFC| > 1$, $FDR < 0.01$) to obtain DEmRNAs. The target genes for the study were identified by combining literature evidence. The upstream transcription factors of the target genes were predicted using hTFtarget (http://bioinfo.life.hust.edu.cn/hTFtarget#!/). The intersection of the predicted transcription fa ctors and the differentially upregulated mRNAs was taken to obtain the differentially expressed transcription factors. Pearson correlation analysis was conducted between the transcription factors and the target genes, and highly correlated transcription factors were selected as the research subjects. Subsequently, the motif binding sites upstream of the target genes (2000 bp) were predicted using MoLoTool (https://molotool.autosome.org/). Further investigations

were carried out through literature review, GSEA enrichment analysis, and correlation analysis to study the pathways through which the target genes influence BC.

Cell culture

The human BC cell lines MCF7 (BNCC100137), HCC1937 (BNCC354617), and ZR-75-1 (BNCC338655), the normal mammary epithelial cell line MCF10A (BNCC337734), and the human embryonic kidney cell line 293T (BNCC353535) were all purchased from BeNa Culture Collection (China). After thawing and resuscitation, HCC1937 and ZR-75-1 cells were cultured in a medium containing 10% fetal bovine serum (FBS, BNCC373008) and 90% RPMI-1640 (BNCC338362). MCF10A cells were cultured in LL-0023 complete medium supplemented with 100 ng/ml cholera toxin. MCF7 and 293T cells were cultured in DMEM-H medium containing 10% FBS. All cells were maintained in a 37 °C incubator with 5% CO_2 for subsequent passaging. All cell lines were authenticated by STR profiling and confirmed to be free of mycoplasma contamination.

Cell transfection

As per the Lipofectamine 2000 transfection kit guidelines (Invitrogen, USA), we transfected sh-SQLE, oe-SQLE, and sh-FOXM1, along with their respective negative controls (Ribobio, China), into ZR-75-1, HCC1937, and 293T cells. Post-transfection, cellular RNA was extracted to assess the expression of SQLE and FOXM1 and to determine the transfection efficacy.

qRT-PCR

Trizol (Invitrogen, USA) was used to extract RNA, which was then reverse transcribed to cDNA using the PrimeScript™ RT reagent Kit (Takara, Japan). qRT-PCR was performed with TB Green Premix Ex Taq II (Takara, Japan) on the Applied Biosystems™ 7500 Real-Time PCR System. PCR primers were designed according to sequences retrieved from the NCBI database (Table 1). The baseline was adjusted as per the instrument's guidelines, and the threshold was set in the linear phase of the fluorescence curve. Ct values were obtained using the software, and the expression levels of the target gene were calculated by the $2^{-\Delta\Delta Ct}$ method, normalized to β-actin.

Table 1 qPCR primers

Gene	Primer sequence	
SQLE	Forward Primer	5'-GATGATGCAGCTATTTTCGAGGC-3'
	Reverse Primer	5'-CCTGAGCAAGGATATTCACGACA-3'
FOXM1	Forward Primer	5'-CGTCGGCCACTGATTCTCAAA-3'
	Reverse Primer	5'-GGCAGGGGATCTCTTAGGTTC-3'
b-actin	Forward Primer	5'-GACCTGACTGACTACCTCATGAAGAT-3'
	Reverse Primer	5'-GTCACACTTCATGATGGAGTTGAAGG-3'

CCK-8

Cells were dispensed at a rate of 2000 per well in a 96-well plate and allowed to grow for 0, 24, 48, and 72 h. Subsequently, 10 µL of CCK-8 was added to each well. Following a 4-h incubation, the absorbance at 450 nm was assessed with a plate reader.

Flow cytometry

To elicit anoikis, cells are cultured on specialized ultra-low attachment plates (BeyoGold™, FULA061-1pc, Beyotime). Following a 16-h cultivation period, apoptosis was assessed by measuring cell death. A total of 10,000 cells were suspended in 0.35% agarose and layered on top of a 0.6% agar base in six-well plates (lids included, individually packaged) to grow without anchorage. Colony counts were performed after four weeks. Cells were collected, washed three times, and centrifuged at 3000 r/min for 5 min with a 10 cm radius, the supernatant was then removed; 500 µl of binding buffer was added to resuspend the cells, followed by the addition of 5 µl of Annexin V and incubated in the dark for 15 min; 5 µl of PI was then added and incubated in the dark for an additional 15 min. A flow cytometer (with an excitation wavelength of 488 nm and an emission wavelength of 530 nm) was used to detect apoptotic cells. Each experiment was repeated three times to obtain mean values.

Western blot (WB)

Cells were lysed to extract total protein using radioimmunoprecipitation assay (RIPA) buffer (Beyotime, China) for 30 min. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Beyotime, China). Proteins were then separated by SDS-PAGE and transferred to a membrane. The membrane was blocked in 5% BCA for 2 h before incubation with the primary rabbit anti-human antibodies overnight at 4 °C. On the next day, the membrane was washed with TBST (5 min × 3 times) and then incubated with the secondary goat anti-rabbit IgG H&L (HRP) for 2 h. The immunoreactive bands were visualized with the Western Blotting Luminol Reagent (Santa Cruz, USA) using the ChemiScope 6000 system. Primary antibodies: HK-2, PKM2, PFKP, C-PARP, C-Caspase3, β-actin

(Abcam, UK) (1:500); secondary antibody: goat anti-rabbit IgG H&L (HRP) (Abcam, UK).

Extracellular acidification rate (ECAR)

sh-SQLE, sh-FOXM1, and oe-SQLE, along with their respective negative controls sh-NC and oe-NC, were transfected into HCC1937 cells. The Seahorse XFe 96 Extracellular Flux Analyzer was employed to measure cellular ECAR. Cells were collected, counted, and seeded at a density of 10,000 cells/well in Seahorse XF 96-well plates for cultivation to achieve a cell confluence of 80-90%. Afterward, the Seahorse XF Glycolysis Stress Test Kit (Agilent, USA) was used to test ECAR. During the ECAR measurement, 10 mM glucose, 1 μM oligomycin, and 50 mM 2-deoxyglucose (2-DG) were sequentially added at specified time points to evaluate the glycolytic activity of the cells.

Determination of glucose, lactate, and adenosine triphosphate (ATP) levels

The Glucose Uptake Assay Kit, Lactate Assay Kit, and Luminescent ATP Detection Assay Kit from Solarbio were deployed to quantify glucose uptake, lactate release, and ATP synthesis in cells.

Luciferase reporter gene assay

We created the pGL3-SQLE-promoter-WT and pGL3-SQLE-promoter-MUT luciferase reporter gene constructs (Promega) and collected around 5×10^5 293T cells in the logarithmic growth phase. These cells were then plated into a 6-well plate and transfected with sh-NC and sh-FOXM1 using a standard lipid-mediated transfection method. Luciferase activity was analyzed with the GloMaxTM 20/20 luminometer from Promega (USA).

Chromatin Immunoprecipitation (ChIP)

The SimpleChIP® Plus Sonication Chromatin IP Kit (CST, USA) was applied for ChIP assays. We harvested the transfected cells and cross-linked them with 1% formaldehyde at 37 °C for 10 min. Subsequently, we quenched the cross-linking reaction with 1 M glycine. The cells were washed with PBS and then resuspended in SDS lysis buffer with PMSF protease inhibitors. Chromatin was sheared to a size of 2,000 bp by sonication. Chromatin fragments were immunoprecipitated using anti-FOXM1 (CST, USA) and anti-IgG (CST, USA) antibodies, followed by extraction and purification of chromatin DNA. The expression of SQLE was assessed by qPCR with primers designed for the SQLE promoter sequence (Table 2).

Table 2 ChIP qPCR primers

Primer	Primer sequence	
Site	Forward Primer	5'- CACTTTTGCTCCCTCGGACT-3'
	Reverse Primer	5'- TCGCTTTGAGGAGAAGCCTG-3'

Statistical analysis

All experiments were replicated three times. *T*-tests were conducted to assess the variance in the quantitative data obtained in the data from qRT-PCR, luciferase assays, and CCK-8. One-way ANOVA was applied to compare differences between two groups. $P<0.05$ indicates statistical significance. All statistical analyses were carried out using GraphPad 8.0 software.

Results

SQLE overexpression in BC

Leveraging TCGA data, we identified high SQLE expression in BC tumor tissues (Fig. 1A). qPCR and WB analyses of SQLE expression in both normal breast epithelial cells and BC cells showed a marked increase in SQLE in BC cell lines, peaking in HCC1937 and bottoming out in ZR-75-1 (Fig. 1B-C). Collectively, SQLE expression is elevated in BC.

SQLE impedes BC cells from anoikis

To explore the impact of aberrant SQLE expression on BC, we analyzed SQLE and found its enrichment in the anoikis pathway (Fig. 2A). Correlation analysis revealed an inverse relationship between SQLE and key genes that promote anoikis, including FAS, PDCD4, and PTEN (Fig. 2B). It has been established that resistance to anoikis can lead to the progression of BC (Fanfone et al. 2022). To substantiate the effect of SQLE on anoikis in BC, sh-SQLE and its control were transfected into HCC1937 cells (displaying the highest SQLE expression), and oe-SQLE and its control were transfected into ZR-75-1 cells (displaying the lowest SQLE expression). qPCR and WB showed a notable decrease in SQLE expression in sh-SQLE-transfected cells and a marked increase in oe-SQLE-transfected cells (Figs. 2C-E). Subsequent discovery suggests a role for SQLE in BC cell survival. Specifically, knocking down SQLE (sh-SQLE) could impair cell vitality, while its overexpression (oe-SQLE) had a revitalizing effect (Fig. 2F-G). Flow cytometry data revealed that sh-SQLE hastened apoptosis in HCC1937 cells, but oe-SQLE slowed it down in ZR-75-1 cells (Fig. 2H-I). As Caspase3 activation is a sign of anoikis, we performed WB to track Caspase3 and PARP cleavage.

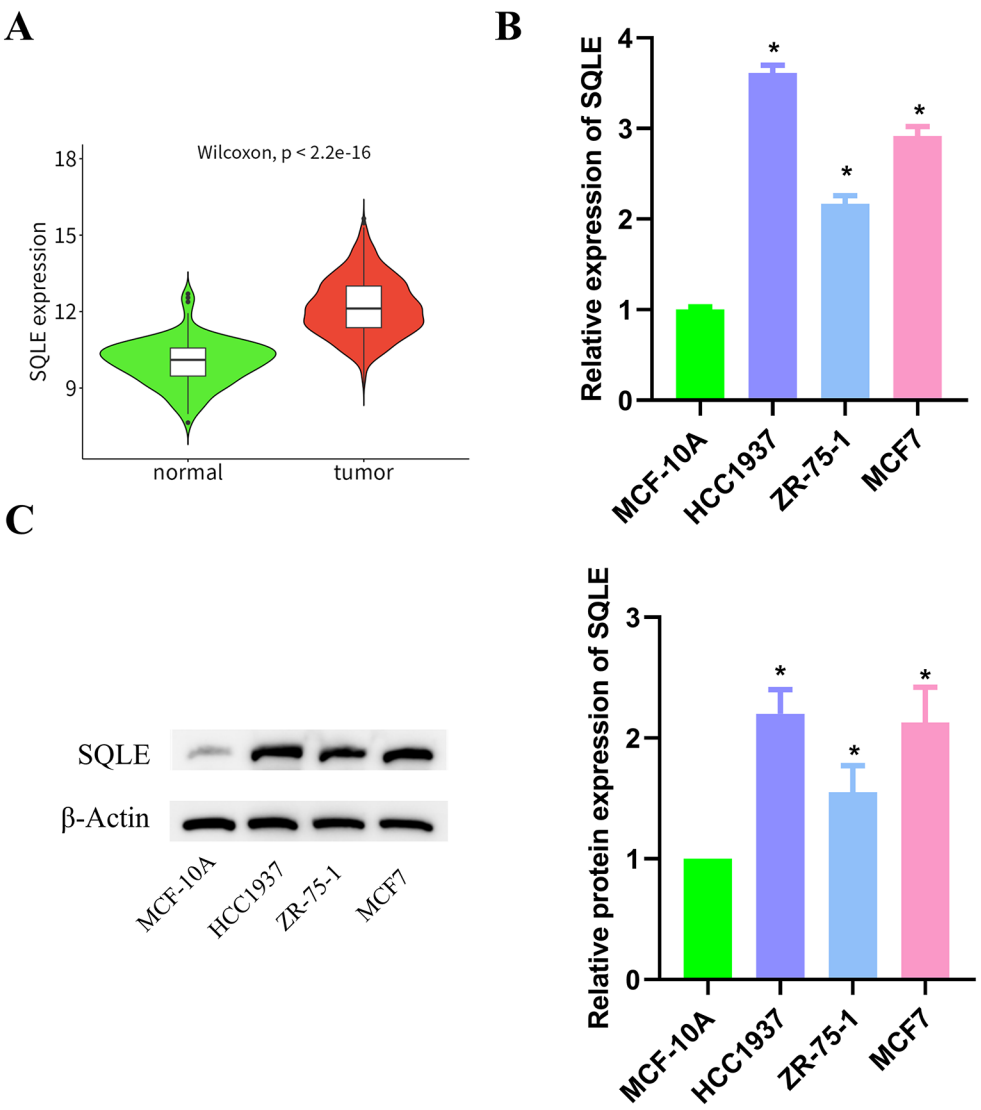

Fig. 1 SQLE overexpression in BC. **A**: Expression of SQLE in BC tissues as per TCGA data; **B**: qPCR analysis of SQLE expression across various cell types; **C**: WB analysis for protein expression. * denotes $P<0.05$

The findings indicate that sh-SQLE drives Caspase3 and PARP cleavage, while oe-SQLE countered these processes (Fig. 2J). The expression of key proteins for glycolysis was dose-dependently reduced in ZR-75-1 cells after treatment with different concentrations (0,1,5,10 mM) of 2-DG, while the levels of Caspase3 and PARP cleavage were significantly inhibited (Supplementary Fig. 1A-B). Overall, SQLE inhibits anoikis in BC cells.

SQLE inhibits anoikis in BC through Glycolysis

Subsequent analysis uncovered that SQLE is mainly found along the glycolytic signaling pathway (Fig. 3A), and positively correlates with genes associated with glycolysis (Fig. 3B). To elucidate the relationship between SQLE and glycolysis, this study examined the glycolytic process in BC cells transfected with sh-SQLE. Initially, WB was conducted to assess the expression levels of proteins involved in glycolytic metabolism and SQLE, showing that, in contrast to the control group, there was a marked decrease in the expression of key glycolytic proteins and SQLE in HCC1937 cells with sh-SQLE transfection (Fig. 3C). A reduction was observed in ECAR levels following sh-SQLE treatment (Fig. 3D). Additionally, sh-SQLE treatment led to a decrease in glucose uptake, lactate secretion, and ATP synthesis in BC cells (Fig. 3E-F). Existing literature has reported that heightened glycolysis elevates lactate levels (Zhang and Li 2020), and lactate could augment the resistance of cancer cells to anoikis (Jin et al. 2017). To confirm the role of SQLE in mediating the impact of aerobic glycolysis on BC anoikis, we employed the glycolytic inhibitor 2-DG in our experiments. It was found that 2-DG treatment counteracted the cell viability increase induced by oe-SQLE (Fig. 3G). oe-SQLE promoted the levels of glucose consumption, lactate and ATP production in ZR-75-1 cells, whereas 2-DG treatment attenuated the promotion

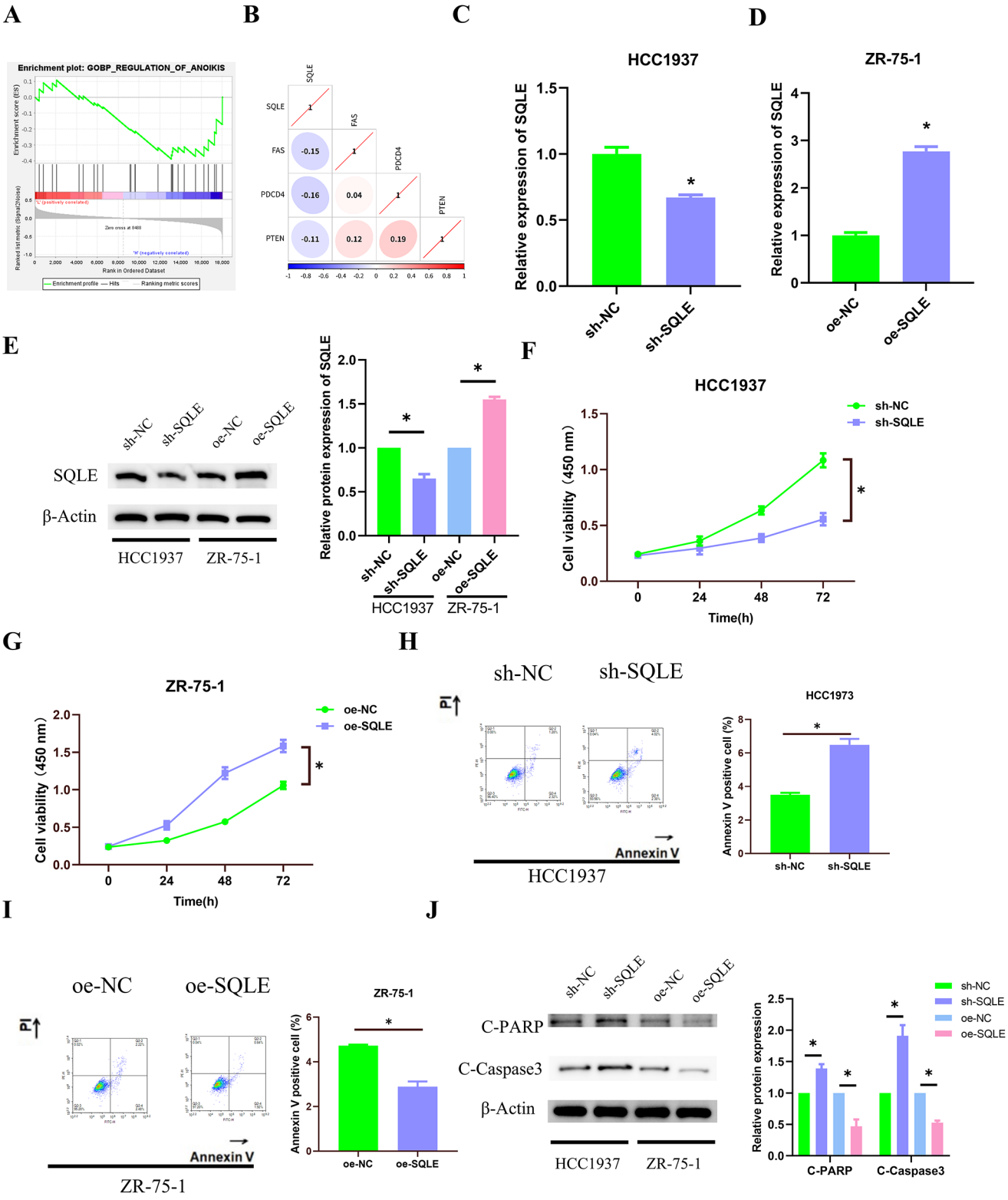

Fig. 2 SQLE impedes BC cells from anoikis. **A**: GSEA profiling for SQLE activity; **B**: Correlation between SQLE and key genes in anoikis; **C-D**: qPCR detection of SQLE levels; E: WB analysis for protein expression; **F-G**: CCK-8 tests for cell viability; **H-I**: Flow cytometry for cell apoptosis; **J**: WB analysis for protein expression. * denotes $P<0.05$

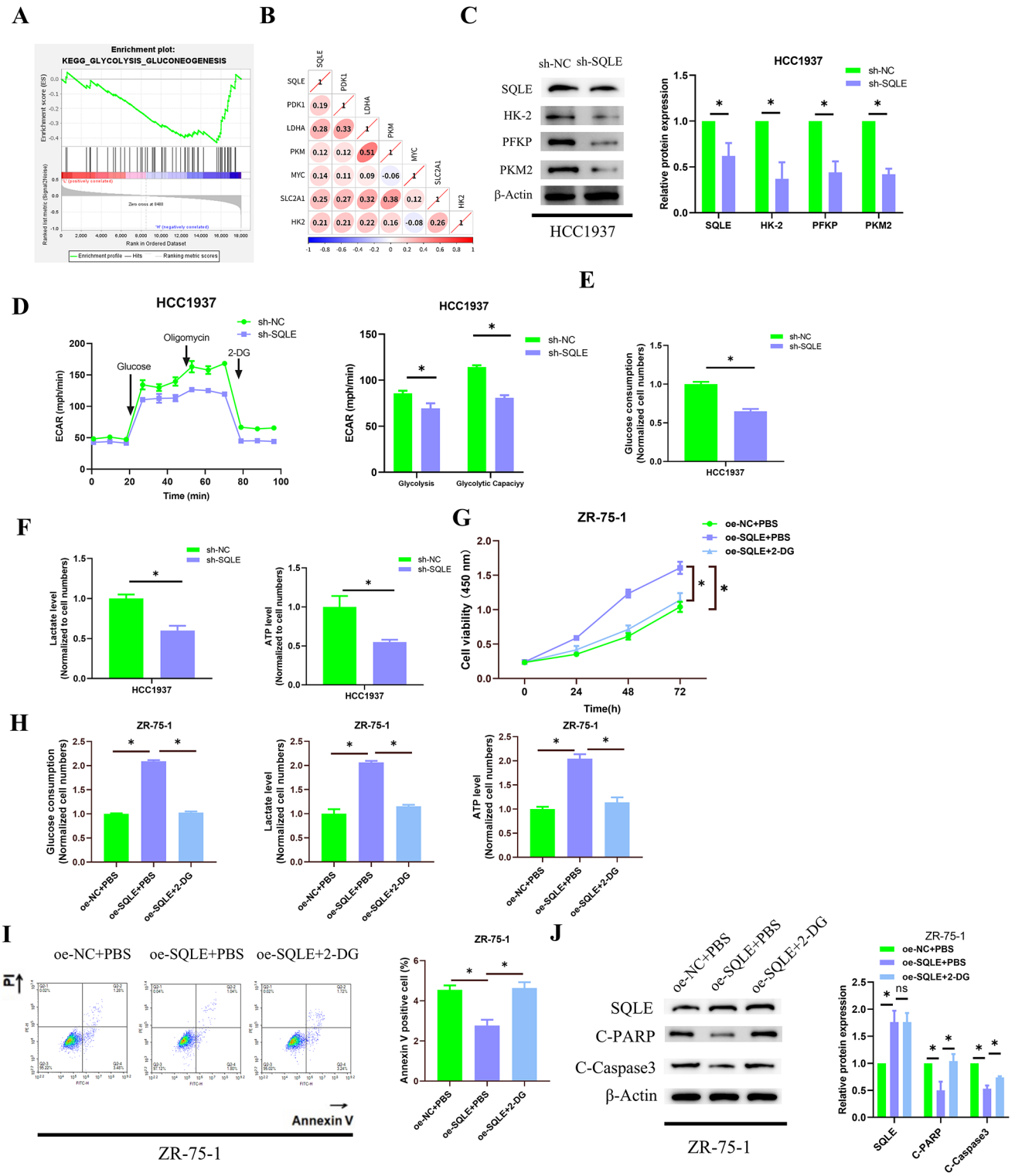

Fig. 3 SQLE inhibits anoikis in BC through glycolysis. **A**: GSEA enrichment analysis for SQLE; **B**: Correlation of SQLE with glycolytic marker genes; **C**: WB analysis of protein expression; **D**: Measurement of ECAR; **E**-**F**: Assessment of glucose consumption, lactate production, and ATP generation levels; **G**: CCK-8 assay for cell vitality; **H**: Assessment of glucose consumption, lactate production, and ATP generation levels; **I**: Flow cytometry for cell apoptosis; **J**: WB analysis of protein expression. * denotes $P<0.05$

of cellular glycolysis levels by oe-SQLE (Fig. 3H). Flow cytometry showed that oe-SQLE remarkably inhibited apoptosis in ZR-75-1 cells, but this effect was diminished with 2-DG treatment (Fig. 3I). WB for caspase-3 and PARP cleavage indicated that 2-DG treatment reduced the inhibitory effect of oe-SQLE on these markers of apoptosis, and oe-SQLE promoted SQLE expression (Fig. 3J). In short, SQLE mediates the inhibition of BC anoikis through aerobic glycolysis.

FOXM1 as an upstream TF of SQLE in BC

Bioinformatics tools were employed to identify potential TFs upstream of SQLE. After intersecting with the upregulated DEmRNAs, 26 potential TFs upstream of SQLE were identified. We discovered a positive correlation between FOXM1 and SQLE (Fig. 4A) as well as their potential binding sites (Fig. 4B). Further investigation into FOXM1 revealed its pronounced upregulation in BC tumor tissues (Fig. 4C). qPCR and WB confirmed high FOXM1 expression in BC cells (Fig. 4D-F). Dual-luciferase reporter assays and ChIP experiments showed that the enzyme activity of the SQLE wild-type sequence group was notably inhibited in FOXM1 knockdown 293T cells, with no significant difference in the mutant sequence group, and the specific anti-FOXM1 antibody, in contrast to IgG, substantially increased the enrichment of SQLE (Figs. 4G-H), illustrating that FOXM1 targets SQLE.

FOXM1 as an upstream TF of SQLE in BC

Cell groups were designed to examine the influence of FOXM1-mediated SQLE upregulation on BC anoikis. qPCR assessment of FOXM1 and SQLE expression indicated that sh-FOXM1 caused a notable reduction in FOXM1 and SQLE levels, while oe-SQLE countered this reduction, without effect on FOXM1 (Fig. 5A). CCK-8 data indicated that sh-FOXM1 led to diminished cell viability, an effect that was abrogated by oe-SQLE (Fig. 5B). WB showed that sh-FOXM1 strongly impeded the expression of key proteins in the glycolytic pathway, FOXM1 and SQLE, and oe-SQLE attenuated this suppressive effect, with FOXM1 expression being unchanged (Fig. 5C). ECAR assessments revealed that sh-FOXM1 inhibits glycolysis, while oe-SQLE can lessen the inhibitory effect of sh-FOXM1 on glycolysis (Fig. 5D). Similarly, sh-FOXM1 caused a pronounced reduction in glucose consumption, lactate production, and ATP synthesis in BC cells, which were partially restored by oe-SQLE (Fig. 5E). Flow cytometry analysis subsequently revealed that sh-FOXM1 greatly promoted BC cell apoptosis, a promotion that oe-SQLE could reverse (Fig. 5F). WB demonstrated that sh-FOXM1 increased caspase-3 and PARP cleavage, and oe-SQLE averted this increase (Fig. 5G). Collectively, FOXM1 activates the transcription of SQLE, which in turn suppresses the anoikis of BC cells through the aerobic glycolysis pathway.

Discussion

Globally, millions of women are diagnosed with BC every year, as reported by the World Health Organization (Prager et al. 2018). Investigating the root causes of BC and developing innovative treatment targets can help make a real difference in the patients' lives. SQLE, an enzyme that facilitates the synthesis of cholesterol and other crucial sterols from squalene by catalyzing the formation of 2,3-oxidosqualene (Gill et al. 2011), is significantly implicated in different types of cancer. Researchers have noted that downregulating SQLE can markedly suppress liver cancer cell growth and metastasis (Sui et al. 2015). Similarly, in pancreatic cancer, SQLE knockdown is associated with inhibited tumor progression and increase sensitivity to chemotherapy in vitro (Zhao et al. 2022). Our study first confirmed the high expression of SQLE in BC cells through bioinformatics analysis and cellular experiments. However, the impact of its high expression on BC cell anoikis remained unclear. Therefore, we designed experimental groups: oe-NC and oe-SQLE. Flow cytometry results demonstrated that high expression of SQLE inhibited BC cell apoptosis, and WB results showed that overexpression of SQLE suppressed Caspase3 cleavage and PARP cleavage levels, thereby confirming that high SQLE expression inhibited BC cell anoikis. Evidence points to that acquired resistance to anoikis is a prerequisite for cancer metastasis and progression. For instance, in intrahepatic cholangiocarcinoma, the downregulation of 14-3-3σ has been shown to inhibit cancer metastasis by suppressing cell migration, invasion, and resistance to anoikis (Yang et al. 2017a, b). In colorectal cancer, the miR-124/ITGA3 axis facilitates cancer metastasis by modulating susceptibility to anoikis (Sa et al. 2018).

In this study, we explored the role of SQLE in regulating anoikis in BC and discovered through GSEA analysis that SQLE is enriched in glycolytic pathways. To investigate whether SQLE inhibits BC cell anoikis through the glycolysis pathway, we designed experimental groups: oe-NC + PBS, oe-SQLE + PBS, and oe-SQLE + 2-DG. Further cellular experiments confirmed that treatment with the glycolysis inhibitor 2-DG attenuated the promoting effect of SQLE overexpression on cell viability and its inhibitory effect on anoikis. An elevated level of glycolysis is one of the important characteristics of tumor cells (Wu et al. 2020). Relevant studies have indicated that inhibiting the expression of key glycolysis genes not only promotes

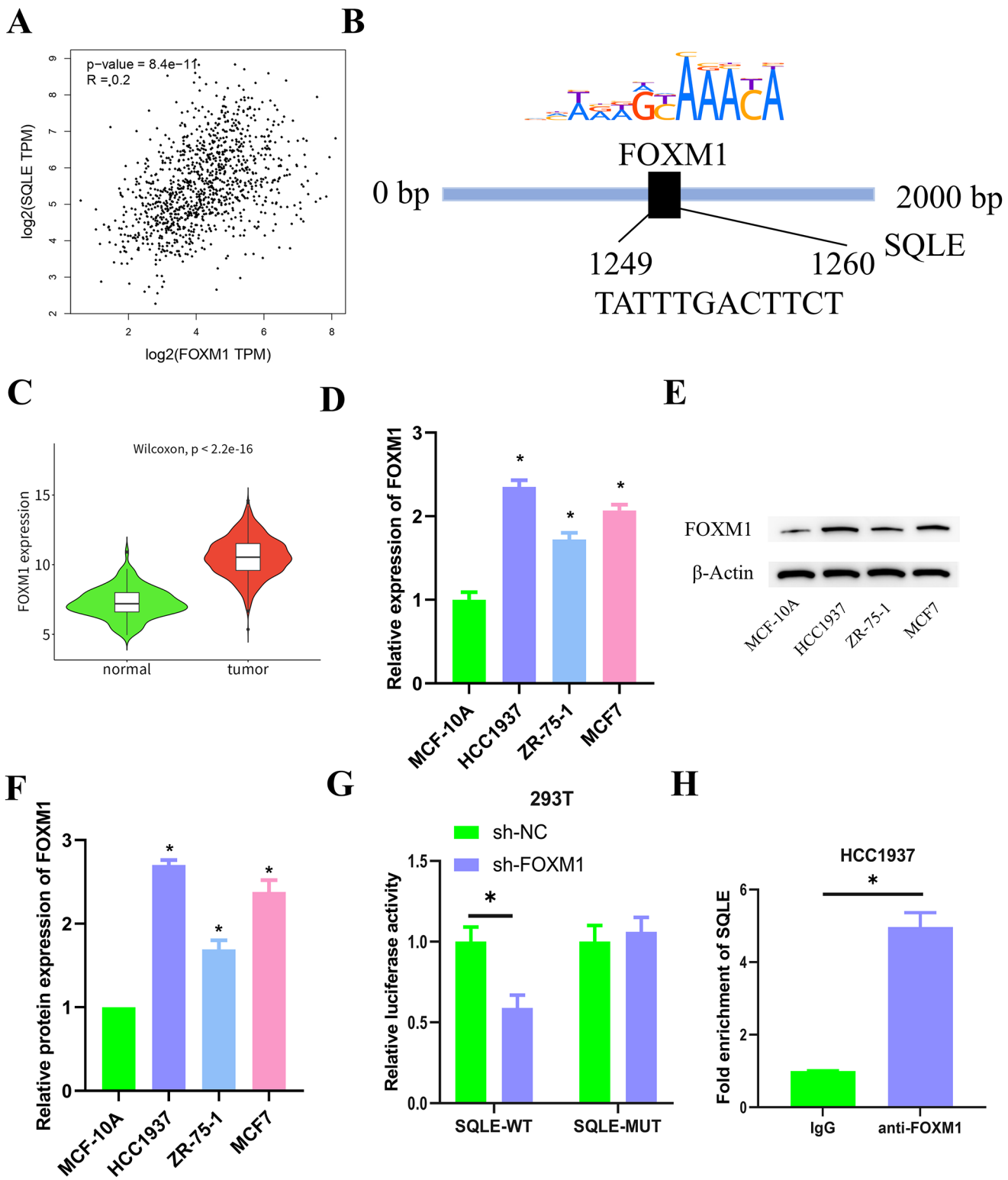

Fig. 4 FOXM1 as an upstream TF of SQLE in BC. **A**: Pearson correlation analysis; **B**: Binding sites of FOXM1 and SQLE; **C**: Bioinformatics analysis of FOXM1 expression in BC tissues; **D**: qPCR detection of FOXM1 expression; **E-F**: WB analysis for protein expression; **G-H**: Dual-luciferase reporter assay and ChIP assay. * denotes $P<0.05$

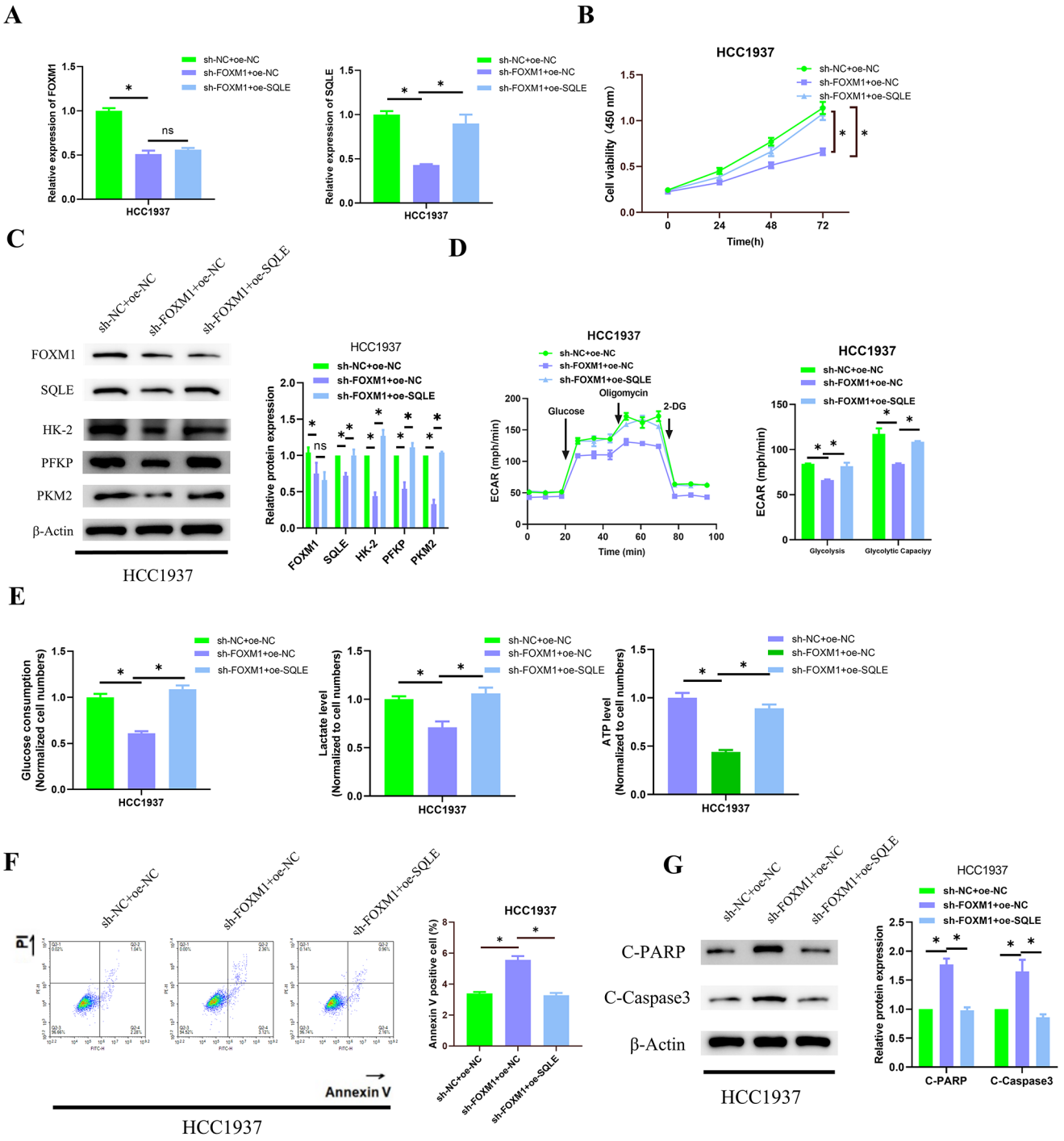

Fig. 5 FOXM1 as an upstream TF of SQLE in BC. **A**: qPCR analysis of transfection efficiency; **B**: CCK-8 assay for cell vitality; **C**: WB analysis for protein expression; **D**: Measurement of ECAR; **E**: Assessment of glucose consumption, lactate production, and ATP generation levels; **F**: Apoptosis detection in various treatment groups by flow cytometry; **G**: WB for protein expression. * denotes $P<0.05$

anoikis of cancer stem cells but also accompanies a decrease in stemness characteristics (Oliveira et al. 2024). Inhibiting glycolytic metabolism can also weaken the anoikis resistance of triple-negative BC cells with strong migratory and invasive capabilities (O'Neill et al. 2019). Combined with our research findings, the activation of glycolysis inhibits anoikis in BC cells, which is consistent with previous literature reports. Furthermore, the role of glycolysis in immune evasion (Lin et al. 2023) and chemoresistance (Ma and Zong 2020) is gradually being substantiated. Therefore, future research should focus on elucidating the specific mechanisms of glycolysis in tumor cell survival, thereby

further uncovering the multifaceted roles of SQLE in the progression of BC

Of note, this study has confirmed the transcriptional activation role of transcription factor FOXM1 on SQLE by dual-luciferase and ChIP assays. FOXM1 belongs to the forkhead box protein superfamily and has been shown to play a role in various cellular processes, including proliferation, differentiation, DNA damage repair, cell apoptosis, and redox signaling (Kalathil et al. 2020). Multiple studies have indicated that FOXM1 can promote tumor development, metastasis, recurrence, and stemness (Khan et al. 2023; Sher et al. 2022). Mechanistically, FOXM1 can act as a transcriptional activator, regulating the expression of various genes such as KIF20A (Khongkow et al. 2016), Beclin-1, LC3 (Hamurcu et al. 2019), AURKA (Yang et al. 2017a, b), PDGF-A (Yu et al. 2015), and others. However, the transcriptional regulatory role of FOXM1 on SQLE has not been reported in previous studies. In this study, to investigate the impact of FOXM1 transcriptionally activating SQLE to regulate glycolysis on anoikis in BC cells, experimental groups were designed as follows: sh-NC + oe-NC, sh-FOXM1 + oe-NC, and sh-FOXM1 + oe-SQLE. The experimental results confirmed that overexpression of SQLE alleviated the inhibitory effects of FOXM1 knockdown on the expression of key glycolysis-related proteins and glycolytic metabolic levels. Additionally, it mitigated the promoting effects of FOXM1 knockdown on the levels of Caspase3 cleavage and PARP cleavage. Our study not only confirms that FOXM1 transcriptionally upregulates SQLE expression but also reveals that the FOXM1/SQLE axis inhibits BC cell anoikis through the glycolysis pathway, filling a gap in current research. This provides new insights into the mechanisms by which the FOXM1/SQLE axis functions in various cancers, including BC. Although existing studies have explored the role of FOXM1 in tumor biology, its specific mechanisms in regulating glycolysis and anoikis have not been systematically elucidated. Additionally, SQLE, as a potential therapeutic target, may provide new strategies for BC treatment. In combination with current BC treatment methods, such as targeted therapy and immunotherapy, interventions targeting SQLE may enhance treatment efficacy and overcome resistance to existing therapies. Therefore, we consider incorporating intervention experiments targeting SQLE in subsequent studies to validate its effectiveness and feasibility as a therapeutic target.

This study presents novel insights into the role of SQLE in BC anoikis, proving that SQLE curbs anoikis in BC via aerobic glycolysis. There is also a nascent signal that other metabolic pathways, including fatty acid oxidation (Choi et al. 2020; Tian et al. 2022), could be at play here, but more work is needed to flesh out these connections, which could be key to unlocking new avenues for treating BC. In the future, we will employ bioinformatics analysis methods combined with designed cellular functional experiments to investigate the impact of the FOXM1/SQLE axis on the regulation of fatty acid oxidation pathways and their influence on anoikis in BC cells. Our study demonstrates that high expression of FOXM1 and SQLE can serve as biomarkers for BC, indicating their potential use in the diagnosis and prognosis of BC. This suggests that detecting FOXM1 and SQLE expression levels could more accurately predict patient treatment responses and survival rates in the future.

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Declarations

Ethics approval and consent to participate Not applicable.

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References

Burguin A, Diorio C, Durocher F (2021) Breast cancer treatments: updates and new challenges. J Pers Med 11(8). https://doi.org/10.3390/jpm11080808From NLM PubMed-not-MEDLINE

Choi HJ, Jhe YL, Kim J, Lim JY, Lee JE, Shin MK, Cheong JH (2020) FoxM1-dependent and fatty acid oxidation-mediated ROS modulation is a cell-intrinsic drug resistance mechanism in cancer stem-like cells. Redox Biol 36:101589. https://doi.org/10.1016/j.redox.2020.101589From NLM Medline

Dai Y, Zhang X, Ou Y, Zou L, Zhang D, Yang Q, Qin Y, Du X, Li W, Yuan Z et al (2023) Anoikis resistance–protagonists of breast cancer cells survive and metastasize after ECM detachment. Cell Commun Signal 21(1):190. https://doi.org/10.1186/s12964-023-01183-4From NLM Medline

Fanfone D, Wu Z, Mammi J, Berthenet K, Neves D, Weber K, Halaburkova A, Virard F, Bunel F, Jamard C et al (2022) Confined migration promotes cancer metastasis through resistance to Anoikis and increased invasiveness. Elife 11. https://doi.org/10.7554/eLife.73150. From NLM

Ge H, Zhao Y, Shi X, Tan Z, Chi X, He M, Jiang G, Ji L, Li H (2019) Squalene epoxidase promotes the proliferation and metastasis of lung squamous cell carcinoma cells though extracellular signal-regulated kinase signaling. Thorac Cancer 10(3):428–436. https://doi.org/10.1111/1759-7714.12944From NLM

Gill S, Stevenson J, Kristiana I, Brown AJ (2011) Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. Cell Metab 13(3):260–273. https://doi.org/10.1016/j.cmet.2011.01.015From NLM

Grossmann J (2002) Molecular mechanisms of detachment-induced apoptosis–Anoikis. Apoptosis 7(3):247–260. https://doi.org/10.1023/a:1015312119693From NLM

Hamurcu Z, Delibasi N, Nalbantoglu U, Sener EF, Nurdinov N, Tasci B, Taheri S, Ozkul Y, Donmez-Altuntas H, Canatan H et al (2019) FOXM1 plays a role in autophagy by transcriptionally regulating Beclin-1 and LC3 genes in human triple-negative breast cancer cells. J Mol Med (Berl) 97(4):491–508. https://doi.org/10.1007/s00109-019-01750-8From NLM Medline

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013From NLM Medline

Icard P, Shulman S, Farhat D, Steyaert JM, Alifano M, Lincet H (2018) How the Warburg effect supports aggressiveness and drug resistance of cancer cells? Drug Resist Updat 38:1–11. https://doi.org/10.1016/j.drup.2018.03.001From NLM

Jin L, Chun J, Pan C, Alesi GN, Li D, Magliocca KR, Kang Y, Chen ZG, Shin DM, Khuri FR et al (2017) Phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumour metastasis. Oncogene 36(27):3797–3806. https://doi.org/10.1038/onc.2017.6From NLM

Kalathil D, John S, Nair AS (2020) FOXM1 and cancer: faulty cellular signaling derails homeostasis. Front Oncol 10:626836. https://doi.org/10.3389/fonc.2020.626836From NLM PubMed-not-MEDLINE

Khan MA, Khan P, Ahmad A, Fatima M, Nasser MW (2023) FOXM1: A small Fox that makes more tracks for cancer progression and metastasis. Semin Cancer Biol 92:1–15. https://doi.org/10.1016/j.semcancer.2023.03.007From NLM Medline

Khongkow P, Gomes AR, Gong C, Man EP, Tsang JW, Zhao F, Monteiro LJ, Coombes RC, Medema RH, Khoo US et al (2016) Paclitaxel targets FOXM1 to regulate KIF20A in mitotic catastrophe and breast cancer Paclitaxel resistance. Oncogene 35(8):990–1002. https://doi.org/10.1038/onc.2015.152From NLM Medline

Li FL, Liu JP, Bao RX, Yan G, Feng X, Xu YP, Sun YP, Yan W, Ling ZQ, Xiong Y et al (2018) Acetylation accumulates PFKFB3 in cytoplasm to promote Glycolysis and protects cells from cisplatin-induced apoptosis. Nat Commun 9(1):508. https://doi.org/10.1038/s41467-018-02950-5From NLM

Li C, Wang Y, Liu D, Wong CC, Coker OO, Zhang X, Liu C, Zhou Y, Liu Y, Kang W et al (2022) Squalene epoxidase drives cancer cell proliferation and promotes gut dysbiosis to accelerate colorectal carcinogenesis. Gut 71(11):2253–2265. https://doi.org/10.1136/gutjnl-2021-325851From NLM

Lin J, Fang W, Xiang Z, Wang Q, Cheng H, Chen S, Fang J, Liu J, Wang Q, Lu Z et al (2023) Glycolytic enzyme HK2 promotes PD-L1 expression and breast cancer cell immune evasion. Front Immunol 14:1189953. https://doi.org/10.3389/fimmu.2023.1189953From NLM Medline

Ma L, Zong X (2020) Metabolic symbiosis in chemoresistance: refocusing the role of aerobic Glycolysis. Front Oncol 10. https://doi.org/10.3389/fonc.2020.00005From NLM PubMed-not-MEDLINE

O'Neill S, Porter RK, McNamee N, Martinez VG, O'Driscoll L (2019) 2-Deoxy-D-Glucose inhibits aggressive triple-negative breast cancer cells by targeting Glycolysis and the cancer stem cell phenotype. Sci Rep 9(1):3788. https://doi.org/10.1038/s41598-019-39789-9From NLM Medline

Oliveira G, Mota SI, Oliveira PJ, Marques R (2024) Adenine nucleotide translocase 2 silencing promotes metabolic adaptations and anoikis in P19 embryonal carcinoma stem cells. *bioRxiv*

Paoli P, Giannoni E, Chiarugi P (2013) Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta 1833(12):3481–3498. https://doi.org/10.1016/j.bbamcr.2013.06.026From NLM

Prager GW, Braga S, Bystricky B, Qvortrup C, Criscitiello C, Esin E, Sonke GS, Martínez GA, Frenel JS, Karamouzis M et al (2018) Global cancer control: responding to the growing burden, rising costs and inequalities in access. ESMO Open 3(2):e000285. https://doi.org/10.1136/esmoopen-2017-000285From NLM

Qin Y, Hou Y, Liu S, Zhu P, Wan X, Zhao M, Peng M, Zeng H, Li Q, Jin T et al (2021) A novel long Non-Coding RNA lnc030 maintains breast cancer stem cell stemness by stabilizing SQLE mRNA and increasing cholesterol synthesis. Adv Sci (Weinh) 8(2):2002232. https://doi.org/10.1002/advs.202002232From NLM

Sa KD, Zhang X, Li XF, Gu ZP, Yang AG, Zhang R, Li JP, Sun JY (2018) A miR-124/ITGA3 axis contributes to colorectal cancer metastasis by regulating Anoikis susceptibility. Biochem Biophys Res Commun 501(3):758–764. https://doi.org/10.1016/j.bbrc.2018.05.062From NLM

Schreier A, Zappasodi R, Serganova I, Brown KA, Demaria S, Andreopoulou E (2022) Facts and perspectives: implications of tumor Glycolysis on immunotherapy response in triple negative breast cancer. Front Oncol 12:1061789. https://doi.org/10.3389/fonc.2022.1061789From NLM PubMed-not-MEDLINE

Sher G, Masoodi T, Patil K, Akhtar S, Kuttikrishnan S, Ahmad A, Uddin S (2022) Dysregulated FOXM1 signaling in the regulation of cancer stem cells. Semin Cancer Biol 86(Pt 3):107–121. https://doi.org/10.1016/j.semcancer.2022.07.009From NLM Medline

Sui Z, Zhou J, Cheng Z, Lu P (2015) Squalene epoxidase (SQLE) promotes the growth and migration of the hepatocellular carcinoma cells. Tumour Biol 36(8):6173–6179. https://doi.org/10.1007/s13277-015-3301-xFrom NLM

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249. https://doi.org/10.3322/caac.21660From NLM Medline

Tang W, Xu F, Zhao M, Zhang S (2021) Ferroptosis regulators, especially SQLE, play an important role in prognosis, progression and immune environment of breast cancer. BMC Cancer 21(1):1160. https://doi.org/10.1186/s12885-021-08892-4From NLM Medline

Tian T, Lu Y, Lin J, Chen M, Qiu H, Zhu W, Sun H, Huang J, Yang H, Deng W (2022) CPT1A promotes Anoikis resistance in esophageal squamous cell carcinoma via redox homeostasis. Redox Biol 58:102544. https://doi.org/10.1016/j.redox.2022.102544From NLM Medline

Wang S, Dong L, Ma L, Yang S, Zheng Y, Zhang J, Wu C, Zhao Y, Hou Y, Li H et al (2022) SQLE facilitates the pancreatic cancer progression via the lncRNA-TTN-AS1/miR-133b/SQLE axis. J Cell Mol Med 26(13):3636–3647. https://doi.org/10.1111/jcmm.17347From NLM

Wu Z, Wu J, Zhao Q, Fu S, Jin J (2020) Emerging roles of aerobic Glycolysis in breast cancer. Clin Transl Oncol 22(5):631–646. https://doi.org/10.1007/s12094-019-02187-8From NLM Medline

Yang Z, Jin Q, Hu W, Dai L, Xue Z, Man D, Zhou L, Xie H, Wu J, Zheng S (2017a) 14-3-3σ downregulation suppresses ICC metastasis via impairing migration, invasion, and Anoikis resistance of ICC cells. Cancer Biomark 19(3):313–325. https://doi.org/10.3233/cbm-160476From NLM

Yang N, Wang C, Wang Z, Zona S, Lin SX, Wang X, Yan M, Zheng FM, Li SS, Xu B et al (2017b) FOXM1 recruits nuclear Aurora kinase A to participate in a positive feedback loop essential for the self-renewal of breast cancer stem cells. Oncogene 36(24):3428–3440. https://doi.org/10.1038/onc.2016.490From NLM Medline

Yu G, Zhou A, Xue J, Huang C, Zhang X, Kang SH, Chiu WT, Tan C, Xie K, Wang J et al (2015) FoxM1 promotes breast tumorigenesis by activating PDGF-A and forming a positive feedback loop with the PDGF/AKT signaling pathway. Oncotarget 6(13):11281–11294. https://doi.org/10.18632/oncotarget.3596From NLM Medline

Yu B, Gu D, Zhang X, Liu B, Xie J (2022) Regulation of pancreatic cancer metastasis through the Gli2-YAP1 axis via regulation of Anoikis. Genes Dis 9(6):1427–1430. https://doi.org/10.1016/j.gendis.2022.05.010From NLM

Zhang L, Li S (2020) Lactic acid promotes macrophage polarization through MCT-HIF1α signaling in gastric cancer. Exp Cell Res 388(2):111846. https://doi.org/10.1016/j.yexcr.2020.111846From NLM

Zhang R, Deng Y, Lv Q, Xing Q, Pan Y, Liang J, Jiang M, Wei Y, Shi D, Xie B et al (2020) SQLE promotes differentiation and apoptosis of bovine skeletal Muscle-Derived mesenchymal stem cells. Cell Reprogram 22(1):22–29. https://doi.org/10.1089/cell.2019.0077From NLM

Zhao S, Guan B, Mi Y, Shi D, Wei P, Gu Y, Cai S, Xu Y, Li X, Yan D et al (2021) LncRNA MIR17HG promotes colorectal cancer liver metastasis by mediating a glycolysis-associated positive feedback circuit. Oncogene 40(28):4709–4724. https://doi.org/10.1038/s41388-021-01859-6From NLM

Zhao F, Huang Y, Zhang Y, Li X, Chen K, Long Y, Li F, Ma X (2022) SQLE Inhibition suppresses the development of pancreatic ductal adenocarcinoma and enhances its sensitivity to chemotherapeutic agents in vitro. Mol Biol Rep 49(7):6613–6621. https://doi.org/10.1007/s11033-022-07504-zFrom NLM