Pfkfb3 Regulates Epithelial-To-Mesenchymal Transition in Tumor Cells

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Research Article/Araştırma Makalesi

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ABSTRACT

Background and aim: Reprogramming of energy metabolism is suggested to play a key role in promotion of the epithelial-to-mesenchymal transition (EMT) program associated with neoplastic features such as metastasis and chemoresistance. Given the effect of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase-3 (PFKFB3) on regulation of glycolysis, we sought to determine whether PFFKB3 is required to maintain the steady-state expressions of EMT genes in tumor cell lines.

Materials and methods: Tumor cell lines that were used as in vitro models included HCT116 (colon), S2VP10 (pancreas), MCF-7 (breast), MDA-MB-231 (breast), and HeLa (cervix). Endogenous PFKFB3 expression was silenced by transfecting cells with a combination of two different siRNA molecules specific for the coding region of the PFKFB3 mRNA. Real-time quantitative PCR and Western blot were used to measure mRNA and protein levels, respectively. Glucose consumption and lactate production were determined spectrophotometrically using commercial kits. HCT116 cells were stably transfected with an expression vector containing PFKFB3 cDNA for oncosphere formation assays. The Cancer Genom Atlas (TCGA) datasets from colorectal adenocarcinoma patients were analyzed using cBioportal tool to study the correlation between PFKFB3 and EMT gene expressions.

Results and conclusions: We demonstrated that silencing of PFKFB3 resulted in changes in expressions of EMT genes, such as E-cadherin, Vimentin and Snail in various tumor cell lines and that PFKFB3 mRNA expression correlates with mRNA levels of mesenchymal genes in colorectal adenocarcinoma patients. We further show that ectopically expressed PFKFB3 increases the ability of HCT116 cells to form oncospheres. Manipulation of PFKFB3 activity may be considered a viable approach to target malignant traits such as metastasis and chemoresistance that is associated with EMT.

Keywords: Epithelial-to-mesenchymal transition, glycolysis, PFKFB3

PFKFB3 Tümör Hücrelerinde Epitelyal-Mezenkimal Geçişi Düzenler

ÖZET

Amaç: Tümör hücrelerinde enerji metabolizmasının yeniden programlanmasının, metastaz ve kemorezistans dahil olmak üzere malign özelliklerle ilişkili epitelyal-mezenkimal geçiş (EMT) programının desteklenmesinde önemli bir rol oynadığı bilinmektedir. 6-fosfofrukto-2-kinaz/fruktoz-2,6-bifosfataz-3 (PFKFB3)'ün glikolitik aktivitenin düzenlenmesindeki rolü nedeniyle bu çalışmada, PFFKB3'ün EMT genlerinin ekspresyonundaki olası rolünü belirlemeyi amaçladık.

Yöntem: HCT116 (kolon adenokarsinomu), S2VP10 (SUIT-2'nin metastatik bir varyantı, pankreatik adenokarsinom), MCF-7 (meme karsinomu), MDA-MB-231 (meme karsinomu) ve HeLa (serviks karsinomu) hücre hatları in vitro modeller olarak kullanıldı. Endojen PFKFB3 ekspresyonu, hücrelerin PFKFB3 mRNA'nın kodlama bölgesine özgü iki farklı siRNA molekülünün kombinasyonu ile transfekte edilmesiyle susturuldu. mRNA ve protein ekspresyon seviyeleri, sırasıyla gerçek-zamanlı kantitatif PCR ve Western blot kullanılarak ölçüldü. Glikoz tüketimi ve laktat üretimi, ticari kitler kullanılarak spektrofotometrik olarak belirlendi. HCT116 hücreleri, onkoküre oluşum analizleri için PFKFB3 cDNA içeren bir ekspresyon vektörü ile stabil bir şekilde transfekte edildi. Kolorektal adenokarsinom hastalarına ait Kanser Genom Atlası (TCGA) veri kümeleri, PFKFB3 ve EMT gen ifadeleri arasındaki korelasyonu incelemek için cBioPortal aracı kullanılarak analiz edildi.

Bulgular ve Sonuç: PFKFB3'ün susturulmasının çeşitli tümör hücre hatlarında E-cadherin, Vimentin ve Snail adlı EMT genlerinin ifadelerinde değişikliklere yol açtığını ve PFKFB3 mRNA ifadesinin kolorektal adenokarsinom hastalarında mezenkimal genlerin mRNA seviyeleri ile korelasyon gösterdiğini gözlemledik. Ayrıca, PFKFB3'ün ektopik ifadesinin, HCT116 hücrelerinin onkoküre oluşturma yeteneğini arttırdığını raporladık. PFKFB3 aktivitesinin manipülasyonu, metastaz ve EMT ile ilişkili kemorezistans gibi habis özellikleri hedeflemek için geçerli bir yaklaşım olarak kabul edilebilir.

Anahtar Kelimeler: Epitelyal-mezenkimal geçiş, glikoliz, PFKFB3

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INTRODUCTION

The epithelial-to-mesenchymal transition (EMT) is an essential cellular event through which epithelial cells convert into a mesenchymal phenotype and plays a critical function in embryonic development, cellular differentiation, and tissue regeneration(Huang et al., 2022). In addition, the EMT endows carcinoma cells with invasive and metastatic capabilities, as well as stem cell properties with heightened drug resistant phenotype (Roche, 2018). At molecular level, during EMT, expressions of epithelial markers, such as the E-cadherin protein, are lost or decreased whereas expressions of mesenchymal markers, such as Vimentin and N-cadherin proteins increase (Serrano-Gomez et al., 2016). The EMT is triggered and promoted by several EMT-associated transcription factors, such as the zinc finger transcription factors Snail (encoded by *SNAI1*) and Slug (encoded by *SNAI2*), and the ZEB family of transcription factors, such as ZEB1 (Lambert et al., 2017; Nieto et al., 2016). The EMT in tumor cells can be induced by a variety of stimuli, e.g. cytokines, transforming growth factor β, and hypoxia (Lambert et al., 2017).

Tumor cells reprogram their energy metabolism to fulfill energetic and biosynthetic requirements of cell division and rapid proliferation, to favor a permissive microenvironment for invasion and metastasis, as well as to burst antioxidant capacity of the invasive and metastatic tumor cells, causing resistance to death signals (Marcucci & Rumio, 2022). For example, acidification of the tumor microenvironment due to increased lactate export by upregulation of the glycolytic activity has been shown to promote an EMT that promotes invasion and metastasis (Pérez-Tomás & Pérez-Guillén, 2020). Thus, elucidation of key enzymes and regulators of glycolysis that contribute to the EMT in tumor cells may help to identify targets that can be utilized for therapeutic purposes.

6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (PFKFB) family of proteins (PFKFB1, PFKFB2, PFKFB3, and PFKFB4) are bifunctional enzymes with kinase and bisphosphatase functions that interconvert fructose 6-phosphate (F6P) and fructose 2,6-bisphoshate (F2,6BP), a powerful allosteric activator of a rate-limiting enzyme of glycolysis, 6-phoshofructo-1-kinase (PFK1) (Chesney, 2006; Okar et al., 2001). PFKFB3 expression is upregulated in multiple tumor types (Atsumi et al., 2002) and its expression has been linked to EMT-associated malignant features including invasiveness and stem cell properties (Cieślar-Pobuda et al., 2015), suggesting that PFKFB3 may contribute to acquisition and/or maintenance of a mesenchymal phenotype in tumor cells. Although PFKFB3 has been shown to be induced by the EMT inducing Transforming Growth Factor βeta-1 (TGFβ1) (Yalcin et al., 2017), whether PFKFB3 expression at steady-state levels is required to maintain an EMT phenotype in tumor cells is yet to be explored.

The objective of this study is to investigate the effect of endogenous PFKFB3 on the steady-state expressions of EMT genes in tumor cell lines of various origins.

MATERIALS AND METHODS

Cell Culture and Treatment

HeLa (ATCC, CCL-2), HCT116 (ATCC, CCL-247), MCF-7 (ATCC, HTB-22), MDA-MB-231 (ATCC, CRM-HTB-26), and S2VP10 cells (a subclone of the SUIT-2 pancreatic adenocarcinoma cell line; a gift from Lacey McNally, Wake Forest School of Medicine, NC) were cultured in DMEM containing 10% fetal bovine serum (FBS). Cells were grown at 37 °C in 5% CO_2.

Real-time Quantitative PCR

Real-time quantitative PCR (qPCR) analyses were carried out on StepOne Plus (ThermoFisher, U.S.A) using gene-specific TaqMan probes (ThermoFisher) following the manufacturer's instructions. The following TaqMan probes were used: PFKFB3, Hs00998698_m1; SNAI1, Hs00195591_m1; SNAI2, Hs00161904_m1; ZEB1, Hs01566408_m1; CDH1, Hs01023895_m1; VIM, Hs00958111_m1; and β-actin, Hs99999903_m1). β-actin was used as endogenous control.

Western Blot Analysis

Cells were washed twice in PBS and lysed with RIPA buffer. Proteins were run on a 10% SDS-PAGE gel and transferred onto PVDF membrane. The membrane was blocked in 5% nonfat dry milk dissolved in Tris-buffered saline and were incubated with primer antibodies against PFKFB3 (Proteintech, 13763-1-AP), E-cadherin (Cell Signaling, 3195), Vimentin (Cell Signaling, 5741) and β-actin (Cell Signaling, 3700) overnight at 4 °C. HRP-conjugated goat anti-rabbit or anti-mouse (Cell Signaling) secondary antibodies were used. Immunoreactive bands were visualized using chemiluminescence (ECL, Amersham) in ChemiDoc™ MP Imaging System (Bio-Rad).

Immunofluorescence

The cells seeded on coverslides (BD Biosciences) were fixed with %4 formaldehyde and processed for immunofluorescence as described in antibody manufacturer's instructions (Cell Signaling). The fixed cells were incubated with the primary antibody against E-cadherin (Cell Signaling, 3195) overnight at 4°C followed by Alexa-Fluor 488-conjugated goat anti-rabbit antibody (Cell Signaling, 4412). Images were analyzed and captured with a FluoView 500 confocal laser microscope (Olympus).

Glucose and lactate assays

Glucose and lactate levels in the media were analyzed spectrophotometrically using commercially available enzyme-based assay kits following the manufacturer's protocol (Biovision Cat. #K606-100 and K607-100) with BioTek Epoch microplate reader.

PFKFB3 cDNA construct and plasmid transfection

The mammalian expression construct on the pIRESneo3 plasmid backbone for the human PFKFB3 was previously created using protocols described according to the Yalcin et al. (Yalcin et al., 2009). Transfections were conducted using Lipofectamine (ThermoFisher). Cells were grown to about 80% confluency before transfection. HCT116 cells that stably express PFKFB3 were selected in media containing the neomycin analog G418 (Gibco).

Oncosphere formation assay

Sphere formation assays were done as described elsewhere (Sato et al., 2019). Briefly, 1000 cells were cultured in ultralow attachment plates (Corning) in 50% DMEM and 50% Ham's F12 with the following supplements: EGF (20 ng/ml, PeproTech), bFGF (20 ng/ml, PeproTech) and B27 (Invitrogen). Fourteen days later, visible spheres were counted using EVOS imaging system (ThermoFisher).

Database analysis

The TCGA Colorectal Adenocarcinoma PanCancer dataset (594 samples) was analyzed using cBioPortal tool (Cerami et al., 2012; Gao et al., 2013) to determine correlations between mRNA levels of PFKFB3 and EMT genes.

Statistical analysis

The experiments were carried out at least three times and all data were expressed as the mean ± SD Statistical significance was assessed by unpaired t-tests and $p<0,05$ was considered to be statistically significant.

RESULTS

SiRNA-mediated silencing of PFKFB3 leads to differential changes in expressions of EMT markers and transcription factors in tumor cell lines

Although PFKFB3 has recently been shown to induce an EMT phenotype when ectopically expressed in gastric cancer cells (Lei et al., 2021), it remains to be determined if the steady-state levels of PFKFB3, i.e., the endogenously expressed PFKFB3, is needed to maintain the expression levels of EMT genes. We, therefore, reduced endogenous PFKFB3 expression in HCT16 (colon adenocarcinoma), S2VP10 (a metastatic variant of SUIT-2, pancreatic adenocarcinoma), MCF-7 (breast carcinoma), MDA-MB-231 (breast carcinoma), and HeLa (cervix carcinoma) cell lines by siRNA-mediated silencing using a combination of two individual validated siRNA molecules, or non-targeting control siRNA, and 48 h later, mRNA expressions of the prototypical epithelial marker E-cadherin, the mesenchymal marker Vimentin, and the EMT transcription factors Snail, Slug, and ZEB1 were analysed by real-time qPCR. As shown in Figure 1, PFKFB3 mRNA levels were markedly

diminished in siRNA-transfected cells relative to control siRNA transfected cells, as expected, validating the efficacy of the siRNA molecules in depleting the endogenously expressed PFKFB3. E-cadherin mRNA levels (encoded by the *CDH1* gene) was reduced in HCT116 cells but increased in HeLa and MDA-MB-231 cells, whereas Vimentin mRNA levels was reduced in HCT116 and S2VP10 cells, upon PFKFB3 silencing (Figure 1). While PFKFB3 silencing led to decreases in both Snail and Slug mRNA levels in S2VP10 cells, it led to decreases in Snail mRNA in HeLa cells and Slug mRNA levels in HCT116 cells. PFKFB3 silencing resulted in a reduction in ZEB1 mRNA levels only in S2VP10 cells (Figure 1). These results suggest a differential effect of the endogenous PFKFB3 expression on mRNA expression levels of EMT genes.

SiRNA-mediated silencing of PFKFB3 leads to an increase in E-cadherin and a decrease in Vimentin protein levels in cell lines

To determine if siRNA-mediated silencing of PFKFB3 leads to changes in protein levels of E-cadherin and Vimentin, consistent with the effect on mRNA levels (Figure 1), we conducted Western blot analyses on total cell lysates prepared from PFKFB3 siRNA and control siRNA-transfected cells using antibodies specific for E-cadherin, Vimentin and PFKFB3. As shown in Figure 2A, there was an increase in E-cadherin protein in HCT116 and S2VP10 cells, and a decrease in Vimentin protein levels in HeLa, S2VP10 and MDA-MB-231 cells, suggesting that the steady-state expression level of the endogenous PFKFB3 is required to maintain a reduced E-cadherin and increased Vimentin protein levels, consistent with its involvement in the mesenchymal phenotype in these cells. The observation that PFKFB3 silencing caused an increase in E-cadherin protein levels (Figure 2A) with no corresponding change in E-cadherin mRNA levels may suggest an effect on stability of the E-cadherin protein. We note that in all the cell lines studied, we were unable to detect the E-cadherin protein in control siRNA-transfected cells, which may suggest a highly mesenchymal phenotype in these cells, except for HCT116 cells (Ieda et al., 2019). We, however, cannot rule out a relatively low sensitivity of the E-cadherin antibody used.

Given that the expression of the E-cadherin protein on the cell membrane is critical for its function and that total E-cadherin protein levels may not always correlate with EMT (Wagoner et al., 2008), we analyzed E-cadherin protein localization in cells using immunofluorescence and observed an increased E-cadherin protein localization to the cell membrane in both HCT116 and S2VP10 cells transfected with PFKFB3 siRNA compared with control siRNA-transfected cells (Figure 2B), confirming the effect of PFKFB3 on E-cadherin expression on the cell membrane.

SiRNA-mediated silencing of PFKFB3 reduces glucose consumption

For the remainder of the study, we chose HCT116 cells as model, because we previously showed that PFKFB3 expression is essential to proliferation of HCT116 cells (Yalcin et al., 2009). Given that PFKFB3 is postulated to play a key role glycolytic activity in tumor cells (Atsumi et al.,

2002), we set out to assess the effect of PFKFB3 depletion on glucose consumption and lactate production as a surrogate for glycolytic activity. PFKFB3 silencing led to a substantial decrease in glucose consumption ($p<0.01$) (Figure 3A), while it had no effect on lactate production in HCT116 cells (Figure 3B).

PFKFB3 expression potentiates the oncosphere formation of HCT116 cells

The EMT has been suggested to contribute to three-dimensional growth of tumor cells as oncospheres in non-adherent and low-mitogen conditions (Ishiguro et al., 2017). To determine if PFKFB3 stimulates the ability of HCT116 cells to grow as oncospheres, we stably expressed PFKFB3 in HCT116 cells and performed sphere formation assays. We first confirmed increased expression of the PFKFB3 protein in transfected cells (Figure 4A). We found that both the size and number of spheres were significantly higher in cells with PFKFB3 overexpression compared with vector-transfected control cells (Figure 4B, C), suggesting that PFKFB3 contribute to the oncogenicity of HCT116 cells.

PFKFB3 expression positively correlates with E-cadherin and negatively correlates with mesenchymal gene expressions in colorectal tumor samples

Because *in vitro* data do not always reflect the actual phenotype in patient tumors, we went on to analyse The Cancer Genom Atlas (TCGA) datasets (encompassing 594 samples) to assess if PFKFB3 mRNA correlates with key EMT genes in colorectal adenocarcinoma patients. As shown in Figure 5, PFKFB3 mRNA levels inversely correlate with E-cadherin mRNA (spearman: -0.24), and positively correlates with Vimentin mRNA (spearman: 0.43), Snail mRNA (spearman: 0.25), Slug mRNA (spearman: 0.33) and ZEB1 mRNA (spearman: 0.24) expressions. These data indicate that PFKFB3 expression may play a role in the mesenchymal phenotype of colorectal cancers, although whether this correlation exists at protein levels remains to be determined.

DISCUSSION

Given the recent evidence indicating a requirement for increased glycolytic activity in the EMT and related phenotypes such as stemness (Marcucci & Rumio, 2022), studies that are aimed at determining whether PFKFB3, a key glycolytic stimulator, contributes to the EMT in tumor cells are warranted. Although our previous study (Yalcin et al., 2017) showed that PFKFB3 is induced by TGFβ1, a potent EMT promoter (Xu et al., 2009), and is required for TGFβ1-induced Snail expression that is associated with invasion in a pancreatic adenocarcinoma cell model, whether the steady-state expression of PFKFB3 is required to maintain the expression levels of EMT markers and transcription factors is unknown.

Our current report demonstrates a differential effect of PFKFB3 on expressions of EMT genes (Figure 1) in different tumor cell lines. For example, PFKFB3 depletion led to decreases in mRNA

expressions of the EMT transcription factors Snail and Slug in HeLa and HCT116 cells, respectively, ZEB1 in S2VP10 cells and Vimentin expression in S2VP10 cells and increases in E-cadherin expression in HeLa and MDA-MB-231 cells. *In-silico* correlation analyses between PFKFB3 mRNA and EMT genes in colorectal cancer patients using TCGA PanCancer datasets suggest that the observed effect of the silencing of endogenous PFKFB3 on EMT genes may not a merely *in vitro* phenomenon, rather this effect is maintained in tumor samples, indicating a translational relevance. Collectively presented data suggest that steady-state levels of PFKFB3 affects expressions of several EMT genes and may be involved in maintenance of the mesenchymal phenotype of tumors, although this effect appears to tumor type-dependent. Despite a marked decrease in Snail mRNA expression, we did not observe a corresponding decrease in Vimentin mRNA expression in HeLa cells upon PFKFB3 silencing under the conditions used in this study (Figure 1). Further, the increase in E-cadherin mRNA in PFKFB3-depleted MDA-MB-231 cells was not accompanied by a corresponding suppression of Snail, Slug or ZEB1 transcription factors, which are known repressors of the *CDH1* gene and activators of the *VIM* gene (encoding Vimentin) (Stemmler et al., 2019). We speculate that additional EMT transcription factors, such as Twist, which is known to have overlapping functions with Snail, Slug and ZEB1 with regard to E-cadherin and Vimentin expression (Jung & Yang, 2015), may play a dominant role in the expression of E-cadherin and Vimentin in these cells. Whether Twist or other EMT transcription factos, e.g. ZEB2, may be involved in mediating the effects of PFKFB3 on E-cadherin and Vimentin expressions in tumor cells remains to be investigated.

A recent study (Thirusangu et al., 2022) published during the preparation of this study showed that, in a small-cell lung cancer model, PFKFB3 inhibition led to decreases in the Snail and Vimentin expression and suppressed oncosphere formation, a trait of cancer stem cells associated with EMT. Our current study also supports the postulation that PFKFB3 regulates EMT in tumor cells and oncosphere formation in a colorectal adenocarcinoma model, although the *in vitro* models used in these studies are different. Another recent study (Lei et al., 2021) has shown a correlation of high PFKFB3 expression with mesenchymal genes and induction of mesenchymal genes upon ectopic expression of PFKFB3 in gastric cancer cells. Combined with our current study where the effect of PFKFB3 on EMT genes were analysed in tumor cells of various origin, these recent and current data lend support to the hypothesis that high PFKFB3 expression in tumor cells may be essential to maintaining a quasi-mesenchymal phenotype that is needed to acquire malignant traits such as invasion, stemness and chemoresistance, and add to the increasing body of evidence implicating key enzymes of glucose metabolism and regulators in endowing tumor cells with mesenchymal traits (Sciacovelli & Frezza, 2017). In the current study, PFKFB3 depletion did not result in a decrease in the glycolytic activity as assessed by lactate release (Figure 3). Although this observation appears to run counter to the presumed role of PFKFB3 in glycolysis, the lack of an obvious effect on glycolysis supports previous studies conducted by others (Li et al., 2018) and us (Yalcin et al., 2009) that suggest a role for PFKFB3 in the nucleus for coordination of glucose metabolism with cell cycle and cell

proliferation. Whether the effect of PFKFB3 on EMT is connected to its role in regulation of glucose metabolism remains unknown.

CONCLUSION AND RECOMMENDATIONS

In conclusion, using siRNA-mediated approach, we demonstrated that PFKFB3 is involved in maintaining steady-state expression levels of EMT markers in tumor cells of various origin and that PFKFB3 expression correlates with mesenchymal gene expressions in colorectal carcinoma tumors. Given that EMT is linked to malignant features such as metastasis and chemoresistance, targeting of PFKFB3 may be an effective strategy in management of tumor progression. Further studies are needed to determine the mechanism by which PFKFB3 regulates the expression of EMT genes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure 1.

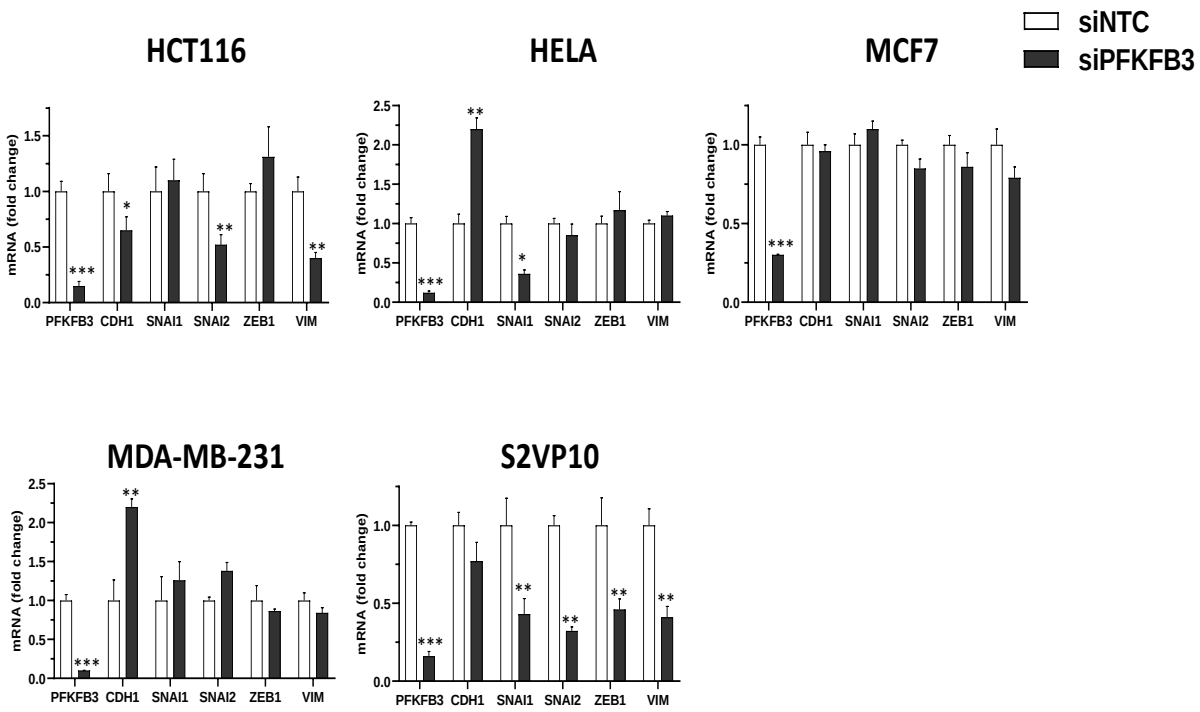
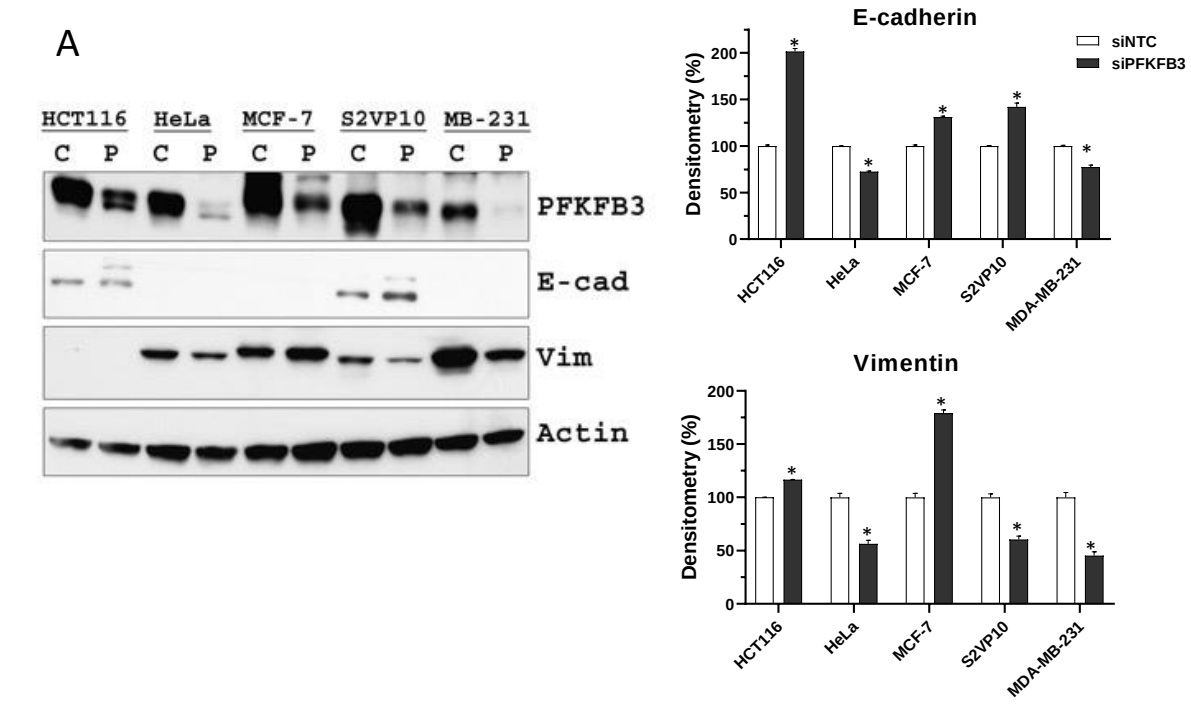

HCT116
HELA
MCF7
siNTC
siPFKFB3
MDA-MB-231
S2VP10
mRNA (fold change)
PFKFB3 CDH1 SNAI1 SNAI2 ZEB1 VIM

Figure 2.


A
HCT116
HeLa
MCF-7
S2VP10
MB-231
C P C P C P C P C P
PFKFB3
E-cad
Vim
Actin
E-cadherin
siNTC
siPFKFB3
Densitometry (%)
0
50
100
150
200
HCT116
HeLa
MCF-7
S2VP10
MDA-MB-231
Vimentin
Densitometry (%)
0
50
100
150
200
HCT116
HeLa
MCF-7
S2VP10
MDA-MB-231

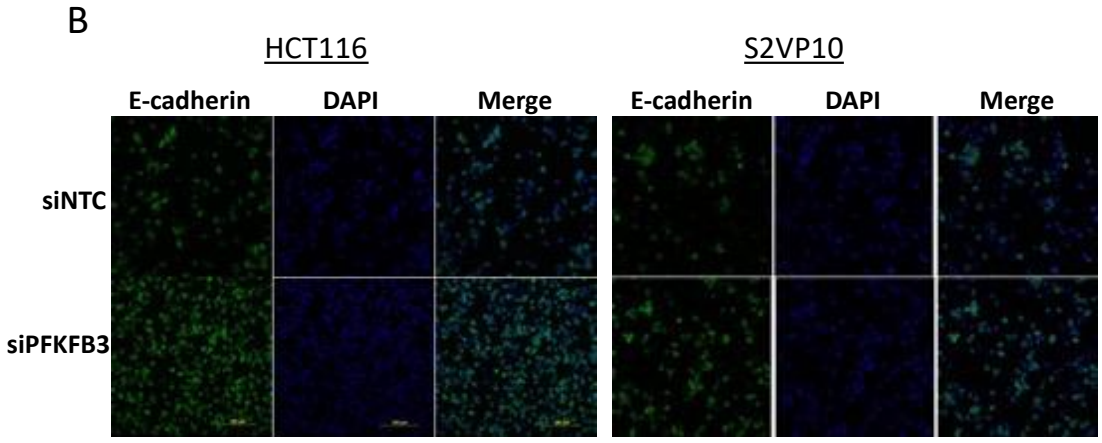

B
HCT116
S2VP10
E-cadherin
DAPI
Merge
E-cadherin
DAPI
Merge
siNTC
siPFKFB3

Figure 3.

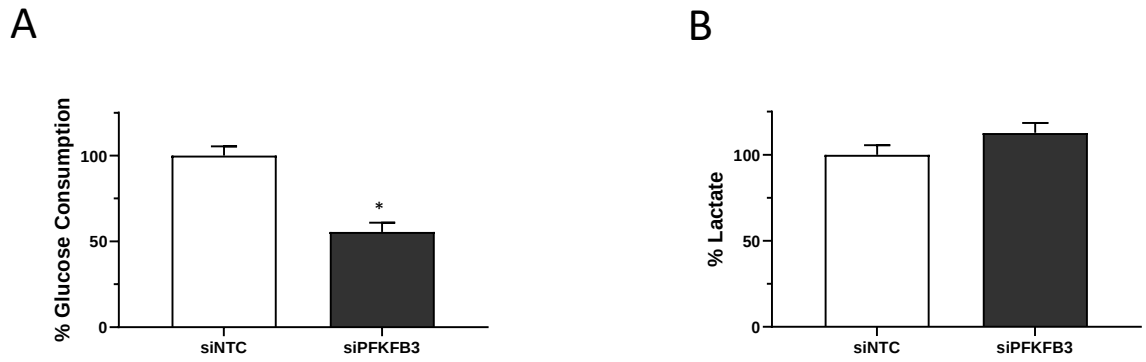


Figure 4.

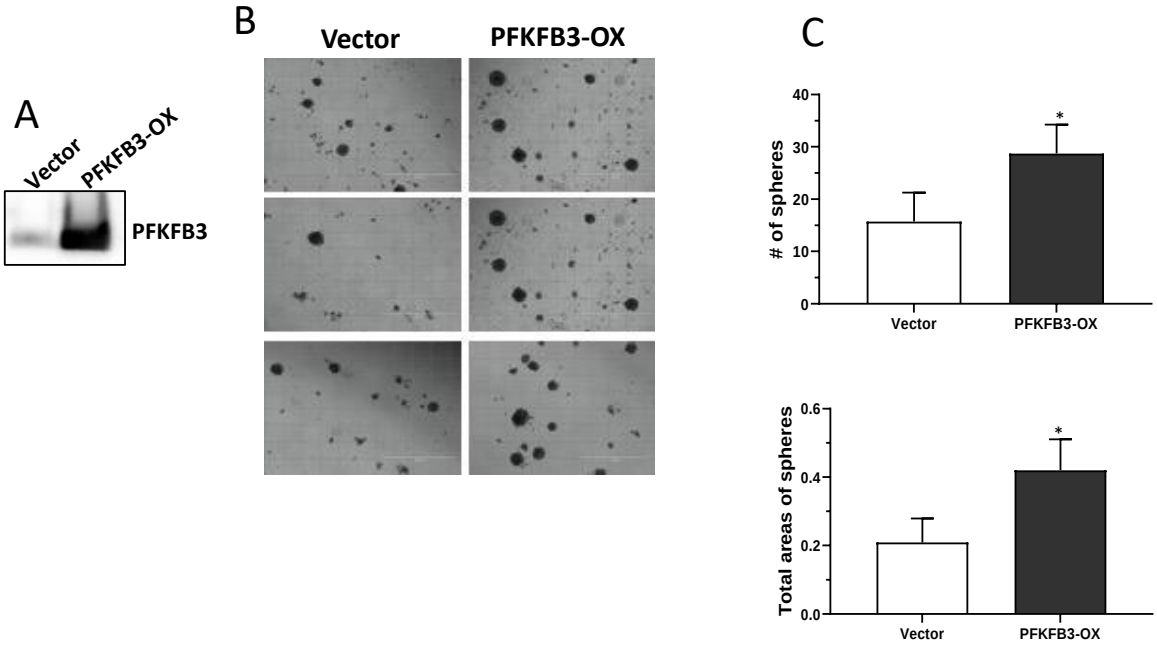
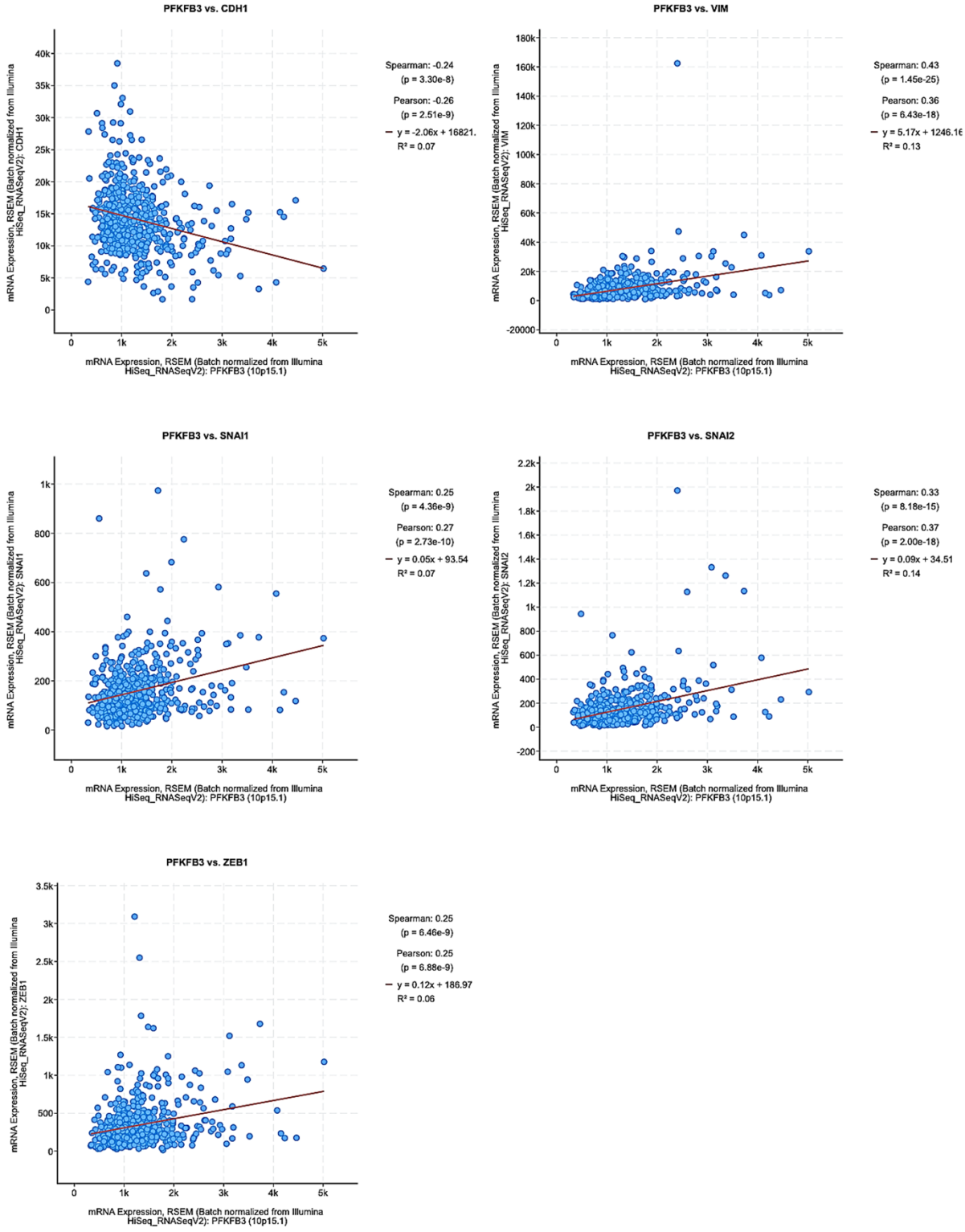


Figure 5.


PFKFB3 vs. CDH1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): CDH1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): PFKFB3 (10p15.1)
Spearman: -0.24
(p = 3.30e-8)
Pearson: -0.26
(p = 2.51e-9)
y = -2.06x + 16821.
R² = 0.07
PFKFB3 vs. VIM
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): VIM
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): PFKFB3 (10p15.1)
Spearman: 0.43
(p = 1.45e-25)
Pearson: 0.36
(p = 6.43e-18)
y = 5.17x + 1246.1
R² = 0.13
PFKFB3 vs. SNAI1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): SNAI1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): PFKFB3 (10p15.1)
Spearman: 0.25
(p = 4.36e-9)
Pearson: 0.27
(p = 2.73e-10)
y = 0.05x + 93.54
R² = 0.07
PFKFB3 vs. SNAI2
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): SNAI2
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): PFKFB3 (10p15.1)
Spearman: 0.33
(p = 8.18e-15)
Pearson: 0.37
(p = 2.00e-18)
y = 0.09x + 34.51
R² = 0.14
PFKFB3 vs. ZEB1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): ZEB1
mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2): PFKFB3 (10p15.1)
Spearman: 0.25
(p = 6.46e-9)
Pearson: 0.25
(p = 6.88e-9)
y = 0.12x + 186.97
R² = 0.06