UDP-glucose dehydrogenase expression is upregulated following EMT and differentially affects intracellular glycerophosphocholine and acetylaspartate levels in breast mesenchymal cell lines

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Metabolic rewiring is one of the indispensable drivers of epithelial–mesenchymal transition (EMT) involved in breast cancer metastasis. In this study, we explored the metabolic changes during spontaneous EMT in three separately established breast EMT cell models using a proteomic approach supported by metabolomic analysis. We identified common proteomic changes, including the expression of CDH1, CDH2, VIM, LGALS1, SERPINE1, PKP3, ATP2A2, JUP, MTCH2, RPL26L1 and PLOD2. Consistently altered metabolic enzymes included the following: FDFT1, SORD, TSTA3 and UDP-glucose dehydrogenase (UGDH). Of these, UGDH was most prominently altered and has previously been associated with breast cancer patient survival. siRNA-mediated knock-down of UGDH resulted in delayed cell proliferation and dampened invasive potential of mesenchymal cells and downregulated expression of the EMT transcription factor SNAI1. Metabolomic analysis revealed that siRNA-mediated knock-down of UGDH decreased intracellular glycerophosphocholine (GPC), whereas levels of acetylaspartate (NAA) increased. Finally, our data suggested that platelet-derived growth factor receptor beta (PDGFRB) signalling was activated in mesenchymal cells. siRNA-mediated knock-down of PDGFRB downregulated UGDH expression, potentially via NFkB-p65. Our results support an unexplored relationship between UGDH and GPC, both of which have previously been independently associated with breast cancer progression.

Abbreviations
AGC, automatic gain control; ATP2A2, sarcomplasmic/endoplasmic reticulum calcium ATPase 2; CD44, CD44 antigen; CDH1, E-cadherin; CDH2, N-cadherin; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; FDFT1, squalene synthase; FDR, false discovery rate; FLNA, filamin-A; FSCN1, fascin; GEMs, genome-scale metabolic network reconstructions; GPC, glycerophosphocholine; HBP, hexosamine biosynthesis pathway; JUP, junction plakoglobin; LGALS1, galectin-1; LMNB1, Lamin-B1; MSN, moesin; MTCH2, mitochondrial carrier homolog 2; NAA, acetylglucosamine; NDRG1, N-myc downstream regulated 1; PDGFRB, platelet-derived growth factor receptor; pERK, extracellular signal-regulated kinase (phosphorylated); PKP3, plakophilin-3; PLA2G15, phospholipase A2 group XV; PLOD2, procollagen-lysine,2-oxoglutarate; PPARy, peroxisome proliferator-activated receptor gamma; PSM, peptide-spectrum matches; RELA(NF-kB-p65), nuclear factor NF-kappa-B p65 subunit; RPL2L1, 60S ribosomal protein L26-like 1; S100A2, S100 calcium-binding protein A2; SERPINE1, plasminogen activator inhibitor 1; SNAI1, Snail family transcriptional repressor 1; SORD, sorbitol dehydrogenase; TLN1, Talin-1; TSTA3, GDP-L-fucose synthase; UDP-Glc, UDP-glucose; UDP-GlcA, UDP-glucuronate; UDP-GDH, UDP-glucose dehydrogenase; VIM, vimentin.
1. Introduction

Epithelial–mesenchymal transition (EMT) is a core developmental process that allows a polarized epithelial cell to assume mesenchymal phenotypes through a series of morphological, molecular, regulatory and functional changes [1]. EMT is part of normal tissue development, organ/tissue fibrosis, wound healing and cancer malignancy. Partial activation of EMT drives tumour metastasis and dissemination to distant organs [2,3].

The biological plasticity and molecular heterogeneity of the EMT programme indicate that EMT is context-specific, which has resulted in discrepancies in its description in the literature [4]. One factor causing EMT heterogeneity in cell line models is the EMT induction method. Growth factors, transcription factors and microRNAs can be manipulated in cells to trigger EMT, such as TGF-β, EGF, Snail1/2, ZEB1 and Twist [3,5–8]. Genetic manipulation may lead to effects that diminish the flexibility and plasticity of the EMT programme. In the present study, we used three breast EMT cell models related to basal mammary gland development and have contributed significantly to the understanding of the molecular regulatory machinery in EMT [16–18].

Metabolic reprogramming is an indispensable driver of EMT in cancer [19]. A better understanding of the metabolism of EMT may facilitate the development of new therapeutics for breast cancer treatment. Glucose metabolism, lipid metabolism, an acidic microenvironment, nucleotide metabolism and amino acid metabolism have been related to EMT in cancer malignancy [20,21]. In our previous studies on EMT-related metabolic dysregulation limited to the D492 EMT model, we observed different preferences for reductive/oxidative carboxylation, glycolysis and amino acid anaplerosis along with an altered lipid profile and shifted glutathione homeostasis [22–24]. To move towards a system’s understanding of how metabolism is influenced following EMT, we compared the metabolic phenotypes of EMT within genome-scale metabolic network reconstructions (GEMs) that allow the integrated analysis of gene expression, proteomic and metabolomic data. These analyses revealed increased dependency on argininosuccinate lyase (ASL) and enhanced activities of the pentose phosphate pathway, hexosamine biosynthesis and one-carbon metabolism post-EMT in the D492 EMT model [23,25]. More recently, we confirmed that metabolic flux through the hexosamine biosynthesis pathway (HBP) increases significantly in mesenchymal cells and that glutamine-fructose-6-phosphate transaminase 2 (GFPT2) in the HBP is associated with breast cancer malignancy [26].

In this study, we further explored the metabolic changes in EMT using shotgun proteomics and expanded our analysis to include three breast cell models descriptive of spontaneous EMT (Fig. 1 and Fig. S1). Several metabolic enzymes were commonly changed after EMT, with UDP-glucose dehydrogenase (UGDH) being most altered. UGDH catalyses conversion of UDP-glucose (UDP-Glc) to UDP-glucuronate (UDP-Glca), both of which are essential metabolites with diverse cellular functions [27,28]. UGDH is involved in a variety of regulatory events. SP1, TGF-β, Slit2, p38MAPK and PI3K/Akt regulate UGDH expression, which in turn influences the downstream targets ERK/MAPK, PPARγ and SNAI1 [27–34]. Several studies have recently reported that UGDH is involved in tumour growth, metastasis and patient survival [27,32,34–37]. To understand the roles of UGDH in EMT in the breast gland, we knocked down UGDH in breast mesenchymal cells with siRNAs and studied effects on cell function and metabolism. Importantly, the three EMT models studied are noncarcinogenic. To account for UGDH in cancer progression and oncogenesis, we compared results from the EMT models to UGDH functions in the tumorigenic breast mesenchymal cell lines D492HER2 and MDA-MB-231. These investigations suggest that the tumour promoting effects of UGDH may in part be attributed to changes in choline metabolism.

2. Materials and methods

2.1. Cell culture

D492 was isolated from primary cultures of reduction mammoplasties with immortalization [9]. D492M was generated via 3D coculture of the D492 cells with endothelial cells to induce EMT [10]. D492HER2 was established by the overexpression of HER2 receptors on D492 [38]. The D492 cell lines (D492, D492M and D492HER2) were cultured in serum-free H14 medium (DMEM/F-12 without glutamine; Thermo Fisher Scientific (TFS), Waltham, MA, USA; 21331020) supplemented with 250 ng·mL⁻¹ insulin (Merck, Kenilworth, NJ,
USA; I6634), 10 µg·mL⁻¹ transferrin (Merck; T2252), 10 ng·mL⁻¹ EGF (PeproTech, Cranbury, NJ, USA; AF-100-15), 2.6 ng·mL⁻¹ Na-selenite (BD Biosciences, San Jose, CA, USA; 354201), 10⁻¹⁰ M estradiol (Sigma, St.Louis, MO, USA; E2758), 1.4 × 10⁻⁶ M hydrocortisone (Sigma; H0888), 0.15 IU prolactin (PeproTech; 100-07), 100 IU penicillin and 0.1 mg·mL⁻¹ streptomycin (Gibco™, TFS; 15140122) and 2 mM glutamine (TFS; 25030024). The passage numbers for both D492 and D492M were from 31, while D492HER2 was cultured from passage 65. HMLE was isolated from reduction mammoplasties[12], while HMLEM was generated from HMLE via differential trypsinization[7]. The HMLE cell lines (HMLE with passage number from 16 and HMLEM with passage number from 28) were cultured in serum-free DMEM/F-12 medium supplemented with 10 µg·mL⁻¹ insulin, 10 ng·mL⁻¹ EGF, 1.4 × 10⁻⁶ M hydrocortisone, 100 IU penicillin and 0.1 mg·mL⁻¹ streptomycin and 2 mM glutamine. PMC42ET was originally established from pleural effusion from the metastatic site in a breast cancer patient[15], and PMC42LA was then generated via mesenchymal–epithelial transition by hormone treatments[13]. The PMC42 cell lines (PMC42LA and PMC42ET with passage numbers from 9) were cultured in RPMI 1640 Medium (TFS; 52400025) supplemented with 10% FBS (Gibco™ 10270106) and 100 IU penicillin and 0.1 mg·mL⁻¹ streptomycin. All cell lines were cultured at 37 °C with 5% CO₂ for routine maintenance, and cells were routinely checked for mycoplasma contamination. All cell lines used in this study were kindly provided by the Stem Cell Research Unit, Biomedical Center, University of Iceland.

2.2. LFQ proteomics and SILAC phosphoproteomic analysis

The proteomic experimental set-up was illustrated in Fig. S1A.

2.2.1. LFQ protein and peptide sample preparation

Cells were lysed with 4% sodium dodecyl sulfate (SDS; MP Biomedicals™, Irvine, CA, USA) in

| Tissue                | Immortalization | Cell type            | Induce EMT                       | Tumorigenicity   |
|-----------------------|-----------------|----------------------|----------------------------------|------------------|
| Breast reduction      | HPV-16 E6/E7 gene transduction | Basal-like; with stem cell properties | Spontaneous EMT; Co-culture with endothelial cells | Non-tumorigenic  |
| mammaryplasty         |                 |                      |                                  |                  |
| Breast reduction      | hTERT and SV-40 Large T antigen expression | Luminal and basal epithelial cells | Spontaneous EMT; Trypsinization | Non-tumorigenic  |
| mammaryplasty         |                 |                      |                                  |                  |
| Breast; derived from metastatic site: pleural effusion | | | Spontaneous MET; Treatment with a combination of hormones to induce MET | Non-tumorigenic  |

Fig. 1. Summary of the three breast EMT cell models. Comparison of the three breast EMT cell models with respect to tissue origin, immortalization methods, cell markers, EMT induction methods and tumorigenicity.
100 mM Tris (Sigma) and kept on ice for 10 min and then transferred to 1.5-mL Eppendorf tubes. After five freeze/thaw (−80 °C/room temperature) cycles, the samples were centrifuged at 20,817 g for 20 min at 4 °C. The supernatants were collected and aliquoted in new tubes and stored at −80 °C. Total protein was quantified with the PierceTM BCA protein assay (TFS). A volume containing 12–15 µg total protein was precipitated by chloroform/methanol precipitation and reconstituted in 50 mM ammonium bicarbonate. The protein sample was reduced with 1 M dithiothreitol (DTT) for 20 min at 70 °C and then alkylated by 200 mM iodoacetamide (IAM) at room temperature in the dark for 30 min, followed by quenching the extra IAM with 1 M DTT for 20 min at room temperature in the dark. Samples were digested overnight with 1.5 µg trypsin at 37 °C. Tryptic peptides were desalted using C-18 StageTips as described [39], after which peptides were dried in a SpeedVac centrifuge and resuspended in 0.1% formic acid.

2.2.2. LFQ LC-MS/MS analysis

Peptides were analysed on an LC-MS/MS platform consisting of an Easy-nLC 1200 UHPLC system (TFS) interfaced with a QExactive HF Orbitrap Mass Spectrometer (TFS) via a nanospray ESI ion source (TFS). Peptides were injected into a C-18 trap column (Acclaim PepMap100, 75 µm i. d. × 2 cm, C-18, 3 µm, 100 Å; TFS) and further separated on a C-18 analytical column (Acclaim PepMap100, 75 µm i. d. × 50 cm, C-18, 2 µm, 100 Å; TFS) using a multi-step gradient with buffer A (0.1% formic acid) and buffer B (80% CH₃CN, 0.1% formic acid): from 2% to 10% buffer B in 10 min, 10% to 50% buffer B in 130 min, 50% to 100% buffer B in 20 min and 20 min with 100% buffer B. The HPLC was re-equilibrated with 2% buffer B before the next injection. The flow rate was 250 nL·min⁻¹. Peptides eluted were analysed on QExactive HF mass spectrometer (TFS) operating in positive ion- and data-dependent acquisition mode using the following parameters: electrospray voltage 1.9 kV, HCD fragmentation with normalized collision energy 29, automatic gain control (AGC) target value of 3 × 10⁶ for Orbitrap MS and 1 × 10⁵ for MS/MS scans. Each MS scan (m/z 350–1650) was acquired at a resolution of 120,000 FWHM, followed by 15 MS/MS scans triggered for AGC targets above 2 × 10⁴, at a maximum ion injection time of 100 ms for MS and 100 ms for MS/MS scans. The proteomic method has been described previously [40].

2.2.3. LFQ protein and peptide identification and quantification

Proteins were identified and quantified by processing MS data using Thermo Scientific™ PROTEOME DISCOVERER™ (PD, version 2.3; TFS). PREVIEW version 2.3.5 (Protein Metrics Inc.) [41] was used to inspect the raw files to determine optimal search criteria, and the following search parameters were used: (a) enzyme specified as trypsin with maximum of two missed cleavages allowed; (b) acetylation of protein N-terminal including loss-of-methionine; (c) oxidation of methionine; (d) deamination of asparagine/glutamine as dynamic post-translational modification; (e) carbamidomethylation of cysteine as static; Precursor mass-tolerance of 10 PPM while fragment mass-tolerance of 0.02 Dalton. PD’s node, Spectrum file RC, was set up to query the raw files against the human proteome downloaded from UniProt (Homo sapiens, UP000005640, October 2018) with the static modification to recalibrate and detect features with the Minora node. Further, the internal contaminant database was also queried along with the human proteome using Sequest [42] search engine available in PD. For downstream analysis of these peptide-spectrum matches (PSM), both protein and peptide identifications/PSM false discovery rate (FDR) were set to 1%; thus, only the unique peptides with high confidence were used for final protein group identification. Peak abundances were extracted by integrating the area under the peak curve. Each protein group abundance was normalized by the total abundance of all identified peptides at FDR < 1%. Summed up median values for all unique peptide ion abundances mapped to respective protein using label-free quantification scaled on all average with Precursor Ion Quantifier node [43] for PD were used.

The mass spectrometry proteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE [44] partner repository with the data set identifier PXD024164.

The protocol for SILAC phosphoproteomic analysis was thoroughly described in the other study [26]. Briefly, protein sample equivalent to 4 mg was dissolved and fractionated, followed by phosphorylated peptide enrichment with MagReSyn-TiIMAC beads (Resyn Biosciences, Edenvale, Gauteng, South Africa) and Magnetic Rack (Dynacheck-2; Life Technologies, Carlsbad, CA, USA). Analyses of peptides for total proteome and phosphorylated proteome were carried out on a Velos-Pro Orbitrap (TFS) mass spectrometer coupled with a Dionex UltiMate 3000 RS (TFS). The raw data files obtained from the mass spectrometric outputs for each experiment were merged into a single quantitated data set using MxQuant (version 1.5.2.8).
MeOH : dH2O (80 : 20) containing an internal stan-
(0.9%), and the metabolites were extracted with
Fig. S1A. Cells were washed with saline solution
The metabolomic experimental set-up was illustrated in
2.3. Metabolomic analysis
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2.3. Metabolomic analysis
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Fig. S1A. Cells were washed with saline solution
(0.9%), and the metabolites were extracted with
MeOH : dH2O (80 : 20) containing an internal stan-
dard mix (Table S1). After adding MeOH : dH2O
(80 : 20), samples were centrifuged, and the supernatant
was taken and vacuum dried. The extracts were anal-
ysed on UPLC mass spectrometry (SYNAPT G2;
Water) according to published protocols [47]. Metabo-
lites were identified and quantified in Masslynx software
(version 4.2) from waters. For untargeted data analysis,
xcms [48] was used for automatic peak-picking (cen-
tWave) [49] and retention time alignment (OBI-Warp)
[50]. All features that eluted in the first 66 s were omit-
ted from further analysis. Feature intensities were nor-
malized using quality control sample-based robust
LOESS (locally estimated scatterplot smoothing) signal
correction (QC-RLSC) [51] which was implemented
using the R-package NormalizeMets [52]. For quality
assurance, all features with over 25% relative standard
development in the QC samples were omitted from further
analysis. Generalized logarithmic transformation (glog)
[53] and autoscaling were used to obtain mean-centred,
normally distributed feature intensity values with equal
variance. The expression of metabolites was normalized
to cell numbers estimated by crystal violet assays. For
the normalization of the metabolic measurements in the
metabolomic experiment, cells were counted using a
crystal violet assay. In short, cells were fixed with 100%
cold MeOH and stained with 0.25% crystal violet (Mer-
ck; C.I. 42555). After washing, stained cells were dis-
solved into 100 µL of 10% acetic acid and measured at
570 nm in the microplate reader (SpectraMaxª M3;
Molecular Devices LLC, San Jose, CA, USA).

2.4. siRNA transient knock-down and
quantitative reverse transcription PCR
Cells were seeded either at 60 000 cells/well in 48-well
plates or at 480 000 cells/well in 6-well plates. Before
seedling, plates were coated with respective control
siRNA (Silencer™ Select Negative Control, 4390843)
and target siRNA (Silencer™ Select siUGDH: s409
and s410; siPDGFRB: s10240; siRELA: s11914 and
s11915) as well as Lipofectamine™ RNAiMAX
Transfection Reagent (TFS). Cells were transfected at
37 ºC and 5% CO2 for 48 h with the final siRNA con-
centration of 10 nm.

In the RT-qPCR experiments, cells were cultured in
48-well plates for 72 h, followed by total RNA extrac-
tion with TRI Reagent™ Solution (Invitrogen™, TFS).
RNA concentration was determined in NanoDrop One
(TFS). 500–1000 ng of RNA was used for cDNA syn-
thesis on the thermal cycler (Peltier Thermal Cycler, MJ
research, PTC-225, Alameda, CA, USA) using High-
Capacity cDNA Reverse Transcription Kit (TFS). Gene
expression was measured with SYBR Green (Luna®,
Universal qPCR Master Mix; New England BioLabs,
Ipswich, MA, USA) on Bio-Rad CFX384 Touch™
Real-Time PCR Detection System (Bio-Rad, Hercules,
CA, USA). Primers were either selected from Primer-
Bank, designed on the Primer3Plus website, or based on
the literature. Primer sequences for genes studied in this
study were listed in Table S2.

2.5. Cell proliferation assay
Cells in quadruplicate were seeded at 10 000 cells/well
in 96-well plates. UGDH knock-down followed the
methods described above. For D492M, 24 h after
seeding (48 h for D492HER2), cells were placed under
the microscope (LEICA CTR 6500, bright field, 10×)
at 37 ºC with 5% CO2 for real-time monitoring and
multiple data acquisition. The microscope was con-
trolled by software micro-manager (version 1.4.22,
Vale’s laboratory, San Francisco, CA, USA). Three
spots were chosen in each well, and photographs were
taken every 6 h. Cell growth was monitored for 66 h
for D492M while 42 h for D492HER2. Photographs
were batch-processed with Macro in software IMAGEJ
1.52p (NIH, Bethesda, MD, USA), and cell numbers
were normalized to the starting point.

2.6. Transwell invasion assay
The D492M and D492HER2 cells were cultured with
siRNA transfection (Scramble and siUGDH) for 48 h
in 6-well plates. UGDH knock-down followed the
methods described above. Cells were then reseeded
into filter units (Falcon® Permeable Support for 24-
well Plate with 8.0-µm Transparent PET Membrane,
353097, Corning, NY, USA) coated with Matrigel
(Corning® Matrigel® Matrix, 356234) at a density of
30 000 cells/well. First, the filter inserts were coated
with 100 µL 1 : 10 diluted Matrigel for 20–30 min at
37 ºC. Next, 300 µL of cell suspension was added on
top of the filter units. Then, 500 µL of H14 medium
with 10% FBS was added to the wells in the 24-well
plates below the filters. Finally, cells were incubated at 37 °C and 5% CO₂ for 48 h. Noninvasive cells on top of the filters were removed with cotton swabs, followed by fixation with paraformaldehyde (PFA, 3.7%, Sigma; 252549) and DAPI staining (1: 5000; Sigma, D9542). Ten images per filter unit were taken by the EVOS® FL Auto Imaging System (10×; TFS), followed by the batch analysis of the images in Macro IMAGEJ 1.52p. For normalization of the different cell numbers in the filter units, cells were seeded into a 24-well plate along with the filter units and cultured and treated in the same way as cells in the filter units.

2.7. Statistical analysis and bioinformatics

All experiments performed in this study were in at least triplicates. The metabolomic analysis of the UGDH knock-down treatment was in six replicates. The proteomic data were processed in PERSEUS (version 1.6.14.0, data imputation based on normal distribution, width = 0.3, downshift = 1.8, permutation-based FDR < 0.05) [54] and R (version 4.0.0, the University of Auckland, New Zealand). Plots in this study were generated in R software. The statistical significance for all two-sample comparisons was based on the two-sided Student’s t-test (Welsch, P < 0.05). Gene Ontology (GO) functional annotation was conducted in DAVID (DAVID Bioinformatics Resources 6.8) with default settings [55,56]. Reactome pathway analysis was performed with Reactome (Pathway browser version 3.7; Reactome database release: 75) with default settings [57]. Proteins with permutation-based FDR < 0.05 were used for the GO annotation and Reactome pathway analysis. Patient survival was plotted in KM plotter (kmplot.com) with basal breast cancer patients (split patients by autoselect best cut-off) [58]. The phosphoproteomic data were analysed in the INGENUITY PATHWAY ANALYSIS (IPA) (QIAGEN, Germantown, MD, USA, version from 2018) for pathway enrichment and PERSEUS for motif enrichment analysis.

All the R codes used for figure plotting in this study could be found on https://github.com/QiongW56/UGDH_Publication_2021.

3. Results

3.1. The proteomic differences based on cell-of-origin outweigh proteomic changes that accompany EMT

Three breast EMT cell models consisting of epithelial and mesenchymal breast cell line pairs were used in this study (Fig. 1), namely, D492/D492M (D492 EMT model), HMLE/HMLEM (HMLE EMT model) and PMC42LA/PMC42ET (PMC42 EMT model). All three epithelial cell lines presented a typical cobblestone-shaped epithelial cell phenotype, while all the mesenchymal cells showed flattened mesenchymal morphology with undefined cell contour (Fig. S1B). The three EMT cell models presented different luminal/myoepithelial/basal phenotypes, with all three models possessing certain degrees of basal breast cell properties.

Irrespective of being epithelial or mesenchymal, cell lines of the same origin were grouped on the proteomic level (Fig. 2A). The PMC42 model shared the least similarities with the other EMT models (Fig. 2B). To confirm the epithelial and mesenchymal phenotypes on the molecular level, we quantified the EMT markers captured by the proteomic analysis from an EMT marker database [59]. VIM, LGALS1 and SERPINEd1 were consistently upregulated in the mesenchymal cells, while PKP3 was downregulated (Fig. 2C–F). Not all EMT markers found in this study were, however, consistently altered among all three models, that is CD44, LMBN1, MSN, FLNA, TTN1, FSCN1, EGFR, S100A2 and NDRG1 (Fig. S2). Since E-cadherin (CDH1) and N-cadherin (CDH2), two typical EMT markers [60], were not covered in the proteomic analysis, we checked the expression of these by real-time PCR. CDH1 was significantly downregulated, while CDH2 was significantly upregulated in all EMT models (Fig. 2G,H).

3.2. Cell–cell and cell–extracellular matrix interactions are altered in EMT, and a diversity of pathways and molecular activities are changed in D492 as opposed to protein translation in HMLE and PMC42

Heterogeneity and plasticity are two intrinsic characteristics of EMT. To further define the epithelial and mesenchymal cells in all three EMT models, we compared their proteomes with respect to the number and profile of the significantly altered proteins along with their biological function and identified consistent EMT markers.

In total, 873 proteins were deemed valid proteins in identification and quantification (Table S3). In the D492 model, 188 out of the 873 valid proteins (21.5%) were significantly changed after EMT (permutation-based FDR < 0.05). In the HMLE model, 436 out of 873 proteins (49.9%) were significantly altered, while 200 proteins (22.9%) were significantly changed in the PMC42 model (Fig. 3A). Out of the significantly altered proteins, 55.9% (105/188) in the D492 model, 18.8% (82/436) in the HMLE model, while 63.5% (127/200) in the PMC42 model were upregulated after EMT.
To ensure reproducibility of the proteomic data used in this study (Table S4), we compared the current proteomic data set with the previously generated data for the D492 EMT model [26]. The correlation coefficient of these two data sets was 0.936 (Fig. S3). We next filtered the identified proteins based on their log2 fold changes and −log10 P-values (Fig. 3C–E) and summarized the consistently altered proteins in all three EMT models (Fig. 3F and Table 1) to identify common changes in EMT. A literature search for each EMT target revealed that all had been associated with EMT previously, albeit to a different extent (Table 1). To evaluate these consistently altered EMT markers in the context of cancer progression, we confirmed the expression of these markers in the tumorigenic breast mesenchymal cell line D492HER2. All the targets detected in D492HER2 showed the same trends in changes (Table 1).

To define functional changes in EMT, we annotated the GO terms for the significantly changed proteins (Table S3) and observed that the Biological Process (BP) ‘cell–cell adhesion’ was altered in all three EMT models (Fig. 4A–C). The D492 model had the least similarity compared with the other two models, with only one common BP term (i.e. cell–cell adhesion) out of the top 10 enriched BP terms (Fig. 4A). In contrast, the PMC42 model shared its top seven terms with HMLE (Fig. 4B, C). The same trend was observed using enriched...
Reactome pathway analysis (Fig. 4D–F). The altered Reactome pathways in the D492 model were related to response to cell stress, IGF signalling and interleukin-12 signalling (Fig. 4D). In both the HMLE and PMC42 models, changes were, however, mainly to pathways involved in the protein translational process (Fig. 4E,F). Comparison of changes to cellular components (Fig. S4A–C) and molecular function (Fig. S4D–F) was similarly indicative of more similarities in changes to protein function following EMT in the HMLE and PMC42 models as compared to the D492 model.

3.3. UGDH is negatively correlated with patient survival and affects cell proliferation, cell invasion and SNAI1 expression

Next, we focused on the metabolic changes during EMT. Out of the thirteen identified targets listed in
Table 1. The EMT targets were significantly different (Student’s t-test, \( P < 0.05 \)) in all EMT models. The literature related to each target in terms of EMT was also listed. Changes in these targets in another breast mesenchymal cell line with tumorigenicity were consistent with the findings in this study.

| UniProt ID | Protein name                      | Gene name | Log2 (D492/D492M) | Log2 (HMLE/HMLEM) | Log2 (PMC42LA/PMC42ET) | −Log (P-value D492s) | −Log (P-value HMLEs) | −Log (P-value PMC42s) | EMT-related literature | Gene expression in mesenchymal cells with tumorigenicty (Log2 ratio) |
|------------|-----------------------------------|-----------|-------------------|-------------------|-------------------------|---------------------|---------------------|---------------------|-----------------------|-------------------------|
| P05121-1   | Plasminogen activator inhibitor 1 | SERPINE1  | −4.236            | −3.442            | −3.993                  | 3.416               | 3.313               | 3.780               | [70,72,97]            | −4.739                  |
| Q9UNX3     | 60S ribosomal protein L26-like 1 | RPL26L1   | −2.367            | −1.123            | −1.346                  | 1.981               | 2.349               | 1.925               | [98]                  | Not detected            |
| Q00469-1   | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 | PLOD2 | −1.817            | −1.504            | −1.121                  | 5.005               | 3.411               | 2.072               | [70,71]               | −1.687                  |
| O60701     | UDP-glucose 6-dehydrogenase       | UGDH      | −1.193            | −2.550            | −0.779                  | 3.101               | 3.962               | 2.285               | [27,28,32,34,75]       | −0.828                  |
| P09382     | Galectin-1                        | LGALS1    | −0.623            | −2.912            | −0.444                  | 1.769               | 3.035               | 1.799               | [67,69,70]            | −0.422                  |
| P08670     | Vimentin                          | VIM       | −0.407            | −1.979            | −3.343                  | 5.493               | 4.802               | 5.970               | [4]                   | −0.957                  |
| Q13630     | GDP-L-fucose synthase             | TSTA3     | 0.553             | 1.009             | 0.508                   | 2.871               | 2.517               | 1.789               | [99,100]              | 0.562                   |
| Q00796     | Sorbitol dehydrogenase            | SORD      | 0.599             | 1.459             | 1.452                   | 2.457               | 2.957               | 2.806               | [101]                 | 0.111                   |
| P37268     | Squalene synthase                 | FDFT1     | 0.683             | 1.536             | 0.491                   | 3.510               | 3.215               | 1.462               | [102]                 | 0.722                   |
| P16815     | Sarco(endo)plasmic reticulum calcium ATPase 2 | ATP2A2 | 0.919             | 0.523             | 0.641                   | 2.388               | 1.551               | 2.316               | [103]                 | 0.299                   |
| Q9Y6C9     | Mitochondrial carrier homolog 2   | MTCH2     | 1.239             | 0.725             | 0.899                   | 2.219               | 1.624               | 3.015               | [99]                  | 0.198                   |
| Q9Y446     | Plakophilin-3                     | PKP3      | 2.958             | 2.067             | 3.478                   | 2.786               | 3.077               | 1.850               | [66,68]               | 3.458                   |
| P14923     | Junction plakoglobin              | JUP       | 4.343             | 1.541             | 1.214                   | 1.783               | 1.356               | 3.611               | [104,105]             | 4.344                   |
Table 1, four proteins were involved in metabolism: FDFT1, SORD, and TSTA3 were downregulated while UGDH was upregulated (Fig. 5A–D). We further tested the RNA expression of UGDH, which showed the most changes to protein expression in all EMT models and was associated with cancer aggressiveness. Though there was no significance in the D492 and PMC42 models, the upregulating trends in all EMT

Fig. 4. Functional annotation of the GO terms (BP) and Reactome pathway analysis for the three EMT models. (A–C) Functional annotation of the GO terms (BP) was conducted on the DAVID platform (DAVID Bioinformatics Resources 6.8) for the D492 model (A), HMLE model (B), and PMC42 model (C). The GO terms were listed according to the $-\log_{10} P$-value in descending order. The numbers of genes in each GO term were also plotted as dots/line plots. (D–F) Reactome pathway analysis (Pathway browser version 3.7; Reactome database release: 75) for the D492 model (D), HMLE model (E) and PMC42 model (F). Data used for both the GO annotation and the pathway analysis (Table S3) were proteins significantly different in each EMT model (permutation-based FDR < 0.05). Default settings in the DAVID and Reactome platforms were used. BP, biological process.
models were seen (Fig. 5E). To relate these findings to breast cancer, we tested the protein level of UGDH in the tumorigenic breast mesenchymal cell line D492HER2. UGDH was upregulated in D492HER2 as observed in nontumorigenic mesenchymal cell line D492M (Fig. 5F).

Recent studies have reported that UGDH affects patient survival [34], cell proliferation [32,37], cell invasion [27], cell migration [34,37] and SNAI1 expression [28]. We set out to confirm these effects of UGDH in our EMT cell lines. High UGDH level was associated with worse patient survival in basal breast cancer patients based on KM plotter (Fig. 6A). Based on this, we analysed effects of UGDH on cell morphology, proliferation, invasion and SNAI1 expression in two types of breast mesenchymal cells: nontumorigenic mesenchymal cell line D492M (Fig. 5F).

Recent studies have reported that UGDH affects patient survival [34], cell proliferation [32,37], cell invasion [27], cell migration [34,37] and SNAI1 expression [28]. We set out to confirm these effects of UGDH in our EMT cell lines. High UGDH level was associated with worse patient survival in basal breast cancer patients based on KM plotter (Fig. 6A). Based on this, we analysed effects of UGDH on cell morphology, proliferation, invasion and SNAI1 expression in two types of breast mesenchymal cells: nontumorigenic D492M and tumorigenic D492HER2 via siRNA-mediated knock-down of UGDH (Fig. S5). Knock-down of UGDH did not yield observable morphological changes but slowed down cell growth (Fig. 6B,C) and invasion (Fig. 6D,E and Fig. S6A–C) in both cell lines. SNAI1 RNA expression was downregulated after UGDH knock-down, which was also consistent with the literature (Fig. 6F,G and Fig. S6D,E).

3.4. GPC is downregulated while NAA is upregulated following UGDH knock-down in the mesenchymal cells

UGDH catalyses the conversion of UDP-Glc to UDP-GlcA that are constituents of glycosaminoglycans and N- and O-linked glycans [61]. To confirm the metabolic impacts of UGDH in mesenchymal cells, we knocked down UGDH with siRNAs and performed metabolomic analysis in all three mesenchymal cell lines. Samples from the same cell line clustered together at the metabolic level despite UGDH knock-down (Fig. 7A,B). As with the proteome, the metabolome of D492M was closer to that of HMLEM than the metabolome of PMC42ET. Knock-down of UGDH did not confer a distinct metabolic phenotype compared with the scramble control in any of the
mesenchymal cell lines (Fig. 7B). An increasing trend of UDP-Glc was observed in all the mesenchymal cell lines with all the siUGDH treatments, although non-significant for one of the siRNAs (Fig. 7C). UDP-GlcA decreased in all the mesenchymal cell lines in all the siUGDH treatments, although nonsignificantly with one siRNA in D492M (Fig. 7D).

To better evaluate the systemic changes of UGDH on metabolism, we carried out an untargeted metabolomics analysis. Knocking down UGDH significantly decreased the intracellular glycerophosphocholine (GPC) level and increased acetylaspartate (NAA) in all the mesenchymal cell lines (Fig. 7E,F), which was confirmed in the aggressive D492HER2 and MDA-
Fig. 7. Metabolomic changes after siRNA knock-down of UGDH in the mesenchymal cell lines. (A, B) Metabolomic clustering of the mesenchymal cell lines with different treatments. Valid metabolite identification and quantification from the negative, positive and basic modes were integrated into the analysis. Samples clustered together based on the differences of their metabolome among different EMT models (A), and the D492 mesenchymal cells were closer to the HMLE mesenchymal cells than PMC42 at the metabolic level (B). (C) The UGDH substrate UDP-glucose was increased following siRNA knock-down of UGDH in all cell lines with two siRNAs (n = 3). (D) The UGDH product UDP-glucuronate was decreased with siRNA knock-down of UGDH in all the cell lines with two siRNAs (n = 3). (E, F) siRNA knock-down of UGDH significantly decreased GPC (n = 5) and increased acetylaspartate (n = 4) in all the cell lines with two siRNA treatments. (G) GPC level was higher in the nontumorigenic D492M than the epithelial D492, and it was the highest in the tumorigenic mesenchymal D492HER2 (n = 3). Student’s t-test, *P < 0.05; **P < 0.01; ***P < 0.001. The error bars indicate standard deviation. UGDH, UDP-glucose 6-dehydrogenase.
MB-231 cell lines (Fig. S7A–D). To investigate whether GPC and NAA were associated with the UGDH level and differently expressed regardless of tumorigenicity, we tested the GPC and NAA levels in the epithelial D492, nontumorigenic mesenchymal D492M and tumorigenic mesenchymal D492HER2 cells. GPC was higher in both D492M and D492HER2 compared with D492 (Fig. 7G). We further looked into the connection between GPC and the mesenchymal state based on published data sets in the literature [62,63] but did not observe any significant correlation (Fig. S7E and Table S5). siRNA-mediated knock-down of UGDH did not yield significant and consistent changes to choline and phosphocholine (Fig. S7F,G).

Glycerophosphocholine is part of the choline synthetic pathway from phosphatidylcholine (PtdCho), and NAA is closely associated with acetyl-CoA and central carbon metabolism. To query how changes to UDP-GlcA might relate to GPC and NAA processing via changes in metabolic flux, we performed in silico knock-down of UGDH using tailored genome-scale metabolic models of D492 [23,25]. Changes to metabolic flux were observed within keratan metabolism, hyaluronan processing, pentose phosphate pathway and the central carbon metabolic pathways (Table S6). Negligible changes were, however, observed to GPC production and consumption.

3.5. PDGFRB signalling regulates UGDH potentially via NFkB-p65

We next investigated the upstream regulation of UGDH by analysing the secretome of the D492 model [64]. IGF, TGF-β and PDGFD signalling regulators were highly presented in the culture medium of D492M cells (Fig. 8A). PDGFRB was highly expressed in the nontumorigenic D492M (Fig. 8B) and the tumorigenic D492HER2 mesenchymal cell lines (Fig. S8A) [65], and PDGFD was secreted by D492M (Fig. 8C). We thus focused on the role of PDGF signalling in UGDH regulation. In addition, the motif enrichment analysis of the phosphorylation sites within the phosphoproteomic data (Table S7) revealed potentially altered kinases in the D492 EMT model, including the downstream target of the PDGF signalling PKC kinase (Fig. 8D). siRNA-mediated knock-down of PDGFRB decreased both the PDGF signalling downstream regulator RELA (NFkB-p65) and UGDH in D492M (Fig. 8E–G) and D492HER2 (Fig. S8B–D). We further investigated the impact of RELA on UGDH and found that siRNA-mediated knock-down of RELA decreased the UGDH RNA level in D492M (Fig. S8H,I and Fig. S8E,F). We observed the same effect of RELA knock-down on UGDH in D492HER2 with only one siRNA (Fig. S8G–J).

4. Discussion

Herein we set out to determine common metabolic changes in cell models used to study EMT in breast epithelium. We chose the D492, HMLE and PMC42 EMT cell models on account of the spontaneous EMT induction approaches and the nontumorigenic properties of these cell lines to ensure the intrinsic characteristics and plasticity of EMT (Fig. 1). First, we validated and compared the EMT cell models on the proteomic level (Fig. 2A,B). Cell lines clustered based on their origin instead of their epithelial or mesenchymal characteristics, indicating that the spontaneous epithelial–mesenchymal switches during EMT/MET are subtle compared with the imprinted intrinsic genetic differences among these cell models.

VIM, LGALS1, SERPINE1, PKP3 and the CDH1-CDH2 switch were consistently altered in all the EMT models (Fig. 2C–H) and have all been related to EMT in different cancer types [4,60,66–72]. Vimentin, a type III intermediate filament and well-known EMT marker, shapes the cell structure and modifies cell movements and cell adhesion [73]. SERPINE1, a key player in endothelial homeostasis, is highly upregulated in EMT. However, the function of SERPINE1 in EMT...
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is poorly understood. The possible role of SERPINE1 in EMT is to affect the function of urokinase-type plasminogen activator receptor (uPAR) to regulate extracellular matrix degradation [72]. LGALS1 is a carbohydrate-binding protein. One study shows that upregulation of LGALS1 decreases CDH1 and increases SNAI1 [67]. PKP3 is an epithelial marker and is under the control of the EMT transcriptional regulator ZEB1 [66,68]. All these EMT markers were consistently altered in the three EMT models (Fig. 2C–F). However, inconsistencies in EMT markers were also observed indicative of their different roles in EMT with respect to cell type. The PMC42 model was different from the other EMT cell models (Fig. S2), potentially reflecting the cell heterogeneity and partially expressed mesenchymal marker CDH2 in the epithelial cells (Fig. 2H) [74]. The consistently altered EMT markers were also confirmed in the tumorigenic breast mesenchymal cell line D492HER2 (Fig. 3F and Table 1), indicating that these makers are not only crucial for EMT but also potentially involved in tumorigenicity and malignancy, even though they are not critical for tumour initiation. Moreover, many of the consistently altered proteins identified in this study remain unexplored in the context of EMT (Table 1).

Our findings confirmed that changes to cellular morphology, cell–cell communication and cell–extracellular matrix interaction are among the main characteristics of EMT (Fig. 4 and Fig. S4). Even though the proteomes of the D492 cell lines were closer to HMLE (Fig. 2B), they shared the least similarity in the altered pathways post-EMT. The changed translational activities in HMLE and PMC42 and the altered responses to stress and signalling regulation in D492 suggest that in HMLE and PMC42, the epithelial or mesenchymal switch may largely be mediated by altered expression of proteins involved, whereas in D492, post-translational control of existing proteins may play a more important role. This may also reflect the more stem-like properties of the D492 epithelial cells that confer cell flexibility. Our findings indicate that distinct and dominant cell properties (e.g. stem cell properties) outweigh similar genetic backgrounds for EMT induction, while cells with disparate genetic backgrounds can rely on similar machineries to induce EMT.

Recently, a growing number of studies have focused on UGDH in cancer, and the roles of UGDH in tumour growth, metastasis and patient survival have been well documented [27,32,34–37]. Additionally, UGDH has been connected to EMT [27,28,32,34,75]. Arnold et al. [27] reported that UGDH was highly expressed in mesenchymal cells and mesenchymal-like breast cancers and connected UDP-GlcA (the enzymatic product of UGDH) to extracellular matrix remodelling and mesenchymal-like properties. Furthermore, UGDH regulates SNAI1, a well-known EMT transcription factor, via UDP-Glc (the enzymatic substrate of UGDH) [28]. We confirmed the upregulation of UGDH in both nontumorigenic and tumorigenic mesenchymal cell lines, suggesting UGDH is associated with the mesenchymal feature in tumorigenic cell lines (Fig. 5F). Interestingly, even though the high expression of UGDH was associated with worse survival in basal breast cancer patients (Fig. 6A) and decreased UGDH jeopardized cell proliferation (Fig. 6B–C) and invasion (Fig. 6D–E and Fig. S6A–C), all the mesenchymal cells in this study possess upregulated UGDH and are nontumorigenic. Thus, elevated UGDH expression is likely not a trigger for tumour initiation, but tumorigenic cells may rely on UGDH to facilitate tumorigenicity and malignancy. UGDH may induce resistance to chemotherapy via drug elimination. This was supported by a recent study demonstrating that high levels of UGDH are correlated with worse prognosis in triple-negative breast cancer patients receiving chemotherapy, likely by promoting UDP-GlcA-mediated detoxification and elimination of epirubicin [76]. The effect of UGDH on SNAI1 supports that UGDH has a regulatory role in EMT and that its function may exceed its catalytic role, perhaps via nonconventional signalling regulatory effects such as glycosylation (Fig. 6F–G and Fig. S6D–E).

The D492 EMT model metabolome was more similar to HMLE than PMC42 (Fig. 7A,B), consistent with the proteomic analysis (Fig. 2A,B). In agreement with the literature, knock-down of UGDH decreased UDP-Glc and decreased UDP-GlcA (Fig. 7C,D), both of which are important metabolites with wide impact on cells [27,28]. The most prominently altered metabolite was, however, GPC (Fig. 7E). Increased GPC in tumours indicates changes to choline metabolism, which has emerged as a hallmark of cancer progression [77]. GPC is negatively correlated with patient survival [78] and is high in basal-like breast cancer xenograft and oestrogen receptor-negative breast cancer patients [79,80]. Reduced GPC levels after chemotherapy are associated with better survival in breast cancer patients [78]. D492 and D492M are basal-like breast cell lines, while D492HER2, deemed as HER2-positive breast cell line, is more closely associated with the aggressive claudin low than other breast cancer types [26,81]. Claudin low is not a distinct intrinsic breast tumour subtype but may permeate various breast cancer types including HER2-positive [82]. The higher levels of GPC along with UGDH in
basal-like mesenchymal D492M and claudin-low D492HER2 are in congruence with the clinical observations. GPC may be involved in EMT, but the connection between GPC and EMT in cancer is unclear [83,84]. Li and colleagues detected GPC in 928 cell lines and performed different types of metabolite-gene association analyses. They reported various genes associated with GPC where the EMT master regulator TWIST1 was one of the top hits [62]. The insignificant association analyses. They reported various genes associated with GPC and mesenchymal cells (Fig. S7E) suggests that the increased GPC levels observed in the D492 mesenchymal cells are results of one or several regulators independent of mesenchymal traits. Our results support that GPC in part is regulated by the mesenchymal metabolic enzyme UGDH, but the molecular mechanisms underlying this warrant further investigation. In silico knock-down of UGDH in the genome-scale metabolic models revealed several metabolic changes (Table S6) primarily on account of rerouting of glucose flux away from UDP-GlcA formation and into glycolysis and associated pathways (e.g. PPP and TCA), which may be partially responsible for the increased NAA (Fig. 7F). It, however, imparted no changes in GPC, implying that the changes to metabolic fluxes encircling GPC due to a mass-action effect of UGDH are likely secondary to changes that arise through altered glycosylation. Cell osmotic pressure balance is vital for normal cell functions and cell survival. GPC is a well-known intracellular osmotic regulator, and proteoglycans serve as extracellular osmolytes [85]. The decreased intracellular GPC may thus counterbalance the decreased extracellular osmotic pressure induced by the reduced proteoglycans caused by the knock-down of UGDH.

Recently, studies have shown that UGDH regulates signalling factors and lipid metabolic genes, such as SNAI1-, SIP-1-, ERK/MAPK-, SIX1- and PPARγ-targeted genes [27,28,32,34]. PPARγ is a nuclear transcription factor regulating genes linked to lipid metabolism [86,87] that interacts with choline/PtdCho metabolism [88]. UGDH has been proposed to inhibit PPAR signalling and affect lipid metabolism [27]. Consistent with this, we observed a negative association between UGDH expression and PPARγ signalling (Fig. S7H), suggesting the UGDH knock-down decreases intracellular GPC level via PPARγ. Moreover, phospholipase A2 group XV (PLA2G15), which belongs to Cytosolic phospholipase A2 (cPLA2), is an enzyme catalysing the hydrolysis of phospholipids, potentially involved in the formation of GPC from PtdCho, and is under the control of ERK signalling [89,90]. Knock-down of UGDH has been reported to downregulate the phosphorylation of ERK (pERK) in highly invasive ovarian cancer cells [32]. We observed that both GPC and PLA2G15 were higher in the mesenchymal cell lines D492M and D492HER2 (Fig. 7G and Fig. S7I), implying UGDH may regulate GPC via pERK-PLA2G15. Taken together, UGDH may indirectly affect GPC via signalling regulations and/or lipid metabolism to retain the osmotic balance across the cell membrane, although further investigations are needed. Furthermore, the absence of UGDH in the list of genes associated with GPC reported in the literature indicates UGDH may not be a dominant GPC regulator [62].

Slit2, SP1, TGF-β, hypoxia, p38MAPK, LMP2A and PI3K/Akt affect and/or regulate UGDH expression, which highlights that UGDH is under complex regulation network control [29–31,33]. These regulators are potentially mediated by PDGF signalling that, along with the downstream transcription factor NFkB, is dysregulated in cancer progression and EMT [7,91,92]. Tam et al. [7] reported a switch from EGFR to PDGFR signalling in cancer stem cell formation and EMT. The higher expression of PDGFRB and secretion of PDGF in D492M compared with D492 suggest PDGFRB signalling is upregulated in mesenchymal cells (Fig. 8A–C and Fig. S8A) supported by the increased phospholipase C, PI3K/Akt and PKCα signalling (Fig. S7H and Fig. 8D) since these are well-known downstream targets of PDGFR [7,93]. This is consistent with PDGFD-PDGFRB signal regulation of EMT [94,95]. NFkB-p65 is a downstream regulator of PDGFR signalling [92,96]. Downregulating either PDGFRB or NFkB-p65 decreased UGDH expression on the RNA level in both D492M and D492HER2. However, the impacts of PDGFRB and NFkB-p65 on UGDH were dampened in D492HER2 compared with D492M (Fig. 8E–I and Fig. S8B–J). We have previously noticed that D492HER2 is a less complete mesenchymal cell line than D492M, suggesting that the regulations of PDGFRB and NFkB-p65 on UGDH are more dominant in complete mesenchymal cells [26]. It thus appears that UGDH is part of an interactive signalling and metabolic network in which PDGFRB differentially regulates UGDH via NFkB-p65 depending on specific cell types.

5. Conclusions

In conclusion, we used three breast EMT cell models to study proteomic changes in EMT, focusing on metabolic reprogramming. We further studied the downstream functions of the metabolic enzyme UGDH in cancer progression and metabolism, and finally, we explored the upstream signalling regulating UGDH (Fig. 9). Several proteins were found to be
involved in the EMT programme and likely to participate in normal human breast gland development, that is SERPINE1, RPL26L1, PLOD2, UGDH, LGALS1, VIM, TSTA3, SORD, FDFT1, ATP2A2, MTCH2, PKP3 and JUP, within which, UGDH, TSTA3, SORD and FDFT1 were metabolic enzymes with UGDH possessing the biggest difference between the epithelial and mesenchymal cell lines. UGDH regulated SNAI1, affected cell proliferation and invasion and is associated with patient survival potentially via regulation of the intracellular GPC level. PDGFRB was involved in the regulation of UGDH in mesenchymal cells, likely through NFKB-p65. Further studies on understanding the roles of UGDH on GPC and its relationship with EMT could be valuable in developing novel therapeutics against breast cancer.

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

QW conceived the study, carried out the experiments, performed the data analysis, designed the figures and wrote the manuscript. STK performed the metabolomic...
untargeted analysis and the flux analysis in the genome-scale metabolic network reconstructions (GEMs); FJ conducted the metabolomic experiments; AIV carried out experiments; LH, DMF and AS performed the proteomic analysis under the supervision of GS. OR conceived, supervised and funded the study, as well as analysed the data and wrote the manuscript. All authors provided critical feedbacks on the manuscript and data analysis.

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Data accessibility
The mass spectrometry proteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE [44] partner repository with the data set identifier PXD024164. The mass spectrometry phosphoproteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE [44] partner repository with the data set identifier PXD025858.

References
1 Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol. 2019;20:69–84. https://doi.org/10.1038/s41580-018-0080-4
2 Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018;18:128–34. https://doi.org/10.1038/nrc.2017.118
3 Krebs AM, Mitschke J, Lasierra Losada M, Schmalhofer O, Boerries M, Busch H, et al. The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat Cell Biol. 2017;19:518–29. https://doi.org/10.1038/nccb3513
4 Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, et al. Guidelines and definitions for research on epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2020;21:341–52. https://doi.org/10.1038/s41580-020-0237-9
5 Aharonov A, Shakked A, Umansky KB, Savidor A, Genzelinakh A, Kain D, et al. ERBB2 drives YAP activation and EMT-like processes during cardiac regeneration. Nat Cell Biol. 2020;22:1346–56. https://doi.org/10.1038/s41556-020-00588-4
6 Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor Snail controls epithelial–mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol. 2020;22:76–83. https://doi.org/10.1038/s41556-020-00502-0
7 Tam WL, Lu H, Buikhuizen J, Soh BS, Lim E, Reinhardt F, et al. Protein kinase C alpha is a central signaling node and therapeutic target for breast cancer stem cells. Cancer Cell. 2013;24:347–64. https://doi.org/10.1016/j.ccr.2013.08.005
8 Yu Y, Luo W, Yang ZJ, Chi JR, Li YR, Ding Y, et al. miR-190 suppresses breast cancer metastasis by regulation of TGF-beta-induced epithelial-mesenchymal transition. Mol Cancer. 2018;17:70. https://doi.org/10.1186/s12943-018-0818-9
9 Gudjonsson T, Villadsen R, Nielsen HL, Rønnov-Jessen L, Bissell MJ, Petersen OW. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev. 2002;16:693–706. https://doi.org/10.1101/gad.952602
10 Sigurðsson V, Hilmarsson T, Sigmundsdóttir H, Fridriksdóttir AJR, Ringné M, Villadsen R, et al. Endothelial induced EMT in breast epithelial cells with stem cell properties. PLoS One. 2011;6:e23833. https://doi.org/10.1371/journal.pone.0023833
11 Bierie B, Pierce SE, Kroeger C, Stover DG, Pattabiraman DR, Thiru P, et al. Integrin-beta4 identifies cancer stem cell-enriched populations of partially mesenchymal carcinoma cells. Proc Natl Acad Sci USA. 2017;114:E2337–46. https://doi.org/10.1073/pnas.1618298114
12 Elenbaas B, Sprio L, Koerner F, Fleming MD, Zimonic DB, Donaher JL, et al. Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. Genes Dev. 2001;15:50–65. https://doi.org/10.1101/gad.828901
13 Ackland ML, Michalczyk A, Whitehead RH. PMC42, a novel model for the differentiated human breast. Exp Cell Res. 2001;263:14–22. https://doi.org/10.1006/excr.2000.5106
14 Cursons J, Leuchowius KJ, Waltham M, Tomaskovic-Crook E, Foroutan M, Bracken CP, et al. Stimulus-dependent differences in signalling regulate epithelial-mesenchymal plasticity and change the effects of drugs in breast cancer cell lines. Cell Commun Signal. 2015;13:26. https://doi.org/10.1186/s12943-015-0106-x
15 Whitehead RH, Bertonecco I, Webber LM, Pedersen JS. A new human breast carcinoma cell line (PMC42) with stem cell characteristics. 1. Morphologic characterization. J Natl Cancer Inst. 1983;70:649–61.
16 Bhatia S, Monkman J, Bick T, Duijf PH, Nagaraj SH, Thompson EW. Multi-omics characterization of the spontaneous mesenchymal-epithelial transition in the PMC42 breast cancer cell lines. J Clin Med. 2019;8:1253. https://doi.org/10.3390/jcm8081253
17 Briem E, Inghotthson S, Traustadottir GA, Hilmarsson T, Gudjonsson T. Application of the
D492 cell lines to explore breast morphogenesis, EMT and cancer progression in 3D culture. J Mammary Gland Biol Neoplasia. 2019;24:139–47. https://doi.org/10.1007/s10911-018-09424-w

18 Kröger C, Afeyan A, Mraz J, Eaton EN, Reinhardt F, KhodorYL, et al. Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells. Proc Natl Acad Sci USA. 2019;116:7353–62. https://doi.org/10.1073/pnas.1812876116

19 Morandi A, Taddei ML, Chiarugi P, Giannoni E. Targeting the metabolic reprogramming that controls epithelial-to-mesenchymal transition in aggressive tumors. Front Oncol. 2017;7:40. https://doi.org/10.3389/fonc.2017.00040

20 Li M, Bu X, Cai B, Liang P, Li K, Qu X, et al. Biological role of metabolic reprogramming of cancer cells during epithelial-mesenchymal transition (Review). Oncol Rep. 2019;41:727–41. https://doi.org/10.3829/or.2018.6882

21 Williams ED, Gao D, Redfern A, Thompson EW. Controversies around epithelial-mesenchymal plasticity in cancer metastasis. Nat Rev Cancer. 2019;19:716–32. https://doi.org/10.1038/s41568-019-0213-x

22 Eiriksson FF, Rolfsson O, Ogmundsdottir HM, Haraldsson GG, Thorsteinsdottir M, Halldorsson S. Altered plasmalogen content and fatty acid saturation following epithelial to mesenchymal transition in breast epithelial cell lines. Int J Biochem Cell Biol. 2018;103:99–104. https://doi.org/10.1016/j.biocel.2018.08.003

23 Halldorsson S, Rohatgi N, Magnusdottir M, Choudhary KS, Gudjonsson T, Knutsen E, et al. Metabolic re-wiring of isogenic breast epithelial cell lines following epithelial to mesenchymal transition. Cancer Lett. 2017;396:117–29. https://doi.org/10.1016/j.canlet.2017.03.019

24 Karvelsson ST, Sigurdsson A, Seip K, Grinde MT, Wang Q, Johannsson F, et al. EMT-derived alterations in glutamine metabolism sensitize mesenchymal breast cells to mTOR inhibition. Mol Cancer Res. 2021;19:1546–58. https://doi.org/10.1158/1541-7786.MCR-20-0962

25 Karvelsson ST, Wang Q, Hilmarsdottir B, Sigurdsson A, Moestue SA, Maelandsmo GM, et al. Argininosuccinate lyase is a metabolic vulnerability in breast development and cancer. NPJ Syst Biol Appl. 2021;7:36. https://doi.org/10.1038/s41540-021-00195-5

26 Wang Q, Karvelsson ST, Kotronoula A, Gudjonsson T, Halldorsson S, Rolfsson O. Glutamine-fructose-6-phosphate transaminase 2 (GFPT2) is upregulated in breast epithelial-mesenchymal transition and responds to oxidative stress. Mol Cell Proteomics. 2022;21:100185. https://doi.org/10.1074/jmcp.2021.100185

27 Arnold JM, Gu F, Ambati CR, Rasaily U, Ramirez-Pena E, Joseph R, et al. UDP-glucose 6-dehydrogenase regulates hyaluronic acid production and promotes breast cancer progression. Oncogene. 2020;39:3089–101. https://doi.org/10.1038/s41388-019-0885-4

28 Wang X, Liu R, Zhu W, Chu H, Yu H, Wei P, et al. UDP-glucose accelerates SNAI1 mRNA decay and impairs lung cancer metastasis. Nature. 2019;571:127–31. https://doi.org/10.1038/s41586-019-1340-y

29 Bontemps Y, Vuillermoz B, Antonicelli F, Perreau C, Danan JL, Maquart FX, et al. Specific protein-I is a universal regulator of UDP-glucose dehydrogenase expression: its positive involvement in transforming growth factor-beta signaling and inhibition in hypoxia. J Biol Chem. 2003;278:21566–75. https://doi.org/10.1074/jbc.M209366200

30 Clarkin CE, Allen S, Kuiper NJ, Wheeler BT, Wheeler-Jones CP, Pitsillides AA. Regulation of UDP-glucose dehydrogenase is sufficient to modulate hyaluronan production and release, control sulfated GAG synthesis, and promote chondrogenesis. J Cell Physiol. 2011;226:749–61. https://doi.org/10.1002/jcp.2293

31 Fernando R, Smith TJ. Slit2 regulates hyaluronan & cytokine synthesis in fibrocytes: potential relevance to thyroid associated ophthalmopathy. J Clin Endocrinol Metab. 2021;106:e20–33. https://doi.org/10.1210/clinem/dgaa684

32 Lin LH, Chou HC, Chang SJ, Liao EC, Tsai YT, Wei YS, et al. Targeting UDP-glucose dehydrogenase inhibits ovarian cancer growth and metastasis. J Cell Mol Med. 2020;24:11883–902. https://doi.org/10.1111/jcmm.15808

33 Pan YR, Vatsyanay J, Chang YS, Chang HY. Epstein-Barr virus latent membrane protein 2A upregulates UDP-glucose dehydrogenase gene expression via ERK and PI3K/Akt pathway. Cell Microbiol. 2008;10:2447–60. https://doi.org/10.1111/j.1462-5822.2008.01221.x

34 Teoh ST, Ogrodzinski MP, Lunt SY. UDP-glucose 6-dehydrogenase knockout impairs migration and decreases in vivo metastatic ability of breast cancer cells. Cancer Lett. 2020;492:21–30. https://doi.org/10.1016/j.canlet.2020.07.031

35 Goodwin CR, Ahmed AK, Xia S. UDP-α-D-glucose 6-dehydrogenase: a promising target for glioblastoma. Oncotarget. 2019;10:1542–3. https://doi.org/10.18632/oncotarget.26670

36 Huang D, Casale GP, Tian J, Lele SM, Pisarev VM, Simpson MA, et al. UDP-glucose dehydrogenase as a novel field-specific candidate biomarker of prostate cancer. Int J Cancer. 2010;126:315–27. https://doi.org/10.1002/ijc.24820

37 Oyinlade O, Wei S, Lal B, Laterra J, Zhu H, Goodwin CR, et al. Targeting UDP-alpha-D-glucose 6-dehydrogenase inhibits glioblastoma growth and migration. Oncogene. 2018;37:2615–29. https://doi.org/10.1038/s41388-018-0138-y
38 Ingthorsson S, Andersen K, Hilmarsson B, Maelandsmo GM, Magnusson MK, Gudjonsson T. HER2 induced EMT and tumorigenicity in breast epithelial progenitor cells is inhibited by coexpression of EGFR. *Oncogene*. 2016;35:4244–55. https://doi.org/10.1038/onc.2015.489

39 Rappisilber J, Ishihama Y, Mann M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal Chem*. 2003;75:663–70. https://doi.org/10.1021/ac026117i

40 Aass KR, Mjelle R, Kastnes MH, Tryggestad SS, van den Brink LM, Westhrin M, et al. IL-32 is a metabolic regulator promoting survival and proliferation of malignant plasma cells. *bioRxiv*. 2021. https://doi.org/10.1101/2021.02.22.431638

41 Kil YJ, Becker C, Sandoval W, Goldberg D, Bern M. Preview: a program for surveying shotgun proteomics tandem mass spectrometry data. *Anal Chem*. 2011;83:5259–67. https://doi.org/10.1021/ac200609a

42 Eng JK, McCormack AL, Yates JR. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom.* 1994;5:976–89. https://doi.org/10.1016/1044-0305(94)80016-2

43 Horn M, Uecket T, Fritzemeier K, Tham K, Paschke C, Berg F, Pfaff H, Jiang X, Li S, Lopez-Ferrer D. New method for label-free quantiti cation in the Proteome Discoverer framework. 2016.

44 Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res*. 2018;47:D442–50. https://doi.org/10.1093/nar/gky1106

45 Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol*. 2008;26:1367–72. https://doi.org/10.1038/nbt.1511

46 Cox J, Neuhauser N, Michalksi A, Scheltema RA, Olsen JV, Mann M. Andromeda: a peptide search engine integrated into the MaxQuant environment. *J Proteome Res*. 2011;10:1794–805. https://doi.org/10.1021/pr101065j

47 Rolffson O, Johannsson F, Magnusdottir M, Paglia G, Sigurjonsson OE, Bordbar A, et al. Mannose and fructose metabolism in red blood cells during cold storage in SAGM. *Transfusion*. 2017;57:2665–76. https://doi.org/10.1111/trf.14266

48 Smith CA, Want EJ, O’Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem*. 2006;78:779–87. https://doi.org/10.1021/ac051437y

49 Tautenhahn R, Böttcher C, Neumann S. Highly sensitive feature detection for high resolution LC/MS. *BMC Bioinformatics*. 2008;9:504. https://doi.org/10.1186/1471-2105-9-504

50 Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, et al. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods*. 2015;12:523–6. https://doi.org/10.1038/nmeth.3393

51 Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc*. 2011;6:1060–83. https://doi.org/10.1038/nprot.2011.335

52 De Livera AM, Oshansky G, Simpson JA, Creek DJ. NormalizeMets: assessing, selecting and implementing statistical methods for normalizing metabolomics data. *Metabolomics*. 2018;14:54. https://doi.org/10.1007/s11306-018-1347-7

53 Durbin BP, Hardin JS, Hawkins DM, Rocke DM. A variance-stabilizing transformation for gene-expression microarray data. *Bioinformatics*. 2002;18:SI05–10. https://doi.org/10.1093/bioinformatics/18.suppl_1.SI05

54 Tyanova S, Temu T, Sinitcyn P, Carlson A, Hei MY, Geiger T, et al. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat Methods*. 2016;13:731–40. https://doi.org/10.1038/nmeth.3901

55 Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37:1–13. https://doi.org/10.1093/nar/gkn923

56 Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4:44–57. https://doi.org/10.1038/nprot.2008.211

57 Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, et al. The reactome pathway knowledgebase. *Nucleic Acids Res*. 2019;48:D498–503. https://doi.org/10.1093/nar/gkz1031

58 Györrfy B, Lanzczky A, Eklund AC, Denkert C, Budezjesi J, Li Q, et al. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Res Treat*. 2010;123:725–31. https://doi.org/10.1007/s10549-009-0674-9

59 Zhao M, Liu Y, Zheng C, Qu H. dbEMT 2.0: an updated database for epithelial-mesenchymal transition genes with experimentally verified information and precalculated regulation information for cancer metastasis. *J Genet Genomics*. 2019;46:595–7. https://doi.org/10.1016/j.jgg.2019.11.010
60 Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, et al. The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. *Cells*. 2019;8:1118. https://doi.org/10.3390/cells8101118

61 Freeze HH, Hart GW, Schnaar RL. Glycosylation precursors. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, et al. editors. Essentials of glycobiology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2015. p. 51–63.

62 Li H, Ning S, Ghandi M, Kryukov GV, Gopal S, Deik A, et al. The landscape of cancer cell line metabolism. *Nat Med*. 2019;25:850–60. https://doi.org/10.1038/s41591-019-0404-8

63 Shaul YD, Freinkman E, Comb WC, Cantor JR, Tam WL, Thiru P, et al. Dihydropyrimidine accumulation is required for the epithelial-mesenchymal transition. *Cell*. 2014;158:1094–109. https://doi.org/10.1016/j.cell.2014.07.032

64 Steinhaeuser SS, Morera E, Budkova Z, Schepsky A, Wang Q, Rolfsson O, et al. ECM1 secreted by HER2-overexpressing breast cancer cells promotes formation of a vascular niche accelerating cancer cell migration and invasion. *Lab Invest.* 2020;100:928–44. https://doi.org/10.1038/s41374-020-0415-6

65 Barkovskaya A, Goodwin CM, Seip K, Hilmarsdottir B, Pettersen S, Stalnecker C, et al. Detection of phenotype-specific therapeutic vulnerabilities in breast cells using a CRISPR loss-of-function screen. *Mol Oncol*. 2021;15:2026–45. https://doi.org/10.1002/1878-0261.12951

66 Aigner K, Descovich L, Mikula M, Sultan A, Dampier B, Bonné S, et al. The transcription factor ZEB1 (deltaEF1) represses Plakophilin 3 during human cancer progression. *FEBS Lett*. 2007;581:1617–24. https://doi.org/10.1016/j.febslet.2007.03.026

67 Bacigalupo ML, Manzi M, Espelt MV, Gentilini LD, Compagno D, Laderach DJ, et al. Galectin-1 triggers epithelial-mesenchymal transition in human hepatocellular carcinoma cells. *J Cell Physiol*. 2015;230:1298–309. https://doi.org/10.1002/jcp.24865

68 Bedi U, Mishra VK, Wasilewski D, Scheel C, Johnsen SA. Epigenetic plasticity: a central regulator of epithelial-to-mesenchymal transition in cancer. *Oncotarget*. 2014;5:2016–29. https://doi.org/10.18632/oncotarget.1875

69 Li H, Zhong A, Li S, Meng X, Wang X, Xu F, et al. The integrated pathway of TGFB/Snail with TNFalpha/NFkappaB may facilitate the tumor-stroma interaction in the EMT process and colorectal cancer prognosis. *Sci Rep*. 2017;7:4915. https://doi.org/10.1038/s41598-017-05280-6

70 Pavón M, Parreño M, Téllez-Gabriel M, Sancho F, López M, Céspedes M, et al. Gene expression signatures and molecular markers associated with clinical outcome in locally advanced head and neck carcinoma. *Carcinogen.* 2012;33:1707–16. https://doi.org/10.1093/carcin/bgs207

71 Xu F, Zhang J, Hu G, Liu L, Liang W, Hypoxia and TGF-b1 induced PLOD2 expression improve the migration and invasion of cervical cancer cells by promoting epithelial-to-mesenchymal transition (EMT) and focal adhesion formation. *Cancer Cell Int*. 2017;17:54. https://doi.org/10.1186/s12935-017-0420-z

72 Yamagami Y, Kawami M, Ojima T, Futatsugi S, Yumoto R, Takano M. Role of plasminogen activator inhibitor-1 in metotrexate-induced epithelial-mesenchymal transition in alveolar epithelial A549 cells. *Biochem Biophys Res Comm*. 2020;525:543–8. https://doi.org/10.1016/j.bbrc.2020.02.131

73 Mendez MG, Kojima S-I, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *FASEB J*. 2010;24:1838–51. https://doi.org/10.1096/fj.09-151639

74 Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, Williams ED, et al. Epithelial-mesenchymal and mesenchymal-epithelial transitions in carcinoma progression. *J Cell Physiol*. 2007;213:374–83. https://doi.org/10.1002/jcp.21223

75 Vergara D, Simeone P, Latorre D, Causone F, Leperotti S, Trerotola M, et al. Proteomics analysis of E-cadherin knockdown in epithelial breast cancer cells. *J Biotechnol*. 2015;202:3–11. https://doi.org/10.1016/j.jbiotec.2014.10.034

76 Vitale DL, Caon I, Parmigiani A, Sevic I, Spinelli FM, Icardi A, et al. Initial identification of UDP-glucose dehydrogenase as a prognostic marker in breast cancer patients, which facilitates epirubicin resistance and regulates hyaluronan synthesis in MDA-MB-231 cells. *Biomolecules*. 2021;11:246.

77 Sonkar K, Ayyappan V, Tressler CM, Adelaja O, Cai R, Cheng M, et al. Focus on the glycerophosphocholine pathway in choline phospholipid metabolism of cancer. *NMR Biomed*. 2019;32:e4112. https://doi.org/10.1002/nbm.4112

78 Cao MD, Sitter B, Bathe T, Bofin A, Lenning PE, Lundgren S, et al. Predicting long-term survival and treatment response in breast cancer patients receiving neoadjuvant chemotherapy by MR metabolic profiling. *NMR Biomed*. 2012;25:369–78. https://doi.org/10.1002/nbm.1762

79 Giskeodérgård GF, Grinde MT, Sitter B, Axelson DE, Lundgren S, Fjosne HE, et al. Multivariate modeling and prediction of breast cancer prognostic factors using MR metabolomics. *J Proteome Res*. 2010;9:972–9. https://doi.org/10.1021/pr9008783

80 Moestue SA, Borgan E, Huuse EM, Lindholm EM, Sitter B, Børresen-Dale A-L, et al. Distinct choline UGDH affects GPC and NAA under PDGFRB regulation.
UDGH affects GPC and NAA under PDGFRB regulation

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metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. *BMC Cancer*. 2010;10:433. https://doi.org/10.1186/1471-2407-10-433

81 Pommier RM, Sanlaville A, Tonon L, Kielbassa J, Thomas E, Ferrari A, et al. Comprehensive characterization of claudin-low breast tumors reflects the impact of the cell-of-origin on cancer evolution. *Nat Commun*. 2020;11:3431. https://doi.org/10.1038/s41467-020-17249-7

82 Fougnier C, Bergholtz H, Norum JH, Sorlie T. Redefinition of claudin-low as a breast cancer phenotype. *Nat Commun*. 2020;11:1787. https://doi.org/10.1038/s41467-020-15574-5

83 Bharti SK, Mironchik Y, Wildes F, Penet MF, Goggins E, Krishnamachary B, et al. Metabolic consequences of HIF silencing in a triple negative human breast cancer xenograft. *Oncotarget*. 2018;9:15326–39. https://doi.org/10.18632/oncotarget.24569

84 Koch K, Hartmann M, Schröter F, Suwala AK, Maciaczyk D, Krüger AC, et al. Reciprocal regulation of the cholinic phenotype and epithelial-mesenchymal transition in glioblastoma cells. *Oncotarget*. 2016;7:73414–31. https://doi.org/10.18632/oncotarget.12337

85 Okazaki Y, Nakamura K, Takeda S, Yoshizawa I, Yoshida F, Ohshima N, et al. GDE5 inhibition accumulates intracellular glycerophosphocholine and suppresses adipogenesis at a mitotic clonal expansion stage. *Am J Physiol Cell Physiol*. 2019;316:C162–74. https://doi.org/10.1152/ajpcell.00305.2018

86 Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPARgamma signaling and metabolism: the good, the bad and the future. *Nat Med*. 2013;19:557–66. https://doi.org/10.1038/nm.3159

87 Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008;77:289–312. https://doi.org/10.1146/annurev.biochem.77.061307.091829

88 Iorio E, Mezzanzanica D, Alberti P, Spadaro F, Ramoni C, D’Ascenzo S, et al. Alterations of choline phospholipid metabolism in ovarian tumor progression. *Cancer Res*. 2005;65:9369–76. https://doi.org/10.1158/0008-5472.CAN-05-1146

89 Menzel N, Fischl W, Hueging K, Bankwitz D, Frentzen A, Haid S, et al. MAP-kinase regulated cytosolic phospholipase A2 activity is essential for production of infectious hepatitis C virus particles. *PLoS Pathog*. 2012;8:e1002829. https://doi.org/10.1371/journal.ppat.1002829

90 Ulisse S, Cinque B, Silvano G, Rucci N, Biordi L, Cifone MG, et al. Erk-dependent cytosolic phospholipase A2 activity is induced by CD95 ligand cross-linking in the mouse derived sertoli cell line TM4 and is required to trigger apoptosis in CD95 bearing cells. *Cell Death Differ*. 2000;7:916–24. https://doi.org/10.1038/sj.cdd.4400716

91 Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, et al. NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Investig*. 2004;114:569–81. https://doi.org/10.1172/JCI21358

92 Naidu S, Shi L, Magee P, Middleton JD, Laganá A, Sahoo S, et al. PDGFR-modulated miR-23b cluster and miR-125a-5p suppress lung tumorigenesis by targeting multiple components of KRAS and NF-kB pathways. *Sci Rep*. 2017;7:15441. https://doi.org/10.1038/s41598-017-14843-6

93 Wang H, Yin Y, Li W, Zhao X, Yu Y, Zhu J, et al. Over-expression of PDGFR-beta promotes PDGF-induced proliferation, migration, and angiogenesis of EPCs through PI3K/Akt signaling pathway. *PLoS One*. 2012;7:e30503. https://doi.org/10.1371/journal.pone.0030503

94 Wang Z, Ahmad A, Li Y, Kong D, Azmi AS, Banerjee S, et al. Emerging roles of PDGF-D signaling pathway in tumor development and progression. *Biochem Biophys Acta*. 2010;1806:122–30. https://doi.org/10.1016/j.bbcan.2010.04.003

95 Wu Q, Wang R, Yang Q, Hou X, Chen S, Hou Y, et al. Chemo-resistance to gemcitabine in hepatoma cells involves epithelial-mesenchymal transition and involves activation of PDGF-D pathway. *Oncotarget*. 2013;4:1999–2009.

96 Shimamura T, Hsu TC, Colburn NH, Bejeck BE. Activation of NF-kappaB is required for PDGF-B chain to transform NIH3T3 cells. *Exp Cell Res*. 2002;274:157–67. https://doi.org/10.1006/excr.2001.5449

97 Xu B, Bai Z, Yin J, Zhang Z. Global transcriptomic analysis identifies SERPINE1 as a prognostic biomarker associated with epithelial-to-mesenchymal transition in gastric cancer. *PeerJ*. 2019;7:e7091. https://doi.org/10.7717/peerj.7091

98 Piskareva O, Harvey H, Nolan J, Conlon R, Alcock L, Buckley P, et al. The development of cisplatin resistance in neuroblastoma is accompanied by epithelial to mesenchymal transition in vitro. *Cancer Lett*. 2015;364:142–55. https://doi.org/10.1016/j.canlet.2015.05.004

99 Ren B-J, Zhou D-J, Ju Y-L, Wu J-H, Ouyang M-Z, et al. Alisertib induces cell cycle arrest, apoptosis, autophagy and suppresses EMT in HT29 and Caco-2 cells. *Int J Mol Sci*. 2015;17:41. https://doi.org/10.3390/ijms17010041

100 Wang Y, Zeng G, Jiang Y. The emerging roles of miR-125b in cancers. *Cancer Manag Res*. 2020;12:1079–88. https://doi.org/10.2147/cmar.S232388
Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Study workflow and the phenotypes of the cell lines. (A) Workflow of the proteomic analysis of the three breast EMT cell models and metabolomics analysis after siRNA knock-down of the metabolic target UGDH in all the mesenchymal cell lines. Three breast EMT cell models (epithelial and mesenchymal cell line pairs) were used in this study, D492&D492M, HMLE&HMLEM, and PMC42LA&PMC42ET. The proteomic strategy was label-free quantification (LFQ) with each cell line in triplicates. The metabolomic strategy was untargeted metabolomics in negative, positive, and basic modes with six replicates. The metabolomic experiments for D492M, HMELM, and PMC42ET were 90 % (n = 5). KD: Knock-down. Student’s T-test, ***: P < 0.001; **: P < 0.01; *: P < 0.05. Default settings were used for the analysis. The GO terms were listed according to the -log10 p value in descending order. The numbers of genes in each GO term were also plotted as dots/line plots. CC: Cellular Component; MF: Molecular Function.

**Fig. S2.** Inconsistent EMT markers. A list of known EMT markers (based on the public EMT database dbEMT) was inconsistently altered among the three EMT models. Student’s T-test, *: P < 0.05; **: P < 0.01; ***: P < 0.001; n = 3. CD44, CD44 antigen; LMNB1, Lamin-B1; MSN, Moesin; FLNA, Filamin-A; TLN1, Talin-1; FSCN1, Fascin; EGFR, Epidermal growth factor receptor; S100A2, S100 calcium binding protein A2; NDRG1, N-myc downstream regulated 1.

**Fig. S3.** Accuracy and validity of the proteomic analysis. The accuracy and validity of the proteomic analysis in this study were confirmed by comparing the current data to our previously generated proteomic data for the D492 EMT model [26]. The correlation between these two datasets was 0.936. The high correlation coefficient (Pearson correlation, 0.936) of the datasets ensures good accuracy and validity of the proteomic analysis in this study. It laid the foundation for valid conclusions deducted from this study.

**Fig. S4.** Functional annotation of the GO terms (CC and MF) for the three EMT models. Functional annotation of the GO terms (CC and MF) was conducted on the DAVID (DAVID Bioinformatics Resources 6.8) platform for each EMT model. Data used for the GO annotation analysis (Supplementary Table 3) were proteins significantly altered in each EMT model (Permutation-based FDR < 0.05). Default settings were used for the analysis. The GO terms were listed according to the -log10 p value in descending order. The numbers of genes in each GO term were also plotted as dots/line plots. CC: Cellular Component; MF: Molecular Function.

**Fig. S5.** Knock-down efficiency of UGDH with two siRNAs. (A-D) The knock-down efficiency of UGDH with two siRNAs compared to the scramble control was around 80 % in D492M (n = 7 for the first siRNA; n = 9 for the second siRNA) (A-B) and 60 % in D492HER2 (n = 5) (C-D). (E) The knock-down efficiency of UGDH with two siRNAs in the metabolomics experiments for D492M, HMELM, and PMC42ET was 90 % (n = 5). KD: Knock-down. Student’s T-test, ***: P < 0.001. UGDH, UDP-glucose 6-dehydrogenase.

**Fig. S6.** Functional analysis of UGDH in EMT. (A) Photos of the D492M and D492HER2 cells following knock-down of UGDH via two siRNAs in the invasion assay. Cells were stained with DAPI and observed under the objective 10x. (B-C) Cell invasion decreased with the second siRNA knock-down of UGDH in both non-tumorigenic D492M (B) and tumorigenic D492HER2 (C) compared with the scramble control. Student’s T-test, *: P < 0.05; **: P < 0.01; ***: P < 0.001; n = 3.
D492HER2 (C). n equals 3, and ten spots were chosen for each replicate during the cell counting process. (D-E) One of the main EMT transcription factors SNAI1 was downregulated following the second siRNA knock-down of UGDH in both non-tumorigenic D492M (n = 5) (D) and tumorigenic D492HER2 (n = 4) (E). (F-H) The Kaplan-Meier plots of FDFT1, SORD, and TSTA3 in basal breast cancer patients were downloaded from kmplot.com. Student’s T-test, **: P < 0.01; ***: P < 0.001. UGDH, UDP-glucose 6-dehydrogenase; SNAI1, Snail Family Transcriptional Repressor 1; FDFT1, Squalene synthase; SORD, Sorbitol dehydrogenase; TSTA3, GDP-L-fucose synthase.

Fig. S7. GPC and NAA were altered with the siUGDH treatment in D492HER2 and MDA-MB-231. (A-D) The glycerophosphocholine (GPC) level was decreased, and the acetylaspaticate (NAA) level was increased after the siUGDH treatment in the tumorigenic D492HER2 (n = 3) and malignant MDA-MB-231 (n = 6) cell lines. (E) There were no significant differences to the GPC levels between mesenchymal cells and non-mesenchymal cells based on published datasets in literature [62, 63] (Supplementary Table 5). (F-G) The expression levels of choline (F) and phosphocholine (G) with UGDH knock-down in the three EMT cell models. No significant and consistent changes were observed for both metabolites in all the cell lines (n = 5). (H) UGDH has been reported to downregulate PPARγ [27]. To test if there was a negative correlation between UGDH and PPAR signaling, we performed a phosphoproteomic analysis on the D492 EMT cell model (Supplementary Table 7) and noticed that the PPAR signaling was downregulated in the mesenchymal cells where UGDH was highly expressed. The IPA pathways were listed based on the -log10(p value), and the z-scores for the pathways were represented by the dots/line plot. Red: higher in the mesenchymal D492M; blue: higher in the epithelial D492. (I) The enzyme PLA2G15 potentially involved in the hydrolysis of phosphatidylcholine (PtdCho) into GPC was higher in both D492M and D492HER2. UGDH has been reported to regulate the phosphorylation of ERK (pERK) [32]. cPLA2 is responsible for GPC synthesis from PtdCho in choline metabolism and is under the control of ERK/MAPK [89, 90]. We also observed that PLA2G15 was highly expressed in D492M and D492HER2 compared to D492 (I), suggesting the knock-down of UGDH may downregulate GPC via pERK-PLA2G15 (n = 3). Student’s T-test, *: P < 0.05; **: P < 0.01; ***: P < 0.001. n.s: not significant. UGDH, UDP-glucose 6-dehydrogenase; PLA2G15, Phospholipase A2 group XV.

Fig. S8. PDGFRB regulates UGDH via RELA (NFkB-p65). (A) PDGFRB was highly expressed in the tumorigenic mesenchymal cell line D492HER2 based on the RPPA analysis (n = 3) [65]. (B) The knock-down efficiency of PDGFRB with siRNA in the D492HER2 cell line was about 90% (n = 6). (C) RELA (NFkB-p65) was downregulated after the siRNA knock-down of PDGFRB in D492HER2 (n = 6). (D) UGDH was downregulated after the siRNA knock-down of PDGFRB in D492HER2 (n = 6). (E) The knock-down efficiency of RELA with the second siRNA in D492M was around 70% (n = 6). (F) UGDH was downregulated after the knock-down of RELA in D492M with the second siRNA (n = 6). (G) The knock-down efficiency of RELA with the first siRNA in D492HER2 was around 90% (n = 6). (H) No significant change in UGDH was observed after the knock-down of RELA with the first siRNA in D492HER2 (n = 6). (I) The knock-down efficiency of RELA with the second siRNA in the D492HER2 cell line was about 90% (n = 6). (J) UGDH was downregulated after the knock-down of RELA in D492HER2 with the second siRNA (n = 6). Student’s T-test, *: P < 0.05; **: P < 0.01; ***: P < 0.001. UGDH, UDP-glucose 6-dehydrogenase; PDGFRB, Platelet-derived growth factor receptor beta; RELA (NFkB-p65), Nuclear factor NF-kappa-B p65 subunit.

Table S1. The internal standard mix used in the metabolomics analysis.
Table S2. A list of primers used in this study.
Table S3. Perseus output data.
Table S4. Raw data of proteomics.
Table S5. Publicly available data on the GPC levels of mesenchymal cells.
Table S6. In silico knockdown of UGDH in GEMs.
Table S7. Data of phosphoproteomics.