



# SIRT7–SREBP1 restrains cancer cell metabolic reprogramming by upregulating IDH1

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## Abstract

SIRT7 plays critical roles in tumorigenesis and tumor progression; however, the underlying mechanisms are poorly understood. Here, we aimed to identify downstream targets of SIRT7 to help delineate its precise function. In this study, we demonstrate that SIRT7 is essential to regulate IDH1 expression in various cancer cell types. Interestingly, both SIRT7 and IDH1 levels are downregulated in breast cancer lung metastases and are useful for predicting disease progression and prognosis. Mechanistically, SIRT7 enhances *IDH1* transcription, and this process is mediated by SREBP1. *SIRT7* insufficiency reduces cellular  $\alpha$ -ketoglutarate, a metabolite product of IDH1, and suppresses lipogenesis and gluconeogenesis. Moreover,  $\alpha$ -ketoglutarate decline increases HIF1 $\alpha$  protein levels and, thus, promotes glycolysis. This effect permits cancer cells to facilitate Warburg effect and undergo fast proliferation. Overall, the SIRT7–IDH1 axis regulates cancer cell metabolic reprogramming and, thus, might serve as a point of therapeutic intervention.

**Keywords** SIRT7 · IDH1 · SREBP1 · Glycolysis ·  $\alpha$ -KG · Metastasis

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## Introduction

The essence of a tumor can be characterized as uncontrolled cell proliferation (Hanahan and Weinberg 2000). To support that, cancer cells frequently adjust their energy metabolism to respond to cell growth and division signals (Xie and Simon 2017). Via a process known as the Warburg effect, most cancer cells utilize glycolysis to generate energy and metabolites, even when sufficient oxygen is present for the oxidative phosphorylation pathway (Warburg 1956; Liberti and Locasale 2016). To support such neoplastic metabolic reprogramming, several metabolic enzymes exhibit dysregulated functions or gain unexpected mutations (Sreedhar and Zhao 2018). The isocitrate dehydrogenase enzyme, IDH1, is one such enzyme that frequently carries a gain-of-function mutation resulting in abnormal expression in certain cancers (Suzuki et al. 2015; Yan et al. 2009). IDH1 localizes in the cytoplasm and peroxisomes, catalyzing the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate ( $\alpha$ -KG) and NADPH (Geisbrecht and Gould 1999; D'Adamo and Haft 1965). IDH1 reversibly converts  $\alpha$ -KG into isocitrate and NADPH via reductive carboxylation (Ochoa 1948) and participates in lipid biosynthesis from glutamine in some cancers (Filipp et al. 2012; Metallo et al. 2011). As a co-factor,  $\alpha$ -KG is indispensable for the enzymatic activity of

( $\alpha$ -KG)-dependent dioxygenases, such as histone demethylases, prolyl hydroxylases (PHDs), collagen prolyl-4-hydroxylases, and the TET (ten-eleven translocation) family of 5-methylcytosine (5mC) hydroxylases (Chowdhury et al. 2011; Tsukada et al. 2006; Tahiliani et al. 2009). Moreover, IDH1 regulates cellular histone methylation to manipulate tumorigenesis. A somatic heterozygous mutation, IDH1<sup>R132</sup>, leads to the generation of D-2-hydroxyglutarate (2-HG), which competes with  $\alpha$ -KG and subsequently blocks ( $\alpha$ -KG)-dependent dioxygenases, resulting in global DNA or histone hypermethylation and hypoxia-inducible factor (HIF1 $\alpha$ ) stabilization; this effect can accelerate glioma and leukemia development (Clark et al. 2016).

IDH1 mutations, albeit at low frequency, have been reported in other solid cancers, and emerging evidence suggests that non-mutated IDH1 also has important biological functions and clinical effects in breast cancers and some gliomas (Calvert et al. 2017; Liu et al. 2015, 2018). IDH1 mRNA and protein levels are elevated in primary glioblastomas (GBMs), promoting growth and therapeutic resistance (Calvert et al. 2017). IDH1 loss during breast cancer progression promotes metastasis via SLUG upregulation and HIF1 $\alpha$  stabilization (Liu et al. 2018). However, the mechanisms of non-mutated IDH1 regulation remain elusive in cancers.

SIRT7 is a member of the sirtuin family of proteins that have NAD<sup>+</sup>-dependent deacetylase activity (Blank and Grummt 2017). Recent findings suggest that SIRT7 is involved in ribosomal RNA (rRNA) transcription (Ford et al. 2006), chromatin remodeling (Vazquez et al. 2016), DNA repair and cell survival (Tang et al. 2017a, b), lipid metabolism (Tang 2015), and cancer metastasis (Li et al. 2018; Tang et al. 2017a, b). Here, we aimed to understand the roles of SIRT7 in regulation of tumor associated metabolism reprogramming. To do so, we analyzed the link between SIRT7 and metabolism-related enzymes and identified IDH1 as a new downstream target of SIRT7, which modulates cancer lipogenesis, gluconeogenesis and glycolysis via  $\alpha$ -KG.

## Materials and methods

### Cell cultures

Human normal hepatic L-O2 cells; human hepatocellular carcinoma HepG2 and CRL-8024 cells; human glioma U-118 MG, U87, and U251 cells; and human breast cancer MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose) (Thermo Fisher, USA) supplemented with 10% fetal bovine serum (FBS, PAN-Biotech GmbH, Germany), 100 U/ml penicillin, and 100 g/ml streptomycin (Thermo Fisher), at 37 °C under a 5% CO<sub>2</sub> atmosphere.

### Constructs, siRNAs, and transfection

The human *IDH1* promoter was amplified from genomic DNA extracted from HEK293 cells, with the following primer sequence. Forward primer: 5'-CCGCTCGAGCCG TTTTCTAAGGCTTCACATC-3'; reverse primer: 5'-GGA AGATCTTCCGATGATATGCTGGCGAAGAG-3'. Then, the promoter region was cloned into the luciferase reporter system vector pGL-4.20 (Promega, USA). To mutate the SREBP1-recognizing motif on *IDH1* promoter, site mutagenesis was performed with the following primers. Forward primer: 5'-CGGGGCTGGGGGAGGTTTCGT GAGGAGGCGGGGCT-3'; reverse primer: 5'-AGGCC CGCCTCCTCACGAAACCTCCCCAGCCCCG-3'. For gene silencing, we synthesized specific custom siRNAs purchased from GenePharma Company (Shanghai, China). All oligo RNA sequences used in this manuscript are listed below: human *SIRT7* siRNA-1, 5'-CUCACCGUAUUU CUACUACUAdTdT-3' and.

human *SREBP1* siRNA-1, 5'-GCUCCUCUCUUGAAG CCUUTT-3'. For plasmid and oligo RNA transient transfections, we employed Lipo3000 reagent (Thermo Fisher), following the operational guidelines.

### RNA isolation and qRT-PCR

Whole RNA extracts were isolated using Trizol reagent RNAiso Plus (TaKaRa, Japan). To transcribe RNA into cDNAs, 1000 ng fresh RNA was incubated with the PrimeScript RT Master Mix (TaKaRa), according to the manufacturer's instructions. To quantify gene expression, qRT-PCR was performed using 2 × SYBR Green Mix (TaKaRa, RR064) in the Bio-Rad detection system. All primers are shown in Supplementary Table 1.

### Cellular $\alpha$ -KG measurement

HepG2 and L-O2 cells with indicated treatments were cultured in 6-well-plates to 70% confluence. Then, the cells were washed twice with cold PBS and lysed with the indicated elution buffer. The levels of  $\alpha$ -KG were measured using a commercial kit (K677-100, BioVision, USA), according to the manufacturer's instructions.

### Dual luciferase reporter assays

HEK293 cells were cultured in 24-well plates to 60% confluence. The indicated plasmids were cotransfected with Lipo3000 reagent. After 48 h, luminescence was measured using a Dual-Luciferase Reporter Assay System

(Promega), according to the manufacturer's instructions. The relative luciferase activity was calculated as the ratio of firefly to *Renilla* luciferase activity (Fluc/Rluc).

### Protein extraction and immunoblotting

To prepare protein samples, cells were lysed in RIPA buffer. Then, protein extracts were resolved in Laemmli sample buffer, separated using SDS-PAGE, transferred to a PVDF membrane (Millipore), blocked with 5% non-fat milk, and probed with the respective primary antibodies. Immunoblotting images were acquired using a ChemiDoc system (Bio-Rad, USA). All antibodies used are described in Supplementary Table 2.

### Immunoprecipitation (IP)

The indicated samples were prepared in IP lysis buffer (200 mM NaCl, 20 mM Tris-HCl (pH 7.9), 5 mM MgCl<sub>2</sub>, 10% glycerol, 0.2 mM EDTA, and 0.1% NP-40) supplemented with protease inhibitors (Sigma, complete™ Protease Inhibitor Cocktail and PhosSTOP™). Clarified cell supernatants were incubated with the respective antibodies for 4 h at 4 °C. After the beads were washed with IP lysis buffer and eluted in Laemmli sample buffer, the immunoprecipitates were subjected to western blotting analysis.

### Kaplan–Meier survival analysis of patients with breast cancer

The online tool, PROGene V2, was employed to analyze correlations between levels of IDH1 with clinical outcomes (relapse-free survival (RFS) or overall survival (OS) of breast cancers. All Kaplan–Meier survival diagrams shown in the manuscript were analyzed according to previously published methods (Goswami and Nakshatri 2014).

### Statistical analysis

All results are presented as means ± SEM, based on at least three independent experiments. Data were analyzed by the Student's *t* test using Prism 7.0 software (GraphPad Prism). A *p* value < 0.05 was considered to be statistically significant.

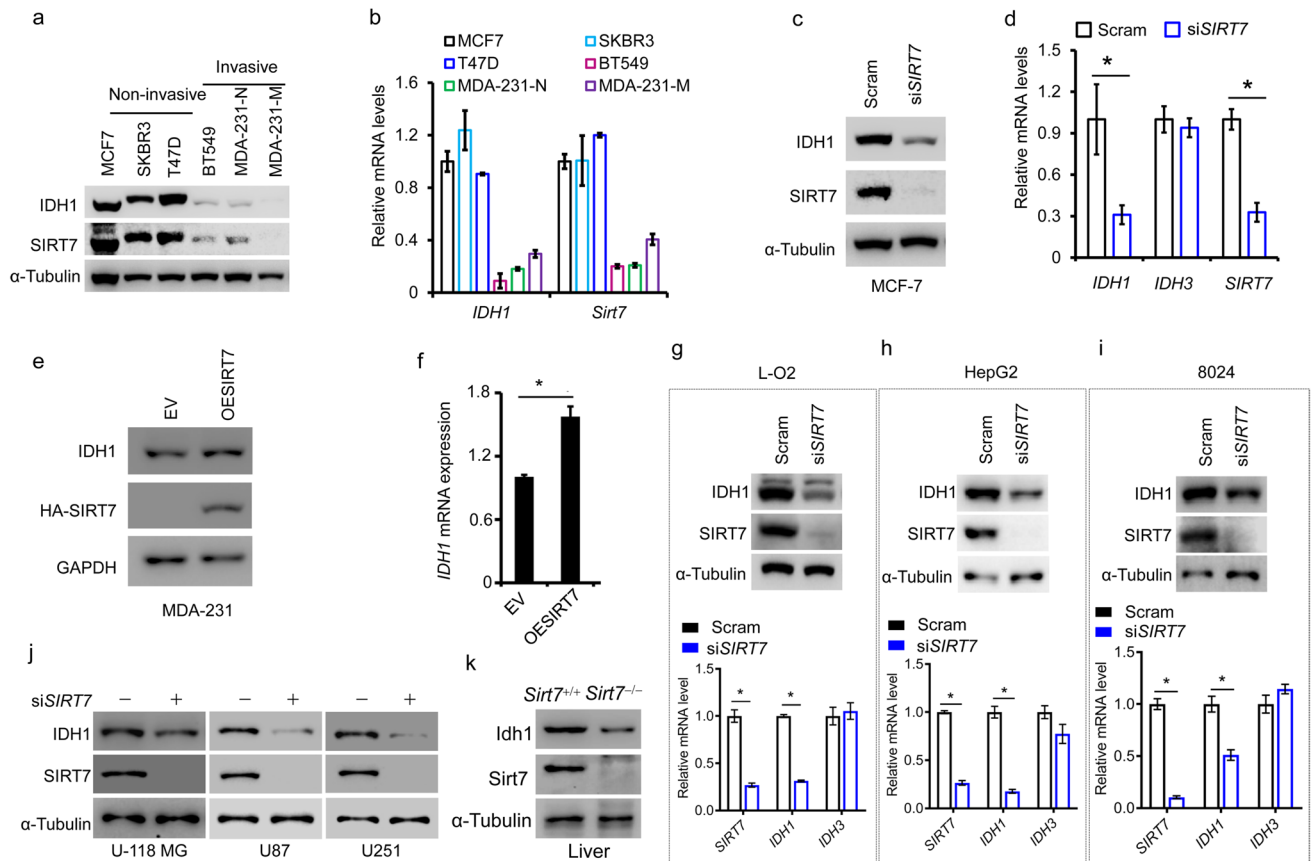
## Results

### SIRT7 positively regulates IDH1 expression in breast cancers

In our first analyses, we determined the IDH1 protein and mRNA expression profile in various breast cancer cell lines. We found that, consistent with previous reports (Liu et al. 2018), IDH1 was decreased at the mRNA and protein level, in invasive breast cancer cells (Fig. 1a, b). These findings suggest that IDH1 is downregulated at the level of its transcriptional regulation. We previously have found that SIRT7 expression is significantly downregulated in breast cancer lung metastases (Tang et al. 2017a, b). Interestingly, similarly to SIRT7, we found that IDH1 protein expression was remarkably decreased in invasive breast cancer cells compared to the non-invasive ones (Fig. 1a). The mRNA levels of *SIRT7* and *IDH1* also displayed consistent tendencies as the protein expression levels (Fig. 1b), suggesting the transcriptional regulation. To explore whether SIRT7 mediates IDH1 expression, we knocked down SIRT7 expression in human breast cancer MCF-7 cells, which have high endogenous SIRT7 and IDH1 levels. *SIRT7* knockdown significantly downregulated IDH1 expression, both in terms of protein and mRNA levels (Fig. 1c, d). By contrast, other isocitrate dehydrogenase enzymes, including IDH2, IDH3α, IDH3β and IDH3γ, were affected little by SIRT7 knockdown (Supplementary Figure 1). We, thus, randomly employed IDH3α as a negative control in the following experiments. Next, we overexpressed SIRT7 in human breast cancer MDA-MB-231 cells (MDA-231), which usually express low levels of SIRT7 and IDH1. Boosting SIRT7 expression increased *IDH1* transcription and protein levels (Fig. 1e, f). Taken together, our results indicate that SIRT7 might positively regulate IDH1 expression in breast cancer cells.

### SIRT7 regulates IDH1 expression in multiple cancers

To date, there have been few reports describing the regulation of IDH1 expression. We, thus, assessed whether the modulation of IDH1 by SIRT7 was dependent on the cell or the cancer context. To this end, we analyzed IDH1 expression changes with or without *SIRT7* knockdown in L-O2 human normal hepatic cells; human hepatocellular carcinoma HepG2 and CRL-8024 cells; and human glioma U-118 MG, U87, and U251 cells. As shown, *SIRT7* knockdown impaired IDH1 protein and mRNA levels, regardless of the cellular context (Fig. 1g, j). SIRT7 regulates lipogenesis and is abundant in liver tissues (Yoshizawa et al. 2014). Consistently, we found that *Idh1* was markedly decreased in the liver lysate of *Sirt7*<sup>-/-</sup> mice (Fig. 1k). Together, these results



**Fig. 1** SIRT7 positively regulates IDH1 expression in multiple cancers. Immunoblotting (a) and qRT-PCR (b) analysis of IDH1 and SIRT7 levels in various breast cancer cells classified into non-invasive and invasive groups. Immunoblotting (c) and qRT-PCR (d) analysis of IDH1 expression in human breast cancer MCF-7 cells with or without *SIRT7* knockdown (si*SIRT7*). Scram, scrambled siRNAs taken as a negative control. IDH3 is an isoenzyme of IDH1 and here was considered to be a control. Immunoblotting (e) and qRT-PCR (f) analysis of IDH1 expression in human breast cancer MDA-MB-231 cells with or without overexpression of ectopic *SIRT7*. EV (empty

vector), cells were transfected with empty vector plasmid as a control. (g, i) Immunoblotting (upper panel) and qRT-PCR (lower panel) analysis of IDH1 levels in human normal hepatic L-O2 cells and human liver cancer HepG2 and 8024 cells, with or without knockdown of *SIRT7*. (j) Immunoblotting analysis of IDH1 levels in human glioma U-118 MG, U87, and U251 cells, with or without knockdown of *SIRT7*. (k) Idh1 protein levels were detected in liver tissues of *Sirt7*<sup>+/+</sup> or *Sirt7*<sup>-/-</sup> mice. Representative data are shown as means ± SEM. \**p* < 0.05; Student's *t* test

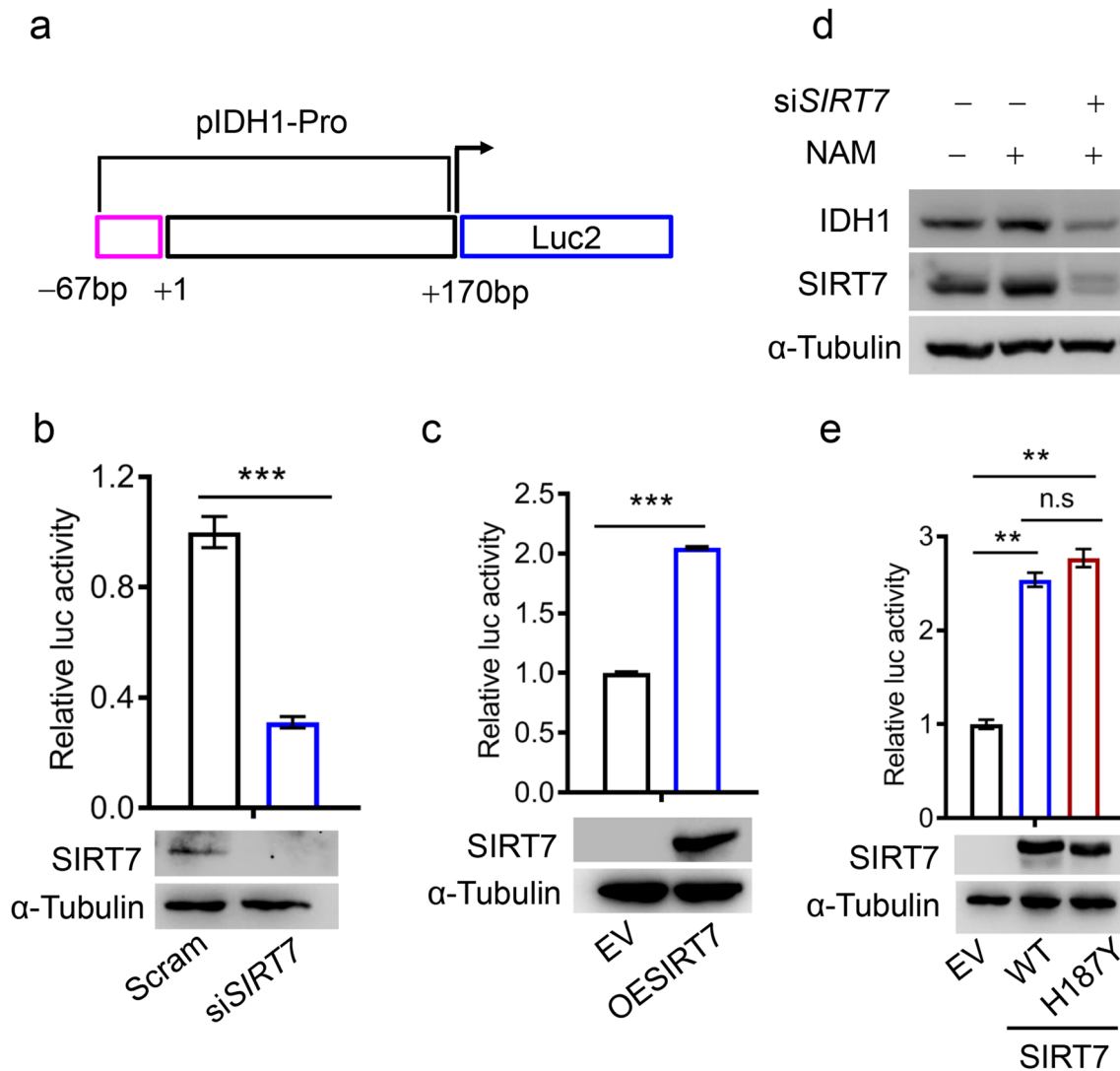
indicate that SIRT7 likely regulates IDH1 expression in multiple cancers and tissues.

### SIRT7 is required to sustain IDH1 transcription

Based on our findings thus far, we assumed that SIRT7 might regulate IDH1 transcription. To test this hypothesis, we cloned the *IDH1* promoter into the pGL-4.20 luciferase reporter system (pIDH1-Pro, Fig. 2a), as previously described (Shechter et al. 2003). We saw that *IDH1* promoter activity was significantly decreased in *SIRT7* knockdown cells (Fig. 2b). By contrast, *SIRT7* overexpression seemed to facilitate *IDH1* transcription (Fig. 2c). These results confirm that SIRT7 sustains *IDH1* transcription.

As SIRT7 possesses deacetylase activity, we next asked whether such regulation of *IDH1* relies on its enzymatic

activity. Surprisingly, compared to the untreated control only with solvent, nicotinamide (NAM; a pan-sirtuin inhibitor) enhanced rather than suppressed the level of IDH1 protein, implying that SIRT7 deacetylase activity had a less profound impact on IDH1 transcriptional activity (Fig. 2d). We presumed that this NAM-induced increase in IDH1 levels could be attributed to elevated SIRT7 levels (Fig. 2d, the middle lane), as *SIRT7* knockdown reversed the effects of NAM on IDH1 (Fig. 2d, the last lane). To exclude any non-specific effects of NAM, we evaluated whether a SIRT7 catalytically inactive mutant (H187Y) was sufficient to promote *IDH1* transcription. Wild-type (WT) SIRT7 and H187Y had similar effects on *IDH1* promoter activity: both causing ~2.5-fold increase of IDH1 promoter activity (Fig. 2e). Additionally, we observed that overexpression of either wild-type SIRT7 or SIRT7-H187Y sufficiently increased endogenous IDH1



**Fig. 2** SIRT7 positively regulates *IDH1* transcription. **a** A schematic diagram of the *IDH1* promoter (pIDH1-Pro). **b** A luciferase reporter assay showing *IDH1* promoter activity (upper panel) in HEK293 cells transfected with *SIRT7* siRNAs (si*SIRT7*) or the control siRNAs (Scram). The lower panel shows SIRT7 protein levels. **c** A luciferase reporter assay detecting *IDH1* promoter activity (upper panel) in HEK293 cells expressing ectopic *SIRT7* (OESIRT7) or empty vector plasmid (EV). SIRT7 levels were monitored by immunoblot-

ting, shown in the lower panel. **d** Immunoblotting analysis of *IDH1* expression in MCF-7 cells treated or not treated with NAM. NAM, nicotinamide (10 mM). **e** A luciferase reporter assay showing *IDH1* promoter activity (upper panel) in HEK293 cells transfected with wild-type (WT) *SIRT7* or catalytically inactive mutant (H187Y). Representative data are shown as means  $\pm$  SEM. \*\* $p < 0.001$ , \*\*\* $p < 0.001$ ; Student's *t* test

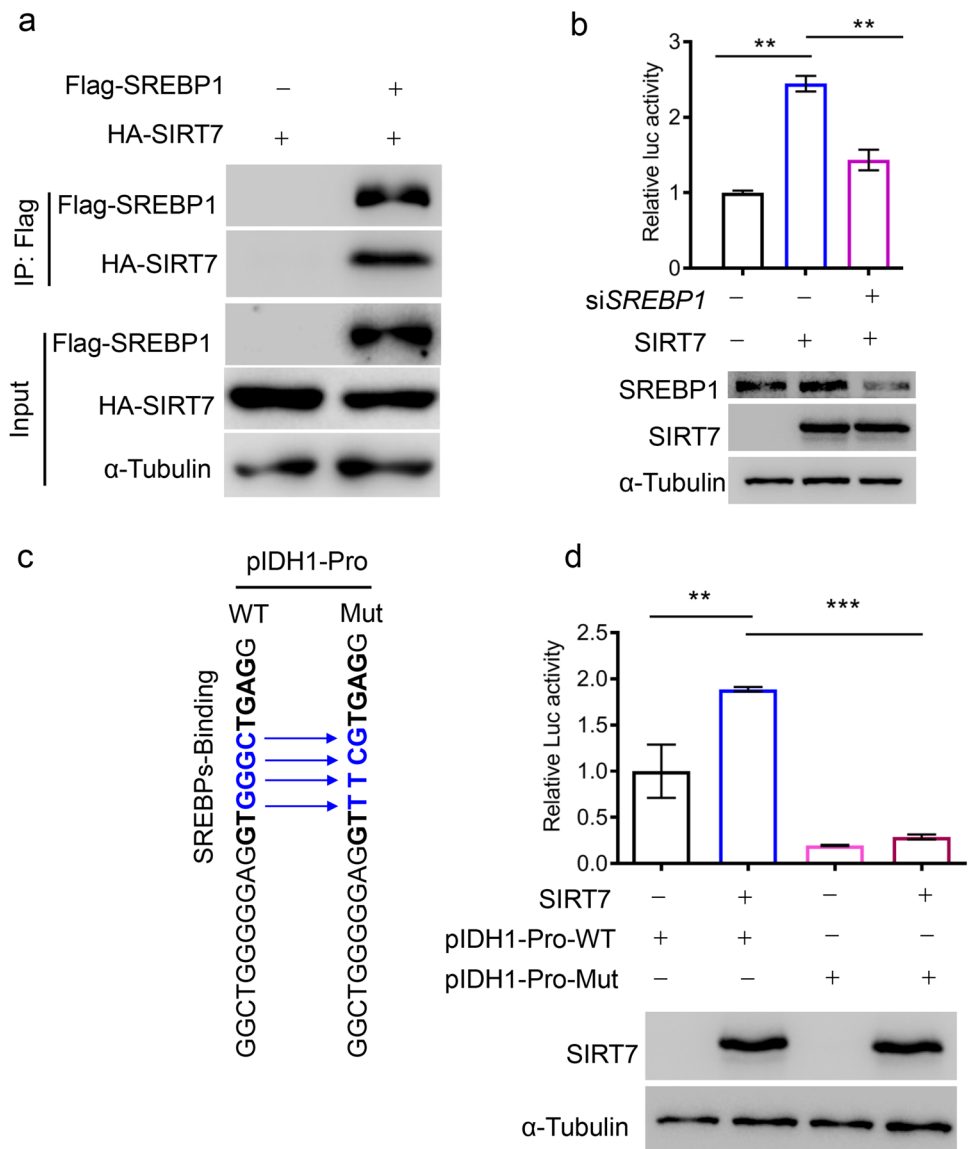
protein levels (Supplementary Figure 2). These findings support that SIRT7 promotes *IDH1* transcription in a deacetylase-independent manner.

### SREBP1 underlies SIRT7-mediated transcriptional regulation of *IDH1*

Previous research has shown that the sterol regulatory element-binding protein 1 (SREBP1) also regulates *IDH1* transcription (Ricoult et al. 2016; Shechter et al. 2003). We, thus, decided to test whether SIRT7 worked with SREBP1

to regulate *IDH1* expression. Interestingly, we observed an apparent protein interaction between SIRT7 and SREBP1 (Fig. 3a). We, thus, investigated whether SIRT7-mediated *IDH1* transcription is SREBP1 dependent. Indeed, the SIRT7 overexpression-induced increase in *IDH1* transcription was significantly attenuated by *SREBP1* knock-down (Fig. 3b). To gain direct evidence of such SREBP1-dependent regulation, we mutated a SREBP1-recognizing motif, GTGGGCTGAG (pIDH1-Pro-WT), to GTTTCG TGAG (pIDH1-Pro-Mut) in the *IDH1* promoter (Fig. 3c). As shown by the luciferase reporter assay, pIDH1-Pro-mut

**Fig. 3** SREBP1 underlies SIRT7-induced *IDH1* transcription. **a** Immunoblotting analysis of HA-SIRT7 in anti-Flag-SREBP1 immunoprecipitates derived from HEK293 cells expressing the indicated plasmids. **b** Analysis of *IDH1* promoter activity in HEK293 cells after *SREBP1* knock-down or *SIRT7* overexpression (upper panel). SREBP1 and SIRT7 levels were analyzed by immunoblotting (lower panel). **c** A schematic diagram of the SREBP1-recognizing motif within the *IDH1* promoter (pIDH1-Pro-WT). Blue indicates the mutated sequence (pIDH1-Pro-Mut) to disrupt SREBP1 binding. **d** Analysis of *IDH1* promoter activity in HEK293 cells transfected with the indicated plasmids (upper panel). Immunoblotting showing the expression of ectopic SIRT7 (lower panel). Data are presented as means  $\pm$  SEM,  $n=3$ , \*\* $p < 0.001$ , \*\*\* $p < 0.001$ ; Student's *t* test



significantly attenuated luciferase activity compared with pIDH1-Pro-WT and failed to respond to *SIRT7* overexpression (Fig. 3d). Together, these results support that SIRT7 regulates *IDH1* transcription via SREBP1.

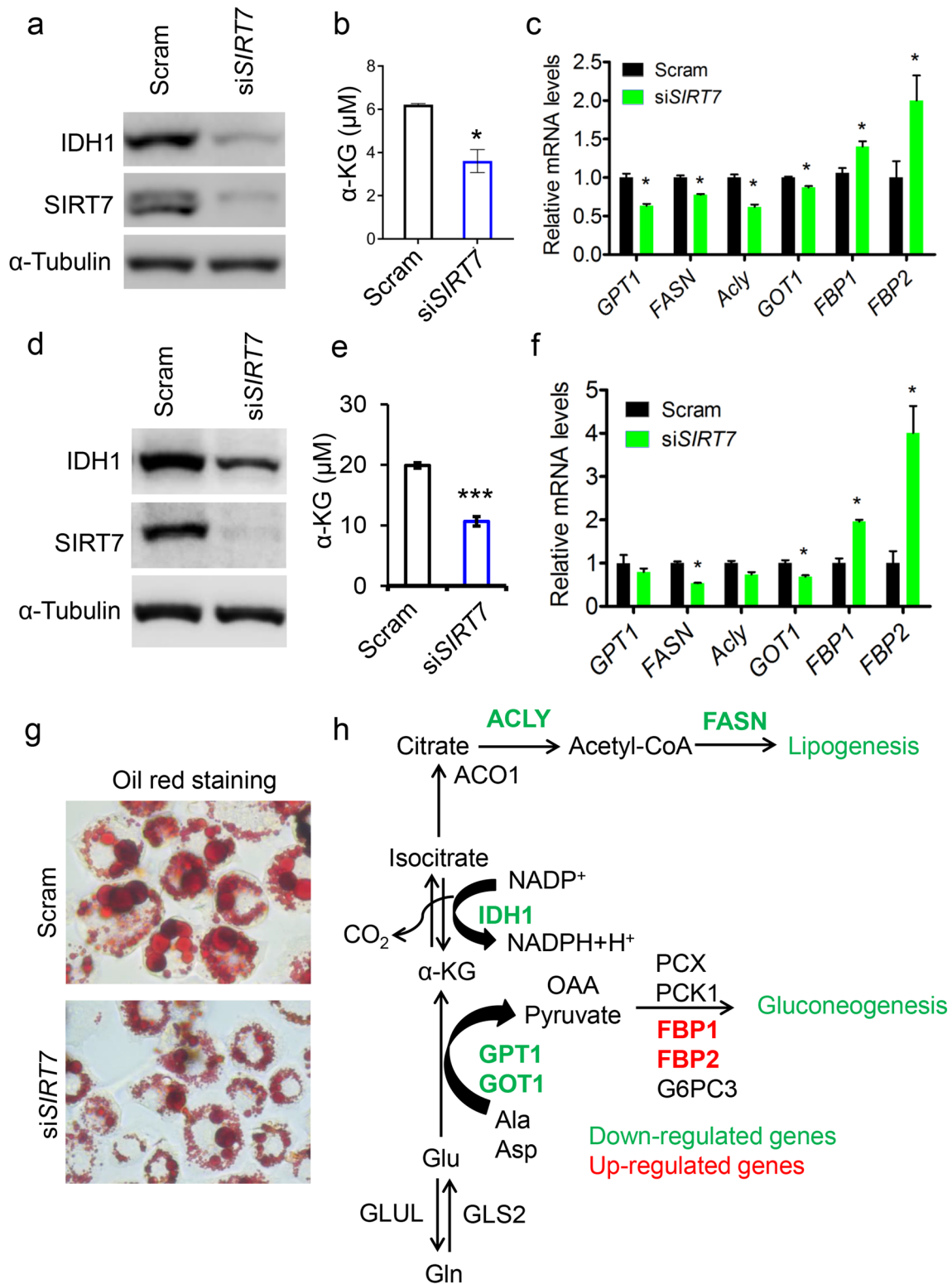
### SIRT7 regulates lipogenesis and gluconeogenesis

IDH1 produces non-mitochondrial  $\alpha$ -KG (Bogdanovic 2015), and as a result, an *IDH1* deficiency attenuates lipogenesis and gluconeogenesis via  $\alpha$ -KG (Shechter et al. 2003; Ye et al. 2017). We, thus, studied whether SIRT7 affected cellular  $\alpha$ -KG levels. We found that *SIRT7* knockdown significantly decreased cellular  $\alpha$ -KG levels in human hepatocellular carcinoma HepG2 cells (Fig. 4a, b). We, thus, investigated whether SIRT7 is involved in lipid biosynthesis in cancer cells. *SIRT7* knockdown in HepG2 and L-O2 cells resulted in the downregulation of *FASN*, *Acly*, *GPT1*, and

*GOT1* mRNA levels, suggesting suppression of gluconeogenesis and lipogenesis (Fig. 4c, f). Furthermore, reduced SIRT7 expression impaired lipid droplet generation in L-O2 cells, indicative of inhibited lipogenesis (Fig. 4g). Notably, *FBP1* and *FBP2* were upregulated (Fig. 4c, f), likely because of the feedback from decreasing glucogenic substrates giving rise to the inhibition of lipid biosynthesis (Fig. 4h). Together, these results indicate that SIRT7-IDH1 axis regulates lipogenesis and gluconeogenesis in cancer cells via modulating  $\alpha$ -KG production.

### SIRT7 regulates HIF1 $\alpha$ stability and glycolysis

Most cancer cells utilize glycolysis to support fast proliferation rate and HIF1 $\alpha$  plays pivotal roles in glycolytic metabolism (Xie and Simon 2017; Liberti and Locasale 2016). Prolyl hydroxylases (PHDs) which target HIF1 $\alpha$



**Fig. 4** SIRT7 regulates lipogenesis and gluconeogenesis. **a** Immunoblotting analysis of IDH1 levels in scramble-(Scram) and siSIRT7-treated human hepatocellular carcinoma HepG2 cells. **b** Quantitative analysis of  $\alpha$ -KG levels in **(a)**. **c** qRT-PCR analysis of gene expression related to lipogenesis and gluconeogenesis in **(a)**. **d** Immunoblotting analysis of IDH1 levels in scramble- (Scram) and siSIRT7-treated human hepatic L-O2 cells. **e** Quantitative analysis of  $\alpha$ -KG levels in

**(d)** **f** qRT-PCR analysis of genes related to lipogenesis and gluconeogenesis in **(d)**. **g** Representative images showing Oil Red O staining in L-O2 cells with or without SIRT7 knockdown. **h** A schematic diagram illustrating changes in metabolic flow in response to SIRT7 deficiency. Green: downregulation; red: upregulation. Representative data are shown as means  $\pm$  SEM. \* $p < 0.05$ , \*\*\* $p < 0.001$ ; Student's  $t$  test

for degradation are  $\alpha$ -KG-dependent dioxygenases (Selak et al. 2005). Given that SIRT7 regulates IDH1 and cellular  $\alpha$ -KG levels, we asked whether SIRT7 affects HIF1 $\alpha$  expression in cancer cells. As shown, *SIRT7* knockdown elicited a prominent increase of HIF1 $\alpha$  protein in breast cancer MCF-7 cells, while IDH1 restoration nearly reversed such effect (Fig. 5a). Moreover, *SIRT7* knockdown facilitated cancer cell glycolysis, which was evidenced by the generation of apparent acidic medium during culture (Fig. 5b). Together, our results indicate that the SIRT7–IDH1 axis contributes to metabolic alterations in cancer cells.

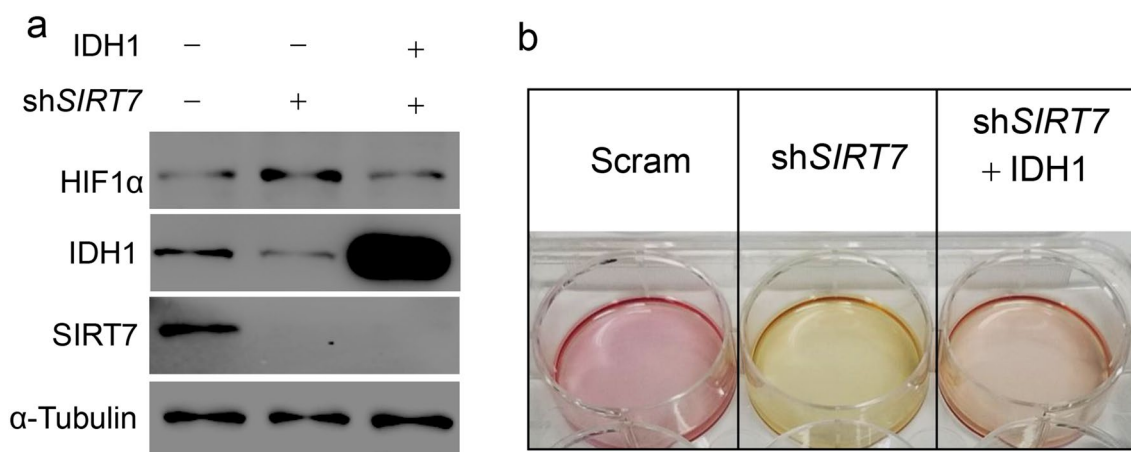
### The SIRT7–IDH1 axis predicts breast cancer progression

Finally, we explored the clinical relevance of the SIRT7–IDH1 axis. First, *IDH1* expression was significantly downregulated in patients with breast cancer who had low *SIRT7* levels (Fig. 6a). Second, supporting the SIRT7–IDH1 axis, the level of IDH1 was notably decreased in lung metastasis tumors compared with its level in paired primary tumors, reflecting the decline in the level of SIRT7 (Fig. 6b). Importantly, low *IDH1* levels were associated with poor prognosis in breast cancers (Fig. 6c, d), which was similar to findings for *SIRT7* (Tang et al. 2017a, b). Taken together, these results indicate that the SIRT7–IDH1 axis might be useful for predicting breast cancer progression.

## Discussion

SIRT7 seems to assume various roles during tumorigenesis that are most likely dependent on the cancer context. On the one hand, SIRT7 is aberrantly increased in colorectal cancers (CRCs), hepatocellular carcinomas (HCCs), and certain stages of thyroid cancers (Wu et al. 2018). Here, SIRT7 serves as an oncogenic regulator that promotes tumor growth and ensures tumor survival (Barber et al. 2012; Wu et al. 2018). However, SIRT7 can also act as a tumor suppressor; for example, SIRT7 antagonizes metastasis in breast cancers and oral squamous cell carcinomas (Li et al. 2018; Tang et al. 2017a, b). SIRT7 acts as a tumor growth suppressor through the deacetylation of WDR77 (Qi et al. 2018), and the loss of *SIRT7* is considered to protect breast cancer cells from chemotherapy-induced death via AKT activation (Yu et al. 2017). *Sirt7* knockout mice exhibit accelerated development of colon cancer, highlighting its tumor suppressor characteristics (Liu et al. 2020). Here, we have shown that *SIRT7* insufficiency downregulates IDH1 expression, resulting in a decline in cellular  $\alpha$ -KG, and, thus, suppression of lipogenesis and contributed to HIF1 $\alpha$  stabilization. We conclude that the SIRT7–IDH1 axis regulates metabolic reprogramming during cancer progression. Future studies should consider whether the SIRT7–IDH1 axis has distinct functions when cells undergo various oncogenic signals.

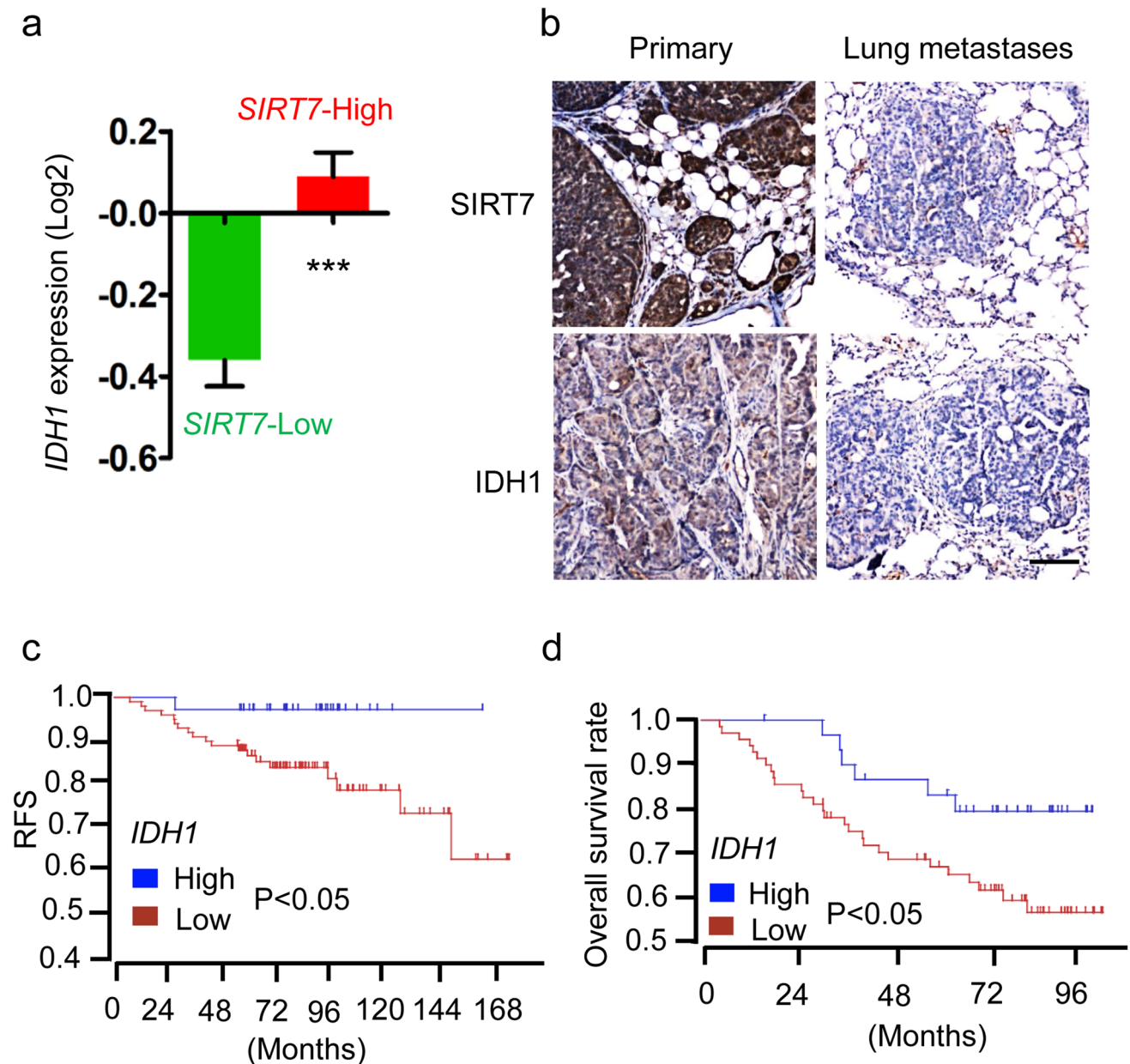
The recent published article unravels a novel finding that SIRT2 directly deacetylates IDH1 and inhibits colorectal cancers (Wang et al. 2020). Both SIRT7 and SIRT2 belong to Sirtuin family and are expected to have the NAD<sup>+</sup>-dependent deacetylase activity. Thus, to explore whether SIRT7 has similar roles for IDH1 deacetylation is interesting. However, we found that SIRT7 was hardly



**Fig. 5** SIRT7 regulates HIF1 $\alpha$  expression via IDH1. **a** Immunoblotting analysis of HIF1 $\alpha$  protein levels in human breast cancer MCF-7 cells subjected into *SIRT7* knockdown or (and) *IDH1* restoration. **b**

Representative images showing the culture medium of MCF-7 cells in (a), noting that the medium showing yellow indicated a higher glycolytic rate





**Fig. 6** IDH1–SIRT7 axis is associated with breast cancer prognosis. **a** Analysis of *IDH1* expression in breast cancer patients stratified by *SIRT7*-Low ( $n = 316$ ) and *SIRT7*-High ( $n = 269$ ). Data were collected from The Cancer Genome Atlas (TCGA) database. **b** Representative images showing immunohistochemistry staining of *SIRT7*

and *IDH1* in primary and paired lung metastasized tumors. Scale bar, 100  $\mu\text{m}$ . **c**, **d** Low *IDH1* expression predicted poor prognosis in breast cancers. Data were collected from two independent cohorts, as described in the “Materials and methods” section

detected in the anti-IDH1 immunoprecipitates (Supplementary Fig. 3), suggesting the lack of direct interaction between *SIRT7* and *IDH1*. Additionally, unlike *SIRT2* which majorly locates at cytosol, *SIRT7* is considered as a nuclear protein. Therefore, *IDH1* as a cytoplasmic enzyme was less likely deacetylated by *SIRT7*. Altogether, our findings highlight the novel mechanism that *SIRT7* regulates *IDH1* expression in a transcriptional-dependent manner.

*SIRT7* possesses deacetylase activity, yet here we found that *SIRT7* regulates *IDH1* transcription via *SREBP1* in a deacetylase-independent manner, suggesting the existence of unexplored mechanisms. Indeed, when compared with other intimate nuclear-localized sirtuins (*SIRT1* and *SIRT6*), *SIRT7* exhibits much weaker deacetylase activity (Michishita et al. 2005). Furthermore, *SIRT7* generally tends to form complexes with other effectors to participate in physiological activity. For instance, *SIRT7* is recruited

by ETS-like transcription factor 4 to deacetylate H3K18 (Barber et al. 2012). SIRT7 interacts with SIRT1 to prevent SIRT1 autodeacetylation and manipulate adipogenesis (Fang et al. 2017). Interestingly, SIRT1 directly deacetylates SREBP1 and, thus, inhibits SREBP-1C activity in regulation of hepatic lipid metabolism (Ponugoti et al. 2010). Considering that IDH1 expression is dependent on SREBP1 (Shechter et al. 2003), we propose the model that SIRT7 recruited by SREBP1 might interact with SIRT1 to inhibit the SIRT1 autocatalytic activation, which impairs the deacetylation of SREBP1 by SIRT1. In the future study, it would be interesting to explore the detailed mechanisms about SIRT7/SREBP1-mediated IDH1 regulation.

IDH1 is an isocitrate dehydrogenase, and belongs to a family that also includes IDH2 and IDH3 (Yan et al. 2009). We observed no apparent change in IDH2 or IDH3 expression following the knockdown of *SIRT7*, suggesting that SIRT7 uniquely regulates IDH1. Much work has focused on the functional relevance of an IDH1 mutation (R132C/H), which produces D-2-hydroxyglutarate (D-2-HG), a competitor of  $\alpha$ -KG, and contributes to tumorigenesis via the suppression of Fe (II)/ $\alpha$ -KG-dependent dioxygenases in low-grade gliomas and secondary GBMs (Yang et al. 2012; Yan et al. 2009). However, comparatively less is known about the regulation of non-mutated IDH1. Indeed, non-mutated IDH1 also has important roles in tumorigenesis. For example, in GBMs IDH1 is upregulated to support macromolecular synthesis, aggressive growth, and therapy resistance (Calvert et al. 2017); conversely, breast cancers that express low levels of IDH1 have invasive phenotypes and undergo epithelial-to-mesenchymal (EMT) transition (Liu et al. 2018). Additionally, dioxygenases such as JmjC domain-containing histone demethylases (KDMs) require  $\alpha$ -KG as co-substrate to catalyze reactions (Tsukada et al. 2006). Given that SIRT7 regulates IDH1 and cellular  $\alpha$ -KG levels, SIRT7 might affect epigenetic modifications, e.g., histone methylation, in cancer cells. Future studies to test that possibility would suggest novel roles of SIRT7 in epigenetic reprogramming besides its H3K18 deacetylation activity (Barber et al. 2012).

HIF1 $\alpha$  plays critical roles in most of solid tumors. In this study, we did not provide many more results to show that SIRT7 regulates HIF1 $\alpha$  protein stability. Actually, SIRT7 regulating the HIF1 $\alpha$  expression is not novel and has been well addressed, i.e., SIRT7 promoting HIF1/2 $\alpha$  degradation is independent of its deacetylase activity (Hubbi et al. 2013). However, the relevant mechanism is not clearly elucidated. Our findings that SIRT7 sustains IDH1 expression and maintains  $\alpha$ -KG production somehow address that issue, i.e., the  $\alpha$ -KG linking to PHDs activity contributing to HIF1/2 $\alpha$  degradation is at least partially dependent on SIRT7.

Overall, our data highlight the potential of SIRT7 to modulate metabolic reprogramming in cancer via IDH1-mediated  $\alpha$ -KG and provide a novel point to evaluate the role of non-mutated IDH1 during tumorigenesis and progression.

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**Availability of data and material** Any data and material in this study are available upon reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare no potential conflicts of interest.

**Consent for publication** All authors have approved the final submission and consented to publication.

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