SETD5 Binding to EP300/HIF1 α is Required for Glycolysis in Breast Cancer Stem-Like Cells

Zhaoting Yang

Yanbian University

Huazi Li

Haici Hospital

Chengye Zhang

Yanbian University

Nan Che

Yanbian University

Ying Feng

Yanbian University

Xingzhe Liu

Yanbian University

Mengxuan Li

Yanbian University

Yanhua Xuan (✉ xuanyh1@ybu.edu.cn)

Yanbian University

Research

Keywords: Breast cancer, SETD5, Cancer stem cell-like, Glycolysis, EP300/HIF1 α

Posted Date: October 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-95100/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Glycolysis plays a pivotal role in breast cancer stem-like cell reprogramming. The SET-domain containing 5 (SETD5) is a previously uncharacterized member of the histone lysine methyltransferase family. Yet, the molecular mechanisms underlying the promotion of stem-like and glycolysis activation traits of SETD5 have not been elucidated.

Methods

Basing on public datasets, we explored clinicopathological and survival analysis of SETD5 on breast cancer (BC) patients. Spheroid formation, transfection experiments and measurement of glucose uptake and lactate production analyzed the regulatory function of SETD5 on glycolysis in breast cancer stem-like cells (BCSC). The impact of SETD5 on tumor growth was studied in a murine xenograft model. Immunohistochemistry, immunofluorescence, western blot, preparation of cytoplasmic and nuclear extracts and co-immunoprecipitation were used to determine the molecular mechanisms of SETD5 in cancer cell glycolysis.

Results

Our data displayed that overexpression of SETD5 in BC tissues is positively associated with progression. SETD5 overexpression is associated with poor post-progression survival in BC patients. SETD5 expression was enriched in spheroid cells. Downregulation of SETD5 significantly decreased BCSC properties and glycolysis *in vitro* and *in vivo*. Interestingly, SETD5 and glycolytic enzymes were accumulated in the central hypoxic regions of subcutaneous tumor tissues. Our mechanistic study found that SETD5 binding to EP300/hypoxia-inducible factor 1 α (HIF1 α) and work as an upstream effector. SETD5 knockdown reduced the expression of HIF1 α , hexokinase-2, and 6-phosphofructo-2-kinase in the nucleus after treatment with cobalt chloride (CoCl₂), a chemical hypoxia mimetic agent, which activates HIF1 α to accumulate in the nucleus.

Conclusion

SETD5 is required for glycolysis in BCSCs through binding to EP300/HIF1 α and could be a potential therapeutic target for BC patients.

Introduction

Breast cancer (BC) is one of the most common incident cancers. Approximately 2.0 million new cases have increased worldwide, pertaining to population growth, change in the age structure of the population, and increase in age-specific incidence rates [1]. Among various therapeutic approaches, cancer cell metabolism is a promising target [2]. To ensure survival, tumor cells usually select their metabolism to avoid host immune attack and to continue to proliferate [3]. Even when oxygen is present, tumor cells

metabolize glucose to generate sufficient ATP to produce lactate, which is much faster than oxidative phosphorylation, and does not require the process of mitochondrial oxidative phosphorylation [4]. This is known as the Warburg effect (aerobic glycolysis). Identifying novel genes involved in BC metabolism and elucidating the potential molecular mechanism of BC are essential for the development of effective therapeutic agents.

Cancer stem-like cells (CSCs) represent small heterogeneous undifferentiated cell populations with a hierarchical organization. Breast cancer stem-like cells (BCSC), a small population of CSC with BC, are suggested to contribute to poor prognosis by malignant progression. Recent studies suggest that CSCs undergo metabolic alterations that include low mitochondrial respiration and high glycolytic activity [4]. Exploiting the regulator of CSCs' metabolism may provide new effective therapies and diminish the risk of recurrence and metastasis [5, 6].

Hypoxia, nutrient starvation, and low pH within the tumor microenvironment play a central role in regulating CSC progression, metastasis, and patient mortality [7]. Hypoxia-inducible factor 1 α (HIF1 α) promotes cell glycolysis by directly upregulating the expression of glycolytic genes, including hexokinase 2 (HK2) [8], 6-phosphofructo-2-kinase (PFKFB3) [9], pyruvate kinase M2 (PKM2) [10], lactate dehydrogenase A (LDHA) [11], and pyruvate dehydrogenase kinase 1 (PDK1) [12] to promote glycolysis in tumors.

p300 (also called EP300) is a transcriptional/epigenetic regulator [13], and involved in a series of physiological and pathological processes [13]. In addition, as EP300 is a key co-activator of hypoxia-inducible transcription [14], some oncogenes cooperate with EP300/HIF1 α pathway to promote hypoxic tumor cell metabolic adaptation and proliferation. Therefore, exploring regulators of EP300/HIF1 α activity will advance our understanding of basic biological processes and suggest future therapeutic strategies.

As a member of the SET-domain containing gene family, the SET-domain containing 5 (SETD5) gene encodes histone-modifying proteins [15]. SETD5 is a previously uncharacterized member of the histone lysine methyltransferase family [16]. De novo heterozygous mutations in SETD5 are considered responsible for intellectual disability and autism spectrum disorders [17, 18]. Overexpression of SETD5 is positively associated with poor prognosis in lung [19] and esophageal cancer [20]. A meta-analysis by Liu et al. previously found that genetic alteration of histone lysine methyltransferases is closely linked to overall survival of BC patients [21]. Previous studies have shown that glycolytic regulators lead to DNA damage responses, contributing to drug resistance [22]. SETD5 is also involved in DNA damage response.

Here, we demonstrated that SETD5 plays a positively critical role in BC progression. Further, we identified the regulatory role of SETD5 in BCSCs. We also found that SETD5 is required for activating glycolysis in BCSCs. Mechanistically, we explored SETD5 binding to EP300/HIF1 α through protein-protein associations (PPI) using the STRING database. SETD5 is an upstream effector of EP300/HIF1 α . Next, SETD5 knockdown reduced the expression of HIF1 α and glycolytic enzymes after treatment with cobalt

chloride (CoCl_2), a chemical inducer of hypoxia. The purpose of this research was to examine the feasibility of SETD5 as a treatment strategy for BC.

Material And Methods

Tissue specimens

TMA containing formalin-fixed and paraffin-embedded 117 human BC tissue specimens were obtained from Shanghai Outdo biotech Co. Ltd. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All mouse experiments were approved by Yanbian University Animal Care & Use Committee. 100 μL of spheroid cells suspension MDA-MB-231 cells (1×10^5 in 100 μL PBS containing 50% Matrigel) were injected subcutaneously into the left or right flank of 5-wks-old BALB/c nude female mice (Vital Rivers, Beijing, China) to establish tumor model. Tumors harvested at 6 weeks after inoculation.

Cell culture, reagents and transfection

MCF-7 and MDA-MB-231 cells (American-Type Culture Collection, Manassas, VA) were cultured under conditions specified by the supplier. CoCl_2 was from Sigma-Aldrich (St. Louis, MO, USA). Negative control and SETD5 endoribonuclease-prepared short interfering RNAs (esi-SETD5) was transfected into cells with Lipofectamine 3000 (Invitrogen, Carlsbad, USA) as per the methods described by the manufacturer. Small interfering RNA (siRNA)-universal negative control and esi-SETD5 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Supplementary table 1 listed the sequence of esi-SETD5.

Immunohistochemistry, western blot, immunofluorescence, and spheroid formation analysis

According to previous protocols, immunohistochemistry staining and evaluation [23], western blot [23], immunofluorescence [24] and spheroid formation assay [24] procedures were performed. The antibodies used are listed in Supplementary table 2.

Measurement of glucose uptake and lactate production

Intracellular glucose was measured according to the Glucose Colorimetric Assay Kit (BioVision, San Francisco, USA) protocol and extracellular lactate was measured according to the Lactate Colorimetric Assay Kit (BioVision, San Francisco, USA) instructions.

Co-immunoprecipitation

Co-immunoprecipitation was performed using the Thermo Scientific Pierce co-IP kit (Thermo scientific, Rockford, USA) following the manufacturer's protocol.

Preparation of cytoplasmic and nuclear extracts analysis

Cytoplasmic and nuclear extracts for western blot analysis were prepared with a Nuclear and Cytoplasmic Protein Extraction Kit was from Beyotime Institute of Biotechnology (Nanjing, China) according to the manufacturer's protocol.

BC patient cohort analysis based on public datasets

GEO (<https://www.ncbi.nlm.nih.gov/geo/>) database were analyzed using GSEA using the GSEA v2.2.4 software (<http://www.broadinstitute.org/gsea/>) to identify the association of SETD5 expression with hypoxia and glycolysis genes. The GSEA analysis includes three key statistics: false discovery rate (FDR), normalized enrichment score (NES), and nominal p-value. Based on CPTAC, UALCAN (<http://ualcan.path.uab.edu>) was used for clinicopathological analysis of SETD5. The Kaplan–Meier Plotter online tool (<http://www.kmplot.com>) was performed to evaluate the correlation between the presence of SETD5 and post-progression survival (PPS) of BC patient. STRING database (version 11.0) (<https://string-db.org/>) was used for mapping protein interaction network. Based on The Cancer Genome Atlas (TCGA), Spearman's correlation analysis was performed using the online web portal GEPIA (<http://gepia.cancer-pku.cn>).

Statistical analysis

Correlations were tested using Spearman correlation analysis as appropriate. Comparisons between groups were performed using Student's t-test with SPSS version 25.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 5.01 (GraphPad Inc., San Diego, CA, USA). All tests were two sided, Results are expressed as mean $\pm$ standard deviation as indicated, and $p < 0.05$ was considered significant.

Results

SETD5 expression was positively related to BC progression

Firstly, we analyzed the clinical pathological diagnosis of SETD5 expression in normal breast ($n = 18$) and BC tissues ($n = 125$) in the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database through the UALCAN web portal. As shown in Fig. 1a, SETD5 protein expression significantly increased in BC tissues relative to normal tissues ($p < 0.001$). CPTAC data also showed that SETD5 was over-expressed in BC tissues with major subclass (Luminal vs Normal, $p < 0.001$; HER2 vs Normal, $p < 0.01$; TNBC vs Normal, $p < 0.05$), patient's age (21–40 years vs Normal, $p < 0.01$; 41–60 years vs Normal, $p < 0.001$; 61–80 Years vs Normal, $p < 0.001$; 81–100 years vs Normal, $p < 0.05$), and individual cancer stages (stage 1 vs Normal, $p < 0.001$; stage 2 vs Normal, $p < 0.01$; stage 3 vs Normal, $p < 0.001$) (Fig. 1b-d). Post-progression survival (PPS) was considered as the median overall survival minus the median progression-free survival for each

trial arm [25]. Furthermore, the Kaplan-Meier Plotter online tool analysis showed that, SETD5 expression was positively related to a poor PPS in BC patients using all Affymetrix ID (Fig. 1e). These data suggest that overexpression of SETD5 in BC tissues could be used as a potential predictor of survival.

SETD5 is a positive regulator of BCSC

Our data provide evidence that SETD5 expression was predictive of poor prognosis and contributed to BC progression. Next, we explored the effect of SETD5 on the properties of CSCs. The protein levels of SETD5 in adherent monolayers and spheroid body-forming cells were determined by western blot analysis. SETD5 were obviously higher in BC spheroid body-forming cells than in adherent monolayer cells (Fig. 2a). The role of SETD5 in BC spheroid body-forming cells was then further investigated. Knockdown of SETD5 expression significantly decreased the number and size of spheroid body-forming cells of BC (Fig. 2b). Immunofluorescent (IF) analysis demonstrated that knockdown of SETD5 resulted in a significant reduction in LGR5 staining in BC spheroid body-forming cells (Fig. 2c).

To further explore how SETD5 acted as a crucial regulator of BCSC maintenance *in vivo*, subcutaneous injections of MDA-MB-231 cells dissociated from the spheroid body were administered in nude mice for 6 weeks. Then, we measured the xenograft tumor size and tumor volume per week by visual analysis (n = 4 tumors/group). Xenograft tumor growth indicated that inhibition of SETD5 expression could significantly reduce the weight and volume *in vivo* (Fig. 3a). Immunohistochemical (IHC) staining of CSC markers was performed in BC xenograft tumor samples. esi-SETD5 downregulated the IHC score of CSC markers, CD133, and LGR5 (Fig. 3b). Additionally, we used IF to examine the correlation between SETD5 expression and CSC markers. Figure 3c showed that SETD5 was co-located with CD133 or LGR5 in BC patient tissues. These findings support a positive functional role of SETD5 in the maintenance of BCSCs.

SETD5 activated BCSC glycolysis

To test the effect of SETD5 on the cellular metabolic reprogramming of BCSC, glucose uptake and lactate production of BC spheroid body-forming cells were assessed. SETD5 depletion inhibited glucose uptake and lactate production in BC spheroid body-forming cells (Fig. 4a, b). Consistently, blocking SETD5 expression significantly reduced glycolytic genes HK2 and PFKFB3 expression in BC spheroid body-forming cells, which was assessed using IF (Fig. 4c). A similar result was obtained *in vivo* by IHC examination (Fig. 4d). SETD5 depletion also downregulated HK2 and PFKFB3 protein expression in xenograft tumor samples. Thus, SETD5 may be a potential regulator of aerobic glycolysis in BCSCs.

SETD5 binding to EP300/HIF1 α in BC cells

Hypoxia is a pervasive environmental stimulus of glucose metabolic reprogramming. Recently, Huang et al. reported that histone methyltransferases SETD8 contribute to HIF1 α regulation and mediate the metabolism process [22]. Thus, the central (hypoxic) and peripheral (normoxic) regions from xenograft tumor tissues were analyzed using hematoxylin-eosin staining (Fig. 5a). IHC staining results showed that SETD5-positive cells mostly gathered centrally with highly hypoxic cells as revealed by HIF1 α , when

compared with the peripheral regions (Fig. 5b). HK2 and PFKFB3 were highly expressed in the central positions compared with the peripheral positions (Fig. 5c). Gene Set Enrichment Analysis (GSEA) and used the GEO database to find an enriched expression of *SETD5* gene in BC tissues. We found a positive correlation between RNA levels of *SETD5* and the *hypoxic* gene (NES = 2.07, FDR q = 0.14, p < 0.001) (Fig. 5d), as well as positive regulatory effects of *SETD5* on *glycolysis* (NES = 1.39, FDR q = 0.23, p = 0.04) (Fig. 5e). Thus, these results indicate that SETD5 activates BCSC glycolysis which is a hypoxia stimulus.

To explore the functional connection of SETD5-HIF1 α and glycolysis, we constructed the PPI network using the STRING database. Nine of these genes comprised a PPI network (PPI enrichment p-value < 0.001) (Fig. 6a). Co-expression analysis revealed tight co-expression across the tissue types between SETD5 and EP300 genes (Fig. S1). Confocal microscopy IF experiments also measured co-expression of SETD5 and EP300 in BC tissues (Fig. 6b).

Furthermore, Tissue Microarray (TMA) analysis was performed for the association between SETD5 and EP300/HIF1 α protein expression in 117 cases of human BC specimens by IHC staining (Fig. S2). In 90 cases of the EP300-positive group, 76 cases (76/90, 84.4%) were positive for the SETD5 gene (p = 0.008) (Fig. 6c). In 76 cases of the HIF1 α -positive group, 63 cases (63/76, 82.9%) were positive for the SETD5 gene (p = 0.018) (Fig. 6c). There was a positive correlation between SETD5 expression and EP300 and HIF1 α detected in BC samples by Gene Expression Profiling Interactive Analysis (GEPIA) (Fig. 6d). Importantly, knocking down SETD5 expression using SETD5 specific esiRNA dramatically decreased the expression of EP300 and HIF1 α in BC cells (Fig. 6e). Therefore, SETD5 expression strongly correlated with EP300/HIF1 α and worked as an upstream effector in BC cells.

SETD5 facilitates glycolysis via HIF1 α in the nucleus

The above-mentioned results prompted us to test the hypothesis that SETD5 regulates HIF1 α protein stability to activate glycolysis. Firstly, we exposed BC cells to CoCl₂ (100, 200, and 400 μ mol/L) for 6 and 12 hours each. CoCl₂ significantly induced the expression of HIF1 α protein in a time- and dose-dependent manner (Fig. S3). HIF1 α protein expression was most effectively upregulated after 200 μ mol/L of CoCl₂-induced hypoxia treatment for 12 hours. This treatment condition was then used in the ensuing hypoxia mimetic experiments. To get insight into the molecular mechanism of SETD5 in contributing to glycolysis in BC cells, the subcellular localization of SETD5 and its putative target genes HIF1 α involved in hypoxia-induced glycolysis were investigated using cytoplasmic and nuclear extract assays. The nuclear expression of HIF1 α was stronger than the cytoplasmic expression after treating with CoCl₂ (Fig. 7a). SETD5 expression levels increased under chemically-induced hypoxia than under normoxic conditions in the total cell lysate. However, there was no obvious change in SETD5 nuclear and cytoplasmic distribution (Fig. 7a). Immunoprecipitation confirmed that SETD5 could bind HIF1 α in the nuclear extracts of BC cells under chemically-induced hypoxia conditions (Fig. 7b). Interestingly, IF staining showed that SETD5 knockdown reduced HIF1 protein expression in the nuclear extracts of BC cells (Fig. 7c). Glycolytic enzymes were further examined in the nucleus of BC cells transfected with esi-SETD5 under chemically-

induced hypoxia conditions. Figure 7d showed that SETD5 silencing decreased the expression of HK2 and PFKFB3 proteins.

Discussion

It is considered that cancer cells can adjust their metabolic phenotypes to adapt to the microenvironment. Indeed, aerobic glycolysis is considered the dominant metabolic phenotype in cancer. Here, for the first time, we correlated SETD5 expression in BCSCs with aerobic glycolysis, which in turn boosts tumor progression and poor prognosis.

Firstly, the association between SETD5 expression and the clinical pathological diagnosis of BC was analyzed with an online tool. The expression of SETD5 in BC was higher than in normal breast tissues, which is consistent with previous results [21]. Furthermore, higher SETD5 expression was associated with worse PPS in BC patients. SETD5 may be an oncogenic factor, as SETD5 was upregulated in non-small cell lung cancer and was a prognostic determinant in prior studies [19].

Activation of an epithelial stem cell (ESC)-like transcriptional program in differentiated adult cells may induce pathologic self-renewal, a characteristic of CSCs [26]. Osipovich et al. reported that SETD5 plays a vital role in mammalian embryonic development, cell cycle progression, and the co-transcriptional regulation of histone acetylation [27]. Recently, Sessa et al. found that SETD5 silencing alters the dynamics of proliferation and differentiation of cortical progenitor cells [28]. This study showed that SETD5 may positively regulate the stem-like properties of BC cells. Furthermore, SETD5 knockdown in BC spheroid body-forming cells significantly downregulated spheroid diameters, the number of cells, and CSC marker expression *in vitro*. Inhibition of SETD5 expression in MDA-MB-231 spheroid cells markedly suppressed tumor growth *in vivo*. Inhibiting SETD5 expression led to downregulation of CD133 and LGR5 proteins in tumor xenograft tissues. Specifically, as shown in Fig. 3, the SETD5-positive cell populations interacted with CSC markers. Moreover, our results showed that SETD5 depletion inhibited glucose uptake while favoring lactate production. The expression of key glycolytic enzymes in BC spheroid body-forming cells were downregulated *in vitro* and *in vivo*. Thus, we suggest that SETD5 is a positive regulator of aerobic glycolysis in BCSCs. Accordingly, we speculated that SETD5 expression is involved in regulating BCSC properties, as well as glycolysis of BCSCs.

EP300 works as histone acetyltransferase and regulates transcription via chromatin remodeling. Histone acetylation gives an epigenetic tag for transcriptional activation. SETD5 is a probable transcriptional regulator that acts via the formation of large multiprotein complexes that modify and/or remodel the chromatin which acts as a regulator of histone acetylation during gene transcription. Our data showed that SETD5 was not only positively associated with EP300 but also co-expressed EP300. A series of epigenetic factors has been shown to exert regulatory roles in HIF1 α protein stability control [22]. Moreover, Finley et al. have shown that regulation of HIF1 α protein stability is necessary for the maintenance of aerobic glycolysis and malignant properties in BC [29]. These results are highly consistent with Huang's report that histone methyltransferase SETD8 reprograms BC cell metabolism

through an HIF1 α -mediated process[22]. GSEA results showed a positive correlation between SETD5, glycolysis, and hypoxia. *In vivo*, HIF1 α , SETD5, HK2, and PFKFB3 were discovered to be increasingly expressed in tumor hypoxic regions than in normoxic regions, which further implied the results *in vitro*. Figure 6 verifies SETD5 as an upstream effector of EP300/HIF1 α in BC tissues. Therefore, we suggest that SETD5 is a positive regulator of EP300/ HIF1 α . Moreover, as Fig. 7d shows that SETD5 silencing decreased the expression of HK2 and PFKFB3 proteins, our findings suggest that SETD5 expression induces glycolysis by binding HIF1 α in the nucleus of BC cells under hypoxia stress.

The functions of HIF1 α depend on its protein stability and subcellular localization. Under hypoxic conditions, ubiquitination of HIF1 α is suppressed, and increased concentrations of HIF1 α bind to HIF1 β to form the HIF1 transcription complex [30]. HIF1 then translocates to the nucleus, where it binds to the hypoxic response element within the promoters of its target genes [30]. Our results confirm that hypoxia mimetic agent CoCl₂ induces HIF1 α nuclear translocation. Co-IP showed that hypoxic stress enhanced HIF1 α and SETD5 interaction in the nucleus of BC cells. More importantly, SETD5 knockdown decreased HIF1 α expression in the nucleus of BC cells and the expression of key glycolytic enzymes under hypoxia mimetic agent treatment. SETD5 indirectly binds HIF1 α in the nucleus and promotes BC cell glycolysis under hypoxic conditions.

Conclusions

SETD5 expression was high in BC tissues and had a strongly positive relationship with poor survival. SETD5 regulated glycolysis in BCSCs, which in turn contributed to BCSC maintenance through binding to EP300/HIF1 α . Thus, targeting may be a potential therapeutic strategy for BC aberrant metabolism treatment.

Abbreviations

BCSC
breast cancer stem-like cell; SETD5:SET-domain containing 5; BC:breast cancer; HIF1 α :hypoxia-inducible factor 1 α ; CSCs:Cancer stem-like cells; HK2:hexokinase 2; PFKFB3:6-phosphofructo-2-kinase; PKM2:pyruvate kinase M2; LDHA:lactate dehydrogenase A; PDK1:pyruvate dehydrogenase kinase 1; PPI:protein-protein associations; CoCl₂:cobalt chloride; siRNA:Small interfering RNA; esi-SETD5:SETD5 endoribonuclease-prepared short interfering RNAs; FDR:false discovery rate; NES:normalised enrichment score; PPS:post-progression survival; TCGA:The Cancer Genome Atlas; CPTAC:Clinical Proteomic Tumor Analysis Consortium; PPS:Post-progression survival; IF:Immunofluorescent:IHC:Immunohistochemical; GSEA:Gene Set Enrichment Analysis; TMA:Tissue Microarray; GEPIA:Gene Expression Profiling Interactive Analysis;

Declarations

Acknowledgments

We'd like to thank *Editage* (Beijing, China) for language editing.

Authors' contributions

Y.X. and Z.Y. was responsible for all data, figures, and text. Z.Y., N.C., Y.F., X.L. and M.L. performed the experiments. H.L., C.Z. and N.C. contributed technical assistance for animal experiments. Y.X., Z.Y. and C.Z. analyzed the data. Z.Y. drafted the manuscript. Y.X. Supervised the work. All authors read, commented and approved final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81760531] to Y.H.X.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Ethical Approval and Consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All mouse experiments were approved by Yanbian University Animal Care & Use Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

¹Department of Pathology, Yanbian University Medicine College, Yanji 133002, China. ²Institute for Regenerative Medicine, Yanbian University Medicine College, Yanji 133002, China. ³Department of Medical Imaging, Haici Hospital Affiliated to Medical College of Qingdao University, Qingdao 266033, Shandong Province, China. ⁴Air Force Medical Center of the Chinese PLA, Beijing 100142, China.

References

1. Fitzmaurice C, Abate D, Abbasi N, Abbastabar H, Abd-Allah F, Abdel-Rahman O, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017. *JAMA Oncol.* 2019; 5(12):e192996-193015.
2. Muñoz-Pinedo C, El Mjiyad N, Ricci JE. Cancer metabolism: Current perspectives and future directions. *Cell Death Dis.* 2012; 3(1): e248-257.
3. Villalba M, Rathore MG, Lopez-Royuela N, Krzywinska E, Garaude J, Allende-Vega N. From tumor cell metabolism to tumor immune escape. *Int J Biochem Cell Biol.* 2013;45:106–13.
4. Deshmukh A, Deshpande K, Arfuso F, Newsholme P, Dharmarajan A. Cancer stem cell metabolism: A potential target for cancer therapy. *Mol. Cancer.* 2016; 15(1):69-78.
5. Tu L, Foltz G, Lin E, Hood L, Tian Q. Targeting Stem Cells-Clinical Implications for Cancer Therapy. 2009;4:147–53.
6. Yuan S, Wang F, Chen G, Zhang H, Feng L, Wang L, et al. Effective elimination of cancer stem cells by a novel drug combination strategy. 2013;31:23–34.
7. Heddleston JM, Li Z, Lathia JD, Bao S, Hjelmeland AB, Rich JN. Hypoxia inducible factors in cancer stem cells. *Br J Cancer.* 2010;102:789–95.
8. Jiang S, Zhang LF, Zhang HW, Hu S, Lu MH, Liang S, et al. A novel miR-155/miR-143 cascade controls glycolysis by regulating hexokinase 2 in breast cancer cells. 2012;31:1985–98.
9. Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, et al. An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: Role in tumor cell glycolysis and the Warburg effect. *Proc Natl Acad Sci U S A.* 1999;96:3047–52.
10. Azoitei N, Becher A, Steinestel K, Rouhi A, Diepold K, Genze F, et al. PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. *Mol Cancer.* 2016;15:3–17.
11. Cui XG, Han ZT, He SH, Wu X Da, Chen TR, Shao CH, et al. HIF1/2α mediates hypoxia-induced LDHA expression in human pancreatic cancer cells. *Oncotarget.* 2017;8:24840–52.
12. Dupuy F, Tabariès S, Andrzejewski S, Dong Z, Blagih J, Annis MG, et al. PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. *Cell Metab.* 2015;22:577–89.
13. Wang F, Marshall CB, Ikura M. Transcriptional/epigenetic regulator CBP/p300 in tumorigenesis: Structural and functional versatility in target recognition. *Cell. Mol.* 2013;70(21):3989–4008.
14. Viziteu E, Grandmougin C, Goldschmidt H, Seckinger A, Hose D, Klein B, et al. Chetomin, targeting HIF-1α/p300 complex, exhibits antitumour activity in multiple myeloma. *Br J Cancer.* 2016;114:519–23.
15. Green C, Willoughby J, Balasubramanian M. De novo SETD5 loss-of-function variant as a cause for intellectual disability in a 10-year old boy with an aberrant blind ending bronchus. *Am J Med Genet Part A.* 2017;173:3165–71.
16. Xiao B, Wilson JR, Gamblin SJ. SET domains and histone methylation. *Curr. Opin. Struct. Biol.* 2003;13(6):699-705.

17. Knock E, Pereira J, Lombard PD, Dimond A, Leaford D, Livesey FJ, et al. The methyl binding domain 3/nucleosome remodelling and deacetylase complex regulates neural cell fate determination and terminal differentiation in the cerebral cortex. *Neural Dev.* 2015;10:13–32.
18. Hu X, Hicks CW, He W, Wong P, MacKlin WB, Trapp BD, et al. Bace1 modulates myelination in the central and peripheral nervous system. *Nat Neurosci.* 2006;9:1520–5.
19. Yu H, Sun J, Zhao C, Wang H, Liu Y, Xiong J, et al. SET domain containing protein 5 (SETD5) enhances tumor cell invasion and is associated with a poor prognosis in non-small cell lung cancer patients. *BMC Cancer.* 2019;19:736–45.
20. Piao L, Li H, Feng Y, Yang Z, Kim S, Xuan Y. SET domain-containing 5 is a potential prognostic biomarker that promotes esophageal squamous cell carcinoma stemness. *Exp Cell Res.* 2020;389:111861–70.
21. Liu L, Kimball S, Liu H, Holowatyj A, Yang ZQ. Genetic alterations of histone lysine methyltransferases and their significance in breast cancer. *Oncotarget.* 2015;6:2466–82.
22. Huang R, Yu Y, Zong X, Li X, Ma L, Zheng Q. Monomethyltransferase SETD8 regulates breast cancer metabolism via stabilizing hypoxia-inducible factor 1 α . *Cancer Lett.* 2017;390:1–10.
23. Yang Z, Cui Y, Ni W, Kim S, Xuan Y. Gli1, a potential regulator of esophageal cancer stem cell, is identified as an independent adverse prognostic factor in esophageal squamous cell carcinoma. *J Cancer Res Clin Oncol.* 2017;143:243–54.
24. Yang Z, Zhang C, Qi W, Cui Y, Xuan Y. GLI1 promotes cancer stemness through intracellular signaling pathway PI3K/Akt/NF κ B in colorectal adenocarcinoma. *Exp Cell Res.* 2018;373:145–54.
25. Saad ED, Katz A, Buyse M. Overall survival and post-progression survival in advanced breast cancer: A review of recent randomized clinical trials. *J. Clin. Oncol.* *J Clin Oncol*; 2010; 28(11):1958–62.
26. Wong DJ, Liu H, Ridky TW, Cassarino D, Segal E, Chang HY. Module Map of Stem Cell Genes Guides Creation of Epithelial Cancer Stem Cells. *Cell Stem Cell.* 2008;2:333–44.
27. Osipovich AB, Gangula R, Vianna PG, Magnuson MA. Setd5 is essential for mammalian development and the co-transcriptional regulation of histone acetylation. *Dev.* 2016;143:4595–607.
28. Sessa A, Fagnocchi L, Mastrototaro G, Massimino L, Zaghi M, Indrigo M, et al. SETD5 Regulates Chromatin Methylation State and Preserves Global Transcriptional Fidelity during Brain Development and Neuronal Wiring. *Neuron.* 2019;104:271-289.e13.
29. Finley LWS, Carracedo A, Lee J, Souza A, Egia A, Zhang J, et al. SIRT3 Opposes Reprogramming of Cancer Cell Metabolism through HIF1 α Destabilization. *Cancer Cell.* 2011;19:416–28.
30. Cockman ME, Masson N, Mole DR, Jaakkola P, Chang GW, Clifford SC, et al. Hypoxia inducible factor- α binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J Biol Chem.* 2000;275:25733–41.

Figures

Figure 1

SETD5 was over-expressed in clinical breast cancer (BC) patients and positively correlated with the survival time. (a) SETD5 expression was detected in normal tissues (n = 18) and BC tissues (n = 125); (b) in normal tissues (n = 18) and major subclass of BC tissues: (Luminal, n = 64; human epidermal growth factor receptor 2, HER2 Positive, n = 10; triple negative breast cancer, TNBC, n = 16); (c) in normal (n = 18) and different patient age groups (21– 40 years, n = 10; 41 – 60 years, n = 50; 61 – 80 Years, n = 53; 81 – 100 years, n = 7); (d) in normal (n = 18) and different individual cancer stages: (stage 1, n = 4; stage 2, n = 74; stage 3, n = 32) using the online web portal, UALCAN. (e) Post-progression survival analysis of the correlation between survival time and SETD5 in BC was performed using the online tool Kaplan–Meier Plotter at four Affymetrix ID including 1569105_at, 1569106_at, 222575_at, and 221806_at.

Figure 1

SETD5 was over-expressed in clinical breast cancer (BC) patients and positively correlated with the survival time. (a) SETD5 expression was detected in normal tissues (n = 18) and BC tissues (n = 125); (b) in normal tissues (n = 18) and major subclass of BC tissues: (Luminal, n = 64; human epidermal growth factor receptor 2, HER2 Positive, n = 10; triple negative breast cancer, TNBC, n = 16); (c) in normal (n = 18) and different patient age groups (21– 40 years, n = 10; 41 – 60 years, n = 50; 61 – 80 Years, n = 53; 81 – 100 years, n = 7); (d) in normal (n = 18) and different individual cancer stages: (stage 1, n = 4; stage 2, n = 74; stage 3, n = 32) using the online web portal, UALCAN. (e) Post-progression survival analysis of the correlation between survival time and SETD5 in BC was performed using the online tool Kaplan–Meier Plotter at four Affymetrix ID including 1569105_at, 1569106_at, 222575_at, and 221806_at.

Figure 1

SETD5 was over-expressed in clinical breast cancer (BC) patients and positively correlated with the survival time. (a) SETD5 expression was detected in normal tissues (n = 18) and BC tissues (n = 125); (b) in normal tissues (n = 18) and major subclass of BC tissues: (Luminal, n = 64; human epidermal growth factor receptor 2, HER2 Positive, n = 10; triple negative breast cancer, TNBC, n = 16); (c) in normal (n = 18) and different patient age groups (21– 40 years, n = 10; 41 – 60 years, n = 50; 61 – 80 Years, n = 53; 81 – 100 years, n = 7); (d) in normal (n = 18) and different individual cancer stages: (stage 1, n = 4; stage 2, n = 74; stage 3, n = 32) using the online web portal, UALCAN. (e) Post-progression survival analysis of the correlation between survival time and SETD5 in BC was performed using the online tool Kaplan–Meier Plotter at four Affymetrix ID including 1569105_at, 1569106_at, 222575_at, and 221806_at.

Figure 2

SETD5 promotes breast cancer stem-like cell (BCSC) properties. (a) The protein expression of SETD5 in spheroid cells compared to that in adherent monolayer counterparts were analyzed using western blot. GAPDH was used as a loading control. (b) Analysis of spheroid formation ability in control and SETD5-silenced MDA-MB-231 and MCF7 cells. (c) Immunofluorescence examined the expression of LGR5 in control and SETD5-silenced spheroid cells. *** $p < 0.001$ versus controls.

Figure 2

SETD5 promotes breast cancer stem-like cell (BCSC) properties. (a) The protein expression of SETD5 in spheroid cells compared to that in adherent monolayer counterparts were analyzed using western blot. GAPDH was used as a loading control. (b) Analysis of spheroid formation ability in control and SETD5-silenced MDA-MB-231 and MCF7 cells. (c) Immunofluorescence examined the expression of LGR5 in control and SETD5-silenced spheroid cells. *** $p < 0.001$ versus controls.

Figure 2

SETD5 promotes breast cancer stem-like cell (BCSC) properties. (a) The protein expression of SETD5 in spheroid cells compared to that in adherent monolayer counterparts were analyzed using western blot. GAPDH was used as a loading control. (b) Analysis of spheroid formation ability in control and SETD5-silenced MDA-MB-231 and MCF7 cells. (c) Immunofluorescence examined the expression of LGR5 in control and SETD5-silenced spheroid cells. *** $p < 0.001$ versus controls.

Figure 3

esi-SETD5 suppresses breast tumorigenicity in vivo. (a) MDA-MB-231-spheroid-cells-control and MDA-MB-231-spheroid-cells-esi-SETD5 (1×10^5 cells per mice) were injected subcutaneously into immunodeficient mice ($n = 4$), respectively. Tumor weights and tumor volumes were monitored at the indicated times post-injection. (b) Expression of CD133 and LGR5 in xenograft tumors transfected with esi-SETD5 were detected by immunohistochemistry. (c) Immunofluorescence analyzed the co-localization of SETD5 and cancer stem-like cell markers CD133 and LGR5 in breast cancer tissues.

Figure 3

esi-SETD5 suppresses breast tumorigenicity in vivo. (a) MDA-MB-231-spheroid-cells-control and MDA-MB-231-spheroid-cells-esi-SETD5 (1×10^5 cells per mice) were injected subcutaneously into immunodeficient mice ($n = 4$), respectively. Tumor weights and tumor volumes were monitored at the indicated times post-injection. (b) Expression of CD133 and LGR5 in xenograft tumors transfected with esi-SETD5 were detected by immunohistochemistry. (c) Immunofluorescence analyzed the co-localization of SETD5 and cancer stem-like cell markers CD133 and LGR5 in breast cancer tissues.

Figure 3

esi-SETD5 suppresses breast tumorigenicity in vivo. (a) MDA-MB-231-spheroid-cells-control and MDA-MB-231-spheroid-cells-esi-SETD5 (1×10^5 cells per mice) were injected subcutaneously into immunodeficient mice ($n = 4$), respectively. Tumor weights and tumor volumes were monitored at the indicated times post-injection. (b) Expression of CD133 and LGR5 in xenograft tumors transfected with esi-SETD5 were detected by immunohistochemistry. (c) Immunofluorescence analyzed the co-localization of SETD5 and cancer stem-like cell markers CD133 and LGR5 in breast cancer tissues.

Figure 4

SETD5 positively regulates glycolysis in breast cancer stem-like cells (BCSC). (a) Compared glucose uptake and (b) lactate production of breast cancer (BC) spheroid cells transfected with esi-SETD5 with control. (c) Immunofluorescence showing the protein expression of hexokinase 2 (HK2) and 6-phosphofructo-2-kinase (PFKFB3) in BC spheroid cells and transfected with esi-SETD5. (d) Expression of

HK2 and PFKFB3 in xenograft tumors and transfected with esi-SETD5 were detected by immunohistochemistry. ** $p < 0.05$, *** $p < 0.001$ versus controls.

Figure 4

SETD5 positively regulates glycolysis in breast cancer stem-like cells (BCSC). (a) Compared glucose uptake and (b) lactate production of breast cancer (BC) spheroid cells transfected with esi-SETD5 with control. (c) Immunofluorescence showing the protein expression of hexokinase 2 (HK2) and 6-

phosphofructo-2-kinase (PFKFB3) in BC spheroid cells and transfected with esi-SETD5. (d) Expression of HK2 and PFKFB3 in xenograft tumors and transfected with esi-SETD5 were detected by immunohistochemistry. **p < 0.05, ***p < 0.001 versus controls.

Figure 4

SETD5 positively regulates glycolysis in breast cancer stem-like cells (BCSC). (a) Compared glucose uptake and (b) lactate production of breast cancer (BC) spheroid cells transfected with esi-SETD5 with

control. (c) Immunofluorescence showing the protein expression of hexokinase 2 (HK2) and 6-phosphofructo-2-kinase (PFKFB3) in BC spheroid cells and transfected with esi-SETD5. (d) Expression of HK2 and PFKFB3 in xenograft tumors and transfected with esi-SETD5 were detected by immunohistochemistry. **p < 0.05, ***p < 0.001 versus controls.

Figure 5

SETD5 is positively associated with hypoxia and glycolysis in breast cancer (BC). (a) Central and peripheral regions of xenograft tumors were analyzed by hematoxylin and eosin staining (HE). (b) immunohistochemical staining for hypoxia-inducible factor 1α (HIF1α) and SETD5 in the central and peripheral regions of xenograft tumor tissues, as well as (c) glycolysis enzymes, hexokinase 2 (HK2) and PFK-2, 6-phosphofructo-2-kinase (PFKFB3). (d, e) Based on SETD5 expression levels, gene set enrichment analysis (GSEA) was performed on BC datasets from GEO database using the gene sets regulated by hypoxia and glycolysis.

Figure 5

SETD5 is positively associated with hypoxia and glycolysis in breast cancer (BC). (a) Central and peripheral regions of xenograft tumors were analyzed by hematoxylin and eosin staining (HE). (b) immunohistochemical staining for hypoxia-inducible factor 1 α (HIF1 α) and SETD5 in the central and peripheral regions of xenograft tumor tissues, as well as (c) glycolysis enzymes, hexokinase 2 (HK2) and PFK-2, 6-phosphofructo-2-kinase (PFKFB3). (d, e) Based on SETD5 expression levels, gene set enrichment analysis (GSEA) was performed on BC datasets from GEO database using the gene sets regulated by hypoxia and glycolysis.

Figure 5

SETD5 is positively associated with hypoxia and glycolysis in breast cancer (BC). (a) Central and peripheral regions of xenograft tumors were analyzed by hematoxylin and eosin staining (HE). (b) immunohistochemical staining for hypoxia-inducible factor 1 α (HIF1 α) and SETD5 in the central and peripheral regions of xenograft tumor tissues, as well as (c) glycolysis enzymes, hexokinase 2 (HK2) and PFK-2, 6-phosphofructo-2-kinase (PFKFB3). (d, e) Based on SETD5 expression levels, gene set enrichment analysis (GSEA) was performed on BC datasets from GEO database using the gene sets regulated by hypoxia and glycolysis.

Figure 6

SETD5 co-expressed EP300 and bound to hypoxia-inducible factor 1α (HIF1α) in breast cancer (BC) cells. (a) STRING analysis of SETD5 genes indicating Protein-Protein Interaction network (PPI). Colored nodes: query proteins and first shell of interactors; Blue-green line: known interactions from curated databases, purple line: known interactions from experiments, green line: predicted interactions form gene neighborhood, red line: predicted interactions form gene fusions, dark blue: predicted interactions form gene co-occurrence, yellow line: interactions form text mining, black line: interactions form co-expression, light blue: interactions form protein homology. (b) Co-expression of SETD5 and EP300 was analyzed using immunofluorescence in BC tissues. (c) The protein expression ratio of SETD5 in EP300 positivity (orange) and HIF1α (blue) positivity was examined in 117 BC tissue microarray using immunohistochemistry staining. (d) Gene Expression Profiling Interactive Analysis (GEPIA) analyzed the correlation between SETD5 and HIF1α mRNA levels in BC tissues. (e) The expression of SETD5, EP300, and HIF1α were analyzed in BC cells transfected with esi-SETD5 using western blot. GAPDH was used as the loading control.

Figure 6

SETD5 co-expressed EP300 and bound to hypoxia-inducible factor 1α (HIF1α) in breast cancer (BC) cells. (a) STRING analysis of SETD5 genes indicating Protein-Protein Interaction network (PPI). Colored nodes: query proteins and first shell of interactors; Blue-green line: known interactions from curated databases, purple line: known interactions from experiments, green line: predicted interactions form gene neighborhood, red line: predicted interactions form gene fusions, dark blue: predicted interactions form gene co-occurrence, yellow line: interactions form text mining, black line: interactions form co-expression, light blue: interactions form protein homology. (b) Co-expression of SETD5 and EP300 was analyzed using immunofluorescence in BC tissues. (c) The protein expression ratio of SETD5 in EP300 positivity (orange) and HIF1α (blue) positivity was examined in 117 BC tissue microarray using immunohistochemistry staining. (d) Gene Expression Profiling Interactive Analysis (GEPIA) analyzed the correlation between SETD5 and HIF1α mRNA levels in BC tissues. (e) The expression of SETD5, EP300, and HIF1α were analyzed in BC cells transfected with esi-SETD5 using western blot. GAPDH was used as the loading control.

Figure 6

SETD5 co-expressed EP300 and bound to hypoxia-inducible factor 1α (HIF1α) in breast cancer (BC) cells. (a) STRING analysis of SETD5 genes indicating Protein-Protein Interaction network (PPI). Colored nodes: query proteins and first shell of interactors; Blue-green line: known interactions from curated databases, purple line: known interactions from experiments, green line: predicted interactions form gene neighborhood, red line: predicted interactions form gene fusions, dark blue: predicted interactions form gene co-occurrence, yellow line: interactions form text mining, black line: interactions form co-expression, light blue: interactions form protein homology. (b) Co-expression of SETD5 and EP300 was analyzed using immunofluorescence in BC tissues. (c) The protein expression ratio of SETD5 in EP300 positivity (orange) and HIF1α (blue) positivity was examined in 117 BC tissue microarray using immunohistochemistry staining. (d) Gene Expression Profiling Interactive Analysis (GEPIA) analyzed the correlation between SETD5 and HIF1α mRNA levels in BC tissues. (e) The expression of SETD5, EP300, and HIF1α were analyzed in BC cells transfected with esi-SETD5 using western blot. GAPDH was used as the loading control.

Figure 7

SETD5 bound hypoxia-inducible factor 1α (HIF1α) to facilitate glycolysis of breast cancer (BC) cells. (a) The expression of SETD5 and HIF1α in the cytoplasm (CE) or nuclear extracts (NE) of BC cells were examined by western blot under normoxia and hypoxia. (b) Co-immunoprecipitation of SETD5 and HIF1α in the nuclear fraction of BC cells under hypoxia. (c) Immunofluorescence showing the location of HIF1α in BC cells under normal condition, treatment with 200 μmol/L CoCl₂ for 12 hours, and transfection with esi-SETD5 for 24 hours and treatment with 200 μmol/L CoCl₂ for 12 hours, respectively. (d) The protein expression of HK2 and PFKFB3 in the cytoplasmic and nuclear fractions of BC cells, transfected with esi-SETD5 under hypoxia were examined by western blot. GAPDH and proliferating cell nuclear antigen (PCNA) were used as loading controls.

Figure 7

SETD5 bound hypoxia-inducible factor 1α (HIF1α) to facilitate glycolysis of breast cancer (BC) cells. (a) The expression of SETD5 and HIF1α in the cytoplasm (CE) or nuclear extracts (NE) of BC cells were examined by western blot under normoxia and hypoxia. (b) Co-immunoprecipitation of SETD5 and HIF1α in the nuclear fraction of BC cells under hypoxia. (c) Immunofluorescence showing the location of HIF1α in BC cells under normal condition, treatment with 200 μmol/L CoCl₂ for 12 hours, and transfection with esi-SETD5 for 24 hours and treatment with 200 μmol/L CoCl₂ for 12 hours, respectively. (d) The protein expression of HK2 and PFKFB3 in the cytoplasmic and nuclear fractions of BC cells, transfected with esi-SETD5 under hypoxia were examined by western blot. GAPDH and proliferating cell nuclear antigen (PCNA) were used as loading controls.

Figure 7

SETD5 bound hypoxia-inducible factor 1α (HIF1α) to facilitate glycolysis of breast cancer (BC) cells. (a) The expression of SETD5 and HIF1α in the cytoplasm (CE) or nuclear extracts (NE) of BC cells were examined by western blot under normoxia and hypoxia. (b) Co-immunoprecipitation of SETD5 and HIF1α in the nuclear fraction of BC cells under hypoxia. (c) Immunofluorescence showing the location of HIF1α in BC cells under normal condition, treatment with 200 μmol/L CoCl₂ for 12 hours, and transfection with esi-SETD5 for 24 hours and treatment with 200 μmol/L CoCl₂ for 12 hours, respectively. (d) The protein expression of HK2 and PFKFB3 in the cytoplasmic and nuclear fractions of BC cells, transfected with esi-SETD5 under hypoxia were examined by western blot. GAPDH and proliferating cell nuclear antigen (PCNA) were used as loading controls.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Fig.S3.tiff](#)
- [Fig.S3.tiff](#)
- [Fig.S3.tiff](#)
- [Fig.S2.tiff](#)

- Fig.S2.tiff
- Fig.S2.tiff
- Fig.S1.tiff
- Fig.S1.tiff
- Fig.S1.tiff
- Supplementarytable.docx
- Supplementarytable.docx
- Supplementarytable.docx