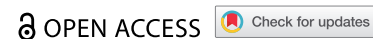


COMMENTARY



Greasy GLUT1 maintains glioblastoma malignancy

Caiyun Liu^{a,b} and Xinjian Li^{a,b}

^aCAS Key Laboratory of Infection and Immunity, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; ^bCollege of Life Sciences, University of Chinese Academy of Sciences, Beijing, China

ABSTRACT

How cancer cells absorb enough glucose to support their rapid growth is poorly understood. We have recently demonstrated that palmitoyl transferase DHHC9 palmitoylates glucose transporter GLUT1 at Cys207 to maintain GLUT1 plasma membrane localization. DHHC9-mediated GLUT1 palmitoylation supports glycolysis, proliferation, colony formation, and tumorigenicity of glioblastoma cells.

ARTICLE HISTORY

Received 7 November 2021
Revised 14 November 2021
Accepted 17 November 2021

KEYWORDS

GLUT1; palmitoylation;
DHHC9; glioblastoma

Glucose is the major source of energy in human cells. One molecule of glucose is converted to two molecules of pyruvate and generates two molecules of adenosine triphosphate (ATP) during glycolysis in mammalian cells. Pyruvate produced from glycolysis is either transported into mitochondria to be further converted to carbon dioxide and water through the tricarboxylic acid (TCA) cycle coupled with the electron transport chain pathway or reduced to lactate, a secretory metabolite, by lactate dehydrogenase in the cytosol. Tumor cells, but not healthy cells, produce ATP by high rate of glycolysis rather than TCA cycle and secrete plenty of lactate, even when oxygen is available, this metabolic phenotype was named Warburg effect.¹ Our previous works indicate that tumor-specific Warburg effect promotes tumor progression.^{2,3} Given that glycolysis depends on glucose consumption, it is still enigmatic how cancer cells absorb glucose to support their rapid proliferation. As reported previously, GLUT1, a widely expressed glucose transporter, has elevated expression levels in many types of human cancer.^{4,5} Recently, a study reported in *Nature Communications* by our group addressed this problem by proposing a model in which glucose uptake supporting aerobic glycolysis of glioblastoma (GBM) cells is facilitated by palmitoylated-GLUT1 (Figure 1). We observe that GLUT1 is palmitoylated at Cys207, and this S-palmitoylation is required for maintaining GLUT1 plasma membrane (PM) localization, which is essential for glucose transporter activity of GLUT1. Mechanistically, DHHC9 is identified as the palmitoyl transferase responsible for GLUT1 palmitoylation. Knockout (KO) of DHHC9 or mutation of GLUT1 Cys207 to serine abrogates palmitoylation and GLUT1 PM distribution, thereby impairing glycolysis, cell proliferation, and GBM tumorigenesis.⁶

S-palmitoylation, a posttranslational modification formed by connecting the carboxyl group of palmitic acid to the sulfhydryl group of cysteine in proteins, is catalyzed by palmitoyl transferases in eukaryotic cells. Twenty-three palmitoyl transferases have been identified in mammalian cells and characterized to regulate membrane localization, inter-membrane

translocation, interaction, and stability of intracellular proteins.^{7,8} To identify the potential palmitoyl acyltransferases (PATs) catalyzing GLUT1 palmitoylation, we constructed a set of lentiviral plasmids expressing a hspCas9 nuclease and guide RNAs targeting PATs to perform a clustered regularly interspaced short palindromic repeats (CRISPR) screen. Our data demonstrate that DHHC9 is required for GLUT1 palmitoylation in GBM cells, and this result is validated by an *in vitro* palmitoylation assay indicating that purified catalytically active DHHC9 palmitates GLUT1. Endogenous DHHC9 KO rendered GLUT1 largely cytoplasmic distribution, and this effect was abrogated by reconstituted expression of wild-type DHHC9, but not the catalytically inactive mutant DHHC9 C169S. These results suggest that DHHC9 palmitoylates GLUT1 to maintain its PM localization.

To determine the site of GLUT1 palmitoylation, we performed the alkynyl palmitic acid incorporation and acyl-polyethylene glycol (PEG) exchange assays. GLUT1 palmitoylation is abolished by mutating GLUT1 Cys207 to serine. Similarly, C207S mutation abrogates DHHC9-mediated GLUT1 palmitoylation in an *in vitro* palmitoylation assay. Notably, molecular structure analysis indicates that GLUT1 C207 is highly conserved among different species and located in the intracellular loop adjacent to the PM. Consistent with DHHC9 KO, GLUT1 C207S mutant displayed cytoplasmic distribution in GBM cells. These data suggest that GLUT1 C207 palmitoylation mediated by DHHC9 is required to keep PM localization of GLUT1.

Functional studies demonstrate that DHHC9-mediated GLUT1 palmitoylation supports glycolysis, proliferation, and colony formation of GBM cells. In addition, studies using a murine orthotopic GBM xenograft model show that disruption of DHHC9-mediated GLUT1 palmitoylation suppresses GBM tumorigenesis. Immunohistochemical staining analyses of human GBM specimens demonstrate that DHHC9 expression positively correlates with GLUT1 PM localization and indicates a poor prognosis in GBM patients.

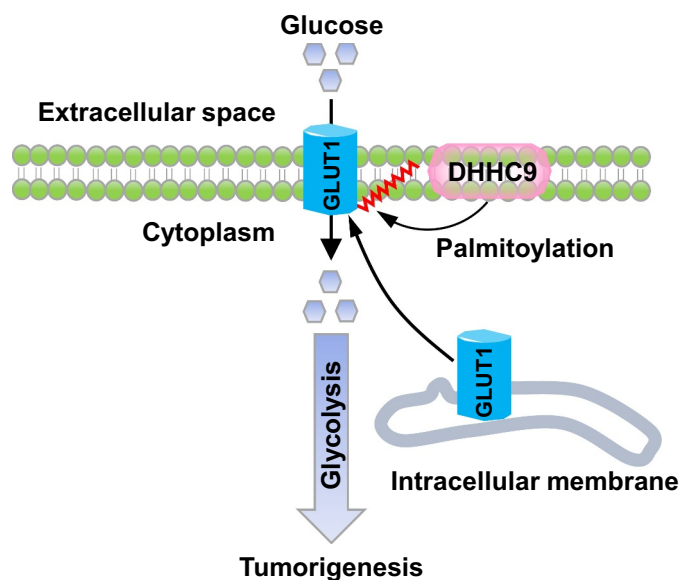


Figure 1. DHHC9-mediated GLUT1 palmitoylation promotes glioblastoma glycolysis and tumorigenesis. DHHC9-mediated GLUT1 palmitoylation maintains GLUT1 plasma membrane (PM) localization, leads to enhanced glucose uptake, and subsequently promotes glycolysis, proliferation, colony formation, and tumorigenicity of GBM cells.

In summary, this study demonstrates that DHHC9 palmitoylates GLUT1 at Cys207. Palmitoylated GLUT1 maintains its PM localization to promote glucose uptake and subsequently enhances glycolysis, proliferation, colony formation, and tumorigenicity of GBM cells. These findings advance our concept that protein subcellular localization, which is independent of gene expression levels, is instrumental for metabolic rewiring of cancer cells, highlighting the importance of protein spatial modulation during cancer progression.

Disclosure statement

No potential conflict of interest were disclosed.

Funding

This work was supported by the Key Program of the Chinese Academy of Sciences (Grant no. KJZD-SW-L05 to X.L.); the National Natural Science Foundation of China (Grant no. 82073060 to X.L.); and the National Key R&D Program of China (Grant no. 2020YFC2002700 to X.L.).

References

1. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324:1029–1033. doi:10.1126/science.1160809.
2. Li X, Jiang Y, Meisenhelder J, Yang W, Hawke DH, Zheng Y, Xia Y, Aldape K, He J, Hunter T, et al. Mitochondria-Translocated PGK1 functions as a protein kinase to coordinate glycolysis and the TCA cycle in tumorigenesis. *Mol Cell*. 2016;61:705–719. doi:10.1016/j.molcel.2016.02.009.
3. Qian X, Li X, Shi Z, Xia Y, Cai Q, Xu D, Tan L, Du L, Zheng Y, Zhao D, et al. PTEN suppresses glycolysis by dephosphorylating and inhibiting autophosphorylated PGK1. *Mol Cell*. 2019;76(3):516–27 e7. doi:10.1016/j.molcel.2019.08.006.
4. Adekola K, Rosen ST, Shanmugam M. Glucose transporters in cancer metabolism. *Curr Opin Oncol*. 2012;24:650–654. doi:10.1097/CCO.0b013e328356da72.
5. Ancey PB, Contat C, Meylan E. Glucose transporters in cancer - from tumor cells to the tumor microenvironment. *FEBS J*. 2018;285:2926–2943. doi:10.1111/febs.14577.
6. Zhang Z, Li X, Yang F, Chen C, Liu P, Ren Y, et al. DHHC9-mediated GLUT1 S-palmitoylation promotes glioblastoma glycolysis and tumorigenesis. *Nat Commun*. 2021;12:5872. doi:10.1038/s41467-021-26180-4.
7. Linder ME, Deschenes RJ. Palmitoylation: policing protein stability and traffic. *Nat Rev Mol Cell Biol*. 2007;8:74–84. doi:10.1038/nrm2084.
8. Chamberlain LH, Shipston MJ. The physiology of protein S-acylation. *Physiol Rev*. 2015;95:341–376. doi:10.1152/physrev.00032.2014.