Received: 23 July 2021 | Accepted: 17 August 2021

DOI: 10.1002/cac2.12211

CANCER COMMUNICATIONS Open Access

EDITORIAL

A moonlighting function of choline kinase alpha 2 in the initiation of lipid droplet lipolysis in cancer cells

Cancer cells in the tumor microenvironment, where nutrient availability is consistently changing during tumor progression, harness lipid metabolism to support their proliferation, survival, migration, invasion, and metastasis [1]. When nutrients in the tumor microenvironment are sufficient, lipids such as triglycerides, steryl esters, and retinyl esters are stored in lipid droplets, which are dynamic lipid-rich cellular organelles surrounded by a single layer of polar and amphipathic phospholipids and structural proteins in the perilipin (PLIN) family. Among these family members, PLIN2 and PLIN3 are ubiquitously expressed, whereas PLIN1 is primarily expressed in adipocytes. These droplet-stored lipids are accessed for energy production via fatty acid oxidation and membrane biogenesis during cell growth. They are also involved in protein modification, redox balance, autophagy modulation and signaling, and can be secreted with lipoproteins. Lipid droplet accumulation has been observed in many types of human cancers, including breast cancer, glioblastoma (GBM), hepatocellular carcinoma (HCC), lung cancer, cervical cancer, prostate cancer, colon cancer, melanoma, cholangiocarcinoma, clear-cell renal cell carcinoma, ovarian cancer, and pancreatic cancer [2]. Accumulated lipid droplets confer the metabolic capacity of tumor cells to strive against severe energy stresses.

Aberrantly activated lipid metabolism in cancer cells drives lipid droplet biogenesis through several ways. First, cancer cells consistently upregulate *de novo* lipid synthesis. Receptor tyrosine kinase activation and activating *KRAS* mutations induce AKT-mediated phosphorylation of cytosolic phosphoenolpyruvate carboxykinase 1 (PCK1) at S90, leading to the inhibition of PCK1 gluconeogenic activity and its translocation to the endoplasmic reticulum (ER). PCK1 binds to the ER membrane proteins INSIG1/2 and acts as a protein kinase to phosphorylate INSIG1 S207 and INSIG2 S151. Phosphorylated INSIGs exhibit reduced binding to oxysterols, thereby, activating SREBP-dependent lipogenesis gene expression [3]. Second, cancer cells upregulate lipid uptake. They increase fatty acid uptake by upregulating the expression of fatty acid transporters, which include the cluster of differentiation 36 (CD36, also known as fatty acid translocase), the family of fatty acid transport proteins (FATPs, also known as SLC27), and plasma membrane fatty acid-binding proteins (FABPs). Similarly, increased uptake of cholesterol in the form of low-density lipoproteins (LDLs) is mediated by LDL receptors (LDLRs) that are transcriptionally upregulated by activated SREBP1 and PTEN loss-induced PI3K/AKT activation [1]. Third, cancer cells mobilize endogenous structural lipids. This is achieved via the breakdown of membranous organelles by autophagy and membrane remodeling by phospholipases [2].

In response to energy stress, accumulated lipid droplets in cancer cells are hydrolyzed through a process known as lipolysis, enabling cancer cell survival and growth. Lipolysis is regulated by two different mechanisms at a later stage of hydrolysis: (1) lipolysis by cytosolic lipases, such as adipose triglyceride lipase (ATGL), which binds to the surface of lipid droplets and hydrolyzes triglycerides to fatty acids and glycerol, and (2) lipolysis by autophagy (macrolipophagy), which leads to the translocation of lipid droplets to lysosomes where they undergo hydrolysis [4]. Although these pathways have been characterized, the mechanisms through which tumor cells initiate the lipolysis of lipid droplets to fuel tumor growth remain unclear.

We recently revealed a critical mechanism by which tumor cells sense energy stress and initiate the lipolysis of lipid droplets through a moonlighting function of choline kinases (CHKs) [4]. The canonical function of CHK is the phosphorylation of choline to produce phosphocholine, leading to the production of the critical

Abbreviations: 2-DG, 2-deoxy-d-glucose; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; CD36, cluster of differentiation 36; CHK, choline kinase; CMA, haperone-mediated autophagy; ER, endoplasmic reticulum; FABP, fatty acid-binding protein; FATP, fatty acid transport protein; GBM, glioblastoma; HCC, hepatocellular carcinoma; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; PC, phosphatidylcholine; PCK1, phosphoenolpyruvate carboxykinase 1; PKM2, M2 isoform of pyruvate kinase; PLIN, perilipin

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. *Cancer Communications* published by John Wiley & Sons Australia, Ltd. on behalf of Sun Yat-sen University Cancer Center

membrane component phosphatidylcholine (PC). CHK is expressed in 3 isoforms as homodimers or heterodimers: CHKα1 and CHKα2 are produced by alternate splicing of the *CHKA* gene, resulting in exon 3-containing CHKα2 and exon 3-excluding CHKα1, whereas CHKβ is encoded by *CHKB*. CHKα2 is the predominant isoform expressed in multiple types of human cancers. In response to glucose deprivation, a small fraction of cytosolic CHKα2 binds to lipid droplets in an AMP-activated protein kinase (AMPK)-dependent manner. Glucose deprivation induces the association between endogenous AMPK and CHKα2, and AMPK phosphorylates CHKα2 at S279A both *in vitro* and *in vivo*. Inhibition or deficiency of AMPK or CHKα2 S279A expression abrogates glucose deprivation-induced binding of CHKα2 to lipid droplets in GBM and HCC cells, whereas AMPK activation promotes the binding of CHKα2 to lipid droplets. Importantly, CHKα2 depletion blocks glucose deprivation-induced lipolysis of lipid droplets. These results suggest that AMPK activation mediates the binding of CHKα2 to lipid droplets and subsequent lipid droplets lipolysis.

Intriguingly, mass spectrum analyses revealed that CHKα2 is not only phosphorylated at S279 but is also acetylated at K247 and that KAT5 acetyltransferase is a CHKα2-associated protein. A GST pull-down assay showed that AMPK-mediated phosphorylation of CHKα2 is required for KAT5 to bind CHKα2, leading to KAT5-mediated CHKα2 K247 acetylation. Importantly, the *CHKα2* K247R mutant, which is phosphorylated at S279 upon glucose deprivation, fails to be translocated to lipid droplets. These results suggest that energy stress elicits a cascade of protein interactions and posttranslational modifications, and that AMPK activation and subsequent binding to CHKα2 and phosphorylation of CHKα2 are required for the association of CHKα2 with KAT5, which subsequently mediates CHKα2 K247 acetylation; a signaling pathway that is indispensable for the translocation of CHKα2 to lipid droplets.

Structural analyses of dimerized CHKα2 showed that the positively charged K247 of one CHKα2 molecule interacts with the negatively charged E97 of another molecule of CHKα2 molecule. As expected, KAT5-mediated K247 acetylation, which adds a negatively charged acetyl group to the K247 residue and neutralizes the Lys charge, disrupted CHKα2 dimerization. Similarly, the substitution of E97 with a positively charged Arg residue inhibited the dimerization of CHKα2 and disabled the binding of CHKα2 to PLIN2 or PLIN3, suggesting that the conversion of CHKα2 from a dimer to a monomer, exposing the dimer interface, enables CHKα2 binding to PLIN2 or PLIN3. Among multiple mutations to the hydrophilic and hydrophobic residues of the CHKα2 dimer interface 175-EAMVLESVMFAILAER-190, I186A/L187A substitutions abolished the glucose deprivation-induced interaction between CHKα2 and PLIN2 or PLIN3, suggesting that CHKα2 K247 acetylation-mediated disruption of CHKα2 dimerization exposes the binding residues I186 and L187 of the α helix in the dimerized interface, enabling their interaction with PLIN2/3.

Our previous studies demonstrated that the M2 isoform of pyruvate kinase (PKM2), phosphoglycerate kinase 1, ketohexokinase-A, and PCK1, which are metabolic enzymes, can also function as protein kinases and phosphorylate a variety of protein substrates to modulate critical cellular functions [3, 5–14]. *In vitro* phosphorylation and mass spectrometric analysis demonstrated that CHKα2 uses ATP as a phosphate donor and phosphorylates PLIN2 at Y232 and PLIN3 at Y251. Computational docking analyses demonstrated that only monomeric CHKα with its expanded choline-binding pocket, which may contribute to reduced choline-binding affinity and canonical activity, enables the PLIN2 Y232 or PLIN3 Y251 peptide to interact with the catalytic domain of CHKα2 for phosphate group transfer. These results suggest that K247 acetylation-mediated monomerization of CHKα alters the structure of the catalytic domain, enabling the phosphorylation of PLIN2/3.

It was previously reported that Hsc70 interacts with PLIN2/3 to promote their chaperone-mediated autophagy (CMA)- and lysosome-dependent degradation, leading to lipid droplet association with cytosolic ATGL and macrolipophagy [15]. Expression of phosphorylation-resistant CHKα2 S279A, acetylation-resistant CHKα2 K247R, or PLIN2/3 phosphorylation-resistant mutants disrupts PLIN2/3 association with Hsc70 under glucose deprivation conditions; blocks the glucose deprivation-induced removal of PLIN2/3 from lipid droplets, the association of ATGL, beclin1, or LC3B with lipid droplets, and the engulfment of lipid droplets by autophagosomes; and consequently abolishes the reduction of lipid droplets in both GBM and HCC cells. Consistently, the expression of these mutants inhibits glucose deprivation-induced increases in glycerol and free fatty acid levels and β-oxidation metabolic flux, decreases cellular acetyl-CoA and ATP levels and cell proliferation under 2-deoxy-d-glucose (2-DG) treatment, and further accelerates glucose deprivation-induced cell apoptosis. In addition, knock-in expression of these mutants inhibits brain tumor growth with prolonged mouse survival time and enhances 2-DG treatment-induced inhibition of the growth of tumors, with corresponding inhibition of lipid consumption and longer mouse survival time. Notably, the expression of these mutants in combination with temozolomide treatment enhances the growth inhibition of tumors.

The clinical significance of CHKα2-regulated lipolysis of lipid droplets in human GBM progression was shown by immunohistochemical analyses of human

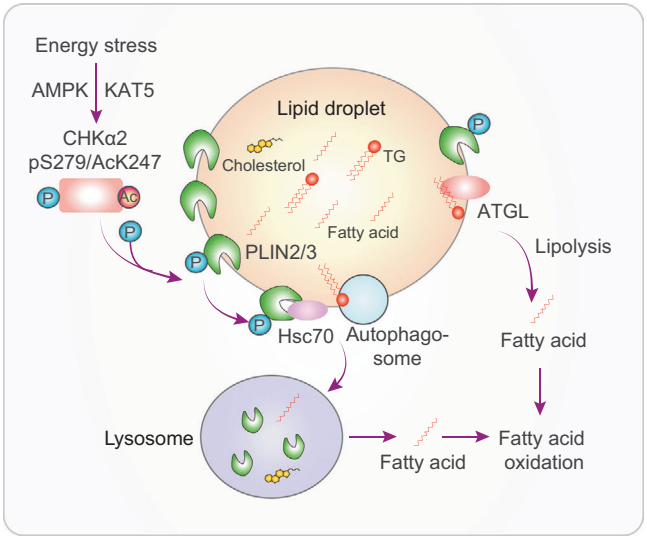

FIGURE 1 Schematic depicting the mechanism underlying CHKα2-mediated lipolysis of lipid droplets in cancer cells
Abbreviations: AMPK, AMP-activated protein kinase; CHK, choline kinase, TG, triglyceride; ATGL, adipose triglyceride lipase (ATGL); P, phosphate group, Ac, acetyl group.

primary GBM specimens which revealed a positive correlation between the levels of AMPK substrate acetyl-CoA carboxylase (ACC) S79 phosphorylation, CHKα2 S279 phosphorylation, CHKα2 K247 acetylation, PLIN2 Y232 phosphorylation and PLIN3 Y251 phosphorylation. In addition, CHKα2 and PLIN2/3 phosphorylation and CHKα2 K247 acetylation were associated with poor survival in GBM patients [4].

CHKα2 is overexpressed in many types of cancer and is associated with tumor prognosis. Therefore, CHKα2 has been targeted for cancer treatment. Nevertheless, all proliferating normal cells and cancer cells require the canonical function of CHKα2 for phosphatidylcholine production and subsequent cell membrane composition. Thus, targeting the canonical function of CHKα2 in cancer treatment is predicted to unavoidably induce severe side effects. Here, we demonstrated that CHKα2 protein kinase activity-dependent PLIN2/3 phosphorylation was required for the energy stress-induced initiation of lipid droplet lipolysis in cancer cells (Figure 1). This finding paves the way for specifically intervening tumor growth by inhibiting the moonlighting function of CHKα2 in lipid metabolism in cancer cells. Together with the clinical association of specific CHKα2 phosphorylation and acetylation and CHKα2-mediated PLIN2/3 phosphorylation with aggressive human GBM, the discovery of CHKα2 as a new member of the human protein kinome indicates that CHKα2 is a novel and promising diagnostic and therapeutic target for cancer.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
Not applicable.

COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS
Z.L. and R.L. conceived and designed the report. All authors contributed to the discussion and composition of the content and helped write the manuscript.

ACKNOWLEDGMENTS
Not applicable.

Qingxia Ma[1,†]
Zhaoyuan Meng[1,†]
Ying Meng[2,3]
Rui Liu[4]
Zhimin Lu[1,2,3]

[1] *Qingdao Cancer Institute, The Affiliated Hospital of Qingdao University, Qingdao, Shandong 266071, P. R. China*
[2] *Zhejiang Provincial Key Laboratory of Pancreatic Disease, the First Affiliated Hospital, Institute of Translational Medicine, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310029, P. R. China*
[3] *Cancer Center, Zhejiang University, Hangzhou, Zhejiang 310029, P. R. China*
[4] *State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Chinese Academy of Medical Sciences Research Unit of Oral Carcinogenesis and Management, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, P. R. China*

Correspondence
Rui Liu, State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Chinese Academy of Medical Sciences Research Unit of Oral Carcinogenesis and Management, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, P. R. China.
Email: liurui_scu@hotmail.com

Zhimin Lu, Zhejiang Provincial Key Laboratory of Pancreatic Disease, the First Affiliated Hospital, Institute of Translational Medicine, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310029, P. R. China
Email:zhiminlu@zju.edu.cn

†These authors contributed equally to this work.

ORCID

Zhimin Lu https://orcid.org/0000-0002-2859-2736

REFERENCES

1. Bian X, Liu R, Meng Y, Xing D, Xu D, Lu Z. Lipid metabolism and cancer. J Exp Med. 2021;218(1).
2. Petan T, Jarc E, Jusovic M. Lipid Droplets in Cancer: Guardians of Fat in a Stressful World. Molecules. 2018;23(8).
3. Xu D, Wang Z, Xia Y, Shao F, Xia W, Wei Y, et al. The gluconeogenic enzyme PCK1 phosphorylates INSIG1/2 for lipogenesis. Nature. 2020;580(7804):530–5.
4. Liu R, Lee JH, Li J, Yu R, Tan L, Xia Y, et al. Choline kinase alpha 2 acts as a protein kinase to promote lipolysis of lipid droplets. Mol Cell. 2021;81(13):2722–35 e9.
5. Lu Z, Hunter T. Metabolic Kinases Moonlighting as Protein Kinases. Trends Biochem Sci. 2018;43(4):301–10.
6. Li X, Qian X, Peng LX, Jiang Y, Hawke DH, Zheng Y, et al. A splicing switch from ketohexokinase-C to ketohexokinase-A drives hepatocellular carcinoma formation. Nat Cell Biol. 2016;18(5):561–71.
7. Li X, Jiang Y, Meisenhelder J, Yang W, Hawke DH, Zheng Y, et al. Mitochondria-Translocated PGK1 Functions as a Protein Kinase to Coordinate Glycolysis and the TCA Cycle in Tumorigenesis. Mol Cell. 2016;61(5):705–19.
8. Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D, et al. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell. 2012;150(4):685–96.
9. Xu D, Shao F, Bian X, Meng Y, Liang T, Lu Z. The Evolving Landscape of Noncanonical Functions of Metabolic Enzymes in Cancer and Other Pathologies. Cell Metab. 2021;33(1):33–50.
10. Xu D, Li X, Shao F, Lv G, Lv H, Lee JH, et al. The protein kinase activity of fructokinase A specifies the antioxidant responses of tumor cells by phosphorylating p62. Sci Adv. 2019;5(4): eaav4570.
11. Qian X, Li X, Shi Z, Xia Y, Cai Q, Xu D, et al. PTEN Suppresses Glycolysis by Dephosphorylating and Inhibiting Autophosphorylated PGK1. Mol Cell. 2019;76(3):516–27 e7.
12. Jiang Y, Li X, Yang W, Hawke DH, Zheng Y, Xia Y, et al. PKM2 Regulates Chromosome Segregation and Mitosis Progression of Tumor Cells. Mol Cell. 2014;53(1):75–87.
13. Jiang Y, Wang Y, Wang T, Hawke DH, Zheng Y, Li X, et al. PKM2 phosphorylates MLC2 and regulates cytokinesis of tumour cells. Nat Commun. 2014;5:5566.
14. Jiang H, Zhu L, Xu D, Lu Z. A newly discovered role of metabolic enzyme PCK1 as a protein kinase to promote cancer lipogenesis. Cancer Commun (Lond). 2020;40(9):389–394.
15. Kaushik S, Cuervo AM. Degradation of lipid droplet-associated proteins by chaperone-mediated autophagy facilitates lipolysis. Nat Cell Biol. 2015;17(6):759–70.