

代谢酶的非经典功能及代谢感知

雷群英*

(复旦大学基础医学院, 上海 200032)

摘要: 代谢是基本的生命活动, 代谢网络以代谢酶和代谢物为中心, 为细胞的生命活动提供物质和能量基础。一方面, 代谢酶发挥经典的功能, 催化不同代谢通路中的代谢物, 并受到严密调控, 维持代谢稳态。另一方面, 近年来国内外的研究, 包括我们研究团队的工作证实了某些代谢酶和代谢物还可发挥非经典的兼有功能(moonlighting functions), 参与信号通路调控和/或作为一个信号分子, 对代谢进行更精细的调控, 在机体的生理和病理过程中发挥关键作用。

关键词: 代谢; 代谢感知; 代谢酶; 代谢物

Non-canonical functions of metabolic enzymes and metabolic sensing

LEI Qunying*

(School of Basic Medical Sciences, Fudan University, Shanghai 200032, China)

Abstract: Metabolism is the fundamental activity in the body, and the metabolic network composed of metabolic enzymes and metabolites provides the basis of biomass and energy for the life. On one hand, metabolic enzymes-catalyzed metabolites, as substrates and/or products in different metabolic pathways, are tightly regulated and display canonical functions to maintain metabolic homeostasis. On the other hand, accumulating evidence from our research team and others have demonstrated that some metabolic enzymes and metabolites acquire moonlighting functions. They can participate into regulation of cell signaling transduction and/or act as signaling molecules to precisely control metabolism, playing a critical role in the physiological and pathological processes of the organism.

Key Words: metabolism; metabolic sensing; metabolic enzymes; metabolites

邹承鲁先生是我国生物化学研究领域的先驱和奠基人之一。自二十世纪五十年代归国后, 在艰苦的科研环境下, 邹先生带领团队致力于研究酶的化学性质和生物学功能, 在酶催化机理、酶空间结构和动力学等方面取得了诸多原创性成果。其中, 邹先生建立的“邹氏公式”和“邹氏作图法”被同行广泛采用并收入教科书。邹先生的科研成就不仅引领了生物化学领域的发展, 也为我国在生命科学领域崛起提供了重要支撑。此

外, 邹先生为我国生物学界培养了一大批杰出的人才, 其中不少现在已经是国内外知名的科学家。邹先生的卓越贡献激励了更多的年轻学者加入到酶学工作的研究中, 继续推动代谢酶研究领域的发展。我也有幸附骥, 从事研究代谢酶和代谢物的生理病理效应, 并得到邹先生门生们诸多的指导、支持和鼓励。

随着科学技术的发展, 越来越多的研究工作证实了代谢酶除了发挥经典功能之外, 也发挥着

*通信作者: E-mail: qli@fudan.edu.cn

非经典功能。值此先生百年诞辰之际，为缅怀这位新中国杰出的爱国科学家和九三学社的先辈，传承其勇于创新、严谨求实的科学精神，在此，浅谈代谢酶/物的非经典功能，以及代谢感知的生理、病理效应和国内研究进展。

1 代谢酶的非经典功能

传统上，人们一直认为，代谢酶的功能就是催化生物反应，如蛋白质合成分解、糖代谢产生能量等。随着分子生物学的研究深入，人们对代谢酶的认识也在不断扩大。代谢酶也具有许多非经典功能，包括基因表达调控、细胞信号转导和细胞凋亡等多种生物学过程。以下简述几个重要代谢酶的非经典功能。

1.1 M2型丙酮酸激酶

丙酮酸激酶(pyruvate kinase, PK)是调节糖酵解的最终限速步骤，催化磷酸烯醇丙酮酸(phosphoenolpyruvate, PEP)转化为丙酮酸并产生三磷酸腺苷(adenosine triphosphate, ATP)。丙酮酸激酶有四种亚型，分别是PKR、PKL、PKM1和PKM2。PKR、PKL和PKM1在特定组织中表达；PKM2在大多成人组织中下降，但在发育中的胚胎和肿瘤中显著上调^[1,2]。PKM2的细胞质功能和调控对肿瘤生长至关重要^[3]。细胞内PKM2存在二聚体(低活性)、四聚体(高活性)两种形式。我们课题组前期研究除发现PKM2感受葡萄糖，第305位赖氨酸(K305)发生乙酰化修饰促进分子伴侣介导的自噬降解，从而增强肿瘤细胞糖代谢的经典功能外，还发现其感受生长信号发生K433乙酰化，获得了蛋白激酶活性^[4,5]。具体来讲，研究发现，生长因子和癌基因信号引起乙酰基转移酶p300乙酰化PKM2的K433位点，从而促进其四聚体向二聚体转变。二聚体形式的PKM2会在细胞核中积累，发挥蛋白激酶的功能，使信号转导子和转录激活子蛋白3(signal transducer and activator of transcription 3, STAT3)蛋白第705位酪氨酸(Y705)磷酸化，激活下游信号通路，促进肿瘤细胞增殖。有趣的是，Hosios等^[6]在基础条件下未发现PKM2激酶活性；他们的研究虽与我们的研究相似，但我们在研究中通过表皮生长因子(epidermal growth factor, EGF)刺激来检测其蛋白激酶活性^[5]。因

此，很多代谢酶可能在不同的刺激下发挥蛋白激酶的作用。PKM2作为代谢激酶和蛋白激酶的双重功能对肿瘤的发展至关重要，因此，PKM2抑制剂和激活剂被开发出来以不同的机制来治疗肿瘤^[7-9]。

1.2 乳酸脱氢酶A

乳酸脱氢酶A(lactate dehydrogenase isoform A, LDHA)是糖酵解过程中的关键代谢酶，催化丙酮酸生成乳酸的可逆反应。LDHA催化活性受蛋白磷酸化及乙酰化调控。我们前期工作发现，LDHA去乙酰化通过促进其蛋白积累和酶活，进而促进胰腺癌发生的经典功能外，还发现LDHA响应肿瘤细胞内氧化应激信号，由四聚体分子转变为二聚体形式，并影响了LDHA在亚细胞结构的再定位，促进其入核^[10,11]。值得注意的是，细胞核内的LDHA获得了非经典酶活，催化 α -丁酮酸(α -ketobutyrate, α -KB)生成 α -羟丁酸(α -hydroxybutyrate, α -HB)，造成 α -HB在细胞内积累，进而发挥表观调控，增强了组蛋白的甲基化修饰，最终影响抗氧化相关基因的转录，增强细胞应对活性氧(reactive oxygen species, ROS)损伤，维持细胞生存，此外，还上调了Wnt信号通路相关基因的表达，促进肿瘤细胞增殖^[11]。

1.3 磷酸烯醇式丙酮酸羧激酶1

磷酸烯醇式丙酮酸羧激酶1(phosphoenolpyruvate carboxykinase 1, PCK1)是糖异生的限速酶之一；它催化草酰乙酸和三磷酸鸟苷(guanosine triphosphate, GTP)生成磷酸烯醇丙酮酸和二氧化碳^[12]。PCK1及其亚型PCK2具有63.4%的序列同源性，分别定位于细胞质和线粒体^[13]。快速增殖的肿瘤细胞需要大量的氨基酸、核苷和脂肪酸来合成蛋白质、脱氧核糖核酸(deoxyribonucleic acid, DNA)和脂类。据报道，PCK1具有蛋白激酶活性并促进脂肪生成。具体而言，丝/苏氨酸蛋白激酶AKT磷酸化PCK1的第90位丝氨酸位点(S90)，并促进其向内质网转运。在内质网中，以GTP为磷酸供体，分别磷酸化胰岛素诱导基因1(insulin-induced gene 1, INSIG1)和INSIG2，这导致胆固醇调节元件结合蛋白(sterol-regulatory element binding proteins, SREBPs)的激活和脂肪生成所需基因的表达，从而促进了肝细胞癌的增殖和肿瘤的发生^[14]。

1.4 果糖-1,6-二磷酸酶1

果糖-1,6-二磷酸酶1(fructose-1,6-bisphosphatase 1, FBP1)是糖异生的限速酶之一,可将果糖-1,6-二磷酸转化为果糖-6-磷酸。然而,FBP1在肿瘤代谢中具有双重功能。它通过降低葡萄糖摄取、减少糖酵解和限制癌细胞增殖,发挥肿瘤抑制因子的作用^[15-17]。核FBP1结合并抑制缺氧诱导因子1 α (hypoxia-inducible factor 1 α , HIF1 α)和HIF2 α 的活性,从而降低HIF靶基因的表达,进而抑制肿瘤代谢。此外,FBP1可以与Notch1结合,促进其蛋白酶体降解,从而降低Notch1靶基因的表达,抑制乳腺肿瘤的发生^[18]。然而,在肺肿瘤微环境中,自然杀伤(natural killer, NK)细胞中FBP1的高表达会降低糖酵解的作用和生存能力,导致NK细胞功能障碍,抑制FBP1可逆转这一现象^[19]。

1.5 果糖-1,6-二磷酸酶2

果糖-1,6-二磷酸酶2(fructose-1,6-bisphosphatase 2, FBP2)是FBP1的同工酶,与主要表达在肝脏和肾脏中的FBP1不同,FBP2表达范围更广。除了在糖异生中的作用外,FBP2也抑制肿瘤的进展,这两种作用涉及两种不同的机制。一方面,FBP2抑制细胞质糖酵解从而抑制Warburg效应和细胞增殖。此外,FBP2易位进入细胞核,发挥转录调控作用,抑制c-Myc介导的线粒体转录因子A(mitochondrial transcription factor A, TFAM)的基因表达,进而抑制线粒体生物发生和呼吸,而与其酶活性无关^[20]。

1.6 磷酸甘油酸激酶1

磷酸甘油酸激酶1(phosphoglycerate kinase 1, PGK1)在糖酵解过程中催化1,3-二磷酸甘油酸转化为3-磷酸甘油酸并产生ATP^[21]。PGK1的活性和功能受翻译后修饰的调控。PGK1作为线粒体中的蛋白激酶,磷酸化丙酮酸脱氢酶激酶1(PDK1)的第338位苏氨酸(T338),从而阻断丙酮酸进入三羧酸(tricarboxylic acid, TCA)循环^[22]。此外,PGK1还可以在第324位酪氨酸(Y324)自磷酸化并激活自身,从而促进糖酵解。磷酸酶PTEN使PGK1去磷酸化,从而抑制糖酵解和ATP产生^[23]。细胞核中的PGK1与细胞分裂周期蛋白激酶7(cell division cycle 7-related protein kinase, CDC7)结合,调控细胞周

期^[24]。PGK1易位进入细胞核,通过抑制上皮细胞钙黏蛋白(E-cadherin)表达,从而促进胰腺癌的转移^[25]。此外,PGK1 K323位点的乙酰化被p300/CBP相关因子(PCAF)和Sirtuin7(SIRT7)可逆调控,促进PGK1的酶活性和肿瘤代谢,从而支持肿瘤生长^[26]。

2 代谢酶(物)感知调控和组织器官稳态

生命进化产生了复杂而精细的代谢感受机制^[27]。哺乳细胞中除了经典的腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)和哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)代谢物感知通路^[28]。近年来,厦门大学Huangyang等^[29]醛缩酶感知细胞内果糖-1,6-二磷酸调控AMPK激活的新机制。有研究发现,进食后血液中上调的胰岛素和葡萄糖作为信号,激活mTORC1,通过mTORC1-USP20-HMGR通路诱导胆固醇合成^[30]。此外,低氧状态下累积的乳酸被NDRG3感知并促进细胞在低氧状态下存活^[31]。乳酸还可以被类泛素化特异性肽酶1(SUMO specific peptidase 1, SENP1)感知,抑制其酶活,调控细胞周期^[32]。我们课题组也发现,腺苷高半胱氨酸水解酶样蛋白1(adenosylhomocysteinase like 1, AHCYL1)可作为代谢物S-腺苷-L-高半胱氨酸(S-adenosylhomocysteine, SAH)的感受器(sensor),调控自噬等生物学过程^[33]。

真核细胞内外环境中代谢物被细胞感知后可进行信号传递,在表观遗传、基因转录、蛋白合成降解、蛋白修饰等多水平进行调控^[27]。一方面,表观调控中绝大多数表观遗传修饰蛋白均以代谢物作为底物或辅酶,代谢组分的改变对表观遗传组带来全景式的影响。如“ATP 柠檬酸裂解酶(ATP citrate lyase, ACLY)”感知葡萄糖的变化,改变组蛋白乙酰化修饰状态调控细胞的生理活动^[34];胚胎干细胞从原始态向始发态转化中,下调S-腺苷甲硫氨酸水平对完成表观代谢重塑至关重要^[35]。而转运核糖核酸(tRNA)来源的小分子RNA修饰在精子中直接参与糖脂代谢调控的跨代遗传^[36,37]。代谢网络还可通过增强子枢纽调控基因多元表达,具体表现形式有超级增强子等^[38]。另一方面,蛋白质是功能的执行者。代谢物调控蛋白

功能除了经典的蛋白质互作如mTOR和AMPK信号通路, 还有代谢物共价结合蛋白质进行修饰, 如乙酰化、甲基化等。目前这种翻译后修饰大约有600多种, 赋予了蛋白质的功能多样化和精准化^[39]。总之, 细胞内部如细胞核、内质网、线粒体、溶酶体等重要细胞器可感知代谢物浓度波动来调整信号转导、细胞代谢等过程应对的环境变化, 在组织器官稳态维持中发挥关键作用^[40]。

3 国内研究现状和水平

近些年来, 我国在代谢酶非经典功能、代谢感知与应激研究方面, 在老一辈科学家奠定的基础上取得了系列原创性成果, 并在国际舞台占有一席之地。Shan等^[41]发现, 在营养过剩状况下肌醇酶1 α 通过阻遏机体的能量消耗促进肥胖和2型糖尿病等代谢性疾病的发生发展。Huangyang等^[29]发现, 果糖1,6-二磷酸和醛缩酶介导AMPK感知葡萄糖^[29]。我们课题组阐明了PKM2和LDHA等代谢酶的非经典功能以及代谢感知异常在肿瘤发生中的关键作用机制^[4,5,11,42-44]。Liu等^[45]提出了一个概念, 称之为“电子转移势能(potential of electron transfer, PET)”, 揭示了乏氧条件下肿瘤代谢重编程的化学本质。Zhang等^[46]发现, 烯醇酶1(enolase 1, ENO1)结合并降解RNA的新功能, 从而调控细胞内铁离子的代谢稳态, 影响铁死亡而促进肝癌的发生发展。上述一系列工作充分体现了我国在代谢酶非经典功能、代谢感知应激研究领域前沿的创新进展, 为我国在该领域持续创新打下了坚实的基础。

综上所述, 我认为一代代科研工作者对邹先生勇于创新、严谨求实科学精神的传承就是最好的缅怀, 这将激励晚辈勇于开拓创新, 潜心教书育人。

参考文献

- [1] Mazurek S. Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. *Int J Biochem Cell Biol*, 2011, 43(7): 969-980
- [2] Dayton TL, Jacks T, Vander Heiden MG. PKM 2, cancer metabolism, and the road ahead. *EMBO Rep*, 2016, 17(12): 1721-1730
- [3] Christofk HR, Vander Heiden MG, Harris MH, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*, 2008, 452(7184): 230-233
- [4] Lv L, Li D, Zhao D, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. *Mol Cell*, 2011, 42(6): 719-730
- [5] Lv L, Xu YP, Zhao D, et al. Mitogenic and oncogenic stimulation of K433 acetylation promotes PKM2 protein kinase activity and nuclear localization. *Mol Cell*, 2013, 52(3): 340-352
- [6] Hosios AM, Fiske BP, Gui DY, et al. Lack of Evidence for PKM2 Protein Kinase Activity. *Mol Cell*, 2015, 59(5): 850-857
- [7] Vander Heiden MG, Christofk HR, Schuman E, et al. Identification of small molecule inhibitors of pyruvate kinase M2. *Biochem Pharmacol*, 2010, 79(8): 1118-1124
- [8] Chen J, Xie J, Jiang Z, et al. Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2. *Oncogene*, 2011, 30(42): 4297-4306
- [9] Anastasiou D, Yu Y, Israelsen WJ, et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nat Chem Biol*, 2012, 8(10): 839-847
- [10] Zhao D, Zou SW, Liu Y, et al. Lysine-5 acetylation negatively regulates lactate dehydrogenase A and is decreased in pancreatic cancer. *Cancer Cell*, 2013, 23(4): 464-476
- [11] Liu Y, Guo JZ, Liu Y, et al. Nuclear lactate dehydrogenase A senses ROS to produce α -hydroxybutyrate for HPV-induced cervical tumor growth. *Nat Commun*, 2018, 9(1): 4429
- [12] Burgess SC, He TT, Yan Z, et al. Cytosolic phosphoenolpyruvate carboxykinase does not solely control the rate of hepatic gluconeogenesis in the intact mouse liver. *Cell Metab*, 2007, 5(4): 313-320
- [13] Méndez-Lucas A, Hyroová P, Novellademunt L, et al. Mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) is a pro-survival, endoplasmic reticulum (ER) stress response gene involved in tumor cell adaptation to nutrient availability. *J Biol Chem*, 2014, 289(32): 22090-22102
- [14] Xu D, Wang Z, Xia Y, et al. The gluconeogenic enzyme PCK1 phosphorylates INSIG1/2 for lipogenesis. *Nature*, 2020, 580(7804): 530-535
- [15] Dong C, Yuan T, Wu Y, et al. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell*, 2013, 23(3): 316-331
- [16] Zhang J, Wang J, Xing H, et al. Down-regulation of FBP1 by ZEB1-mediated repression confers to growth and invasion in lung cancer cells. *Mol Cell Biochem*, 2016,

- 411(1-2): 331-340
- [17] Hirata H, Sugimachi K, Komatsu H, et al. Decreased expression of fructose-1,6-bisphosphatase associates with glucose metabolism and tumor progression in hepatocellular carcinoma. *Cancer Res*, 2016, 76(11): 3265-3276
- [18] Lu C, Ren C, Yang T, et al. A noncanonical role of fructose-1, 6-bisphosphatase 1 is essential for inhibition of notch1 in breast cancer. *Mol Cancer Res*, 2020, 18(5): 787-796
- [19] Cong J, Wang X, Zheng X, et al. Dysfunction of natural killer cells by FBP1-induced inhibition of glycolysis during lung cancer progression. *Cell Metab*, 2018, 28(2): 243-255
- [20] Huangyang P, Li F, Lee P, et al. Fructose-1,6-bisphosphatase 2 inhibits sarcoma progression by restraining mitochondrial biogenesis. *Cell Metab*, 2020, 31(1): 174-188
- [21] Zhang Y, Yu G, Chu H, et al. Macrophage-associated PGK1 phosphorylation promotes aerobic glycolysis and tumorigenesis. *Mol Cell*, 2018, 71(2): 201-215
- [22] Li X, Jiang Y, Meisenhelder J, et al. Mitochondria-translocated PGK1 functions as a protein kinase to coordinate glycolysis and the TCA cycle in tumorigenesis. *Mol Cell*, 2016, 61(5): 705-719
- [23] Qian X, Li X, Shi Z, et al. PTEN suppresses glycolysis by dephosphorylating and inhibiting autophosphorylated PGK1. *Mol Cell*, 2019, 76(3): 516-527
- [24] Li X, Qian X, Jiang H, et al. Nuclear PGK1 alleviates ADP-dependent inhibition of CDC7 to promote DNA replication. *Mol Cell*, 2018, 72(4): 650-660
- [25] Liang C, Shi S, Qin Y, et al. Localisation of PGK1 determines metabolic phenotype to balance metastasis and proliferation in patients with SMAD4-negative pancreatic cancer. *Gut*, 2020, 69(5): 888-900
- [26] Hu H, Zhu W, Qin J, et al. Acetylation of PGK1 promotes liver cancer cell proliferation and tumorigenesis. *Hepatology*, 2017, 65(2): 515-528
- [27] Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. *Cell*, 2015, 161(1): 67-83
- [28] Hardie DG. AMPK sensing energy while talking to other signaling pathways. *Cell Metab*, 2014, 20(6): 939-952
- [29] Zhang CS, Hawley SA, Zong Y, et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature*, 2017, 548(7665): 112-116
- [30] Lu XY, Shi XJ, Hu X, et al. Feeding induces cholesterol biosynthesis via the mTORC1-USP20-HMGCR axis. *Nature*, 2020, 588(7838): 479-484
- [31] Lee DC, Sohn HA, Park ZY, et al. A lactate-induced response to hypoxia. *Cell*, 2015, 161(3): 595-609
- [32] Liu W, Wang Y, Bozi LHM, et al. Lactate regulates cell cycle by remodeling the anaphase promoting complex. *Nature*, 2023. doi: 10.1038/s41586-023-05939-3
- [33] Huang W, Li N, Zhang Y, et al. AHCYL1 senses SAH to inhibit autophagy through interaction with PIK3C3 in an mTORC1-independent manner. *Autophagy*, 2022, 18(2): 309-319
- [34] Wellen KE, Hatzivassiliou G, Sachdeva UM, et al. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science*, 2009, 324(5930): 1076-1080
- [35] Sperber H, Mathieu J, Wang Y, et al. The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. *Nat Cell Biol*, 2015, 17(12): 1523-1535
- [36] Chen Q, Yan M, Cao Z, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science*, 2016, 351(6271): 397-400
- [37] Sharma U, Conine CC, Shea JM, et al. Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science*, 2016, 351(6271): 391-396
- [38] Hnisz D, Abraham BJ, Lee TI, et al. Super-enhancers in the control of cell identity and disease. *Cell*, 2013, 155(4): 934-947
- [39] Gut P, Verdin E. The nexus of chromatin regulation and intermediary metabolism. *Nature*, 2013, 502(7472): 489-498
- [40] Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. *Nature*, 2015, 517(7534): 302-310
- [41] Shan B, Wang X, Wu Y, et al. The metabolic ER stress sensor IRE1 α suppresses alternative activation of macrophages and impairs energy expenditure in obesity. *Nat Immunol*, 2017, 18(5): 519-529
- [42] Lin R, Tao R, Gao X, et al. Acetylation stabilizes ATP-citrate lyase to promote lipid biosynthesis and tumor growth. *Mol Cell*, 2013, 51(4): 506-518
- [43] Gao X, Lin SH, Ren F, et al. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. *Nat Commun*, 2016, 7(1): 11960
- [44] Wang YP, Zhou W, Wang J, et al. Arginine methylation of MDH1 by CARM1 inhibits glutamine metabolism and suppresses pancreatic cancer. *Mol Cell*, 2016, 64(4): 673-687
- [45] Liu M, Wang Y, Yang C, et al. Inhibiting both proline biosynthesis and lipogenesis synergistically suppresses tumor growth. *J Exp Med*, 2020, 217(3): e20191226
- [46] Zhang T, Sun L, Hao Y, et al. ENO1 suppresses cancer cell ferroptosis by degrading the mRNA of iron regulatory protein 1. *Nat Cancer*, 2022, 3(1): 75-89