Received: 14 April 2024 | Revised: 15 July 2024 | Accepted: 17 July 2024

DOI: 10.1002/cac2.12599

CANCER COMMUNICATIONS Open Access

RESEARCH HIGHLIGHTS

Abrogation of nuclear entry of TERT by fructose 1,6-bisphosphatase 1-mediated dephosphorylation

Pengbo Yao[1] | **Gaoxiang Zhao**[1] | **Min Li**[2] | **Wensheng Qiu**[1] | **Zhimin Lu**[2,3,4]

[1]Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao Cancer Institute, Qingdao, Shandong, P.R. China

[2]Zhejiang Provincial Key Laboratory of Pancreatic Disease, The First Affiliated Hospital, and Institute of Translational Medicine, Zhejiang University School of Medicine, Hangzhou, Zhejiang, P. R. China

[3]Cancer Center, Zhejiang University, Hangzhou, Zhejiang, P. R. China

[4]Institute of Fundamental and Transdisciplinary Research, Zhejiang University, Hangzhou, Zhejiang, P. R. China

Correspondence

Wensheng Qiu, Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao Cancer Institute, Qingdao, Shandong, P.R. China.
Email: wsqiuqd@163.com

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

Funding information

the Ministry of Science and Technology of the People's Republic of China, Grant/Award Number: 2020YFA0803300; National Center of Technology Innovation for Biopharmaceuticals, Grant/Award Number: NCTIB2022HS02006; Taishan Scholar Project from Shandong province, Grant/Award Number: tsqn202312174; National Natural Science Foundation of China, Grant/Award Numbers: 82030074, 82103351, 82188102

Telomeres maintain chromosome integrity. Loss of telomere function, which is attributed to progressively shortened telomeres in each round of DNA replication, induces end-to-end fusion of chromosomes, anaphase bridges with subsequent chromosome breakage, and eventually leads to senescence and apoptosis in normal cells [1]. In cancer cells, highly activated telomerase synthesizes telomere repeats to promote telomere elongation. For assembling catalytically active telomerase, cytoplasmic telomerase reverse transcriptase (TERT), which is the catalytic protein subunit of telomerase, needs to translocate into the nucleus. This translocation requires AKT-mediated TERT S227 phosphorylation and subsequent binding of the nuclear localization signal (NLS) of TERT to importin α [2]. However, whether cancer cells and normal cells differentially regulate TERT phosphorylation and telomere functions remain largely unknown.

We recently demonstrated that fructose 1,6-bisphosphatase 1 (FBP1), the rate-limiting gluconeogenic enzyme that converts fructose 1,6-bisphosphate (F-1,6-BP) to fructose 6-phosphate (F-6-P), acts as a protein phosphatase and dephosphorylates TERT [3]. Through analyses of FBP1 immunoprecipitants from hepatocellular carcinoma (HCC) Huh7 cells by mass spectrometry, TERT was identified as an FBP1-associated protein, and this interaction was primarily in the cytosol. An in vitro glutathione S-transferase (GST) pulldown assay showed that FBP1 directly bound to TERT, and truncation

List of Abbreviations: ALT, alternative lengthening of telomere; ccRCC, clear cell renal cell carcinoma; F-1,6-BP, fructose 1,6-bisphosphate; F-6-P, fructose 6-phosphate; FBP1, fructose 1,6-bisphosphatase 1; GST, glutathione S-transferase; HCC, hepatocellular carcinoma; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; mRNA, messenger RNA; NF-κB, nuclear factor κB; NLS, nuclear localization signal; TERT, telomerase reverse transcriptase.

Pengbo Yao, Gaoxiang Zhao and Min Li contributed equally to this work.

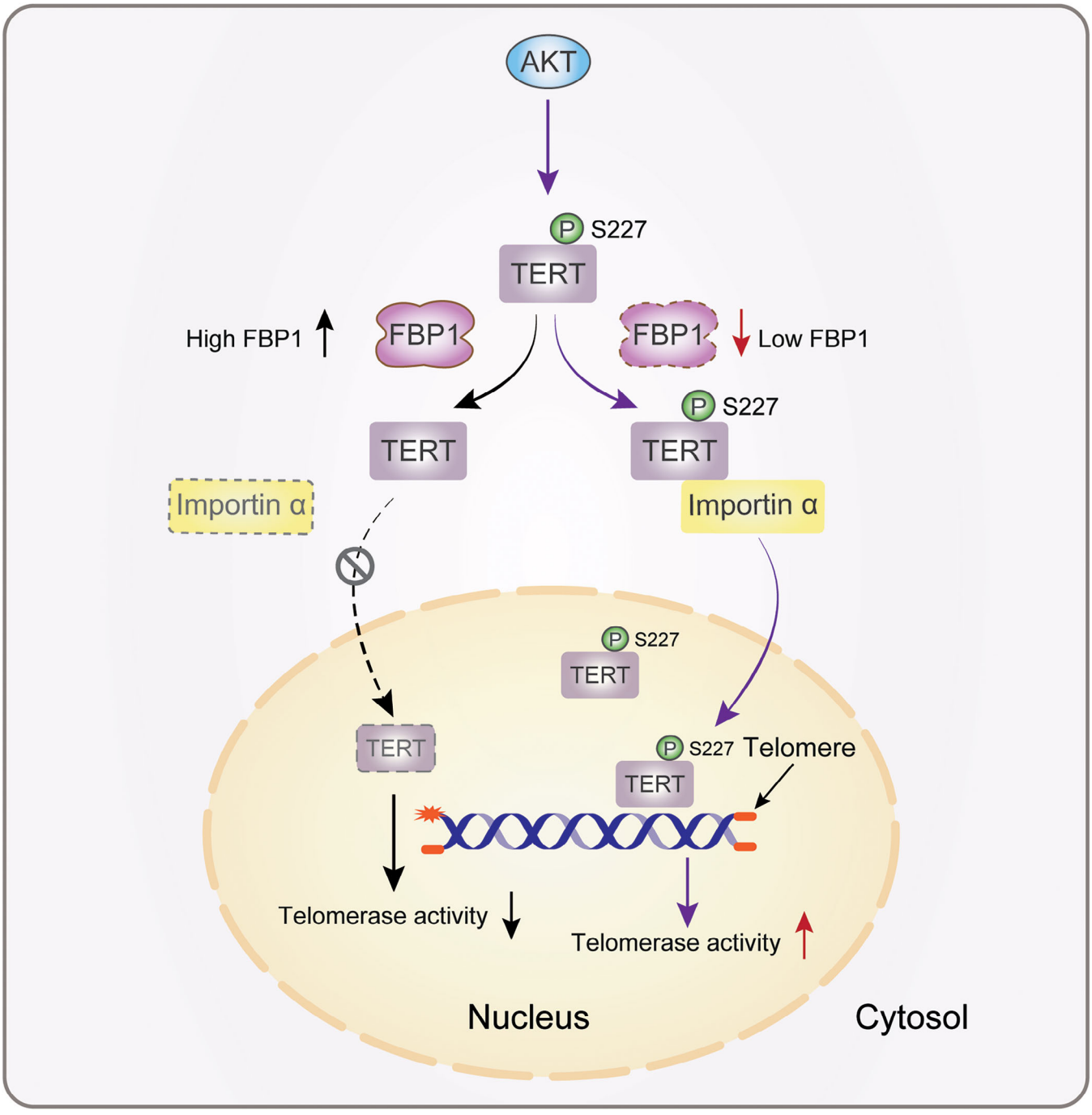

FIGURE 1 A schematic depicting the role of FBP1 in TERT dephosphorylation and tumor growth inhibition. FBP1 dephosphorylates TERT S227 in the cytosol. This dephosphorylation induces cell senescence. FBP1 deficiency promotes tumor growth. Abbreviations: FBP1, fructose 1,6-bisphosphatase 1; TERT, telomerase reverse transcriptase; CDS, coding sequence.

and mutagenesis analyses identified that asparagine (N)273 of FBP1 is a key residue involving in binding to TERT. Importantly, a protein dephosphorylation assay showed that wild-type (WT) FBP1, but not FBP1 N273A mutant, dephosphorylated AKT1-phosphorylated TERT at S227. Notably, FBP1 G260R, a metabolically inactive mutant defected in its binding to F-1,6-BP, was still able to dephosphorylate TERT, indicating that FBP1 dephosphorylates TERT independent of its gluconeogenic activity.

The catalytic domain of conventional protein phosphatases contains a conserved and reduced cysteine (C), which is critical for the dephosphorylation of protein substrates. Molecular docking analyses showed that the phosphorylated S227 residue of TERT was in close proximity to C129 of FBP1 [3]. In addition, during the process of the

dephosphorylation, FBP1 formed a covalent phospho-C129 intermediate. FBP1 C129S mutant, which had comparable metabolic activity to its WT counterpart, lost its ability to dephosphorylate TERT pS227 both in vitro and in vivo. Structural analyses revealed that a binding pocket was formed by FBP1 C129, R244 and D128, which interact with the phosphate group of pS227 of TERT. Notably, mutations of FBP1 R244 or D128 also decreased TERT pS227 dephosphorylation by FBP1.

As expected, FBP1 depletion enhanced the nuclear translocation of TERT and telomerase activity, leading to increased telomere lengths in normal human renal cells [3]. In contrast, in clear cell renal cell carcinoma (ccRCC) cells with deficiency in FBP1 expression, ectopic expression of WT FBP1, but not FBP1 C129S or FBP1 N273A reduced nuclear TERT levels and activity of telomerase, resulting in decreased telomere lengths and increased cell senescence. In addition, the expression of these proteins did not alter the alternative lengthening of telomere (ALT) pathway. Mouse studies showed that WT FBP1 expression substantially promoted anaphase bridge formation, a feature of dysfunctional telomere, enhanced cell senescence in tumors, and inhibited tumor growth that accompanied with decreased levels of TERT S227 phosphorylation. In contrast, the expression of FBP1 C129S and FBP1 N273A, to a large extent, failed to inhibit tumor growth and tumor cell senescence (Figure 1). In addition, FBP1 expression levels and TERT pS227 levels were inversely correlated with each other in ccRCC and HCC specimens and poor prognosis of the patients. These results indicate that FBP1 acts as a tumor suppressor and inhibits tumor growth through TERT pS227 dephosphorylation.

To explore the therapeutic potential of employing FBP1's tumor-suppressing function to treat cancer, animal studies were conducted using lipid nanoparticles (LNPs) to deliver *FBP1* mRNA [3]. Intravenous delivery of LNP-*FBP1* mRNA, which contained an Arg-Gly-Asp (*RGD*) tripeptide on the surface of LNP for binding to integrin overly expressed in tumor cell membrane, markedly reduced glycolytic flux in the tumor tissues and inhibited tumor growth. Of note, treatment with the LNPs did not elicit obvious adverse effects.

Tumor cell proliferation relies on substantially increased glycolysis regardless of oxygen availability, which is known as the Warburg effect [4]. As a gluconeogenic enzyme, FBP1 inhibited growth of acute myeloid leukemia through anti-Warburg effect [5]. Glycolytic enzymes, including hexokinase, phosphoglycerate kinase 1, pyruvate kinase M2, as well as other metabolic enzymes such as ketohexokinase isoform A, phosphoenolpyruvate carboxykinase 1, choline kinase α2, and creatine kinase B, exhibit protein kinase activity. They phosphorylate diverse protein substrates critical for various cellular activities [6, 7]. The finding of FBP1's protein phosphatase activity in dephosphorylation of histone H3 and TERT reshapes the understanding of the moonlighting functions of metabolic enzymes, involving both phosphorylation and dephosphorylation of proteins [8]. The deficiency in expression of gluconeogenic enzyme FBP1 in tumor cells promotes cell proliferation not only by facilitating the Warburg effect but also through the loss of its protein phosphatase activity. FBP1 has been shown to be able to dephosphorylate histone H3 at T11 and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα), resulting in suppression of histone H3T11 phosphorylation-dependent gene transcription and nuclear factor κB (NF-κB) activity, respectively [9, 10]. The finding that FBP1 plays a critical role in the dephosphorylation of TERT and the inhibition of telomere function underscores its previously unknown role in regulating cell senescence and immortality. The proof-of-concept studies showing that LNP-FBP1 mRNA effectively attenuated tumor growth provide an attractive strategy for cancer treatment.

AUTHOR CONTRIBUTIONS

Zhimin Lu and Wensheng Qiu conceptualized the writing. Zhimin Lu, Wensheng Qiu, Pengbo Yao, Gaoxiang Zhao, and Min Li wrote the manuscript.

ACKNOWLEDGMENTS

This study was supported by grants from the National Natural Science Foundation of China (82188102, 82030074, Z.L.; 82103351, M.L.), the Ministry of Science and Technology of the People's Republic of China (2020YFA0803300, Z.L.), National Center of Technology Innovation for Biopharmaceuticals (NCTIB2022HS02006, Z.L.), and Taishan Scholar Project from Shandong province (tsqn202312174, P.Y.). Z.L. is the Kuancheng Wang Distinguished Chair.

CONFLICT OF INTEREST STATEMENT

Dr. Zhimin Lu owns shares in Signalway Biotechnology (Pearland, TX, USA). Dr. Lu's interest in this company had no bearing on its being chosen to supply the reagents. The remaining authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request. The CDS sequence of human liver FBP1 is available in the National Center of Biotechnology Information (NCBI) under accession code NM_000507.4. Mass spectrometry data have been deposited in ProteomeXchange with the accession code PXD050213.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The use of human samples in this study was approved by the Research Ethics Committee of The First Affiliated

Hospital, Zhejiang University School of Medicine and complied with all relevant ethical regulations. All tissue samples were collected in compliance with the informed consent policy. Animal experiments were performed according to the institutional guidelines for the care and use of laboratory animals approved by the Institutional Review Board at The First Affiliated Hospital, Zhejiang University School of Medicine.

ORCID

Pengbo Yao https://orcid.org/0000-0003-2091-7142
Zhimin Lu https://orcid.org/0000-0002-2859-2736

REFERENCES

1. Sahin E, DePinho RA. Axis of ageing: telomeres, p53 and mitochondria. Nat Rev Mol Cell Biol. 2012;13(6):397-404.
2. Jeong SA, Kim K, Lee JH, Cha JS, Khadka P, Cho HS, et al. Akt-mediated phosphorylation increases the binding affinity of hTERT for importin alpha to promote nuclear translocation. J Cell Sci. 2015;128(12):2287-301.
3. Li M, Wang Z, Tao J, Jiang H, Yang H, Guo D, et al. Fructose-1,6-bisphosphatase 1 dephosphorylates and inhibits TERT for tumor suppression. Nat Chem Biol. 2024.
4. Thompson CB, Vousden KH, Johnson RS, Koppenol WH, Sies H, Lu Z, et al. A century of the Warburg effect. Nat Metab. 2023;5(11):1840-3.
5. Xu Y, Hino C, Baylink DJ, Xiao J, Reeves ME, Zhong JF, et al. Vitamin D activates FBP1 to block the Warburg effect and modulate blast metabolism in acute myeloid leukemia. Biomark Res. 2022;10(1):16.
6. Guo D, Tong Y, Jiang X, Meng Y, Jiang H, Du L, et al. Aerobic glycolysis promotes tumor immune evasion by hexokinase2-mediated phosphorylation of IkappaBalpha. Cell Metab. 2022;34(9):1312-24 e6.
7. Wu K, Yan M, Liu T, Wang Z, Duan Y, Xia Y, et al. Creatine kinase B suppresses ferroptosis by phosphorylating GPX4 through a moonlighting function. Nat Cell Biol. 2023;25(5):714-25.
8. Bian X, Jiang H, Meng Y, Li YP, Fang J, Lu Z. Regulation of gene expression by glycolytic and gluconeogenic enzymes. Trends Cell Biol. 2022;32(9):786-99.
9. Wang Z, Li M, Jiang H, Luo S, Shao F, Xia Y, et al. Fructose-1,6-bisphosphatase 1 functions as a protein phosphatase to dephosphorylate histone H3 and suppresses PPARalpha-regulated gene transcription and tumour growth. Nat Cell Biol. 2022;24(11):1655-65.
10. Zhu W, Chu H, Zhang Y, Luo T, Yu H, Zhu H, et al. Fructose-1,6-bisphosphatase 1 dephosphorylates IkappaBalpha and suppresses colorectal tumorigenesis. Cell Res. 2023;33(3):245-57.

How to cite this article: Yao P, Zhao G, Li M, Qiu W, Lu Z. Abrogation of nuclear entry of TERT by fructose 1,6-bisphosphatase 1-mediated dephosphorylation. Cancer Commun. 2024;44:1102–1105.
https://doi.org/10.1002/cac2.12599