RESEARCH HIGHLIGHT

Repurposing FBP1: dephosphorylating IκBα to suppress NFκB

Gang Zhang[1], Jingjing Tao[2], Liming Lin[2], Wensheng Qiu[1]✉ and Zhimin Lu[2,3]✉

Cell Research (2023) 33:419–420; https://doi.org/10.1038/s41422-023-00785-4

Metabolic enzymes can function as protein kinases and protein phosphatases to govern the phosphorylation status of protein substrates, thereby regulating pivotal cellular activities. A new study published in *Cell Research* demonstrated that fructose 1,6-bisphosphatase 1 directly dephosphorylates IκBα to suppress NFκB activation and modulate the tumor immune microenvironment.

As a critical protein post-translational modification that plays an important role in the regulation of fundamental cellular activities, protein phosphorylation is controlled by two groups of enzymes with opposite activities: protein kinases and protein phosphatases.[1] The human genome possesses > 500 protein kinases for protein phosphorylation and < 200 identified protein phosphatases for dephosphorylation,[2] suggesting that protein dephosphorylation is less specifically regulated by protein phosphatases and that cells may use unknown and classic protein phosphatase-irrelevant mechanisms to dephosphorylate proteins. In the human genome, 1653 of ~2700 human enzymes are metabolic enzymes, much more than the total number of protein kinases and phosphatases.[3] Importantly, some metabolic enzymes possess moonlighting functions using proteins as substrates for phosphorylation.[4–6] As the rate-limiting gluconeogenic enzyme that catalyzes the hydrolysis of fructose 1,6-bisphosphate (F-1,6-BP) to fructose 6-phosphate (F-6-P), fructose 1,6-biphosphatase 1 (FBP1) has been reported as a protein phosphatase that dephosphorylates histone H3 at T11 and suppresses peroxisome proliferator-activated receptor α (PPARα)-mediated β-oxidation and subsequent fatty acid oxidation in mitochondria.[7] Given that protein phosphatases dephosphorylate different substrates in a cell signaling context-dependent manner, whether FBP1 can dephosphorylate a nonhistone protein remains unclear.

A recent study by Zhu et al. demonstrated that IκBα can be dephosphorylated by FBP1.[8] By screening 57 metabolic phosphatases with molecular docking of phosphorylated serine, threonine, and tyrosine dipeptides followed by molecular dynamic simulations, FBP1 was identified to have strong binding free energy. An in vitro phosphatase assay showed that recombinant FBP1 reduced the phosphorylation of 72 proteins in the lysate of colon cancer cells, among which IκBα, a key inhibitory regulator of NFκB signaling, was dephosphorylated at S32/36. In addition, recombinant wild-type FBP1, but not its metabolically inactive mutant FBP1 G260R, dephosphorylated a synthesized S32/36-phosphorylated peptide, inhibitor of nuclear factor kappa-B kinase subunit β (IKKβ)-phosphorylated recombinant IκBα at S32/36, and tumor necrosis factor α (TNFα)-induced IκBα S32/36 phosphorylation in colon cancer cell lysates. Molecular dynamic simulations and docking analyses showed that pS32 in an IκBα S32/36 peptide is likely located at the Mg^{2+} catalytic reaction center of FBP1. In addition, FBP1 had a higher catalytic rate, substrate affinity, and efficiency toward F-1,6-BP than toward phosphorylated IκBα S32/36. These findings suggest that FBP1 catalyzes the dephosphorylation of IκBα pS32/36 and F-1,6-BP in a similar manner with a lower energy barrier toward F-1,6-BP.

Intriguingly, TNFα stimulation induced the binding of FBP1 to IκBα. Luciferase reporter assays showed that FBP1 depletion enhanced TNFα-induced NFκB activity accompanied by enhanced IκBα S32/36 phosphorylation and its subsequent degradation in tumor cells. Molecular docking and binding free energy analyses and subsequent mutagenesis showed that FBP1 N213K, which had similar F-1,6-BP-binding affinity and gluconeogenic activity to wild-type FBP1, lost its binding with IκBα, its ability to dephosphorylate IκBα S32/36, and its inhibition of TNFα-induced NFκB activation. In addition, FBP1 N213K expression promoted TNFα-induced and NFκB-mediated expression of prosurvival genes, such as *BCL2*, *BIRC2*, *BIRC3*, *CFLAR*, *TRAF1*, *TRAF2*, and *XIAP*, and attenuated H_2O_2-, TNFα- or Fas ligand-induced tumor cell death. These results indicate that FBP1 dephosphorylates IκBα pS32/36 and suppresses NFκB-mediated gene expression, thereby promoting tumor cell death under oxidative and inflammatory stresses.

Consistent with the cell-based studies, FBP1 N213K knock-in mice with no altered embryonic viability or sex ratio developed much more and larger tumors with a much shorter survival duration than *Fbp1*$^{+/+}$ mice following treatment with azoxymethane and the inflammatory agent dextran sodium sulfate. In addition, FBP1 N213K knock-in expression reduced IκBα expression in tumor tissues and tumor cell apoptosis accompanied by decreased infiltration of $CD8^+$ T cells and enhanced infiltration of immunosuppressive myeloid-derived suppressor cells (MDSCs) with increased expression of MDSC mobilization-associated cytokines, such as *Tgfb1*, *Csf1*, *Csf3*, and *Kitl*. These results suggest that deficiency in Fbp1-mediated IκBα dephosphorylation in tumor cells leads to the recruitment of MDSCs and promotes tumor immune evasion and tumor growth. These findings were further supported by analysis of human colorectal cancer specimens, revealing a positive correlation between FBP1 expression and IκBα expression, the level of cell apoptosis, and MDSC abundance (Fig. 1).

Metabolic enzymes exhibit substantial plasticity in the regulation of metabolic or pivotal nonmetabolic activities in a manner dependent on their canonical metabolic activities or noncanonical activities.[8] Importantly, metabolic enzymes are often differentially regulated in normal and tumor cells, resulting in unique metabolic features in

[1]Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao Cancer Institute, Qingdao, Shandong, 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, China. [3]Cancer Center, Zhejiang University, Hangzhou, Zhejiang, China. ✉email: wsqiuqd@163.com; zhiminlu@zju.edu.cn

Published online: 24 February 2023

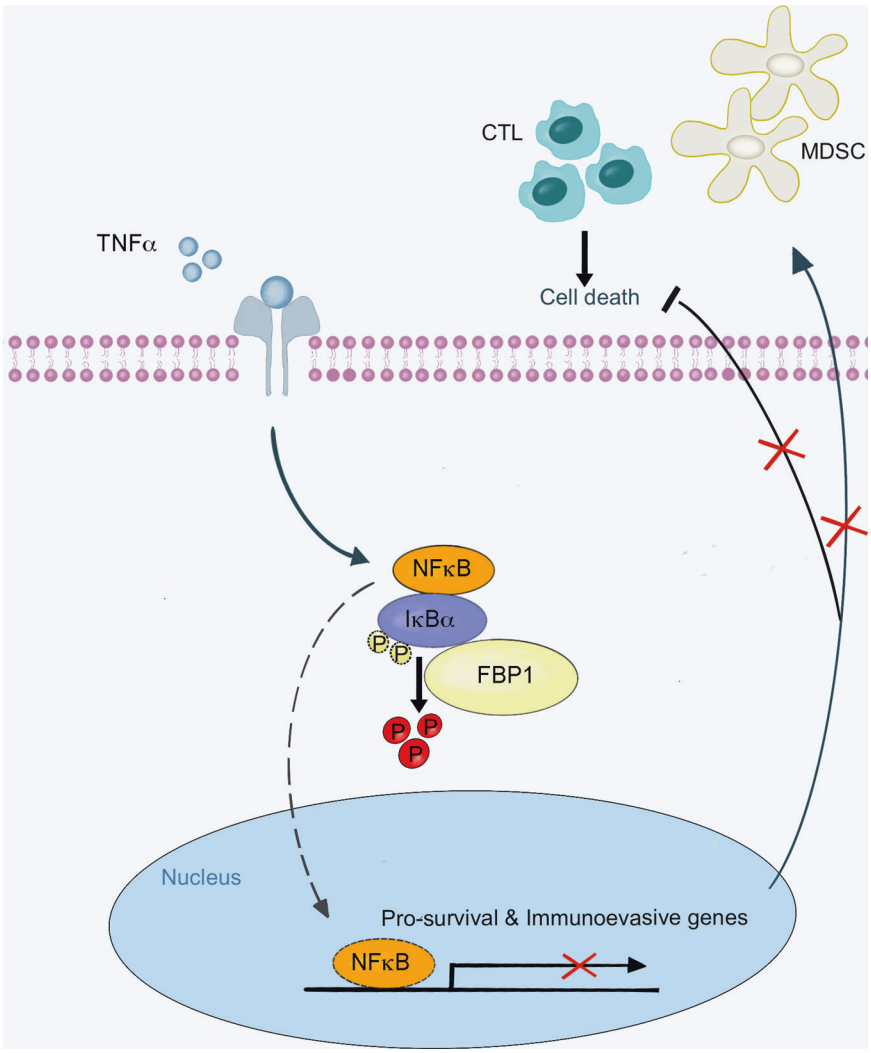

Fig. 1 Schematic illustration depicting the role of FBP1-regulated IκBα dephosphorylation in tumor growth. TNFα stimulation induces the binding FBP1 to IκBα, resulting in FBP1-mediated IκBα dephosphorylation, suppression of NFκB-mediated expression of prosurvival genes and cytokines, inhibition of the mobilization of MDSCs, and enhanced tumor infiltration of cytotoxic T lymphocytes (CTLs). Dashed lines indicate decreased signaling.

cancer cells to support tumor growth and counteract metabolic, inflammatory, and genotoxic stress during cancer progression.[9] Under energy stress, FBP1 was previously shown to translocate into the nucleus with the conversion from a tetramer to monomers and act as a protein phosphatase to dephosphorylate histone H3, in which C129 in a reduced state in the catalytic domain forms a covalent phospho-C129 intermediate.[7] Under cytokine stimulation or oxidative stress, FBP1 binds and dephosphorylates IκBα pS32/36 to suppress NFκB activation.[8] Consequently, deficiency or low expression of FBP1, which frequently occurs in many types of cancer, including clear cell renal cell carcinoma and hepatocellular carcinoma, leads to tumor cell advantages in growth, counteraction to metabolic stresses, and genotoxic stress, and immune evasion.

Multiple metabolic enzymes, such as pyruvate kinase M2, phosphoglycerate kinase 1, phosphoenolpyruvate carboxykinase 1, ketohexokinase isoform A, hexokinase 2, and choline kinase α2, have been shown to function as protein kinases, phosphorylating various protein substrates and modulating diverse cellular functions, including gene expression, cell cycle progression, de novo nucleotide synthesis, glycolysis, lipid metabolism, autophagy, and tumor immune evasion.[4, 10] The findings that FBP1 can dephosphorylate histone H3 and IκBα expand our understanding of the diverse regulation of protein phosphorylation and dephosphorylation governed by metabolic enzymes.

REFERENCES

1. Lu, Z. & Hunter, T. *Annu. Rev. Biochem.* **78**, 435–475 (2009).
2. Chen, M. J., Dixon, J. E. & Manning, G. *Sci. Signal.* **10**, eaag1796 (2017).
3. Romero, P. et al. *Genome Biol.* **6**, R2 (2005).
4. Xu, D. et al. *Cell Metab.* **33**, 33–50 (2021).
5. Li, X. et al. *Nat. Rev. Mol. Cell Biol.* **19**, 563–578 (2018).
6. Bian, X. et al. *Trends Cell Biol.* **32**, 786–799 (2022).
7. Wang, Z. et al. *Nat. Cell Biol.* **24**, 1655–1665 (2022).
8. Zhu, W. et al. *Cell Res.* https://doi.org/10.1038/s41422-022-00773-0 (2023).
9. Wang, Y., Xia, Y. & Lu, Z. *Cancer Commun.* **38**, 65 (2018).
10. Guo, D. et al. *Cell Metab.* **34**, 1312–1324.e6 (2022).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Wensheng Qiu or Zhimin Lu.