JCB Journal of Cell Biology

SPOTLIGHT

Ubiquitinating the way to T cell metabolism

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In this issue, Harris et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202203095) show that phosphofructokinase is a substrate for ubiquitination by Fbxo7, a key protein in the ubiquitination pathway. Their findings point to a new interplay between metabolic enzyme degradation in the regulation of T cells.

F-box protein only 7 (Fbxo7) is a multifunctional protein that facilitates various biochemical reactions between tissues. Consequently, Fbxo7 has been implicated in a wide range of human pathologies, including Parkinson's disease and cancer (1, 2). Fbxo7 is most well-known for playing a critical role in the ubiquitination pathway, as it acts as a substrate-docking subunit of Skp1-Cullin1-F-box protein (SCF)–type E3 ubiquitin ligases, which catalyze the final modification of protein substrates by ubiquitin (3). Beyond its role in ubiquitination, Fbxo7 has been shown to interact with and dictate the function of two critical cell cycle proteins: the G1-phase cell cycle regulators Cdk6 and p27 (4). Although Fbxo7 has been shown to play a dominant role in two essential cellular processes, its role in metabolism, another critical cellular process that is intricately linked to cell cycle regulation, remains poorly understood.

In this issue, Harris et al. (5) uncovered a novel link between metabolism and Fbxo7 by identifying phosphofructokinase (PFKP) as a substrate for ubiquitination by Fbxo7. PFKP is a key regulatory enzyme that catalyzes the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate in glycolysis (6). The authors discovered a novel interaction between Fbxo7 and PFKP using a yeast two-hybrid screen. Moreover, ubiquitination assays showed that SCF^{Fbxo7} ubiquitinates PFKP and that this reaction was dependent on both the N- and C-termini of Fbxo7 (Fig. 1).

Cdk6 phosphorylates PFKP and Fbxo7 activates Cdk6 (7, 8). Given this and the new findings that PFKP ubiquitination relies on Fbxo7, intriguing questions arise about the relationship between these three proteins, and consequently the direct interplay between metabolic, cell cycle, and ubiquitination pathways. To answer these questions, the authors inhibited Cdk6 production and examined the effect on PFKP–Fbxo7 interaction and PFKP ubiquitination. They found that neither Cdk6 activity or presence were required for PFKP interaction with, or ubiquitination facilitated by Fbxo7. Conversely, the authors found that knockdown of Fbxo7 eliminated both PFKP interaction with and phosphorylation by Cdk6, indicating the Fbxo7 was required for Cdk6 phosphorylation of PFKP.

Next, to investigate if Fbxo7-mediated ubiquitination of PFKP was targeting PFKP for degradation by the 26S proteasome, Harris et al. (5) investigated if PFKP levels were different in Fbxo7 knockdown CCRF-CEM cells compared to WT CCRF-CEM cells. Surprisingly, they found no difference in PFKP steady-state levels in the cell. However, since PFKP exists in catalytically distinct complexes, the authors performed immunoblot analyses to determine if levels of distinct PFKP complexes changed in Fbxo7 knockdown CCRF-CEM cells. Interestingly, the authors found that the inactive forms of PFKP, the dimer/monomer forms, decreased by 75% in knockdown cells, confirming a shift to the active tetramer form in Fbxo7 knockdown CCRF-CEM cells.

Harris et al.'s (5) findings that knockdown of Fbxo7 in CCRF-CEM cells increases the catalytically active form of PFKP prompted the authors to ask: What are the effects of Fbxo7 knockdown on glycolysis in cancer cell lines? The authors found that Fbxo7 knockdown CCRF-CEM and Jurkat E6 cells had significantly increased compensatory and basal glycolysis rates. Wanting to confirm these findings in primary T cells, the authors investigated glycolysis levels in activated CD4 T cells isolated from Fbxo7-deficient and WT mice. They found that glycolysis levels were upregulated in Fbxo7 knockout CD4 T cells (Fig. 1). Importantly, these results were further confirmed in the mutant cells by metabolomics analysis and stable isotope tracing studies, which both identified lactate as an upregulated metabolite upon Fbxo7 knockout. There was also a significant decrease in viability of these mutant cells, which was in line with previous data that highlighted the essential role of Fbxo7 in T cells (9).

Several key experiments provided the crucial linkage between Fbxo7-mediated ubiquitination and metabolism. Here, metabolomics analysis on the mutant activated CD4 T cells revealed alterations in arginine metabolism, increases in purines and decreases in pyrimidines. However, supplementation with pyrimidines did not rescue viability in the mutant CD4 T cells, implying that another compensatory pathway dictates how Fbxo7 controls metabolism.

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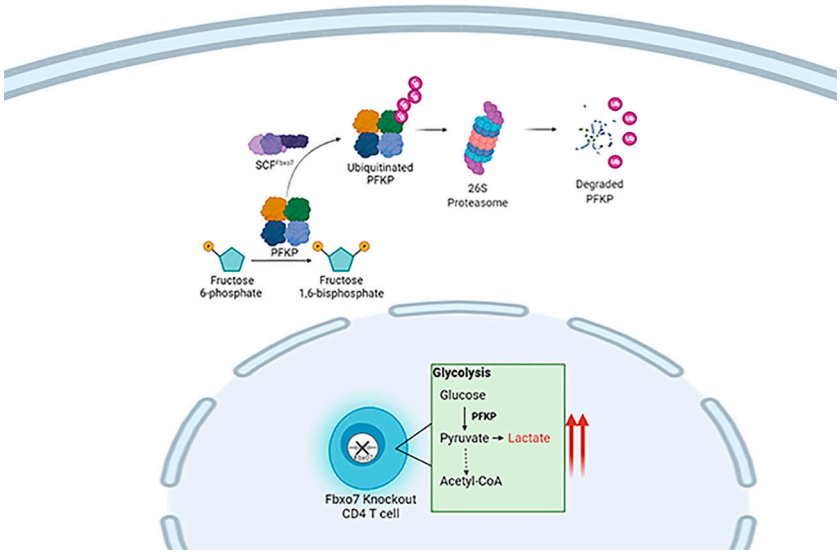

Figure 1. **Fbxo7 regulates T cell metabolism by ubiquitinating the glycolytic enzyme PFKP.** The catalytically active tetramer of PFKP is ubiquitinated by SCF^{Fbxo7} and degraded by the 26S proteasome (cytoplasm). Glycolysis levels are upregulated in Fbxo7 knockout CD4 T cells (nucleus). Created with BioRender.com.

Expression of glycolytic enzymes in T cells varies based on extracellular glucose levels (10). Indeed, there was a dose-dependent change in Fbxo7 protein levels in response to varying levels of glucose, where Fbxo7 was reduced following glucose starvation. The decrease was due to Fbxo7 degradation by the autophagy pathway, as treatment with BafA1 rescued Fbxo7 protein levels during glucose starvation. These results indicated that the autophagy pathway was responsible for the decreased Fbxo7 levels in glucose-starved T acute lymphoblastic leukemia cells.

Much of the T cell metabolism field has focused the transcriptional and post-translational modifications as the primary regulator of metabolic enzyme expression. This elegant paper adds a new dimension to the field by putting a spotlight on the ubiquitin degradation pathway as a crucial mechanistic component of how T cells tune metabolism to meet cell cycle requirements. The new links in this study provides a different angle into how drugs like pomalidomide, which act on the ubiquitin/proteosome pathway, may be acting on metabolism as its mode of action. Such knowledge could open up other avenues of directly targeting specific metabolic enzymes, where metabolism is the underlying cause of a pathological condition.

Acknowledgments

Funding provided by the Canadian Institutes of Health Research (J.J. Lum) and jointly by IRICoR and Ovarian Cancer Canada (J.J. Lum). S. McPhedran is funded by scholarships from University of Victoria and BC Regenerative Medicine Network.

The authors declare no competing financial interests.

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