CELL CYCLE
2016, VOL. 15, NO. 7, 875–876
http://dx.doi.org/10.1080/15384101.2016.1151728

Taylor & Francis
Taylor & Francis Group

EDITORIALS: CELL CYCLE FEATURES

The 26S proteasome: A cell cycle regulator regulated by cell cycle

Xing Guo[a] and Jack E. Dixon[b,c,d]

[a]Life Sciences Institute, Zhejiang University, Hangzhou, China; [b]Department of Pharmacology, University of California-San Diego School of Medicine, La Jolla, CA, USA; [c]Department of Chemistry and Biochemistry, University of California-San Diego School of Medicine, La Jolla, CA, USA; [d]Department of Cellular and Molecular Medicine, University of California-San Diego School of Medicine, La Jolla, CA, USA

ARTICLE HISTORY Received 27 January 2016; Accepted 31 January 2016

KEYWORDS cell cycle; DYRK2; phosphorylation; proteasome

Since the discovery of the 26S proteasome nearly three decades ago, its role in executing protein degradation and regulating almost every cellular function, especially cell cycle, has been well established and widely accepted. Inhibition of the 26S proteasome invariably leads to cell cycle block which can eventually cause cell death, underlying the proven clinical usage of proteasome inhibitors (such as Bortezomib or Velcade^{TM}) for treating cancers.[1] We recently published a study[2] showing that the proteasome itself is phosphorylated in a cell cycle-dependent manner, and that blocking a single phosphorylation event can alter proteasome activity, perturb cell cycle and reduce tumor growth, in the absence of proteasome inhibitors.

The 26S proteasome consists of a 20S core particle (CP) and one or two 19S regulatory particles (RP). The 19S RP performs a number of activities including capturing, unfolding and translocating the substrate into the 20S for proteolysis. Most of these activities depend on six AAA-type ATPase subunits of the 19S RP, namely Rpt1-6. The biological importance and biochemical complexity of the 26S proteasome demand that it must be tightly regulated.[3]

One particularly important regulatory mechanism of the proteasome is reversible phosphorylation. Previously we identified the first known proteasome-resident phosphatase, UBLCP1.[4] In our efforts to identify the target sites of UBLCP1 using quantitative mass spectrometry, we noticed phosphorylation at Thr25 of the ATPase subunit Rpt3. Although this site was later found not to be dephosphorylated by UBLCP1, changing the Thr residue to Val (phospho-deficient) or to Asp (phospho-mimetic) did alter proteasome activity in opposite directions. This suggested to us that Rpt3-T25 phosphorylation might be a functionally relevant event to the proteasome. A number of phospho-proteomics studies have documented this phosphorylation, with a hint for its association with cell cycle.[5] We wondered whether the proteasome itself is dynamically phosphorylated during cell cycle, which may affect its proteolytic function that in turn regulates cell cycle progression. This was an intriguing question that had not been rigorously addressed.

We began by generating a phospho-T25-specific antibody, and confirmed that this phosphorylation is prevalent in various cell types and mouse tissues. Notably, pT25 level is low in G1-phase cells but is markedly elevated upon S phase entry and persists throughout G2 and M phases. This is the first clear evidence that the 26S proteasome undergoes dynamic, cell cycle-dependent phosphorylation. The result not only verifies previous mass spectrometry findings but also suggested a novel regulatory mechanism of the proteasome that may be important for cell cycle progression.

To test this notion, we introduced homozygous Rpt3-T25A mutation to different cell lines using CRISPR/Cas9-mediated gene editing. The mutant cells, which lack T25 phosphorylation, displayed weakened proteasome activity as well as reduced proliferation, indicating a positive role of T25 phosphorylation in regulating proteasomal degradation and cell cycle progression. Indeed, our quantitative mass spectrometry study revealed that degradation of nearly 20% of all detected proteins was impaired due to loss of T25 phosphorylation, many of which are involved in cell cycle regulation (unpublished). Two clear examples were the CDK inhibitors, $p21^{Cip1}$ and $p27^{Kip1}$, whose stabilization in the T25A knock-in cells fits nicely with the prolonged transition from S to G2/M phases (Fig. 1). Thus, Rpt3-T25 phosphorylation coordinates proteasome activity with cell cycle progression, and mutation of this single site reshapes the cellular proteome.

Through a kinome-wide screen, we found DYRK2 (dual-specificity tyrosine-regulated kinase 2) as the primary kinase phosphorylating Rpt3-T25. Interestingly, we show that DYRK2 itself is a cell cycle-regulated kinase and its upregulation at the start of S phase explains the dynamics of T25 phosphorylation. DYRK2 directly activates the proteasome by enhancing substrate translocation to the 20S CP, although other mechanisms

CONTACT Xing Guo xguo@zju.edu.cn 866 Yuhangtang Rd., Nano Bldg., Rm 487, Life Sciences Institute, Zhejiang University, Hangzhou, Zhejiang, P. R. China 310058; Jack E. Dixon jedixon@ucsd.edu 9500 Gilman Drive, LBR, Rm 283, University of California-San Diego School of Medicine, La Jolla, CA 92093-0721.

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/kccy.

Feature to: Guo X, et al. Site-specific proteasome phosphorylation controls cell proliferation and tumorigenesis. Nat Cell Biol 2016; 18(2):202-12; PMID: 26655835; http://dx.doi.org/10.1038/ncb3289

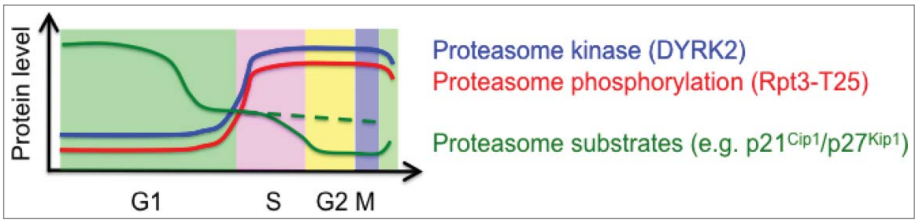

Figure 1. DYRK2-mediated proteasome phosphorylation at Rpt3-T25 regulates cell cycle progression. Upon S phase entry, increased Rpt3-T25 phosphorylation (red) caused by DYRK2 upregulation (blue) leads to enhanced proteasomal degradation of cell cycle inhibitors such as $p21^{Cip1}$ and $p27^{Kip1}$ (green). Loss of T25 phosphorylation stabilizes $p21^{Cip1}$ and $p27^{Kip1}$ (dotted line) and delays cell cycle progression. We want to emphasize that blocking Rpt3-T25 phosphorylation also affects many other proteins involved in cell cycle and several different cellular processes.

may exist. CRISPR/Cas9-mediated DYRK2 knockout phenocopied T25A knock-in in difference cell types, and also sensitized a basal-like triple-negative breast cancer cell line, MDA-MB-468, to low-dose Bortezomib treatment. Importantly, either DYRK2 disruption or T25A knock-in alone was able to significantly reduce the tumorigenic growth of MDA-MB-468 cells in nude mice.

Together, our findings represent the first detailed study of proteasome phosphorylation during cell cycle. We argue that proteasome activity can be fine-tuned by post-translational modifications, resulting in an optimal level of protein degradation that is necessary for certain physiological processes. In the case of breast cancer, proteasome addiction has been found to be a vulnerability of the basal type of tumor cells.[6] Our data demonstrate that blocking proteasome phosphorylation alone (without using any proteasome inhibitors) can effectively thwart tumor growth. To our satisfaction, a very recent large-scale study has placed Rpt3 (also known as PSMC4) and DYRK2 in the same "essential gene set" of basal breast cancers.[7] These findings, plus the negative correlation between DYRK2 expression and breast cancer patient prognosis, suggest the possibility of targeting DYRK2 for cancer treatment. In a broader sense, we postulate that a considerable number of existing kinase inhibitors may indirectly act upon the proteasome and synergize with proteasome inhibitors to more effectively (and perhaps more selectively) eliminate cancer cells that show a strong dependence on proteasome function and/or aberrant proteasome phosphorylation. Testing this hypothesis obviously requires a better understanding of proteasome phosphorylation, a goal that we will continue to pursue.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Hoeller D, Dikic I. Nature 2009; 458:438-44; PMID:19325623; http://dx.doi.org/10.1038/nature07960
[2] Guo X, et al. Nat Cell Biol advance online publication; 2015; 18 (2):202-12; PMID:26655835; http://dx.doi.org/10.1038/ncb3289
[3] Finley D. Annu Rev Biochem 2009; 78:477-513; PMID:19489727; http://dx.doi.org/10.1146/annurev.biochem.78.081507.101607
[4] Guo X, et al. Proc Natl Acad Sci 2011; 108:18649-54; PMID:21949367; http://dx.doi.org/10.1073/pnas.1113170108
[5] Dephoure N, et al. Proc Natl Acad Sci U S A 2008; 105:10762-7; PMID:18669648; http://dx.doi.org/10.1073/pnas.0805139105
[6] Petrocca F, et al. Cancer Cell 2013; 24:182-96; PMID:23948298; http://dx.doi.org?/10.1016/j.ccr.2013.07.008
[7] Marcotte R, et al. Cell 2016; 164:293-309; PMID:26771497; http://dx.doi.org/10.1016/j.cell.2015.11.062