AUTOPHAGY
2017, VOL. 13, NO. 7, 1091–1092
https://doi.org/10.1080/15548627.2017.1321723

Taylor & Francis
Taylor & Francis Group

EDITOR'S CORNER

A novel role for a glycolytic pathway kinase in regulating autophagy has implications in cancer therapy

Aileen R. Ariosa and Daniel J. Klionsky

Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA

ABSTRACT

When it comes to cancer initiation and progression, macroautophagy/autophagy seemingly acts in a contradictory fashion, serving either as a suppressive factor that functions to protect against tumor formation or as a support mechanism that sustains the disease itself through its cytoprotective functions. In tumor suppression, autophagy assists by restricting oxidative stress and curbing genomic instability that could possibly cause oncogenic mutations. However, in certain circumstances, autophagy can also promote cancer by providing nourishment and by limiting stress-response pathways, leading to cancer cell survival and rapid proliferation. Thus, autophagy's role in oncogenesis is highly context-dependent and varies from one cancer type to another. As a consequence, identifying the mechanisms that alter and rewire autophagic regulation and flux is extremely crucial to target autophagy as a possible avenue for anticancer treatment. In a recent study, Qian et al. endeavored to identify one such key regulatory pathway in hypoxia- and glutamine deprivation-induced autophagy in tumorigenic cells. In this pathway, phosphatidylinositol 3-phosphate (PtdIns3P) production by the class III phosphatidylinositol 3-kinase (PtdIns3K) complex is greatly improved through a cascade of posttranslational modifications that culminates in the phosphorylation of the scaffolding protein BECN1 by the glycolytic pathway kinase PGK1.

ARTICLE HISTORY
Received 18 April 2017
Revised 18 April 2017
Accepted 18 April 2017

KEYWORDS
autophagy; BECN1; cancer; glutamine; PGK1; stress

Many cancer cells are dependent on glutamine metabolism—this particular amino acid is a good source of both carbon and nitrogen, is readily used and is exploited to drive uncontrolled proliferation and rapid growth.[1,2] In their manuscript, Qian et al.[3] show that the deprivation of glutamine in U87 human glioblastoma cells results in increased autophagy that is independent of AMPK- and ULK1-mediated phosphorylation of BECN1. Through mass spectrometry, the authors identified the kinase directly responsible for activating BECN1—PGK1. This role of PGK1 was successively verified through a series of immunoprecipitation experiments wherein the authors clearly showed that PGK1 can bind to BECN1 and ATG14 when glutamine is depleted.

The authors next explored the molecular trigger that allows for this interaction. Again using mass spectrometry it was discovered that upon glutamine deprivation PGK1 becomes acetylated at residue Lys388 by the NAA10/ARD1 acetyltransferase. When this lysine is mutated to an arginine, acetylation is abolished, resulting in PGK1's inability to bind and subsequently phosphorylate BECN1 at Ser30. The authors further explored the effect of this acetylation on autophagy. Compared to the wild type, the Lys388Arg variant exhibits decreased PtdIns3P production, reduced SQSTM1/p62 degradation, and lower LC3B-II levels. In contrast, when Lys388 is mutated to a Glu to mimic acetylation, enhanced BECN1 binding is observed. Moreover, the authors probed NAA10 and PGK1 binding and showed that even though these 2 proteins can readily interact with each other during glutamine-rich conditions, as exhibited by several complimentary immunoprecipitation experiments, their interaction, upon glutamine starvation, is enhanced. To further substantiate their claim that NAA10 is indeed the acetyltransferase that activates PGK1, the authors performed an in vitro acetylation experiment using recombinantly purified proteins. This result was verified in vivo, as PGK1 is not acetylated when NAA10 is knocked down using RNA interference. NAA10 also proves to be crucial to glutamine starvation-induced autophagy in U87 cells as its depletion or replacement with a transferase-deficient variant results in reduced SQSTM1 degradation. Overall, these results indicate that PGK1 is indeed an NAA10 substrate and this acetylation reaction is responsible for autophagy induction during glutamine deprivation.

In the next part of their analysis the authors probed how NAA10-facilitated acetylation of PGK1 is regulated. It was previously shown that NAA10 is directly phosphorylated by MTOR, and the authors determined that Ser228 is the site relevant to glutamine deprivation-induced autophagy. More importantly, when U87 cells are treated with the MTOR inhibitor Torin1 PGK1 and NAA10 interaction is enhanced, PGK1 Lys388 acetylation is evident, and PGK1-BECN1 binding is observed despite there being ample amounts of glutamine in the medium. These enhanced molecular interactions result in high levels of LC3B-I to LC3B-II conversion, suggesting that overall autophagic flux is improved upon Torin1 treatment. Together, these results paint a clear picture that MTOR inhibition brought about by glutamine deprivation results in the dephosphorylation of NAA10, which leads to the acetylation of

CONTACT Daniel J. Klionsky klionsky@umich.edu University of Michigan; Life Sciences Institute; Rm. 6036; 210 Washtenaw Ave; Ann Arbor, Michigan 48109–2216 USA.

PGK1 and its subsequent binding to BECN1, and together these events induce autophagy.

The authors then investigated how PGK1-dependent phosphorylation of BECN1 affects the function of the PtdIns3K complex. To this end, the activity of PIK3C3/VPS34 was examined. Compared to the wild type, U87 cells bearing the phospho-deficient BECN1^{S30A} mutant, exhibit lower production of PtdIns3P upon glutamine starvation. This result is due to lower binding of PIK3C3 to its substrate, PtdIns, and not due to an unstable PIK3C3-ATG14-BECN1 complex. Protease digestion patterns also revealed a probable conformational change in the PtdIns3K complex upon BECN1 Ser30 phosphorylation mediated by PGK1. This conformational change provides a likely explanation for the increase in PIK3C3's ability to bind PtdIns, which results in an upsurge in PtdIns3P synthesis.

The authors then asked whether this regulatory pathway is specific to glutamine deprivation by testing other forms of stressors including glucose starvation and hypoxia. Interestingly, depletion of glucose does not induce the PGK1-dependent phosphorylation of BECN1 whereas hypoxia does. In a similar fashion to glutamine deprivation, U87 cells cultured in hypoxic conditions also exhibit PtdIns3P puncta formation.

To test the significance of their findings, the authors looked at how PGK1-mediated autophagy induction affects cancer cell growth. First, U87 cell proliferation is reduced when hypoxia-induced autophagy is inhibited by PGK1 or BECN1 depletion. Moreover, this effect is reversed when the respective wild-type proteins are added back to the cells. Significantly, when either an acetylation-deficient variant of PGK1 or a phospho-deficient BECN1 at Ser30 is reintroduced into the respective depletion strains cell proliferation is inhibited similar to the original depletion strains. Second, tumorigenesis was studied in mice cranially injected with U87 cells. The results show that pronounced tumor growth is seen in mice treated with cells recombinantly expressing wild-type PGK1 or BECN1, whereas tumor growth is stalled in mice implanted with PGK1- or BECN1-depleted cells. Similarly, tumor growth rate is considerably less in mice receiving U87 cells containing either PGK1^{K388R} or BECN1^{S30A}. Last, the authors looked at dozens of human glioblastoma samples to determine whether or not there is a correlation between PGK1 acetylation and/or BECN1 S30 phosphorylation with regard to cancer prognosis and patient survivability. Using specific antibodies it was determined that higher levels of both PGK1 Lys388 acetylation and BECN1 Ser30 phosphorylation strongly correlate with decreased survivability in patients. However, when acetylation and phosphorylation levels are low, average survival duration more than doubles. Taken together, these results strongly indicate that under hypoxic conditions the NAA10-mediated acetylation of PGK1 and the PGK1-dependent phosphorylation of BECN1 induce autophagy and trigger brain tumor development, proliferation and aggressiveness.

In conclusion, the work presented by Qian et al. provides a thorough mechanistic look into how PGK1 can act as a protein kinase to regulate autophagy, a novel role distinct from its canonical glycolytic function, which has significant implications in anticancer therapy. Although autophagy can suppress cancer formation,[4] it can also facilitate oncogenesis and aid cancer cell survival in response to stress in a particular microenvironment.[5] One main drawback of inactivating autophagy as a cure for cancer is that normal, healthy cells still require autophagic function. The work discussed here could present a way to circumnavigate this dilemma by allowing autophagy to be specifically inhibited only in cancerous tissues, sparing healthy ones from detrimental consequences. Thus, the NAA10-dependent PGK1-mediated phosphorylation of BECN1 is an attractive regulatory pathway to pursue to cause autophagy inactivation as a form of cancer therapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by NIH grant GM053396 to DJK.

ORCID

Daniel J. Klionsky http://orcid.org/0000-0002-7828-8118

References

[1] Souba WW. Glutamine and cancer. Ann Surg 1993; 218:715-28; PMID:8257221; https://doi.org/10.1097/00000658-199312000-00004

[2] Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer 2016; 16:619-34; PMID:27492215; https://doi.org/10.1038/nrc.2016.114 10.1038/nrc.2016.71 10.1038/nrc.2016.131

[3] Qian X, Li X, Cai Q, Zhang C, Yu Q, Jiang Y, Lee JH, Hawke D, Wang Y, Xia Y, et al. Phosphoglycerate kinase 1 phosphorylates beclin1 to induce autophagy. Mol Cell 2017; 65:917-31.e6; PMID:28238651; https://doi.org/10.1016/j.molcel.2017.01.027

[4] Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, Bray K, Reddy A, Bhanot G, Gelinas C, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell 2009; 137:1062-75; PMID:19524509; https://doi.org/10.1016/j.cell.2009.03.048

[5] Altman BJ, Jacobs SR, Mason EF, Michalek RD, MacIntyre AN, Coloff JL, Ilkayeva O, Jia W, He YW, Rathmell JC. Autophagy is essential to suppress cell stress and to allow BCR-Abl-mediated leukemogenesis. Oncogene 2010; 30:1855-67; PMID:21151168; https://doi.org/10.1038/onc.2010.561