compuscript

Acta Materia Medica

COMMENTARY

The 'New (Nu)-clear' evidence of the tumor-driving role of PI3K

Franklin Mayca Pozo[a], **Tony Hunter**[b], **Youwei Zhang**[a,*]

[a]Department of Pharmacology, Case Comprehensive Cancer Center, School of Medicine, Case Western Reserve University, 2123 Adelbert Road, Wood Building W343A, Cleveland, OH 44106, USA
[b]Molecular & Cell Biology Laboratory, The Salk Institute, La Jolla, CA 92037, USA

***Correspondence:** yxz169@case.edu (Y. Zhang)
Tel.: +1-216-368-7588; **Fax:** +1-216-368-1300

Received: 07 May 2022; Revised: 10 May 2022; Accepted: 10 May 2022

Published online: 16 May 2022

DOI: 10.15212/AMM-2022-0013

ABSTRACT

The classical phosphatidylinositol 3-kinases (PI3Ks) are heterodimers of p110 and p85. *PIK3CA*, the gene encoding the catalytic p110α subunit, is one of the most frequently mutated oncogenes in human cancers. Mutations have been observed to occur in hotspots in the helical domain or kinase domain. Tumors with these two types of *PIK3CA* mutations show overlapping yet distinct phenotypes; however, the underlying mechanisms driving these phenotypes remain unclear. In a recent publication [1], Hao et al. have revealed exciting findings indicating that the PI3K p85β regulatory subunit promotes cancer progression driven by *PIK3CA* helical-domain mutations. The authors found that p85β disassociates from the PI3K complex and translocates into the nucleus only in cancer cells bearing *PIK3CA* helical-domain mutations. Disrupting the nuclear localization of p85β suppresses tumor growth of cancer cells with *PIK3CA* helical-domain mutation in mice. Mechanistically, the authors have elegantly shown that nuclear p85β recruits the deubiquitinase USP7, which stabilizes the EZH1/2 histone methyltransferases and consequently enhances H3K27 trimethylation and the transcription of genes. Combining an EZH inhibitor with a PI3K inhibitor specifically resulted in regression of mouse xenograft tumors with *PIK3CA* helical-domain mutations. These findings illustrate a previously uncharacterized function of p85β in tumor development and suggest an effective approach to targeting tumors with *PIK3CA* helical-domain mutations.

Keywords: PI3K, p110, p85, cancer mutation, nuclear translocation, colorectal cancer

The phosphatidylinositol 3-kinases (PI3Ks) are a group of highly conserved lipid kinases with important roles in determining cellular fate. Although they are associated with the protein kinases that phosphorylate proteins, PI3Ks phosphorylate lipids by adding a phosphate group, thereby converting phosphatidylinositol-4, 5-triphosphate (PIP2) to phosphatidylinositol-3, 4,5-triphosphate (PIP3), the second messenger that recruits AKT to the plasma membrane through its interaction with the pleckstrin-homology domain of AKT [2]. Subsequently, downstream pathways, including the mechanistic target of rapamycin 1 (mTORC1) pathway, are activated. PI3K can be activated by receptor tyrosine kinases (RTKs), cytokine/chemokine receptors, G-protein coupled receptors and B/T-cell receptors. Therefore, the PI3K pathway regulates cell growth, nutrition and metabolism, cytoskeletal remodeling, cell proliferation and even cell death [3], thereby controlling normal cell physiology and cellular transformation.

The PI3K family is divided into three classes (classes 1–3) according to structural differences. Class 1 is the most widely studied and is further subcategorized into 1A and 1B subtypes, which transduce RTK and G-protein-coupled-receptor signaling, respectively. Type 1A PI3Ks are heterodimers consisting of a catalytic subunit (p110) and a regulatory subunit (p85 or p55). Four distinct genes encode PI3K catalytic subunits, p110α/β/γ/δ, whereas the regulatory subunit is encoded by three genes that are translated into p85α, p85β, p55γ and splice variants. In basal state or in the absence of stimuli, the p85 subunit binds the p110 subunit and inhibits its catalytic activity [4]. After stimulation, such as by growth factors, the p110/p85 complex is recruited to the plasma membrane, where p85, via its SH2 domains, binds tyrosine-phosphorylated RTKs or adaptor proteins, such as

insulin receptor substrate 1 (IRS1) [5], thus leading to the displacement of p85 from the PI3K complex and subsequent p110 activation. Hence, the expression level of p85 inside cells is critical in regulating the activation of p110. In parallel, cells have evolved a checkpoint system to turn off or limit PI3K-AKT activation, in which several lipid phosphatases, particularly phosphatase and tensin homolog (PTEN), convert PIP3 back to PIP2 by removing its 3′-phosphate [2].

The link between the PI3K pathway and cancer can be traced back to the original discovery of the first PI3K activity associated with oncogene-induced transformation, for instance, by Src and polyomavirus middle T antigen [6]. *PIK3CA*, which encodes the p110α catalytic subunit of PI3K, is among the most frequently mutated oncogenes in human cancers [7]. The negative regulator, the phosphatase PTEN, is also frequently mutationally inactivated in tumors; consequently, the normal kinase/phosphatase balance is disrupted, thus resulting in constitutive elevation of PIP_3 and activation of the PI3K-AKT pathway driving transformation and tumor progression [8]. While the p85α subunit is considered a tumor suppressor, the p85β subunit actually promotes tumor development [9]. In various cancers, *PIK3R1*, the gene encoding p85α, contains mutations that disrupt its binding with p110. In contrast, *PIK3R2*, the gene encoding p85β, is overexpressed in tumors [9]. Furthermore, p85 functions in a p110-independent manner, largely through acting as an adaptor protein that facilitates processes including signal transduction regulating the insulin response, senescence, unfolded-protein stress, cytoskeletal rearrangement and endocytic trafficking [10]. However, understanding of exactly how these PI3K isoforms regulate tumor progression remains incomplete.

The authors have previously reported that p110α helical-domain hotspot mutations lead to direct association of the protein with IRS1, independently of p85, thus resulting in recruitment of the mutant p110α to the plasma membrane, conversion of PIP_2 to PIP_3 and promotion of tumor growth [11]. However, tumors with *PIK3CA* helical-domain mutations rather than hotspot mutations in the catalytic domain are believed to be less responsive to inhibitors targeting PI3K, AKT or mTOR [12]. These results have also suggested the existence of yet-unidentified mechanisms driving the distinct phenotypes of the p110 helical-domain mutants. In the current study, Hao et al. have reported an unexpected, intriguing observation regarding the role of the p85β, but not the p85α subunit, in specifically promoting tumor development in cancer cells with p110 helical-domain mutations, but not wild-type or kinase-domain-mutant p110. They first demonstrated a diminished interaction between the p85β, but not the p85α subunit, and p110 with helical-domain mutations, but not wild-type or kinase-domain-mutant p110. By aligning the amino acid sequences of p85β and p85α, the authors found that the N-terminal regions of the two isoforms are less highly conserved than the C-terminal regions containing the two SH2 domains. By swapping the N-terminal regions, which include the SH3 and GAP domains, between p85β and p85α, they confirmed that this region of p85β is indeed responsible for the lower affinity of p85β toward p110, thus leading to higher levels of the free form of p85β.

To determine the importance of p85β in cancer, the authors analyzed TCGA data in colon, bladder, endometrial and breast cancers, and found that high expression of *PIK3R2* (encoding p85β) correlates with poorer prognosis only in cancers bearing p110 helical-domain mutations, thereby indicating a tumor-promoting role of p85β. Consistently, they observed that knockdown of p85β specifically inhibits the growth of tumors derived from colon cancer cells with p110 helical-domain mutations, yet has no effect on tumors from cancer cells with wild-type or kinase-domain-mutant p110.

This result prompts an important question regarding how p85β promotes the growth of tumors bearing p110 helical-domain mutations. The authors ruled out the involvement of canonical PI3K downstream factors such as AKT and mTOR in this process, thus suggesting a previously unknown mechanism. During their analysis, the authors observed an increase in the nuclear signal of p85β in cancer cells expressing the p110 helical-domain mutations, thereby suggesting that free p85β can translocate into the nucleus. They indeed found a non-canonical nuclear localization signal (NLS, amino acids 474–484 with a sequence of LQMKRTAIEAF) in p85β that mediates its nuclear translocation.

To understand how nuclear p85β drives tumor progression, the authors conducted RNA sequencing in isogenic cell lines expressing wild-type p85β or an NLS-disrupted mutant ($K^{477}R^{478}$ to $A^{477}A^{478}$). The results revealed that many tumor-suppressing genes, including *ATM*, *BRCA1/2* and *APC*, were upregulated in p85β NLS-mutant cells, indicating that wild-type p85β in the nucleus downregulates tumor-suppressor genes, thus potentially contributing to its tumor-promoting role. Further analysis indicated that p85β specifically upregulated the level of trimethylated H3K27, but not that of H3K9, H3K36 or H3K76, thus resulting in global gene transcriptional regulation. They then found that p85β increased the protein levels of the EZH1/2 methyltransferases, but not the other subunits of the PRC2 repressor complex. Although p85β acts a global transcriptional regulator, its effect on EZH1/2 levels occurred not through transcriptional upregulation but through protein-stability regulation. Again, these changes were observed only in cancer cells with p110 helical-domain mutations. The authors then demonstrated that p85β stabilized EZH1/2 through recruiting the deubiquitinase USP7 (also called HAUSP), which removed ubiquitin from EZH1/2 and led to the stabilization of these two histone methyltransferases.

The authors further tested whether combining an EZH1/2 inhibitor with a PI3K inhibitor might specifically

Acta
Materia
Medica

target colon cancers with p110 helical-domain mutations. As expected, the combination treatment suppressed the xenograft tumor growth of human colon cancer cell lines bearing helical-domain mutations of p110 more strongly than that of cell lines with kinase-domain-mutant or wild-type p110, as confirmed in colorectal PDX tumors with p110 helical-domain mutation.

In conclusion, Hao et al. [1] have presented compelling and exciting findings regarding a novel function of p85β in the nucleus, thus filling a knowledge gap in the understanding of how the PI3K pathway drives cancer progression specifically in the genetic background of p110 helical-domain mutations (**Figure 1**). These findings also suggest an effective approach to treating tumors with p110α helical-domain mutations by combining an EZH1/2 inhibitor with a PI3K inhibitor.

Although these findings offer innovative insights into PI3K signaling and its effects on tumor progression, they also prompt several questions worthy of further investigation. First, why does p85β demonstrate these effects only in cancer cells with helical-domain-mutant p110? For nuclear translocation, is the binding affinity between p85β and the helical-domain-mutant p110 greatly diminished? X-ray crystallography and cryo-EM studies have shown that the N-terminal ABD domain of p110α binds the internal nSH2 and iSH2 domains of p85α [13, 14]. However, the ABD domain in p110 and the SH3 domain in p85, which are considered to interact with each other, tend to be flexible and therefore have failed to produce high resolution structures. Yet, the finding that the addition of a specific p110 inhibitor stabilizes the structures of these domains suggests that the N-terminal SH3 domain of p85α, which was also responsible for the observed differences in p110 binding in the current study, points toward the catalytic domain but not the helical domain of p110α [13]. Hence, whether the amino terminus of p85β binds the helical domain of p110 remains unknown. Future structural studies will be important to answer these questions. The identified NLS (LQMKRTAIEAF) in p85β meets only the minimum

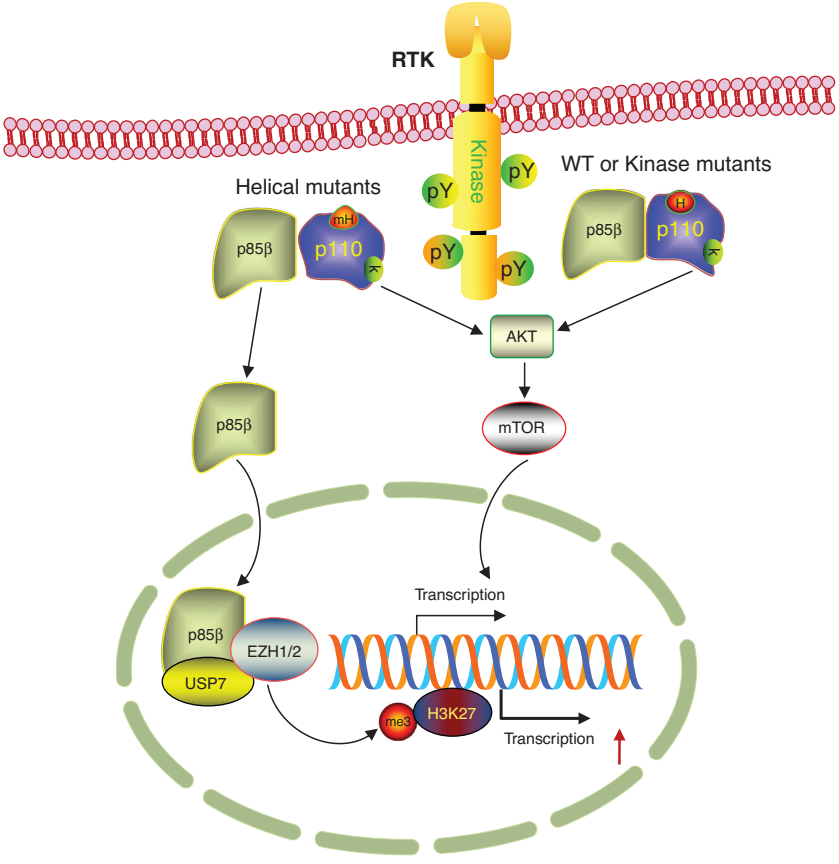

Figure 1 | Model of the novel nuclear function of p85β in promoting tumor growth.
Receptor tyrosine kinase (RTK) activates the p110/p85 complex, which stimulates cell growth through two routes. p85β binds wild-type (WT) or kinase-domain-mutant p110, forming the canonical PI3K complex, which then activates downstream AKT and mTOR, and promotes gene expression and cell growth. However, when p110 has mutations in the helical domain (mH), p85β disassociates from p110 and translocates into the nucleus though its nuclear localization signal. In the nucleus, p85β recruits USP7 to EZH1/2; consequently, the stabilization of EZH1/2 increases H3K27 trimethylation and global transcriptional gene expression.

requirement (*i.e.*, KR) for an NLS; hence, whether post-translational modifications such as phosphorylation on T^{479}, methylation on R^{478} or acetylation on K^{477} might be involved in promoting p85β nuclear shuttling is an interesting question worthy of investigation. In addition, why p85β does not interact with USP7 or EZH1/2 in the nucleus in non-helical p110-mutant cancer cells is unknown, given that the p110 subunit should be irrelevant to the function of p85β in the nucleus. Additionally, USP7 is a widely studied de-ubiquitin enzyme that regulates tumors through stabilizing many important proteins that drive/promote or suppress tumor progression, such as CHK1, MDM2, c-MYC, P53 and PTEN, probably in a context dependent manner [15]. This study adds another protein, EZH1/2, to the long list of USP7 substrates that promote colon tumors. Hence, combining a USP7 inhibitor with a PI3K inhibitor may produce a similar anti-tumor effect to that seen after treatment with the combination of EZH1/2 inhibitor and PI3K inhibitor reported here. Finally, if the nuclear signaling is specific to the helical-domain mutations of p110, an inhibitor that can specifically target helical-domain mutations without acting as a general p110 kinase inhibitor might produce an even better tumor-reduction effect when combined with EZH1/2 inhibitors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

Youwei Zhang is supported partly by NIH/NCI (R01CA230453) and the Case Comprehensive Cancer Center grant (P30CA043703).

AUTHOR CONTRIBUTIONS

Franklin Mayca Pozo, Tony Hunter and Youwei Zhang wrote the manuscript.

REFERENCES

[1] Hao Y, He B, Wu L, Li Y, Wang C, Wang T, et al.: Nuclear Translocation of p85β Promotes Tumorigenesis of PIK3CA Helical Domain Mutant Cancer. *Nature Communications* 2022, 13:1974.

[2] Cantley LC: The Phosphoinositide 3-kinase Pathway. *Science* 2002, 296:1655-1657.

[3] Ghigo A, Morello F, Perino A, Hirsch E: Phosphoinositide 3-kinases in Health and Disease. *Sub-cellular Biochemistry* 2012, 58:183-213.

[4] Vadas O, Burke JE, Zhang X, Berndt A, Williams RL: Structural Basis for Activation and Inhibition of Class I Phosphoinositide 3-kinases. *Science Signaling* 2011, 4:re2.

[5] Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM: Regulation of the p85/p110 Phosphatidylinositol 3'-kinase: Stabilization and Inhibition of the p110alpha Catalytic Subunit by the p85 Regulatory Subunit. *Molecular and Cellular Biology* 1998, 18:1379-1387.

[6] Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM: Association of Phosphatidylinositol Kinase Activity with Polyoma Middle-T Competent for Transformation. *Nature* 1985, 315:239-242.

[7] Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al.: Mutational Landscape and Significance Across 12 Major Cancer Types. *Nature* 2013, 502:333-339.

[8] Cantley LC, Neel BG: New Insights Into Tumor Suppression: PTEN Suppresses Tumor Formation by Restraining the Phosphoinositide 3-kinase/AKT Pathway. *Proceedings of the National Academy of Sciences of the United States of America* 1999, 96:4240-4245.

[9] Vallejo-Diaz J, Chagoyen M, Olazabal-Morán M, González-García A, Carrera AC: The Opposing Roles of PIK3R1/p85α and PIK3R2/p85β in Cancer. *Trends in Cancer* 2019, 5:233-244.

[10] Fox M, Mott HR, Owen D: Class IA PI3K Regulatory Subunits: p110-independent Roles and Structures. *Biochemical Society Transactions* 2020, 48:1397-1417.

[11] Hao Y, Wang C, Cao B, Hirsch BM, Song J, Markowitz SD, et al.: Gain of Interaction with IRS1 by p110α-helical Domain Mutants is Crucial for their Oncogenic Functions. *Cancer Cell* 2013, 23:583-593.

[12] Janku F, Wheler JJ, Naing A, Falchook GS, Hong DS, Stepanek VM, et al.: PIK3CA Mutation H1047R is Associated with Response to PI3K/AKT/mTOR Signaling Pathway Inhibitors in Early-phase Clinical Trials. *Cancer Research* 2013, 73:276-284.

[13] Liu X, Yang S, Hart JR, Xu Y, Zou X, Zhang H, et al.: Cryo-EM Structures of PI3Kα Reveal Conformational Changes During Inhibition and Activation. *Proceedings of the National Academy of Sciences of the United States of America* 2021, 118:e2109327118.

[14] Miller MS, Schmidt-Kittler O, Bolduc DM, Brower ET, Chaves-Moreira D, Allaire M, et al.: Structural Basis of nSH2 Regulation and Lipid Binding in PI3Kα. *Oncotarget* 2014, 5:5198-5208.

[15] Yeasmin Khusbu F, Chen FZ, Chen HC: Targeting Ubiquitin Specific Protease 7 in Cancer: A Deubiquitinase with Great Prospects. *Cell Biochemistry and Function* 2018, 36:244-254.