EBioMedicine 52 (2020) 102634

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Commentary

Molecular insight into the autoinhibition of a master regulator of lipid signalling in human disease

John E. Burke

Department of Biochemistry and Microbiology, University of Victoria, Victoria V8W 2Y2, British Columbia, Canada

ARTICLE INFO

Article History:
Received 2 January 2020
Revised 4 January 2020
Accepted 7 January 2020
Available online xxx

The synthesis and degradation of lipid phosphoinositides are fundamental in mediating signal transduction. Some of the most well studied phosphoinositide metabolising enzymes are the phospholipase C (PLC) family, which can hydrolyse the lipid phosphatidylinositol 4,5 bisphosphate (PIP_2) into the signalling molecules inositol trisphosphate (IP_3) and diacylglycerol [1]. There are multiple isoforms of the PLC family that are variably expressed in different cells/tissues, with each able to be activated downstream of a unique subset of cell surface receptors, including G-protein coupled receptors and tyrosine phosphorylated receptors. Recent years have revealed the myriad roles of a specific class of PLCs (PLCγ, encoded by the genes *PLCG1* and *PLCG2*) in various human pathologies, including cancer [2,3], neurodegeneration [4], and immune disorders [5].

Disease linked mutations or deletions in PLCγ frequently lead to hyperactivation of lipase activity. However, the mechanism by which these mutations mediate activation is unknown. Hindering the ability to understand the molecular mechanism of activation has been a lack of structural information for the regulatory mechanisms that lead to PLCγ auto-inhibition, as well as how it can be activated downstream of tyrosine phosphorylated receptors, including the fibroblast growth factor receptor (FGFR) kinase.

In the recent issue of *EBioMedicine*, Liu et al. [6] has used an integrative structural biology approach to provide the first molecular insight into the regulation of PLCγ, and how mutations or deletions lead to activation. Using a synergy of cryo-electron microscopy, chemical crosslinking, and hydrogen deuterium exchange mass spectrometry, the authors were able to provide insight into how the regulatory domains of PLCγ (composed of C2, PH, and two SH2 domains [referred to as the nSH2 and cSH2]) [7] are able to inhibit the catalytic module of PLCγ (composed of a PH, EF hand, and catalytic TIM barrel domain). They find that the regulatory domains form extended inhibitory contacts with the catalytic module, that putatively prevent binding to lipid substrate present on cellular membranes.

Many disease-linked mutations map to this surface, and likely lead to disruption of the catalytic/regulatory auto-inhibitory interface. In addition, they were able to map the interface of the n-terminal SH2 domain with the soluble kinase domain of phosphorylated FGFR, revealing the molecular interface between PLCγ and its activator. Together, this work provides a breakthrough in our molecular understanding of how disease linked mutations in patients leads to disruption of PLCγ autoinhibitory mechanisms that prevent activation in the absence of upstream stimuli.

From a clinical perspective, this structure provides information on putative mutational hotspots at the interface of the catalytic and regulatory domains of PLCγ that might be expected to lead to activation and pathological levels of PLCγ activity. Due to the large number of PLCγ mutations that have been revealed so far, it is likely that there are still more disease-linked activating mutations in PLCγ to be discovered. For clinicians who have discovered novel mutations in PLCγ, this structure will provide a road map for structure-based hypotheses on the molecular mechanism of these mutations.

From a basic science perspective, this work shows how the integrative structural approach allowed for unique molecular insight even for structures at medium resolution (<6 Å). The application of HDX-MS and XL-MS allowed for the validation of the medium resolution EM model, and also provides insight into the protein dynamics of the complex. This led to the unambiguous definition of both the auto-inhibitory and FGFR interface. There has been controversy in the mechanism by which PLCγ is activated downstream of FGFR [8,9], and this structure provides some clarity into the first steps of PLCγ activation.

While this data does provide an exciting first glimpse into the regulatory mechanisms that control how PLCγ is inhibited by its regulatory domains, and the first step of activation through engagement of the PLCγ nSH2 domain with the phosphorylated FGFR, there are still many important questions that remain to be answered.

First, the medium resolution nature of this structure does not provide atomic details of the interactions that are occurring between the catalytic and regulatory modules. To fully understand how the disease linked mutations at this interface can mediate activation will require a high-resolution structure that reveals atomic level details. An excellent companion piece to this study is a recent report in *Elife* [10], that used X-ray crystallography to capture a higher resolution snapshot (2.5 Å) of an engineered variant of PLCγ that confirms and

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2019.102607.
E-mail address: jeburke@uvic.ca

https://doi.org/10.1016/j.ebiom.2020.102634

provides additional detail on the autoinhibitory interface between the regulatory and catalytic modules.

Second, while this structure captures the first step in activation downstream of FGFR, there are multiple additional steps that are required before the catalytic domain can engage with lipid substrate on the membrane. This includes phosphorylation of PLCγ leading to engagement of the cSH2 domain, disruption of the auto-inhibitory interface, and interaction of the catalytic domain with membranes.

Third, the mutations and deletions biochemically characterised in this study showed different capabilities to activate the lipase activity PLCγ. This leads to an important question of how this will relate to the clinical phenotype seen for different mutations/deletions. Continued cellular and preclinical work will be required to further study this effect.

Overall, this research provides an exciting advance in our fundamental understanding of the regulation of the PLC pathway, and provides a novel framework for future study into disease-linked mutations in both *PLCG1* and *PLCG2*.

Declaration of Competing Interest

The author declares no conflicts of interest.

Acknowledgements

Work in the Burke laboratory is supported by research grants from CIHR (CRN-142393), NSERC (2014-05218), and the Cancer Research Society (CRS 24368) along with salary awards from CIHR (New investigator award), and the Michael Smith Foundation for Health Research (Scholar 17686).

References

[1] Kadamur G, Ross EM. Mammalian phospholipase C. Annu Rev Physiol 2013;75(1):127–54.
[2] Woyach JA, Furman RR, Liu T-M, Ozer HG, Zapatka M, Ruppert AS, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med 2014;370(24):2286–94.
[3] Behjati S, Tarpey PS, Sheldon H, Martincorena I, Van Loo P, Gundem G, et al. Recurrent PTPRB and PLCG1 mutations in angiosarcoma. Nat Genet 2014;46(4):376–9.
[4] Sims R, van der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, et al. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. Nat Genet 2017;49(9):1373–84.
[5] Ombrello MJ, Remmers EF, Sun G, Freeman AF, Datta S, Torabi-Parizi P, et al. Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. N Engl J Med 2012;366(4):330–8.
[6] Liu Y, Bunney TD, Khosa S, Mace K, Beckenbauer K, Askwith T, et al. Structural insights and activating mutations in diverse pathologies define mechanisms of deregulation for phospholipase C gamma enzymes. EBioMedicine 2020. [In this issue.].
[7] Bunney TD, Esposito D, Mas-Droux C, Lamber E, Baxendale RW, Martins M, et al. Structural and functional integration of the PLCγ interaction domains critical for regulatory mechanisms and signaling deregulation. Structure 2012;20(12):2062–75.
[8] Huang Z, Marsiglia WM, Basu Roy U, Rahimi N, Ilghari D, Wang H, et al. Two FGF receptor kinase molecules act in concert to recruit and transphosphorylate phospholipase Cγ. Mol Cell 2016;61(1):98–110.
[9] Bae JH, Lew ED, Yuzawa S, TomE F, Lax I, Schlessinger J. The selectivity of receptor tyrosine kinase signaling is controlled by a secondary SH2 domain binding site. Cell 2009;138(3):514–24.
[10] Hajicek N, Keith NC, Siraliev-Perez E, Temple BRS, Huang W, Zhang Q, et al. Structural basis for the activation of PLC-γ isozymes by phosphorylation and cancer-associated mutations. Elife 2019;8:e51700.