Cyclophilin D palmitoylation and permeability transition: a new twist in the tale of myocardial ischaemia-reperfusion injury

Alice Main[+], Olivia Robertson-Gray[+], William Fuller[*]

Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

+ These authors contributed equally to this work

* to whom correspondence should be addressed. Dr. William Fuller, Sir James Black Building, University of Glasgow, G12 8QQ, UK. Telephone: (+44) 141 330 3228; Fax: (+44) 141 330 3228; E-mail will.fuller@glasgow.ac.uk

WF ORCID: 0000-0002-5883-4433

Haworth and Hunter first described the characteristics of permeability transition in bovine mitochondria in the late 1970s, establishing it to be a consequence of the reversible opening of a pore within the inner mitochondrial membrane. The role of this pore in myocardial ischaemia/reperfusion (I/R) injury has been the subject of considerable interest in the intervening years. During myocardial ischaemia, this pore, now known as the mitochondrial permeability transition pore (PTP) remains closed because metabolic acidosis prevents its opening. Upon reperfusion, PTP opening in response to biochemical changes (restoration of pH_i, generation of reactive oxygen species and mitochondrial calcium (Ca^{2+}) overload), causes significant cellular injury. In part, this injury happens because PTP opening has deleterious effects on the mitochondria themselves: swelling and rupture, membrane depolarisation and uncoupling of oxidative phosphorylation[1]. In part, substances released from the mitochondria through PTP initiate cell death. Together, these events contribute to the overall injury sustained by the heart during myocardial I/R injury. Since the PTP is a key mediator of myocardial I/R injury, much attention is focused on understanding its intrinsic regulatory pathways and how these may be targeted for therapeutic gain. The peptidylprolyl isomerase, cyclophilin D (CypD) is widely recognised as a regulator of the PTP. I/R-induced cell death *in vivo* is reduced in CypD knockout mice, confirming that CypD and PTP contribute to the tissue damage mediated by such injury[2].

Importantly, the CypD inhibitor cyclosporin A (CsA) attenuates infarct size in small and large animal models of I/R. Promisingly, CsA also protected human atrial trabeculae subjected to simulated I/R injury[1] and in a small pilot trial, its intravenous administration attenuated infarct size by approximately 40%[3] in patients with ST-segment elevation myocardial infarction. However, data generated from the

much larger CIRCUS trial proved less promising[4], to the consternation of many. Since CsA affects the mitochondria of all organs it may produce deleterious off-target effects. Gaining a greater insight into CypD regulation and function will therefore allow for the development of alternative and more specific therapies targeted at the cardiac PTP.

Although CypD knock out mice have significantly improved our understanding of its role in cardiac (patho)physiology, more subtle knock in animals are important to identify the key residues involved in its function. In the present issue of *Cardiovascular Research*, Amanakis *et al.* have used this knock in approach to evaluate the importance of CypD cysteine 202 in I/R injury. This residue had already been established to be oxidised during I/R injury, reversibly nitrosylated during ischaemic preconditioning[5] and can also form an intramolecular disulphide[6]. Amanakis and colleagues now demonstrate novel, dynamic S-palmitoylation of this residue. Their study reports substantial protection from I/R injury in C202S hearts compared to wild type, alongside a significantly enhanced resistance of C202S mitochondria to calcium overload-induced permeability transition. Interestingly C202S hearts confer no additional protection following I/R injury from CsA, suggesting PTP assembly requires CypD C202. CypD S-palmitoylation is reduced significantly during ischaemia (in intact hearts) and following calcium overload (in isolated mitochondria). Evidently the signalling poise of this single amino acid has the potential to swing life and death decisions in the mitochondrial matrix.

To further elucidate the role of CypD palmitoylation in PTP assembly, the mechanisms by which the palmitoylation and de-palmitoylation occur warrant further study. S-Palmitoylation is catalysed by Asp-His-His-Cys (DHHC)-palmitoyl acyltransferase enzymes (zDHHC-PATs) which are integral membrane proteins localised throughout the secretory pathway. The activity of this enzyme family has been implicated in I/R injury by others. The cell surface localised zDHHC5 regulates a number of important cardiac substrates[7], and is a key component of the process of massive endocytosis (MEND) following anoxia/reperfusion. The MEND pathway is triggered by PTP opening and acyl-CoA release from mitochondria, which leads to substantial remodelling of the cell surface membrane by zDHHC5[8]. The sensitivity of MEND to PTP suggests CypD palmitoylation may itself regulate this pathway. As such, understanding which zDHHC-PAT palmitoylates CypD will be important to determine the impact of palmitoylation on CypD function and PTP assembly. Of the 23 zDHHC-PATs identified in humans, most are localised in the Golgi apparatus, endoplasmic reticulum and on the cell surface. However, proteomic studies have revealed several mitochondrial proteins are palmitoylated,[9] so there is indirect evidence to suggest that some zDHHC-PATs localise to mitochondria. In particular, zDHHC13 was identified in a quantitative analysis of the liver S-palmitoylome, where its absence significantly

impacted expression of proteins implicated in mitochondrial function[10], and zDHHC8 has been suggested to play an important role in regulating mitochondria in the brain[11]. Although these DHHC-PATs have not been studied in the context of cardiac mitochondria, they could provide an insight into the mechanism of CypD palmitoylation. Conversely, it is noteworthy that as a mitochondrial protein, CypD is likely exposed to high local concentrations of acyl-CoA, and it is conceivable that this could cause it to be auto-palmitoylated, as has been demonstrated with other soluble proteins[12].

In the work of Amanakis and colleagues, exposing cardiac mitochondria to high calcium triggers CypD de-palmitoylation, potentially increasing availability of C202 for oxidation and subsequent PTP-induced injury at reperfusion. Calcium regulated de-palmitoylation is a previously unreported phenomenon that may have important consequences for palmitoylated cardiac substrates – particularly those involved in calcium handling[13]. The mechanism of CypD de-palmitoylation is therefore of greatest interest. Recent work has revealed that APT1, one of seven protein thioesterases identified to date, localises in mitochondria as well as the cytosol[14]. In addition, ABHD10 (recently reported to act as a thioesterase), is exclusively localised in mitochondria where it de-palmitoylates the mitochondrial homeostasis regulator peroxiredoxin-5 (PRXD5)[15]. Both enzymes warrant investigation as the source of CypD de-palmitoylation during ischaemia.

While the significance of C202 in CypD function has been clearly demonstrated in this study, an important outstanding question concerns CypD palmitoylation stoichiometry, to understand what population of the protein is being regulated by palmitoylation. It remains a significant challenge to the field of cysteine post-translational modifications that chemically distinct post-translational modifications can compete for the same residue, complicating our understanding of the phenotypes of knock in models. Typically, it is the same solvent-exposed cysteines that form disulphides and are subjected to S-nitrosylation, S-acylation, oxidation, S-glutathiolation, etc. Since protection of C202 from oxidation may generate significant therapeutic benefit, understanding the cross-talk between modifications will be of vital importance.

Acknowledgements

We acknowledge financial support from the British Heart Foundation

Declaration of Interests

The authors declare no competing interests.

Figure Legends

Figure 1: CypD C202 Modification in Cardiac Mitochondria. Amanakis et al. demonstrate that under basal conditions, the mitochondrial permeability transition pore (PTP) regulator cyclophillin-D (CypD) is reported to be both S-nitrosylated and S-palmitoylated at position cysteine 202 (C202). Under conditions of ischaemia, CypD S-palmitoylation and S-nitrosylation are reduced as C202 undergoes oxidation. This leads to greater association of CypD with the F1-ATPase subunit of the PTP and enhances pore opening and calcium efflux at reperfusion, thus leading to greater myocardial damage and increased infarct size. Mutation of C202 to a serine (S) results in no possible modification by S-nitrosylation, S-palmitoylation but also S-oxidation and transgenic mice have significantly reduced infarct size and enhanced functional recovery as a result.

References

1. Hausenloy, D. J. & Yellon, D. M. Myocardial ischemia-reperfusion injury: A neglected therapeutic target. *Journal of Clinical Investigation* vol. 123 92–100 (2013).

2. Baines, C. P., Kaiser, R. A., Purcell, N. H., Blair, N. S., Osinska, H., Hambleton, M. A., Brunskill, E. W., Sayen, M. R., Gottlieb, R. A., Dorn, G. W., Bobbins, J. & Molkentin, J. D. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**, 658–662 (2005).

3. Piot, C., Croisille, P., Staat, P., Thibault, H., Rioufol, G., Mewton, N., Elbelghiti, R., Cung, T. T., Bonnefoy, E., Angoulvant, D., Macia, C., Raczka, F., Sportouch, C., Gahide, G., Finet, G., André-Fouët, X., Revel, D., Kirkorian, G., Monassier, J.-P., Derumeaux, G. & Ovize, M. Effect of Cyclosporine on Reperfusion Injury in Acute Myocardial Infarction. *N. Engl. J. Med.* **359**, 473–481 (2008).

4. Zografos, T. A. & Katritsis, D. G. Cyclosporine before PCI in Acute Myocardial Infarction. *N. Engl. J. Med.* **374**, 88–90 (2016).

5. Kohr, M. J., Sun, J., Aponte, A., Wang, G., Gucek, M., Murphy, E. & Steenbergen, C. Simultaneous measurement of protein oxidation and S-nitrosylation during preconditioning and ischemia/reperfusion injury with resin-assisted capture. *Circ. Res.* **108**, 418–426 (2011).

6. Linard, D., Kandlbinder, A., Degand, H., Morsomme, P., Dietz, K. J. & Knoops, B. Redox

characterization of human cyclophilin D: Identification of a new mammalian mitochondrial redox sensor? *Arch. Biochem. Biophys.* **491**, 39–45 (2009).

7. Howie, J., Reilly, L., Fraser, N. J., Vlachaki Walker, J. M., Wypijewski, K. J., Ashford, M. L. J., Calaghan, S. C., McClafferty, H., Tian, L., Shipston, M. J., Boguslavskyi, A., Shattock, M. J. & Fuller, W. Substrate recognition by the cell surface palmitoyl transferase DHHC5. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 17534–9 (2014).

8. Lin, M.-J., Fine, M., Lu, J.-Y., Hofmann, S. L., Frazier, G. & Hilgemann, D. W. Massive palmitoylation-dependent endocytosis during reoxygenation of anoxic cardiac muscle. *Elife* **2**, e01295 (2013).

9. Kostiuk, M. A., Corvi, M. M., Keller, B. O., Plummer, G., Prescher, J. A., Hangauer, M. J., Bertozzi, C. R., Rajaiah, G., Falck, J. R. & Berthiaume, L. G. Identification of palmitoylated mitochondrial proteins using a bio-orthogonal azido-palmitate analogue. *FASEB J.* **22**, 721–732 (2008).

10. Shen, L. F., Chen, Y. J., Liu, K. M., Haddad, A. N. S., Song, I. W., Roan, H. Y., Chen, L. Y., Yen, J. J. Y., Chen, Y. J., Wu, J. Y. & Chen, Y. T. Role of S-Palmitoylation by ZDHHC13 in Mitochondrial function and Metabolism in Liver. *Sci. Rep.* **7**, (2017).

11. Maynard, T. M., Meechan, D. W., Dudevoir, M. L., Gopalakrishna, D., Peters, A. Z., Heindel, C. C., Sugimoto, T. J., Wu, Y., Lieberman, J. A. & LaMantia, A. S. Mitochondrial localization and function of a subset of 22q11 deletion syndrome candidate genes. *Mol. Cell. Neurosci.* **39**, 439–451 (2008).

12. Duncan, J. A. & Gilman, A. G. Autoacylation of G protein α subunits. *J. Biol. Chem.* **271**, 23594–23600 (1996).

13. Reilly, L., Howie, J., Wypijewski, K., Ashford, M. L. J., Hilgemann, D. W. & Fuller, W. Palmitoylation of the Na/Ca exchanger cytoplasmic loop controls its inactivation and internalization during stress signaling. *FASEB J.* **29**, 4532–4543 (2015).

14. Kathayat, R. S., Cao, Y., Elvira, P. D., Sandoz, P. A., Zaballa, M. E., Springer, M. Z., Drake, L. E., Macleod, K. F., Van Der Goot, F. G. & Dickinson, B. C. Active and dynamic mitochondrial S-depalmitoylation revealed by targeted fluorescent probes. *Nat. Commun.* **9**, 334 (2018).

15. Cao, Y., Qiu, T., Kathayat, R. S., Azizi, S. A., Thorne, A. K., Ahn, D., Fukata, Y., Fukata, M., Rice, P. A. & Dickinson, B. C. ABHD10 is an S-depalmitoylase affecting redox homeostasis through peroxiredoxin-5. *Nat. Chem. Biol.* **15**, 1232–1240 (2019).

Figure

Click here to access/download;Figure(s);Graphical abstract V1 final landscape.tif