King's Research Portal

DOI:

[10.1016/j.yjmcc.2018.07.254](https://doi.org/10.1016/j.yjmcc.2018.07.254)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Bromage, D. I., Santos, C. X., & Shah, A. M. (2018). Developing potential biomarkers through bedside-to-bench translation. *Journal of Molecular and Cellular Cardiology*. <https://doi.org/10.1016/j.yjmcc.2018.07.254>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Developing potential biomarkers through bedside-to-bench translation

D.I. Bromage, C.X. Santos, A.M. Shah

PII: S0022-2828(18)30688-6

DOI: <https://doi.org/10.1016/j.yjmcc.2018.07.254>

Reference: YJMCC 8857

To appear in: *Journal of Molecular and Cellular Cardiology*

Received date: 21 July 2018

Accepted date: 25 July 2018

Please cite this article as: D.I. Bromage, C.X. Santos, A.M. Shah , Developing potential biomarkers through bedside-to-bench translation. Yjmcc (2018), <https://doi.org/10.1016/j.yjmcc.2018.07.254>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Developing potential biomarkers through bedside-to-bench translation**Bromage DI, Santos CX and Shah AM**

King's College London British Heart Foundation Centre of Excellence, London, UK.

Correspondence: Professor Ajay M Shah, School of Cardiovascular Medicine and Sciences, James Black Centre, King's College London BHF Centre of Excellence, 125 Coldharbour Lane, London SE5 9NU, UK. Tel: 44-207848-5189; Fax: 44-207848-5193. Email: ajay.shah@kcl.ac.uk

Key Words: Biomarkers, acute heart failure, gene-modified animal models, oxidase, ER stress

None of the authors declare any conflicts of interest

The vast majority of therapies for heart failure (HF) are based on randomised clinical trials of highly heterogeneous groups of patients. Mortality and morbidity remain substantial even in patients who are optimally treated with such therapies. There is therefore significant interest in identifying biomarkers that might enable earlier or more refined diagnosis, staging, prognostication and personalisation of therapy. The only biomarker that is routinely employed in clinical practice is NT-proBNP but this is useful mainly in informing the likelihood of HF rather than guiding therapy.

Pre-clinical approaches to identify new biomarkers that have wider utility typically focus on specific molecular signalling pathways or biological processes known to be involved in HF (e.g. inflammation), followed by the testing of markers of such processes in patients. However, bench-to-bedside translation of this type has generally been unsuccessful, possibly because most pre-clinical models that are utilised are too far removed from the clinical HF syndrome [1, 2]. An alternative approach is to undertake global screens (e.g. with 'omics approaches) in patients with HF in the hope that markers that are strongly associated with a particular aspect of HF may lead to clinically useful biomarkers. This approach has also, so far, failed to identify useful biomarkers – possibly because the markers chosen for further study are not necessarily causatively linked to disease pathogenesis or pathophysiology. In a recent article published in the journal, Caillard *et al* employ a reverse translation 'bedside-to-bench' approach to address this gap [3]. These investigators previously used plasma proteomic studies to identify Quiescin Q6 sulfhydryl oxidase 1 (Qsox1) as a potential diagnostic biomarker of acute decompensated HF when used in conjunction with BNP [4]. In the current study, they have now studied the potential biological link(s) between Qsox1 and HF in a pre-clinical model, an approach that may strengthen the relevance of the marker and suggest how it might best be used, before returning to additional clinical studies (**Fig 1**).

An important feature of the heart's response to stress is alterations of redox signalling [5]. In HF, redox homeostasis is perturbed due both to increased ROS generation by several sources (e.g. mitochondria, NADPH oxidases, monoamine oxidase) and altered antioxidant enzymes and molecules (e.g. thioredoxin [Trx], peroxiredoxin [Prx]) [5]. For example, circulating Trx1 is increased in patients with HF and correlates with disease severity [6, 7]. Qsox1 is a redox enzyme that contains a Trx domain (Trp-Cys-Gly-His-Cys) and has FAD-dependent sulfhydryl oxidase activity that is thought to be involved in protein folding in the endoplasmic reticulum (ER). Qsox1 may coordinate in this function with other thiol oxido-reductase isomerases such as protein disulfide isomerase, ER oxidase-1 and Prx [8, 9]. Caillard and colleagues [3] developed and characterised a global Qsox1 knockout mouse model in an attempt to determine the role of Qsox1 in HF. They found that Qsox1^{-/-} mice have cardiac dilatation at baseline, with very mild systolic impairment, a reduced SERCA2a content and altered calcium homeostasis. After induction of acute stress with isoproterenol, Qsox1^{-/-} mice developed worse contractile impairment than wild-type controls and this persisted for longer – suggesting that Qsox1 is involved in the acute stress response.

Protein folding in the ER may be compromised under stress conditions ("ER stress") and triggers a compensatory response known as the unfolded protein response (UPR) which increases the expression of ER chaperones and other proteins that restore normal protein homeostasis. If there is a failure to restore normal protein folding, cells will tend to develop oxidative stress and may enter a downward spiral that leads to cell death [9-11]. Caillard *et al.* [3] found that Qsox1^{-/-} mice had evidence of UPR activation at baseline and that after isoproterenol stress, this activation was enhanced and persistent and was accompanied by oxidative stress and evidence of inflammation.

These data would be consistent with the notion that the absence of Qsox1 impairs protein folding especially during acute stress, and that this in turn leads to prolonged detrimental activation of the UPR, thereby contributing to cardiac impairment. One interesting question in this study is the mechanistic inter-relationship between altered sarcoplasmic reticulum calcium levels and ER stress. It is known that deranged intracellular calcium handling provokes ER stress and activates the UPR but also that ER stress impairs calcium homeostasis [12]. Therefore, the primary defect in Qsox1^{-/-} mice could either be in calcium handling due to the decreased Serca2a levels (with secondary ER stress and UPR activation) or could be a direct general effect on protein folding leading to ER stress (with secondary effects on calcium homeostasis). Further work is necessary to dissect this question. The data in the study by Caillard et al [3] would be explained by the actions of Qsox1 in cardiomyocytes but it is conceivable that other cell types might also be involved. Qsox is expressed during development and, in addition to its cellular location, it is present in foetal serum and the extracellular space, where it plays a role in extracellular matrix formation [8].

This study convincingly suggests an adaptive role of increased Qsox1 in the setting of acute adrenergic crisis and explains why Qsox1 may have emerged as a biomarker in acute HF. Whether circulating Qsox1 in the blood has any pathophysiological role is an intriguing question. It would also be of interest to investigate the possible contribution of Qsox1 to ventricular remodelling.

The study by Caillard and colleagues adds to the growing body of evidence for a role of redox mechanisms in HF and highlights Qsox1 as a potential novel target. It also provides a demonstration of the utility of bedside-to-bench studies to illuminate clinically relevant mechanisms, potentially leading both to novel biomarkers and innovative therapies for HF.

Work in the authors' laboratory is supported by the British Heart Foundation.

References

- [1] W.L. Miller, A.S. Jaffe, Biomarkers in heart failure: the importance of inconvenient details, ESC Heart Fail 3(1) (2016) 3-10.
- [2] T. Ahmad, M. Fiuzat, M.J. Pencina, N.L. Geller, F. Zannad, J.G. Cleland, J.V. Snider, S. Blankenberg, K.F. Adams, R.F. Redberg, J.B. Kim, A. Mascette, R.J. Mentz, C.M. O'Connor, G.M. Felker, J.L. Januzzi, Charting a roadmap for heart failure biomarker studies, JACC Heart Fail 2(5) (2014) 477-88.
- [3] A. Caillard, M. Sadoune, A. Cescau, M. Meddour, M. Gandon, E. Polidano, C. Delcayre, K. Da Silva, P. Manivet, A.M. Gomez, A. Cohen-Solal, N. Vodovar, Z. Li, A. Mebazaa, J.L. Samuel, QSOX1, a novel actor of cardiac protection upon acute stress in mice, J Mol Cell Cardiol 119 (2018) 75-86.
- [4] A. Mebazaa, G. Vanpoucke, G. Thomas, K. Verleysen, A. Cohen-Solal, M. Vanderheyden, J. Bartunek, C. Mueller, J.M. Launay, N. Van Landuyt, F. D'Hondt, E. Verschuere, C. Vanhaute, R. Tuytten, L. Vanneste, K. De Cremer, J. Wuyts, H. Davies, P. Moerman, D. Logeart, C. Collet, B. Lortat-Jacob, M. Tavares, W. Laroy, J.L. Januzzi, J.L. Samuel, K. Kas, Unbiased plasma proteomics for novel diagnostic biomarkers in cardiovascular disease: identification of quiescin Q6 as a candidate biomarker of acutely decompensated heart failure, Eur Heart J 33(18) (2012) 2317-24.
- [5] J.R. Burgoyne, H. Mongue-Din, P. Eaton, A.M. Shah, Redox signaling in cardiac physiology and pathology, Circ Res 111(8) (2012) 1091-106.
- [6] C. Kishimoto, K. Shioji, H. Nakamura, Y. Nakayama, J. Yodoi, S. Sasayama, Serum thioredoxin (TRX) levels in patients with heart failure, Jpn Circ J 65(6) (2001) 491-4.
- [7] A. Jekell, A. Hossain, U. Alehagen, U. Dahlstrom, A. Rosen, Elevated circulating levels of thioredoxin and stress in chronic heart failure, Eur J Heart Fail 6(7) (2004) 883-90.
- [8] V.K. Kodali, C. Thorpe, Oxidative protein folding and the Quiescin-sulfhydryl oxidase family of flavoproteins, Antioxid Redox Signal 13(8) (2010) 1217-30.

- [9] C.X. Santos, L.Y. Tanaka, J. Wosniak, F.R. Laurindo, Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase, *Antioxid Redox Signal* 11(10) (2009) 2409-27.
- [10] C.X. Santos, A.D. Hafstad, M. Beretta, M. Zhang, C. Molenaar, J. Kopec, D. Fotinou, T.V. Murray, A.M. Cobb, D. Martin, M. Zeh Silva, N. Anilkumar, K. Schroder, C.M. Shanahan, A.C. Brewer, R.P. Brandes, E. Blanc, M. Parsons, V. Belousov, R. Cammack, R.C. Hider, R.A. Steiner, A.M. Shah, Targeted redox inhibition of protein phosphatase 1 by Nox4 regulates eIF2 α -mediated stress signaling, *EMBO J* 35(3) (2016) 319-34.
- [11] S. Chakravarthi, C.E. Jessop, M. Willer, C.J. Stirling, N.J. Bulleid, Intracellular catalysis of disulfide bond formation by the human sulfhydryl oxidase, QSOX1, *Biochem J* 404(3) (2007) 403-11.
- [12] E. Castellero, H. Akashi, K. Pendrak, H. Yerebakan, M. Najjar, C. Wang, Y. Naka, D. Mancini, H.L. Sweeney, D.A. J, Z.A. Ali, P.C. Schulze, I. George, Attenuation of the unfolded protein response and endoplasmic reticulum stress after mechanical unloading in dilated cardiomyopathy, *Am J Physiol Heart Circ Physiol* 309(3) (2015) H459-70.

Figure 1

A schematic of forward and reverse translation, using Qsox1 as an example.

Systems biology approaches, including proteomics, can generate hypotheses for investigation in pre-clinical models. This knowledge can then be used to develop new approaches to prognostication, diagnosis and treatment in patients. Reverse translation has the advantage of focusing pre-clinical investigation on mechanisms that may be more likely to have clinical relevance.