Journal of Pathology
J Pathol 2015; **235:** 669–671
Published online 3 February 2015 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4507

INVITED COMMENTARY

CaMKII: do not work too hard in the failing heart†

Min Liu,* You Li and Ruichuan Chen*

State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, 361101, China

**Correspondence to: Ruichuan Chen and Min Liu, State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, 361101, China. E-mail: chenrc@xmu.edu.cn or minliu@xmu.edu.cn*

†Invited Commentary for Awad S, Al-Haffar KM, Marashly Q, *et al.* Control of histone H3 phosphorylation by CaMKII in response to hemodynamic cardiac stress. *J Pathol* 2015; **235**: 606–618.

Abstract

CaMKIIδ, a calcium/calmodulin-dependent protein kinase, plays pivotal roles in the development of heart disease. In this issue of *The Journal of Pathology*, Salma Awad and colleagues demonstrate that CaMKIIδ is engaged in both pathological hypertrophy and heart failure. By analysis of mouse and human heart samples, they found that the level of CaMKIIδ is increased in both pathological processes. Further studies demonstrated that CaMKIIδ mediates the phosphorylation of histone H3 at serine 10 (H3S10), which then tethers the chaperone protein 14-3-3 to promoter regions of fetal cardiac genes to activate their transcription. Combined with recent highlights on transcription regulation, this study revealed a fuzzy boundary between pathological hypertrophy and subsequent heart failure and indicates that current therapeutic strategies towards heart failure may have potential risks to patients.

Keywords: CaMKIIδ; pathological hypertrophy; heart failure

Received 17 December 2014; Revised 17 December 2014; Accepted 2 January 2015

No conflicts of interest were declared.

Heart failure is a major public health concern in the modern world. After chronic or acute pathological changes, the heart fails to pump sufficient blood for the circulatory system, which results in massive cardiomyocyte death due to lack of nutrition, oxygen, and waste removal. Pathological hypertrophy, as a well-known compensatory response process, often induces the accumulation of fetal cardiac proteins in cardiomyocytes and substantial pathological changes of the heart, thereby leading to chronic heart failure. Many studies have focused on explaining the molecular bases of hypertrophy, rather than heart failure itself. In this issue of the journal, Salma Awad and colleagues reveal a novel connection between histone modification and heart failure-related transcriptional reprogramming [1].

It is well known that hypertrophic signals induce rapid activation of immediate-early genes and increase the expression of fetal cardiac genes. Awad *et al* revealed that calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ) is required for these processes [1,2]. According to their observations, CaMKIIδ levels increase not only in hypertrophic cardiomyocytes, but also in failing hearts from heart transplant patients. The enriched CaMKIIδ can be tethered to the promoter regions of fetal cardiac genes such as *ANP*, β-*MHC*, *c-MYC*, and *GATA4*, and is thought to be responsible for phosphorylation of the nucleosomal histone, H3, at serine 10 (H3S10). Further investigation showed that this phosphorylated H3S10 (H3S10ph) is able to bind 14-3-3 chaperone protein, thereby promoting the tethering of specific transcription factors such as GATA4 or MEF2 to allow proper elongation of fetal cardiac genes by RNA polymerase II (RNAPII). In contrast, knockout of CaMKIIδ abolished both the phosphorylation of H3S10 and the reactivation of fetal genes in mouse cardiomyocytes even under pathological stress, further confirming the role of CaMKIIδ in regulating cardiac hypertrophy and end-stage failure through phosphorylating H3S10 [1,2]. Therefore, their studies provide an insight into the mechanism of cardiac hypertrophy and heart failure at the molecular level.

Of note, the functions of CaMKIIδ in regulating cardiac pathological processes are more complicated and beyond those addressed by Awad *et al.* First, besides phosphorylating H3S10, CaMKIIδ is also reported to phosphorylate HDAC4, a member of class II histone deacetylases (HDACs), and thereby drive it out of the nucleus. This not only releases the HDAC4-bound MEF2 for the expression of MEF2-mediated hypertrophic genes, but also enhances the acetylation of nucleosomal histones for remodelling of promoter regions [3,4]. Second, CaMKIIδ has been shown to induce the expression of anti-apoptotic genes such as *BCL-2* through activating GATA4 [5]. Moreover, these

two CaMKIIδ-activated factors, MEF2 and GATA4, can synergistically enhance the expression of calcineurin (PP2B), thereby inducing cardiac hypertrophy by triggering calcineurin–NFAT signalling [6]. Intriguingly, the positive transcription elongation factor P-TEFb and its recruiting partner Brd4 are essential for cardiac hypertrophy [7]. However, in an unstressed state, P-TEFb is sequestrated into an inactive 7SK snRNP complex and Brd4 is locked on chromatin. Our previous studies demonstrated that calcineurin together with PP1α plays a critical role in activating P-TEFb by liberating it from the inactive 7SK snRNP complex [8], whereas PP1α together with class I HDACs plays an essential role in releasing Brd4 from chromatin [9]. Interestingly, to release chromatin-bound Brd4, PP1α is used to dephosphorylate H3S10ph – the opposite action of CaMKIIδ – and this dephosphorylation leads to the deacetylation of nucleosomal histone H4 at H4K5ac/K8ac by HDACs, thereby releasing chromatin-bound Brd4. The released Brd4 subsequently binds to and recruits P-TEFb onto promoters to enhance the expression of inducible genes [9].

As mentioned above, although many studies have revealed the functional mechanisms of CaMKIIδ in cardiac hypertrophy, a compensatory process for cardiomyocyte survival, it remains unclear how CaMKIIδ controls heart failure, an apparently opposite process involving massive cardiomyocyte death. Nevertheless, the connection between CaMKIIδ-mediated phosphorylation of H3S10 and the transcriptional reprogramming in both pathological processes addressed by Awad *et al* has opened a door for glancing at the progression from cardiac hypertrophy to heart failure stage. Combined with the conclusions of other research fields, it can be hypothesized that at the very early stages of cardiac hypertrophy, the first genes subjected to transcription should be the immediate-early genes, such as *FOS*, *MYC*, *GATA4*, *NFAT*, *NFKB*, and *MEF2*. As transcription initiation at the promoter regions of these genes has already occurred, yet RNA polymerase II (RNAPII) is stalled at the promoter-proximal regions in the unstressed state, these genes are ready for rapid transcription elongation by the recruitment of Brd4/P-TEFb in response to stress [10]. Meanwhile, hypertrophic signals trigger the liberation of P-TEFb from inactive 7SK snRNP through calcineurin and PP1α signalling pathways [8], and induce the release of chromatin-bound Brd4 via the dephosphorylation of H3S10ph and the deacetylation of H4K5ac/K8ac by activated PP1α and class I HDAC pathways [9]. Released Brd4 subsequently binds to P-TEFb and loads it onto paused RNAPII to stimulate transcription elongation of the immediate-early genes.

Under the continuing stimulation of pathological stress, the newly synthesized transcription factors encoded by the immediate-early genes trigger transcription of fetal cardiac genes such as *ANP* and β-*MHC*. At this stage, increased CaMKIIδ kinase activity will mediate the phosphorylation of H3S10 at the promoter regions of fetal cardiac genes, inducing chromatin remodelling to facilitate the recruitment of specific transcription factors and assembly of the transcription initiation complex. Of note, during this stage, the kinase activity of CaMKIIδ in cardiomyocytes should be limited and CaMKIIδ-mediated H3S10 phosphorylation restricted to promoter regions of fetal cardiac genes, whereas the majority of nucleosomal H3S10ph is dispersed and available for dephosphorylation by PP1α, thereby aiding the release of chromatin-bound Brd4 and the recruitment of P-TEFb to enhance transcription elongation of fetal cardiac genes. Thus, by the cooperation of specific transcription factors and the transcription elongation factor Brd4/P-TEFb, a large number of hypertrophic genes are expressed, causing the heart to undergo substantial pathological changes. To survive these pathological challenges, GATA4 also mediates the expression of anti-apoptotic genes in a Brd4-dependent manner [11]. Thus, at this hypertrophy stage, the kinase activity of CaMKIIδ towards phosphorylating H3S10 and the phosphatase activity of PP1α for dephosphorylating H3S10ph should be maintained in an equilibrium state to enable cardiomyocytes to survive the environmental challenges.

However, according to the observation of Awad *et al*, both CaMKIIδ and H3S10ph were elevated during the progression of cardiac hypertrophy and reached their highest levels at the stage of heart failure. It is conceivable that with the increase of CaMKIIδ, its kinase activity would finally overwhelm the phosphatase activity of PP1α. In such a case, the H3S10-phosphorylated nucleosomes would spill over the promoter regions and spread to the gene body, even along the whole genome [10]. Moreover, excessive CaMKIIδ kinase activity would cause the export of class I HDACs together with HDAC4 out of the nucleus, thereby indirectly inducing global acetylation of nucleosomal H4K5/K8 [4,12]. As a result, Brd4 would be locked onto chromatin by acetylated H4K5/K8. Since PP1α-mediated H3S10ph dephosphorylation is a prerequisite for the deacetylation of H4K5ac/K8ac, the reduction of PP1α activity would impair the release of chromatin-bound Brd4 and the subsequent recruitment of P-TEFb for a stress response [9]. When Brd4 becomes totally unavailable, the Brd4-mediated stress response, such as the expression of anti-apoptotic genes, would be completely stopped, thereby leading to the massive death of cardiomyocytes. Thus, these events apparently switch CaMKIIδ's original role in compensatory responses to pathological stress, to the inducer of cardiomyocyte death.

Taken together, the underlying functions of CaMKIIδ in different pathological stages are more complicated than has been currently addressed. Therefore, it should be recognized that any therapeutic strategy for cardiac hypertrophy by administering a specific inhibitor may raise the potential risk. In line with this notion, the administration of CaMKII inhibitors at different pathological stages has different, even opposite, effects [13]. Moreover, Brd4 is essential for cardiac hypertrophy and inhibiting Brd4 with the specific inhibitor JQ-1 was able to alleviate cardiac hypertrophy [7]. However, at the later

stages of pathological hypertrophy, Brd4 might also be essential for the survival of cardiomyocytes. If JQ-1 is administered at this stage, it might cause the massive death of cardiomyocytes and heart failure, rather than alleviating hypertrophy, thereby raising potential risks to heart patients. Therefore, broader and more detailed molecular studies are required for deeper insights into the processes involved in the development of cardiac hypertrophy towards heart failure.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81361120386, 31270809, and 30930046 to RC) and the 973 Program (2013CB917802 to RC).

Author contribution statement

ML and RC wrote and revised the manuscript. YL organized materials for writing.

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