

Letter to the Editor

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Response by Zhou et al to Letter Regarding Article, “Loss of Adult Cardiac Myocyte GSK-3 Leads to Mitotic Catastrophe Resulting in Fatal Dilated Cardiomyopathy”

In response:

We thank Drs Karlstaedt and Taegtmeier for their favorable comments on our recent report demonstrating mitotic catastrophe as a key mechanism of fatal dilated cardiomyopathy in glycogen synthase kinase 3 (GSK-3)–deficient hearts (conditional GSK-3 α/β double knockout [DKO]).¹ Considering the complex biology of GSK-3 in regulating numerous biological processes and cellular functions, we completely agree with Drs Karlstaedt and Taegtmeier that there may be additional layer(s) of complexity to be elucidated for the complete understanding of the molecular basis of the observed phenotype. Indeed, our focus on cell cycle regulation and mitotic catastrophe was guided by unbiased microarray analysis.

It is well established that GSK-3 is a central regulator of nutrient and energy homeostasis. In response to the comments of Karlstaedt et al regarding metabolism, now we have examined the metabolic consequences of the complete loss of GSK-3 in adult hearts. As expected, an increased Periodic acid–Schiff–diastase positivity demonstrated enhanced glycogen deposition in the DKO hearts (Online Figure IA). The Glycogen deposition in the DKO hearts was further confirmed by transmission electron microscopy (Online Figure IB). These findings are in complete agreement with our previous reports demonstrating isoform-specific loss of GSK-3 α^2 and GSK-3 β^3 leads to upregulation of glycogen synthase activity. Hence, increased glycogen deposition in DKO hearts was essentially expected.

Karlstaedt et al pointed out a study⁴ from one of our long-term collaborators and coauthor on the original article, Dr Woodgett, which revealed the tissue-specific role of GSK-3 β in glucose metabolism. Patel et al⁴ reported that the liver-specific GSK-3 β knock-out mice displays no metabolic phenotype. However, mice lacking GSK-3 β in skeletal muscle showed improved glucose tolerance and glycogen deposition. In complete agreement with the concept of tissue-specific role of GSK-3 isoforms in glucose metabolism, our unpublished data with cardiomyocyte (CM)-specific GSK-3 β conditional knockout mice indicate that CM-GSK-3 β regulates systemic glucose metabolism. With that said, the oral glucose tolerance was comparable between DKO and littermate controls at 2- and 3-week on tamoxifen timeline (data not shown). Moreover, we have consistently reported the isoform- and tissue-specific role of GSK-3 in diverse biological processes.^{3,5–8} Taken together, the role of GSK-3 in the pathogenesis of glucose tolerance and metabolism is diverse. Although, the contribution of elevated glycogen deposition in the DKO phenotype is an area of future investigation, we do not think it is the primary cause of the observed phenotype. Nevertheless, metabolism was not a primary focus of the study, because the unbiased microarray analysis revealed a maximum dysregulation in the cell cycle regulators and fibrosis-related genes.

Furthermore, chronological analysis of trichrome-stained heart sections at various time point of tamoxifen timeline and experiments with isolated adult cardiac fibroblasts clearly ruled out fibrosis as the primary cause of observed phenotype.

In response to comments of Karlstaedt et al on mitochondrial destabilization and apoptosis, now we have extensively investigated the DKO mitochondrial biology, which includes analysis of mitochondrial membrane potential ($\Delta\psi_m$), oxygen consumption rate, and mitochondrion number (Online Figure II). Herein, we are limited by space to include all the data; however, in summary, mitochondria from DKO hearts demonstrated significantly increased oxygen consumption rate and mitochondrial membrane potential compared with that in the littermate controls. However, the numbers of mitochondria were comparable between DKO and littermate control hearts. These observations are consistent with many previous reports suggesting beneficial effects of GSK-3 inhibition by directly modulating the mitochondrial permeability transition pore complex.^{9,10} Indeed, GSK-3–mediated regulation of mitochondrial function is an interesting area and currently a subject of investigation in numerous laboratories. Thus, loss of GSK-3 indeed causes broad metabolic alterations; however, based on our systematic phenotyping, unbiased array analysis, and mechanistic studies, we still think cell cycle (mitotic catastrophe), instead of metabolism, is the primary cause leading to lethal cardiomyopathy.

We think that clinical implications of our DKO findings are substantial. GSK-3 is reported to be a potential drug target for the treatment of several pathological conditions, including progressive central nervous system disorders, cancer, metabolic disorders, and cardiovascular diseases including ischemic heart disease. To the best of our knowledge, we are the first to report the lethal consequences of targeting both isoforms of GSK-3. Indeed, all currently available GSK-3 inhibitors are not isoform specific. Our studies with DKO indicate that systemic, prolonged, clinical use of nonselective inhibitors might put patients at risk of cardiomyopathy and fibrotic cardiac remodeling. We think that the greatest promise for drug development may lie in better understanding of the isoform-specific GSK-3 biology and the development of isoform-selective, small-molecule GSK-3 inhibitors.

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Disclosures

None.

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