Response to Letter Regarding “Myostatin Regulates Energy Homeostasis in the Heart and Prevents Heart Failure”

In the article referred to in Drs McLean and Oudit’s letter, we analyzed the effects of inactivation of myostatin in adult cardiomyocytes. Drs McLean and Oudit correctly point out that treatment of mice with tamoxifen to induce activation of cre recombinase estrogen receptor fusion proteins causes transient systolic dysfunctions. They expressed concern that the relatively high dosage of tamoxifen administered in our experiments (100 mg/kg daily for up to 5 days) contributed to decreased survival, transient impairment of contractility, and cardiomyocyte hypertrophy in adult cardiomyocyte-specific myostatin mutants. In our experiments, we always directly compared tamoxifen-treated αMyHC-MCM/Mstnfl/fl with tamoxifen-treated αMyHC-MCM mice, which in our view is the best possible control. In tamoxifen-treated αMyHC-MCM mice, we only observed a mild depression of cardiac function without any effect on lethality while tamoxifen-treated αMyHC-MCM/Mstnfl/fl presented with a >50% reduced ejection fraction, nearly 30% lethality and a massive enlargement of the right atria, which was never seen in any tamoxifen-treated control mice described in the literature or analyzed in our laboratory. The majority of tamoxifen related side-effects reported a return to baseline levels 10 days after the first injection in most published studies, which is the reason why we started to analyze our mice at this time point. We would also like to point out that we observed the same impairment of cardiac function in αMyHC-MCM/Mstnfl/fl mice, in which cre recombinase activity was induced by implantation of tamoxifen pellets (50-mg slow-release pellets, unpublished observations). Such pellets have previously been demonstrated to yield only low serum tamoxifen concentrations making cardiotoxic effects of tamoxifen unlikely.

Surprisingly, Drs McLean and Oudit directly compared dosages of tamoxifen delivered by different routes (intraperitoneal injection, oral gavage) and different solvents (corn oil, miglyol), which is not adequate and stirs confusion. Tamoxifen is a hydrophobic prodrug that is insoluble in water and gets activated in the liver. We administered a suspension in miglyol IP, which has a different effect on its bioavailability and pharmacokinetics compared with oral application of the same dose in another solvent. To determine concentrations of tamoxifen that affect cardiac function, it is not appropriate to compare dosages applied by different regimes but required to determine the actual concentration of 4-hydroxytamoxifen within the target tissue. The use of appropriate controls, in our case tamoxifen-treated αMyHC-MCM mice, avoids such efforts and allows correct normalization.

Of course, it is difficult to completely exclude synthetic phenomena caused by the combination of 2 events, which alone do not yield an effect. To cope with this possibility, we used 2 additional mouse strains. (1) We overexpressed myostatin in the heart by removing a stop-flox cassette using αMyHC-MCM mice and the same tamoxifen treatment regimen used to delete the myostatin gene. Tamoxifen-induced overexpression of myostatin slightly improved cardiac contractility arguing for beneficial effects of increased, short-term myostatin expression for the heart ruling out a major cardiotoxicity of our tamoxifen treatment routine. (2) We analyzed αMyHC-Cre/Mstnfl/fl mice, in which the myostatin gene was constitutively inactivated in the myocardial cell lineage. These mice displayed increased lethality and cardiac systolic dysfunction at 4 to 5 months of age without tamoxifen treatment, clearly indicating an important role of myostatin for maintaining heart functions. Furthermore, other studies have reported eccentric hypertrophy and reduced ejection fraction in mice with germ-line inactivation of myostatin.

Drs McLean and Oudit claim that we did not analyze any established markers of myostatin-dependent signaling, such as Smad2/3 phosphorylation or nuclear localization. It might escape their attention that we performed Chromatin IP experiments using a pSmad3 antibody and demonstrated reduced binding of pSmad3 to the Rgs2 promoter after deletion of myostatin in cardiomyocytes of αMyHC-MCM/Mstnfl/fl mice. Irrespective of the reduced binding of pSmad3 to targets promoters after inactivation of myostatin, our data do not argue for a decisive role of Smad2/3 for myostatin signaling in the heart. Furthermore, Drs McLean and Oudit stated that they “would have expected that selective cardiomyocyte deletion of the myostatin receptor ActRIIB may yield a distinct cardiac phenotype” citing 2 studies, in which ActRIIB inhibitors and not a clean genetic deletion of ActRIIB were used. In fact, inactivation of the ActRIIB receptor results in severe cardiac abnormalities. To the best of our knowledge, no cardiomyocyte-specific deletion of ActRIIB has been accomplished yet.

Drs McLean and Oudit rightly point out that our study suggests a significant new direction for the paradigm of myostatin signaling emphasizing the importance of localized autocrine/paracrine functions and local cell–cell communications. The massive increase of myostatin expression in noncardiomyocytes and the cardiac phenotype after inactivation of myostatin in cardiomyocytes indicates that circulating myostatin levels are indeed unable to compensate for the loss of myostatin in cardiomyocytes. We assume that myostatin primarily acts in a paracrine and autocrine manner, at least in the heart. It will be interesting to decode the communication between cardiomyocytes and noncardiomyocytes that results in a dramatic upregulation of myostatin in noncardiomyocytes when cardiomyocytes fail to express myostatin. We also agree that our data raise questions about the use of myostatin antagonists in elderly people with sarcopenia, who often show age-dependent diastolic dysfunction or have attracted severe cardiac diseases. Efficient inhibition of myostatin signaling in the heart might aggravate cardiac dysfunctions arguing for restricted inhibition of myostatin signaling in skeletal muscles.
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Disclosures
None.

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References
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