Reply to “Letter to the editor: ‘KDAC and the regulation of nonnuclear smooth muscle protein acetylation’”

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REPLY: We appreciate the interest expressed by Taggart and colleagues (9) in our article (6) for American Journal of Physiology-Cell Physiology. They have raised several issues regarding the paper. Below are detailed our responses.

To assess the role of histone deacetylase 8 (HDAC8), we used lentivirus-mediated short hairpin RNA (shRNA) against HDAC8 as a major tool. Knockdown of HDAC8 attenuated the agonist-induced cortactin deacetylation, actin polymerization, and contraction at tissue level, which is a key finding in the article. The pharmacological tool was an alternative approach for this study. We agree that the inhibitor is not specific, which is a common problem for many pharmacological agents. It is possible that other HDACs may also exist in human bronchial tissues and mouse tracheal tissues and may have a role in the pathway [see DISCUSSION section in our paper (6)]. However, the inhibitor issue does not substantially affect our conclusion, which is largely based on the results from shRNA against HDAC8. Finally, if we treat the knockdown tissue with the inhibitor as they suggest in their letter, it may generate inconclusive results. The effects of the compound on knockdown tissues are implicated by partial knockdown, dose of the compound, etc. However, we think this is a good thought.

With regard to coimmunoprecipitation control experiment, we recently published a paper to characterize the interaction of cortactin with Pfn-1 in smooth muscle. In this paper, nonimmune IgG does not interact with cortactin as well as Pfn-1 (11). In addition, the IgG does not associate with HDAC8 in this study (data not shown). Finally, nonimmune IgG does not interact with many other proteins in smooth muscle as well as other cell types (1, 3, 8, 10, 12). Thus, it is well documented that nonimmune IgG conjugated with beads does not precipitate specific proteins.

Our laboratory has been focusing on cytoskeletal regulation of smooth muscle contraction. Cortactin has been recently implicated in the regulation of the actin cytoskeleton in vitro biochemical studies and in nonmuscle cells. Thus, we are interested in the role and regulation of cortactin. Our recent studies demonstrate that cortactin is required for actin dynamics and smooth muscle contraction (11). To further understand how cortactin is regulated, we evaluated cortactin acetylation/deacetylation in smooth muscle. This is because our collaborator (Dr. Seto) has recently found that cortactin undergoes deacetylation during cell migration (13), which also involves cytoskeletal processes. We agree that there is a possibility that many other proteins get acetylated or deacetylated in smooth muscle. Functional studies on potentially acetylated/deacetylated proteins are needed to unveil their role in smooth muscle contraction.

Our expertise is cellular and molecular physiology. We are particularly interested in physiological functions and regulation of cytoskeletal proteins in smooth muscle contraction. The large-scale proteomic study (7) is an impressive paper. However, it was unclear whether contractile activation affected the acetylation level of these proteins. Moreover, the functional roles of these potentially acetylated proteins in muscle contraction were not determined in this paper. We feel that this paper is not highly relevant to our paper. Second, we initiated this project a couple of years ago. We did not notice the paper (5) at that time (probably this is because HDAC8 is not included in the article’s title). Actually, we are excited that the independent studies by Taggart’s group (2, 5) and our laboratory (6) have similar findings in terms of the role of HDAC8 (KDAC8) in smooth muscle contraction. We also notice differences between Taggart’s papers and our paper. Taggart’s papers (2, 5) suggest a role of KDAC8 in regulating HSP20. Moreover, several proteins including cortactin were coimmunoprecipitated with KDAC8 as evidenced by immunoblots. Acetylated form of proteins including cortactin was increased after KDAC8 inhibition, which is an intriguing finding. In our study (6), we demonstrate that contractile stimulation induces cortactin deacetylation. Furthermore, by using a dominant negative cortactin mutant, we demonstrate that cortactin deacetylation plays a critical role in mediating smooth muscle contraction. We also show that cortactin deacetylation affects contraction by modulating actin polymerization, not by myosin activation. Moreover, cortactin deacetylation is mediated by HDAC8 as evidenced by shRNA-mediated knockdown. These are new and novel findings, which suggest a clear and novel cytoskeletal signaling during smooth muscle contraction. Third, HDACs including HDAC8 are originally found to mediate histone deacetylation. In our studies (6), HDAC8 is present both in the nucleus and in the cytoplasm of human and mouse airway smooth muscle cells. In addition, HDAC8 has been found in the nucleus of nonmuscle cells (4). We appreciate the original role of this enzyme and call it HDAC8, which is commonly used in the literature and by biotechnology companies. We also think that KDAC8 is an adequate name for this enzyme.

Finally, we appreciate comments made by Taggart and colleagues (9) again. We hope that our research and discussion contribute to our better understanding of the role and mechanisms of HDACs in smooth muscle contraction.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

D.D.T. drafted manuscript; D.D.T. edited and revised manuscript; D.D.T., R.A.C., R.W., and O.J.G. approved final version of manuscript.

REFERENCES


