Important Considerations for Evaluating the Data Presented by Igarashi et al

To the Editor:

The major conclusion drawn in the article by Igarashi et al., that cholesteryl ester hydrolase (gene symbol CES1) does not contribute to neutral cholesterol ester (CE) hydrolase activity, is based on the inability of the authors to detect CE hydrolase activity associated with the cDNA clone that they obtained by PCR amplification and by the inability of the authors to demonstrate change in intracellular CE hydrolase activity by short hairpin (sh)RNA-mediated knockdown of CES1. The authors have not provided the sequence of the clone obtained and do not unequivocally show its identity to the reported CES1 with established role not only in intracellular CE hydrolysis, but also in attenuation of diet-induced atherosclerosis and lesion necrosis. It is noteworthy that the sense primer used in this study also shows a 95% homology to human CES4 (17/18 bp match; accession no. NR_003276). Single amino acid changes in carboxylesterase family of enzymes dramatically affect their substrate specificity where an enzyme with an ability to only hydrolyze p-nitrophenyl esters can acquire CE hydrolytic activity by mutagenesis of key residues. The CES1 clone examined in this study hydrolized PNPB but not CE and no evidence is provided that this effect is not due to changes in critical amino acid residues. In the absence of the validation of the CES1 clone used in these studies, the conclusions drawn from the data presented in Figure 2 of the article, showing no changes in CE content in Ad-CES1-transduced cells, are not valid. The authors show Western blot analyses to demonstrate overexpression of proteins (Figure 2A of the article). However, no characterization of the antibody generated and used, especially with respect to its specificity for CES1 and not other carboxylesterases, is provided. The authors do show that it does not cross-react with KIAA1363. The peptide sequence used to generate these antibodies is homologous to other members of the carboxylesterase family (CES1, -2, -3, -7, Esterase 31, egasyn, and CES hBr2 and CES hBr3), and antibodies raised to this sequence will, therefore, not be specific for CES1. The rationale for not using the antibody generated in their own laboratory (and used for all the Western blot analyses) for immunohistochemical studies and using an antibody raised against a liver carboxylesterase and used to detect carboxylesterase in human brain tissues is not evident. The high degree of homology between the members of carboxylesterase family underscore the importance of exercising caution when drawing conclusions from studies using antibodies raised to a peptide.

The second evidence presented to conclude that CES1 is not involved in intracellular CE hydrolysis is the inability of Ad-shRNA specific for CES1 to decrease CE hydrolytic activity (Figure 4 of the article). However, the authors provide no explanation for an ~50% decrease in activity when cells were transduced with Ad-LacZ. Furthermore, the conclusion that “NCEH is the only enzyme that requires attention when dealing with neutral CE hydrolase activity in human macrophages” is not supported by the data shown in Figure 4 of the article. In cells transduced with Ad-LacZ, there is ~50% decrease in CE hydrolytic activity and compared with this, there is an additional 50% decrease with Ad-NCEH1. Greater than 25% of cellular CE hydrolytic activity is still present in cells with negligible expression of NCEH1 protein (Figure 1A of the article), indicative of the presence of other enzymes and invalidating the major conclusion of this study.

The authors failed to cite a recent study by Buchebner et al demonstrating that CE hydrolytic activity remains unchanged in macrophages lacking KIAA1363 (an enzyme that the authors have rediscovered and named as NCEH1), contradicting the earlier publication from this group. It is also noteworthy that Buchebner et al observed identical CE turnover in Lipe−/− and wild-type macrophages and concluded that “HSL hydrolyzes CE in macrophages together with other enzymes” and that “measurement of CE hydrolyase activities in vitro might not reflect in vivo situation.” Therefore, caution needs to be exercised when drawing conclusions based on in vitro studies alone and, because multiple enzymes hydrolyze CE, the true physiological role of any macrophage CE hydrolase can only be established by demonstrating that macrophage-specific overexpression enhances cholesterol efflux and reverse cholesterol transport resulting in attenuation of atherosclerosis.

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