Response to the Letter by Ghosh

Below, we would like to respond to the criticisms raised by Dr Ghosh1 to our recent article published in Circulation Research.2

1. Authenticity of the cDNA clones of cholesterol ester hydrolase (CES1): We confirmed the nucleotide sequence of the cDNA clones of CES1 that were used for the experiments. In database, 3 variants of CES1 are registered. One of them is the cDNA reported by Dr Ghosh3 (NM_001025194). We made expression vectors of this (NM_001025194) and another variant (NM_001025195). Both vectors gave us the same results. Therefore, it is unlikely that our failure to reproduce the results reported by Dr Ghosh was attributable to amino acid changes in the clones.

2. Specificity of the antibodies against CES1: In our study, we used 2 different antibodies: one provided by Dr Hosokawa and the other generated by ourselves. We confirmed that both antibodies recognized the expressed CES1. As shown in the results of Western blot analysis (Figure 1C in our article), we obtained a single band with molecular weight of CES1 in many human-derived cells and tissues. The pattern of Western blot analyses was essentially identical between the 2 antibodies. The specificity of the antibody from Dr Hosokawa was described in the preceding paper.4 Even though our antibodies are not specific enough, our contention that the expression of CES1 was not as high as that of NCEH1 (Figures 1C and 5D) cannot be refuted. Moreover, as mentioned above, it is unlikely that we expressed another member of the carboxylesterase family, such as CES4, because we confirmed the nucleotide sequence of the expressed cDNA clones very carefully.

3. A significant decrease in neutral cholesterol ester (CE) hydrolase activity in the cells infected with Ad-LacZ: In general, transient transfection of plasmids and/or infection of viral vectors compromise the condition of the cells. Macrophages are known to be notoriously resistant to both plasmid transfection and adenovirus infection. That was why we used a relatively higher multiplicity of infection of the recombinant adenovirus. Infected with such a high multiplicity of infection, many cell types tend to lose viability, thereby decreasing the expression of many genes. We have confirmed, however, that the viability of the cells was not compromised and was similar among 3 vectors (LacZ, NCEH1, and CES1).

4. No reference to a paper reported by Buchebner et al, who showed that neutral CE hydrolase activity was not changed in KIAA1363 mice: This work, which was published during the time when we revised our manuscript,4 contained several major differences from our results. One of them was the claim of the authors that neutral CE hydrolase activity was unchanged in mice lacking KIAA1363. Thus far, no data demonstrating the clean knockout of KIAA1363 of this model by Western and/or Northern blot analyses have been reported.5 We cannot understand how one can conclude that an enzyme is not involved without showing that the knockout is complete and clean.

5. A model of macrophage-specific overexpression is needed to show physiological role of NCEH1: We do not agree with this claim. First, overexpression is not physiological in general. Because carboxylesterases are known to metabolize diverse molecules,9 overexpression of CES1, for example, might affect the development of atherosclerosis via a pathway independent of cholesterol trafficking. Second, it is also well known that a transgene might disrupt and/or transactivate endogenous genes in the chromosome into which it is integrated. In contrast, knockout models are more specific, because they are free from the aforementioned problems. In this regard, Ces3 (mouse ortholog of human CES1) knockout mice7 will be a useful tool to further validate the hypothesis that CES1 is the neutral CE hydrolase. Moreover, we are now generating transgenic mice overexpressing NCEH1, CES1, or LIPE under the same promoter.

6. Finally, naming of genes is sometimes tricky. Since Dr Ghosh named CES1 as cholesterol ester hydrolase (CEH),3 she has never mentioned in her subsequent papers that CEH is identical to CES18,9 or to triacylglycerol hydrolase (TGH).10 For people naïve in this field, it is hard to know that CEH has other names and other assigned functions. Without such knowledge, it would be difficult to judge whether the claims by Dr Gosh are valid. We hope our continuing efforts will settle the controversy and uncover the truth.

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Disclosures

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