LETTER

Reply to Liu et al.: Loss of TGF-β signaling in CARASIL pathogenesis

We thank Liu et al. (1) for their comments on our work on the link between the cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)-related protease high temperature requirement protein A1 (HtrA1) and the TGF-β signaling pathway. To advance our understanding of the mechanisms underlying HtrA1 deficiency, we used dermal fibroblasts from CARASIL patients and *Htra1* knockout mice as experimental systems (2). We agree with Liu et al. that findings from fibroblasts might or might not fully reflect the physiological processes occurring in a vessel wall and that alterations in other cell types such as endothelial and vascular smooth muscle cells (VSMCs) certainly contribute to CARASIL vessel pathology. Nevertheless, fibroblast cultures have been successfully used in the past to study TGF-β dysregulation in other vascular disorders, demonstrating their usefulness in the elucidation of disease-relevant mechanisms (3). Moreover, HtrA1-dependent changes in TGF-β activity were observed not only in fibroblasts but also in mouse brains arguing for their presence in other cell types (2).

We acknowledge (and have already discussed in our publication) that the hypothesis of a loss of TGF-β signaling as a mechanism underlying CARASIL cannot be easily reconciled with the current view that fibrosis, a pathologic process contributing to CARASIL pathogenesis, is promoted by increased rather than decreased TGF-β levels in a variety of vascular and connective tissue disorders. However, conflicting results on the precise role of TGF-β in the normal and diseased vasculature have been reported before and explained by the complexity of the TGF-β system arising from cell type-specific responses, compensatory mechanisms, and noncanonical signaling (4).

We feel that the HtrA1 expression data presented by Liu et al. (1), although representing a valuable contribution toward an understanding of HtrA1 function in vivo, are in their current form too preliminary to draw meaningful conclusions: First, the examined vessels represent small coronary arteries that clearly differ in their structure from small cerebral arteries, most decisively by lacking a blood-brain barrier. Second, no characterization of the different cell types used for HtrA1 expression quantification, e.g., by analyzing specific marker genes, is presented. Third, detection of endogenous HtrA1 protein by available antibodies is known to be difficult, and the authors provide no information about the source and specificity of the anti-HtrA1 antibodies used in Western blotting and ELISA. Fourth and most critically, the effects of elevated HtrA1 expression levels in VSMCs on latent TGF-β binding protein 1 (LTBP-1) processing and TGF-β activity are not addressed. Thus, the major conclusion of our study—the attenuation of TGF-β signaling by the loss of HtrA1-mediated LTBP-1 cleavage—cannot be challenged by the presented data. Cell type-specific analyses of TGF-β protein and pathway activity measurements will be required to elucidate the roles of the different vascular cell types in CARASIL pathogenesis.

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1 Liu J, Dong F, Hoh J (2015) Loss of HtrA1-induced attenuation of TGF-β signaling in fibroblasts might not be the main mechanism of CARASIL pathogenesis. *Proc Natl Acad Sci USA* 112:E1693.

2 Beaufort N, et al. (2014) Cerebral small vessel disease-related protease HtrA1 processes latent TGF-β binding protein 1 and facilitates TGF-β signaling. *Proc Natl Acad Sci USA* 111(46): 16496–16501.

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The authors declare no conflict of interest.

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