Response to Pomozi et al Research Commentary

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Dear Editors,

In the course of studying the effects of ABCC6 deficiency in mice, we observed an enrichment of mitochondrial gene expression signatures. In subsequent studies we found that ABCC6 null mice exhibited abnormal mitochondrial morphology and functional mitochondrial deficiencies.\(^1\) We then carried out subcellular fractionation studies indicating that ABCC6 co-localized with markers of the mitochondria-associated membranes (MAM) in mouse liver and kidney.\(^1\) Our results differed from Le Saux et al (2011), from the Varadi group who had concluded in a recent publication that ABCC6 resided in the plasma membrane.\(^2\) To test whether ABCC6 was localized in plasma membrane, we performed cell surface protein biotin labeling experiments, which were negative for ABCC6.\(^1\)

Pomozi et al\(^3\) of the Varadi group have now challenged our conclusions based, as in their previous report,\(^2\) on immunofluorescence imaging of frozen liver sections and cells in culture showing peripheral cellular localization of antibody binding. They argue that cell disruption and subcellular fractionation in our study\(^1\) may have resulted in artifactual associations of membrane proteins. However, this seems improbable given that the plasma membrane markers that we examined did not fractionate with ABCC6, and MAM constitutes a very small fraction of the total membranes.\(^1\) Cellular fractionation techniques have been utilized almost universally to provide definitive evidence of subcellular localization.\(^4,5\) Pomozi et al\(^3\) also argue that ABCC6 lacks sufficient amine groups on the extracellular surface to allow biotin labeling. This possibility cannot be excluded, although the manufacturer of the surface protein biotin labeling assay (Thermo Pierce) indicates that it is sensitive to even a single amine group, which would be present in the N terminus, or in one of the predicted available lysines.

In our studies, we used the N-terminal binding (S-20) antibody from the same commercial supplier (Santa Cruz Biotechnology) as reported by Pomozi et al.\(^3\) In our hands, this antibody exhibited significant non-specific binding in ABCC6 null mouse tissue sections (not shown) and in Western blots.\(^1\) We note that in their publication,\(^2\) the methods state that the S-20 antibody is made in rabbit and blocking was performed using goat serum. In fact, the antibody is made in goat and blocking with goat serum would produce non-specific labeling (per Santa Cruz Biotechnology). An advantage of our results obtained by subcellular fractionation as compared to immunofluorescence of tissue sections is that, in the former, the protein is detected on Western blots following separation by gel electrophoresis, thus allowing better separation from cross-reacting proteins. A lack of accompanying Western data in the current challenge, in our opinion, significantly weakens the ability to ascertain the specificity of the signal.

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In conclusion, we recognize that localization of proteins can be challenging and is highly dependent on the quality of the antibody reagents. While we cannot exclude the possibility that some of the ABCC6 protein resides on the plasma membrane, low resolution imaging studies of frozen liver sections and cells in culture do not provide convincing evidence against the localization of ABCC6 in the MAM.

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References


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