# Using Proteomic Analysis and MEMRI to Understand Axonal Transport Deficits and Improvements in a Mouse Model of Alzheimer's Disease

Taeko Inoue[1], Tabassum Majid[1,2], and Robia Pautler[1,2]

[1]*Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, United States,* [2]*Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, United States*

**Introduction:** Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive decline in cognitive functions and the deposition of aggregated amyloid  (A) into senile plaques and the protein tau into tangles. In addition, oxidative stress has long been known to be a major hallmark of AD. What is not known, however, are the mechanisms by which oxidative stress contributes to the pathology of AD. In our previous study, we used a mouse model of AD, the Tg2576 mouse[1], and genetically boosted its ability to quench free radicals, specifically superoxide, by overexpressing the antioxidant protein, superoxide dismutase 2 (SOD-2)[2,3]. We found that reducing superoxide levels through SOD-2 overexpression conferred protection against axonal transport deficits as assessed by MEMRI in the Tg2576 mice[2]. Here, we used the proteomic analysis, Differential Gel Electrophoresis (DIGE) in conjunction with our MEMRI data to begin to understand the improvements we observed in the AD mice.

**Materials & Methods:** DIGE analysis is a proteomic strategy to identify protein differences that exist between samples (e.g. a control and a treatment group)[4]. In this strategy, tissue homogenates from the different groups of interest are labeled with different colored fluorescent dyes[4]. These samples are then loaded into the same well on the same gel and separated by size and pH. If the samples run identically, then the fluorescent dyes will overlap. In regions where there are differences, then one of the fluorescent dyes will predominate. We prepared brain homogenates from wildtype, Tg2576, SOD-2 overexpressing and Tg2576/SOD-2 overexpressing mice for DIGE analysis. From the DIGE analysis, we identified spots on the DIGE gels that exhibited at least a two-fold change in expression using commercialized software specialized for DIGE (see **Figure 1** for a sample DIGE gel). We have begun to identify the identity of these proteins from the DIGE data through Mass Spec analysis.

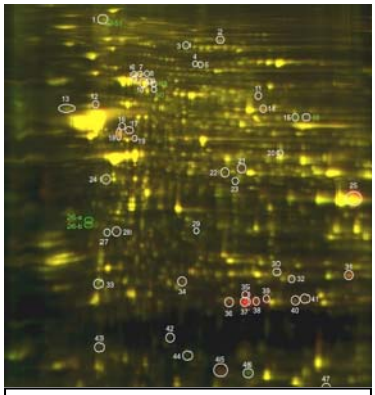

**Figure 1:** This figure is an example of the DIGE datasets used for this analysis. Samples from Tg2576 mice (green) and Tg2576/SOD-2 mice (red) have been loaded. Note the presence of red and green spots in parts of the gel, indicating proteins that are differentially expressed.

**Results and Discussion:** From our DIGE and Mass Spec analysis, we have begun to identify proteins involved in the recovery of AD pathologies in the Tg2576/SOD2 mice compared with Tg2576 mice (**Figure 1**). First, we determined that transgelin -3 gets upregulated by 2 fold in Tg2576/SOD-2 mice compared with Tg2576 mice. Transgelin-3 has been implicated in the cytoskeletal protein, actin, organization and dynamics[5]. Second, we identified that overexpressing SOD-2 in Tg2576 mice results in a 5-fold decrease in Acot7, a protein expressed in activated macrophage during inflammation[6]. Third, we identified that glial fibrillary acidic protein (GFAP) increases by 3 fold upon SOD-2 overexpression in Tg2576 mice indicating that astrocyte activity is upregulated compared with the Tg2576 animals. We are in the process of validating these DIGE and Mass Spec results with Western blot analysis and continuing our Mass Spec analysis of the DIGE data.

**Conclusion:** In this series of studies, we used MEMRI to demonstrate that axonal transport deficits exist in the Tg2576 mouse model of AD and can be recovered by overexpressing SOD-2. However, the mechanism to date, for these improvements is not clear. To better understand these deficits, we have combined our MEMRI data with a proteomic analysis and have shown that overexpressing SOD-2 stimulates transgelin-3 that is involved in actin structural integrity, reduces inflammation by normalizing a macrophage specific protein, Acot7, and promotes the degradation of excess Aβ by stimulating astrocytes as shown by the marked increase in GFAP. Relevant to the axonal transport improvements we observed using MEMRI in the Tg2576/SOD-2 animals, these data implicate actin, Aβ and inflammation as some of the primary contributing factors to the observed improvements in axonal transport. These MEMRI studies in conjunction with additional proteomic analysis will provide insights into the mechanisms of the transport deficits and also the recoveries we have observed. Additionally, this multi-disciplinary approach will pave the way for the understanding of improvements in disease models such as the Tg2576 mouse and will potentially allow for the development of targeted therapeutic development.

**References**:

1. Frautschy, S.A. *et al.* Microglial response to amyloid plaques in APPsw transgenic mice. *Am. J. Pathol.* **152**, 307-317 (1998).
2. Massaad, C.A. *et al.* Mitochondrial superoxide contributes to blood flow and axonal transport deficits in the Tg2576 mouse model of Alzheimer's disease. *PLoS ONE* **5**, e10561 (2010).
3. Massaad, C.A., Washington, T.M., Pautler, R.G. & Klann, E. Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13576-13581 (2009).
4. Unlü, M., Morgan, M.E. & Minden, J.S. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* **18**, 2071-2077 (1997).
5. Ward, M.W. *et al.* The amyloid precursor protein intracellular domain(AICD) disrupts actin dynamics and mitochondrial bioenergetics. *J. Neurochem.* **113**, 275-284 (2010).
6. Forwood, J.K. *et al.* Structural basis for recruitment of tandem hotdog domains in acyl-CoA thioesterase 7 and its role in inflammation. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10382-10387 (2007).