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Grant Title: Identification of glial inhibitory proteoglycans in the neurological movement disorder dystonia

The brain extracellular matrix (ECM) is a complex three-dimensional milieu that has a profound influence on synaptic plasticity and myelination during development. Previous work has established that differentiation of the oligodendrocyte progenitor cells (OPCs) into mature myelinating oligodendrocytes (OLs) is strongly influenced by the ECM composition. This proposal investigates the recently identified role of the OPCs in the generation and secretion of ECM components, a discovery made from studies of a transcriptional pathway dysregulated in the neurological movement disorder, dystonia. The neurodevelopmental disorder DYT-THAP1 (or DYT6) dystonia is caused from the loss-of-function mutations in the transcription factor gene *THAP1*. Recent studies in mouse models of DYT-THAP1 has demonstrated that loss of THAP1 in the OL lineage significantly impairs developmental myelination in the CNS resulting from deficits in the progression of OPC into mature myelinating OLs. Our work demonstrates that the mechanism of dysmyelination observed in the mouse model of dystonia from *Thap1* deletion is the accumulation and secretion of excess glycosaminoglycans (GAGs) by *Thap1* null OPCs. These findings bring attention to role of oligodendrocytes and proteoglycans secreted from these cells as key contributors to dystonia pathogenesis. In these studies we used proteomic studies to profile proteoglycans secreted by OPCs, and identify those differentially regulated during their maturation into myelinating OL and accumulating in dystonia mutants (*Thap1* loss of function). We supplemented the oligodendrocyte culture media with azidoacetylgalactosamine (Ac4GalNAz) to metabolically label PGs. The glycoproteins bearing azides were selectively labeled with biotin alkyne (Click-iT sDIBO Alkyne) using chemoselective ligation (click chemistry) to create a biotin-tagged secretome from both OPCs. Using streptavidin-coated dynabeads, we immunoprecipitated biotinylated secreted proteins, which were further analyzed using LC-MS/MS proteomic analyses. This approach identified 357 unique proteins secreted by OPCs, with 63 significantly enriched in a THAP1-dependent manner. Notably, the secretome of THAP1-deficient OLs was enriched for ECM-binding proteins and protease inhibitors—suggesting a role for these secreted components in ECM remodeling. The results identify candidate ECM-remodeling proteins secreted by the oligodendroglial lineage that will be tested for their relevance in the generation of myelinating oligodendrocytes and dystonia pathology.

Publications resulting from this funding:

Basu A, Archer-Hartmann S, Chopra P, Taherzadeh Ghahfarrokhi M, Dong X, Patel N, et al. Quantitative HILIC-Q-TOF-MS analysis of glycosaminoglycans and non-reducing end carbohydrate biomarkers via glycan reductive isotopic labeling. ChemRxiv. 2025; doi:10.26434/chemrxiv-2025-60p82