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Presentation Abstract

Program#/Poster#:	366.19/UU60
Presentation Title:	Investigation of astrocyte specific responses to MPTP-induced neurotoxicity in the ALDH1L1 BAC-TRAP mouse
Location:	WCC Hall A-C
Presentation time:	Monday, Nov 17, 2014, 8:00 AM -12:00 PM
Presenter at Poster:	Mon, Nov. 17, 2014, 10:00 AM - 11:00 AM
Topic:	++G.01.a. Molecular, biochemical, and genetic techniques
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Abstract:	A central problem in neurotoxicology is detecting the selective and unpredictable damage to specific cells produced by toxic agents and mixtures. Evaluating astrogliosis overcomes this problem as reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Thus, determining the specific in vivo transcriptomic profile of astrocytes under control and reactive conditions will allow for the identification of additional astrogliosis biomarkers. Heintz and Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology that allows the characterization of the actively translating transcriptome of a particular cell type. Using hippocampal and striatal damage due to TMT and MPTP, respectively, we evaluated the localization and response of ALDH1L1 compared to astrogliosis seen by GFAP immunohistochemistry and ELISA. Staining of ALDH1L1 revealed localization to astrocytes after TMT and MPTP, while immunoblots of

ALDH1L1 revealed basal expression of this protein but little enhanced expression after MPTP and TMT, confirming it to be an astrocytic “housekeeping” gene/protein. Thus, ALDH1L1 BAC-TRAP mice can be used to characterize the transcriptome of astrocytes under various conditions. To begin to characterize additional biomarkers of astrogliosis occurring in response to neurotoxic damage ALDH1L1 BAC-TRAP mice were given a single 12.5 mg/kg s.c. dose of MPTP, a well characterized dopaminergic neurotoxicant that induces significant astrogliosis. Phenotypic characterization of the ALDH1L1 BAC-TRAP mice was performed by investigation of morphological changes under both control and neurotoxic conditions. Striatal tissue was obtained at 12, 24, and 48 hrs following a single s.c. dosage of saline or 12.5 mg/kg MPTP. Tissue was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. Changes in the actively translating RNA induced by MPTP damage were determined by microarray (Illumina MouseWG-8 v2 Expression BeadChip) and the dataset interrogated using Ingenuity Pathway Analysis (IPA). MPTP induced robust transcriptome changes in genes previously identified as astrocyte specific (e.g., 400-800 fold increases in TIMP1 from 12 to 48 hrs) as well as others not previously considered astrocyte-specific (e.g., ~200 fold increases in PHOX2A at 12 and 24hrs). Our data indicate the BAC-TRAP technology can be used to identify additional biomarkers of astrogliosis and will aid in characterizing various astrocyte phenotypes.

Disclosures: **K.A. Kelly:** None. **D.B. Miller:** None. **J.P. O'Callaghan:** None.

Keyword (s): ASTROCYTE

BAC-TRAP

MPTP