Staff Writer
February 11, 2020

Research Sheds Light on Optic Nerve Regeneration

A simultaneous analysis of proteomics and lipidomics in developmental growth cones of the optic nerve is providing new information about pathways influencing adult optic nerve regeneration. “We believe insights derived from our analyses will aid in promoting adult nerve regeneration and functional innervation in glaucoma and other devastating neurodegenerative diseases,” said Sanjoy Bhattacharya, Ph.D., M.Tech., professor of ophthalmology at Bascom Palmer Eye Institute at the University of Miami Miller School of Medicine.

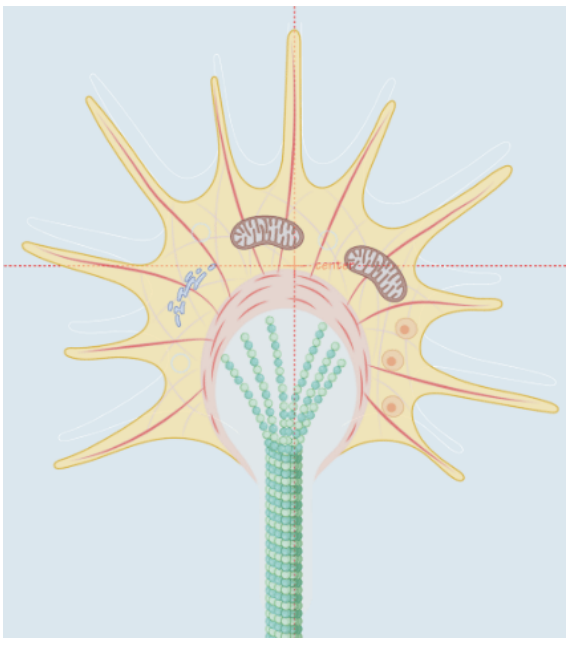

Diagram of growth cone zones.

Dr. Bhattacharya is co-author of a study titled “Multi-omic

Analyses of Growth Cones at Different Developmental Stages Provides Insight into Pathways in Adult Neuro-Regeneration," published in the January issue of the journal iScience.

Growth cones are structures at the tip of a growing axon or dendrite on a neuron. They guide embryonic nerve fibers in their growth. Growth cone membrane expansion, which relies on protein-lipid interactions, is essential for growth cone movement and neuroregeneration.

Researchers used a web-based tool to analyze large amounts of proteomic and lipidomic data and integrate it with data from a separate nerve regeneration study of mice following optic nerve crush.

Dr. Bhattacharya worked with multiple co-authors, including the University of Miami's Abigail Hackam, Ph.D., and Kevin Park, Ph.D.; Jeffrey Goldberg, M.D., Ph.D., Stanford University School of Medicine; Larry Benowitz, Ph.D., Harvard Medical School; and Mohammad Samarah, Ph.D., Carroll University, Wisconsin.

Growth cones are responsible for neuronal expansion toward a target during early development, collateral sprouting resulting in additional or new neuronal connectivity, and enabling regeneration of severed neurites in the central and peripheral nervous systems in adults.

The latter is particularly significant in devising novel intervention strategies for functional recovery in progressive neurodegenerative diseases, such as Parkinson's and spinal cord injuries, and in progressive neuropathies like glaucoma.

Noteworthy findings of this multifaceted, in-depth analysis include:

- The novel data set generated in the first study is the first, and unique, to combine growth cone proteomes and lipidomes across development and fraction.
- Researchers noted growth cone (GC) molecular changes at different developmental time points. For example, significant molecular variability in GCs across developmental ages align with the upregulation and downregulation of lipid metabolic processes and correlate with distinct changes in the lipid composition of the GC plasma membrane.
- For the first time, developmental and regenerative neurobiological lipidomic data were combined to find common lipid trends, species and processes that define the growth permissive state of growth cones in neurons.
- Researchers identified biophysical and chemical properties of the GC plasma membrane that undergo dramatic stage-specific changes, which actively contribute to membrane organization and function.
- Global correlation analysis of GCs with regenerating adult optic nerve revealed that, as a whole, proteomic and lipidomic data were not significantly correlated with each other. However, there were a number of individual proteins that significantly correlated with lipid species in specific lipid classes.

Methodology

Researchers worked with lab models at early fetal, early-mid fetal, and neonatal-early infancy growth cone developmental

stages. They integrated proteomic and lipidomic data to identify GC pathways, cell phenotypes, and lipid-protein interactions.

They then correlated data with separate studies of mice subjected to optic nerve crush, where regeneration was promoted by three independent approaches:

1) Proteomic profiling of GCs across fraction and developmental stage.

2) Similar analyses on mice with regenerating neurons in the optic nerves.

3) High-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of the proteome and lipidome from two growth cone fractions: growth cone membrane (GCM) and growth cone particulate (GCP). These fractions were generated through established differential centrifugation techniques with lipid and protein extraction.

Ultimately, a combined analysis of growth cone lipidome and proteome was compared with the group's regeneration study of optic nerve neurons in mice. This comparison led to the discovery of protein-protein and protein-lipid complexes common to both study groups of mice.

To analyze and integrate the large amounts of data, Bascom Palmer researchers, in collaboration with Dr. Samarah, then at the Florida Polytechnic University, developed an online computational and visualization tool. The web-based tool is called Neuronal Growth Cone Multi-Omics Insight (GC-Insights).

"Our analysis of mass spectrometry data heavily relied on

several new bioinformatics tools, such as lipid ontology tools, and statistical methods," Dr. Bhattacharya says. "For example, we used specific expression analysis (SEA) that utilized expression profiles of targeted neuronal cell types from bacTRAP mouse lines for identified proteomic data. SEA involved a cluster of neurons of layer 5a, layer 5b and layer 6 in the cortex for which bacTRAP data is available.

"The bioinformatics analyses have also taken advantage of existing genomic data in the Gene Expression Omnibus, as well as other resources, such as NCBI Sequence Read Archive. The analysis involved genomics (transcriptomics), proteomics and lipidomics analysis."

Researchers also identified possible molecules (proteins or lipids) that could be important in supporting regeneration in the adult central nervous system. To better elucidate the importance of lipid-correlated proteins in adult regeneration, University of Miami scientists examined how their expressions change in response to knockouts of genes with important roles in axonal regeneration.

Unique aspects of this research included 1) combining the study of both GC proteomes and lipidomes, 2) correlating this information with a separate study on regenerating optic nerve cells in mice, and 3) further integrating existing transcriptomics data in Gene Expression Omnibus and neuronal cell type specific bacTRAP databases.

Dr. Bhattacharya said, "These combined analyses used a plethora of new bioinformatics tools and databases. They provided confirmation that GC plasma expansion lipids correlate with lipids present during adult optic nerve

regeneration, as promoted by different pharmacological agents or other proteins/molecules approaches.

“We have established the importance of these complexes in both development and induced regeneration in the adult central nervous system. The protein-protein complexes and protein-lipid complexes we identified can be targeted in future testing to determine whether they promote regeneration in adults or not.”

Implications for Future Neuroregeneration Research

Dr. Bhattacharya said the insights derived from these analyses may help promote adult regeneration and functional innervation in devastating neurodegenerative diseases.

The conclusion of the article says, “Our analysis thus provides additional leads, molecular complexes and segments of pathways that affect human health and progressive neurodegenerative disease and trauma-induced degeneration. Our visualization may aid in interrogation of such protein and lipid pathways and potential interactions for others to generate their own hypotheses.”