Public Health Science Academy, summer 2023 research project
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Broad topic: Environmental exposure

Project title: Environmental factors affecting brain aging: altering neural cellular populations impacting normal functions.

Background: The environmental factors that we are exposed to during their lifetime, called the exposome, may interact with our genes to affect normal brain aging, but this interaction is poorly understood. Furthermore, exposure to environmental toxicants, such as heavy metals and metalloids, induces changes that affect gene expression without causing any genetic mutations. These epigenetic changes may affect the communication between brain cells. It is crucial that we improve our understanding to address these types of gene-environment interactions.

Environmental arsenic (As) is at the top of the list of environmental toxicants threatening human health (ATSDR 2017 Substance Priority List). Arsenic can easily pass brain barriers and accumulate. Low-level As in water has been associated with poor cognition in adults and induced changes in epigenetic markers. Nearly every cell type secretes substances enclosed by lipid membrane called extracellular vesicles (EVs). EVs contain cargo including lipids (fatty compounds), RNA, and proteins, and actively contribute to cell–cell communication[1]. EVs released by neurons have been shown to change the function of supporting glial cells in the brain[2]. A recent study showed EVs from As-exposed cell culture were enriched with oncogenic (causing cancer) factors. However, we do not understand the potential for As to significantly alter EV cargo in the central nervous system, impacting inflammation or neurodegeneration.

Research Hypothesis: We hypothesize that exogenous environmental factors induce complex pathophysiological mechanisms between neurons and glial cells through alterations in extracellular vesicle (EV) cargo, altering healthy brain aging, bioenergetics, and response to inflammatory stimuli.

Objective 1. Isolate extracellular vesicle (EV) from biological fluid and tissue to determine the impact of environmental determinants.

Rationale: Most EV studies have been performed by using cell lines or body fluids, but the number of studies on tissue-derived EVs is still limited. Furthermore, studies have shown the ability of EVs associated with the periphery to cross the blood brain barrier, possibly impacting brain function.

Approach: We will isolate EVs from animal models exposed to chronic human-relevant arsenic levels in drinking water. We will use the most advanced techniques to isolate EVs from plasma and brain for comparison of how the exposome impacts these different systems[3].

Objective 2. Characterization of extracellular vesicle (EV).

Rationale: EVs, include exosomes, ectosomes, microvesicles, microparticles, apoptotic bodies and other EV subset and it is currently technically challenging to obtain a totally pure EV fraction free from non-vesicular components for functional studies. We will use the minimal experimental requirements for characterization of EVs from the International Society for Extracellular Vesicles[4].

Approach: Isolated EVs from object 1 will undergo nanoparticle tracking analysis (NTA), electron microscopy and western blotting for EV specific proteins.

Objective 3. Initiate small RNA analysis of circulating small extracellular vesicle (EV) cargo.

Rationale: Small non-coding RNAs (18–23 nucleotides long) regulate gene expression at post-transcriptional level and are important in intercellular communication facilitated by EVs. Small non-coding RNAs in EVs released by neurons under stress can impact the response of glial cells in the brain. We have shown that small RNA's in plasma EVs can be a reliable biomarker for neurodegeneration in human patients[5]

Approach: We will initial bioinformatic analysis of small RNA sequencing to define arsenic induced changes in the profile of plasma and brain EV cargo.

References

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3 Konoshenko, M. Y., Lekchnov, E. A., Vlassov, A. V. & Laktionov, P. P. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. *Biomed Res Int* **2018**, 8545347, doi:10.1155/2018/8545347 (2018).

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