Elucidate environmental impact on the establishment of a persistent neurotoxic state via novel engineering tools

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Neurodegenerative disease (ND) is a debilitating neurological disorder characterized by progressive loss of neurons in central nervous system (CNS), resulting in the decline in memory, cognition and motor functions. Alzheimer's disease (AD) and Parkinson's disease (PD) are the two of the most prevalent NDs, affecting millions of individuals in the United States. While hundreds of genetic risk factors have been identified in association with ND, familial cases with genetic origin only account for 10% and 15% of diagnosed AD and PD incidences, respectively. The majority of ND cases occur sporadically. Mounting evidence from epidemiology studies suggests that environmental stressors are one of the key ND associated risk factors where exposure to environmental stressors leads to the on-set of ND years or decades later. Little is known about the molecular mechanism facilitating the establishment of the persistent and potentially permanent neurotoxic state after exposures, particularly at a developmental stage. Hence, there is a pressing need in understanding the cellular machineries involved in establishment of a persistent neurotoxic state resulting from early-life exposure to environmental toxins. Subcellular compartments are crucial for the maintenance of neuronal homeostasis. Alterations in various subcellular compartments, including the nucleus, mitochondria, and lysosomes, have been commonly noted in cases of AD and PD; and are believed to play a crucial role in the establishment of a persistent neurotoxic state. The primary goal of my thesis is thus to uncover the dysregulation in multiple subcellular compartments and their contributes to ND pathogenesis induced by early-in-life exposure to environmental stressors, including atrazine (ATZ), per-and polyfluoroalkyl substances (PFAS), and neurofibrillary tangles.

I started by developing live-cell compatible tools to track cellular and sub-cellular changes. Mitochondria DNA methylation is of particular interest, due to its potential regulatory role in the expression of electron transport chain (ETC) subunits and thus mitochondrial activity. Thus, I started expanding the mitochondria probe tool set by designing a novel probe targeting methylated CpGs of mitochondrial DNA (mtDNA). We demonstrated the capability of our probe to reveal spatial distribution of methylated mtDNA and capture mtDNA methylation change at single cell level. Combined with our previously developed probe for nuclear DNA methylation, we monitored mtDNA and nuclear DNA methylation simultaneously on the single-cell level where unsynchronized dynamics of DNA methylation from nucleus and mitochondria were discovered. Our tool offers a unique opportunity to understand epigenetic regulation of mtDNA and its dynamic response to microenvironment and cellular changes. Later, I further extended these efforts to develop in situ probes for tracking the formation of tau

aggregates based on fluorescence resonance energy transfer (FRET); and demonstrated the superior performance of our engineered probes compared to the current state-of-the-art.

I explored two neuronal culture systems, namely SH-SY5Y- and human induced pluripotent stem cell (hiPSC)-derived neurons; and their feasibility in studying neurotoxic effects of developmental exposure to environmental stressors. Specifically, I used SH-SY5Y derived neuron-like cells to study the impact of pre-differentiation exposure to PFOA, abundant chemical in environment due to its historical uses in consumer products and industrial applications. hiPSC-derived neurons were used to study the effects of developmental exposure to ATZ. Both studies identified cellular changes, for example neurite morphology and expression of enzyme catalyzing the production of neurotransmitters, that last after completion of differentiation. We also identified changes of pathogenic markers aligning with increased PD risks associated with developmental PFOA and ATZ exposure. Compared to SH-SY5Y, hiPSC-derived neurons were more advantageous due to their ability to recapitulate neuronal activity and pathogenic changes related to ND, and thus were used in my follow-up studies.

I adopted hiPSC derived neuron model to study the molecular mechanism of ND using established ND etiology. Patients with neurodegenerative disorders (ND) exhibit varying levels and temporal patterns of aggregated β-amyloid (Aβ) and tau protein. We exposed neurons derived from hiPSC with preformed fibrils (PFFs) of Aβ, tau and Aβ+tau, respectively. These treatments result in significant alterations in neurite network morphology, nuclear morphology, chromatin compactness and synaptic density. Interestingly, Aβ and tau fibrils seem to have opposite effects on mitochondrial membrane potential on neurites. Increased quantity of lysosomes was found in neurons treated with Aβ, tau and Aβ+tau, while decrease of lysosomal acidity was only observed in neurons treated with Aβ and tau sequentially. Collectively, our data suggests the potential synergy between Aβ and tau in establishing a neurotoxic state.

In summary, my thesis work has developed enabling engineering tools to monitor cellular and subcellular changes in neurons; identified hiPSC-derived neurons as a promising platform for studying developmental neurotoxicity; and paved the way towards understanding multi-etiology and its molecular underpinning for ND.