Project Title: Research on Neuron-glia Interactions in the Discovery of Novel Theranostic Exosomal Targets of Alzheimer's Disease(AD)

關於研究腦神經元與膠質細胞之相互作用及探索其應用於阿茲海默症的外泌體生物治療診斷標記

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A key hallmark of Alzheimer's disease (AD) is the accumulation of extracellular Aβ plaques causing neuroinflammation and neurotoxicity. Amyloid precursor protein (APP) is the precursor protein producing Aβ plaques, and its upregulation is identified in AD. Interesting finding is that the detection of APP C-terminal fragments (APP C-99) and Aβ plaques in neurons is highly associated with altered exosome secretion, function, and biophysics, which is an potential biomarker for AD pathogenesis. However, the contributions of APP and Aβ plaques to neurotoxicity via exosome production, release, and component, and the mechanisms evolved in these processes are unclear. Particularly, the application of exosomal biomarker for AD liquid biopsy has not been much studied.

In this project, the team first hypothesised that CD147, a neuroinflammation-related factor, and MCT1, a metabolic remodeling-related factor, in neuron and glia are co-regulated by Aβ plaques, inducing neurotoxicity partly via altered exosome component, production, release, and uptake. Thus, human SH-SY5Y neuroblastoma (NB) cells-differentiated neurons and human HMC3 microglia have been used to determine the effect of Aβ peptide on altered cellular and exosomal phenotypes.

Major achievements in this report are the establishment and validation of Aβ peptide-induced AD model of neuron and microglia, and their co-culture model of AD. When differentiated NB-derived neurons were exposed to low level of 500nM to 1 μM concentration of Aβ peptide, they began to show axon pathology and Wallerian degeneration, such as axon fragmentation and loss, which was detected by both beta-tubulin and APP immunostaining. Eventually, reduced neuron number was found by DAPI staining, further supporting Aβ-induced neurotoxicity in these models of AD.

Secondly, microglia by treatment with 1 μM Aβ peptide significantly changed their morphology into hypertrophic shape without many processes, indicating reactive status. Uptake of Aβ peptide by microglia and the formation of Aβ plaques in the extracellular space were clearly shown, indicating the significant role of microglia in Aβ-induced neurotoxicity and threshold reduction for the formation of Aβ plaques.

Importantly, the upregulation and membrane localisation of MCT1 and CD147 in microglia by uptake of Aβ and presence of Aβ plaques in their surrounding were detected by immunocytochemistry, indicating the close correlation between microglia MCT1/CD147 upregulation and Aβ plaque formation. Thus, MCT1/CD147 in microglia may contribute to Aβinduced neurotoxicity in AD by modulating neurometabolism and neuroinflammation in the legion.

As exosome release could be related with Aβ-induced neurotoxicity, the effect of Aβ peptide on exosome release from neurons and microglia were determined by nanoparticle tracking analysis (NTA). Small amount (e.g. 0.1-, 0.5-, 1-, and 10-nM) Aβ peptide treatment increased exosome release from both cells without significant axonopathy and gliosis, indicating that altered exosome release could be a much sensitive indicator for cellular toxicity. Size of exosome was in range of 160 to 200nm for both neurons and microglia. By treatment of Aβ peptide, exosome size was not changed.

Comprehensive analysis of neurons- and microglia-derived exosomes by treatment of various concentration of Aβ peptide to each cell type has been undergoing using NTA, TEM, ImmunogoldEM, AFM, Western blot, and ELISA for their application as biomarkers in AD liquid biopsy as well as research on their function in AD pathogenesis. It has been found that exosomes are capable of carrying chemokines, cytokines, misfolded proteins (e.g. Aβ plaques), and miRNA to induce oxidative stress and neuroinflammation. Furthermore, exosomes can carry APP and tau protein to induce the accumulation of amylogenic proteins in the neurons, indicating exosomes as toxicity mediators. Indeed, exosomes from 1 μM Aβ peptide-treated microglia induced axonopathy and neuronal apoptosis in NB-derived neurons.

Additionally, exosomes from 1 μM Aβ peptide-treated neurons significantly altered morphology of microglia and upregulated the expression and membrane localization of MCT1 and CD147 as detected by ICC, western blot, and RT-q-PCR, as well as the formation of Aβ aggregation/Aβ plaques, further indicating the crucial role in exosomes in AD pathogenesis through neuron-glia interactions.