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ANALYSING THE ROLE OF AUTOPHAGY IN ALZHEIMER'S DISEASE PATHOGENESIS USING THE ZEBRAFISH MODEL SYSTEM

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Abbreviations

Ab	Antibody
BSA	Bovine Serum Albumin
cDNA	Complementary Deoxyribonucleic acid
Ct	Cycle threshold
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid
gDNA	Genomic DNA
mg	Milligram
ml	Milliliter
mRNA	Messenger Ribonucleic acid
MW	Molecular Weight
nM	Nano molar
°C	Degree Celcius
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Room temperature
µg	Microgram
µL	Microliter
µM	Micromolar

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LIST OF PUBLICATIONS CONTRIBUTED TO DURING

Ph.D. CANDIDATURE

Identification and expression analysis of the zebrafish orthologues of mammalian *MAP1LC3* gene family.

Swamynathan Ganesan, Seyyed Hani Moussavi Nik, Morgan Newman, Michael Lardelli.

Experimental Cell Research, 2014

Hypoxia alters expression of Zebrafish Microtubule-associated protein Tau (*mapta, maptb*) gene transcripts.

Seyyed Hani Moussavi Nik, Morgan Newman, Swamynathan Ganesan, Mengqi Chen, Ralph Martins, Giuseppe Verdile and Michael Lardelli.

BMC Research Notes, 2014

Abstract

Alzheimer's disease (AD) is the most common form of dementia and is characterized by the formation of neuritic plaques and neurofibrillary tangles in the brain. The plaques are composed of β-amyloid peptides resulting from the cleavage of the Amyloid Precursor Protein (AβPP) by β-secretase and then by γ-secretase. Failure in clearance of these peptides results in the formation of these senile plaques. Autophagy is a degradative pathway in cells responsible for protein clearance and its dysfunction has been implicated in AD pathogenesis. Moreover, the PRESENILINs which are central in AβPP processing also have functional roles in autophagy. Other contributing factors of AD progression such as hypoxia, ER stress, and mitochondrial dysfunction are also related to autophagy.

Zebrafish embryos are a suitable system in which to monitor autophagy and investigate its implications for AD pathogenesis. Assays to monitor autophagy can be carried out efficiently in zebrafish embryos. In **Chapter II** of this thesis, we identified the zebrafish orthologues of the mammalian *MAP1LC3* gene family which is comprised of important proteins involved in autophagy. We identified two genes namely *map1lc3a* and *map1lc3b* through phylogeny and conserved synteny analysis. Using the LC3 immunoblot assay, we validated that the LC3II/LC3I ratio is significantly increased in the presence of rapamycin and sodium azide with chloroquine. This was used to confirm that hypoxia induces autophagy in 72 hpf zebrafish larvae. Similarly, results of qPCR assays also showed increased *map1lc3a* transcript levels in the presence of both rapamycin and sodium azide. However transcript levels of *map1lc3b* were reduced

under these same conditions. In **Chapter III** of this thesis, we used the LC3 immunoblot assay to monitor the effects of truncations of PRESENILIN proteins on autophagy. PRESENILINs are critical for the autophagy pathway but in our study, truncations of PRESENILIN proteins (zPsen1Δ4 and zPsen2Δ4) do not affect autophagy in zebrafish larvae. We also showed that rapamycin has the ability to induce autophagy in explanted zebrafish adult brains. In **Chapter IV**, we tested a chemical, Latrepirdine, to see whether it can induce autophagy in zebrafish larvae. Using the LC3 immunoblot assay and TEM analysis we showed that Latrepirdine at a 5µM concentration can induce autophagy in zebrafish larvae. As a continuation, in **Chapter V**, we tested two Latrepirdine-related drugs (Harmol and P7C3) on zebrafish larvae to observe whether they show similar effects. Both these drugs did not appear to have an effect on autophagy or apoptosis in 72 hpf zebrafish larvae. In **Chapter VI** we developed a novel assay based on poly-glutamine repeats fused to GFP (polyQ80-GFP) to monitor autophagy. Using this assay we showed that polyQ degradation in cells occur in an autophagy-dependent manner. This assay will be a useful tool to monitor autophagy in zebrafish larvae in future. In conclusion, zebrafish serves as an excellent tool to analyze autophagy. Various assays to monitor autophagy can be efficiently carried out using zebrafish embryos.