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Abstract

Introduction and aims: The accumulation of misfolded Aβ and phospho-tau are characteristic features of AD pathology. One of the cellular mechanisms responsible for the removal of these aggregates is autophagy, where proteins and organelles are degraded through the formation of autolysosomes. Nutrient status regulates autophagy through crosstalk between several signalling pathways including mTOR and AMPK. Under fed conditions, BCAAs stimulate mTOR activity and downregulate autophagy. Conversely, under stress such as starvation, mTOR is inhibited and autophagy is initiated. In AD, autophagy is considered to be dysregulated and contributes to the build-up of misfolded proteins. As the hBCAT proteins, which metabolise BCAAs, are significantly up-regulated in AD brain, it is important to understand if this increase impacts mTOR and autophagy. Understanding of these mechanisms will offer insight into the clearance of protein aggregates and pathology underpinning AD.

Methods: Using molecular biological investigations, western blot analysis and confocal microscopy, the impact of increased hBCAT expression in neuronal cells was determined in response to nutrient and hormonal stimuli.

Results and discussion: This work demonstrates for the first time the impact of hBCAT overexpression on the autophagy and mTOR pathways. hBCAT overexpression resulted in an increase in mTOR activation, whereas autophagy was significantly increased at the lower concentrations of overexpression plasmid but decreased at the higher concentrations. Furthermore, hBCAT overexpression reduced the level of Aβ in a concentration-dependent manner. These findings indicate a concentration-dependent role for hBCAT in autophagy, consequently impacting Aβ load. The association of hBCATc with the membrane in response to insulin signalling is likely to play a role in this mechanism. Co-immunoprecipitation studies showing that PDI, the protein disulphide isomerase responsible for protein folding, and hBCAT interact offer additional novel roles for hBCAT in protein folding. Although this mechanism requires further interpretation, we anticipate that through its redox-active CXXC motif, hBCAT operates as a bifunctional enzyme switching between regulation of metabolic pathways such as mTOR and protein folding pathways, involving PDI. Developing the understanding of hBCAT’s role in the brain, in the context of AD, reveals new insights into the dysregulated pathways such as mTOR and autophagy. A greater understanding of these pathways has the potential to provide new therapeutic strategies in the future.
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Abbreviations

Aβ - Amyloid-β peptide
AD - Alzheimer's disease
APOE - Apolipoprotein E
APS - Ammonium persulphate
APP - Aβ precursor protein
ATP - Adenosine triphosphate
AV - Autophagic vacuole
BCAA - Branched-chain amino acids
BCKD - Branched-chain α-keto acid dehydrogenase
BSA - Bovine serum albumin
CMA - Chaperone-mediated autophagy
DAPI - 4',6-Diamidino-2-phenylindole
DTT - Dithiothreitol
EBSS - Earle's balanced salt solution
EDTA - Ethylenediaminetetraacetic acid
ER - Endoplasmic reticulum
ERK1/2 - Extracellular signal-regulated protein kinases
GABA - Gamma-aminobutyric acid
GDH - Glutamate dehydrogenase
GLUT - Glucose transporter
Grx - Glutaredoxin
GSNO - S-nitrosoglutathione
GSH - Glutathione reduced

GSSH - Glutathione oxidised

hBCATc - Human branched-chain aminotransferase (cytosolic isoform)

hBCATm - Human branched-chain aminotransferase (mitochondrial isoform)

HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HRP - Horseradish peroxidase

IMR-32 - Human neuroblastoma cell line

LIR - LC3 interacting region

IMS - Intermembrane space

IPTG - Isopropyl β-D-1-thiogalactopyranoside

IR - Insulin receptor

IRS1 - Insulin receptor substrate 1

KIC - Ketoisocaproate

LRS - Leucyl-tRNA-synthetase

MAP4K3 - Mitogen-activated protein kinase kinase kinase kinase 3

mTOR - Mammalian target of rapamycin

mTORC1 - mTOR complex 1

mTORC1 - mTOR complex 2

NMDA - N-methyl-D-aspartic acid

NO - Nitric oxide

PAT1 - Proton-assisted amino acid transporter

PDI - Protein disulphide isomerase

PE - Phosphatidylethanolamine
PI3P - Phosphatidylinositol-3-phosphate

PIP3 - Phosphatidylinositol(3,4,5)phosphate-3

PKB - Protein kinase B (Akt)

PKC - Protein kinase C

PLP - Pyridoxal phosphate

RIPA - Radioimmunoprecipitation assay buffer

RPMI - Roswell park memorial institute medium

SDS - Sodium dodecyl sulphate

SNO-PDI - S-nitrosylated PDI

SNOs - S-nitrothiols

TBST - Tris-buffered saline/tween

TCA - Trichloroacetic acid

TEMED - Tetramethylethylenediamine

Trx - Thioredoxin

TSC1/2 - Tuberin sclerosis complex 1/2