BMB
Reports

BMB Rep. 2024; 57(6): 281-286
www.bmbreports.org

Integrative analysis of microRNA-mediated mitochondrial dysfunction in hippocampal neural progenitor cell death in relation with Alzheimer's disease

A Reum Han[1,3,4], Tae Kwon Moon[2], Im Kyeung Kang[2], Dae Bong Yu[2], Yechan Kim[2], Cheolhwan Byon[2], Sujeong Park[2], Hae Lin Kim[2], Kyoung Jin Lee[2,5], Heuiran Lee[2,5], Ha-Na Woo[3,5,] & Seong Who Kim[3,4,5,*]*

[1]Department of Translational Medicine, [2]Department of Microbiology, [3]Department of Biochemistry and Molecular Biology, Asan Medical Institute of Convergence Science and Technology, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, [4]Stem Cell Immunomodulation Research Center, University of Ulsan College of Medicine, Seoul 05505, [5]Bio-Medical Institute of Technology, University of Ulsan College of Medicine, Seoul 05505, Korea

Adult hippocampal neurogenesis plays a pivotal role in maintaining cognitive brain function. However, this process diminishes with age, particularly in patients with neurodegenerative disorders. While small, non-coding microRNAs (miRNAs) are crucial for hippocampal neural stem (HCN) cell maintenance, their involvement in neurodegenerative disorders remains unclear. This study aimed to elucidate the mechanisms through which miRNAs regulate HCN cell death and their potential involvement in neurodegenerative disorders. We performed a comprehensive microarray-based analysis to investigate changes in miRNA expression in insulin-deprived HCN cells as an *in vitro* model for cognitive impairment. miR-150-3p, miR-323-5p, and miR-370-3p, which increased significantly over time following insulin withdrawal, induced pronounced mitochondrial fission and dysfunction, ultimately leading to HCN cell death. These miRNAs collectively targeted the mitochondrial fusion protein OPA1, with miR-150-3p also targeting MFN2. Data-driven analyses of the hippocampi and brains of human subjects revealed significant reductions in OPA1 and MFN2 in patients with Alzheimer's disease (AD). Our results indicate that miR-150-3p, miR-323-5p, and miR-370-3p contribute to deficits in hippocampal neurogenesis by modulating mitochondrial dynamics. Our findings provide novel insight into the intricate connections between miRNA and mitochondrial dynamics, shedding light on their potential involvement in conditions characterized by deficits in hippocampal neurogenesis, such as AD. [BMB Reports 2024; 57(6): 281-286]

*Corresponding authors. Seong Who Kim, Tel: +82-2-3010-4270; Fax: +82-2-477-4266; E-mail: swhokim@amc.seoul.kr; Ha-Na Woo, Tel: +82-2-3010-2918; Fax: +82-2-3010-4259; E-mail: woohn101@gmail.com

https://doi.org/10.5483/BMBRep.2023-0167

Received 8 September 2023, Revised 23 September 2023, Accepted 28 November 2023, Published online 22 February 2024

Keywords: Adult hippocampal neurogenesis, Bioinformatics, Hippocampal neural stem cell, microRNA, Mitochondria, Neurodegenerative disease

INTRODUCTION

Neurogenesis, defined as the formation of neurons *de novo*, persist during the embryonic developmental period and throughout the entire life of mammals (1). Hippocampal neural stem (HCN) cells proliferate, maintaining the stem cell population and differentiating into new neurons, astrocytes, and oligodendrocytes, which are continuously incorporated into dentate gyrus to replace dead or damaged ones. This process maintains hippocampus-related functions, including learning, memory, and emotional regulation (2). However, the process is gradually impaired over the course of aging, and Kroemer *et al*. proposed stem cell exhaustion as one of the hallmarks of aging (3). The impairment of HCN cells is even more pronounced in patients with neurodegenerative diseases (4). The hippocampus is mainly associated with cognitive function and is one of the regions most affected in patients with Alzheimer's disease (AD). Moreno-Jiménez *et al*. revealed that the number of stem cells markedly declined in AD patients and further reduced as the disease advanced (5). Thus, current research is actively exploring the regulation of adult neurogenesis to prevent its impairment (6).

MicroRNAs (miRNAs), a type of short non-coding RNAs, repress gene expression by inhibiting translation and/or promoting the degradation of target mRNAs (7). miRNAs function as fine-tuners in the regulation of cellular physiology. They have been found to be related to various neuropathological processes, such as oxidative stress, interneuron connectivity, and synaptic plasticity (8). The dysregulation of miRNAs is known to affect hippocampal interneuron function and memory in mice (9), and altered levels of miRNAs have been reported in many neurodegenerative disorders in human and animal models (10). These findings suggest that miRNA-regulated pathways might

ISSN: 1976-670X (electronic edition)

play an important role in neurological disorders. While miRNAs are also reported to play crucial roles in neurogenesis (11), their relevance to the mechanisms underlying neurodegenerative disorders remains unclear. Further studies are needed to elucidate this aspect.

We previously investigated the expression levels of miRNAs in adult rat HCN cells induced for autophagic cell death after insulin withdrawal (12). These cells were isolated from the adult rat hippocampus by Gage *et al*. and were demonstrated to proliferate and differentiate into mature neurons or glial cells, serving as an *in vitro* model for investigating neuronal stem cell biology (13). Exogenous factors, such as fibroblast growth factor and insulin, regulate their differentiation and proliferation. Previous studies showed that insulin withdrawal led them to increase autophagy-dependent cell death (14, 15). Seong-Woon Yu *et al*. demonstrated that triggering autophagy-dependent cell death in mice HCN cells not only repressed hippocampal neurogenesis but also resulted in cognitive and mood impairments (16). Based on these results, we propose that inducing autophagic death in HCN cells through insulin withdrawal could potentially serve as an *in vitro* model for cognitive deficits like neurodegenerative disorders. Expanding upon our previous study, which was limited to a small set of miRNA arrays (12), we conducted a comprehensive microarray analysis to investigate alterations in miRNA expression at multiple time points during HCN cell death triggered by insulin withdrawal. Our study results demonstrate the pathological mechanism of miRNAs in inducing HCN autophagic cell death and provide insight into their potential roles as biomarkers of AD.

RESULTS AND DISCUSSION

Microarray analysis reveals significant changes in miRNA expression in HCN cells following insulin withdrawal

We conducted a comprehensive miRNA array analysis using Genechip, which interrogates 728 mature rat miRNAs and 490 pre-miRNAs annotated from miRBase Release 20 to investigate the sequential changes in miRNA expression during HCN cell death. In our previous study, we observed cytotoxic effects in HCN cells within 24 hours of insulin withdrawal, leading to cell death around 48 hours (12). This promoted us to subsequently assess miRNA expression at intervals of 0, 12, 24, and 48 hours after insulin withdrawal. The quality of the miRNA array was assessed by analyzing density and box plots and measuring the correlation matrix between samples (Supplementary Fig. 1). The heatmap shown in Fig. 1A displays the mean fold-increase or decrease in miRNA expression at 12, 24, and 48 hours after insulin withdrawal compared to controls. Principal component analysis (PCA) of miRNAs differentially expressed during HCN cell death revealed distinct differences in certain miRNAs at each time point (Fig. 1B). The miRNAs were sorted according to their high variance value, and the top 10 miRNAs with the highest variance over time were selected (Supplementary Fig. 2). We performed integrated target prediction analysis using miRWalk and validated the target genes of these 10 miRNAs (selected based on a miRWalk score $\geq$ 1.00) to gain further insight. Subsequently, these target genes were used as input for ontology analysis using the Enrichr metadata searching tool. As shown, their shared functional roles included GTPase regulator activity, protein serine/threonine kinase activity, L-amino acid transmembrane transporter activity, and more (Fig. 1C). The findings suggest that miRNAs whose expression is elevated in HCN cell damage may be involved in regulating intracellular signaling, particularly in cellular events including GTPases.

Upregulated miRNAs lead to the induction of mitochondrial dysfunction, mitochondrial fission, and cell death

Among the 10 miRNAs, we focused on miR-150-3p, miR-323-5p, and miR-370-3p, as their targets are associated with mitochondrial fission, a mechanism we previously linked to HCN cell death without insulin (17). To identify the direct causal relationship between increases in miRNAs and HCN cell death, we transfected HCN cells with miR-150-3p, miR-323-5p, and miR-370-3p. Propidium iodide (PI) staining demonstrated a decrease in the viability of cells treated with these miRNAs, whereas the control group showed no significant change in cell viability (Fig. 2A, B). The cells transfected with miR-150-3p, miR-323-5p, or miR-370-3p all exhibited substantial cell death

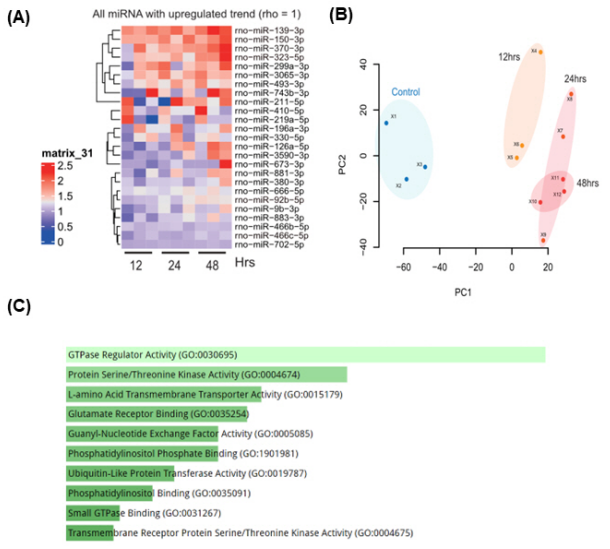

Fig. 1. Profiling of miRNAs differentially regulated in damaged HCN cells. (A) Heatmap of miRNA array data with hierarchical clustering. Color scale illustrates relative expression levels of each miRNA: red represents increased miRNA expression, blue represents decreased miRNA expression. (B) Principal component analysis (PCA) showing the variability between the control group and the insulin-deficient sample at different times of treatment (n = 3, each group). (C) Gene ontology (GO) analysis using the top 10 miRNA target genes obtained from the miRWalk algorithm.

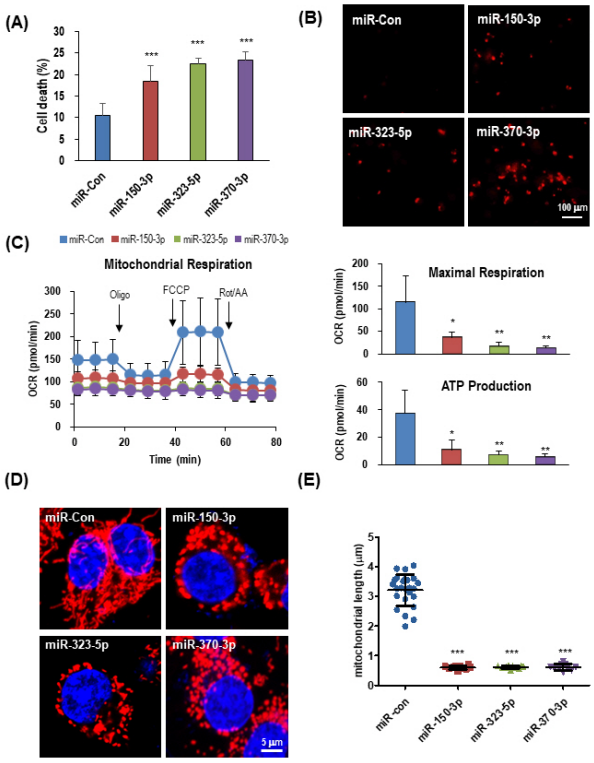

Fig. 2. Effects following overexpression of up-regulated miRNAs. (A) Assessment of cell death in HCN cells treated with miR-150-3p, miR-323-5p and miR-370-3p, measured by PI staining (n > 3). (B) Representative images of PI stained cells after miRNAs transfection. (C) Oxygen consumption rate (OCR), maximal respiration and ATP production indices derived from Seahorse Mito Stress analysis. Analysis of mitochondrial morphology (D) and mitochondrial length (E) affected by miRNAs (n > 15). Data represented as mean ± SD and statistical significance was analyzed by Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

rates exceeding 18% (Fig. 2A, B, Supplementary Fig. 3).

We analyzed mitochondrial function to elucidate the mechanism of miRNA-induced HCN death. The oxygen consumption rate (OCR) test results in both the basal and overall mitochondrial function of the miRNA-transfected groups were significantly reduced compared to the control group (Fig. 2C). When treated with FCCP, the miRNA-transfected groups exhibited significantly lower OCR increments compared to the control group, indicating reduced maximal respiration. Following oligomycin treatment, the miRNA-transfected groups showed pronounced decreases from basal levels, indicating reduced ATP production. We also examined mitochondrial integrity using mitotracker and found that mitochondrial fission was significantly more prominent in the miRNA-transfected groups than in the control group (Fig. 2D). The average mitochondrial length in the miR-150-3p, miR-323-5p, and miR-370-3p groups was 0.6 μm in each, significantly shorter than the control group's average of 3.2 μm (Fig. 2E).

Consistent with these results, our previous study demonstrated that miRNA-351-5p induced mitophagic cell death in HCN cells (17). Additionally, Ash *et al*. demonstrated that miRNA-137, which we also observed to be elevated (data not shown), enhanced mitochondrial fusion and fission in human neural stem cells (18). These findings support that miRNAs regulate stem cell fate by modulating mitochondrial homeostasis. However, our understanding of the mechanisms of miRNAs regulating hippocampal neurogenesis is very limited. Here, we demonstrated that the dysregulation of mitochondrial dynamics and function is an important process contributing to defects in hippocampal integrity.

miRNAs upregulated after insulin withdrawal commonly target OPA1 and MFN2

We refined our focus on their ability to induce mitochondrial fission (Fig. 2D, E) and their potential association with GTPase regulator activity from gene ontology (GO) pathway analysis of the miRNA array results in Fig. 1C to identify the target proteins of upregulated miR-150-3p, miR-323-5p, and miR-370-3p. We identified a total of 117 integrated target genes of these 3 miRNAs through miRWalk analysis by selecting genes with a miRWalk score ≥ 1.00 (Fig. 3A). Among these shared genes, we observed a notable mitochondrial fusion-related gene, OPA1 (optic atrophy protein 1) (Fig. 3A). miR-150-3p was also found to target another mitochondrial fusion-related gene, MFN2 (mitofusin2). The 3′UTR of MFN2 contains one matching sequence to the seed sequence of miR-150-3p (Fig. 3B). The 3′UTR of wild-type (WT) or mutant-type (MT) MFN2 with 7-nt mismatches was inserted into psiCHECK2 downstream of the luciferase coding sequence. The actual binding activity was quantitatively determined through a luciferase assay. miR-150-3p suppressed luciferase expression, resulting in a 30% reduction compared to the miR-Con group, revealing its direct binding to the 3′UTR of MFN2 (Fig. 3C). Reverse transcription-quantitative polymerase chain reaction (qRT-PCR) was performed to quantify the expression of the target gene in each miRNA-transfected cell and verify the authenticity of the above microarray analysis (Fig. 3D, E). Consistent with the miRWalk analysis results, all miR-150-3p, miR-323-5p, and miR-370-3p-transfected cells exhibited a low expression of OPA1, and miR-150-3p-transfected cells also showed decreases in the expression of MFN2. The 3′UTR of OPA1 harbors multiple and remotely separated matching sequences for miR-150-3p, miR-323-5p, and miR-370-3p. Thus, direct evidence of miR-150-3p, miR-323-5p, and miR-370-3p binding to the 3′UTR of OPA1 has not been established. However, as depicted in Fig. 3E, the mRNA expression of OPA1 was substantially decreased in response to miR-150-3p, miR-323-5p, and miR-370-3p, indicating the potential for these miRNAs to bind to OPA1.

Mitochondria are highly dynamic structures that continuously undergo fusion and fission (19). The significant role of these miRNAs in regulating mitochondrial dynamics becomes appa-

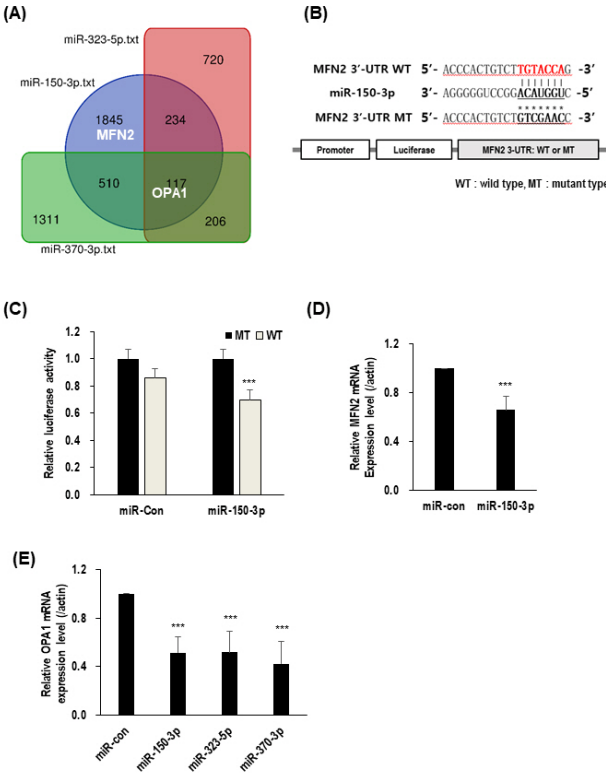

Fig. 3. Target prediction and validation of miRNA-target axis. (A) Venn diagram of common targets for miR-150-3p, miR-323-5p and miR-370-3p. (B) Sequence alignment of miR-150-3p in 3′UTR of MFN2 and (C) direct targeting activity of miR-150-3p by luciferase reporter assay. RT-qPCR analysis of target mRNA MFN2 (D) and OPA1 (E) after transfection with each miRNA. ***P < 0.001.

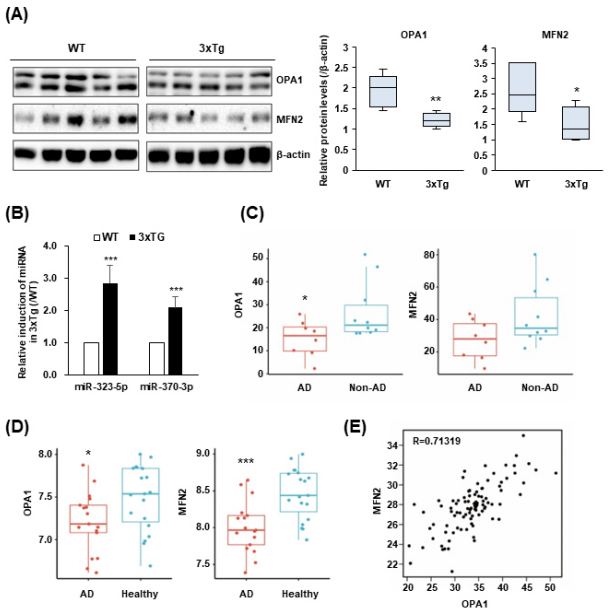

Fig. 4. *In vivo* validation of expression level of miRNAs and their target proteins and genes in the Alzheimer models. (A) Western blot image demonstrates protein expression levels of OPA1 and MFN2 in 3xTg and WT mice. (B) RT-qPCR of miR-323-5p and miR-370-3p in 3xTg and WT mice. (C) Expression level of OPA1 and MFN2 in GSE173955, which contains RNA-seq data from the hippocampus of AD patients (n = 8) and controls (n = 10). The P-value for OPA1 expression is 0.031, while that for MFN2 is 0.058. (D) Expression level of OPA1 and MFN2 in GSE183260, which includes microarray data from the brain of AD patients (n = 19) and healthy controls (n = 21). The P-value for OPA1 expression is 0.029, while that for MFN2 is 0.00042. (E) Correlation matrix of OPA1 and MFN2 from the hippocampus of human brain RNA-seq data in the Allen brain Atlas (n = 94) using Pearson's method. The Pearson's correlation coefficient, denoted by R. *P < 0.05, **P < 0.01, ***P < 0.001.

rent through their substantial involvement in promoting excessive mitochondrial fission, which ultimately leads to HCN cell death. We identified that these miRNAs can target OPA1 and MFN2, which both have a highly conserved dynamin GTPase domain and are essential mediators of mitochondrial membrane fusion (20). Although the underlying mechanisms remain to be elucidated, it is noteworthy that both OPA1 and MFN2 are involved in regulating mitochondrial respiratory chain coupling and oxidative phosphorylation (21, 22), consistent with the results in Fig. 2C. OPA1 and MFN2 have been implicated in families with autosomal dominant optic atrophy and axonal Charcot-Marie-Tooth disease type 2A, respectively, both characterized by neurological deficits (20). Missense mutations in OPA1 exhibit neurodegenerative features, such as parkinsonism and dementia (23), reinforcing the possibility of miR-150-3p, miR-323-5p, and miR-370-3p involvement in neurodegenerative disorders. These findings support the hypothesis that OPA1 and MFN2 could be the integrated target proteins of miR-150-3p, miR-323-5p, and miR-370-3p, leading to HCN cell death.

However, it is reasonable to anticipate that other miRNAs might similarly impact HCN cell death by modulating intracellular signaling pathways through phosphorylation, as shown in the second line in Fig. 1C. This assertion gains support from studies demonstrating that the phosphorylation of p62 by AMP-activated protein kinase and the phosphorylation of ULK1 by GSK3B led to HCN autophagic cell death (24). However, a comprehensive investigation is needed to thoroughly explore this aspect.

OPA1 and MFN2 are significantly reduced in patients with Alzheimer's disease

We then examined whether miR-150-3p, miR-323-5p, and miR-370-3p and their target proteins we identified were up- or downregulated in AD disease models in which hippocampal neurogenesis was notably defective (5, 6). Thus, we investigated the expression of miRNAs and proteins in the brain tissue of triple-transgenic AD (3xTg-AD) model mice, a widely used model of AD (25), compared to WT mice. Both OPA1 and

MFN2 were downregulated in 3xTg-AD mice (Fig. 4A), whereas miR-323-5p and miR-370-3p showed more than 2-fold increases in 3xTg-AD mice compared to WT (Fig. 4B). These findings are consistent with our *in vitro* results. We also investigated the gene expression levels of OPA1 and MFN2 in human samples by analyzing an available open-source database. Through bioinformatic analysis of the GEO dataset (GSE173955), which included RNA sequencing (RNA-seq) data extracted from hippocampal tissues, we found a significant downregulation of OPA1 and MFN2 expression in the AD group compared to the non-AD group (Fig. 4C). Similarly, our investigation of the GSE183260 dataset, which included a microarray of whole brain tissues, demonstrated a significant reduction in both OPA1 and MFN2 gene expression in the AD group (Fig. 4D). A significant positive correlation was found between the expression levels of OPA1 and MFN2 in RNA-seq data from the hippocampi of human brains donated to the Allen Brain Atlas (Fig. 4E). Taken together, these findings imply a strong functional proximity between OPA1 and MFN2 within the hippocampus, reinforcing our findings that the upregulation of miRNAs targeting OPA1 and MFN2 could potentially contribute to the impairment of hippocampal neurogenesis, particularly in relation to AD.

Given the limitations in studying hippocampal neurogenesis *in vivo*, we further extended our analysis to a human brain database to support our findings. However, data-based studies also have limitations, not only focused on hippocampal neural progenitor cells but also included whole brain or hippocampal neurons. More investigations are required to show a direct causal relationship between elevated miR-150-3p, miR-323-5p, and miR-370-3p and defects in hippocampal stem cells, leading to AD aggravation.

MATERIALS AND METHODS

Materials and methods are available in supplementary material.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research program through the National Research Foundation of Korea (NRF) funded by the ministry of Education (NRF-2018R1A5A2020732 to S.W.K., 2021R1I1A1A01048638 to H.N.W., and 2021R 1A2C1093614 to H.L.). And this research was also supported by a grant of the MD-Phd/Medical Scientist Training Program through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Boldrini M, Fulmore CA, Tartt AN et al (2018) Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell 22, 589-599 e585
2. Toda T, Parylak SL, Linker SB and Gage FH (2019) The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry 24, 67-87
3. Lopez-Otin C, Blasco MA, Partridge L, Serrano M and Kroemer G (2013) The hallmarks of aging. Cell 153, 1194-1217
4. Terreros-Roncal J, Moreno-Jimenez EP, Flor-Garcia M et al (2021) Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. Science 374, 1106-1113
5. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J et al (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med 25, 554-560
6. Salta E, Lazarov O, Fitzsimons CP, Tanzi R, Lucassen PJ and Choi SH (2023) Adult hippocampal neurogenesis in Alzheimer's disease: a roadmap to clinical relevance. Cell Stem Cell 30, 120-136
7. Pritchard CC, Cheng HH and Tewari M (2012) MicroRNA profiling: approaches and considerations. Nat Rev Genet 13, 358-369
8. McNeill E and Van Vactor D (2012) MicroRNAs shape the neuronal landscape. Neuron 75, 363-379
9. Daswani R, Gilardi C, Soutschek M et al (2022) MicroRNA-138 controls hippocampal interneuron function and short-term memory in mice. Elife 11, e74056
10. Juźwik CA, S. Drake S, Zhang Y et al (2019) microRNA dysregulation in neurodegenerative diseases: a systematic review. Prog Neurobiol 182, 101664
11. Cheng LC, Pastrana E, Tavazoie M and Doetsch F (2009) miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. Nat Neurosci 12, 399-408
12. Park S, Lee SHS, Lee WI et al (2019) MicroRNA expression profiling of adult hippocampal neural stem cells upon cell death reveals an autophagic cell death-like pattern. Biochem Biophys Res Commun 509, 674-679
13. Gage FH, Coates PW, Palmer TD et al (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci U S A 92, 11879-11883
14. Yu SW, Baek SH, Brennan RT et al (2008) Autophagic death of adult hippocampal neural stem cells following insulin withdrawal. Stem Cells 26, 2602-2610
15. Ha S, Ryu HY, Chung KM, Baek SH, Kim EK and Yu SW (2015) Regulation of autophagic cell death by glycogen synthase kinase-3beta in adult hippocampal neural stem cells following insulin withdrawal. Mol Brain 8, 30
16. Jung S, Choe S, Woo H et al (2020) Autophagic death of neural stem cells mediates chronic stress-induced decline of adult hippocampal neurogenesis and cognitive deficits. Autophagy 16, 512-530
17. Woo HN, Park S, Kim HL et al (2021) miR-351-5p/Miro2 axis contributes to hippocampal neural progenitor cell death via unbalanced mitochondrial fission. Mol Ther Nucleic Acids 23, 643-656

18. Channakkar AS, Singh T, Pattnaik B, Gupta K, Seth P and Adlakha YK (2020) miRNA-137-mediated modulation of mitochondrial dynamics regulates human neural stem cell fate. Stem Cells 38, 683-697
19. Gao S and Hu J (2021) Mitochondrial fusion: the machineries in and out. Trends Cell Biol 31, 62-74
20. Burte F, Carelli V, Chinnery PF and Yu-Wai-Man P (2015) Disturbed mitochondrial dynamics and neurodegenerative disorders. Nat Rev Neurol 11, 11-24
21. Yao CH, Wang R, Wang Y, Kung CP, Weber JD and Patti GJ (2019) Mitochondrial fusion supports increased oxidative phosphorylation during cell proliferation. Elife 8, e41351
22. Mishra P, Carelli V, Manfredi G and Chan DC (2014) Proteolytic cleavage of OPA1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation. Cell Metab 19, 630-641
23. Liskova P, Ulmanova O, Tesina P et al (2013) Novel OPA1 missense mutation in a family with optic atrophy and severe widespread neurological disorder. Acta Ophthalmol 91, e225-231
24. Ha S, Jeong SH, Yi K et al (2017) Phosphorylation of p62 by AMP-activated protein kinase mediates autophagic cell death in adult hippocampal neural stem cells. J Biol Chem 292, 13795-13808
25. Oddo S, Caccamo A, Shepherd JD et al (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409-421