ROLE AND THERAPEUTIC POTENTIAL OF miRNAs TARGETING BACE-1 OR TAU IN ALZHEIMER DISEASE.

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INTRODUCTION

First described in 1993 by Ambros and colleagues, miRNAs are 21-23 nucleotide-long non-coding RNAs, which have been found to be crucial for proper gene regulation, usually at a post- transcriptional level. Since their discovery back in 1993 and hitherto numerous miRNAs have been reported to be essential for proper CNS development and function maintenance. Consequently, their deregulation may lead to neuronal dysfunction and therefore promote the initiation and/or progression of neurological disorders (NDDs) like Alzheimer Disease.

Alzheimer Disease (AD) is a chronic neurodegenerative disease which represents the most habitual cause of dementia in the US and Europe. Its main molecular hallmarks are the following two: presence of amyloid plaques (also known as senile plaques) principally composed of Aß40 and Aß42 peptides, and neurofibrillary tangles (NFTs) mainly consisting of hyperphosphorylated tau protein (Amemori et al., 2015).

In this review, my main aims are the following ones:

(1) Discuss how some specific miRNAs contribute to AD progression by targeting either BACE-1 or tau mRNAs.
(2) Analyze some therapeutic approaches, which attempt to palliate AD symptomatology by either restoring or else overexpressing specific miRNAs.

METHODS

In outline, in first place relevant articles were searched on the DDBBs of the NCBI as well as Nature Publications. Secondly, I thoroughly read through the selected ones and summarized the important information on *Excel* files.

I subsequently proceeded to write the review and select the relevant figures in order to make it more intelligible.

Finally, the poster was designed and new figures were built to help understand the relevant information displayed.

BACE-1

MiR-188-3p, which targets BACE-1, is downregulated in AD patients.

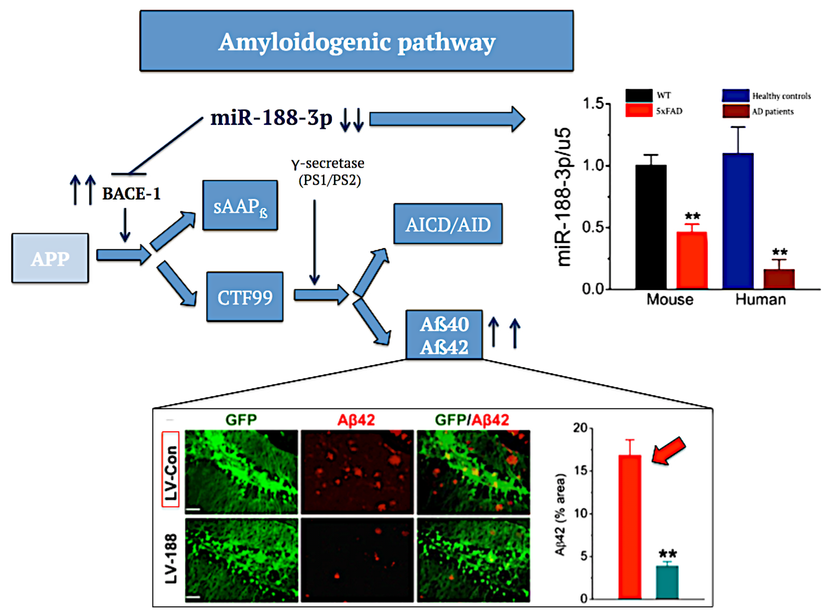

Figure 1: **MiR-188-3p downregulation in AD patients leads to increased BACE-1 protein levels and consequently, incremented Aß42 production**. In this figure a schematic representation of the amyloidogenic pathway is displayed, where miR-188-3p is shown to repress BACE-1 by directly targeting its mRNA. The graphic presented on top (left side) demonstrates that miR-188-3p is found downregulated in both human AD patients and 5xFAD mice compared to healthy controls. In the immunostaining experiment, it can be clearly seen that Aß42 is augmented in LV-scramble treated mice compared to LV-miR-188-3p-treated 5XFAD mice. This miRNA is just one of many regulating BACE-1 protein levels, which are also commonly found downregulated in AD patients. Some of these would include: miR-107, the miR-29 family and miR-124.

Adapted from: (Zhang et al., 2014)

MiR-188-3p overexpression significantly improves spatial memory.

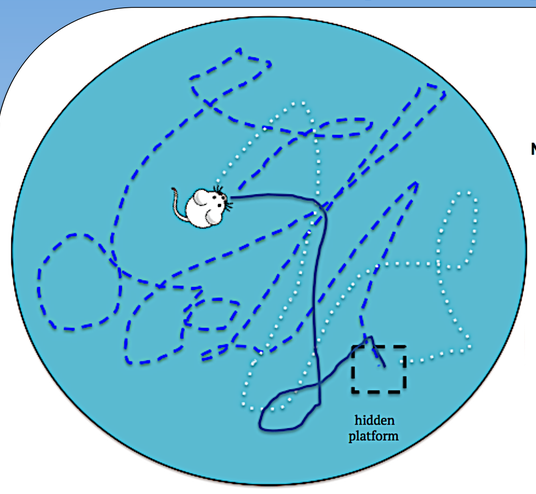

Figure 2: LV-miR-188-3p treatment significantly improves spatial memory in 5XFAD mice. Spatial memory was assessed through the Morris water test. Latency (sec) and distance (cm) are the two parameters exhibited on the right side for each group tested. LV-miR-188-3p-treated 5XFAD mice performed in a similar way to WT-LV-scramble-treated mice.
MiR-188-3p overexpression did not alter sensorimotor function or other behavioral patterns (assessed through rotarod and open field tests – data not shown here).

Inspired in: (Zhang et al., 2014)

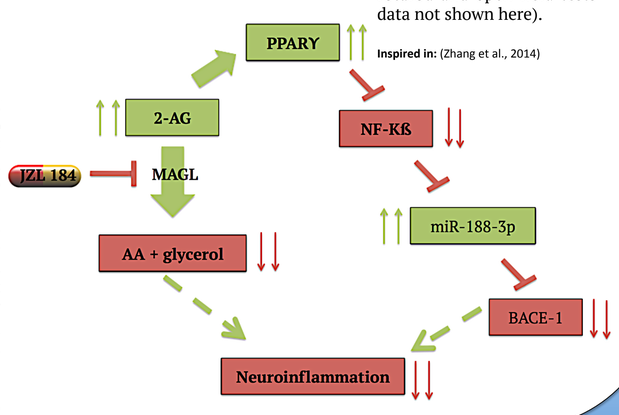

Figure 3: A novel therapeutic approach: restoring endogenous miR-188-3p levels using the MAGL inhibitor JZL 184. I personally deem this therapeutic approach has a great potential, since it does not rely on any vector delivery system to increase miR-188-3p levels. Actually, JZL 184-treated 5XFAD mice showed similar miR-188-3p levels compared to vehicle-treated WT mice (data not shown here). In outline, JZL 184 inhibits MAGL, and hence increases 2-AG levels, which in turn promotes PPARγ activation. This latter fact leads to repression of the NF-Kß pathway, which further enhances miR-188-3p synthesis. Finally, this miRNA represses BACE-1 expression and as a result reduces neuroinflammation.

Inspired in: (Zhang et al., 2014)

AD progression

MiR-132/212 depletion affects long-term memory.

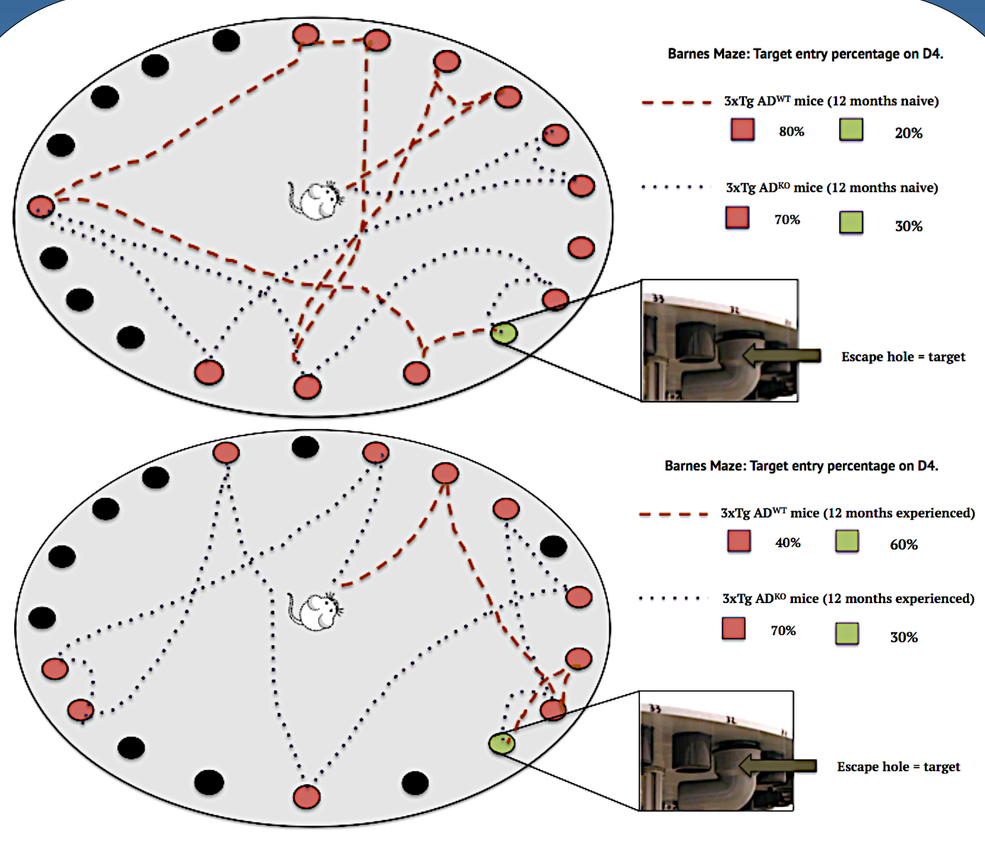

Figure 4: MiR-132/212 depletion impairs long-term memory. In this study, the Barnes maze was employed to assess spatial memory in 3xTg AD^{WT} mice and 3xTg AD^{KO} mice (which lack the miR-132/212 *locus*). As illustrated above, no significant differences were found between both groups using 12-month-old naive mice. However, such differences proved to be relevant when testing 12-month-old experienced mice. In this figure, only one parameter is shown: target entry. Needless to say, other parameters such as latency or path efficiency were also measured. Results obtained suggest this miRNA bicistronic *locus* plays a pivotal role in long-term memory.

Inspired in: (Smith et al., 2015)

Restoring miR-132 levels improves long-term memory and reduces tau phosphorylation.

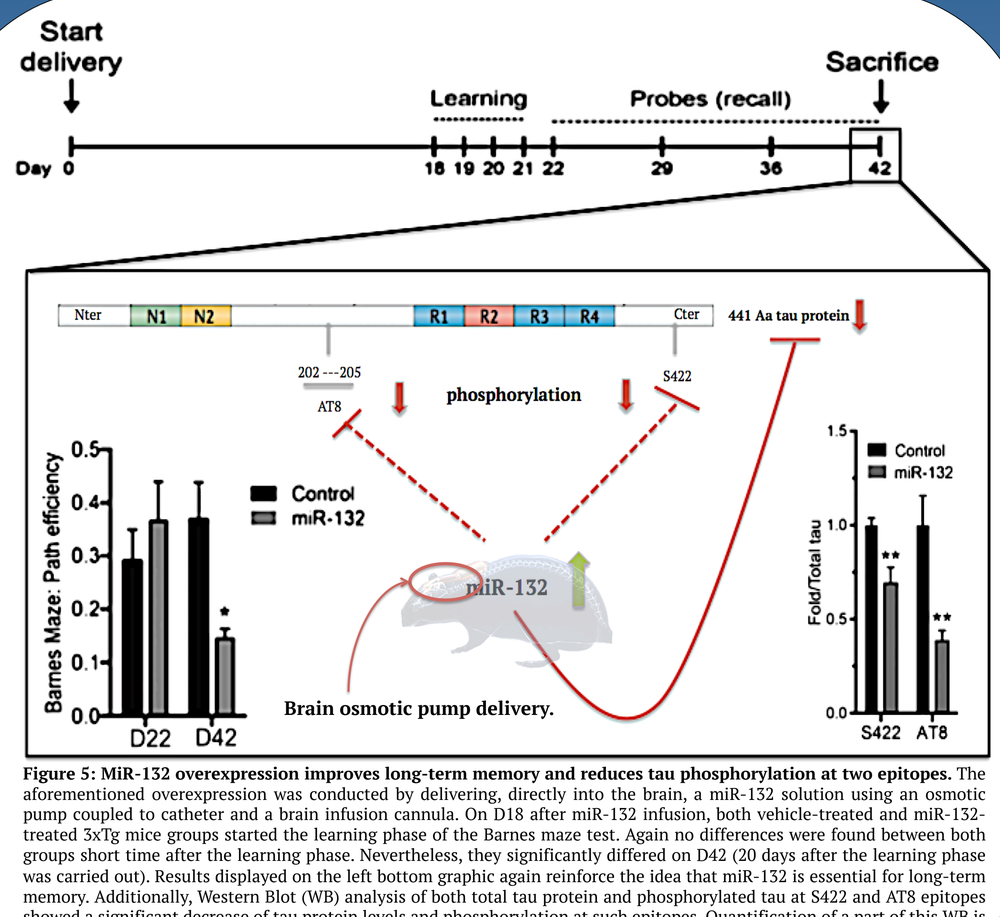

Figure 5: MiR-132 overexpression improves long-term memory and reduces tau phosphorylation at two epitopes. The aforementioned overexpression was conducted by delivering, directly into the brain, a miR-132 solution using an osmotic pump coupled to catheter and a brain infusion cannula. On D18 after miR-132 infusion, both vehicle-treated and miR-132-treated 3xTg mice groups started the learning phase of the Barnes maze test. Again no differences were found between both groups short time after the learning phase. Nevertheless, they significantly differed on D42 (20 days after the learning phase was carried out). Results displayed on the left bottom graphic again reinforce the idea that miR-132 is essential for long-term memory. Additionally, Western Blot (WB) analysis of both total tau protein and phosphorylated tau at S422 and AT8 epitopes showed a significant decrease of tau protein levels and phosphorylation at such epitopes. Quantification of a part of this WB is shown on the right bottom graphic. Statistics: *p<0.05 and **p<0.01 using an unpaired Student's t-test.

Adapted from: (Smith et al., 2015)

TAU

TAKE-HOME MESSAGE

Several miRNAs targeting either BACE-1 or tau mRNAs, among other mRNAs, are commonly found downregulated in AD patients. Additionally, some of these have been associated with axonal transport, spatial learning and memory; thus reinforcing the idea of miRNAs being essential for proper brain function maintenance.

When taking into account all miRNAs found deregulated in AD, it appears to be no miRNA more relevant than another regarding disease progression. This is another hurdle towards finding the ideal miRNA-based therapy to treat AD.

Despite being promising, only two *in vivo* miRNA-based therapeutic approaches, which attempt to restore miRNA levels in order to palliate AD symptomatology, have been reported so far.

These potential therapies have demonstrated that they improve spatial learning and long-term memory by overexpressing either miR-188-3p or miR-132/212, which target BACE-1 and tau respectively. This constitutes additional evidence supporting the relevance of both tau and BACE-1 epigenetic deregulation in contributing to AD progression.

REFERENCES

Amemori, T., Jendelova, P., Ruzicka, J., Urdzikova, L.M., and Sykova, E. (2015). Alzheimer's Disease: Mechanism and Approach to Cell Therapy. Int. J. Mol. Sci. *16*, 26417–26451.

Puzzo, D., Gulisano, W., Arancio, O., and Palmeri, A. (2015). The keystone of Alzheimer pathogenesis might be sought in Aβ physiology. Neuroscience *307*, 26–36.

Schonrock, N., and Götz, J. (2012). Decoding the non- coding RNAs in Alzheimer's disease. Cell. Mol. Life Sci. CMLS *69*, 3543–3559.

Smith, P., Hernandez-Rapp, J., Jolivette, F., Lecours, C., Bisht, K., Goupil, C., Dorval, V., Parsi, S., Morin, F., and Planel, E. et al. (2015). miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. Hum. Mol. Genet. 24, 6721-6735.

Wang, Y., and Mandelkow, E. (2015). Tau in physiology and pathology. Nature Reviews Neuroscience *17*, 22-35.

Zhang, J., Hu, M., Teng, Z., Tang, Y.-P., and Chen, C. (2014). Synaptic and cognitive improvements by inhibition of 2-AG metabolism are through upregulation of microRNA-188-3p in a mouse model of Alzheimer's disease. J. Neurosci. Off. J. Soc. Neurosci. *34*, 14919– 14933.

5xFAD TG mice model: http://www.alzforum.org/research-models/5xfad

3xTg mice model: http://www.alzforum.org/research-models/3xtg