EFFECTS OF LETROZOLE ON MICRO-RNAs: IMPLICATIONS ON MEMORY AND ALZHEIMER’S DISEASE

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ABSTRACT

In the brain, aromatase plays a role in the regulation of synaptic activity and plasticity, neurogenesis and the neuronal response to injury. It may also play a role in controlling behavior, cognition and mood.

Letrozole is a selective, powerful aromatase inhibitor. It binds to the iron in the heme moiety of the enzyme, and partially mimics the steroidal backbone of its natural substrate; androstenedione.

The effects of aromatase inhibitors (AIs), including Letrozole, on memory are controversial. Some human studies show adverse cognitive effects, such as forgetting and concentration difficulty, in women receiving AIs, and breast cancer patients on AIs reported impairments of verbal learning and memory. However, a few reports found no cognitive changes in women given AIs.

Few studies have linked the administration of Letrozole to changes in the expression of multiple microRNAs (miRNAs). Of which, many were also linked to neurodegeneration and Alzheimer’s disease, a chronic illness massively researched especially in regards to theranostics, pharmacological and non-pharmacological interventions, like exercise/physiotherapy as well as molecular mechanisms and relevant pathways.

In this review, we aim to discuss a number of those miRNAs that could be influenced by Letrozole administration and may link to cognitive effects and Alzheimer’s disease.

Key words: Letrozole, MicroRNAs, Alzheimer’s disease

I. INTRODUCTION TO LETROZOLE

Aromatase (estrogen synthase) is an enzyme catalyzing the final step of synthesis of estrogens from androgens, called aromatization (1). Aromatase is expressed in the ovary, brain, muscle, fat, liver, and breast (2,3).

With age, estrogen production from the ovaries declines (4), and the contribution of peripheral estrogens production increases (5). Thus, aromatase inhibitors (AIs) that inhibit estrogen production, have become a stable in the treatment of estrogen-sensitive postmenopausal patients with breast cancer (6).

In the brain, aromatase plays a role in the regulation of synaptic activity and plasticity, neurogenesis and the neuronal response to injury. It may also play a role in controlling behavior, cognition and mood (7,8).

Letrozole is a highly selective, powerful aromatase inhibitor (9). It binds to the iron in the heme moiety of the enzyme, and partly mimics the steroidal backbone of its natural substrate; androstenedione (5,10).
Following oral intake, Letrozole undergoes rapid and complete absorption and distribution to tissues. Approximately 60% of Letrozole is bound to plasma proteins, mainly albumin. The drug is mainly eliminated by cytochrome P450 isoenzymes, which metabolize it into an inactive carbinol metabolite (11).

Steady-state Letrozole concentrations are obtained after 2 – 6 weeks, and can be maintained for extended periods with no evidence of accumulation. Letrozole does not significantly interact with other drugs, and age does not affect its pharmacokinetics (11).

Studies have suggested that Letrozole can cross the blood brain barrier (12,13), and that it may display different pharmacokinetics and dynamics in males and females, such as much higher plasma and tissue concentrations of the drug in females compared to male animals receiving the same dose (14).

Steroid hormones, including estrogens, may have a role in primary and metastatic brain tumor development (15–17). Thus, Letrozole was also investigated for possible use in the management of brain tumors (8,18).

II. LETROZOLE AND MEMORY IMPAIRMENT

The development and preservation of various cognitive capabilities is promoted by estrogen (19,20) which may also act during recovery from stress or brain injury (20–24); it comes as no surprise that drugs that inhibit aromatase could adversely affect the cognitive functions (25).

The effects of aromatase inhibitors (AIs), such as Letrozole, on memory present a point of controversy. Studies with normal male rats propose that AI might actually improve both spatial and working memory (26,27). Conversely, others showed that Letrozole significantly compromised memory in normal rats (28).

Conflicting results have been reported in females as well, studies on females mice and hippocampal slice cultures have shown that Letrozole decreased learning and memory as well as causing synaptic loss and downregulation of hippocampal synaptic proteins (29–33). However, in another study it appeared that inhibition of estrogen synthesis in the brain may be beneficial for spatial memory (34).

Human studies on the relation between aromatase inhibition and memory impairment also have conflicting results. Cognitive complaints, like forgetting and concentration difficulty, are described in women receiving AIs (35), and breast cancer patients on AIs reported impairments in verbal learning and memory (36). Contrariwise, a few reports have found no cognitive changes in women given AIs (37).

It is still uncertain whether the cognitive impairments reported in patients receiving Letrozole are because of neurosteroidal insufficiencies or they are aggravated in presence of an aging-related change.

In their study, Chang et al. examined the effects of Letrozole and oligomeric Aβ42 on hippocampal slice cultures. Each of Aβ42 or Letrozole alone was sufficient to decrease mitochondrial volume, dendritic spine density, and synaptic proteins. Mitochondrial and synaptic deficits were exacerbated when Letrozole was combined with Aβ42. These findings propose that Letrozole may raise neuronal susceptibility to pathological affection, like Aβ42 oligomers in AD (38).

III. EFFECTS OF LETROZOLE ON MICRORNAS

MicroRNAs are small (18–25 nucleotides), non-coding RNAs that posttranscriptionally regulate gene expression via repression of translation or by mRNA degradation (39,40). miRBase-21 (http://www.mirbase.org/) has approximately 2588 mature miRNAs, which have been shown to contribute in the regulation of cellular homeostasis in health and in several diseases, such as cancer, immune disorders, and neurodegenerative diseases (39).

Several studies, as later discussed, have linked the administration of Letrozole to changes in the expression of multiple microRNAs (miRNAs). Of which, several were also linked to neurodegeneration and Alzheimer’s disease.

IV. MICRORNAS IN ALZHEIMER’S DISEASE

Alzheimer’s disease (AD) is a cause of aging-related cognitive impairment and dementia (ARCID) (41) with a prevalence doubling every five years after the age of 65 (42).
Alois Alzheimer was first to describe the disease in a dementia precox patient in 1906. He referred to it as a “peculiar severe disease process of the cerebral cortex” with the presence of “miliary foci” of beta-amyloid plaques, and "fibrils" or neurofibrillary tangles (43).

Regulation of APP by microRNAs (miRNAs) was suggested by several studies (44). For instance, miR-101 inhibition increased APP levels in vivo (45), and several microRNAs were shown to control the translation of β-secretase enzyme (BACE1); including miR-107, miR-328, miR-298, miR-29, and miR-9 (44,46–48).

MicroRNAs were found to also affect other pathways related to AD. For instance, Let-7a overexpression led to augmentation of Aβ40-induced neurotoxicity through regulation of autophagy, and that PI3K/Akt signaling cascade plays a role in this process (49).

V. MICRONRNAS AS POSSIBLE LINKS BETWEEN LETROZOLE AND NEURODEGENERATION

Let-7 microRNAs

Let-7 (lethal-7) was one of the early discovered miRNAs. It was first identified as a developmental timing regulator in the nematode, Caenorhabditis elegans (C. elegans) (50).

Characteristics of the Let-7 family

Let-7 miRNAs are well-conserved across different animal species, including humans (51,52). Using computational analysis, researchers discovered 401 Let-7 miRNA sequences from numerous organisms (53).

Most Let-7s have the “seed sequence”, which is a greatly conserved sequence spanning nucleotides 2 to 8 in some miRNAs (54) and is required for target recognition by the RISC complex (55–57). This conserved feature of Let-7 miRNAs suggests that they have similar targets and functions across diverse animal species (53).

Despite conservation of the Let-7 sequences, several differences are found between the closely related Let-7 family members of different animal species (58). For example, C. elegans has one Let-7 miRNA, while rodents and humans have multiple Let-7 members, including Let-7a, b, c, d, e, f, g, i and miR-98 (53).

Each Let-7 miRNA can be found in multiple copies in the genomes of higher animals. In humans, 12 loci encode the nine mature Let-7 miRNAs. Let-7 members can either be individually encoded or encoded as clusters along with other Let-7 members and/or unrelated miRNAs (53).

Let-7 biogenesis commonly occurs via the canonical miRNA biogenesis pathway, but, some family members need an extra step. Some pri-Let-7 precursors have a bulging adenosine or uridine next to the Drosha processing site, and Drosha may fail to recognize this bulge, resulting in the generation of a one-nucleotide 3’ overhang which is not preferred for the subsequent Dicer activity. Thus, terminal uridylyl transferases (TUTases) must mono-uridylate the 3’ end of the resultant pre-Let-7s, to yield the two-nucleotide 3’ overhang preferred by Dicer (53,59).

Biological roles and mechanisms of action of Let-7 family members

The mechanisms of action of Let-7 miRNAs on target mRNAs are still unclear. Theories suggest that Let-7a may inhibit the translation of its target mRNAs via ribosomal binding and inhibition. Let-7s may also cause deadenylation and decay of mRNA (60).

Evidence suggests that Let-7 miRNAs promote cell differentiation during development and act as tumor suppressors in multiple cancers (53,61–63). This latter action occurs via targeting oncogenes, such as c-Myc, ras, high-mobility group A (HMG A), Janus protein tyrosine kinase (JAK) and signal transducer and activator of transcription 3 (STAT3), and their signaling pathways that regulate cell cycle, apoptosis and cell adhesion and are essential for tumorigenesis, proliferation and invasion (60,64).

Interestingly, recent studies have shown that many genes related to T2DM are also oncogenes or cell cycle regulators (65), and that the JAK/STAT signaling pathway is dysregulated in metabolic diseases including T2DM (66).
Let-7 was shown to be a powerful regulator of glucose metabolism and peripheral insulin resistance by targeting insulin-like growth factor-1 receptors, insulin receptors and insulin receptor substrate-2 in skeletal muscles and liver (67). Let-7a and d were also found to be upregulated in the skeletal muscles of T2DM patients compared with normal controls (68).

Let-7 expression is elevated during embryogenesis and brain development in mammals (69–72) and stays relatively high in adult brain tissue (73).

Let-7 miRNAs are key regulators of different inflammatory processes in the CNS, where they perform either pro-inflammatory, or anti-inflammatory functions (74). For instance, Let-7 can help the activation of microglia and macrophages via acting on Toll-like receptor 7 (TLR7) (75,76).

**Effects of Letrozole on Let-7 miRNAs**

Shibahara *et al.* profiled miRNA expression before and after Letrozole treatment in breast cancer cell cultures. Letrozole significantly increased Let-7f expression, and high let-7f expression correlated with low levels of aromatase protein in breast cancer cases (77).

In addition, Cho *et al.* exposed Ishikawa cells and endometrial stromal cells from endometriosis patients to different concentrations of Letrozole. Their results from Ishikawa cells showed significant increases in expression of Let-7b and Let-7f with a Letrozole dose of 20 μmol/L compared to controls. On the other hand, a significant increase of Let-7f expression was noted in endometrial stromal cells from endometriosis patients, at a 1 μmol/L concentration of Letrozole concentration, which is a typical therapeutic level (78).

**Let-7 miRNAs in AD**

Multiple studies provide evidence of the possible involvement of Let-7 miRNAs in neurodegenerative disorders, particularly AD (Table 1). One study found that overexpression of Let-7a enhanced the neurotoxicity induced by Aβ42 in adrenal pheochromocytoma and human neuroblastoma cell lines via regulation of autophagy, and the PI3K/Akt signaling pathway functions in this process (49).

Furthermore, Lehmann *et al.* injected Let-7b into the cerebrospinal fluid (CSF) of wild-type mice, which led to neurodegeneration, and this effect was mediated via TLR7 activation (75).

Additionally, Derkow *et al.* found that the CSF of AD patients had higher levels of Let-7b and Let-7e than healthy controls (79). Wang *et al.* were first to study global miRNA profiles of AD mice using microarrays. They found 37 differently expressed miRNAs, of which some Let-7 members were upregulated (80). Further profiling of miRNA from the brains of transgenic AD mice was done by Wang *et al.*, who found that the level of Let-7f-5p, among other miRNAs, was higher in the brains of 3-month and 6-month-old mice, while Let-7d was high in 6-months-old ones, which indicates the possible involvement of these miRNAs in AD progression (81). In another study using a cholesterol-based rabbit model of late onset AD, Six miRNAs were upregulated in the brain. Of which, Let-7a and miR-98 were upregulated 12 weeks following cholesterol treatment, while Let-7e was upregulated in both 8 and 12 week samples. Let-7b, however, began to rise only 4 weeks following treatment (82).

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample</th>
<th>Examined Let-7</th>
<th>Finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD patients</td>
<td>CSF</td>
<td>Let-7b &amp; e</td>
<td>Upregulated</td>
<td>(79)</td>
</tr>
<tr>
<td>AD patients</td>
<td>CSF</td>
<td>Let-7f</td>
<td>Upregulated</td>
<td>(83)</td>
</tr>
<tr>
<td>AD patients</td>
<td>CSF</td>
<td>Let-7i</td>
<td>Upregulated</td>
<td>(84)</td>
</tr>
<tr>
<td>Late-onset AD patients</td>
<td>CSF</td>
<td>Let-7a</td>
<td>Downregulated</td>
<td>(85)</td>
</tr>
<tr>
<td>Early onset fAD patients</td>
<td>CSF</td>
<td>Let-7a-3p</td>
<td>Upregulated</td>
<td>(86)</td>
</tr>
<tr>
<td>with PSEN1 G378E mutation</td>
<td>CSF</td>
<td>Let-7f</td>
<td>Upregulated</td>
<td>(87)</td>
</tr>
<tr>
<td>AD patients</td>
<td>Serum</td>
<td>Let-7f</td>
<td>Upregulated</td>
<td></td>
</tr>
</tbody>
</table>

Table (1): Examples of studies examining Let-7 miRNA expression in AD
<table>
<thead>
<tr>
<th>AD patients</th>
<th>Serum</th>
<th>Let-7d</th>
<th>Downregulated</th>
<th>(88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCI &amp; AD patients</td>
<td>Plasma</td>
<td>Let-7b</td>
<td>Upregulated in mild cognitive impairment (MCI)</td>
<td>(89)</td>
</tr>
<tr>
<td>AD patients</td>
<td>Plasma</td>
<td>Let-7d &amp; g</td>
<td>Downregulated</td>
<td>(90)</td>
</tr>
<tr>
<td>AD patients</td>
<td>plasma-derived extracellular vesicles</td>
<td>Let-7i</td>
<td>Downregulated</td>
<td>(91)</td>
</tr>
<tr>
<td>Transgenic APPswe/ PSEN1E9 AD mice</td>
<td>Cerebral cortex</td>
<td>Let-7b, c, d &amp; e</td>
<td>Upregulated at 3 and 6 months</td>
<td>(80)</td>
</tr>
<tr>
<td>Transgenic APP/PSEN1 AD mice</td>
<td>Brain</td>
<td>Let-7f &amp; d</td>
<td>Upregulated at 3 months and 6 months, respectively.</td>
<td>(81)</td>
</tr>
<tr>
<td>Cholesterol-induced AD rabbits</td>
<td>Brain</td>
<td>Let-7a, b &amp; e</td>
<td>Upregulated: *Let-7b: At 4 weeks of cholesterol treatment. *Let-7e: At 8 and 12 weeks. *Let-7a: At 12 weeks.</td>
<td>(82)</td>
</tr>
</tbody>
</table>

**MicroRNA-191 (miR-191)**

MiR-191 is part of the highly conserved MiR-191/425 cluster. It was first discovered in mice then confirmed in the HL-60 human leukemia cell line and in twenty other human tissues afterwards. MiR-191 is a major miRNA in rats’ neurons and its expression rises with time to approximately 2-5 folds in cultures of rat cortical cells, which suggest that it plays a role in neuronal development (92).

Letrozole treatment was found to significantly reduce the expression of miR-191 in glioma cells as well as decrease tumor cell proliferation, an effect that was successfully reversed by transiently transfecting the Letrozole-treated cells with synthetic miR-191 (18).

MiR-191 was significantly downregulated in plasma samples of patients with Alzheimer’s disease compared to normal controls. In their analysis, Kumar et al. stated that miR-191 had a 95% sensitivity and a 76% enriched specificity, and was hence deemed as one of the best standalone miRNA biomarkers for early detection of Alzheimer’s disease (90).

**miR-206**

MiR-206 is one of six members of the miR-1 family, arranged into: 1) the miR-1-2/miR-133a-1 cluster, 2) the miR-1-1/miR-133a-2 cluster and, 3) the miR-206/miR-133b gene cluster. The miR-206/miR-133b cluster is only expressed in vertebrates (93). MiR-206 was initially identified in muscles, where it partakes in myogenesis and NMJ regeneration. However, miR-206 upregulation was reported in the degenerating brains or brain areas in models of cerebral ischemia (94) and neurotoxicity (95). MiR-206 seems to be found at an undetectable / very low level in normal brain tissues but, gets aberrantly elevated in diseased ones (96).

In their study on the effects of interval exercise training and hormonal therapies, including Letrozole, on several miRNAs in mice with breast tumors, Isanejad et al. found that miR-206 was over-expressed in mice treated with Letrozole and/or subjected to exercise compared to the positive control (tumor) group (97).

MiR-206 is heavily implicated in Alzheimer’s disease. Its level was increased in the olfactory mucosa of patients with cognitive impairment (early dementia) (98), temporal cortices of human AD brains, the brains of Tg2576 transgenic AD mice (96) the CSF, plasma and hippocampal and cortical tissues of embryonic amylloid beta precursor protein (APP)/presenilin-1 (PS1) transgenic mice (APP/PS1) transgenic mice (99,100). In addition, increased serum levels of miR-206 could predict the transition from mild cognitive impairment to Alzheimer’s dementia (101). Mir-206 could be exerting its neurotoxic effect via enhancing inflammation and amylloid beta release by targeting microglial insulin-like growth factor 1 (IGF-1) (102), or by downregulating the expression of brain derived neurotropic actor (BDNF) (96,99).

**miR-155**

MiR-155 has been extensively implicated in inflammation-related processes. It undergoes upregulation in activated microglial cells and macrophages, driving them to a pro-inflammatory state (103). MiR-155 inhibition can attenuate
neuroinflammation in the CNS and improve neurological recovery in models of different neurodegenerative conditions (104–109).

Using miRNA deep sequencing approach, Letrozole was found to decrease the expression of miR-155 in the uterus from a letrozole-induced PCOS rat (110). Letrozole also decreased miR-155 expression in xenografts of aromatase inhibitor-sensitive human breast cancer (111).

Mir-155 is heavily studied in pathological processes related to Alzheimer’s disease. Its level was found to be increased in the blood of AD patients (112) and it was found to influence fibrillar beta-Amyloid catabolism by microglial cells as its overexpression decreases beta-Amyloid catabolism and vice versa (113). A significant upregulation of miR-155 was seen in the hippocampi of 10-month-old APPtg (APPSwe/PS1L166P) and TAUtg (THY-Tau22) mice (114) and the brains of 12-month-old 3xTg AD animals with a concurrent increase in microglial and astrocyte activation prior to the appearance of extracellular Beta-Amyloid aggregates, suggesting that early miR-155 upregulation may contribute to production of inflammatory mediators like IL-6 and IFN-β (115). Another study showed an increased miR-155 expression in the hippocampi of AD rats, along with increased IL-1β, IL-6 and TNF-α, and ICV administration of miR-155 inhibitor halted the increases of IL-1β, IL-6 and TNF-α and their receptors’ upregulation and largely restored the AD animals’ impaired learning performance (116).

**miR-125b-5p**

MiR-125b is a brain-enriched microRNA (117) that is part of the miR-125 family. This family resides in two human miRNA clusters along with the miR-99 and let-7 families and thus these microRNAs are likely co-transcribed (118).

In a model of poly cystic ovary syndrome (PCOS) in mice, Letrozole, the PCOS-inducing agent, was found to significantly decrease the expression of miR-125b-5p in the ovaries of the PCOS mice (119).

miR-125b-5p levels were shown to be decreased in diverse studies and models of AD. Examples include: Aβ-treated primary mouse cortical neurons and Neuro2a (N2a) cells (120), Exosome-enriched plasma fractions from AD patients (121) and sera of AD patients (88,122).

In primary cortical neurons, amyloid pathology supresses miR-125b-5p (123) and this downregulation key for the neurotoxicity Aβ in such neurons. (124)

Circulating miR-125b was also decreased in APP/PS1 transgenic AD mice, with a positive correlation between miR-125b expression and the cognitive function of the APP/PS1 mice. (125)

MiR-125b was linked to changes in cortical thickness, cortical glucose metabolism and cognitive performance. Lower serum miR-125b was correlated with decreased thickness of the right temporal lobe and deficient glucose metabolism spreading bilaterally in the cortex and extending throughout the neocortex (126).

Conversely, other studies found increased expression of miR-125b-5p in the CSF and CSF-derived exosomes of AD patients (127,128) and in frozen human postmortem AD brain specimens. MiR-125b was found to significantly enhance the apoptosis of neuronal cells and Tau phosphorylation via activation of cyclin-dependent kinase 5 and p35/25, with Forkhead box Q1 (FOXQ1) being its direct target gene (129).

**Other microRNAs**

Table (2): Additional microRNAs potentially influenced by Letrozole and with possible roles in Alzheimer’s disease.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Effect of Letrozole</th>
<th>Potential role in Alzheimer’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-142</td>
<td>• Downregulation in uterus (PCOS rat model) (110)</td>
<td>– Upregulated in Aβ2-treated neuroblastoma cell line (SH-SY5Y) and its Inhibition rescued the Aβ2-mediated synaptic dysfunctions (130).</td>
</tr>
<tr>
<td></td>
<td>• Downregulation in MCF-7 cells co-cultured with primary breast cancer stromal cells (77)</td>
<td>– Upregulated in blood of AD patients (84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Downregulated in plasma of AD patients (90,131)</td>
</tr>
<tr>
<td>mir-129-5p</td>
<td>• Downregulation in uterus (110) and ovary (132) (PCOS rat model).</td>
<td>– Downregulated in AD and its overexpression alleviates nerve injury and inflammatory response (133).</td>
</tr>
</tbody>
</table>
| mir-34a | • Downregulation in uterus (PCOS rat model) (110). | – Downregulated in Aβ-treated primary mouse cortical neurons and Neuro2a (N2a) cells (120).  
– Downregulated in plasma of AD patients (131)  
– Downregulated in cortical neurons treated with Aβ1-42 peptide and in the cortex of APP/PS1 mice (134)  
– Upregulated in early stage AD in APP/PS1 mice (before the appearance of major pathology: Aβ production and plaque formation, astrogliosis, and cognitive impairments) (135) |
| mir-223 | • Downregulation in uterus (PCOS rat model) (110). | – Downregulated in serum of AD patients (122,136).  
– Downregulated in serum exosomes from dementia patients (137).  
– Upregulated in plasma neural-derived small extracellular vesicles from AD patients (139). |
| mir-193b | • Downregulation in uterus (PCOS rat model) (110). | – Downregulated in Exosome-enriched plasma fractions from AD patients (121).  
– Upregulated in the hippocampi of aged senescence-accelerated mouse prone 8 mice (140). |
| mir-22 | • Downregulation in uterus (PCOS rat model) (110). | – Downregulated in peripheral blood (141) and sera (142,143) of AD patients.  
– Downregulated in the hippocampus of Aβ1-42-induced AD rats and its expression reversed the degradation of hippocampal synaptic structures (144).  
– Upregulated in 6 and 12-month-old hTau mice (145). |
| mir-132 | • Downregulation in uterus (PCOS rat model) (110).  
• Downregulation in ovary (PCOS rat model with insulin resistance) (146). | – Downregulated in brains (147,148) and neural exosomes isolated from plasma from AD patients (147).  
– Downregulated in brains of AD patients and APP/PS1 mice, which aggravates both amyloid and Tau pathology (149).  
– Downregulated in hippocampi of AD rats and its upregulation improved cognitive function (150).  
– Downregulated in hippocampi of Scopolamine-induced AD mice (151). |
| mir-100 | • Upregulation in uterus (PCOS rat model) (110). | – Downregulated in plasma neural-derived small extracellular vesicles from AD patients (miR-100-3p) (139).  
– Downregulated at early stages while upregulated at late stages of APP/PS1 mice (152).  
– Upregulated in CSF of AD patients (153). |
| mir-29a | • Upregulation in uterus (PCOS rat model) (110). | – Upregulated in CSF of AD patients (154,155). – Downregulated miR-29a/b-1 cluster in AD patients displaying abnormally high BACE1 protein (46). |
| mir-26a and b | • Upregulation in uterus (PCOS rat model) (110). | – Mir-26a: Downregulated in APP(swe)/PS1 (ΔE9) transgenic mice AD mice (157) and sera of AD patients (142). – Mir-26b: Upregulated in APP/PS1 AD mice (158) and human postmortem AD brains (159). |
| mir-212 | • Downregulation in ovary (PCOS rat model) (132) and (PCOS rat model with insulin resistance) (146). | – Downregulated in Aβ25-35-treated SH-SY5Y and IMR-32 cells, Plasma (160) and neutrally-derived plasma exosomes from AD Patients (147), and temporal cortex of AD patients (161). |
| mir-200a, b and c | • Mir-200a and b: Downregulation in ovary (PCOS rat model) (132). • Mir-200c: Upregulation in ovary (PCOS rat model with insulin resistance) (146). | – Mir-200a: Downregulated in the hippocampus of APP/PS1 and SAMP8 mice, APPswe cells and plasma from AD patients (162). – Mir-200b: Downregulated in hippocampi, CSF & serum from APP/PS1 AD mice, in SH-SY5Y cells after incubation with Aβ42, and serum and CSF of AD patients (163). – Mir-200c: Upregulated at 4-month old APP/PS1 mice and peaked at 6 months of age, then decreased to baseline and stayed at that level from 9-month old onward, and upregulated in plasma of AD patients (164). |
| mir-135a | • Downregulation in ovary (PCOS rat model) (132). | – Downregulated in hippocampi of APP/PS1 AD mice (163,165). – Upregulated in serum exosomes of AD patients (166). |
| mir-146a | • Upregulation in MCF-7 cells co-cultured with primary breast cancer stromal cells (77). | – Upregulated in human AD superior temporal lobe neocortex (Brodmann area A22) (167), and hippocampus (168), hippocampi of APPtg and TAUtg AD mice (114), brains of 6 and 12-month-old hTau AD mice (145) and Aβ1-42-stimulated SH-SY5Y cells (169). – Upregulated in patients with MCI who later converted to AD, with a negative correlation was with amyloid beta concentration in CSF. Higher levels were also associated with presence of apolipoprotein E ε4 allele and smaller volume of the hippocampus (170). – Downregulated in CSF (155,168), plasma (155) and serum (171) of AD patients. |
VI. CONCLUSION AND FUTURE PROSPECTIVE

The research focusing on the possible effects of Letrozole administration on microRNAs is relatively young, and mostly targeted towards Letrozole-induced poly cystic ovary syndrome model (110), breast cancer research and, specifically, its hormonal therapy (97) and resistance of hormonal therapy (178) as well as some other cancers (18) and conditions such as endometriosis (78). However, the evidence suggesting that Letrozole can cross the blood brain barrier (12,13) along with conflicting reports of its effects on memory, cognitive functions and related circuits and mechanisms (29–37) warrant more research to investigate its specific effects on the brain and brain microRNAs, specifically those previously implicated in neurodegenerative conditions, such as Alzheimer’s disease. Further research into the effects of Letrozole on brain-enriched microRNAs and microRNAs central to memory and cognitive mechanisms could also resolve the controversy around the effect of Letrozole on memory and help explain the seemingly conflicting evidence in animals studies of this matter, possibly on the basis of determining which brain microRNAs are influenced by Letrozole, the degree of influence, whether there are temporal, dose-dependent or spatial effects and the pathways greatly affected by the microRNAs involved.

Conflicts of Interest: The authors report no conflicts of interest.

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