Neurodegeneration: From BDNF to Hyperexcitability

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Abstract

Brain-derived neurotrophic factor (BDNF) plays a pivotal role in cell differentiation, neuronal survival, and synaptic plasticity. Most neurodegenerative disorders are associated with both BDNF deficiency and neuronal hyperexcitability. This paper shows that the two abnormalities can be linked by the microRNA, miR-132, which is critical for regulating Tau expression. According to the MT model for excitability (Paper 2), increased Tau level, especially the 4-repeat (4R) Tau, should enhance excitability. The BDNF-TrkB pathway stimulates miR-132 expression. Hence, BDNF deficiency should lead to miR-132 deficiency, resulting in higher 4R Tau level, and consequently hyperexcitability. Pathologic TAR DNA-binding protein 43 (TDP-43) and C9ORF72 have been shown to reduce miR-132 biogenesis, which may contribute to the development of Tau-positive neurodegeneration.

Introduction

Brain-derived neurotrophic factor (BDNF) plays a pivotal role in cell differentiation, neuronal survival, and synaptic plasticity in the central nervous system (Cunha et al., 2010). Low level of BDNF has been shown to associate with Alzheimer's disease (Yasutake et al., 2006), Huntington's disease (Zuccato and Cattaneo, 2007), amyotrophic lateral sclerosis (ALS) (He et al., 2013), frontotemporal dementia (FTD) (Ventriglia et al., 2013; Zanardini et al., 2016) and Parkinson's disease (Howells et al., 2000). In cognitively normal adults, the cerebrospinal fluid concentration of BDNF decreases with age (Li et al., 2009). This may explain why age is a risk factor for neurodegeneration.

BDNF promotes mTOR activation (discussed below). Paper 3 has presented compelling evidence that hyperactive mTOR may lead to hyperexcitability which is an early sign of neurodegeneration. Then, how can neurodegeneration associate with **low level** of BDNF? This paper will show that the answer may lie in the microRNA, miR-132, which is

down-regulated in Alzheimer's disease (Lau et al., 2013), Huntington's disease (Johnson and Buckley, 2009; Lee et al., 2011), ALS (Freischmidt et al., 2013), FTD (Hébert et al., 2013), and Parkinson's disease (Gillardon et al., 2008).

Biogenesis and Functions of microRNA

A microRNA is a small non-coding RNA molecule (~ 22 nucleotides), generated from the genomic DNA. To date, over 2000 microRNAs have been discovered in humans (Hammond, 2015). They are named with the prefix "miR" followed by a dash and a number. A suffix, -3p or -5p, may also be included, specifying whether the mature microRNA originates from the 3' or 5' arm of its precursor.

Figure 1. The structure of pre-miR-132. Red color indicates its mature segment. [Source: Wanet et al., 2012]

The biogenesis of a microRNA starts from its newly transcribed microRNA, i.e., the primary transcript (pri-miRNA), which is then cleaved by the microprocessor complex consisting of Drosha and DGCR8, resulting in a shorter microRNA-precursor denoted by pre-miRNA (Figure 2). Subsequently, the pre-miRNA is transported from the nucleus to the cytoplasm for further processing by the Dicer complex, which leads to a mature microRNA (Ye et al., 2016).

The mature microRNA interacts with its target (the mRNA of a protein) within a structure called RNA-induced silencing complex (RISC). For perfect or nearly perfect complementarity between a microRNA and its mRNA target, the interaction within RISC will result in mRNA degradation. For partial complementarity, the translation of the target mRNA will be repressed (Ye et al., 2016).

miR-132 targets the mRNA of both Tau protein and a splicing factor, polypyrimidine tract-binding protein 2 (PTBP2). Thus, miR-132 regulates not only the total Tau protein level, but also the ratio between 4-repeat (4R) and 3-repeat (3R) Tau proteins. miR-132 deficiency has been shown to cause increased Tau expression and higher 4R:3R Tau ratio (Smith et al., 2011; Smith et al., 2015).

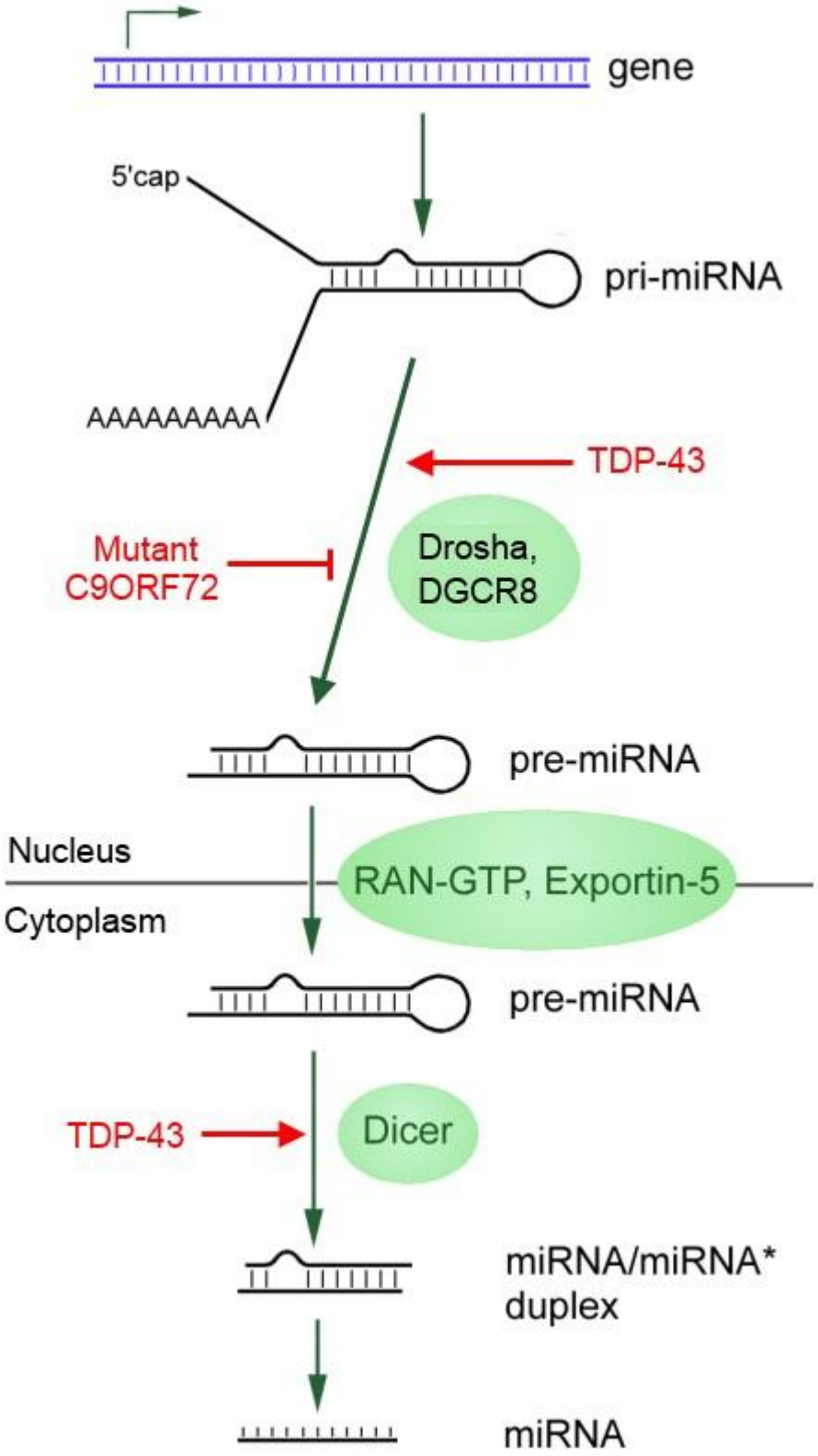

Figure 2. The biogenesis of a microRNA. TDP-43 promotes microRNA biogenesis by interacting with the nuclear Drosha complex and the cytoplasmic Dicer complex. The mutant C9ORF72 disrupts microRNA biogenesis by binding to Drosha. [Modified from: Wikipedia]

BDNF Signaling Pathways

The pathways related to neuronal excitability are triggered by the binding between BDNF and the tropomyosin-related kinase B (TrkB) located on either presynaptic or postsynaptic membranes. Three main pathways have been elucidated: PLCγ, PI3K and ERK (Figure 3). They all lead to the activation of the transcription factor CREB that mediates transcription of genes essential for synaptic plasticity. The PI3K pathway may also activate mTOR for protein translation from mRNA. Transcriptions of both BDNF and miR-132 genes are regulated by CREB (Zheng et al., 2012; Wanet et al., 2012; Yi et al., 2014). Thus, BDNF can stimulate its own production and increase the level of miR-132.

It is important to note that the Tau gene (*MAPT*) is regulated by the transcription factors SP1 and AP2, not CREB (Caillet-Boudin et al., 2015). Based on the hyperexcitability hypothesis for neurodegeneration, the Tau gene cannot be regulated by CREB, for otherwise BDNF would also increase Tau mRNA. As described in Paper 2, excess Tau proteins may cause hyperexcitability, leading to neurodegeneration. Although the BDNF/PI3K/mTOR pathway stimulates Tau protein translation from mRNA, the Tau mRNA can be repressed or even degraded by miR-132. Therefore, miR-132 plays a crucial role in preventing neurodegeneration by suppressing Tau mRNA. If the Tau gene were also regulated by CREB, it would negate the beneficial effects of BDNF via up-regulation of miR-132.

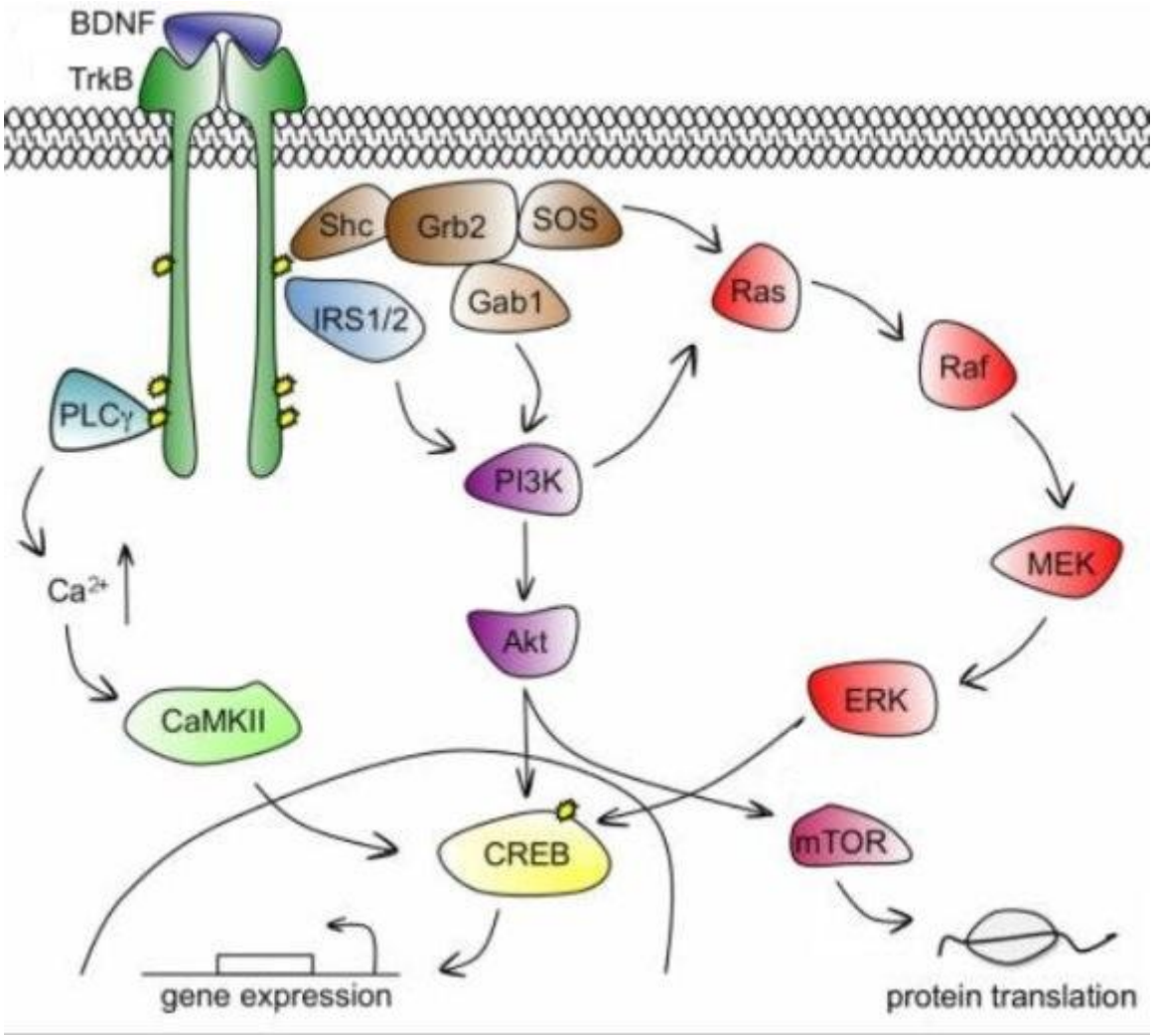

Figure 3. The BDNF-TrkB signaling pathways which induce the production of plasticity-related molecules, including BDNF and miR-132. [Source: Cunha et al., 2010]

The TDP-43 Pathology

TAR DNA-binding protein 43 (TDP-43) is the major component of neuronal inclusions that characterize ALS and FTD (Mackenzie et al., 2010). Pathologic TDP-43 (hyperphosphorylated, ubiquitinated or cleaved) also exists in some inclusions characteristic for Alzheimer's disease (Wilson et al., 2011), Huntington's disease (Schwab et al., 2008) and Parkinson's disease (Nakashima-Yasuda et al., 2007). TDP-43 promotes microRNA biogenesis by interacting with the nuclear Drosha complex and the cytoplasmic Dicer complex (Kawahara and Mieda-Sato, 2012). Hence, the loss of TDP-43 will down-regulate targeted microRNAs. TDP-43 regulates a number of microRNAs. Among them,

miR-132 and miR-9 are most relevant to neurodegeneration (Kawahara and Mieda-Sato, 2012; Freischmidt et al., 2013; Zhang et al., 2013).

The C9ORF72 Pathology

The most common cause of familial ALS and FTD arises from mutations in the *C9ORF72* gene. In a normal person, the gene contains less than 15 GGGGCC repeats, but in ALS/FTD patients, the number of GGGGCC repeats may expand to over 200 (Renton et al., 2011). The C9ORF72 pathology is characterized by the "dipeptide repeat inclusion" which is composed of dipeptide repeat proteins (translated from GGGGCC repeats), ubiquilin and p62 (ubiquilin-binding protein), but rarely TDP-43 (Al-Sarraj et al., 2011).

The biological function of C9ORF72 is not clear. However, it is unlikely to play a role in motor functions, as knock-out of the *C9ORF72* gene in mice does not cause motor neuron degeneration or motor deficits (Koppers et al., 2015). On the other hand, expression of the GGGGCC expanded *C9ORF72* in mice recapitulate disease features such as dipeptide repeat inclusions, TDP-43 pathology, and behavioral abnormalities (Chew et al., 2015). These results demonstrate that the toxicity of C9ORF72 mutation is not due to the loss of its normal function, but arises from the gain of abnormal processes.

In many cases, Drosha proteins are also recruited to the dipeptide repeat inclusions (Porta et al., 2015). Since Drosha is critical for microRNA biogenesis, the mutant C9ORF72 may cause ALS/FTD by binding to Drosha, thereby disrupting the biogenesis of essential microRNAs. Indeed, the C9ORF72 mutation has been shown to result in miR-132 down-regulation (Freischmidt et al., 2013) and hyperexcitability (Geevasinga et al., 2015; Schanz et al., 2016).

Pathogenesis of Neurodegeneration

Most neurodegenerative disorders are associated with both BDNF deficiency and neuronal hyperexcitability, which can be linked by deficient miR-132. Thus neurodegeneration could start from BDNF deficiency to miR-132 reduction and to hyperexcitability. This pathogenic cascade is consistent with experimental observations in specific diseases.

Alzheimer's disease begins in the medial entorhinal cortex, especially the stellate cells in layer II. These cells are normally highly electroresponsive. A small change (1-3 mV) of membrane potential is sufficient to elicit action potentials (Geevasinga et al., 2015). It is conceivable that these neurons are most vulnerable to BDNF deficiency. Hyperexcitability in this region causes Tau hyperphosphorylation, which is a characteristic of disorders collectively referred to as "tauopathy".

Huntington's disease is caused by mutations in the huntingtin gene (*HTT*). The mutant huntingtin contains a larger number of glutamine repeats, capable of interacting with the splicing factor SRSF6, leading to increased 4R Tau and total Tau protein level (Fernández-Nogales et al., 2014). Furthermore, huntingtin plays an important role in enhancing the transport of BDNF-containing vesicles from the cell body to synapses (Gauthier et al., 2004). The mutation of huntingtin will reduce BDNF level at synapses.

FTDP-17 (frontotemporal dementia and parkinsonism linked to chromosome 17) is caused by mutations of the Tau gene located on chromosome 17. In many cases, the mutation increases the 4R Tau level (Hyman et al., 2005), which may account for the comorbidity with epileptic seizures (Sperfeld et al., 1999).

FTD is characterized by the neuronal inclusions with pathologic TDP-43. The von Economo neurons in anterior cingulate cortex (ACC) and frontal insula are the most severely affected (Seeley, 2008). TDP-43 pathology may or may not accompany hyperphosphorylated Tau proteins. As discussed above, the loss of TDP-43 will lead to down-regulation of miR-132 and miR-9. It is possible that miR-132 deficiency may cause Tau-positive FTD while miR-9 is related to Tau-negative FTD.

ALS is characterized by TDP-43 pathology that affects motor neurons in the motor cortex, brain stem, and spinal cord. As in FTD, its neuronal inclusions may be either Tau-positive or Tau-negative (Umahara et al., 1994; Hilton et al., 1995), suggesting distinct pathogenic mechanisms. Hyperexcitability in Tau-positive and Tau-negative degeneration could result from hyperactive mTOR and glutamate over-stimulation (Manuel and Heckman, 2011), respectively.

Parkinson's disease is caused by the degeneration of dopaminergic neurons within the substantia nigra pars compacta. This region rarely exhibit Tau pathology (Wills et al., 2010). Therefore, like Tau-negative ALS, Parkinson's

disease may arise from glutamate over-stimulation of the dopaminergic neurons (Blandini et al., 1996; Ambrosi et al., 2014).