



IS THE EFHD2/SWIPROSIN-1 PROTEIN LINKED TO ALZHEIMER'S DISEASE?

CZY BIAŁKO EFHD2/SWIPROSINA 1 MA ZWIĄZEK Z CHOROBAŁ ALZHEIMERA?

Correspondence to/
Adres do korespondencji:

Marta J. Broniarczyk-Czarniak
Department of Adult Psychiatry
Medical University of Lodz
159 Aleksandrowska St.
91-229 Łódź, Poland
e-mail: martabroniarczyk@op.pl

Submitted/Otrzymano: 16.10.2018
Accepted/Przyjęto do druku: 27.08.2019

Marta J. Broniarczyk-Czarniak, Piotr Gatecki

Department of Adult Psychiatry, Medical University of Lodz, Poland

Klinika Psychiatrii Dorosłych, Uniwersytet Medyczny w Łodzi, Polska

Abstract

Purpose: The article provides information about the current state of knowledge regarding EFhd2/Swiprosin-1, i.e. a protein that may be associated with the process of neurodegeneration and Alzheimer's disease.

Views: Alzheimer's disease is a commonly occurring debilitating disorder, the prevalence of which increases gradually with age – from below 1% at 65 years of age to as high as 40% over the age of 90. Genes are said to have a significant impact on the development of AD. EFhd2, also referred to as Swiprosin-1, is a calcium-binding protein, which is highly expressed in the central nervous system and linked with various pathological forms of tau proteins in tauopathies. EFhd2 is expressed in all sections of the brain. The physiological or pathological roles of EFhd2 have not yet been investigated thoroughly, and hence are not well understood. Studies show that EFhd2 is linked with the microtubule associated protein tau (MAPT) in a tauopathy mouse model (JNPL3), and in humans suffering from tauopathies, such as Alzheimer's disease.

Conclusions: The process of neuronal death which accompanies tauopathies is induced by the abnormal modification and gene expression of the tau protein. Nevertheless, the molecular mechanism responsible for neurotoxicity is still unknown. Various research results demonstrate that EFhd2 is a novel amyloid protein connected with the pathological form of the tau protein in the brain in AD and that the process of calcium binding may regulate the formation of the amyloid structures of EFhd2.

Key words: EFhd2, Swiprosin-1, Alzheimer's disease, tauopathy.

Streszczenie

Cel: W artykule przedstawiono aktualny stan wiedzy na temat Efhd2/swiprosiny 1, białka, którego występowanie może być związane z procesem neurodegeneracji i chorobą Alzheimera.

Poglądy: Choroba Alzheimera to powszechnie występująca, silnie inwalidyzująca jednostka chorobowa o rozpowszechnieniu stopniowo zwiększającym się z wiekiem, od około 1% u osób 65-letnich, do nawet 40% u 90-latków. Geny odgrywają istotną rolę w etiopatogenezie tego schorzenia. Efhd2, nazywane również swiprosiną 1, jest białkiem wiążącym wapń. Wysoką ekspresję tego białka obserwuje się w całym ośrodkowym układzie nerwowym. Efhd2 występuje we wszystkich częściach mózgowia. Jego fizjologiczna rola i udział w patogenezie chorób mózgu zostały słabo poznane. W badaniach wykazano związek występowania Efhd2/swiprosiny 1 z obecnością patologicznej formy białka tau w tauopatiach. Obecność Efhd2 została zidentyfikowana w mysim modelu tauopatii (JNPL3) oraz mózgowiach ludzi z chorobą Alzheimera. Zmieniona ekspresja genu występuje u osób z chorobą Alzheimera, płasawicą Huntingtona, chorobą Parkinsona i schizofrenią.

Wnioski: Postępujący proces obumierania neuronów obserwowany w tauopatiach jest indukowany przez zmienioną ekspresję genu i modyfikacje w białku tau. Molekularny mechanizm leżący u podłoża tej neurotoksyczności nadal pozostaje nieznany. Wyniki badań sugerują, że Efhd2 jest nowym białkiem amyloidowym związanym z patologiczną formą białka tau w mózgowiach osób z chorobą Alzheimera oraz że to właśnie zdolność do wiązania wapnia może regulować tworzenie struktur amyloidowych w mózgu.

Słowa kluczowe: EFhd2, swiprosina 1, choroba Alzheimera, tauopatia.

INTRODUCTION

An inevitable part of the ageing process is the loss of neurons. Besides the death of mature neurons, neurogenesis in adults declines with age. This condition may be intensified in the course of the neurodegenerative diseases, e.g. Alzheimer's disease (AD) [1, 2]. AD is a commonly occurring debilitating disorder, the prevalence of which rises gradually with age – from below 1% at 65 years of age to as high as 40% over the age of 90. Genes are said to have a significant impact on the development of AD [3]. It has been demonstrated that variations in the following four genes result in rare forms of early-onset AD: the amyloid precursor protein (APP) gene, presenilin 1 (PS1), presenilin 2 (PS2), and apolipoprotein E (APOE); all of these increase the general risk of disease development [4]. On the other hand, we still do not fully understand the more common late-onset disorder. Selected screening data for genome-wide linkage disequilibrium (LD) suggest that several chromosomal loci associated with AD exist, which may include new susceptibility genes for late-onset AD [5]. The modified expression of *EFhd2* gene has been documented and confirmed in Alzheimer's disease (AD), as well as in Parkinson's disease, Huntington disease and schizophrenia, which indicates that *EFhd2* gene expression is regulated in response to neuropathological processes; still, our understanding of the specific role of EFhd2 in the pathophysiology of neurological and psychiatric diseases is still rather poor [4]. The main emphasis of this review is placed on the evidence that links EFhd2 to Alzheimer's disease (AD).

SWIPROSIN-1/EFHD2 AT THE PROTEIN LEVEL

The *EFhd2* gene codes for a 26.8 kDa highly conserved calcium-binding protein, which is in humans located in chromosome 1 (1p36.21) [4]. The protein is also referred to as Swiprosin-1, in reference to the Swiss-Prot database used for tandem mass spectrometry data analyses [6]. Subsequently, due to the presence of two EF-hand calcium binding motifs, the name of this novel protein has been changed to EF-hand domain family member D2 (EFhd2). Swiprosin-1/EFhd2 is a calcium-binding protein expressed to the largest extent in the central nervous and the immune systems of mammals [1, 7, 8]. EFhd2 was first found and identified in the immune system in human CD8 lymphocytes [6, 9]. It is still unclear what the physiological function of this newly discovered protein is.

At the protein level, EFhd2 consists of a low complexity N-terminal region with an alanine stretch, a functional Src homology 3 (SH3-) binding motif, two EF hands and a C-terminal coiled-coil (CC) domain, which is a conserved domain among fibrillar proteins and is required for interactions between proteins [1, 9]. EFhd2's EF-

hand motifs are conserved from humans to nematodes, therefore it is suggested that the calcium binding activity of EFhd2 may be crucial for its physiological role [1]. A distinctive polyalanine motif of the N-terminal region is only present in mammals and its size varies from 6 to 9 alanines [1]. In the absence of the N-terminus, EFhd2's thermal stability is restored through calcium binding [1]. Among its orthologs, EFhd2 is the only protein containing a highly conserved Ser residue at position 183, which is known to be phosphorylated [7]. Upon Ca²⁺ binding, EFhd2 dimerizes and bundles F-Actin. EFhd2 phosphorylation at S138 modulates the dynamics of lamellipodia, while EFhd2 phosphorylation was found in a complex with p-TAU in the brain of a transgenic mouse model for tauopathy, as well as in old and diseased brains of humans [2].

Recently conducted studies have also shown that EFhd2 is structurally similar to the amyloid proteins observed in neurological disorders. EFhd2 co-aggregates and interacts with tau protein, which may suggest that EFhd2 plays an essential role in the pathophysiology of neurodegenerative diseases [4].

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL FUNCTIONS OF SWIPROSIN-1/EFHD2 IN THE NERVOUS SYSTEM

A number of studies have revealed that EFhd2 is expressed mainly in the brain. The western blot analytical technique made it possible to determine that EFhd2 is expressed in all parts of the brain (i.e. brain stem, amygdala, cerebellum, hippocampus, striatum, cortex and prefrontal cortex) at similar levels [9]. Furthermore, EFhd2 proteins have been found in the cytosol and proximal to the membrane in neurons of almost all regions of the brain; their higher expression has been observed and confirmed in the deeper layers of the cortex and hippocampus [10]. EFhd2 is also co-localized with neurite markers such as tau, MAP2 or synapsin, which suggests that its neuronal function could be associated with vesicular transport and the homeostasis of synapses [10, 11]. Swiprosin-1 is also involved in the following processes: calcium signaling, actin cytoskeleton, apoptosis, and in the regulation of synapse formation [4, 8]. The formation process of dendrites and synapses depends on differentiation signals, like synaptic input or soluble factors, and requires a complex organization of the neuron's actin and microtubule networks [2]. Synaptic dysfunction and Ca²⁺ dysregulation accompany behavioral disorders and neurodegenerative processes. [9]. It is now increasingly apparent that EFhd2 plays a substantial role in the nervous system, while its function can be associated with a number of neurological disorders [9].

Borger *et al.* and Purohit *et al.* demonstrated that *in vitro* knockdown of EFhd2 resulted in an increase of synapsin 1a/b puncta labeling in neurites, which may suggest that EFhd2 modulation has an impact on the development of functional synapses, yet has no effect on their conversion into mature synapses as determined by the co-localization of synapsin and PSD95 [10, 11]. This process applies to different functions of the brain, like learning and memory [4]. Purohit *et al.* also revealed that elimination of the *EFhd2* gene had no discernible effect on the anatomy or function of the brain. The researchers implied that vesicular transport velocity was enhanced in *EFhd2(-/-)* knockout primary hippocampal neurons and that the EFhd2 protein inhibited kinesin-mediated microtubule gliding in *in vitro* studies [11]. In the synaptosome fraction, EFhd2 may both co-purify with tubulin and take part in actin bundling [12]. These results suggest that EFhd2 may act as a synapse formation modulator through the regulation of vesicular transport velocity and cytoskeleton rearrangement [4].

ALZHEIMER'S DISEASE AS A TAUOPATHY

The group of neurological disorders characterized by the aggregation of hyperphosphorylated and filamentous tau proteins in ultrastructures referred to as neurofibrillary tangles are called tauopathies [13, 14]. The reversible process of physiologic phosphorylation of the microtubule associated protein tau modulates microtubule dynamics. On the other hand, intracellular neurofibrillary tangles comprising insoluble and hyperphosphorylated MAPT (microtubule-associated protein tau, p-TAU) are a typical neuropathological feature of Alzheimer's disease [2]. We still do not fully understand the molecular mechanism that leads to tau-mediated neurodegeneration [8]. AD is the most known and studied disease of all the neurological disorders referred to as tauopathies [15]. Beta-amyloid and tau protein are accumulated in the cortex. According to some researchers, tau protein can also accumulate in the retina. A non-invasive examination which shows early neurodegenerative changes in the retina is optical coherence tomography [16]. p-TAU accumulation is observed in other neurodegenerative diseases as well, including conditions such as progressive supranuclear palsy and frontotemporal dementia [2].

EFhd2, a calcium-binding protein, is highly expressed in the central nervous system and linked with various pathological forms of tau proteins in tauopathies. Earlier studies suggest that EFhd2 may be phosphorylated. Vazquez-Rosa *et al.* examined whether Cdk5, a hyperactivated kinase in tauopathies, phosphorylates EFhd2 and has an impact on its known molecular activities. The results obtained by the researchers indicated that EFhd2 is phosphorylated by brain extract derived from CK-p25 trans-

genic mice, which overexpresses the Cdk5 constitutive activator p25. Moreover, EFhd2 phosphorylation mediated by Cdk-5 affected its calcium binding activity. Results showed that EFhd2 is phosphorylated *in vivo* at S74, which implies that the physiological and pathological function of EFhd2 could be regulated by its phosphorylation state [17].

Additionally, Vega *et al.* identified EFhd2 in the tauopathy mouse model JNPL3. It was found that the EFhd2 protein is associated with human TauP301L in terminally ill JNPL3 mice and enriched in the sarkosyl-insoluble fraction. The link between EFhd2 and tau protein was not confirmed in young JNPL3 mice, hence their association may be connected with the process of neurodegeneration. Notably, the association between EFhd2 and tau was validated in humans and was found to be increased in AD [8]. However, the relationship between the tau-EFhd2 association and the severity of neurodegeneration requires further studies.

It has been shown that the expression of the *EFhd2* gene and protein abundance are altered in AD, which may suggest *EFhd2* gene expression might be regulated as a response to neurodegeneration [4]. Two research studies have demonstrated that late-onset Alzheimer's disease is linked to the chromosome region encompassing the *EFhd2* gene locus [4]. Hiltunen *et al.* revealed that chromosomal loci in 1p36 were associated with AD. The researchers performed a population-based genome-wide search with the use of LD mapping. The data collected by them suggest that several AD-associated chromosomal loci exist, which may include novel susceptibility genes for late-onset AD. One of them is the chromosomal loci in 1p36, which is connected with EFhd2 [5]. Furthermore, Olson *et al.* and Holmans *et al.* provided ambiguous evidence showing that the mean age at onset of an affected sib-pair had an impact on linkage to markers on chromosome 1p [18, 19].

There have been differences reported with respect to EFhd2 regulation in AD. While Borger *et al.* revealed a specific EFhd2 downregulation in the cortex, but not the hippocampus, of human dementia brains from different pathological traits, Ferrer-Acosta *et al.* demonstrated that EFhd2 was upregulated at the protein level in the frontotemporal cortex of human patients suffering from AD [10, 20]. The discrepancy between these two studies might result from different extraction protocols. However, in addition to protein data, Borger *et al.* confirmed the transcriptional downregulation of EFhd2, thereby supporting their protein biochemical data [10].

Whereas the associated downregulation of EFhd2 mRNA protein, specifically in the cortex of patients suffering from dementia, suggests a tissue-specific transcriptional mechanism of EFhd2 regulation, we cannot exclude an additional post-translational mechanism of EFhd2 regulation.

Dysregulation of neuronal calcium has been suggested as a molecular signal observed in neurodegeneration [21].

Ca²⁺ ions constitute crucial second messengers in cytoskeleton function and synaptic transmission [11]. Calcium ions, in influx and efflux, facilitate changes in the proteome, such as the activation of proteases and kinases, which have been said to take part in tau-mediated neurodegeneration pathobiology [22, 23]. It is reasonable to suggest that an increase in the concentration of neuronal calcium ions may promote EFhd2 linkage to tau proteins, thus leading to a shift from a physiological to a pathological function. In their study, Ferrer-Acosta *et al.* showed, conversely, that no calcium ions are required for the formation of amyloid structures or EFhd2 filaments. Therefore, the calcium-binding capacity of EFhd2 proteins may be linked to its physiological function in the nervous system, which suggests that factors disrupting the EFhd2 calcium-binding capacity may cause a conversion of this protein into a pathological molecule [20]. Based on this, it is possible to conclude that Swiprosin-1 might be involved in neurodegenerative diseases and neuronal degeneration.

CONCLUSIONS

In summary, the abnormal expression and modification of the tau protein may induce the process of neu-

ronal death observed during tauopathies. However, the molecular mechanism mediating neurotoxicity is still unknown. The results of studies show that EFhd2 is structurally similar to the amyloid protein linked with pathological tau protein in the brain of patients suffering from AD, and that the process of calcium binding may modulate the formation of EFhd2's amyloid structures. Hence, EFhd2 may be significantly involved in the pathobiology of tau-mediated neurodegeneration [1].

We consider Swiprosin-1 to be an attractive target for research in various pathological conditions, e.g. neurodegeneration and dementia. The challenges for the future include clarifying the complexity and variability of the Swiprosin-1 network in neurodegenerative disorders.

Given the growing incidence of neurodegenerative diseases, it would potentially be important to examine *EFhd2* gene expression in humans in order to determine the correlation between the single-nucleotide polymorphisms of the genes encoding EFhd2 and the role of EFhd2 in humans suffering from Alzheimer's disease. This would provide support for the hypothesis that this calcium-binding protein takes part in the pathogenesis of Alzheimer's disease.

Conflict of interest/Konflikt interesu

Absent./Nie występuje.

Financial support/Finansowanie

Absent./Nie występuje.

References/Piśmiennictwo

1. Ferrer-Acosta Y, Cruz ENY, Vaquer AD, Vega IE. Functional and structural analysis of the conserved EFhd2 protein. *Protein Pept Lett* 2013; 20: 573-583.
2. Regensburger M, Mielenz D, Winner B. Swiprosin-1/EFhd2 – another piece in the puzzle of tauopathy? *Aging (Albany NY)* 2018; 10: 522-523.
3. Bachman DL, Wolf PA, Linn R, Knoefel JE, Cobb J, Belanger A, et al. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. *Neurology* 1992; 42: 115-119.
4. Vega IE. EFhd2, a protein linked to Alzheimer's disease and other neurological disorders. *Front Neurosci* 2016; 10: 150.
5. Hiltunen M, Mannermaa A, Thompson D, Easton D, Pirskanen M, Helisalmi S, et al. Genome-wide linkage disequilibrium mapping of late-onset Alzheimer's disease in Finland. *Neurology* 2001; 57: 1663-1668.
6. Vuadens F, Rufer N, Kress A, Corthesy P, Schneider P, Tissot JD. Identification of swiprosin 1 in human lymphocytes. *Proteomics* 2004; 4: 2216-2220.
7. Park KR, An JY, Kang JY, Lee JG, Lee Y, Mun SA, et al. Structural mechanism underlying regulation of human EFhd2/Swiprosin-1 actin-bundling activity by Ser183 phosphorylation. *Biochem Biophys Res Commun* 2017; 483: 442-448.
8. Vega IE, Traverso EE, Ferrer-Acosta Y, Matos E, Colon M, Gonzalez J, et al. A novel calcium-binding protein is associated with tau proteins in tauopathy. *J Neurochem* 2008; 106: 96-106.
9. Mielenz D, Gunn-Moore F. Physiological and pathophysiological functions of Swiprosin-1/EFhd2 in the nervous system. *Biochem J* 2016; 473: 2429-2437.
10. Borger E, Herrmann A, Mann DA, Spires-Jones T, Gunn-Moore F. The calcium-binding protein EFhd2 modulates synapse formation in vitro and is linked to human dementia. *J Neuropathol Exp Neurol* 2014; 73: 1166-1182.

11. Purohit P, Perez-Branguli F, Prots I, Borger E, Gunn-Moore F, Welzel O, et al. The Ca²⁺ Sensor Protein Swiprosin-1/EFhd2 Is Present in Neurites and Involved in Kinesin-Mediated Transport in Neurons. *PLoS One* 2014; 9: e103976.
12. Kwon MS, Park KR, Kim YD, Na BR, Kim HR, Choi HJ, et al. Swiprosin-1 is a novel actin bundling protein that regulates cell spreading and migration. *PLoS One* 2013; 8: e71626.
13. Ballatore C, Lee VMY, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci* 2007; 8: 663-672.
14. Hernandez F, Avila J. Tauopathies. *Cell Mol Life Sci* 2007; 64: 2219-2233.
15. Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. *Annu Rev Neurosci* 2001; 24: 1121-1159.
16. Torbus-Paluszczak M, Łabuz-Roszak B. Optical coherence tomography in the diagnostics of neurodegenerative diseases. *Adv Psychiatry Neurol* 2018; 27: 334-342.
17. Vazquez-Rosa E, Rodríguez-Cruz EN, Serrano S, Rodríguez-Laureano L, Vega IE. Cdk5 phosphorylation of EFhd2 at S74 affects its calcium binding activity. *Protein Sci* 2014; 23: 1197-1207.
18. Holmans P, Hamshere M, Hollingworth P, Rice F, Tunstall N, Jones S, et al. Genome screen for loci influencing age at onset and rate of decline in late onset Alzheimer's disease. *Am J Med Genet B Neuropsychiatr Genet* 2005; 135B: 24-32.
19. Olson JM, Goddard KAB, Dudek DM. The amyloid precursor protein locus and very-late-onset Alzheimer disease. *Am J Hum Genet* 2001; 69: 895-899.
20. Ferrer-Acosta Y, Rodríguez-Cruz EN, Orange F, De Jesús-Cortés H, Madera B, Vaquer-Alicea J, et al. EFhd2 is a novel amyloid protein associated with pathological tau in Alzheimer's disease. *J Neurochem* 2013; 125: 921-931.
21. Mattson MP. Calcium and neurodegeneration. *Aging Cell* 2007; 6: 337-350.
22. Reinecke JB, DeVos SL, McGrath JP, Shepard AM, Goncharoff DK, Tait DN, et al. Implicating calpain in tau-mediated toxicity in vivo. *PLoS One* 2011; 6: e23865.
23. Wang JZ, Grundke-Iqbal I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci* 2007; 25: 59-68.