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Research Paper

PTK2B/Pyk2 overexpression improves a mouse model of Alzheimer's disease

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ABSTRACT

Pyk2 is a Ca^{2+}-activated non-receptor tyrosine kinase enriched in forebrain neurons and involved in synaptic regulation. Human genetic studies associated *PTK2B*, the gene coding Pyk2, with risk for Alzheimer's disease (AD). We previously showed that Pyk2 is important for hippocampal function, plasticity, and spine structure. However, its potential role in AD is unknown. To address this question we used human brain samples and 5XFAD mice, an amyloid mouse model of AD expressing mutated human amyloid precursor protein and presenilin1. In the hippocampus of 5XFAD mice and in human AD patients' cortex and hippocampus, Pyk2 total levels were normal. However, Pyk2 Tyr-402 phosphorylation levels, reflecting its autophosphorylation-dependent activity, were reduced in 5XFAD mice at 8 months of age but not 3 months. We crossed these mice with $Pyk2^{-/-}$ mice to generate 5XFAD animals devoid of Pyk2. At 8 months the phenotype of 5XFAD x $Pyk2^{-/-}$ double mutant mice was not different from that of 5XFAD. In contrast, overexpression of Pyk2 in the hippocampus of 5XFAD mice, using adeno-associated virus, rescued autophosphorylated Pyk2 levels and improved synaptic markers and performance in several behavioral tasks. Both $Pyk2^{-/-}$ and 5XFAD mice showed an increase of potentially neurotoxic Src cleavage product, which was rescued by Pyk2 overexpression. Manipulating Pyk2 levels had only minor effects on Aβ plaques, which were slightly decreased in hippocampus CA3 region of double mutant mice and increased following overexpression. Our results show that Pyk2 is not essential for the pathogenic effects of human amyloidogenic mutations in the 5XFAD mouse model. However, the slight decrease in plaque number observed in these mice in the absence of Pyk2 and their increase following Pyk2 overexpression suggest a contribution of this kinase in plaque formation. Importantly, a decreased function of Pyk2 was observed in 5XFAD mice, indicated by its decreased autophosphorylation and associated Src alterations. Overcoming this deficit by Pyk2 overexpression improved the behavioral and molecular phenotype of 5XFAD mice. Thus, our results in a mouse model of AD suggest that Pyk2 impairment may play a role in the symptoms of the disease.

Abbreviations: 5XFAD, mice transgenic for human APP and presenilin1 with 5 mutations found in familial Alzheimer's disease; AAV, adeno-associated virus; Aβ, amyloid β; AD, Alzheimer's disease; ANOVA, analysis of variance; APP, amyloid precursor protein; CA1/3, *cornu Ammoni* 1/3; DAPI 4′, 6-diamidine-2′-phenylindole; DG, dentate gyrus; FAK, focal adhesion kinase; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; IB, immunoblotting; IF, immunofluorescence; Inserm, Institut national de la santé et de la recherche médicale; LTM, long term memory; LTP, long term potentiation; mGluR5, metabotropic glutamate receptor 5; NIH, National Institute of Health; NMDA, *N*-methyl-D-aspartate; PLA, proximity ligation assay; PrPc, cellular prion protein; PSD-95, post-synaptic density protein, 95 kDa; PTK2B, protein tyrosine kinase 2B; Pyk2, proline-rich tyrosine kinase 2; SDS, sodium dodecyl sulfate; SEM, standard error of the mean; SFK, Src-family kinase; STEP, striatal enriched phosphatase; STM, short term memory; WT, wild type

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1. Introduction

Late onset Alzheimer's disease (AD) is the most common form of dementia in the aging population accounting for 60–80% of all cases (Alzheimer's Association Report, 2012). AD is a progressive neurodegenerative disorder characterized by plaques composed of β-amyloid protein (Aβ) surrounded by dystrophic neurites and neurofibrillary tangles (Alzheimer's Association Report, 2012). These histopathological hallmarks are accompanied by synaptic and neuronal loss, cerebral atrophy, cerebral amyloid angiopathy (Vinters, 2015), and inflammatory processes (Heneka et al., 2015). The progression of neurodegeneration in AD patients results in memory impairment and decline in other cognitive abilities often combined with non-cognitive symptoms including mood and personality changes (Alzheimer's Association Report, 2012).

A meta-analysis of genome-wide association studies identified 11 new loci associated with AD, among which *PTK2B* was one of the most significant (Lambert et al., 2013). This finding was replicated in other studies (Jiao et al., 2015), which suggested that *PTK2B* was associated with hippocampal sclerosis (Beecham et al., 2014), disease progression (Wang et al., 2015), and cognitive decline (Nettiksimmons et al., 2016). *PTK2B* encodes Pyk2, a Ca^{2+}-activated non-receptor tyrosine kinase closely related to focal adhesion kinase (FAK) (Lev et al., 1995; Sasaki et al., 1995; Avraham et al., 1995). The sensitivity of Pyk2 to increases in intracellular free Ca^{2+} distinguishes it from other tyrosine kinases. In response to Ca^{2+} Pyk2 is autophosphorylated on Tyr-402, which recruits and activates Src-family kinases (SFKs), (Dikic et al., 1996; Walkiewicz et al., 2015). In turn, SFKs phosphorylate other residues in Pyk2 and associated proteins, and initiate multiple signaling pathways. The striatal-enriched protein tyrosine phosphatase (STEP), which is enriched in forebrain regions, dephosphorylates Pyk2 (Xu et al., 2012). Pyk2 plays a role in several cancers and specific inhibitors are under development (Lipinski & Loftus, 2010). Although many cell types express Pyk2, it is highly enriched in forebrain neurons (Menegon et al., 1999) where it is activated by neuronal activity and excitatory neurotransmission (Corvol et al., 2005; Huang et al., 2001; Siciliano et al., 1996). Pyk2 regulates NMDA receptor function and is involved in synaptic plasticity (Huang et al., 2001; Bartos et al., 2010; Hsin et al., 2010). Our recent work with Pyk2 knockout mice shows that Pyk2 in the hippocampus is essential for spatial memory and long-term potentiation (Giralt et al., 2017). It also modulates the density and morphology of spines and the organization of post-synaptic regions (Giralt et al., 2017). Interestingly, heterozygous Pyk2 knockout mice have memory and hippocampal long term potentiation (LTP) deficits similar to homozygous mice and decreased Pyk2 levels contribute to the hippocampal phenotype of a Huntington's disease mouse model (Giralt et al., 2017). Thus, partial loss of Pyk2 function in the hippocampus has clear functional consequences.

The production of Aβ, a particular proteolytic fragment of amyloid precursor protein (APP), is a hallmark of AD (Benilova et al., 2012). It can form plaques and neurotoxic oligomers of various conformation and complexity (Mucke & Selkoe, 2012). Aβ oligomers can bind to cellular prion protein (PrPc) (Lauren et al., 2009) and signaling by PrPc and metabotropic glutamate receptor 5 (mGluR5), which includes Pyk2 activation, is disrupted by Aβ oligomers (Haas & Strittmatter, 2016). Another key molecular abnormality in AD is aberrant phosphorylation of Tau (Mandelkow & Mandelkow, 2012). In *Drosophila* the single orthologue of Pyk2 and FAK interacts with Tau and appears to suppress its toxicity (Dourlen et al., 2016). Moreover, in human AD brain hyperphosphorylated Tau and Pyk2 are colocalized (Dourlen et al., 2016). Finally, inhibitors of STEP enhance Pyk2 phosphorylation in vivo and improve some cognitive deficits in 3xTg-AD mouse model of AD (Xu et al., 2014). Thus, the *PTK2B* locus is reliably associated with AD risk and experimental evidence suggests that Pyk2 has the potential to play a role in the course of the disease, which remains to be characterized.

Here we investigated the role of Pyk2 in the 5XFAD mouse model in which Aβ production is strongly increased, due to expression of mutated forms of human APP and presenilin-1 (Oakley et al., 2006). These mice display age-dependent plaque development with features of AD including hippocampal-related cognitive deficits, neuroinflammation, neuronal loss, and synaptic degeneration (Oakley et al., 2006). We tested the potential role of Pyk2 in this model by genetic deletion and adeno-associated virus (AAV) mediated overexpression. Although Pyk2 levels were not changed in samples from AD or 5XFAD mice, Pyk2 autophosphorylation was reduced in 5XFAD mice indicating a decreased function. Deletion of the Pyk2 gene did not markedly alter the phenotype of 5XFAD mice, whereas overexpression of Pyk2 in the hippocampus rescued some behavioral alterations with correction of deficit in synaptic proteins and Src.

2. Materials and methods

2.1. Mouse lines

5XFAD mice expressing human amyloid precursor protein 695 (APP695) with Swedish, London, and Florida mutations, and M146L/L286V presenilin-1, under the control of the murine Thy-1 promoter (Oakley et al., 2006), were crossed with $Pyk2^{-/-}$ mice (Giralt et al., 2016). Genotyping (Oakley et al., 2006; Giralt et al., 2016) was carried out from tail biopsy (Charles River services, France). Mice were housed at 19–22 °C and 40–60% humidity with ad libitum access to food and water, under a 12:12 h light/dark cycle and used at 8 months, in accordance with ethical guidelines (Declaration of Helsinki and National Institute of Health, publication no. 85-23, revised 1985, European Community Guidelines, and French Agriculture and Forestry Ministry guidelines for handling animals, decree 87,849, license A 75-05-22), approved by the *Charles Darwin* ethical committee. Male and female mice were used.

2.2. Human samples

Prefrontal tissue was from patients with AD Braak grades V-VI (3 females, 2 males, age, 87.6 ± 2.8 years; post-mortem intervals, 8.0 ± 2.4 h, means ± SEM) and control cases (4 females, 1 male, age, 87.2 ± 3.3 years; post-mortem intervals, 7.2 ± 0.9 h) were obtained from the Center of Cognitive Neurology, Lariboisière Hospital (Paris France) with approval by the Ethical Committee of Paris Diderot University Hospitals (CEERB Bichat University Hospital, Paris, France). Hippocampal samples were obtained from two different sources: i) The Lille Neurobank (fulfilling criteria of the French law on biological resources and declared to competent authority under the number DC-2008-642) with donor consent, data protection and ethical committee review, samples managed by the CRB/CIC1403 Biobank, BB-0033-00030, patients with AD Braak stage VI (3 females, 2 males, age, 75.4 ± 3.8 years, post-mortem interval 17.4 ± 3.8 h, means ± SEM) and controls (2 females, 3 males, age, 50.8 ± 9.7 years, post-mortem interval 16.6 ± 4.0 h); ii) the Banc de Teixits Neurològics (Biobanc-HC-IDIBAPS) with the approval of the ethical committee of the University of Barcelona with the reference: IRB00003099, patients with AD Braak grades V-VI (11 females, 4 males, age, 84.7 ± 1.8 years; post-mortem intervals, 9 ± 3.4 h, means ± SEM) and control cases (6 females, 4 males, age, 84.2 ± 3.4 years; post-mortem intervals, 12 ± 1 h). The 2 sets of samples were analyzed separately, results expressed as a % of the control mean in the series and data being similar they were pooled for final comparison (1 aberrant point was discarded). All the patients had an history of progressive dementia and satisfied National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria for probable AD (McKhann et al., 1984) and satisfied neuropathological criteria for AD (Paquet et al., 2012). This research project was.