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Fyn knock-down increases Aβ, decreases phospho-tau, and worsens spatial learning in 3×Tg-AD mice

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Abstract

Fyn kinase phosphorylates tau and exacerbates amyloid beta (Aβ)-mediated synaptic dysfunction. However, Fyn also increases the nonpathological cleavage of amyloid precursor protein (APP), suggesting opposing roles for Fyn in the pathogenesis of Alzheimer's disease (AD). To determine the effect of Fyn on both Aβ and tau pathologies, we crossed homozygous Alzheimer's disease triple transgenic (3×Tg) mice harboring mutations in amyloid precursor protein, presenilin-1, and tau with wild-type or Fyn knockout mice to generate $Fyn^{+/+}3\times Tg^{+/-}$ or $Fyn^{+/-}3\times Tg^{+/-}$ mice. We found that $Fyn^{+/-}3\times Tg^{+/-}$ mice had increased soluble and intracellular Aβ, and these changes were accompanied by impaired performance on the Morris water maze at 18 months. $Fyn^{+/-}3\times Tg^{+/-}$ mice had decreased phosphorylated tau at 15–18 months (as did Fyn knockout mice), but $Fyn^{+/-}3\times Tg^{+/-}$ mice had increased phosphorylated tau by 24 months. In addition, we observed that $Fyn^{+/-}3\times Tg^{+/-}$ males were delayed in developing Aβ pathology compared with females, and displayed better spatial learning performance at 18 months. Overall, these findings suggest that loss of Fyn at early stages of disease increases soluble Aβ accumulation and worsens spatial learning in the absence of changes in tau phosphorylation.

Keywords: Fyn; APP; Aβ; Tau; Phosphorylation

1. Introduction

The pathogenesis of Alzheimer's disease (AD) is defined by the presence of 2 neuropathological lesions—amyloid plaques and neurofibrillary tangles (Duyckaerts et al., 2009). Plaques are extracellular aggregates of the amyloid beta (Aβ) peptide, a fragment of the β-amyloid precursor protein (APP) (Nunan and Small, 2000). Processing of APP to Aβ is altered by extracellular interactions (Hoe and Rebeck, 2008), intracellular adaptor proteins (King and Turner, 2004), and covalent alterations (Suzuki and Nakaya, 2008). The accumulation of neurofibrillary tangles occurs when the microtubule protein tau is hyperphosphorylated under pathological conditions and dissociates from microtubules, forming highly insoluble paired helical filaments which aggregate to form tangles (Duyckaerts et al., 2009). Several kinases have been shown to phosphorylate the serine and threonine residues of tau which are thought to underlie the tangle formation observed in AD (Trojanowski and Lee, 1994), including cyclin-dependent kinase 5 (Cdk5), glycogen synthase kinase 3 (Gsk3α and β), mitogen-activated protein kinase (MAPK), protein kinase A (PKA), and calcium/calmodulin activated kinase II (CaMKII). However, it is unclear which kinases are responsible for initiating the cascade of pathological tau phosphorylation.

Fyn tyrosine kinase is hypothesized to play a dual role in both the Aβ and tau pathologies observed in AD. Fyn colocalizes with pathological serine/threonine phosphorylated forms of tau in AD (Shirazi and Wood, 1993), and interacts directly with tau (Lee et al., 1998), phosphorylating it

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at tyrosine 18 (Lee et al., 2004). Tyrosine 18-phosphorylated tau is found in neurofibrillary tangles of AD brains and AD transgenic mice (Bhaskar et al., 2010; Lee et al., 2004), and Fyn expression increases with increased degree of pathology (Ho et al., 2005). Fyn-tau complexes have been hypothesized to localize to postsynaptic densities to affect glutamate receptor function (Ittner et al., 2010). Fyn also plays a role in Aβ-mediated pathological events, including Aβ-induced disruption of hippocampal network activity (Peña et al., 2010) and impairment of synaptic transmission and plasticity (Venkitaramani et al., 2007). In vivo models show that Fyn exacerbates Aβ-induced neuronal and behavioral deficits, and these effects are blocked by the genetic ablation of Fyn (Chin et al., 2004, 2005). However, our group has found that Fyn causes decreased Aβ production in vitro and Fyn knockout mice have decreased α-secretase APP products (Hoe et al., 2008). These findings suggest antagonistic roles for Fyn in increasing tau phosphorylation and Aβ-mediated neurotoxicity and in decreasing Aβ production, leading to the question of whether Fyn inhibition will ultimately prove beneficial or detrimental for the treatment of AD.

In order to investigate this question, we utilized a triple transgenic model of AD (3×Tg) harboring mutations in human APP, presenilin-1, and tau, which recapitulates both the Aβ and tau pathologies of AD, to determine the effect of Fyn on each (Oddo et al., 2003a, 2003b). We employed a genetic approach by breeding 3×Tg mice with either wild-type or Fyn knockout mice to generate wild-type or heterozygous Fyn mice on a heterozygous 3×Tg background ($Fyn^{+/+}3\times Tg^{+/-}$ and $Fyn^{+/-}3\times Tg^{+/-}$). We found that knock-down of Fyn resulted in both increased Aβ levels and decreased tau phosphorylation accompanied by deficits in spatial learning in the Morris water maze. These findings implicate a greater role for Aβ, and not tau, pathology in mediating cognitive performance at early disease stages in the triple transgenic model of AD. Taken together, our study implicates a harmful effect of long-term reduction of Fyn kinase on Aβ production and cognitive performance.

2. Methods

2.1. Animals and breeding

Fyn knockout mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) at 3 months of age for analysis of phospho-tau. Wild-type B6129SF2/J controls were also purchased from Jackson Laboratories. We crossed male Fyn knockout and wild-type mice with female 3×Tg AD mice originally generated by comicroinjection of human APP (K670M/N671L) and tau (P301L) transgenes under the control of the Thy 1.2 promoter into mutant PS-1 (M146V) knockin mice (Oddo et al., 2003b). Female and male $Fyn^{+/+}3\times Tg^{+/-}$ or $Fyn^{+/-}3\times Tg^{+/-}$ mice were generated and euthanized by rapid cervical dislocation (to eliminate anesthesia-mediated tau phosphorylation; Planel et al., 2007) at 15, 18, 21, and 24 months of age for females and 18, 21, and 24 months of age for males. Brains were quickly isolated, and hemi-brains were either snap-frozen in dry ice for biochemical analyses or immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for histochemical analyses.

2.2. Chemicals and antibodies

We used antibody 6E10 (Signet, Dedham, MA, USA) to detect Aβ, antibodies AT8, AT180, and AT270 (Pierce, Rockford, IL, USA) for phospho-tau epitopes, and Tau46 (Sigma, St. Louis, MO, USA) for total tau. Polyclonal Fyn antibody was purchased from Millipore (Billerica, MA, USA). Antibodies 1A10 (for Aβ1–40), 1C3 (for Aβ1–42), and 82E1 (human-specific Aβ antibody) for Aβ enzyme-linked immunosorbent assays (ELISAs) were obtained from IBL (Gunma, Japan).

2.3. Tissue preparation

Mouse brains were homogenized in a 10-fold volume of 50 mM Tris-HCl buffer, pH 7.6, containing 250 mM sucrose and protease inhibitor cocktail (Sigma). Soluble APP and Aβ were extracted in 0.4% diethylamine (DEA), as previously described (Nishitomi et al., 2006). Briefly, crude 10% brain homogenate was mixed with an equal volume of 0.4% DEA, sonicated, and ultracentrifuged for 1 hour at 100,000*g*. The supernatant was collected and neutralized with 10% 0.5 M Tris base, pH 6.8. The resulting DEA fraction was used for soluble Aβ ELISA analyses. Insoluble Aβ was extracted from the pellet after ultracentrifugation in formic acid (FA), sonicated, and ultracentrifuged for 1 hour at 100,000*g*. The supernatant was collected and neutralized with 1M Tris base and 0.5 M Na_2HPO_4, and the resulting FA fraction was used for insoluble Aβ ELISA analyses.

2.4. Western blot analyses

Proteins were extracted from brain homogenates with radioimmunoprecipitation assay (RIPA) buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, and 1 mM EDTA (Millipore), and probed for phosphorylated tau using antibodies AT8 (Ser202/Thr205), AT180 (Thr231), or AT270 (Thr181) (Hirata-Fukae et al., 2008). Total tau was detected with antibody Tau46 as a band at 60 kDa, and all phosphorylated tau values were normalized to total tau. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels, transferred onto a polyvinylidene fluoride (PVDF) membrane, and blocked with 5% nonfat dry milk. The blots were incubated with antibodies at room temperature overnight. Horseradish peroxidase (HRP)-conjugated secondary antibodies were visualized using an enhanced chemiluminescence detection system and exposed to film. For quantification of Fyn levels, 1 sample was run on every gel to allow for normalization across age, sex, and genotype. Bands were quantified using Bio-Rad QuantityOne Version 4.6.9 software (Hercules, CA,