Archival Report

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β-Amyloid Precursor Protein Intracellular Domain Controls Mitochondrial Function by Modulating Phosphatase and Tensin Homolog–Induced Kinase 1 Transcription in Cells and in Alzheimer Mice Models

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ABSTRACT

BACKGROUND: Mitophagy and mitochondrial dynamics alterations are two major hallmarks of neurodegenerative diseases. Dysfunctional mitochondria accumulate in Alzheimer's disease–affected brains by yet unexplained mechanisms.

METHODS: We combined cell biology, molecular biology, and pharmacological approaches to unravel a novel molecular pathway by which presenilins control phosphatase and tensin homolog–induced kinase 1 (Pink-1) expression and transcription. In vivo approaches were carried out on various transgenic and knockout animals as well as in adeno-associated virus–infected mice. Functional readout and mitochondrial physiology (mitochondrial potential) were assessed by combined procedures including flow cytometry, live imaging analysis, and immunohistochemistry.

RESULTS: We show that presenilins 1 and 2 trigger opposite effects on promoter transactivation, messenger RNA, and protein expression of Pink-1. This control is linked to γ-secretase activity and β-amyloid precursor protein but is independent of phosphatase and tensin homolog. We show that amyloid precursor protein intracellular domain (AICD) accounts for presenilin-dependent phenotype and upregulates Pink-1 transactivation in cells as well as in vivo in a Forkhead box O3a–dependent manner. Interestingly, the modulation of γ-secretase activity or AICD expression affects Pink-1–related control of mitophagy and mitochondrial dynamics. Finally, we show that parkin acts upstream of presenilins to control Pink-1 promoter transactivation and protein expression.

CONCLUSIONS: Overall, we delineate a molecular cascade presenilins–AICD–Forkhead box O3a linking parkin to Pink-1. Our study demonstrates AICD-mediated Pink-1–dependent control of mitochondrial physiology by presenilins. Furthermore, it unravels a parkin–Pink-1 feedback loop controlling mitochondrial physiology that could be disrupted in neurodegenerative conditions.

Keywords: AICD, FOXO3a, γ-Secretase, Mitochondrial dysfunction, Mitophagy, Parkin, Pink-1, Presenilins, 3xTgAD Mice

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It arose recently that besides macroscopic lesions characterizing specific subsets of neurodegenerative diseases, mitochondrial function appears to be consistently affected in brain diseases (1,2). Indeed, mitochondrial deficits are now considered as a major hallmark in Alzheimer's disease (AD) (3). In both familial and sporadic AD cases, early accumulation of structurally abnormal mitochondria has been evidenced (4,5), and such defects also stand in animal models of AD (6). Thus, β-amyloid precursor protein (βAPP) transgenic mice-derived neurons display drastically altered mitochondrial dynamics (6,7).

Exacerbated neuronal autophagy/mitophagy also corresponds to a consistent anatomical stigma in neurodegenerative diseases (2). In AD-affected brains, electron microscopy unraveled neuronal accumulation of autophagic vacuoles and impairment of autophagosomes maturation that ultimately yield amyloid-β (Aβ) overload (8). While both mitochondrial dynamics and mitophagy could well contribute to early phase of AD-linked neurodegeneration, little is known concerning the mechanistic defects that could account for such alterations.

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Phosphatase and tensin homolog (PTEN)-induced kinase 1 (Pink-1) controls both mitochondrial dynamics and mitophagy by selectively enhancing mitochondrial fission (9) and recruiting parkin (PK) to damaged mitochondria (10), respectively. Pink-1 is a PTEN-induced putative kinase 1 (11), and it is noteworthy that PTEN expression is altered in AD brains in a region-specific manner (12) and appears to be involved in the synaptic plasticity and cognition in AD mice models (13). Strikingly, immunological detection also revealed Pink-1 expression in senile plaques (14). Overall, alteration of PTEN-Pink-1 homeostasis could well account for some of the defects taking place in AD.

It is remarkable that presenilin 1 (PS1) and presenilin 2 (PS2), which constitute the catalytic core of γ-secretase responsible for the ultimate cleavage yielding Aβ (15,16) and its C-terminal counterpart APP intracellular domain (AICD) (17) from βAPP, regulate the level of PTEN (18). This led us to question of whether PSs could act as upstream regulators of Pink-1 and its associated functions. Here, we show that PS1 controls Pink-1 by a γ-secretase–dependent and βAPP-dependent but PTEN-independent mechanism. We show that AICD, which was shown to behave as a transcription factor (19–21), indeed controls Pink-1 transactivation and expression in a Forkhead box O3a (FOXO3a)-dependent process. AICD-mediated control of Pink-1 influences its mitochondrial and mitophagic functions. Finally, we demonstrate that PK, which acts as a transactivator of PS1 promoter (22), controls Pink-1 in a fully PS1-dependent but PTEN-independent manner. Thus, our study delineates, for the first time, a molecular cascade linking PS1 and Pink-1. Furthermore, we reveal a PS-dependent molecular link between PK and Pink-1 that could be part of a feedback loop responsible for their cellular homeostasis and mitochondrial health that could be altered in neurodegenerative condition.

METHODS AND MATERIALS

Cellular and animal models, promoter activities assay, messenger RNA (mRNA) analysis, and description of constructs are provided in the Supplement.

Drug Description and Administration Ex Vivo

The mitochondrial uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) and LY294002 (2-(4-morpholinyl)-8-phenyl-1(4*H*)-benzopyran-4-one hydrochloride) (23) (Millipore-Sigma; St Quentin Fallavier, France) were incubated at 10 μM for 6 and 16 hours, respectively, as previously described (24). DFK-167 (MP Biomedical, Santa Ana, CA) was applied on human embryonic kidney 293 (HEK293) and mouse embryonic fibroblast cells at 50 μM for 15 hours. ELND006 (D6) was kindly provided by Elan Pharmaceuticals (South San Francisco, CA) and used in both ex vivo and in vivo studies (see "3xTgAD and In Vivo Drug Treatment" section below) (25). In ex vivo experiences, HEK293 and mouse embryonic fibroblast cells were treated for 15 hours with D6 (5 μM).

Cellular and Mouse Brain Sample Preparation and Western Blot Analysis

Western blot analysis of cellular and mouse brain samples was performed by standard procedures and is described in the Supplement.

3xTgAD Mice and In Vivo Drug Treatment

3xTgAD mice (harboring $PS1_{M146V}$, βAPP_{swe}, and TAU_{P301L} transgenes) and nontransgenic (wild-type) mice (26) were housed with a 12-hour light/dark cycle and were given free access to food and water. All experimental procedures were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and local French legislation. Two groups of 24 wild-type male mice (129/C57BL/6) and 25 3xTgAD male mice were used. Mice were treated daily for 10 days with either vehicle or D6 (30 mg/kg) (Elan Pharmaceuticals) (25) via oral gavage (27). Animals were sacrificed 6 hours after the last administration. Dissected hippocampi were either submerged for 2 days in RNA*later* RNA Stabilization Reagent (Qiagen, Marseille, France) for quantitative polymerase chain reaction mRNA analysis or reserved for membrane fractions preparation devoted to γ-secretase activity measurement (28), and Western blot analysis of AICD and βAPP C-terminal fragments were performed as described in the Supplement.

Adeno-associated Virus-Nuclear Localization Sequence-AICD Production and Mice Injection

Virus production and mice injection were performed according to previously described protocols (27,29) and are resumed in the Supplement.

Mitochondrial Potential Disruption Analysis

Mitochondrial potential was accessed using live imaging and flow cytometry analysis of tetramethyl-rhodamine methyl ester probe as detailed in the Supplement.

Immunohistochemistry

Immunohistochemical analyses of mouse brain slices are described in the Supplement.

RESULTS

PS1 and PS2 Differently Modulate Pink-1 Transcription in a PTEN-Independent Manner

We have examined the ability of PS1 and PS2 to modulate Pink-1 levels at both transcriptional and posttranscriptional levels. We show that Pink-1 expression is poorly detectable in mock-transfected HEK293 cells and could be enhanced on CCCP treatment (Figure 1A, B), in agreement with the well-established stabilizing effect of this uncoupling agent on Pink-1 protein (10). Stable expression of PS1 drastically enhances Pink-1 expression, while PS2 reduces Pink-1 to levels below CCCP-treated mock-transfected cells (Figure 1A, B). Interestingly, PS1 and PS2 also trigger similar opposite effects on Pink-1 promoter transactivation (Figure 1C) and mRNA levels (Figure 1D).

PSs regulate cellular levels of PTEN (18), a tumor suppressor thought to transcriptionally transactivate Pink-1 (11). Therefore, we questioned whether a direct PS–PTEN–Pink-1 cascade could mechanistically account for PS-mediated modulation of Pink-1 or whether alternative pathways could be envisioned. As expected, PS1 overexpression enhances PTEN protein (Supplemental Figure S1A) and mRNA (Supplemental Figure S1C) expressions and transactivates its