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Research Paper

ADAM30 Downregulates APP-Linked Defects Through Cathepsin D Activation in Alzheimer's Disease

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ABSTRACT

Although several ADAMs (A disintegrin-like and metalloproteases) have been shown to contribute to the amyloid precursor protein (APP) metabolism, the full spectrum of metalloproteases involved in this metabolism remains to be established. Transcriptomic analyses centred on metalloprotease genes unraveled a 50% decrease in *ADAM30* expression that inversely correlates with amyloid load in Alzheimer's disease brains. Accordingly, in vitro down- or up-regulation of *ADAM30* expression triggered an increase/decrease in Aβ peptides levels whereas expression of a biologically inactive ADAM30 (ADAM30^{mut}) did not affect Aβ secretion. Proteomics/cell-based experiments showed that ADAM30-dependent regulation of APP metabolism required both cathepsin D (CTSD) activation and APP sorting to lysosomes. Accordingly, in Alzheimer-like transgenic mice, neuronal ADAM30 over-expression lowered Aβ42 secretion in neuron primary cultures, soluble Aβ42 and amyloid plaque load levels in the brain and concomitantly enhanced CTSD activity and finally rescued long term potentiation

Abbreviations: ADAM, A Disintegrin and Metalloproteinase Domain; APP, amyloid precursor protein; BACE, Beta-site APP cleaving enzyme 1; BSA, bovine serum albumin; CamKIIα, Ca2+/calmodulin-dependent protein kinase II alpha; COFRADIC, combined fractional diagonal chromatography; CTSD, cathepsin D; GKAP1, G kinase-anchoring protein 1; IRS4, insulin receptor substrate 4; LTP, long term potentiation; MMP, metalloproteinase; MRI, magnetic resonance imaging; PLA, proximity ligation assay; TD2, Type 2 diabetes.

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alterations. Our data thus indicate that lowering ADAM30 expression may favor Aβ production, thereby contributing to Alzheimer's disease development.

1. Introduction

Alzheimer's disease is a complex, multifactorial, neurodegenerative disease. It is the leading cause of dementia in elderly people. The main pathologic features of Alzheimer's disease are neurofibrillary tangles and senile plaque formation in the brain. The latter is caused by the progressive deposition of mainly 39- to 43-amino acid amyloid β (Aβ) peptides generated by proteolytic cleavage of the amyloid precursor protein (APP). The systematic observation of changes in APP metabolism in monogenic forms of Alzheimer's disease suggested that the Aβ/APP pathway is at the heart of the disease (Hardy and Selkoe, 2002). Even though the key role of APP processing in the etiology of Alzheimer's disease has been challenged in recent years, recent genetic and GWAS studies of sporadic forms of Alzheimer's disease seem to support the importance of dysfunctional APP metabolism and Aβ peptide production/degradation in the physiopathology of Alzheimer's disease (Lambert and Amouyel, 2011; Jonsson et al., 2012; Tian et al., 2013; Young et al., 2015).

The two major Aβ peptide species (Aβx-40 and Aβx-42) are produced by the sequential endoproteolysis of APP by β-secretase and γ-secretase complexes. APP can also undergo non-amyloidogenic cleavage by α-secretase within the Aβ sequence, which thereby precludes Aβ generation. The various enzymes and protein complexes involved in these secretase activities are increasingly well characterized: β-site APP cleaving enzyme 1 (BACE1) accounts for almost all the β-secretase activity, whereas a complex that includes presenilin 1 or 2 is responsible for the γ-secretase activity (De Strooper, 2003). More recently, an additional matrixine (MMP-MT5) has been shown to contribute to APP processing by acting upstream of the β-site (Willem et al., 2015; Baranger et al., 2016). Finally other *N*-terminal truncated Aβ peptide species can be generated by other proteases besides BACE1 (Wang et al., 2006; Schönherr et al., 2016).

In contrast, α-secretase activity is less well characterized even if several research groups have suggested that ADAM10 and ADAM17 are the major enzymes responsible for constitutive and regulated α-secretases-mediated pathways in the brain (Lammich et al., 1999; Kuhn et al., 2010). In addition to these direct cleavages taking place on APP, several additional proteins are likely to modulate APP levels by interfering with secretase activity, APP trafficking and/or APP degradation (Vincent and Checler, 2012). This complex network of protein-protein interactions is however poorly characterized and the identification of its components should improve our understanding of APP biology and fate, and might enable the delineation of therapeutic approaches.

Given this context, we decided to focus on ADAMs and related proteins. Our study was inspired by several observations besides our knowledge of the involvement of ADAMs as α-secretases (Rosenberg, 2009): (i) ADAMs and APP are involved in many different biological processes including brain development, plasticity and repair (Yang et al., 2006), and (ii) several matrix metalloproteases (MMP-2, -3 and -9) can degrade Aβ peptides (White et al., 2006; Reitz et al., 2010; Carson and Turner, 2002). We therefore performed a multi-angle screen for new components of APP metabolism, with a focus on MMPs, ADAMs and related proteins. We postulated that differentially expressed MMPs, ADAMs and related proteins (when comparing expression in Alzheimer's disease brains and control brains) might be clues for their involvement in APP physiology.

2. Materials and Methods

Written informed consent was obtained from study participants or, for those with substantial cognitive impairment, from a caregiver, legal guardian, or other proxy and the study protocols for all populations were reviewed and approved by the appropriate Institutional review boards of each country.

All animal experiments were approved by the local animal care and use committee (*Comité d'Ethique en Experimentation Animale du Nord - Pas de Calais*, Lille, France).

2.1. Study Design

We postulated that uncharacterized MMPS, ADAMs or related proteins may be involved in the APP metabolism. The purpose of this study was thus to explore this possibility. Potential candidates were selected from transcriptomic analyses targeting MMPs/ADAMs expression using total RNAs extracted from the brain of AD cases and controls. The strongest variations in expression were validated in an independent sample of brains using a different technology. Potential correlation between amyloid deposition in the brain of AD cases and expression of our genes of interest were examined. This work allowed us to select ADAM30 for further exploration.

We developed ADAM30 over- or under-expression experiments in different cellular models to assess ADAM30 impact on the APP metabolism. Potential α-, β- or γ-secretase activities of ADAM30 were examined. A without a priori research for ADAM30 substrates was performed using COFRADIC experiments. Impact of ADAM30 on APP metabolism through CTSD activation was tested using pharmacological or siRNA tools. All the experiments have been made at least in triplicates and by two independent manipulators for most of them. This work allowed us to demonstrate that ADAM30 modulates the APP metabolism through CTSD activity.

The in vitro observations were finally extended to an “Alzheimer-like” transgenic mouse model specifically over-expressing ADAM30 in neurons. Primary cultures of adult neurons were used to validate the results obtained from cell lines. Measurement of soluble Aβ42 and amyloid deposition were performed to corroborate the in vitro results in the mouse brains. Electrophysiological analyses were finally performed to extend the results to neuronal activity. All the analyses were performed in a blinded fashion. Our data demonstrated that ADAM30 over-expression led to a decrease in Aβ42 secretion in primary cultures, in soluble Aβ42 and amyloid deposition in the cerebral tissue and to a rescue of LTP in the Alzheimer-like mouse brain.

2.2. Microarray Analyses

The preparation of human brain samples is described in the Supplementary experimental procedures. Total RNA was extracted with a phenol/chloroform protocol (TRIzol® reagent, Invitrogen®, USA) from frozen frontal cortex brain tissue from one hundred fourteen Alzheimer's disease samples and one hundred sixty seven control samples. The quality of the total RNA extract was assessed with an Agilent 2100 Bioanalyzer (Agilent) and the ribosomal 28S/18S RNA ratio was estimated with the system's onboard biosizing software. Twelve Alzheimer's disease cases and twelve controls were selected among the initial samples by applying the following two criteria: (i) a ribosomal 28S/18S RNA ratio of 1.0 or more; (ii) a Braak stage below 2 (for control samples) (Table S1) (Bensemain et al., 2009).

Specific oligonucleotides for one hundred thirty two open reading frames (corresponding to MMPs, ADAMs, ADAMTSs and related proteins) were designed using OLIGOMER software (Mediagen) (Supplementary experimental procedures and Table S2; Bensemain et al.,