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Blood-Based Biomarkers

A novel detection method of cleaved plasma high-molecular-weight kininogen reveals its correlation with Alzheimer's pathology and cognitive impairment

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Abstract

Introduction: Accumulation of β-amyloid is a pathological hallmark of Alzheimer's disease (AD). β-Amyloid activates the plasma contact system leading to kallikrein-mediated cleavage of intact high-molecular-weight kininogen (HKi) to cleaved high-molecular-weight kininogen (HKc). Increased HKi cleavage is observed in plasma of AD patients and mouse models by Western blot. For potential diagnostic purposes, a more quantitative method that can measure HKc levels in plasma with high sensitivity and specificity is needed.

Methods: HKi/c, HKi, and HKc monoclonal antibodies were screened from hybridomas using direct ELISA with a fluorescent substrate.

Results: We generated monoclonal antibodies recognizing HKi or HKc specifically and developed sandwich ELISAs that can quantitatively detect HKi and HKc levels in human. These new assays show that decreased HKi and increased HKc levels in AD plasma correlate with dementia and neuritic plaque scores.

Discussion: High levels of plasma HKc could be used as an innovative biomarker for AD.

Keywords: Alzheimer's disease; Contact system activation; HMW kininogen; Cleaved HMW kininogen; Plasma biomarker; Sandwich ELISA; Diagnosis; Dementia

1. Introduction

High-molecular-weight kininogen (HKi) is a key constituent of the plasma contact-kinin system [1]. Activation of factor XII (FXII) triggers HKi cleavage by plasma kallikrein, resulting in the generation of cleaved high-molecular-weight kininogen (HKc) and the release of the proinflammatory peptide bradykinin. Increased activation of the contact system has been reported in thromboinflammatory diseases such as ischemic stroke [2] and myocardial infarction [3], as well as in autoimmune diseases [4] and hyperlipidemia [5]. More dramatic HKi cleavage is observed in hereditary angioedema [6], systemic lupus erythematosus [7], cancers [8], and sepsis [9].

The Alzheimer's disease (AD)-associated peptide Aβ42 can activate the contact system *in vitro*, leading to FXII-dependent cleavage of HKi [10–13]. *In vivo*, wild-type mice intravenously injected with Aβ42 have increased plasma HKi cleavage, which is not observed in FXII-knockout mice [14]. Furthermore, increased plasma HKi cleavage is found in two different AD mouse models driven by overexpression of human β-amyloid (Aβ) [14,15], suggesting that elevated Aβ expression can lead to increased contact system activation. The idea that plasma FXII activation and HKi cleavage contribute to AD

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pathogenesis is supported by studies showing that depletion of plasma FXII using an antisense oligonucleotide reduces plasma HKi cleavage, lowers brain inflammation, and improves cognitive function in an AD mouse model [15]. Importantly, increased HKi cleavage and elevated plasma kallikrein activity are also found in the plasma of AD patients, with the amount of HKi cleavage correlating with an established marker of AD progression [14]. Because some AD patients show HKi cleavage without detectable FXII activation [14], changes in HKi and HKc levels might be a more sensitive indicator of possible AD status.

Cleavage of HKi to HKc can be detected by Western blot, but this technique lacks the sensitivity needed for a diagnostic tool and is not amenable to a clinical setting. A more quantitative method that can measure HKi and HKc levels in plasma with high sensitivity and specificity would therefore be of interest as a possible tool to aid in the diagnosis of AD.

Here, we developed three classes of monoclonal antibodies: (1) one detecting only HKi; (2) one detecting only HKc, and (3) one detecting both HKi and HKc (HKi/c). We then established two sandwich ELISAs capable of discriminating between HKi and HKc in human plasma. Finally, we demonstrated the ELISAs' ability to show differences in HKi and HKc levels between Aβ-activated plasma versus vehicle-treated plasma and in AD patient plasma versus nondemented (ND) control plasma. These tools may help in the diagnosis of AD and other diseases characterized by contact system activation.

2. Methods

2.1. Production of monoclonal antibodies

For immunization, 50 μg of KLH-conjugated peptide (IQSDDDWIPDIQIDPNGLSC), which maps to the unique light chain of HK but not low-molecular-weight kininogen or HKi- and/or HKc-purified protein from human plasma (molecular innovations), was injected into Armenian hamsters and mice. See Supplementary Methods for further experimental details.

2.2. Antibody screening

Supernatants from hybridomas were screened at The Rockefeller High Throughput Screening Resource Center by direct ELISA with or without a fluorescent substrate using purified human HKi and HKc. See Supplemental Methods for further details.

2.3. Cloning of hybridoma cells and purification of monoclonal antibody

Cloning of hybridoma cells and purification of monoclonal antibody were performed at the Antibody and Bioresource Core Facility at Memorial Sloan Kettering Cancer Center. Details are in Supplementary Methods.

2.4. Sandwich ELISAs for measuring HKi and HKc levels in human plasma

After the purification step, the HKi (2B7) and HKc (4B12) antibodies were biotinylated using EZ-Link® Sulfo-NHS-LC-Biotin (Thermo Scientific) according to the manufacturer's instructions. After coating Fisherbrand™ 96-well plates with HKi/c antibody (3E8) at 100 ng/well in 0.1 M sodium bicarbonate buffer pH 9.6 (binding buffer) overnight at 4°C, the plates were washed with 0.1% Tween-20/PBS and incubated in blocking buffer (1% BSA in 0.1% Tween-20/PBS). Plates were washed three times between each step thereafter. Then, HKi/c protein or human plasma diluted in blocking buffer was incubated for 60 minutes at room temperature. After incubation with biotinylated 2B7 or 4B12 for 60 minutes at room temperature, plates were incubated with peroxidase-conjugated Streptavidin (Jackson ImmunoResearch Laboratories) for 60 minutes at room temperature. Signal was detected with HRP substrate (680) (LI-COR) or with BioFX® One Component HRP Microwell Substrate (SurModics). The ELISA results were normalized to total protein concentration determined by BCA (Thermo Scientific) in each plasma sample before the correlation analysis.

2.5. Pull-down assay

Pull-down assay with Dynabeads™ M-280 Streptavidin (Invitrogen) was performed according to the manufacturer's instructions. Details are in Supplementary Methods.

2.6. Human plasma samples

Experiments with plasma from human donors were approved by The Rockefeller University Institutional Review Board. Blood was drawn from two healthy donors (one 26-year-old male and one 36-year-old male), who giving informed written consent, using 21-gauge 0.75-inch butterfly needles into BD Vacutainer™ (BD) 10-mL plastic tubes with sodium heparin (158 USP Units) at The Rockefeller University Hospital. To obtain platelet-poor plasma, blood was centrifuged twice at 2000× *g* for 10 minutes at RT. Platelet-poor plasma was frozen immediately at −80°C.

Plasma was incubated with or without Aβ42 for 60 minutes at 37°C. Aβ42 (Anaspec) was prepared as described previously [14]. Briefly, Aβ42 was suspended in a minimum amount of 1% NH_4OH and then diluted to 1 mg/mL in PBS. The concentration of Aβ42 was determined by BCA (Thermo Scientific), and the aggregation state was confirmed by transmission electron microscopy at Rockefeller's Electron Microscopy Resource Center.

Plasma from AD patients and ND controls was obtained from University of Kentucky Sanders-Brown Center on Aging (Supplementary Table S1). Blood was drawn into heparinized plastic vacutainer tubes. AD cases were defined by both a clinical diagnosis of AD and a Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuritic