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Isolation and characterization of antibody fragments selective for toxic oligomeric tau

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ABSTRACT

Oligomeric tau species are important in the onset and progression of Alzheimer's disease (AD), as they are neurotoxic and can propagate tau-tangle pathology. Therefore, reagents that selectively recognize different key morphologies of tau are needed to help define the role of tau in AD and related diseases. We utilized a biopanning protocol that combines the binding diversity of phage-displayed antibody libraries with the powerful imaging capability of atomic force microscopy to isolate single-chain antibody fragments (scFvs) that selectively bind toxic oligomeric tau. We isolated 3 different antibody fragments that bind oligomeric but not monomeric or fibrillar tau. The scFvs differentiate brain tissue homogenates of both 3×TG and tau-AD mice from wild-type mice, detecting oligomeric tau at much earlier ages than when neurofibrillary tangles are typically detected. The scFvs also distinguish human postmortem AD brain tissue from cognitively normal postmortem human brain tissue, demonstrating the potential of this approach for developing biomarkers for early detection and progression of AD.

1. Introduction

Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease that causes brain atrophy, memory deterioration, and cognitive loss in affected individuals. It is the sixth leading cause of death in the United States, currently affecting over 5.4 million Americans with annual costs of over $200 billion in medical care (Alzheimer's Association, 2012). Although AD was first discovered over a hundred years ago, and substantial progress has been made in understanding the etiology of the disease, there are still no effective therapeutic or definitive diagnostic approaches available. AD is characterized by the presence of 2two hallmark pathologies, namely: extracellular neuritic plaques containing insoluble fibrillar aggregates of amyloid-beta (Aβ) and intracellular neurofibrillary tangles (NFTs) containing fibrillar aggregates of tau. Although these insoluble aggregated species have long been considered as the primary toxic elements of AD, increasing evidence indicates that small soluble oligomeric forms of both Aβ and tau play more critical roles in the onset and progression of AD than the fibrillar aggregates (Glabe, 2006; Lambert et al., 1998; Ward et al., 2012). The role of Aβ aggregation in AD in particular has been extensively studied (Gestwicki et al., 2004; Hirohata et al., 2007; Ono et al., 2002, 2004, 2006; Sinha et al., 2011, 2012), but despite very promising results in animal models, various therapeutic routes to target Aβ aggregation have had only very limited success in clinical trials (Check, 2002; Gilman et al., 2005; Salloway et al., 2009; Sperling et al., 2010). The role of tau in the progression of AD is gaining more attention, including studies to elucidate the roles of different variants and aggregate forms of tau (Berger et al., 2007; Blair et al., 2013; Cohen et al., 2013; Cowan and Mudher, 2013; Gerson and Kayed, 2013; Lasagna-Reeves et al., 2010, 2012b; Ward et al., 2012; Yanamandra et al., 2013).

Tau is a microtubule-associated protein, generally located in the axons of neurons, where it is involved in the assembly and stabilization of microtubules from tubulin. Although human tau is encoded by a single gene on chromosome 17q21, 6 major tau isoforms can be formed by alternative posttranscriptional splicing of exons 2, 3, and 10. Tau can also be posttranslationally modified by phosphorylation, glycosylation, ubiquitinylation, or glycation among others (Avila et al., 2004; Hernandez and Avila, 2007; Wang et al., 2007) resulting in a wide variety of different tau species that exist in vivo.

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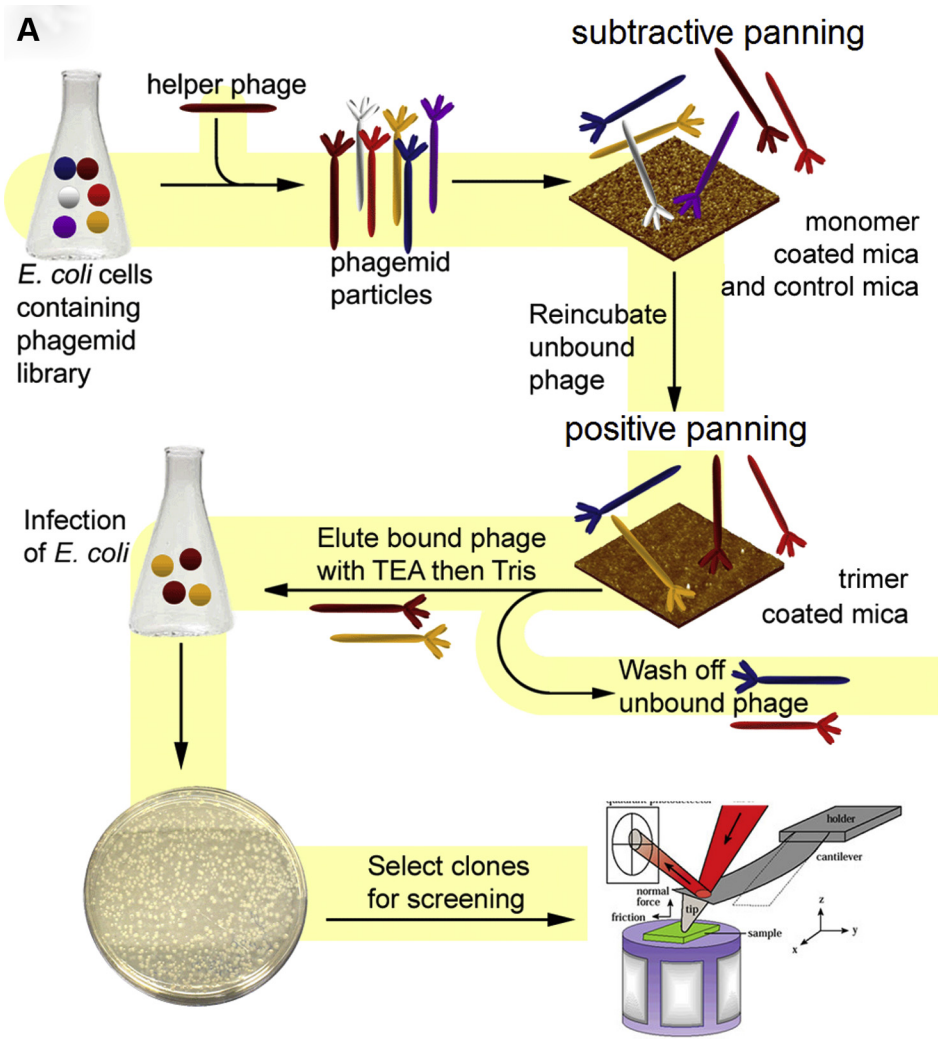

Fig. 1. The novel biopanning process combines subtractive panning and positive panning from phagemid scFv library with screening using AFM. (A) Schematic representation of the AFM-based panning process. (B) Subtractive panning against BSA after 1 (left), 3 (middle) and 5 (right) rounds of negative panning. The absence of phage after 5 rounds of negative panning indicated that the BSA subtractive panning step was complete. The scale bar of 1 μm applies to all 3 images. (C) Positive panning against tau trimer was performed and imaged with AFM. Left image: purified trimeric tau 1N4R immobilized on mica; right image: the same sample after addition of the remaining phage pool from subtractive panning and rinse, and it shows phage bound to target antigen. Large irregular shaped white particles in samples with added phage reflect contaminants present in the phage preparation. The scale bar of 1 μm applies to both images. Abbreviations: AFM, atomic force microscopy; BSA, bovine serum albumin; scFv, single-chain antibody fragment. (For interpretation of the references to color in this Figure, the reader is referred to the Web version of this article.)

Because tau hyperphosphorylation is associated with AD, it has been extensively studied, and inhibition of kinases involved in tau phosphorylation has been pursued as a potential therapeutic approach (Alonso et al., 2001; Hanger et al., 2009; Schneider et al., 1999). Levels of phosphorylated variants of tau correlate well with AD and other tauopathies including FTD (Alonso Adel et al., 2004). Hyperphosphorylation of the microtubule-binding domain of tau has been suggested to be one of the factors that promotes misfolding and loss of physiological function (Morris et al., 2011). However, phosphorylation of tau may also be required for some cellular functions including adult neurogenesis, as new adult-born granule neurons contain a significant amount of hyperphosphorylated 3 repeat tau variants (Bullmann et al., 2007). Therapeutic strategies aimed at regulating kinase activity bear the risk of interrupting normal phosphorylation dependent functions of tau along with other cellular functions. Given the complexity of the many different potential isoforms of tau that can occur in vivo and the uncertainty as to the physiological effects of tau hyperphosphorylation and aggregation, the roles of different hyperphosphorylated and aggregated tau variants in AD require clarification (Congdon and Duff, 2008; Cowan and Mudher, 2013; Schneider et al., 1999) for diagnostic or therapeutic development.

Numerous studies indicate that soluble aggregates of tau play an important role in the pathology of AD (Berger et al., 2007; Yoshiyama et al., 2007). Both brain derived and recombinant oligomeric tau aggregate species disrupt intracellular calcium levels and are toxic to cultured human neuronal cells when added extracellularly (Demuro et al., 2005; Gomez-Ramos et al., 2006, 2008). In animal models expressing human tau, neurodegeneration-related phenotypes including behavioral impairments, neuronal loss, and synapse lesions correlate better with the presence of soluble tau oligomers and prefilament species than with fibrillar NFT levels (Andorfer et al., 2005; Berger et al., 2007; Brunden et al., 2008; Polydoro et al., 2009). Neuronal loss also precedes NFT formation suggesting involvement of other species such as oligomeric tau variants (Brunden et al., 2008; Kayed et al., 2009; Marx, 2007; Meraz-Rios et al., 2010). In postmortem human brains, high oligomeric tau levels were detected in the frontal lobe cortex at early stages of AD