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Research Focus

En route to early diagnosis of Alzheimer's disease – are we there yet?

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As therapeutics for Alzheimer's disease (AD) become available, it will become increasingly important to develop accurate and reliable tools for its early diagnosis. A recent paper by Georganopoulou *et al.* suggests that nanobiotechnology could help to overcome the limitations with the current diagnostic methods. The paper introduces a nanoparticle-based assay using antibodies that are specific for amyloid β-protein (Aβ) oligomers with sub-femtomolar sensitivity. This assay appears to be more specific for AD than previous ELISA-based work and, if specificity of the assay for Aβ oligomers can be established, the method developed might provide a sensitive, reliable diagnostic tool for AD.

Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting ~4.5 million Americans yearly [1]. By 2050 the number of people with AD is expected to triple [2], placing an enormous burden on health care and social care systems. AD is a progressive neurodegenerative disorder that first manifests as short-term memory deficits, progresses to language problems, social withdrawal, deterioration of executive functions and eventually culminates in death [3]. Definitive identification of AD requires both a clinical diagnosis of the disease and post mortem detection of amyloid plaques and neurofibrillary tangles [4]. A probable diagnosis of AD can be established pre mortem with a confidence level of ~80%, based on clinical criteria, including medical history, physical examination, laboratory tests, radiology and neuropsychological evaluation [5]. Accurate, early diagnosis of AD is currently impossible because the early symptoms of the disease are shared by a variety of disorders, including other types of dementia and depression. Therefore, clinical assessment of AD often requires multiple examinations and the results are not always accurate. The need for accurate and early diagnosis of AD is increasingly important as therapeutics become available. Early therapeutic intervention, before severe cellular damage, would greatly improve the prognosis and quality of life of AD patients. Following recommendations by consortium groups for the Alzheimer's Association, research towards successful AD diagnostics has focused on two avenues – brain imaging techniques (http://www.alz.org) and disease-specific biological markers [6].

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Imaging techniques

The use of imaging techniques for the diagnosis of AD has been an area of extensive research [7]. Despite the high cost of instrumentation and limited availability, these methods are attractive because they are relatively noninvasive. Magnetic resonance imaging and computerized tomography are used primarily to rule out dementia due to causes other than AD and to detect hippocampal atrophy, a strong predictor for the conversion of patients from mild cognitive impairment (MCI) to AD [8]. However, atrophy reflects significant neuronal loss and is preceded by molecular and cellular changes. Thus, these methods are insufficient for early diagnosis and intervention. Metabolic imaging techniques, including fluorine-18-fluorodeoxyglucose-positron emission tomography (FDG-PET), show that patients with MCI have substantial changes in cortical metabolism. In fact, regional cerebral metabolic changes can be observed with FDG-PET before symptomatic manifestation of the disease, making PET a useful predictor of conversion from normal cognitive function to MCI [9]. Recent imaging research has been focusing on developing dyes that target fibrillar Aβ, thus enabling visualization of amyloid plaques, which are a pathological hallmark of AD [10]. However, the usefulness of such reagents for early diagnosis of AD is questionable because plaque accumulation happens relatively late in the disease and does not correlate well with cognitive deficits [11,12]. Currently, it is believed that soluble Aβ oligomers, rather than Aβ fibrils, are the primary effectors of AD [13]. Current imaging techniques cannot detect Aβ oligomers, which are transient, metastable species. Thus, despite encouraging progress, the information gleaned from imaging techniques is predictive and cannot definitively distinguish AD from other forms of dementia.

Cerebrospinal fluid biomarkers

A promising area of research in the pursuit of diagnostic tools for AD is the analysis of cerebrospinal fluid (CSF) for potential biomarkers [6]. Humans have ~150 ml of CSF that surrounds the brain and spinal cord. CSF can be sampled using lumbar puncture, a moderately invasive procedure, enabling analyses such as cell count, protein concentration and glucose level [14]. Disease-induced cellular and biochemical changes in the brain are often echoed in the CSF, making it an attractive repository of disease biomarkers. CSF-based tests are available for infective, inflammatory, ischemic and degenerative central nervous system diseases [14]. Several brain-derived

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molecules have been investigated as potential CSF makers for AD, including interleukins, isoprostanes, 3-nitrotyrosine, Aβ, tau and apolipoprotein E [15]. Of these, the most promising CSF biomarkers for AD are Aβ and tau, the major components of the two pathological hallmarks of AD, amyloid plaques and neurofibrillary tangles, respectively. Aβ exists predominantly as either a 40-amino acid (Aβ40) or a C-terminally extended 42-amino acid peptide (Aβ42). Several ELISA-based studies have shown that total tau and phosphorylated-tau (P-tau) levels are increased, whereas Aβ42 levels are decreased in the CSF of patients at early stages of AD, relative to healthy controls [14]. However, these changes are not unique to AD and have been observed in other forms of dementia [14]. To increase the specificity of tau-based assays for AD, ELISAs for specific epitopes of P-tau were developed. A study using specific P-tau epitopes demonstrated discrimination not only between AD and healthy controls, but also between AD and other types of dementia [16]. In another study, patients with AD, non-AD dementia and healthy controls were better distinguished using assays for Aβ42:Aβ40 ratio, compared with assays for Aβ42 alone, and the specificity was further increased when the Aβ42:Aβ40 ratio was combined with total tau [17]. However, these assays do not significantly improve the accuracy of present tests that diagnose ~80% of AD cases correctly.

A key requirement for reliable biomarkers is to detect fundamental disease features [6]. Soluble Aβ oligomers are thought to be fundamental to AD pathogenesis. Aβ oligomers have been detected in the brain of AD patients but not in age-matched controls [18–20]. In addition, using fluorescence correlation spectroscopy, soluble Aβ assemblies were detected in the CSF of AD patients but not in age-matched controls [21]. Therefore, a reliable assay for Aβ oligomers would be extremely helpful for accurate diagnosis of AD. However, detection of individual Aβ oligomers is difficult because the oligomers are transient and metastable and their concentration levels in CSF are below the detection limits of most current analytical methods.

Nanotechnology tools for AD diagnostics

Nanobiotechnology represents the convergence of the fields of engineering and molecular biology and offers new tools for disease diagnostics. Nanoparticle-based assays can detect target protein levels in the attomolar concentration range, six orders of magnitude lower than concentrations detected by ELISA [22]. Therefore, these assays have the potential to improve substantially our ability to detect early metabolic changes associated with disease. Such assays would enable physicians to properly diagnose disease at very early stages and begin treatment before severe cellular damage, improving patient prognosis. Because damage to the brain is irreversible, this is particularly important for neurodegenerative disorders such as AD.

A nanoparticle oligonucleotide bio-barcode assay developed by Nam *et al.* has been used to detect attomolar levels of the cancer marker prostate-specific antigen in serum [23]. This assay detects a protein of interest by its capture on magnetic microparticles coated with a monoclonal antibody, specific for the target protein. The microparticle–target protein complexes are then reacted with gold nanoparticles, which are coated with barcode DNA and a second (polyclonal) antibody for the target protein. The ternary microparticle–target protein–nanoparticle complexes are magnetically captured, and then the barcode DNA is released and detected using highly sensitive silver amplification [23]. This assay was adapted by Georganopoulou *et al.* to detect the presence of Aβ in human CSF using oligomer-specific monoclonal and polyclonal antibodies [24]. The study of Georganopoulou *et al.* demonstrated an eightfold increase in the mean Aβ oligomer immunoreactivity in AD patients relative to controls. These data are consistent with the study by Pitschke *et al.*, who used fluorescence correlation spectroscopy to detect CSF Aβ oligomers [21], and contradict previous ELISA studies in which total Aβ concentration levels were negatively correlated with AD [14]. A plausible explanation for the discrepancy between the ELISA data and the studies of Pitschke *et al.* and Georganopoulou *et al.* is that the oligomers detected in the latter studies might represent a small percentage of the total Aβ found in CSF. These studies indicated that assays for Aβ oligomers are more specific for AD than assays for total Aβ. The new study [24] offers advantages over the study by Pitschke *et al.* because it is more specific to oligomeric assemblies and will translate readily to high-throughput diagnostics of AD.

Future work

The protocol used by Georganopoulou *et al.* raises concerns regarding the assembly state of the Aβ species detected in their assay, which will need to be addressed in future studies of this system. Georganopoulou *et al.* used oligomer-specific antibodies to capture Aβ in the CSF. However, conceivably, these antibodies might cross-react with monomeric and/or polymeric Aβ assemblies. Other than binding to the antibodies, no evidence is provided for the assembly state of Aβ in the preparation. This concern is exacerbated in the experimental method used by Georganopoulou *et al.*, which involves incubations of Aβ with vigorous shaking at 37°C, a procedure known to induce rapid fibrillogenesis. Thus, data demonstrating the actual assembly state of Aβ are necessary to establish the specificity of the assay for Aβ oligomers.

Despite the need for additional evidence, the authors successfully demonstrated that the nanoparticle-based assay is a sensitive method to detect Aβ in CSF and their data suggest that this assay could be specific for AD. Two of the 15 patients tested in the study showed an overlap in their CSF Aβ immunoreactivity with controls [24]. In both cases, evidence suggests that these patients might represent a false-positive diagnosis of AD by current diagnostic methods. It will be important to evaluate the ability of the new assay to distinguish AD from other dementias and to determine whether it can detect AD before the appearance of clinical disease symptoms. The data from the study by Georganopoulou *et al.* are encouraging and position nanoparticle-based assays as a

promising technology for sensitive and reliable diagnosis of AD.

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