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NANOTECHNOLOGY FOR ALZHEIMER'S DISEASE DETECTION

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Alzheimer's disease (AD) is the most common cause of dementia among people of age 65 and older. The diagnosis of sporadic AD is based on clinical exclusion criteria and is only definite at necropsy. So, biochemical markers for AD would be of great value for its early diagnosis. During the last decade, research efforts have focused on developing cerebrospinal fluid (CSF) biomarkers for AD. The diagnostic performance of the CSF biomarkers: Tau protein, the 42-amino acid form of beta amyloid (Aβ42) and Amyloid Precursor Protein are of great importance. One possible biomarker for Alzheimer's is amyloid beta-derived diffusible ligands (ADDL). The correlation of CSF ADDL levels with disease state offers promise for improved AD diagnosis and early treatment. This is made possible by combining ADDL-specific monoclonal antibodies with an ultrasensitive, nanoparticle-based protein detection strategy termed biobarcode amplification (BCA). This review article explains how this BCA strategy makes clever use of nanoparticles as DNA carriers to improve the sensitivity of detection of Alzheimer's biomarker.

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1. Introduction

Alzheimer's disease (AD), first described by Alois Alzheimer in 1907, is the leading cause of dementia, accounting for more than half of all dementias in old age [1]. Memory loss is typically the earliest sign of AD. Besides memory loss, AD may present other neurologic symptoms, such as impairment of judgement, language, learning, abstract thinking, visuo-spatial skills and praxis. AD may further present changes in personality disorientation, sleep disturbances and hallucinations. At the onset of disease some motor symptoms may also be present, including rigidity or myoclonus, snout reflex or increased jaw jerk [2,3]. Dementia results from disorders of cerebral neuronal circuits and is a result of the total quantity of neuronal loss combined with specific location of such loss. The components of the medial temporal lobe memory system include the hippocampus and adjacent cortex, including the entorhinal, perirhinal and parahippocampal regions. This includes a circular pathway of neurons from the entorhinal cortex to the dentate gyrus, CA3 and CA1 neurons of the hippocampus to the subiculum and back to the entorhinal cortex, this pathway is heavily damaged in AD [4]. The characteristic histopathological features of the disease are extracellular amyloid plaques, formed by amyloid β-peptide (Aβ) depositions and intracellular neurofibrillary tangles (NFTs), which are, paired helical filaments of the hyperphosphorylated tau protein [5,6].

Both genetic and environmental factors are believed to play an important role in the causation and progression of AD [7]. Overproduction of Aβ, or failure to clear this peptide, leads to AD primarily through amyloid deposition, which produces neurofibrillary tangles; these lesions are associated with cell death, which is reflected in memory impairment, the hallmark of AD [8]. Familial AD is genetically heterogeneous and three different genes have been identified by genetic

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studies, amyloid precursor protein (APP), presenilin 1 (PS-1) and presenilin 2 (PS-2). Mutations in these genes lead to familial forms of AD. AD is usually divided into early-onset (presenile) dementia and late-onset (senile) dementia, and is also divided into familial and sporadic forms of disease according to family history [9]. Polymorphism of ApoE (apolipoprotein E) gene has been demonstrated and ApoE ε4 allele has been identified as a risk factor in late-onset AD patients [9]. Tau protein is predominantly expressed in axons, where it binds to and stabilizes microtubules [10] and is also the main component of paired helical filaments (PHFs). Phosphorylation on at least twenty five serine and threonine residues has been reported in tau isolated from an Alzheimer brain [11,12,13]. Tau in PHF is abnormally hyperphosphorylated, and it is hypothesized that this hyperphosphorylation contributes to neurodegeneration through the destabilization of microtubules [14].

2. Problems in the diagnosis of Alzheimer's disease

The diagnosis of AD during life remains difficult and a definite diagnosis of AD relies on histopathological confirmation at post-mortem or by cerebral biopsy [15]. Inspite of the fact that it is so common, AD often goes unrecognized or is misdiagnosed in its early stages. Some disorders that can result in dementia – such as – depression and poor nutrition- are curable, but AD is not. Therefore, it is very important to diagnose the cause of the dementia early and correctly. However, through thorough testing and a "process of elimination", a condition referred to as "probable AD" can be diagnosed with almost 90% accuracy. An early clinical diagnosis can be made if patients are tested by trained neuropsychologists. The great problem is not that mild cognitive impairment (MCI) cannot be diagnosed, but that the patients do not see doctor until severely affected. MCI may be defined as a transitional state between normal aging and AD in which memory impairment is greater than expected for age, but general cognitive function and daily living activities are preserved [16]. While diagnostic accuracy for the disease has improved, the differential diagnosis is still problematic. In the very early stages of disease, frequently classified as MCI, delineating disease process from "normal ageing" may also be difficult. Furthermore, the disease progression is slow and there is variability of performance on clinical measures, making it difficult to monitor change effectively [17].

3. Need for biomarkers

While diagnostic accuracy for the disease has improved, differential diagnosis for the disorder is still problematic [17]. In the very early stages, classified as MCI, delineating disease process from "normal aging" may be difficult. In later stages of the disease distinguishing AD from other neurological diseases associated with dementia may also be difficult. Moreover the disease progression is slow and there is variability of performance on clinical measures, making it difficult to monitor change effectively. Early diagnosis is highly desirable, for neurodegradation becomes severe and widespread in later age groups. Thus there is a great need for biomarkers that could substantially aid early diagnosis of AD.

4. Amyloid β peptide

The major component of neuritic plaques is the amyloid beta (Aβ) protein, a small 42 residue protein fragment derived through proteolytic processing of a large membrane bound glycoprotein, the amyloid precursor protein (APP) [18]. In AD brain, Aβ protein ending at residue 42 ($A\beta_{42}$) is deposited first and is the prominent form of senile plaques; whereas Aβ protein ending at residue 40 ($A\beta_{40}$) is deposited later in the disease [19]. Of all Aβ normally released from cells, $A\beta_{40}$ accounts for approximately 90% while $A\beta_{42}$ accounts for approximately for 10%[20]. $A\beta_{42}$ concentrations are decreased in CSF of AD patients and a number of studies have confirmed the finding [20]. The levels were also found to be lower in AD patients with ApoE ε4 allele than those without ε4 allele [21].

5. Tau Protein

As the intercellular space in the brain is in direct contact with CSF, biochemical changes in the brain may be reflected by CSF analyzed. So, analysis of neuronal proteins in CSF may function as biochemical biomarkers for the neuronal degeneration in AD. One such neuronal protein is tau protein, a normal human brain phospho protein, which binds to microtubules in neuronal axons, thereby promoting microtubule assembly and stability [22]. A pronounced increase in CSF tau protein (CSF-tau) is found in most patients with AD [23]. Total tau (t-tau) and truncated form of monomeric and phosphorylated tau (p-tau) can be measured in the CSF [17]. Using antibodies that detect all isoforms of tau proteins independent of phosphorylation, or specific phosphorylated sites, ELISA have been developed to measure total (t-) and p-CSF tau proteins concentrations [24,25].

Tau has been demonstrated to be the major protein component of the Alzheimer's neurofibrillary tangles' (NFTs) paired helical filaments (PHFs) and tau is abnormally hyperphosphorylated in tangles. The six isoforms of tau have been found in hyperphosphorylated state in PHF. It is believed that hyperphosphorylated tau can no longer interact properly with microtubules, leading to cellular dysfunction and subsequent neuronal death [15].

6. Amyloid Precursor Protein (APP)

The characteristic histopathologic features of the disease are extracellular amyloid plaques, formed by amyloid-β peptide (Aβ) deposition [7]. Although these plaques contain multiple proteins, their cores are composed primarily of β amyloid, a 39-42 amino acid proteolytic fragment derived from the amyloid precursor protein[47]. APP is a single-transmembrane protein with a 590-680 amino acid (aa) long extracellular amino acid terminal domain and approximately 55 amino acid cytoplasmic tail which contains intracellular trafficking signals [26]. Alternatively splicing of APP mRNA yields eight possible isoforms, three of which (695, 751 and 770) predominate in the brain [27,28].

In principle, if APP mismetabolism underlying AD, then indices of sAPP (secretory APP) could have diagnostic utility. In addition, measuring CSF-APP could provide indices of APP processing [17]. Although APP has been measured by Western blot in several studies [29,30], this method is not truly quantitative, lacks the precision and reliability of other methods, and does not permit the precise analysis of the large number of samples. The CSF concentration of APP appears to decrease with advancing severity of dementia, consistent with the smaller pool of surviving neuronal Sapp [17].

7. Apolipoprotein E (ApoE)

ApoE is a glycoprotein containing 299 aa, with a relative molecular mass of 34200 Da [31]. There are three major forms of ApoE (E2, E3 and E4) that are the product of three allelic forms (e2, e3 and e4) of this single gene [32]. The risk of developing AD seems to be allele dose dependent. Individuals carrying two e4 alleles are at higher risk and have an earlier onset of disease than those with 1 or no e4 allele [33]. In general, the small e2 allele at the ApoE locus may be protective against AD [34,35]. Numerous studies have established that the APOE genotype perhaps the most significant biological marker for susceptibility for AD, account for 45% - 60% of the genetic component [33]. However, in the patient with the clinical diagnoses of AD, the addition of APOE testing increase the positive predictive value of a diagnosis of AD by approximately 4% (from 90% – 94%) if an APOE e4 allele was present. In the patient with a clinical diagnosis of non-AD, the absence of an APOE e4 allele increased the negative predictive value by 8% (from 64% - 72%).

8. Nanomedicine: Introduction

Nanomedicine is defined as the application of nanotechnology to health. It exploits the improved and novel physical, chemical and biological properties of materials at the nanometric scale. Nanomedicine has potential impact on the prevention, early and reliable diagnosis and treatment of disease. It encompasses three interrelated themes of:

- nanodiagnostics including imaging
- targeted drug delivery and controlled release
- regenerative medicine.

The ultimate goal of **nanodiagnostics** is to identify disease at the earliest stage possible. Nanotechnology can offer diagnostic tools of better sensitivity, specificity and reliability. The objective of **drug delivery systems** is to target selected cells or receptors within the body. This technique is driven by the need on one hand to more effectively target drugs to the site of disease, to increase patient acceptability and reduce healthcare costs; and on the other hand to deliver new classes of pharmaceuticals that cannot be effectively delivered by conventional means. The **regenerative medicine** is focused to work with the body's own repair mechanisms to prevent and treat disabling chronic disorders of cardiovascular and central nervous system. Rather than targeting the symptoms or attempting to delay the progress of disease, future therapies will be designed to rectify chronic conditions using body's own healing mechanisms. For example, promoting self-repair mechanisms in the areas of the central nervous system [36].

The brain represents one of the most complex systems in biomedicine. With an improved understanding of brain functioning, nanotechnology offers better diagnosis and treatment for neurodegenerative disorders like multiple sclerosis, Alzheimer's disease and Parkinson's disease.

9. Nanoscience as Alzheimer's biomarker detector

One of the most promising applications of nanoscience is in Alzheimer's disease. As patients can be definitely diagnosed after they pass way and their brain is examined for the teltale damage, scientists are hunting for tests that would help make a diagnosis in living patients. One possible biomarker for Alzheimer's is a protein called amyloid beta-derived diffusible ligands (ADDL). Support for the role of ADDLs comes from their neurotoxicity [37], and presence at elevated levels in the brains of AD patients as compared with the age-matched controls [38]. The correlation of CSF ADDL levels with disease state offers promise for improved AD diagnosis and early treatment. This finding was made possible by combining ADDL-specific monoclonal antibodies [39,40] with an ultrasensitive, nanoparticle-based protein detection strategy termed biobarcode amplification (BCA) [41]. The BCA strategy used by Klein, Mirkin and coworkers [42,41] makes clever use of nanoparticles as DNA carriers to enable millionfold improvements over ELISA sensitivity. CSF is first exposed to monoclonal anti-ADDL antibodies bound to magnetic microparticles. After ADDL binding, the microparticles are separated with a magnetic field and washed before addition of secondary antibodies bound to DNA:Au nanoparticle conjugates. These conjugates conatin covalently bound DNA as well as complementary "barcode" DNA that is attached via hybridization. Unreacted antibody:DNA:Au nanoparticle conjugates are removed during second magnetic separation, after which elevated temperature and low-salt conditions release the barcode DNA for analysis [43]. Because the pathology of AD is thought to begin decades before the first symptoms, it would be very interesting to learn at what stage of disease progression ADDL levels in the CSF rise above those in healthy individuals.

10. Ethical Issues

Nanotechnology offers great promise for medicine, but much of this lies in future. This future orientation has made nanotechnology vulnerable to the current zeitgeist of over claiming in science, either the harm or benefit. There is a need to be careful about placing premature weight on speculative hopes or concerns about nanotechnologies raised ahead of evidence. Concern for economic competitiveness and other economic values may come into conflict with respect for

human dignity. The unrestricted freedom of some may endanger the health and safety of others. Therefore a balance has to be struck between values that are all legitimate in our culture. Even though the technology is based on the tiniest of particles, the possible payoff is anything but small.

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