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Research report

# Probing amyloid beta-induced cell death using a fluorescence-peptide conjugate in Alzheimer's disease mouse model

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ABSTRACT

With the increasing worldwide incidence of Alzheimer's disease (AD), there is a critical need for the discovery of more effective diagnostic methods. However, development of diagnostic tools in AD has been hindered by obstacles such as the absence of exact biomarkers. Apoptosis caused by amyloid-β (Aβ) plays an important role in AD pathology; therefore, provides an attractive biological target for the diagnosis of AD. The present study aimed to evaluate the potential of small peptide, named ApoPep-1 (Apoptosis-targeting peptide-1) as a new apoptosis imaging agent in AD. The fluorescein-conjugated ApoPep-1, but not the control peptide, targeted apoptotic cells in the brain of amyloid precursor protein (APP)/presenilin 1 (PS1) mice. We also observed fluorescence signals during *in vivo* imaging of apoptotic cells using ApoPep-1, and fluorescence levels increased in an age-dependent manner in APP/PS1 mice. *Ex vivo* imaging of isolated brains in APP/PS1 mice further confirmed the targeting of ApoPep-1 to apoptotic cells. The fluorescein-labeled ApoPep-1 co-localized with brain cells such as neurons, astrocytes, and microglia, all of which undergo apoptosis in the APP/PS1 mice brain. These findings demonstrate that ApoPep-1 can target apoptotic brain cells, and be used for experimental investigations relevant to apoptosis in AD.



## 1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in the elderly. This neuropathological condition is characterized by a progressive loss of cognitive function, and is defined by two established pathophysiological hallmarks in the brain. These are extracellular accumulations composed primarily of the amyloid-β (Aβ) peptide, and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein that promote neuronal apoptosis (Hardy and Selkoe, 2002; Querfurth and LaFerla, 2010).

Millions of people are currently affected by this disease. It is predicted that within the next few decades, AD will exert a huge social and economic impact if no efficient therapeutics and/or early diagnosis approaches become available (Brambilla et al., 2011). Therefore, an important challenge in the management of AD is to establish a method for effective diagnosis, in order to identify patients with AD prior to the actual onset of dementia (Forlenza et al., 2010). Due to the serious clinical need to predict adverse outcomes in patients at high risk of AD, there have been many developments in AD biomarker research. These include the development of cerebrospinal fluid (CSF) biomarkers (Blennow and Hampel, 2003; Clark et al., 2003; Buerger et al., 2006) and structural/functional neuroimaging protocols (Herholz et al., 2002; Singh et al., 2006). However, some limitations need to be overcome before these tools can be introduced into clinical practice. For example, fluid biomarkers are needed assay standardization and anatomical precision in the measurements. Magnetic resonance imaging and positron emission tomography are relatively

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expensive and require experienced personnel. Therefore, there is still a need for better diagnostic approaches to detect AD. Recently, although optical imaging by peptide-probe has the limitation of depth in tissue penetration, it is one of the most widely used imaging modality in clinical practice and in research. Compared to other imaging systems, optical imaging has many advantages, as it enables non-invasive, and safe detection using readily available instruments at moderate cost. Also, due to their advantages of high sensitivity, optical imaging plays a central role in the investigation of disease diagnosis and relevant drug development (Edgington et al., 2009).

Apoptosis caused by Aβ has been shown to play an important role in AD pathology. Although some researchers have suggested that there is a poor correlation between amyloid plaque load and the presence of dementia in AD (Engler et al., 2006; Holmes et al., 2008), many studies indicate that Aβ triggers a cascade of pathogenic events that culminate in neuronal apoptosis/death, neuritic dystrophy, and oxidative stress (Behl and Moosmann, 2002; Butterfield et al., 2010; Yang et al., 2009). Therefore, cellular apoptosis driven by Aβ provides an attractive biological target to better predict AD in individual patients. Although peptide-based probes have brief serum half-lives caused by degradation or excretion, small peptides as imaging probes have many potential advantages including more efficient penetration into tissues, easier conjugation with imaging agents and higher specificity to targets compared to proteins and antibodies. Additionally, small peptides have a low production cost and low immunogenicity (Lee et al., 2010). ApoPep-1 (Apoptosis-targeting peptide-1), six-amino-acid CQRPPR peptide, recognizes apoptotic and necrotic cells by binding to histone H1 exposed on the cell surface and located at the nucleus (Wang et al., 2010). ApoPep-1 has been successfully used for *in vivo* imaging of cell death in tumor cells and myocardial cells (Wang et al., 2010; Acharya et al., 2013).

The present study was designed to evaluate the feasibility of ApoPep-1, as an imaging tool for apoptosis in AD. Here, we have shown that ApoPep-1 bound to primary cultured apoptotic cells under conditions simulating AD, as well as to apoptotic brain cells from AD mice. Our findings demonstrate that ApoPep-1 could

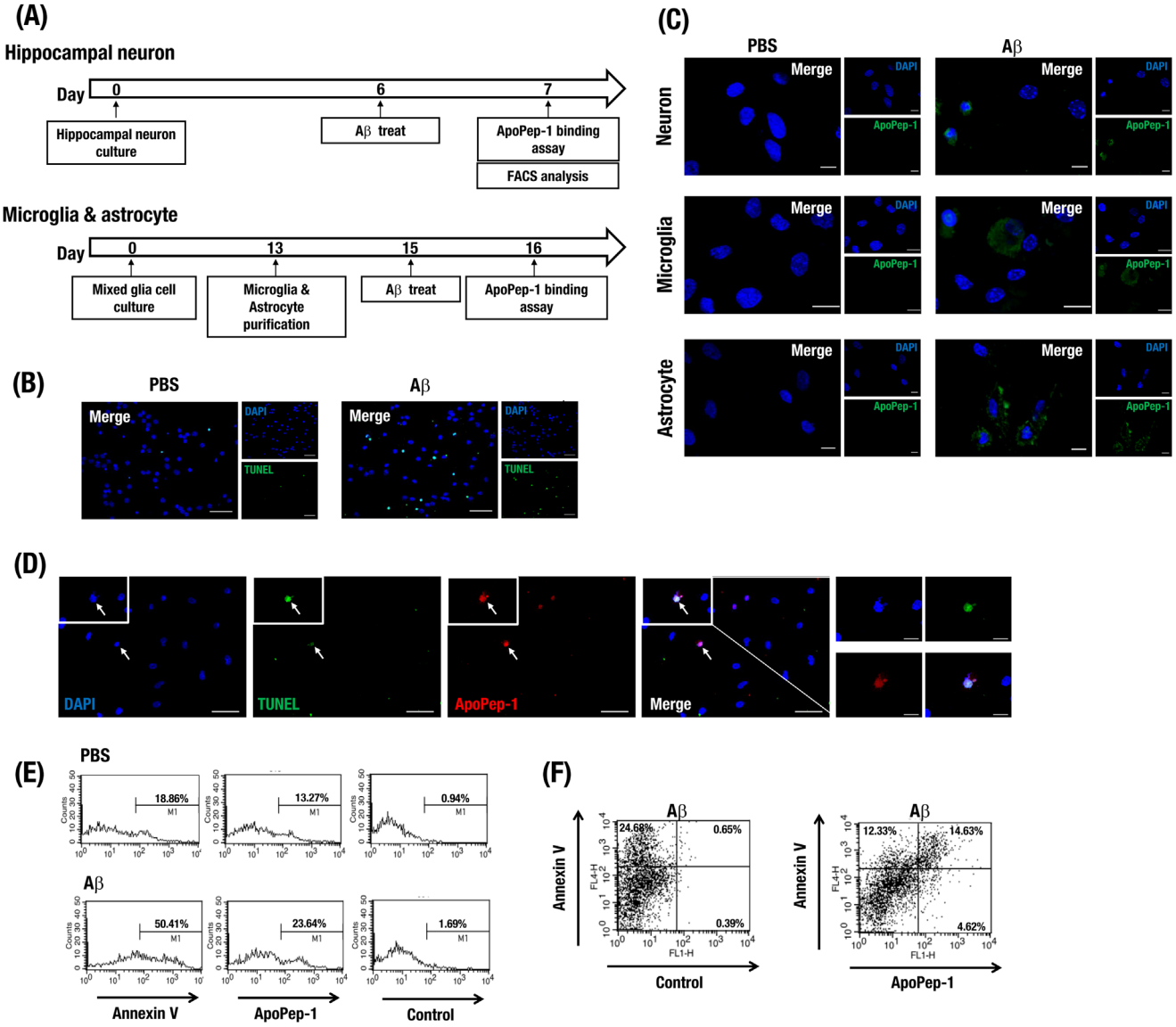


**Fig. 1.** ApoPep-1 can detect Aβ-induced apoptosis in primary cultured cells. (A) Protocol of the ApoPep-1 binding assay in primary cultured cells. (B) Hippocampal neurons were treated with 10 μM aggregated Aβ42 for 24 h and then stained with TUNEL/DAPI ($n=4$, scale bar, 50 μm). (C) Cells were stained with FITC-ApoPep-1 peptide (green) and DAPI (blue), and images were merged ($n=5$, scale bar, 10 μm). (D) Hippocampal neurons were treated with 10 μM aggregated Aβ42 for 24 h and then stained with Cy 5.5-conjugated ApoPep-1 and TUNEL/DAPI ($n=5$, low magnification scale bar, 50 μm; high magnification scale bar, 20 μm). (E, F) Flow cytometry analysis of hippocampal neurons detected by Annexin V, ApoPep-1, and control peptide ($n=4$–5). Aβ42 was used to induce apoptosis. Data are shown the representative images.