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Studying the relationship between cell cycle and Alzheimer's disease by gold nanoparticle probes

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ABSTRACT

In this study, a simple gold nanoparticle (GNP)-based colorimetric assay has been developed for studying the relationship between cell cycle and β-amyloid peptide (Aβ, the biomarker of Alzheimer's disease [AD]) expression level. It was found that Aβ expression of neuronal cells (e.g., SHG-44 cell line) is strongly dependent on cell cycle phases; that is, the Aβ expression level was highest when cells were arrested in the G1/S phase by thymidine and was lowest when they were arrested in the G2/M phase by nocodazole. This finding may improve the understanding of AD pathology and provide a new tool for anti-dementia drug development.

Alzheimer's disease (AD) is clinically characterized by progressive memory loss and impairments in behavior and language, and it is the most common cause of dementia [1,2]. AD is characterized by deposition of extracellular amyloid plaque, with the major component being the β-amyloid peptide (Aβ). Aβ is the enzymatic degradation product of amyloid precursor protein (APP) [3,4]. Currently, there is no definitive diagnosis of AD other than postmortem identification of senile plaques in the brain [3,5–7]. There are obstacles in treating the disease and designing an efficient therapeutic protocol because of the lack of understanding the pathogenesis of the disease [7–9]. During the past few years, more and more evidence has indicated that cell cycle abnormality plays a crucial role in AD pathogenesis [2,10–12]. For example, it is known that cell cycle-dependent mechanisms regulate phosphorylation and metabolism of APP in neuronal-like tissues [13]. Cell cycle regulators dramatically reduce the risk of AD in APP transgenic mice [14]. Human epidemiological study also indicates that individuals with a history of long-term use of a cell cycle regulator (e.g., a nonsteroidal anti-inflammatory drug) have reduced risk of AD [15]. Therefore, the cell cycle hypothesis may not only promote the understanding of AD pathology but also inspire a new potential therapeutic approach to cure AD. Recently, a range of methods/techniques, including DNA and protein electrophoresis, fluorescence spectrometry, and polymerase chain reaction (PCR) assay, have been employed to study the relationship between cell cycle and AD [11,16]. However, these assays have several potential drawbacks such as being time-consuming, requiring expensive or harmful reagents (e.g., fluorescent or radiolabeled antibodies), and having complex operation.

Recently, gold nanoparticle (GNP)-based colorimetric sensors have been extensively explored and applied in chemical and biological sensing such as detecting wide ranges of analytes, discriminating cancer and normal cells, and studying the interactions of metal ions with Aβ [17–20]. Here, the GNPs were employed as the signal indicators to unravel the relationship between cell cycle and AD. Semi-quantitative analysis of Aβ expression of SHG-44 cells (the SHG-44 cell line is a kind of neurocyte that derived from human frontal lobe astrocytoma) was demonstrated. The GNP-based colorimetric assay circumvents the need for expensive fluorescent antibodies, sophisticated equipment, and complicated experimental procedures.

Materials and methods

Materials and reagents

Peptides {GK(biotin)G DAEFR HDSGY EVHHQ K [$A\beta_{1-16}$(biotin)], CALNN, CALNN GK(biotin)G} were purchased from Scilight

Abbreviations: AD, Alzheimer's disease; Aβ, β-amyloid peptide; APP, amyloid precursor protein; GNP, gold nanoparticle; SA, streptavidin; HRP, horseradish peroxidase; pAb, polyclonal antibody; IgG, immunoglobulin G; DMEM, Dulbecco's modified Eagle's medium; UV–visible, ultraviolet–visible; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter.

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Biotechnology (Beijing, China). Thymidine, nocodazole, and hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Sigma–Aldrich (USA). Skim milk was purchased from Dingguo (Beijing, China). Streptavidin (SA) was purchased from Promega (USA). Rabbit polyclonal antibody (pAb) specific to human β-amyloid 1 to 42 ($A\beta_{1-42}$) (bs-0107R), pAb specific to cyclin A (bs-5739R), pAb specific to cyclin B (bs-0572R), pAb specific to cyclin E (bs-8929R), pAb specific to APP (bs-0112R), and horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) were obtained from Bioss (Beijing, China). The human glioma cell line (SHG-44), human glioma cell line (U-251), and human cervical cancer cell line (HeLa) were obtained from the Shanghai cell bank of the Chinese Academy of Sciences (Shanghai, China). Propidium iodide, RNase, cell lysis kit, and TMB color development solution were obtained from Beyotime (Haimen, China). Other chemicals were analytical grade and were used as received. Milli-Q water (18.2 MΩ cm) was used in all experiments.

Synthesis of SA-functionalized GNPs

The SA-functionalized 13-nm GNPs were prepared by a previously reported method [18] and were named as SA–GNPs. All experiments were carried out at room temperature unless mentioned otherwise.

Cell experiment

SHG-44, U-251, and HeLa cell lines were cultured with fresh Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum in a 6-well microtiter plate (for cell cycle-dependent assay) or a 48-well microtiter plate (for colorimetric assay) (10^5 cells/ml) under a humidified 5% CO_2 atmosphere at 37 °C for 12 h following interaction of 2 mM thymidine for 24 h. After removing the thymidine, the cells were cultured with fresh DMEM for another 12 h; subsequently, 2 mM thymidine or 100 ng/ml nocodazole was added and incubated for another 12 h. After removing the thymidine and nocodazole, the cells were washed with fresh DMEM (200 μl, three times) and interacted with 10 μg/ml $A\beta_{1-16}$(biotin) and 5 μM Cu^{2+} for 12 h. Then, the cell culturing media were removed; the cells were washed with Hepes buffer (200 μl, three times) and interacted with SA–GNPs (150 μl, 2.4 nM) for another 2 h. Finally, the cells were washed with Hepes buffer (200 μl, three times) carefully to avoid the nonspecific adsorption of GNPs and imaged by a Soif 37XB microscope (Shanghai Optical Instrument Factory, China) equipped with an A470 IS digital camera (Canon, Japan).

For cell cycle-dependent assay, the thymidine- or nocodazole-pretreated SHG-44 and U-251 cells were continually cultured in fresh DMEM supplemented with 10% (v/v) fetal bovine serum for another 0, 12, 24, 36, and 48 h, respectively. The cell culturing media were collected, and cells at the bottom of the microtiter plate were (i) interacted with 10 μg/ml $A\beta_{1-16}$(biotin), 5 μM Cu^{2+}, and 2.4 nM SA–GNPs as described previously for microscopic observation and ultraviolet–visible (UV–visible) spectral measurements or were (ii) lysed using the cell lysis kit following the manufacturer's instructions for traditional enzyme-linked immunosorbent assay (ELISA).

ELISA experiment

The collected cell culturing media and lysates were centrifuged at 13,000 rpm for 30 min to remove the insoluble aggregates and used immediately. The $A\beta_{1-42}$, APP, cyclin A, cyclin B, or cyclin E expression level in SHG-44 cells was determined by ELISA. Briefly, 100 μl of cell culturing media or lysates was added into the 96-well ELISA plate (Greiner, Germany). After being incubated at 4 °C overnight, the samples were removed and 200 μl of 5% (w/w) skim milk was used to block the plate well. After being incubated at 37 °C for 1 h, the skim milk solution was removed and the plate was washed with PBS-T (10 mM NaH_2PO_4/Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl, and 0.05% [v/v] Tween 20, pH 7.4). Then, 100 μl of $A\beta_{1-42}$ antibody (bs-0107R), cyclin A antibody (bs-5739R), cyclin B antibody (bs-0572R), cyclin E antibody (bs-8929R), or APP antibody (bs-0112R) (dilution ratio is 1:5000) was added into each well. After being incubated at 37 °C for 1 h, the antibodies were removed and the plate was washed with PBS-T. Subsequently, 100 μl of HRP-labeled goat anti-rabbit IgG (dilution ratio is 1:10,000) was added into each well. After being incubated at 37 °C for 1 h, the second antibody was removed and the plate was washed with PBS-T. Finally, 200 μl of TMB solution was added into each well and incubated for 10 min, and then 50 μl of H_2SO_4 solution (2 M) was added into each well and incubated for 1 min. After being treated by H_2SO_4, the maximum absorbances of the UV–visible spectra of solutions (OD_{max}) were recorded.

UV–visible absorption of GNP-stained cells

UV–visible absorption spectra of GNP-stained cells were measured by the microplate reader at room temperature. In general, after the cells were incubated with SA–GNPs as described previously, they were washed with Hepes buffer carefully (200 μl, three times) to remove the unbound nanoparticles, scraped from the microtiter plate, and redispersed in Hepes buffer (2.25×10^5 cells/ml). Then, 100 μl of cell solution was transferred to a 96-well microtiter plate, and UV–visible spectra of solutions were recorded by the microplate reader (BioTek PowerWave XS2, BioTek Instruments, USA).

FACS experiment

A fluorescence-activated cell sorter (FACS) experiment was employed to determine the cell cycle phases of SHG-44 cells. The thymidine- or nocodazole-pretreated SHG-44 cells were continually cultured in fresh DMEM supplemented with 10% (v/v) fetal bovine serum for another 0, 24, and 48 h, respectively. The cells at the bottom of the microtiter plate were first washed with PBS and then detached using trypsin–EDTA (ethylenediaminetetraacetic acid) solution. The cells were centrifuged at 800 rpm for 3 min. After removing the supernatant, the precipitate was mixed with 200 μl of PBS and then 800 μl of iced ethanol was added and mixed adequately. After being fixed at −20 °C for 8 h, cells were centrifuged to remove the ethanol, the precipitate was dispersed using PBS, and then RNase was added to make the final concentration 50 μg/ml. After being incubated at 37 °C for 30 min, the cells were mixed with propidium iodide and the final concentration of propidium iodide was 50 μg/ml. After being incubated at 0 °C in a dark place for 30 min, the cells were detected using a BD Accuri C6 flow cytometer (BD, USA).

Results and discussion

Here, biotinylated $A\beta_{1-16}$ [$A\beta_{1-16}$(biotin)] was selected as probe molecule. According to the previous report, Cu^{2+} can induce Aβ aggregates, and in the presence of Cu^{2+} $A\beta_{1-16}$(biotin) can interact with neural cell produced Aβ species on the cellular surface [21]. Through this reaction, together with the peptide, a biotin site is transferred to the cellular surface. Subsequently, SA–GNPs have been used as indicators to detect the Aβ expression level of SHG-44 cells because the SA has very high affinity with biotin [22]. In this case, 2.4 nM SA–GNPs were used for cell staining because the