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Au nanoclusters suppress chronic lymphocytic leukaemia cells by inhibiting thioredoxin reductase 1 to induce intracellular oxidative stress and apoptosis

Qiong Li [a,c,1], Qing Yuan [b,c,d,1], Mohan Zhao [e], Yawen Yao [d], Liang Gao [c], Ru Liu [c], Yaling Wang [c], Yong Gong [e], Fuping Gao [c,*], Xueyun Gao [c,d,*]

[a] Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[d] Department of Chemistry and Chemical Engineering, Beijing University of Technology, Beijing 100124, China
[e] Synchrotron Radiation Facility, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Chronic lymphocytic leukaemia (CLL) is a rare blood cancer that always relapses as refractory disease and eventually leads to death. To date, therapeutic options for CLL patients are scarce and there is an urgent need to develop novel chemotherapeutics that are both effective and safe. Gold-containing compounds induce a lethal oxidative and endoplasmic reticulum stress response in cultured and primary CLL cells via inhibition of thioredoxin reductase (TrxR). However, traditional gold-containing medicines have revealed side effects during clinical applications. Therefore, safer gold-containing drugs are needed to overcome this challenge. In this study, a novel peptide templated gold cluster $Au_{25}Sv_9$ was synthesized and its therapeutic effect on CLL cells was evaluated. This nanocluster could induce cell apoptosis in MEC-1 cells in a dose-dependent manner which correlated with the uptake amount of clusters in cells. As expected, increasing intracellular reactive oxidative species (ROS) in MEC-1 cells was exhibited with the increase of cluster dosage. Further analyses demonstrated the underlying mechanism that the nanoclusters suppress the activity of TrxR1, increase the level of intracellular ROS, destroy the mitochondrial membrane potential and finally trigger the mitochondrial apoptotic pathway in MEC-1 cells. Furthermore, the direct interaction between $Au_{25}Sv_9$ clusters and TrxR1 was confirmed for the first time by isothermal titration calorimetry. These findings explored the preclinical efficacy and potential mechanism of gold clusters in CLL therapy and provided a fundamental reference for the development of other novel gold-containing chemotherapeutics to treat CLL.

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1. Introduction

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia among the four major types of leukaemia in Western countries [1]. This rare blood cancer is usually associated with a long-period progression and typically affects aged people [1,2]. Although the initial chemoimmunotherapy has high remission rates, the typical outcome is relapse as refractory disease that ultimately leads to death [3,4]. To date, therapeutic options for CLL patients are limited and patients usually become resistant to current chemotherapies [2]. Therefore, there is an urgent need for the development of novel, effective and safe treatments for CLL.

Recent studies have reported higher levels of reactive oxygen species (ROS) in CLL cells than in normal lymphocytes [5–7]. As a result, the imbalanced redox status induces oxidative stress in CLL cells and the cells are more sensitive to agents that further increase oxidative stress [6]. Thioredoxin reductase (TrxR) is a homodimeric protein that catalyses the NADPH-dependent reduction of thioredoxin and other oxidized substrates to regulate the intracellular redox status and ROS levels [8,9]. Therefore, TrxR contributes to redox homeostasis as a general reducing enzyme, which is essential for many cellular processes including tumour cell growth and apoptosis [8–13]. Moreover, biomedical and clinical evidence have revealed that TrxR plays a central role in pathophysiologic

* Corresponding authors at: CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.

E-mail addresses: gaofp@ihep.ac.cn (F. Gao), gaoxy@ihep.ac.cn (X. Gao).

[1] Qiong Li and Qing Yuan have equal contribution.

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progression of chronic diseases, such as certain tumours and rheumatoid arthritis [8,14].

Human TrxR contains a selenocysteine residue at its active site and gold (I) shows strong affinity to selenol groups. Thus, the strategy to inhibit TrxR by gold-containing drugs has attracted great interest [8,13,15–17]. Auranofin (AF), an U.S. Food and Drug Administration (FDA) approved oral gold-containing small molecule drug that was used for rheumatoid arthritis treatment was previously found to have anti-cancer potential [12–14,18,19]. The mechanism of AF in tumour suppression involves TrxR inhibition and intracellular ROS induction, and the selenocysteine of TrxR has been identified as the drug target [13,14,18,19]. Based on these results, the in vitro and in vivo activity of AF in CLL treatment has also been examined. Studies showed that AF could induce a lethal oxidative and endoplasmic reticulum stress response in cultured and primary CLL cells via TrxR inhibition and intracellular ROS increase, as in other tumour cells [12]. These results suggested that TrxR could be a potential therapeutic target for CLL and that gold-containing agents could be effective drugs for CLL treatment [12,14]. However, one remaining challenge is that traditional gold-containing medicines have shown non-negligible side effects during clinical applications [14,20–22]. Therefore, safer gold-containing drugs need to be developed.

Gold clusters are novel nanomaterials that bridge the gold atoms/molecules and gold nanoparticles with atomic precision and ultra-small size [23–25]. The sizes of gold clusters are usually smaller than 2 nm and comparable with the Fermi wavelength of electrons [24,25]. Because of the ultra-small size, gold clusters exhibit some unique physical and chemical properties, such as a well-defined molecular structure and strong photoluminescence [24,25]. In recent studies, gold clusters synthesized with functional biomolecules revealed excellent biocompatibilities and synergistic effects on biomedical properties [23–25]. These functional biomolecules include natural or rationally designed proteins, peptides and nucleotides [24,25]. Owing to the advanced physicochemical properties of these biomolecules-protected gold clusters, such as strong photoluminescence and X-ray absorption, extensive studies have examined the applications of these clusters in bioimaging, biosensing and radiotherapy [24,25]. However, studies on their potential biomedicine applications are extremely rare.

We previously synthesized the Au nanocluster with a designed tridecapeptide (H_2N-CCYGGPKKKRKPG-COOH, abbreviated as Sv) comprised of 25 Au atoms and 9 tridecapeptides in each cluster molecule, named $Au_{25}Sv_9$ [23]. The highly positively charged (+5) tridecapeptides were not only used to stabilize the structure of clusters but also ensure good water dispersion as well as easier membrane translocation [23]. The synthesized $Au_{25}Sv_9$ clusters could emit red fluorescence after being excited, which can be used to track their location in cells, and the emission spectra peak is located at 645 nm [23]. The $Au_{25}Sv_9$ clusters could efficiently penetrate the membrane and specifically bind to TrxR in the cytoplasm and suppress the activity of TrxR [23]. In view of the high level of ROS in CLL cells, we hypothesized that $Au_{25}Sv_9$ can specifically suppress CLL by inhibition of TrxR activity. Therefore, in this study, we examined the in vitro suppressing activity of $Au_{25}Sv_9$ clusters in CLL and studied the underlying mechanism of action. Our findings will help contribute to the development of a potential therapeutic agent for CLL.

2. Materials and methods

2.1. Materials and cell line

All chemical reagents were purchased from Sigma-Aldrich (USA). The peptides were purchased from China Peptides Co. Ltd. (purity: 95%). All the primary antibodies were purchased from Cell Signaling Technology Inc., (USA) and the secondary antibody was bought from Beyotime Biotechnology (China). The blocking buffer and primary antibody dilution buffer for western blot were obtained from Beyotime Biotechnology. All cell culture reagents were purchased from Thermo Fisher Scientific Inc. (Gibco™, USA). The human chronic lymphocytic leukaemia (CLL) cell line, MEC-1, was obtained from the DSMZ (Braunschweig, Lower Saxony, Germany). And the mouse macrophage RAW 264.7 cell was obtained from the American Type Culture Collection (ATCC).

2.2. Preparation of $Au_{25}Sv_9$ clusters

An aqueous solution of $HAuCl_4$ (25 mmol/L, 16 μL) was slowly added to peptide solution (1.06 mmol/L, 376 μL) in a 5 mL vial under vigorous stirring, and then NaOH (0.5 mol/L, 8 μL) was added within 30 s to give a final pH of 10. The sample was sealed and stored in darkness for 15 h avoiding any disturbance to produce the Au_{25}peptide$_9$ products. The as-synthesized products were dialyzed for 12 h (Dialysis Tube MWCO:500) to remove free $HAuCl_4$ and NaOH. Then the sample was further concentrated by using an ultrafiltration tube (Millipore, USA, MWCO3000) to remove free peptides. The purified Au_{25}peptide$_9$ was sterilized by filtration with 0.22 μL filter for cell treatment. An aliquot was detected by ICP-MS to quantify the Au content and the rest was sealed and stored in dark under 4 °C.

2.3. Characterization of $Au_{25}Sv_9$ clusters

High-resolution transmission electron microscopy (HRTEM) was used to observe of synthetic $Au_{25}Sv_9$ and Nano Measurer 1.2 software was used to calculate the size distribution. Samples were prepared by casting and evaporating on a 300-mesh holey carbon-coated copper grid (Electron Microscopy Sciences, Washington, USA). High resolution images were obtained by TENCAI F20 high resolution transmission electron microscopy (FEI, USA) at 200 kV accelerating voltage. A PerkinElmer (LS-55) fluorescence spectrometer (PerkinElmer, USA) with Xe lamp was used to detect the excitation/emission spectra of $Au_{25}Sv_9$. The excitation and emission slits were both 10 nm and the scanning speed was 400 nm/min. Detection was repeated for three times.

2.4. Cell culture and cytotoxicity detection

MEC-1 cells and RAW264.7 cells were cultured in Iscove's modified Dulbecco's medium (IMDM) and Dulbecco's modified Eagle medium-F12 (DMEM/F12 1:1) (Hyclone) respectively supplemented with 10% heat-inactivated fatal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator.

Cytotoxicity was evaluated by Cell Counting Kit-8 system (Dojindo Laboratory, Kumamoto, Japan). Cells were seeded into 96-well plates at a density of 1×10^4 cells in 100 μL of the appropriate medium per well. After 24 h, cells were treated with different doses of $Au_{25}Sv_9$ or equivalent Sv peptide for another 48 h. The Cell Counting Kit-8 reagent was added and cells were incubated for 1 h at 37 °C. Absorbance was measured at 450 nm with a SpectraMax® M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The cell viability was calculated and shown as the mean ± standard error of triplicate experiments.

2.5. Observation of $Au_{25}Sv_9$ uptake in cells

Before observation, MEC-1 cells (5×10^4 cells/dish) were incubated with a series of doses of $Au_{25}Sv_9$ (0.5–20 μmol/L) for 48 h in confocal dishes. Cells were washed twice with PBS buffer after the culture media was discarded. Fresh culture media was added

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