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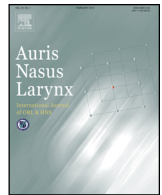

Induction of apoptosis by high-dose gold nanoparticles in nasopharyngeal carcinoma cells

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ABSTRACT

Objective: Nasopharyngeal carcinoma (NPC) is a rare malignancy in most parts of the world, but is a common cancer in southern Asia. Local recurrent disease and distant metastasis of NPC are still the unsolved problems. Recently, gold nanoparticles (AuNPs) have been developed as potential *in vivo* diagnostic and therapeutic agents. However, their role on nasopharyngeal cancer remains unknown. The object of this study is to investigate if AuNPs can be used as a new therapeutic agent for NPC by evaluating their anti-tumor effect *in vitro.*
Methods: The AuNPs were prepared by the reduction of chloroauric acid to neutral gold. Their size distribution and microstructures were characterized by transmission electron microscopy (TEM). To evaluate their cytotoxic effect, NPC cell line TW01 and Human Nasal Epithelial Cells (HNEpC) were cultured in various concentrations of AuNPs for 3 days. Cell viability was evaluated by Trypan Blue viability assay while morphologic findings were observed *via* light microscopy. Terminal deoxynucleotidyltransferase-mediated dUPT nick end labeling (TUNEL) assay was used to detect apoptosis.
Results: AuNPs prepared in this study had an average diameter of 20.5 nm and they were observed under light microscopy as dark material aggregated in the cells after treatment. Contrary to the HNEpC, the AuNPs reduced cell viability of NPC cell in a concentration-dependant manner by Trypan Blue assay, especially at high concentration. Besides, cell apoptosis was demonstrated by positive TUNEL assay.
Conclusions: The AuNP possesses specific imaging properties and is cytotoxic to NPC cells at high concentrations.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is a rare malignancy in most parts of the world, but is one of the most common cancers among Chinese or Asian ancestry. Its annual incidence in southern China is >20 cases per 100,000, which is much higher compared to other populations not as risk [1]. Its etiology is thought to be associated with a complex interaction of genetic, environmental and dietary factors, as well as Epstein–Barr virus exposure. Definitive diagnosis is made by biopsy of the nasopharyngeal tumor under direct visualization with the endoscope. However, NPC is often diagnosed late due to its deep location and vague symptoms, such that 60–70% of cases present with neck lymph node metastasis, and 5–11% of these present with distant metastasis [2].

Radiotherapy is the foundation of curative treatment although chemotherapy has been used in cases with advanced stages. However, advanced NPC patients who have concurrent chemoradiation therapy often suffer from radiation- or chemotherapy-related complications [2]. Contemporary NPC research, including genomics, proteomics, metabolomics and bioinformatics, has unraveled possible molecular mechanisms of this malignancy, and many molecular biomarkers have been discovered, enabling the development of specific molecular targeted therapies as potential therapeutic agents [1,3–5].

Nanotechnology has engendered a range of novel materials with unique properties and raised the possibility of designing molecularly targeted therapeutic or diagnostic agents. Nanoparticles can be effectively endocytosed by the cells, resulting in a high cellular uptake of entrapped biomarkers or therapeutic agents. Gold nanoparticles (AuNPs) have distinct advantages due to their relatively high biocompatibility, photo-stability, and ease of conjugation to biomolecules [6].

Recently, AuNPs have been developed as potential *in vivo* diagnostic and therapeutic agents, as X-ray contrast agents, drug delivery vehicles and radiation enhancers [7]. Although several studies have shown that AuNPs conjugated with certain

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biomolecules or drugs are potent against some cancer cell lines like prostate cancer [8,9] and ovarian cancer [10], their role on nasopharyngeal cancer remains unknown. The present study investigated whether or not AuNPs have cytotoxic effects on NPC cells *in vitro*.

2. Materials and methods

2.1. Preparation of AuNPs

AuNPs were prepared by the reduction of chloroauric acid (Au^{3+}, $HAuCl_4$) to neutral gold (Au^0) by sodium citrate. Briefly, water solutions of hydrogen tetrachloroaurate trihydrate and trisodium citrate dihydrate were mixed under heavy boiling, resulting in gold particles with a net negative charge from the citrate ions, which stabilized the particles. Varying the citrate to $HAuCl_4$ concentration ratio produced particles with an average size of about 20.5 nm. The AuNPs formed were then filtered through a 0.22-μm and used for experiments.

2.2. Transmission electron microscopy (TEM) observations

Transmission electron microscopy (TEM) was employed using a JEOL 2100, operating at 200 kV. The TEM was attached to a high-angle annular dark-field (HAADF) detector for *z*-contrast imaging. The AuNPs were dropped on a copper mesh coated with an amorphous carbon film for TEM observations. TEM images were used to determine the size distribution and microstructures of the AuNPs.

2.3. Cell culture and AuNPs treatment

NPC cell lines TW01 was kindly provided by Dr. Lin CT (Department of Pathology and Graduate Institute of Pathology, College of Medicine, National Taiwan University, Taiwan). The cell line was derived from primary nasopharyngeal tumors of Chinese patients with *de novo* NPC and was cultured with RPMI 1640 with 10% FBS. Human Nasal Epithelial Cells (HNEpC) (PromoCell Bioscience Alive, Heidelberg, Germany) were cultured with Airway Epithelial Cell Growth Medium (PromoCell Bioscience Alive, Heidelberg, Germany). Cultures were maintained at 37 °C under 5% CO_2. After being plated in 24-well plates (2×10^4 cells/well) for 24 h, the cells were rinsed with PBS buffer and then immersed into various concentrations of AuNPs solution (0 μM, 50 μM, 100 μM, and 200 μM) for three days, each concentration for 3 times, in a humidified atmosphere (37 °C and 5% CO_2).

2.4. Trypan Blue viability test

The dye exclusion test was used to determine the number of viable cells present in a cell suspension based on the principle that live cells possessed intact cell membranes that excluded certain dyes like Trypan Blue, Eosin, and propidium, whereas dead cells did not. The aliquots of cell suspension treated with AuNPs were centrifuged for 5 min at 100 × *g* and supernatant was discarded. The cell pellet was re-suspended in 1 ml serum-free medium. One part of 0.4% Trypan Blue and one part cell suspension were mixed and incubated for 3 min at room temperature. A drop of the Trypan Blue/cell mixture was applied to a hemacytometer. The unstained (viable) and stained (nonviable) cells were counted separately by microscopy. The percentage of viable cells was calculated as follows:

$$\text{Viable cells}\,(\%) = \frac{\text{Total number of viable cells per ml of aliquot}}{\text{Total number of cells per ml of aliquot}} \times 100$$

2.5. TUNEL stain

Terminal deoxynucleotidyltransferase-mediated dUPT nick end labeling (TUNEL) was performed according to the manufacturer's instructions (*in situ* cell death detection kit, TMR red; Roche Products, Hertforshire, UK). Briefly, after treatment with AuNPs solution for 48 h, the NPC cells were fixed for 1 h in 4% paraformaldehyde at room temperature. Cells underwent two washes in PBS and were incubated in permeabilization solution (0.1% Triton X-100 and 0.1% sodium citrate) for 2 min at 4 °C. The cells were then washed twice in PBS followed by incubation for 60 min in the dark at 37 °C in the "TUNEL reaction mix" from the Roche Products kit. The cells underwent two more washes in PBS and were immediately analyzed under fluorescence microscope (Olympus IX-70). Positive control was performed by incubating cells for 60 min at 37 °C in DNase solution before incubation in the TUNEL reaction mix. Negative control was performed by incubating cells in reaction mix that contained only label solution and no TUNEL enzyme components. All cells exhibited TUNEL staining in the positive control while no cellular staining was observed in the negative control.

2.6. Statistical analysis

Results were presented as means ± SD. Statistical comparisons of multi-group data were analyzed by ANOVA, followed by Sheffe's

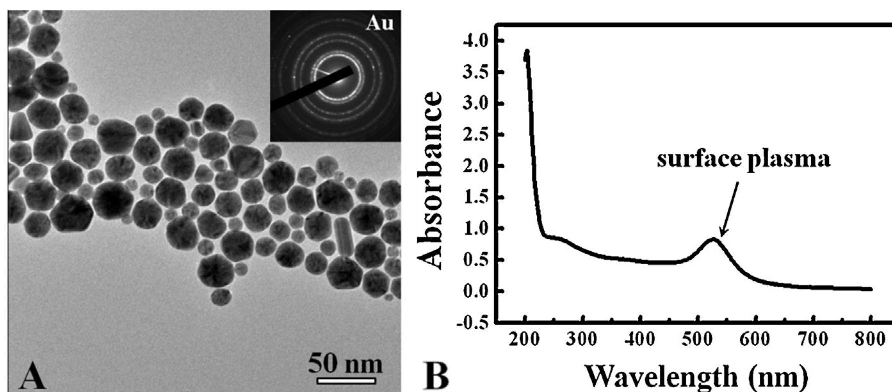

Fig. 1. TEM image and absorption spectrum of AuNPs. (A) TEM image of the prepared AuNPs showed the size of the nanoparticles followed a normal distribution with an average diameter of 20.5 nm. The inset showed the corresponding diffraction pattern, which identifies these nanoparticles to be composed of gold. (B) Absorption spectrum showed the AuNPs were characterized by an absorption maximum at 527 nm with a full width at half-maximum of approximately 58 nm.

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