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Extended Abstract

Colloidal Gold Nanoparticles Induce Apoptosis in MCF-7 Human Breast Adenocarcinoma Cells, Bhuiyan S, Jarvis Christian College, USA

Bhuiyan S

Abstract

Nanoparticles have been widely used as remedies for disorders for a long time. They are 10^{-9} m specks of substances that can be found both naturally and synthesized in the laboratory with metal and non-metal materials. In this study, gold nanoparticles (AuNPs) were synthesized using the citrate reduction method, and the 35 nm size of the nanoparticles was determined using a UV-Vis Spectrophotometer at 525 nm wavelength. The synthesized nanoparticles were further studied on MCF-7 breast cancer cells to understand how various genes are expressed in the induction of apoptosis in signal transduction pathways. The results obtained from the anticancer activity of the gold nanoparticles showed approximately 90% inhibition of cell growth after 72 hours of treatment. Western blot analysis demonstrated the down regulation of p44/42 MAPK (ERK1/2) protein due to gold nanoparticle treatment. Moreover, reverse transcription-polymerase chain reaction (RT-PCR) analysis of apoptotic genes revealed the up regulation of the p53 tumor suppressor gene, Bax, and caspase-9. The results assembled from this study further indicates that p44/42 MAPK, p53, caspase 9 and Bax play a major role in the mechanism of apoptosis in the MCF-7 breast cancer cells.

Introduction

Nanotechnology has become a very rampant research topic over the past years ranging from in vitro to inVivo clinical research using various metal and nonmetal particles targeting cancer cells and tumors . One of the established advantages of the nanoparticles is its size and its potential to be programmed to specifically target

organelles or tumors. Gold is popularly known as an element on the periodic table with an oxidation number of +3 and +1. It has an atomic number of 79 and an atomic mass of 197 amu. It is a transition metal and is solid at room temperature. Gold is mostly naturally occurring but can also be synthesized, which is rare. Though gold has been used for a while in orthodontic medicine, electronics, and aeronautic engineering, a small size (nanoparticle) has been accepted by multiple researchers as a probable agent for cancer remedy in biomedicine. A popular method for gold nanoparticle synthesis is a reduction reaction of tetrachloroauric acid (HAuCl4) by citrate commonly called the Turkeivich method. Other reducing agents such as plants as illustrated by Raghunandan et al. and Geetha et al. and fungi have been used as well. Researchers have also reported how the possible sizes and shapes of synthesized gold nanoparticles

Cells were purchased from ATCC (American Type Culture Collection) and maintained in 5% DMSO at -80°C until use. For culture, cells were maintained in RPMI 1460 Medium (Gibco, CA) enriched with 10% FBS (Fetal Bovine Serum) and 1% antibiotic/ antimycotic at 37°C with 5% CO_2 in a humidified atmosphere. Cells were plated at 5×10^4 cells/well to obtain a pattern of growth for further studies.

The nanoparticles were synthesized using a modification of the Turkevich method by Cesbron et al. Briefly, 0.01 g of $HAuCl_4$ was added to 50 ml of heated water. 1% of sodium citrate (Fisher Scientific) was then added to the heated stirring solution.

A reduction reaction was noticed when the solution turned into a wine color. The solution was purified using a 0.2 μm filter, protected from light and stored at 4°C.

MCF-7 cells were seeded at 3 × 105 cells per plate, allowed to attach for 24 hours and incubated in a humidified atmosphere at 37°C with 5% CO_2. The cells were treated with 0, 5, 10, 15 and 20 μg/ml of AuNPs in culture media. Cells were counted using the improved Neubauer hemocytometer chamber for 5 consecutive days.

The MCF-7 cells were plated at a density of 1×10^4 cells per well in a 96 well plate and allowed to attach. Thereafter, the cells were treated with AuNPs at 0, 5, 10, 15 and 20 μg/ml for 72 hours. Manufacturer's (Promega) instructions were followed. Briefly, 20 μl of

Bhuiyan S, Jarvis Christian College,USA, E-mail: sbhuiyan@jarvis.edu

MTS solution was added to each well and incubated for 4 hours. The plates were read at 492 nm using a SkanIt plate reader. The data obtained were statistically analyzed.

The MCF-7 cells (5×10^5) were incubated in petri dishes and allowed to attach for 24 hours. The cells were treated with similar concentrations as stated above of AuNPs for three consecutive days, trypsin zed using EDTA-Trypsin (Gibco), washed with PBS and stored at -20°C.

Statistical data for the reverse transcription-polymerase chain reaction was obtained in reference to β-actin. The method of calculation was obtained from Kannan [12]. The difference between the Ct values of subtracted and treated samples were obtained for all of the targeted genes (p53, caspase-9, bcl2, bax, and β-actin). The difference between these two newly obtained values was calculated to the negative power of two, for acquiring the relative fold change.

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