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Genomic instability of gold nanoparticle treated human lung fibroblast cells

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ABSTRACT

Gold nanoparticles (AuNPs) are one of the most versatile and widely researched materials for novel biomedical applications. However, the current knowledge in their toxicological profile is still incomplete and many on-going investigations aim to understand the potential adverse effects in human body. Here, we employed two dimensional gel electrophoresis to perform a comparative proteomic analysis of AuNP treated MRC-5 lung fibroblast cells. In our findings, we identified 16 proteins that were differentially expressed in MRC-5 lung fibroblasts following exposure to AuNPs. Their expression levels were also verified by western blotting and real time RT-PCR analysis. Of interest was the difference in the oxidative stress related proteins (NADH ubiquinone oxidoreductase (NDUFS1), protein disulfide isomerase associate 3 (PDIA3), heterogeneous nuclear ribonucleus protein C1/C2 (hnRNP C1/C2) and thioredoxin-like protein 1 (TXNL1)) as well as proteins associated with cell cycle regulation, cytoskeleton and DNA repair (heterogeneous nuclear ribonucleus protein C1/C2 (hnRNP C1/C2) and Secernin-1 (SCN1)). This finding is consistent with the genotoxicity observed in the AuNP treated lung fibroblasts. These results suggest that AuNP treatment can induce oxidative stress-mediated genomic instability.

1. Introduction

The current knowledge of the toxicological profile of gold nanoparticles (AuNPs) is incomplete and this is inhibiting their use in many clinical applications including diagnostic imaging [1], drug delivery [2,3] and photothermal therapy [4]. Bulk gold is a yellow solid and relatively inert, while AuNPs at nanosize appear wine red in solution and their biological activity is still not entirely understood. The unique characteristics and properties of the nanosized particles also make it hard to predict their biological reactivity. Some studies have suggested that AuNPs may cause toxicity *in vitro*, citing oxidative stress and DNA damage as results of AuNP treatment [5,6,7]. *In vivo* studies also reflect similar observations; AuNPs exhibited pulmonary toxicity and genotoxicity in mice as well as oxidative stress in aquatic species [8,9]. One study on inhalation exposed rats to AuNPs also showed differential expression in global gene analysis in various organ tissues [10]. As we had previously reported, AuNP treatment also induces oxidative stress, autophagy and DNA damage *in vitro* [5,11]. It would seem that, there is still a need to perform in depth investigations on the toxicity profile of AuNPs for its use in bio-applications to be safe and meaningful.

Proteomic techniques, such as two dimensional gel electrophoresis (2D-GE), are useful tools in the field of drug and toxicity studies. In 2D-GE, proteins are separated according to 2 measures, firstly by their isoelectric points and secondly by molecular weight through SDS-PAGE gel electrophoresis. It is one of the most common tools currently used in toxicity studies today. Coupling it with mass spectrometry, specifically the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS), allows the identification of protein biomarkers of disease progression or predictive markers of toxicogenesis [12]. The use of proteomic techniques in toxicological studies is steadily growing as the field relies more heavily on molecular data to identify critical protein changes and pathways to provide a reliable predictive platform for drug development and toxicological profiling [13,14]. A number of

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researches have classified the use of proteomics in toxicity studies into two levels. Tier I analysis refers to global protein mapping and profiling for differential expression while Tier II involves elucidating the protein functions and interactions as well as how specific post-translational modifications and their threedimensional structure affect these processes [14].

The primary focus of the current study was on the quantification and identification of proteins (Tier I analysis) and its differential expression upon AuNP treatment since nanomaterial-induced toxicological profiles are still largely unknown. We performed a comparative analysis of the protein expression profile of AuNP treated and control human fetal MRC-5 lung fibroblasts. To confirm the proteomic findings, selected protein expression results were verified by western blotting and real time RT-PCR analysis. In addition, we further correlated the above investigations with the alkaline single-cell gel electrophoresis assay (comet assay) and the fluorescence *in situ* hybridization (FISH) assay to assess DNA damage and chromosomal aberrations caused by *in vitro* exposure to AuNPs.

2. Materials & methods

2.1. Cell culture

The cells used were MRC-5 human fetal lung fibroblast cells (ATCC No.: CCL-171) cultured in RPMI 1640 media and supplemented with 10% fetal bovine serum (FBS) in 100 μg/ml penicillin/streptomycin in a 37 °C, 5% CO_2 incubator.

2.2. AuNP synthesis and preparation

Gold nanoparticles (AuNPs) of 20 nm in diameter, were prepared in citrate reduction from gold salts. The nanoparticles were spun down to remove the citrate buffer and subsequently coated with fetal bovine serum, washed and reconstituted in phosphate buffer saline (PBS) solution to form the stock solution. The AuNP stock solution was then sterile filtered before addition into treatment media.

2.3. AuNP treatment

MRC-5 cells were seeded in 6 wells cell culture plates (NUNC) at a seeding density of 4×10^4 cells/ml and treated with 1 nM concentration of AuNP in growth media the following day. Control cells were cultured in growth media. Treated and control cells were then incubated for 72 h before harvesting.

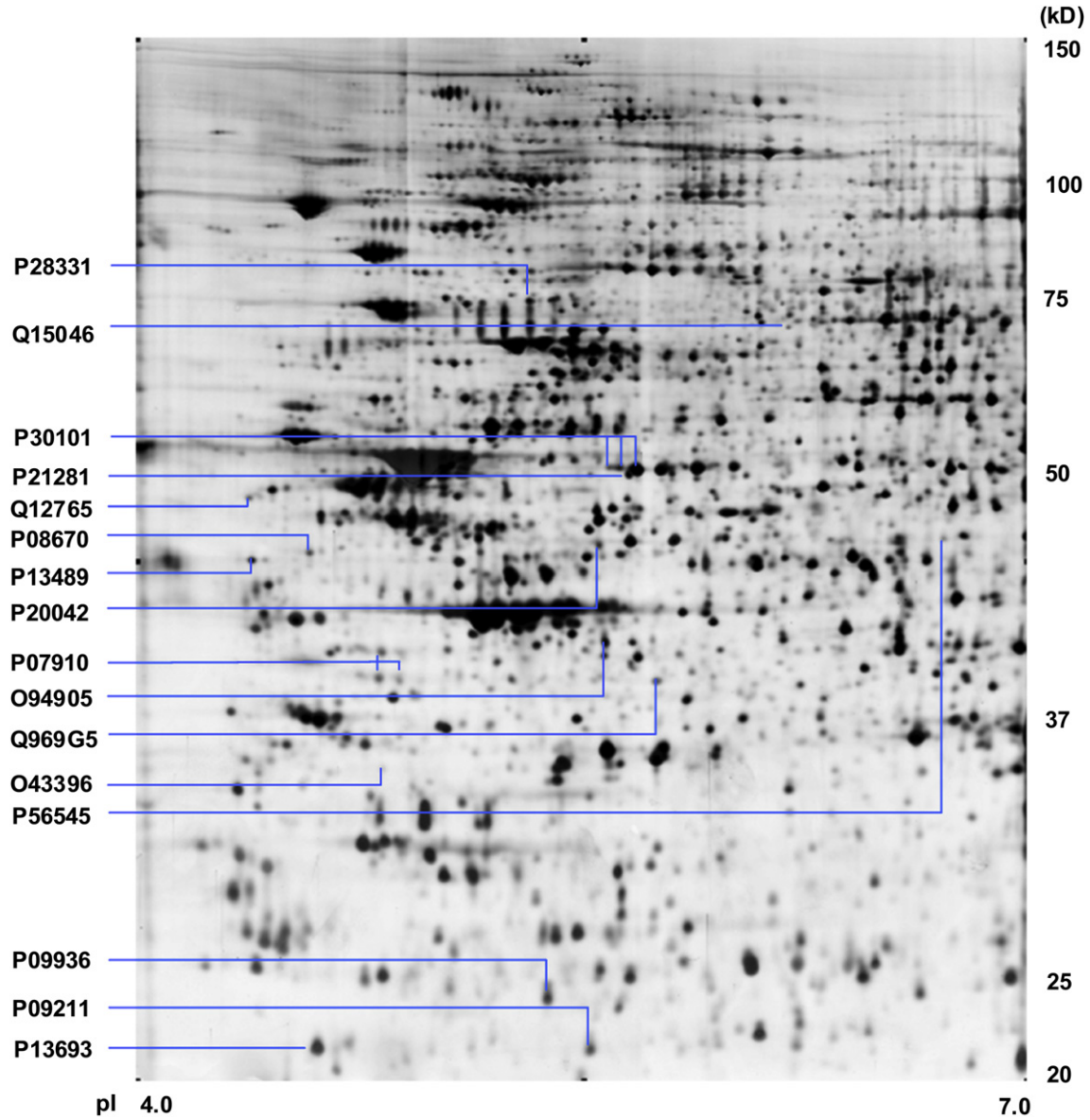

Fig. 1. Representative map of silver-stained two dimensional electrophoresis from MRC-5 whole cell lysate focused on a non-linear pH 4–7 IPG strip. Sixteen proteins identified were labeled with their respective Swiss-Prot accession numbers.