Biomaterials 37 (2015) 13–24

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

MicroRNA sequencing and molecular mechanisms analysis of the effects of gold nanoparticles on human dermal fibroblasts

Yan Huang, Xiaoying Lü*, Yinghua Qu, Yamin Yang, Si Wu

State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, 2# Si Pailou, Nanjing 210096, PR China

ARTICLE INFO

Article history:
Received 21 August 2014
Accepted 2 October 2014
Available online 22 October 2014

Keywords:
Gold Nanoparticles
Human dermal fibroblasts
Molecular mechanism
MicroRNA sequencing
Bioinformatics analysis

ABSTRACT

The aim of this study is to investigate the mechanism of the effects of gold nanoparticles (GNPs) on human dermal fibroblasts (HDFs) at the microRNA level. First, 20-nm GNPs were synthesized and their effect on HDF proliferation was assayed. SOLiD sequencing technology was then utilized to obtain the microRNA expression profile after GNP treatment. The microRNA expression data were compared with previously obtained mRNA and protein expression data to identify the microRNA target mRNAs/proteins. Moreover, bioinformatics analyses and validation experiments were conducted. Lastly, the roles of GNPs and silver nanoparticle (SNPs) on HDFs were compared at the microRNA level. The results showed that GNPs were not cytotoxic as 202 microRNAs were differentially expressed after treatment with 200 μM GNPs for 1, 4 and 8 h. Bioinformatics analyses revealed that these dysregulated miRNAs mainly functioned in metabolic processes and participated in 71 biological pathways, including two key pathways in which the differentially expressed miRNA, target mRNAs and proteins were simultaneously joined, the mRNA processing pathway and MAPK signaling pathway. Biological experiments in cells confirmed that GNPs affected energy metabolism but did not induce apoptosis, destroy the cytoskeleton or induce reactive oxygen species (ROS) production. Comparing the mechanism of the effects of GNPs and SNPs on HDFs at the microRNA level, it was found that, unlike SNPs, GNPs impacted the cell cycle, weakened the ATP synthesis inhibition and cytoskeleton damage, suppressed apoptosis, and did not lead to cytotoxicity. The difference in ROS production by these two nanoparticles might partially explain the fact that GNPs showed no cytotoxic effects on HDFs, unlike SNPs.

1. Introduction

Gold is an inert, noble metal that has long been considered to have therapeutic and even medicinal value in its bulk form. Hence, gold nanoparticles (GNPs) are also thought to be relatively non-cytotoxic [1–3]. GNPs have important applications, including gene and drug delivery, medical imaging, and rheumatoid arthritis and cancer therapy, due to their unique physical and chemical properties [4–7]. Although an increasing number of studies have examined the potential toxicity of GNPs prior to clinical application, the reported results are not consistent. While many studies found that GNPs are nontoxic and nonreactive to cells [1–3], others found a non-negligible toxicity of GNPs. Pan et al. found that the toxicity of GNPs was size-dependent, with the smallest nanoparticles (1.4 nm in size) having the greatest toxicity [8]. Yen et al. reported that GNPs (2.8, 5.5, and 38 nm in size) were toxic and induced up-regulation of the pro-inflammatory genes interleukin-1, interleukin-6 and tumor necrosis factor [9]. GNPs have also been found to decrease extracellular matrix protein synthesis [10], affect the actin cytoskeleton, cell migration and adhesion [10,11], increase reactive oxygen species (ROS) generation [12], trigger the stress response [13,14], induce DNA damage, cause apoptosis [15,16] and even exhibit cytotoxicity [17]. Hence, it is important to confirm the toxicity as well as the safety and risks involved in the use of GNPs in therapeutics and future research [18].

The development of high-throughput biomics approaches has made it possible to investigate the molecular biocompatibility of GNPs. With DNA microarray analysis, Khan et al. found that GNPs might induce a stress response but did not cause significant cytotoxicity [13]. Tedesco et al. investigated the oxidative stress and toxicity induced by GNPs in *Mytilus edulis* using proteomics technology. The results revealed that GNPs could increase protein ubiquitination and carbonylation, and decrease the prevalence of thiol-containing proteins; furthermore, oxidative stress occurred within 24 h [19,20]. With the appearance of systems biology concepts, the comprehensive analysis of the interaction between GNPs

* Corresponding author. Tel.: +86 25 83793430; fax: +86 25 83792882.
E-mail address: luxy@seu.edu.cn (X. Lü).

http://dx.doi.org/10.1016/j.biomaterials.2014.10.042
0142-9612/

and cells from multiple molecular levels and the systematic integration of obtained biomics data are very important. Our research group has investigated the molecular mechanism of the interactions between GNPs and human dermal fibroblasts (HDFs) based on gene expression profile microarray and proteomics at the mRNA and protein levels [21,22]. It was observed that the expression patterns of one gene at the mRNA level did not agree with that at the protein level and the obtained mechanisms of GNPs on HDFs at the mRNA and protein levels are not completely consistent. Prior to the current study, the reasons for the above differences and the key mechanism of the role of GNPs on cells were unknown. Despite being an important part of systems biology, the influence of nanoparticles on the expression profile of microRNAs (miRNAs) is rarely reported.

Medical studies have begun to explore the reasons for the differences in mRNA and protein expression levels in cells *via* gene expression regulation [23]. In the process of transferring genetic information from DNA to RNA to protein, RNA serves as more than just a bridge. A subset of small non-coding RNAs, including microRNA, siRNA, piRNA and esiRNA, *etc.*, constitute a regulatory network that regulates gene expression at the transcriptional and post-transcriptional levels and is involved in a wide range of physiological processes such as cell proliferation, differentiation, and apoptosis, and so on [24]. Elkan-Miller et al. investigated the mRNA, miRNA and protein expression profiles in cochlear and vestibular hair cells and identified miRNA-target pairs, and reporting that the greatest differentially expressed miRNA, miR-135b, regulated PSIP1-P75 by translational suppression [23].

In the RNA regulatory network, miRNAs have attracted increasing attention due to their important roles in cell differentiation, biological development, and the genesis and development of disease, *etc*. More than 1000 miRNAs have been found in the human genome, and they have been shown to regulate 50% of protein-coding genes [25]. The simultaneous down-regulation of many target genes can be achieved by miRNAs *via* mRNA degradation or translational repression. In this way, the global mRNA and protein expression profiles of a cell are drastically modified, accompanied by changes in the miRNAs themselves [23,25,26]. Our previous research revealed that silver nanoparticles (SNPs) altered mRNA, miRNA and protein expression profiles in cells. Furthermore, miRNAs regulated mRNA and protein expression levels and also played important roles in SNP-cell interactions [27]. Therefore, an integrated study at the mRNA, miRNA, and protein levels is beneficial for identifying the key mRNAs, miRNAs, and proteins that play important roles in the effect of GNPs on cells as well as the key pathways involved. However, analyses of the influence of GNPs on cells at the miRNA level and comprehensive studies of mRNA and protein expression profiles have not been performed.

Similar to GNPs, SNPs have been widely used in clinical settings as an efficient antimicrobial material. Several comparative studies have found important differences between the effects of GNPs and SNPs on cells. Farkas et al. revealed that SNPs caused a significantly reduced membrane integrity and cellular metabolic activity in a concentration-dependent manner. GNPs increased ROS levels but not cytotoxicity. The adverse effects of GNPs and SNPs on cells might be related to the release of silver or gold ions [12]. Bachand et al. reported that occupational exposure to GNPs and SNPs might trigger a significant inflammatory response in the alveolar epithelium, although acute toxicity was not produced. Significant up-regulation of interleukin (IL)-8 was observed, and the increase in IL-8 secretion was strongly dependent on both nanoparticle size and concentration [28]. The above studies showed that the physicochemical properties of nanoparticles (such as surface charge and particle size) affected their cellular effects, but the molecular mechanism has not been explained. Our research group has systematically studied the mechanism of cytotoxicity of SNPs at the mRNA, miRNA, and protein levels [27,29]. Thus, studying the similarities and differences between the effects of GNPs and SNPs on cells at the molecular level is of great significance for better understanding the interaction between nanoparticles and cells.

The purpose of this study is to determine the miRNA expression profile in HDFs after treatment with GNPs and to identify miRNA targets by comparison with previously obtained mRNA and protein expression data. The functions of the dysregulated miRNAs are analyzed with bioinformatics tools. The key pathways in which differentially expressed miRNAs, target mRNAs and proteins participated simultaneously are determined, and the mechanisms of GNPs on HDFs are discussed. By comparing the effects of GNPs with those of SNPs at the miRNA level, the different effect of these two nanoparticles on cells are illustrated.

2. Materials and methods

2.1. GNP synthesis and characterization

GNPs were prepared using a previously described method [30]. Briefly, 0.2 mL of 1 wt% chloroauric acid was added to 19.8 mL of redistilled water. The solution was heated to boiling, and 0.26 mL of 1 wt% sodium citrate was added under constant stirring. The mixture was boiled for an additional 5 min after turning wine-red in color. The morphology and size of GNPs were determined by transmission electron microscopy (TEM, JEOL JEM-2100, Japan).

2.2. Cell culture and cytotoxicity evaluation of GNPs

HDFs were cultured in low-glucose DMEM (HyClone, USA) supplemented with 10% fetal calf serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd, China) and 1% penicillin-streptomycin (Hyclone, USA) in a humidified 37 °C incubator with 5% CO_2.

The cytotoxicity of GNPs on HDFs was measured using a real-time cell electronic sensing system (RT-CES). Briefly, 50 μL of cell culture medium was first added to each well of 16 × e-plates to obtain a steady impedance value. Next, 6×10^3 cells in 100 μL of medium were added, and the cell index (CI) was measured every 30 min. GNPs were dissolved in culture medium at a series of concentrations (10, 50, 100, 200, and 300 μM). After 24 h, the medium was changed to GNP-containing medium. Cells cultured in medium without GNPs were used as a negative control, and cells exposed to 0.7% acrylamide were used as a positive control. After cells were cultured for another 72 h, the cell proliferation percentage (P) of GNP-treated cells was calculated using the following equation:

$$P = \frac{\overline{CI}_{\text{GNP-treated}}}{\overline{CI}_{\text{Negative control}}} \quad (1)$$

2.3. mRNA and protein expression profile analysis following treatment with GNPs

mRNA and protein expression data were obtained from our previous studies on the interaction between GNPs and HDFs using gene expression profile microarray and proteomics approaches. HDFs were treated with 200 μM GNPs for 1, 4 and 8 h; 1794 and 24 differentially expressed mRNAs and proteins were identified, respectively (data not shown) [21,22]. The emphasis of the previous studies was analyzing all differentially expressed mRNAs and proteins, while the present study focused on filtering mRNA and protein targets of miRNAs.

2.4. MicroRNA sequencing of GNP-treated HDFs

Small RNAs from HDFs treated with 200 μM GNPs for 1, 4 and 8 h were extracted using the mirVana™ miRNA isolation kit (Ambion, USA). Untreated HDFs were used as a control. Small RNAs were then converted into a double-stranded cDNA library, and sequencing experiments were performed on an Applied Biosystems SOLiD platform (Life Technologies, USA) at Agene Bioinformative Technologies (Wuxi) Co., Ltd (China).

The detected nucleotide sequences were compared with known miRNAs in the miRbase database (http://www.mirbase.org) to identify the miRNAs in the untreated and GNP-treated HDFs. The differentially expressed miRNAs were filtered by fold-change analysis: an miRNA was considered to be up-regulated when the fold-change value was >2 and down-regulated when the fold-change value was <0.5 [27].

2.5. Target gene prediction of GNP-induced differentially expressed miRNAs and miRNA-mRNA/protein target pair identification

The target genes of differentially expressed miRNAs were predicted using the public database miRecord (http://mirecords.biolead.org/prediction_query.php), and only those predicted by miRanda, PicTar, and TargetScan were filtered.