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The effects of silver nanoparticles on mouse embryonic stem cell self-renewal and proliferation

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ABSTRACT

Silver nanoparticles (AgNPs) are gaining rapid popularity in many commonly used medical and commercial products for their unique anti-bacterial properties. The molecular mechanisms of effects of AgNPs on stem cell self-renewal and proliferation have not yet been well understood. The aim of the work is to use mouse embryonic stem cells (mESCs) as a cellular model to evaluate the toxicity of AgNPs. mESC is a very special cell type which has self-renewal and differentiation properties. The objective of this project is to determine the effects of AgNPs with different surface chemical compositions on the self-renewal and cell cycle of mESCs. Two different surface chemical compositions of AgNPs, polysaccharide-coated and hydrocarbon-coated, were used to test their toxic effects on self-renewal and proliferation of mESCs. The results indicated that both polysaccharide-coated and hydrocarbon-coated AgNPs changed the cell morphology of mESCs. Cell cycle analysis indicated that AgNPs induced mESCs cell cycle arrest at G1 and S phases through inhibition of the hyperphosphorylation of Retinoblastoma (Rb) protein. Furthermore, AgNPs exposure reduced Oct4A isoform expression which is responsible for the pluripotency of mESCs, and induced the expression of several isoforms OCT4B-265, OCT4B-190, OCT4B-164 which were suggested involved in stem cell stresses responses. In addition, the evidence of reactive oxygen species (ROS) production with two different surface chemical compositions of AgNPs supported our hypothesis that the toxic effect AgNPs exposure is due to overproduction of ROS which altered the gene expression and protein modifications. Polysaccharide coating reduced ROS production, and thus reduced the AgNPs toxicity.

1. Introduction

Nanotechnology is a rapidly growing field with many uses in medicine and in the manufacture of commonly used products [1–3]. Among the hundreds of products that contain nanomaterials, the most widely used are AgNPs. AgNPs are most commonly used in medical and commercial products for their unique anti-bacterial properties and can be found in medical wound dressing, surgical instruments, and medical face masks to reduce microbial populations [4–6]. They are also used as filtering agents in humidifiers and water purification treatments [7].

AgNPs are highly chemically reactive due to their small size and high surface area, which produces a great amount of reactive oxygen species (ROS) [8]. ROS and free radical production cause oxidative stress, inflammation, and protein, DNA, and membrane damage. Studies on fibroblast cells have shown that AgNPs can enter human fibrosarcoma, skin carcinoma cells and primary neural cells and induce ROS and cell death while causing oxidative stress

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and DNA damage [9–12]. Another study suggested that AgNPs induce cell death via up-regulation of p53-mediated apoptotic pathways [13]. AgNPs caused an increase in expression of p53, which is a tumor suppressor, p21, Noxa, Bax, and DNA damage repair proteins Rad 51 and H2AX [11,14,6,15].

The objective of this project is to determine the effects of AgNPs with and without coating on the self-renewal and cell cycle of mESCs. Polysaccharide-coated is the commonly employed coating/stabilizing agents for AgNPs applications. It has been shown that the coating will influence the particles shapes, sizes and surface properties will contribute to the AgNPs toxicity [16]. Many studies have shown toxic effects associated with nanoparticles introduced into a variety of human somatic cells and non-mammalian cells such as those of the zebrafish. However, little work has been done involving the effects that AgNPs have on embryonic stem cells [17]. Embryonic stem cells (ESCs) are clonal cell lines derived from the inner cell mass of a developing blastocyst [18,19]. They can proliferate indefinitely in vitro and also have the ability to differentiate into cells of all three germ layers (endoderm, mesoderm, and ectoderm), and thus ESCs is an excellent cellular model for classifying Engineered Nanoparticles (NPs) according to their toxic potential [19,20]. Our long-term objectives for the research are to understand the AgNPs toxic effects on self-renewal and proliferation of mouse mESCs and develop an Embryonic Stem Cell Test (EST) as a tool for classifying Engineered Nanoparticles (NPs) according to their toxic potential. We hypothesize that overproduction of ROS after AgNPs exposure can induce oxidative stress, which lead to alter key regulated genes expression and protein modification, and thus inhibition of cell renewal and cell growth. Furthermore, the proper AgNPs capping can reduce the toxicity by preventing the ROS production.

ESCs self-renewal properties and pluripotency characteristics are regulated by the interacting network of several transcription factors including Oct4 and Nanog. Oct4 is known as a master regulator and is exclusively found in ES cells. The OCT4 gene can generate at least two transcripts (OCT4A, OCT4B) by alternative splicing [21,22]. OCT4A is the most commonly described transcript, which is translated into a full-length nuclear-localized OCT4 protein with N- and C-terminal transactivation domains separated by a POU DNA-binding domain. This transcript is a transcription factor responsible for the pluripotency of ESCs. In contrast, the Oct4B transcript is truncated without exon 1. Because Oct4B cannot sustain ES cell self-renewal, it was suggested that it may be responsible to cellular stresses. Furthermore, a single OCT4B transcript may encode at least three protein isoforms, OCT4B-265, OCT4B-190 and OCT4B-164, by alternative translation through an internal site of OCT4B mRNA [23]. However, the function of Oct4B and how its alternative translation is regulated are unclear. Our results provided the evidence to support the function of Oct4B in stem cell stress response.

The cell cycle regulation is monitored by a cell cycle control system that responds to various intracellular and extracellular signals. If a cell is under stress, the control system will shut down the cell cycle at one of its several checkpoints [21]. We have examined the expression of Retinoblastoma (Rb) which is a critical protein plays a protective role in response to genotoxic stress by inhibiting the cell cycle at the G_1/S checkpoint. Hyperphosphorylation of Rb inactivates the protein which allows the E2F transcription factor to detach and push the cells into the S phase of the cell cycle. If Rb is hypophosphorylated or not phosphorylated at all, it is active and remains attached to the E2F transcription factor, causing cell cycle arrest. Studying the protein modification in response to AgNPs treatment will offer better understanding of the molecular mechanisms of nanotoxicity and provide the biomarkers for nanotoxicity analysis.

2. Materials and methods

2.1. Characterization of silver nanoparticles (AgNPs)

The two types of AgNPs used in this study differed primarily in their surface chemical composition. The 20 nm hydrocarbon-coated AgNPs were processed with hydrocarbons that prevent sintering, but leave a non-uniform hydrocarbon surface layer. Polysaccharide-coated AgNPs were a generous gift from Dr. Dan Goia (Clarkson University, Center for Advanced Materials Processing, Potsdam, NY). The 20 nm polysaccharide-coated AgNPs were synthesized with surface capping using a polysaccharide (acacia gum). The sizes of AgNPs in the solution were characterized using transmission electron microscopy (TEM) on a Hitachi H-7600 tungsten-tip instrument at an accelerating voltage of 100 kV. The AgNPs were suspended in deionized water at 1 mg/ml and then sonicated using a Branson-1510 sonicator bath at room temperature for 15 min at 35–40 W to aid in mixing and forming a homogeneous dispersion. For size measurements, sonicated 1 mg/ml AgNPs stock solution was diluted to a 50 μg/ml working solution. AgNPs were examined after NPs suspensions were deposited in carbon film-coated Cu grids. The advance microscopy techniques (AMT) software for the digital TEM camera was calibrated for size measurements of the nanoparticles. Information on mean size and SD was calculated using the point-to-point method as described elsewhere [25]. The result showed that the size of the majority AgNPs are between 5.0 and 20 nm (Supplementary Fig. 1a and b). Polysaccharide-coated nanoparticles tend to be individually distributed, whereas hydrocarbon-coated particles tend to agglomerate.

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.toxrep.2015.05.005.

2.2. mESCs cell culture and AgNPs treatments

J11 mESCs cells were a gift from Dr. Peter Stambrook (College of Medicine, University of Cincinnati). The cells were cultured on DMEM supplemented with 5% penicillin-streptomycin, 1X non-essential amino acids (NEAA, GIBCO Cat # 11140) and 1X Glutamax (GIBCO Cat # 35050), 10% embryonic stem cell qualified FBS (USA Scientific Cat # 98375200), 0.1 μM β-mercaptoethanol (Sigma Cat # M6250) and 50 μM leukemia inhibitory factor (LIF, Millipore Cat # ESG 1107). AgNPs solutions were prepared