THE JOURNAL OF GENE MEDICINE
J Gene Med 2014; **16**: 331–335.
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jgm.2810

RESEARCH ARTICLE

Designing a gold nanoparticle-based nanocarrier for microRNA transfection into the prostate and breast cancer cells

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Received: 4 September 2014
Revised: 11 October 2014
Accepted: 14 October 2014

Abstract

Background Cancer is one of the most common causes of human deaths worldwide. Nanotechnology has the potential to facilitate the detection, diagnosis, and treatment of cancer cases. Successful delivery of nucleic acids into cancer cells with the use of nanoparticles would be a significant improvement for medical and cellular biology. The use of nanoparticle-based vehicles in clinical treatment is considerably important for treating genetic disorders. Gold nanoparticles (AuNPs) have been suggested as therapeutic delivery tools for cancer. Because microRNAs (miRNAs), which induce post-transcriptional gene silencing, are deregulated in cancer cells, they are also considered as strong candidates for cancer therapy applications. In prostate and breast cancer, miR-145, a well-known tumor suppressor miRNA, is strongly downregulated in tumor tissues compared to their corresponding normal tissues.

Methods In the present study, we aimed to use engineered AuNPs as nanocarrier platforms to deliver miRNAs to prostate/breast cancer cells. 13-nm AuNPs were modified with thiolated RNAs and then the miR-145 was hybridized to the RNAs that were chemically attached to the AuNPs.

Results The results obtained in the present study demonstrate the efficient delivery of miR-145 to prostate/breast cancer cells. We also show that delivery was more efficient when the AuNP-RNA-miRNA carrier complex was formed at an elevated temperature of 72 °C.

Conclusions In conclusion, we show that AuNPs help the effective *in vitro* delivery of miR-145 into cancer cells.

Keywords gold nanoparticle; hybridization; microRNA; mir-145; oligonucleotide

Introduction

Cancer is one of the leading causes of death worldwide. Prostate and breast cancer, being among the most frequent cancer types, attract much attention [1]. Recently, several studies have focused on the potential role of nanoscience in cancer diagnosis and therapy, which led to the born of a new discipline; nano-oncology [2,3]. Nanomaterials have been shown to facilitate important advances in the detection, diagnosis and treatment of human cancers [3,4].

Gold nanoparticles (AuNPs) have been used as a useful tool for cancer therapy applications and the treatment of human diseases [5]. AuNPs, with a high affinity for biomolecules, can be chemically functionalized with alkyl-thiol-terminated oligonucleotides, which become highly stable in saline solution and specifically bind their complementary nucleic acids [6]. In addition, AuNPs have a number of outstanding properties, such as reduced cytotoxicity, easy size control and well-developed surface chemistry. The use of AuNPs for the delivery of small interfering (si)RNA into cells with the help of polyethyleneimine (PEI) has been described and AuNPs have also been used for effective siRNA delivery into cells [7,8].

MicroRNAs (miRNAs) are a large class of philogenetically conserved single-stranded RNA molecules of approximately 20–22 bp in length. They are responsible for the regulation of post-transcriptional gene expression and are implicated in diverse human diseases [9–12]. Most studies have shown that miRNAs are involved in a variety of essential biological processes, including development, cell proliferation, apoptosis and tumorigenesis [13,14]. Studies of individual miRNAs have shown that they can act as oncogenes or tumor suppressor genes [14,15]. Hsa-miR-145, one of the well-characterized tumor suppressor miRNAs, has been reported to be significantly downregulated in several cancer types, including prostate and breast cancer [16,17].

In the present study, we aimed to utilize engineered AuNPs as nanocarrier platforms to deliver miRNAs to prostate/breast cancer cells *in vitro*. 13-nm AuNPs were modified with thiolated RNAs and then the hsa-miR-145 was hybridized to the RNAs that were chemically attached to the AuNPs to facilitate the effective *in vitro* delivery of hsa-miR-145 into cancer cells.

Materials and methods

Synthesis of AuNPs

AuNPs were prepared by reducing $HAuCl_4 \cdot 3H_2O$ with sodium citrate (Merck, Whitehouse Station, NJ, USA). This procedure produces AuNPs with an average size of 13 nm. Briefly, 100 ml (1 mM) of $HAuCl_4 \cdot 3H_2O$ (Sigma, Munich, Germany) solution was heated until boiling. Then, 10 ml of 38.8 mM citrate solution was added immediately into the boiling solution and the mix was continually boiled for 15 min. The solution color changes from yellow to deep red.

Characterization of AuNPs

The synthesized AuNPs and AuNP-oligonucleotides were characterized with an ultraviolet-visible spectrophotometer (PerkinElmer, Boston, MA, USA). The zeta potential and size distribution of AuNPs in suspension were evaluated using a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, UK) at 25 °C with a 173° scattering angle and a 4-mW He-Ne laser.

Modification of AuNPs

AuNPs were modified with thiolated oligonucleotide (3'-AAAAAAAAAAAUCUACGAUUCUACC-5'; SynGen, Inc., Sacramento, CA, USA). Two microlitres of 200 μM oligonucleotide were added to 1 ml of AuNP suspension and then the oligonucleotide and AuNP mix was left for incubation for 24 h at 20 °C on a shaker for the successful attachment of oligonucleotides onto the AuNPs. A schematic representation of 13-nm AuNPs modified with thiolated oligonucleotide is provided in Figure 1.

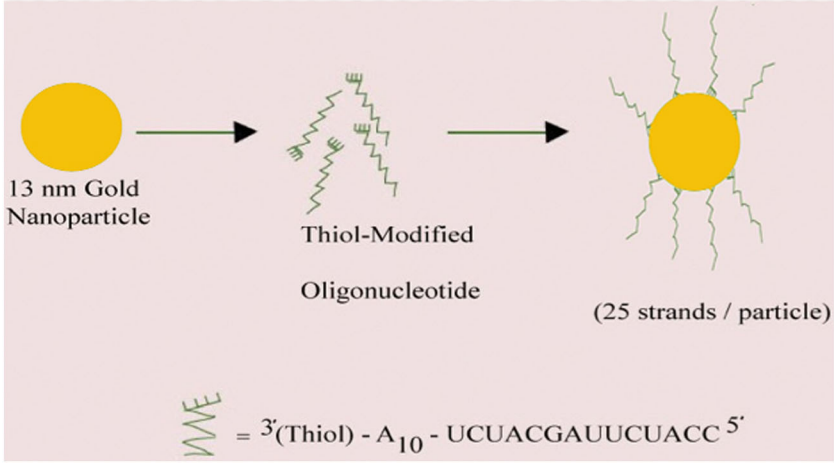

Figure 1. Schematic representation of 13-nm AuNPs modified with thiolated oligonucleotides.

DOI: 10.1002/jgm

Hybridization of miRNAs on AuNPs in suspension

Ten microlitres of precursor-miR-145 (pre-miR-145), 150 µl of AuNP-modified with thiolated oligonucleotide and 40 µl of Gene Expression Hybridization Kit 2X GE Hybridization buffer (Agilent Technologies Inc., Santa Clara, CA, USA) were mixed and vortexed vigorously for 10 s. Samples were subjected to hybridization either at 72 °C for 2.5 min and 37 °C for 16 h or 94 °C for 1 min, 72 °C for 2.5 min, and 37 °C for 16 h in a thermal cycler.

Cell culture and transfection of cells with miRNAs

PC3, a human prostate cancer cell line, and MCF-7, a human breast cancer cell line, were used for the experiments. PC3 and MCF7 cells were grown within RPMI medium (Gibco-BRL, Bethesda, MD, USA) and Dulbecco's modified Eagle's medium (Invitrogen, Paisley, UK), respectively, containing 10% fetal bovine serum, 1% L-glutamine and 1% prostate-specific antigen at 37 °C in a humidified and 5% CO_2 incubator. Pre-miR-145 was purchased from Ambion Inc. (Austin, TX, USA). Transfection experiments were optimized and performed with an X-treme Gene Transfection Kit (Roche, Basel, Switzerland) in accordance with the manufacturer's instructions. When nontargeting control miRNAs are used as a control for miR-145 transfection, the expression of miR-145 is not affected by the delivery of control miRNA. Therefore, we only prepared and utilized AuNP-oligo-miR-145.

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Pre-mir-145 transfected PC3 cells, MCF7 cells and control cells were collected 24 h after transfection. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. RNA samples were kept refrigerated at –80 °C until the qRT-PCR. Equal amounts of total RNA were used for the first-strand DNA (cDNA) synthesis with miRNA specific primers purchased from Applied Biosystems and a TaqMan MicroRNA reverse transcription Kit in accordance with the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). qRT-PCR was carried out in a Roche LightCycler480-II real-time thermal cycler (Roche). A TaqMan Universal Master Mix was used for qRT-PCR and microRNA specific probes were purchased from Applied Biosystems (Naerum, Denmark). Each experiment was performed in duplicate and expression data were normalized to RNU43. The relative quantification analysis was performed using the delta-delta-Ct method as described previously [18].

Results

After the synthesis of 13-nm AuNPs, we characterized them in terms of their sizes and zeta potential in standard Malvern zeta potential disposable capillary cells and polystyrene cuvettes and confirmed the average size of AuNPs as 13 nm. Figure 2A demonstrates the hydrodynamic radii image of 13-nm AuNPs. Because AuNPs modified with oligonucleotides were exposed to a high temperature when they were hybridized to miR-145 molecules, we aimed to ensure that the AuNPs were still stable after the heating step. Accordingly, a temperature gradient was imposed on the colloidal suspension containing AuNPs and then the zeta potential and size distribution of AuNPs at temperatures from 30 °C to 90 °C were evaluated with a Zetasizer Nano ZS instrument. Figure 2B shows the dynamic light scattering spectrum of AuNPs exposed to different temperatures.

To explore the feasibility of engineered AuNPs as nanocarrier platforms for delivery of miRNAs to the prostate/breast cancer cells, we transfected both PC3 and MCF7 cells with pre-miR-145. First, the production

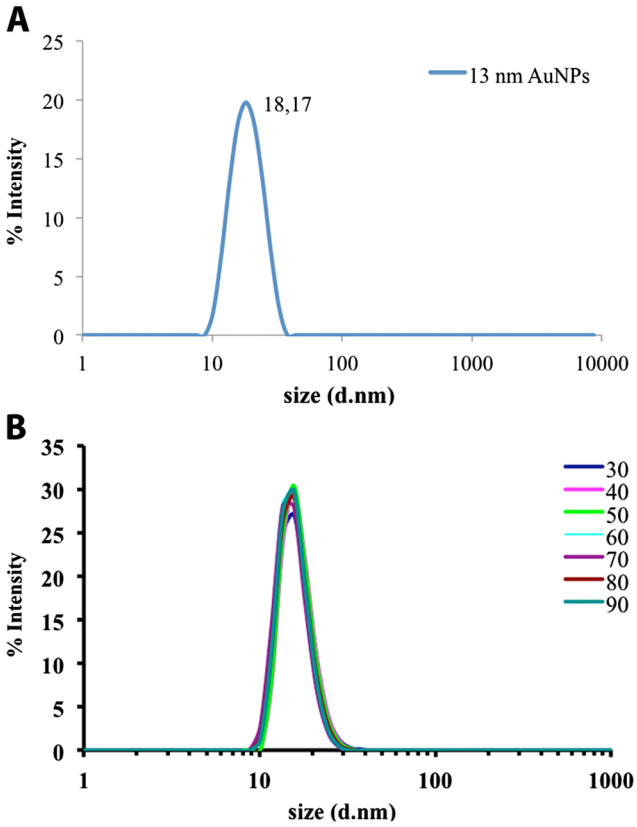

Figure 2. Hydrodynamic radii image of 13-nm AuNPs (A). Dynamic light scattering spectrum of 13-nm AuNPs (B).

DOI: 10.1002/jgm

of mature miR-145 molecules and the duration of their stability were checked with microRNA qRT-PCR, which demonstrated that the mature miR-145 molecules are produced effectively in pre-miR-145 transfected cells but not in blanks or the control group (Figures 3 and 4).

Then, we incubated both PC3 and MCF7 cells with AuNPs modified with thiolated oligonucleotides to determine whether this alters the miR-145 expression level. No significant increase in both PC3 and MCF7 cells was found compared to blank or mock controls. To investigate the nanocarrier potential of AuNPs, we hybridized pre-miR-145 molecules to AuNPs modified with oligonucleotides at either 72 °C AuNP-oligo-miR-145 (72) or 94 °C AuNP-oligo-miR-145 (94). Incubation of cells with AuNP-oligo-miR-145 (72) resulted in a significant increase in miR-145 levels in both PC3 and MCF7 cells. The increase in ectopic miR-145 expression in cells was quite strong, highlighting the success and feasibility of using AuNPs as nanocarrier platforms for the delivery of nucleic acids into cells. We have shown downregulation of SOX2 (i.e. one of the direct targets of miR-145) with respect to both RNA and protein levels via ectopic expression of miR-145 (Karatas OF, Yuceturk B, Suer I, Yilmaz M, Cansiz H, Ittmann M, and Ozen M).

Temperature was used to increase the efficiency of the hybridization of miRNAs to the complementary oligonucleotides on the surface of the AuNPs. We explored

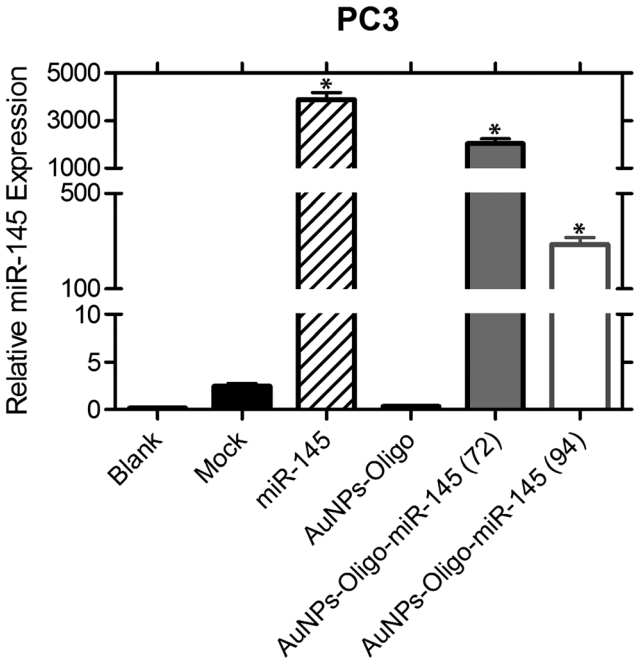

Figure 3. The relative expression of hsa-miR-145 in blank, mock and miR-145 transfected PC3 cells or in AuNP-oligo (AuNPs modified with thiolated oligos), AuNP-oligo-miR-145 (72) (AuNPs modified with thiolated oligos and hybridized with miR-145 via heating at 72 °C) and AuNP-oligo-miR-145 (94) (AuNPs modified with thiolated oligos and hybridized with miR-145 via heating at 72 °C and then 94 °C) delivered PC3 cells. *$p < 0.05$.

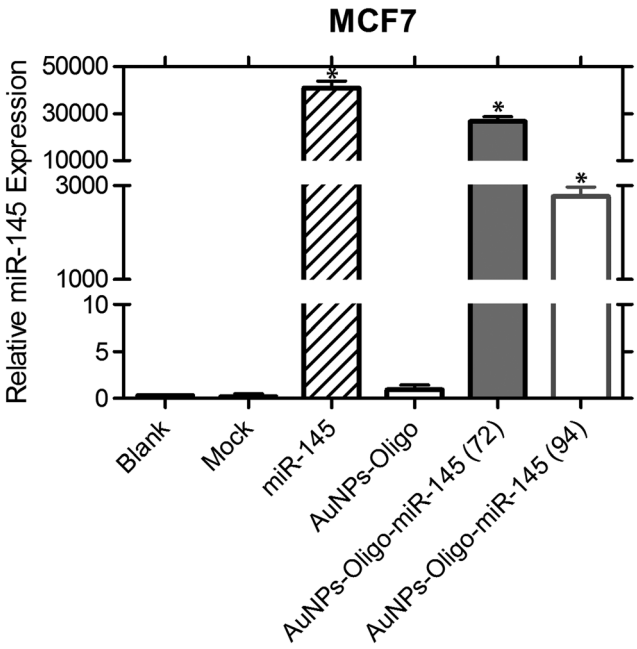

Figure 4. The relative expression of hsa-miR-145 in blank, mock and miR-145 transfected MCF7 cells or in AuNP-oligo (AuNPs modified with thiolated oligos), AuNP-oligo-miR-145 (72) (AuNPs modified with thiolated oligos and hybridized with miR-145 via heating at 72 °C) and AuNP-oligo-miR-145 (94) (AuNPs modified with thiolated oligos and hybridized with miR-145 via heating at 72 °C and then 94 °C) delivered MCF7 cells. *$p < 0.05$.

whether the hybridization temperature affected the efficiency of miRNA delivery and compared two different temperatures for hybridization. When the temperature is below 70 °C, plausible transfection was not observed. Therefore, the heating temperature was maintained at 72 °C. Hybridization of miR-145 molecules when heated at 72 °C resulted in the more efficient delivery of miR-145 molecules to cells. Heating at 94 °C and then 72 °C still resulted in strong expression of ectopic miR-145 in both PC3 and MCF7 cells (Figures 3 and 4).

Discussion

Hsa-miR-145 is downregulated in prostate and breast tumors. In the present study, we aimed to generate a specific nanoparticle platform from AuNPs that carries miR-145 molecules and transfers them into cells. We modified 13-nm AuNPs with thiolated RNAs and hybridized them with pre-miR-145 molecules. The incubation of these nanoparticles with PC3 and MCF7 cells for 24 h resulted in the effective and successful delivery of miR-145 molecules into target cells and induced the overexpression of ectopic miR-145 in both PC3 and MCF7 cells.

There is increasing interest in the utilization of AuNPs in biomedical imaging, photothermal therapy and targeted gene/drug delivery applications as a result of their

DOI: 10.1002/jgm

extraordinary optical features and reduced cytotoxicity [19]. There are numerous studies using AuNPs in oligonucleotide delivery applications. For example, Li *et al*. [20] prepared oligonucleotide AuNP complexes where thiolated oligonucleotide molecules are attached to pegylated AuNPs. In addition, Lee *et al*. [8] prepared an oligonucleotide delivery platform composed of poly β-amino ester (PBAE), siRNA and AuNPs. PBAE–siRNA–AuNP complexes were introduced into HeLa cells, and their delivery was confirmed by visualization of nanoparticle aggregates confined within endosomes in the cytosol via transmission electron microscopy.

Moreover, Giljohann *et al*. [21] reported that flow cytometry analysis confirmed the uptake of RNA-AuNPs by >99% of the cell population. The persistent knockdown of the target gene through utilization of the RNA-AuNPs was evaluated as the result of the stabilization of RNA molecules on the nanoparticle complex [21]. Furthermore, successful release from endosomes is necessary for oligonucleotide carrier platforms to be able to improve siRNA/miRNA-silencing efficiencies. Guo *et al*. [22] demonstrated that siRNA molecules delivered by the AuNP complex (modified PEI/siRNA/PEI/AuNP complex) were effectively transferred inside the cells, whose penetration has been investigated by confocal laser scanning microscopy and tracked via cy5 labeled siRNA molecules [22].

Furthermore, for *in vivo* utilization of AuNPs in cancer management, Lim *et al*. [23] demonstrated that antibody-conjugated hollow gold nanospheres could be used as drug delivery agents for the introduction of drugs or oligonucleotides into tumor cells. To ensure the specific targeting of cancer cells at the same time as sparing noncancerous cells and tissues, engineered AuNPs were modified with specific surface ligands to be directed only to tumor cells. AuNPs are also proposed to be used as sensitive probes in the detection and imaging of tumors for diagnostic purposes [23].

In the present study, we demonstrated that the introduction of miR-145 molecules into target cells by AuNPs modified with thiolated RNAs without any further modification is an efficient method in breast cancer and prostate cancer cell lines. Hybridization of pre-miR-145 molecules to the RNA oligonucleotides bound on the AuNPs provides the opportunity to carry miRNAs into the cells by endocytosis. Hybridization of pre-miR molecules during their delivery is assumed to prevent the degradation of miRNA molecules.

In conclusion, we suggest that AuNPs can be considered as putative microRNA and small oligonucleotide delivery systems, considering their low cytotoxicity and their potential for surface modifications, when performing gene silencing in targeted cell populations.

Acknowledgements

The authors declare that there are no potential conflicts of interest.

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