Gold Naoparticles for Improved Cancer Therapeutics

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ABSTRACT

Recently, gold nanoparticles (GNPs) have been used as sensitizers in radiation therapy research. GNPs used in these studies were untargeted and were localized in the cell cytoplasm. we report the use of nuclear targeting of GNPs to further enhance radiation damage to cancer cells. Irradiated cells with GNPs targeted into nucleus showed higher cell damage compared to the ones with NPs localized in the cytoplasm. There was a modest increase in the radiation-induced DNA double-strand breaks for irradiated cell populations with nuclear targeted GNPs. These studies support the hypothesis that optimizing the distribution of GNPs within the cell could lead to higher sensitization effects. With nuclear targeting, there is a possibility in the production of additional low-energy secondary electrons within the nucleus causing more damage to DNA. The outcome of this research will enable the optimization of GNP-based sensitizers for use in therapy.

Keywords: Nanoparticles, radiation therapy, DNA damage

1 INTRODUCTION

The flexibility of cancer nanotechnology allows the development of safer yet more effective therapeutic modalities for cancer therapy [1, 2]. Despite recent progress in conventional methods such as, Surgery, radiation and chemotherapy, there is still a great need for further improvement in existing therapeutics and also for development of novel therapeutics. Recently, NP-based platforms have been introduced to overcome some of the challenges. For example, NPs are being explored for the targeted delivery of therapeutics to tumors for minimizing side effects [3-7]. In addition, NP-based technology has the capability to develop novel multiplex systems to combine more than one treatment and imaging modality for creating a more aggressive and effective approach in eradicating cancer [8].

Among other NP systems, GNPs have been receiving significant attention for use in cancer diagnosis and treatment [8-11]. Recently, GNPs are being used as sensitizers in radiation therapy [12-17]. The concept of using high- Z materials to increase the dose given to a tumor during radiation therapy was advanced over 20 years ago when iodine was used by Matsudaira et al. to sensitize cultured cells [18]. However, the use of gold as a radiosensitizer seems more promising than the earlier attempts using iodine since gold has a higher Z number than iodine and has greater biocompatibility [18-23]. Herold et al. went one step further and demonstrated the dose enhancement for cells suspended in a solution of gold microspheres as well as for tumors injected with gold microspheres [12]. The major drawback of using gold microspheres was that it was very difficult to deliver them uniformly throughout the tumor due to their larger size. To overcome these difficulties, smaller gold NPs with dimensions of 1.9 nm were used [17, 24]. However, based on recent experimental and theoretical studies, NP cell uptake is dependent on the size of the NPs. For example, NPs of size 50 nm have the highest while smaller particles (2– 10 nm) or larger particles (micrometer dimensions) would be expected to have a very low cell uptake [25, 26]. In accordance with these findings, our previous study has shown that GNPs with size 50 nm showed the highest sensitization enhancement as compared larger or smaller NPs. However, in most of these studies, GNPs were localized in the cytoplasm of the cell. The exact mechanisms of cell damage when GNPs are localized away from DNA (either when they are in the media or in the cytoplasm of the cell) are not known yet.

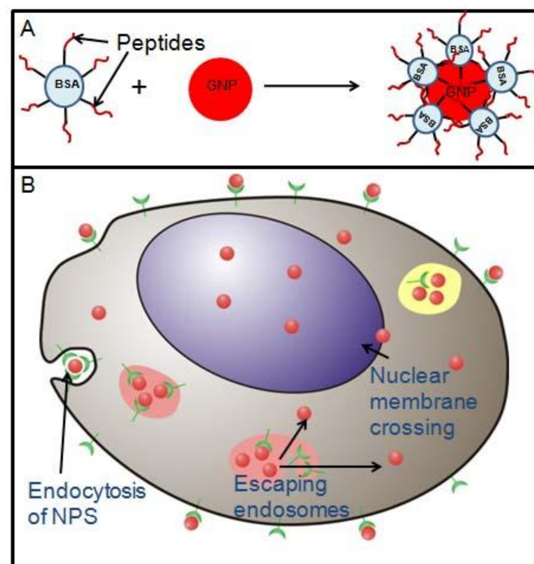

Figure 1: Nuclear targeting of GNPs. A) Preparation of petide-GNP complexes.B) Schematic explaning the path of GNPs. NPs eneters the cell via endocytosis process, escape the endosomes for nuclear localization.

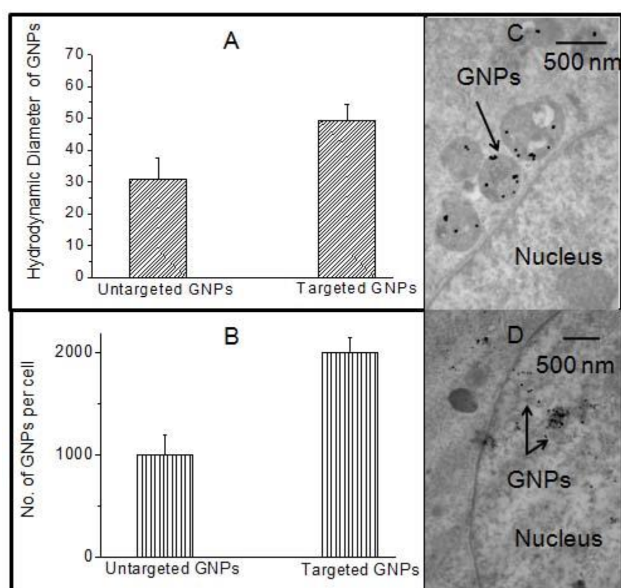

Figure 2: Cellular uptake of GNPs. A) Hydrodynamic radius of uncoated 14nm GNPs and petide-GNP complexes.B) Dependence of uptake for targeted and non-targeted GNPs,C) TEM image showing that untargeted NPs trapped in endosomes. D) TEM image showing that targetd NPs could escape the endosomes and reach the nucleus.

As a step forward to understand the mechanism of sensitization due to gold, several studies have performed using GNPs in close proximity to DNA. Based on these studies, it is believed that enhanced localized absorption of X-rays by NPs could lead to effective release of low-energy electrons from GNPs, and the electrons released could create more radicals. Most of these studies were done either in water or using dry DNA-GNP films. In order to fully elucidate the mechanism of sensitization, it is necessary to target GNPs into the nucleus of cells to investigate interactions between GNPs and DNA with incoming radiation. Hence, in this study, we have targeted the GNPs to nucleus in order to investigate for the first time the enhancement in radiation damage when GNPs are localized within the nucleus as compared to cytoplasm.

For successful targeted nuclear delivery the NP complexes must satisfy the following requirements: a) small enough to enter cells and cross the nuclear membrane (<100 nm for uptake by receptor-mediated endocytosis (RME) and <30 nm for import through nuclear pores), (b) penetrate cellular membranes or bind to cell-specific plasma membrane receptors, (iii) bypass or escape endosomal/lysosomal pathways, (iv) penetrate nuclear membranes or access importins to pass through the nuclear pore complex, and (v) low toxicity (see Fig.1). In the past, viruses have been used to deliver genes to cell nuclei and the design of nonviral vectors capable of performing all of these functions is still a challenge. In order to investigate radiosensitization enhancement effects when GNPs are localized within the nucleus, we used synthetic cellular targeting peptides complexed to a nanometer-sized gold particle for effective nuclear targeting (see Fig.1).

2 MATERIALS AND METHODS

Synthesis of GNPs

GNPs of size 14nm were synthesized using the citrate reduction method (34). First, 300 ml of 1% HAuCl4.3H2O (Sigma-Aldrich) was added to 30 ml of double-distilled water and heated on a hot plate while stirring. Once it reached the boiling point, 600 μl of 1% anhydrous citric acid (Sigma-Aldrich) was added to form nanoparticles 14 nm in diameter, respectively.

Nanoparticle-peptide bioconjugation

Peptide-GNP complexes were assembled by conjugating peptides to bovine serum albumin (BSA) and then attaching BSA-peptide conjugates to GNPs (Figure 1). To prepare BSA-peptide conjugates, BSA was mixed with 3-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBS) in a 1:50 ratio [27].

Cell Culture and Particle Delivery

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. For investigating the cellular localization of peptides, cells were incubated with NP conjugates (10 nM) for various times.

Quantification of NP uptake:

After 8 h of incubation with gold nanoparticles, the cells were washed three times with PBS and trypsinized for quantification of the number of GNPs present per cell. Cells were counted and then treated with HNO_3 at 200°C in an oil bath for ICP-AES analysis.

TEM Analysis of Cells with Internalized Nanoparticles

Cells incubated with nanoparticles as above were washed three times with PBS and fixed (2.5% paraformaldehyde, 0.5% glutaraldehyde) for 8 h. The cells were then postfixed in 1% osmium tetroxide for 2 h, washed and dehydrated in graded concentrations of ethanol (25%, 50%, 70% and 100%) and propylene oxide. Cell samples were then embedded in Epon (Polysciences Inc.) and sectioned. The grids were visualized using an H7000 TEM (Hitachi Corp., Japan).

3 RESULTS AND DISCUSSION

Peptide-GNP complexes were prepared by conjugating peptides to bovine serum albumin (BSA) and then attaching BSA-peptide conjugates to GNPs (Fig. 1). In this study, we focus on the peptide nuclear localization signal (NLS) derived from a peptide derived from the HIV Tat protein (M1), and adenovirus fiber protein (M2), as well as a modified integrin binding domain peptide (M3). An initial

ratio MBS:BSA of 50:1 was used and it resulted in 23 ±5 MBS linkers per BSA molecule as measured by a fluorescamine assay [27]. BSA-peptide conjugates were complexed to 14 nm diameter gold particles in 25 mM pH 11 carbonate buffer to prevent colloidal gold aggregation caused by positively charged peptides. Dynamic light scattering revealed that there is an increase in size of the GNPs due to the complexation with BSA-peptide conjugates (Fig. 2). A surface coverage of 140 ± 10 BSA per 14 nm GNP was found using a protein assay.

The path of the multipeptideconjugated NPs was studied using TEM. In general, nanoparticle uptake followed one of three pathways: (i) cell entry followed by exocytosis; (ii) cell entry and transport transport up to the nuclear membrane; (iii) cell entry and nuclear translocation. In this case NP complexes were able to a) enter cell via mostly through endocytosis process, b) escape endosomes and release into the cytoplasm, and c) reach the nucleus through nuclear pores as illustrated in Fig.1.

Cellular uptake of uncoated and peptide coated GNP complexes were characterized using HeLa cell line. It was found that targetd NPs internalized more as compared to uncoated GNPs as shown in Fig. 2B. The fate of nanoparticles inside HeLa cells depended upon the targeting peptide complexed to the gold surface. Cellular localization was probed by transmission electron microscopy (TEM) and optical microscopy. After 6 h of incubation with HeLa cells, untargeted NPs were found to be trapped in the endosomes and lysosmes while peptide-GNP conjugates were found to escape endosmes and reach the nucleus.. GNPs carrying peptide M1 showed minimal nuclear localization. NPs carrying peptide M2 were also found inside the nucleus of over 70% of the observed cells. Nuclear targeting was also observed during the delivery of nanoparticles carrying peptide M3 and it did not have any previously known NLS function, carrying only the integrin binding domain plus six lysine residues. The peptide M3 is largely synthetic in nature, containing only the integrin binding domain and a segment of basic lysine residues. The integrins are a family of transmembrane glycoproteins exploited by a number of viruses (adenovirus, echovirus, and foot-and-mouth-disease virus), as well as bacterial intracellular pathogens, for cell entry [28]. We found that the combination of M3 and M2 gave the best result as illustrated in Fig. 2D.

We used the combination of peptides M2 and M3 for investigating the therapeuticc enhancement due to nuclear targeted NPs. There was a three fold enhancement in the therapeutic ratio for nuclear targeted cells as compared to untargeted case (Fig. 3B). The exact mechanisms of cell damage when GNPs are localized in the cytoplasm or in the nucleus of the cell are not known yet. Based on previous studies, it is believed that enhanced localized absorption of X-rays by NPs could lead to effective release of low-energy electrons from GNPs, and the electrons released could create more radicals as illustrated in Figure 4A. Most of these studies were done either in water or using dry DNA-GNP films. The exact mechanisms of cell damage when GNPs are localized in the cytoplasm of the cell are not known yet. Based on several studies performed using GNPs in close proximity to DNA, the enhancement in cell damage for cells with targeted GNPs may be due to the close proximity to DNA. GNPs can enhance the number of low energy electrons that can damage the DNA. Figure 5 shows that there is higher DNA double strand breaks in the cells with nuclear targeted NPs.

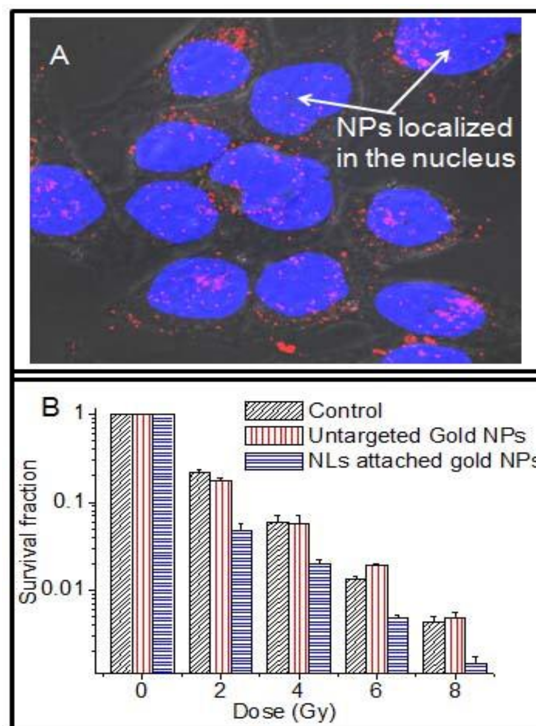

Figure 3: Improved therapeutics using targetd GNPs. A) Optcal image showing GNPs localized both in the cyotoplasm and nucleus. B) Increased in cell death for cells with NPs targeted within the nucleus.

These studies support the hypothesis that optimizing the distribution of GNPs within the cell could lead to improved therapeutic effects. With nuclear targeting, there is a possibility in the production of additional low-energy secondary electrons within the nucleus causing more damage to DNA. The outcome of this research will enable the optimization of GNP-based systems for use in cancer radiotherapy. It has demonstrated recently that the addition of gold nanoparticles to anticancer drugs such as cisplatin and other platinum agents enhanced the cell damage. Therefore, GNPs in combination with radiations and chemotherapeutic drugs provide interesting avenues to further improve the treatment of cancer. Based on this study it can be concluded that it is possible to develop a

nanoparticle based multiplex system to improve therapeutic ratio in cancer therapy.

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