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Targeting heat shock protein 70 using gold nanorods enhances cancer cell apoptosis in low dose plasmonic photothermal therapy

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ABSTRACT

Plasmonic photothermal therapy (PPTT) is a promising cancer treatment where plasmonic nanoparticles are used to convert near infrared light to localized heat to cause cell death, mainly via apoptosis and necrosis. Modulating PPTT to induce cell apoptosis is more favorable than necrosis. Herein, we used a mild treatment condition using gold nanorods (AuNRs) to trigger apoptosis and tested how different cell lines responded to it. Three different cancer cell lines of epithelial origin: HSC (oral), MCF-7 (breast) and Huh7.5 (liver) had comparable AuNRs uptake and were heated to same environmental temperature (under 50 °C). However, Huh7.5 cells displayed a significant increase in cell apoptosis after PPTT as compared to the other two cell lines. As HSP70 is known to increase cellular resistance to heat, we determined relative HSP70 levels in these cells and results indicated that Huh7.5 cells had ten-fold decreased levels of HSP70 as compared with HSC and MCF-7 cells. We then down-regulated HSP70 with a siRNA and observed that all three cell lines displayed significant reduction in viability and an increase in apoptosis after PPTT. As an enhancement to PPTT, we conjugated AuNRs with Quercetin, an inhibitor of HSP70 which displayed anti-cancer effects via apoptosis.

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1. Introduction

In the last decade, many publications associated with the use of gold nanorods (AuNRs) in plasmonic photothermal therapy (PPTT) have shown great promise for combating cancer [1–3]. PPTT relies on the principle that AuNRs show distinct physicochemical properties compared to other gold nanoparticles [4–6]. AuNRs absorb near infrared light and convert it into heat rapidly and efficiently by non-radioactive processes, including electron-electron and electron-photon interactions [7–10]. Thus, when AuNRs are targeted to cancer cells, electromagnetic irradiation with an optical laser induces enough heat to selectively destroy cancerous cells [11–15].

PPTT causes cancer cell death mainly via necrosis and apoptosis [16,17]. One of the main challenges in PPTT is the overheating caused by large dose of nanoparticles or laser exposure, which can easily cause necrosis. During necrosis the plasma membrane is broken causing cytoplasmic components to leak out and inflammation which is known to induce metastasis and cancer growth [18–20]. Additionally, the overheating could hurt the neighboring healthy cells. On the other hand, PPTT induced apoptosis occurs through a programming cell death, which does not cause inflammation [21]. Therefore, developing a new strategy targeting cancer cell apoptosis and avoiding necrosis would be of great importance for the next generation of PPTT.

It has been reported that different intracellular locations of AuNRs or laser strength regulate the switch between necrosis and apoptosis in PPTT [12,22]. However, lower heating would decrease the efficacy of PPTT, and might not be very effective for cells that exhibit thermo-resistance. Therefore, it is important to know the major players that contribute to thermo-resistance. Based on this, we can develop new PPTT strategies that prevent the thermo-resistance and enhance apoptosis. Many factors could contribute to thermo-resistance such as: the inhibition of cell death signals, inhibition of endocytosis or increased exocytosis of nanoparticles [16,17]. Heat induced apoptosis can also be resisted by a class of proteins called Heat Shock Proteins (HSPs) [23–26]. When binding to denatured proteins or partially unfolded intermediates, HSPs prevent their aggregation and initiate protein refolding [27,28]. Interestingly, PPTT induces the expression of most HSPs [29–31],

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and multiple studies have shown the importance of the HSP in enabling cancer cells to resist heat-induced apoptosis [32–37]. However, the specificity of these inhibitors to individual heat shock proteins has not been tested [36].

As HSP70 inhibits cell apoptosis directly by preventing the cytochrome c/dATP-mediated caspase activation [38] and binds with multiple other HSPs to mediate protein refolding [29], we conducted PPTT on multiple cell lines and positively correlated HSP70 levels with PPTT response. We then used siRNA to remove HSP70 and observed an enhancement in PPTT. These results led us to fabricate novel HSP70 inhibitor (Quercetin) conjugated AuNRs and compared to non-conjugated AuNRs, the new conjugate displayed a superior ability to combat thermo-resistance, causing a higher percentage of cells to undergo apoptosis. Therefore, AuNRs act as integrally in delivering of Quercetin to cancer cells as well as acting as an inducer of apoptosis-associated PPTT.

2. Results

To better define which cancers might be best treated with PPTT and to determine if and how PPTT displays more or less effectiveness against different cancer types, we created nanorods (AuNRs) using a novel technique [7,39]. We performed transmission electron microscopy (TEM) to determine the quality and homogeneity. These AuNRs were uniformly shaped (Fig. 1a) with an approximate length and width of 25 ± 3 nm × 5.5 ± 0.8 nm (Supplementary Fig. 1a, b). Additionally, these AuNRs displayed an excitation at 800 nm (Fig. 1b). AuNRs were then conjugated with bovine serum albumin (BSA), to block nonspecific interactions, arginylglycylaspartic acid (RGD) to enable integrin-mediated endocytosis, and Rifampicin (RF), an inhibitor of drug exocytosis [39]. To confirm conjugation with RF we measured the absorption spectra of AuNRs before and after conjugation with RF. AuNRs conjugated only with BSA absorb at 530 nm and 800 nm (Fig. 1b). RF alone has two absorption wavelengths: 330 nm and 470 nm (Fig. 1b). When the AuNRs were conjugated with RF two new peaks appeared at the same absorption wavelength as RF, suggesting a successful conjugation with RF [39]. The effectiveness of these AuNRs was then tested on three different cancer cell lines: HSC (human squamous carcinoma), MCF-7 (human breast cancer) and Huh7.5 (human hepatocellular carcinoma) cells. To ensure each cell line endocytosed similar amounts of AuNRs the absorbance of the AuNRs in the media before and after PPTT was measured by ultraviolet–visual spectroscopy (UV–vis). We observed a similar loss in AuNR absorbance from the media in all three cell lines before laser treatment, suggesting that all three cell lines endocytosed similar amounts of AuNRs (Fig. 1c). When performing PPTT we chose an 808 nm laser with a power of 5.8 W/cm^2 for duration of 2 min. The duration and strength of these laser settings have been shown not to harm cells in culture [9,10,38–42]. Under these laser settings we observed that the media from all three cell lines consistently reached approximately 50 °C after PPTT treatment as measured by a hypodermic thermocouple.

Noticing no differences in the amount of AuNRs uptake or temperature of the media we used two different methods to measure the effectiveness of PPTT. To measure apoptosis and necrosis we labeled cells with Annexin V and propidium iodide and then performed flow cytometry. Similar percentages of apoptotic cells (AnnexinV+) were observed between AuNR treated without laser and AuNR with laser treated HSC and MCF-7 cells (Fig. 2a). However, PPTT treated Huh7.5 cells displayed a significant increase in apoptosis after PPTT as compared to HSC and MCF-7 cells. We did not observe propdidium iodide staining under any conditions suggesting necrosis was not occurring (Supplementary Fig. 2a), as has been previously observed for other cell lines [43]. To measure

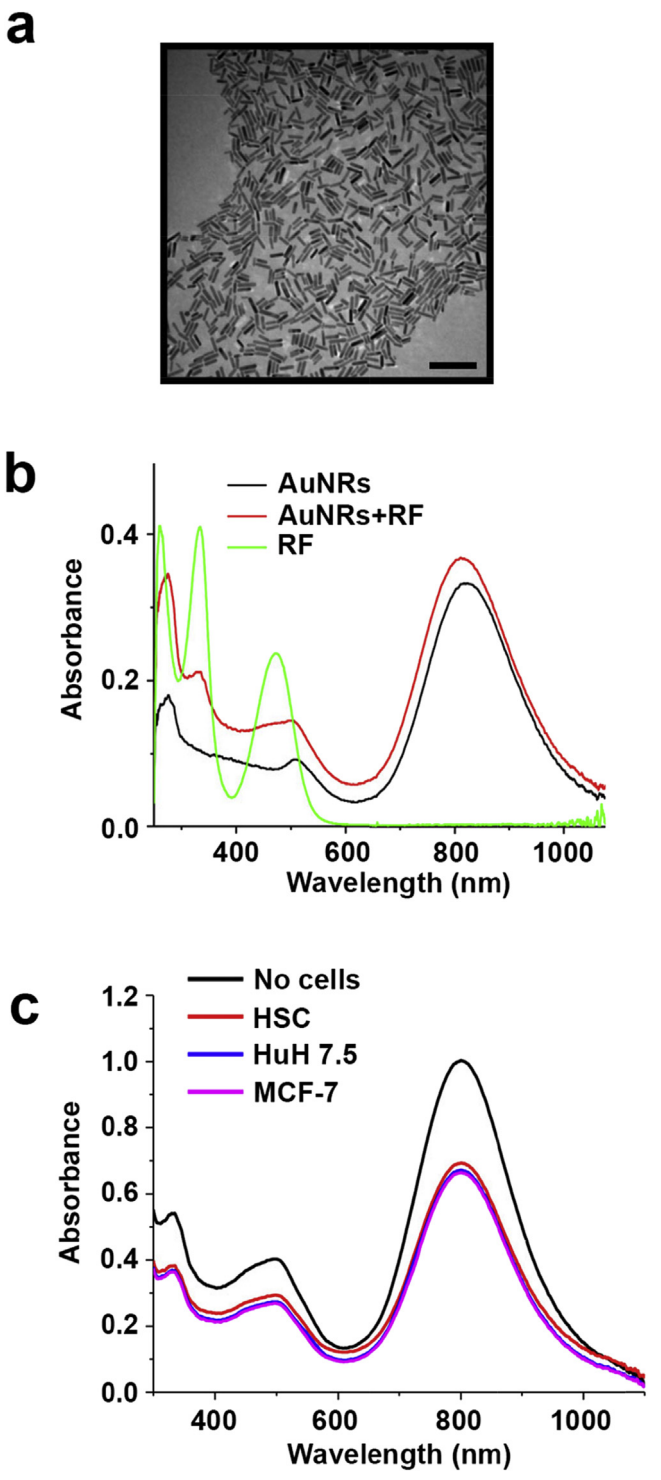

Fig. 1. Characterization of conjugated AuNRs and measurement AuNRs of endocytosis. (a) Transmission Electron Microscopic (TEM) image of conjugated nanorods (AuNRs). Scale bar = 100 nm. (b) UV–vis absorption spectra of the unconjugated AuNRs or the nanorods conjugated with Rifampicin (AuNRs + RF-RGD). (c) The UV–vis spectra for the AuNRs in cell culture media before and after incubation with HSC (human squamous carcinoma), MCF-7 (human breast cancer) and Huh7.5 (human hepatocellular carcinoma) cells for 24 h.

the effectiveness of PPTT by another method we performed an XTT assay. All three cell lines displayed similar levels of cell viability without laser treatment after AuNRs treatment, indicating the AuNRs alone were not toxic to the cells. Consistent with the flow