Multimodality therapy: Enhancement of melanoma cell death with combination of heat shock protein 90 inhibitor, 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG), and gold nanoparticles in a noninvasive radiofrequency field

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ABSTRACT

Melanoma is the most serious form of skin cancer. Hyperthermia has been attempted as a viable option to treat advanced stage melanoma. However, solid tumors often respond minimally to hyperthermic treatment partly due to the thermo-tolerance induced by chaperons of the heat shock protein family (Hsp). 17-AAG, a Hsp90 inhibitor, is currently being studied in clinical trials as a direct cancer therapeutic agent. We have shown that gold nanoparticles heat cancer cells in a noninvasive radio frequency (RF) field. Here, we demonstrate the enhancement of melanoma cell death with the tandem treatment based on Hsp90 inhibition and heat therapy using gold nanoparticles in a noninvasive RF field.

Keywords: hyperthermia, hsp, nanoparticles, radiofrequency

1 INTRODUCTION

Melanoma is a cancer that evolves in melanocytes and has a very high tendency to metastasize in its advanced stages. While early stage melanoma has a high curability rate, not many effective treatment options are currently available for the advanced stages of the disease. One of the most common chemotherapy drugs used for advanced stage melanoma is Dacarbazine that is often combined with several other drugs [1]. It has been suggested that hyperthermia increases blood flow and can facilitate optimum distribution of chemotherapeutic agents in cancer cells [2].

In general, the treatment options available for cancer are invasive and toxic, with acute and chronic side effects. Hyperthermia, which at low levels is considered to have minimal side effects, has been used to treat cancer for thousands of years. Cells start to undergo apoptosis when heated to temperatures in the range 41-47^{0}C and temperatures above 55^{0}C kill cells mostly through necrosis rather than clinically desirable apoptotic processes [2] [3]. Currently, administered clinical hyperthermic ablation of cancer cells is still relatively invasive as the probe or needle has to be inserted into the tumor. Regardless of the conventional heat source used, this treatment lacks a high specificity for cancer cells and brings about a higher proportion of necrotic cell death due to poor controllability of heating. Gold nanoparticles specifically delivered to cancer cells and subjected to a radio frequency field provide more controlled heating to therapeutic temperatures to bring about cancer specific apoptotic cell death [4]. However, the specificity of gold nanoparticle uptake by the cancer cells has been a limiting factor in this approach.

Heat shock proteins (Hsp) play a vital role in protecting normal tissues during stress. Early studies have shown that the Hsp90 plays a crucial role for stability and function of a wide variety of oncogenic kinases and signaling intermediates [5]. In addition, Hsp90 is also essential for normal cell viability and growth. Inhibition of heat shock proteins has been considered as a potential target to enhance cancer cell death. Furthermore, inhibition of Hsp activity may enhance the apoptosis of cancer cells induced by gold nanoparticle mediated hyperthermia. In this study we investigated the efficacy of a simultaneous hyperthermic and chemotherapeutic melanoma treatment.

2 MATERIALS AND METHODS

Two human melanoma cell lines, A375 and AAB527, were cultured according to AATC guidelines. Each cell line was treated with 20nm gold nanoparticles (Ted Pella Inc. Redding, CA) at a 0.1ug/ml concentration for a 24 hour period at 37^0 C in a 5% carbon dioxide (CO_2) incubator. The treated cells and the control cells were washed with PBS just before adding the heat shock protein inhibitor 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG). The IC_{50} (half-maximal inhibitory concentration) value for 17-AAG was determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) cell proliferation assay (data not shown). One hour after the incubation, cells were exposed to a focused noninvasive 13.56 MHz radio frequency field at a power of 500W for 3 minutes. The final temperature of the media was kept below 42^0 C. The cells were exposed to the same RF field once more for 1.5 hours after the initial RF exposure. Uncertainties and standard error of the mean was assessed using triplicates of samples. The cellular uptake of gold nanoparticles was measured by transmission electron microscope (TEM). Three days after the RF treatment, cells were analyzed in an LSRII flow cytometer (BD

Bioscience, San Jose, CA). Apoptosis, necrosis, viable and dead cells were determined by Annexin V –FITC and PI assay.

3 RESULTS

TEM images indicated a good uptake of 20 nm gold nanoparticles by both melanoma cell lines A375 and AAB527 (Figure 1). The viability of A375 cells decreased from 95.5% to 87.5% (± 2.5%) after treating with 17-AAG alone, and the cell viability further decreased to 65% (± 3.2%) when the drug treatment was combined with exposure to RF field in the presence of gold nanoparticles. However, any significant cell viability reduction was not observed in any of the corresponding samples of AAB527 cells. Although RF field exposure in the presence of gold nanoparticles increased the cell death via apoptosis of A375 cells, the highest contribution to cell death came from necrosis due to the Hsp90 inhibitor. The necrotic cell death due to 17-AAG seemed to have increased in the presence of gold nanoparticle mediated heating in the RF field.

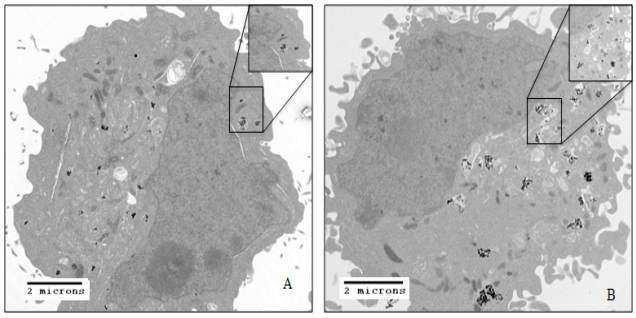
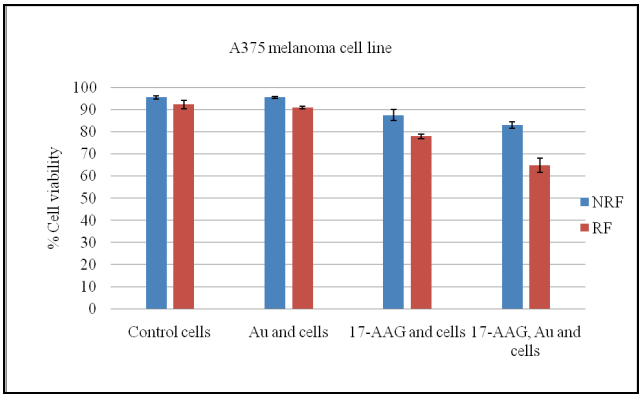


Figure 1: The TEM image (7500 X magnification) of dense populations of 20nm gold nanoparticles scattered in vesicles in the cytoplasm. A375 cells (A) and AAB527 cells (B).



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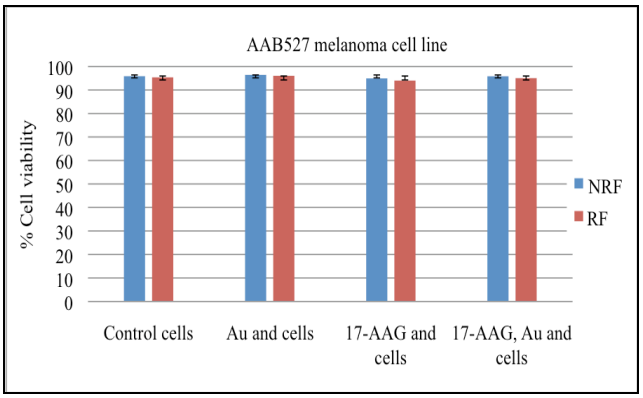

B

Figure 2: Both cell lines A375 and AAB527 were treated with 0.1uM 17-AAG. Viable cell population of A375 (A) cells and AAB527 (B) cells without RF exposure (blue) and after RF exposure (red).

4 DISCUSSION

Although cell death via apoptosis was clearly increased by combination of hyperthermic and chemotherapeutic approaches implemented in this study, the Hps90 inhibitor dependent cell necrosis was the prominent contributor to overall cell death. Hyperthermic treatment of cancer using gold nanoparticles is still in its infancy and the cell death via apoptosis needs to be optimized by further regulating the dosing of the heat shock protein inhibitor in combination with RF field exposure. The enhancement of cell death seen here is comparable to that in a published study using another Hsp90 inhibitor, geldanamycin (GA), in combination with ferromagnetic particle-mediated hyperthermia [3]. However, the 17-AAG is far less cytotoxic than GA, while the concentrations of ferromagnetic particles required to induce hyperthermia in magnetic fields are significantly higher than the low dose of gold nanoparticles needed in the RF field [3]. Also, the delivery of ferromagnetic particles selectively into cancer cells in sufficient quantities is considered to be significantly challenging [3]. In this project we have demonstrated the successful delivery of 20 nm gold nanoparticles into melanoma cells and demonstrated a promising approach to cancer treatment with far less severe side effects. It will be possible to enhance delivery of gold nanoparticles to cancer cells through conjugation with targeting antibodies, peptides, or drugs.

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