A novel therapeutic intervention to treat Squamous Cell Cancer – Cytoplastic/nuclear-directed gold nanospheres (CGNS) and radiation to enhance malignant cell death
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BACKGROUND

Nanotechnology offers exciting new approaches to combat cancer with novel forms of drug delivery, cancer cell diagnostics, and therapeutics. The size match of nanoparticles and cellular structures such as proteins, DNA, and organelles enables unique subcellular targeting therapies. Solid gold nanospheres (GNS) are attractive nanotechnology due to easy preparation, bioconjugation, and relative nontoxicity. It has been found in vitro that GNS targeted to the nucleus selectively kills some oral cancer cells via direct mitotic inhibition. Interestingly, when ionizing radiation is applied to elements with a high atomic number (Z), such as gold (Z=79), the local effect of the radiation is increased. Preliminary research suggests GNS may locally enhance the radiation effect in the treatment of tumors up to 300%. Initially, GNS are coated with citrate that aggregates in biologic fluids and requires an immunopassivating coating, such as polyethylene glycol (PEG). While GNS-PEG can target tumors when injected intravascularly, improved intracellular targeting may be achieved with additional attachment of bioactive ligands. Since ionizing radiation therapy (IRT) kills tumor cells by damaging DNA, we hypothesized that GNS delivered to the nucleus would enhance effect of injected coating, are coated with citrate that aggregates in biologic fluids and requires an immunopassivating coating, such as polyethylene glycol (PEG). While GNS-PEG can target tumors when injected intravascularly, improved intracellular targeting may be achieved with additional attachment of bioactive ligands. Since ionizing radiation therapy (IRT) kills tumor cells by damaging DNA, we hypothesized that GNS delivered to the nucleus would enhance effect of IRT and would add to the independent toxic effect of targeted nuclear GNS.

METHODS

GNS are surface modified with (1) PEG (2) a cytoplasmic localizing sequence (RGD), and/or (3) a nuclear localizing sequence (NLS) and conjugated into cell cultures. Two squamous cancer cell lines (oral cancer HSC-3, and a primary derived skin cancer line, SCC) were compared to an immortalized epithelial cell line (HaCat). Cells are treated with ionizing radiation with and without GNS. Cell localization of GNS, DNA fragmentation (a measure of RT damage) and cell survival was measured.

Synthesis of 30nm gold nanospheres

HaCo2 was tested to boiling while stirring. Trisodium citrate solution was then quickly added to the acid solution. The solution changed color within several minutes from yellow to brown and then purple color. The amount of citrate added controls the size of the nanospheres. All experiments were performed with 30nm gold nanosphere. A transmission electron microscope was used to confirm particle size as indicated in Figure 1A. It should be noted that there is some variation in particle size (Figure 1B).

RESULTS

Ligand Toxicity Determination

First, cell survival was measured comparing 4 ligands in the HSC-3 cell line and noncancerous cell line HaCat (Figure 4). The ligands included PEG, RGD, NLS, and RGD combined with NLS. While there was no statistical difference in survival within the HaCat cell line among the ligands, decreased cell survival was observed with NLS and RGD/NLS in the HSC-3 cell line (p<.05). RGD/NLS was the only ligand to also have a significant difference between non-irradiated and irradiated HSC-3 cell populations. For all further experiments, RGD/NLS conjugated nanospheres were used.

Nanostructure Localization

Location of the GNS was confirmed by darkfield microscopy. As seen in Figure 5, uptake of the conjugated gold particles was much more prevalent in the cancer cell lines (HSC-3 and SCC) relative to the noncancerous cell line (HaCat). The HaCat cell line did not appreciably take the RGD/NLS-GNS into the nucleus. RGD/NLS-GNS localized to the nucleus in the HSC-3 cell line and predominately in the cytoplasm for the human derived epithelial SCC line.

Cell viability with nuclear targeted nanosphere treatment

Combined RGD/NLS-GNS and Radiation on SCC Breaks DNA Double-Strand Breaks

This study reveals that nuclear targeted GNS are preferentially cytotoxic to the two squamous carcinoma lines compared with immortalized nonmalignant skin cells (HaCat). Selective targeting of malignant cells may result from poorly restricted access to the cells nuclei in malignancy; the results may be extendable to other cancer cell types. The cell toxicity is increased in combination with the effect of ionizing radiation. When analyzed individually (data not shown) the survival effect appears additive and not synergistic in this model at these doses with ~30nm RGD/NLS-GNS. However evaluation of DS breaks reveals GNS enhanced the effect of ionizing radiation.

This study demonstrates that RGD/NLS-GNS may offer a novel combined therapy with radiation for enhancing malignant cell killing at lower doses of radiation due to a selective impact on cancer cells.