Determining the Effect of Nano-Based Therapeutics in Cancer Vs Normal Cells

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Introduction

Radiation dose enhancers such as gold nanoparticles (GNPs) encapsulated in cancer cells has been tested to enhance the deposited radiation dose. However, the tumor microenvironment is a combination of stromal cells, such as normal fibroblasts (NFs) and cancer associated fibroblasts (CAFs) and simply targeting the cancer cells with GNPs will not be enough to eliminate a malignancy. While FBs have anti-tumourigenic properties, CAFs promote the proliferation of tumours and are related to metastasis and treatment resistance. The goal of this study was to understand the extent of GNP uptake and retention in normal vs cancers cells, and to elucidate the outcome when treated in combination with radiation.

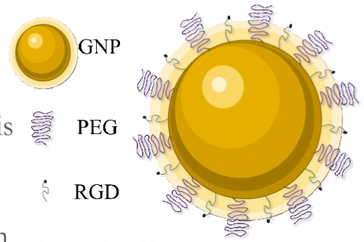

Figure 1. Gold Nanoparticles. Gold nanoparticles (GNPs) act as radiosensitizers and anti-cancer drug carriers. GNPs used in this study are conjugated with polyethylene glycol (PEG) and an integrin binding domain RGD peptide.

Methods

For evaluation of the GNP uptake, HeLa, FBs, and CAFs were incubated with GNPs of 15 nm diameter over a period of 24hrs. Gold content was measured using inductively coupled plasma mass spectrometry (ICP-MS). A photon-based radiation dose of 2 Gy was given using a clinical linear accelerator to ascertain radiation effects in the presence of GNPs. Assessment was completed using a resazurin-based proliferation assay over a 14-day time period and an immunofluorescent-based 53BP1 foci assay.

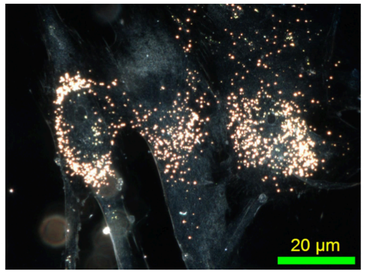

Figure 2. GNPs in CAFs. The uptake of GNPs can be imaged using darkfield microscopy.

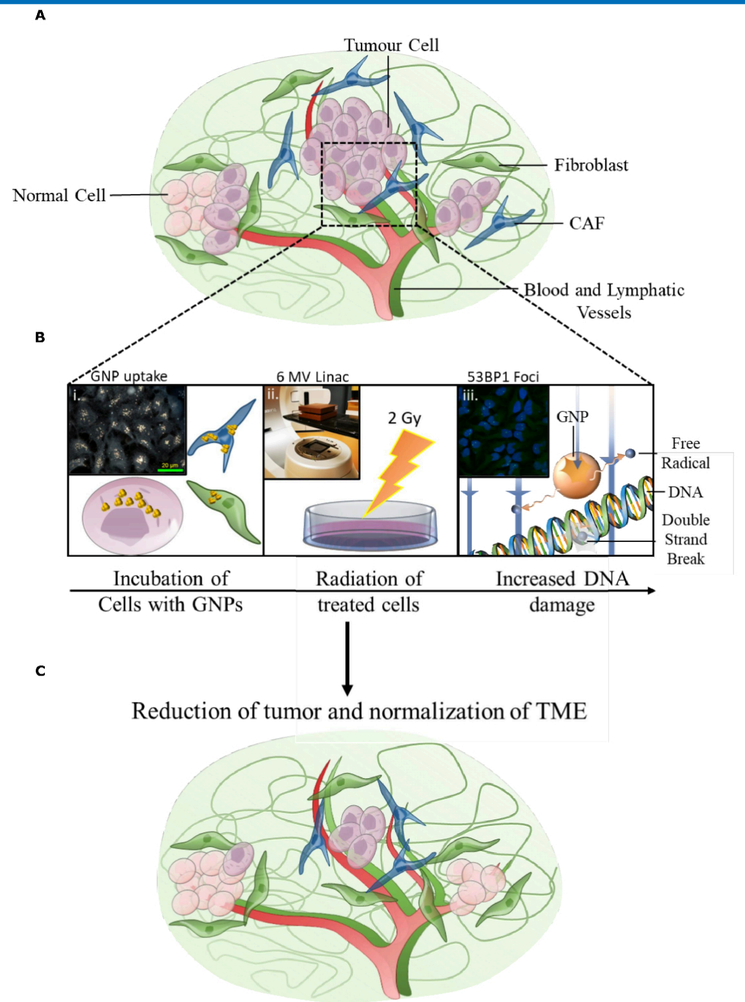

Figure 3. Normal and cancer associated fibroblasts in cancer therapeutics. (A) In normal tissue, FBs have anti-tumourigenic properties that supress proliferation of the tumour. However, when CAFs are introduced, a largely pro-tumourigenic influence is now exerted on the tumour microenvironment, leading to growth and eventual metastasis. (B) i) Cells uptake GNPs at differing rates, which is directly related to the efficacy of the treatment. ii) Cells are irradiated with a 2 Gy dose using a 6 MV linac. iii) GNPs have been shown to have a radiosensitization effect on cancer cells, through improved formation of free radicals (C) Upon treatment with the dual combination of GNPs and radiation, the TME is more normalized and there is a reduction in tumour growth as well as invasive and migratory behaviour.

Results

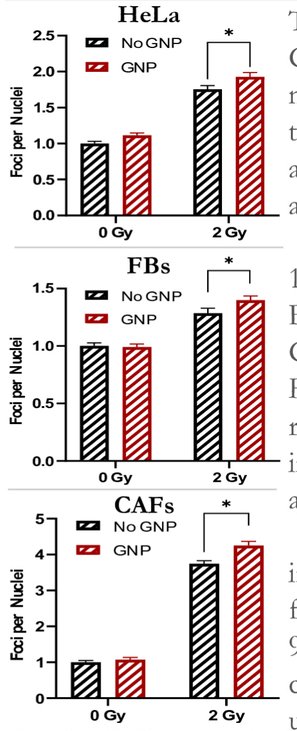

Figure 4. 53BP1 DNA damage foci. Quantitative data of the number of foci per nuclei, gathered from a minimum 50 cells.

The CAFs had the largest uptake of the GNPs per cell, with on average 265% as many GNPs relative to HeLa, which had the second largest GNPs/cell. FBs had, on average, only 7.55% the uptake of HeLa and 2.87% the uptake of CAFs.

There was a significant decrease of 13.0% and 24.7% in survival in HeLa and FBs upon treatment with radiation and GNPs, compared to irradiated control. However, CAFs saw no significant reduction. This was most likely due to inherit radioresistance of the CAFs. Thus, a DSB assay was employed.

There was a 13.5% increase over irradiated control in 53BP1 foci formation following radiation with GNPs for CAFs, 9.8% for HeLa, and 8.8% in FBs. This corresponded with the increased GNP uptake. Increase in DNA DSBs is likely due to the enhancement in the absorbed dose due to GNPs.

Conclusions

CAFs had the largest uptake of cells and largest increase in 53BP1 foci-related radiation damage. This DNA damage due to selective targeting of cancer associated cells over normal cells allows GNPs to be an effective tool in future cancer radiotherapy. However, further effects of radiation on CAFs will have to be explored in the future.