Targeted gold nanoparticles as nanosonosensitizers in ultrasound based cancer treatment

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Gold nanoparticles (AuNPs) have attracted a great deal of attention in recent years because of their enhanced and tunable optical properties, easy production and functionalization as well as their good biocompatibility (Shukla et al, 2005). Moreover, gold nanoparticles set themselves apart from other nanoplatforms thanks to the unique localized surface plasmon resonance which can be used in the multimodal treatment of cancer, an example of which is photothermal therapy (Huang et al, 2008). AuNPs are then front-runners for use as targeting moieties in external physical trigger based cancer treatment.
The aim of this study is to investigate cancer targeted AuNPs in their role as nanosonosensitizers which increase the acoustic cavitation effect, typically associated with sonodynamic therapy, a developing non-invasive anticancer approach where ultrasound (US) is used to trigger sonosensitizer cytotoxicity leading to cancer cell death (Tachibana et al, 2008).
In this work, AuNPs have been produced via the reduction of tetrachlorauric acid with sodium citrate and decorated with 2 kDa mPEG-SH, to provide stealth features, and 3.5 kDa folate-PEG-SH (FA-PEG-AuNPs), which acts as a targeting agent for the biorecognition of folate receptor expressing cancer cells. AuNPs have been characterized by TEM, UV-Vis and photon correlation spectroscopy in order to assess size, morphology and colloidal stability. *In vitro* experiments were performed on human cell lines that express a folate receptor, i.e. epidermoid carcinoma KB and colon carcinoma HCT116, and that do not express a folate receptor, i.e. breast cancer MCF-7. Cell association has been investigated using confocal microscopy and atomic absorption spectrometry. FA-PEG-AuNP's ability to selectively kill cancer cells under US field exposure has been studied at 1.8 MHz and two different energy densities inside the cell tube; $8x10^{-6}$ J cm^{-2} for 5 minutes at 30°C and $8x10^{-5}$ J cm^{-2} for 5 minutes at 45°C. The treatment's effect on cell growth has been investigated using a WST-1 proliferation assay, cell death and reactive oxygen species (ROS) production studied using flow cytometric analysis (FCA). Experiments have also been carried out with the free radical scavenger N-acetylcysteine (NAC) to assess the role of ROS in cancer cell death.
FA-PEG-AuNPs possess a hydrodynamic size of about 30 nm and selectively target folate receptor expressing cancer cells, KB and HCT-116, and do not associate with MCF-7 cells that do not express folate receptors. A 60% reduction in cancer cell growth was only observed in folate receptor expressing cells, KB and HCT116, upon incubation with FA-PEG-AuNPs and US treatment, as compared to control conditions, 72 hours from sonodynamic treatment, confirming our hypothesis that gold nanoparticles can act as nanosonosensitizers. Moreover, FCA only showed a significant increase in late apoptotic/necrotic cells in KB and HCT116 cells that had been treated with FA-PEG-AuNPs and US at the two different energy densities. A specific ROS production pattern was also observed after FA-PEG-AuNPs and US treatment at the two different energy densities. Interestingly, NAC only suppresses cyototoxicity and ROS production when used with the lower energy density sonodynamic treatment, while it seems to play a minor role in suppressing these effects at the higher energy density, which displayed a moderate hyperthermic condition. In conclusion, the simultaneous exploitation of the targeting capacity of the gold nanoparticles and the sensitizing effect afforded by a localized external stimulus, namely ultrasound, make them promising candidates for the site-specific treatment of cancer. Furthermore, this *in vitro* study can be

considered a proof of concept for AuNP use as nanosonosensitizers in the ultrasound based treatment of cancer.

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