Improving proton therapy by gold nanoparticles: study of internalization and subcellular localization.

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One of the promising clinical cancer treatments is proton therapy, a type of external beam radiotherapy that uses a beam of protons instead of conventional X-rays to irradiate diseased tissue. It makes use of ionizing radiation to kill cancer cells while trying to cause the least possible damage to the surrounding healthy tissue. Because of their relatively large mass, protons have little lateral side scatter in the tissue; the beam does not broaden much, stays focused on the tumour shape and delivers only low-dose side effects to surrounding tissue. All protons of a given energy have a certain range; very few protons penetrate beyond that distance. Furthermore, the dose delivered to tissue is maximized only over the last few millimeters of the particle's range; this maximum is called the Bragg peak.

Recently, it is of particular interest to find methods that increase the biological effectiveness of proton therapy: increasing the dose deposition selectively within the tumoral targets. In this regard, a large number of studies have emerged on the possible use of radiosensitizers and tumor-specific materials. Among these, the addition of metallic nanoparticles in the tumour has been proposed as a new strategy to enhance selective cell killing and, thus, overcome the limitation of proton therapy. In particular, gold nanoparticles (GNPs) may be particularly promising tools. Many *in vivo* and *in vitro* studies demonstrated a dose enhancement effect induced by GNPs, at megavoltage and kilovoltage energies. The use of GNPs during a proton therapy protocol is advantageous since gold nanoparticles, because of their optical resonance properties, release X-rays when affected by protons. In this way, X-rays will be generated directly in the tumor site.

The current work presents preliminary data about the *in vivo* biodistribution of GNPs and the subcellular localization after intravenous injection in mouse caudal vein. The average size of the synthetized GNPs was 12±5 nm and the maximum absorption peak at 530 nm. In order to follow *in itinere* the biodistribution of GNPs by microPET-CT, NPs were funzionalized with a contrast agent, i.e. ^{18}F-FDG. A complete body distribution of the GNPs was observed after three hours from the blood injection. At the end of experiment, organs were dissected and analyzed by ICP-MS, TEM and STEM analysis. GNPs are retained by brain, kidneys, liver, intestine, spleen and hearth, thus suggesting that our GNPs are able to also pass biological barriers. Interestingly a different accumulation of gold was measured in the various organs: liver the highest and stomach the lowest. A diversified subcellular localization of GNPs was observed: i.e. cytoplasm, nearby and inside

mitochondria, endoplasmic reticulum, lysosomes and nucleus. Our *in vivo* data showing the GNPs ability to pass physiological barriers, reach different organs and localize in intracellular organelles highlights the potentiality to use such nanostructures in proton therapy. Furthermore, the radiosensitizing effect of GNPs, combined with their unique physico-chemical proprieties (such as good biocompatibility, and simple synthesis process), make them powerful tool in biomedical research.