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Indocyanine Green-Loaded Ferritin Nanoparticles for Intraoperative Detection of Cancer Tissue

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Extended Abstract

Background: Nanotechnology represents one of the most promising field of study in this century, considering that its application to medicine is becoming fundamental in the diagnosis and treatment of several diseases such as cancer. Since chemotherapy often suffers from limited effectiveness and specificity against cancer cells, causing important side effects and toxicity in healthy tissues, the main clinical option is still usually represented by surgery. In this context, the development of nano-tracers with tumor specificity to achieve an accurate and timely diagnosis could be of crucial importance. In recent years, several studies have been carried out using nanoparticles of ferritin (HFn), that offer new therapeutic options, potentially allowing the specific drug delivery to cancer cells, as well as the possibility of exploiting them as nano-tracers for tumor detection and fluorescence-guided surgery (FGS) applications. Indeed, one of the most important features of HFn is its natural homing towards cancer cells thanks to the specific recognition of the transferrin-1 receptor (TfR1), which is abundantly expressed in most tumor subtypes [1-4].

Objectives: To support surgeons during intraoperative tumor and metastasis localization, we proposed the use of ferritin nanoparticles loaded with indocyanine green (ICG), a fluorescent dye currently used in clinics. This nanosystem is constituted by 24 monomers of H-Ferritin (HFn), which self-assembles in a spherical cage structure enclosing ICG. In order to demonstrate its suitability for FGS approaches, we characterized the *in vivo* behaviour of the nanotracer in a murine model of breast cancer and assessed its ability to reach cancer cells, discriminating them from healthy tissues.

Methods: ICG was loaded in endotoxin-free HFn nanocages [5] (HFn-ICG) exploiting the ability of HFn to disassemble and reassemble its quaternary structure in response to changes in pH [6]. Then we evaluated HFn−ICG tumor accumulation and off-target biodistribution in 4T1 tumor-bearing mice. The administration of HFn-ICG has been compared with the injection of the same amount of free ICG. Mice were sacrificed at 6h and 24h and analyzed in order to detect the fluorescent signal by coupling the use of the KARL STORZ NIR/ICG endoscopic system to the IVIS Lumina II system [7]. Moreover, we investigated if HFn could maintain the ICG fluorescence signal longer and protect it from rapid metabolism and degradation performing a UHPLC-MS/MS quantification on organ homogenates

Results and discussion: Our study demonstrated that HFn-ICG was able to deliver ICG to the tumor more efficiently than the free dye in a murine model of breast cancer. Moreover, the evaluations of ICG and HFn-ICG accumulation in off-target organs, evidenced that the nanoformulation seemed to slow down the tracer's kinetic. UHPLC-MS/MS quantifications on organ homogenates reported an increased fluorescence signal in off-target organs from HFn-ICG treated mice. This is reasonably associated with improved ICG stability and increased biodistribution achieved by nanoformulation. HFn−ICG demonstrated potentiality as a nanotracer for cancers where fluorescence-guided surgery is a valuable resource. However, further efforts are required to accelerate HFn-ICG wash-out from off-target organs and obtain more specific signals at cancer to also allow the detection of tumors with unknown localization and micrometastases.

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