Engineered ferritin nanoparticles for the bioluminescence tracking of nanodrug delivery in cancer.

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Nanoparticles (NPs) have long been identified as appealing delivery system of anticancer drugs and the identification of a reliable and sensitive method to monitor the delivery of administered nanodrugs into the tumor cells is a key aspect of the development of new nanoformulated therapies. Among them, apoferritin represents one of the most promising tool and an ideal candidate for clinical translation.

Concerning NPs for cancer imaging, no data have been reported about apoferritin NPs for bioluminescence imaging (BLI). Bioluminescence, defined as the production and emission of light via an enzymatic reaction, in which a chemical substrate (luciferin) is oxydized by an enzyme (luciferase), represents a cost-effective method that enables real-time analysis of biological process in intact organisms, but it has some limitations that nanoformulation of luciferin could overcome.

In the present work we developed a glutathione-sensitive NP for stimuli-responsive release of luciferin within cancer cells. H-Ferritin (HFn), composed by 24 heavy chain subunits of human apoferritin, was chosen as nanovector, thanks to its tumor-specific targeting. Luciferin was conjugated on HFn surface by means of a disulfide containing linker, which undergoes an intramolecular cyclization reaction that results in the release of free luciferin, in the presence of a reducing agent.

First, a stimuli-sensitive self-immolative linker was synthesized in order to load, transport and selectively release luciferin inside tumor cells, in response to a change in gluthatione concentration and the mechanism of release, providing high luminous signal in the presence of luciferase, was verify via cell-free *in vitro* test. Then, the luciferin-linker was conjugated to HFn NPs, leading to Luc-linker@HFn, and bioluminescence kinetics of Luc-linker@HFn and free luciferin were studied *in vitro* and *in vivo* using 4T1 cell line, which is a murine TfR-1+ and luciferase+ breast cancer cell line. Bioluminescence was monitored by IVIS imaging at different time of incubation. For both *in vitro* and *in vivo* experiments the BLI resulting from the nanoformulated luciferin showed a different kinetics compared to free luciferin. The long-lasting BLI signal would be due to the TfR-1 mediated internalization of Luc-linker@HFn and to the slow luciferin release from HFn by endo-lysosomal pathway.

In conclusion, Luc-linker@HFn could be a useful tool for cancer imaging and for monitoring the delivery of administered nanodrug into the tumor cells exploiting the tumor-specific targeting and the internalization pathway of HFn, leading a long-term bioluminescence signal.