PEG-masked ferritin-based multifunctional nanoparticles in melanoma murine model

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Target audience- All oncologists and radiologists involved in new therapeutic strategies.

Introduction - Melanoma, within skin cancers, is the most life-threatening type of skin cancer. Despite the promising effects of the recently-approved immunological therapies, new therapeutic strategies are still needed for metastatic melanoma. Nanoparticle (NP)-based materials are promising agents for enhancing cancer diagnosis and treatment. NPs can be selectively delivered to tumors by passive and/or active targeting. Active targeting is mediated by NP-conjugated ligands, able to bind with high affinity and selectivity target molecules over-expressed by tumor cells as compared to healthy tissues. Our recently developed melanoma-targeting NPs, based on the human protein ferritin (HFt) functionalized with melanoma stimulating hormone (MSH) moiety and poly(ethylene glycol) (PEG) molecules, were shown to selectively bind and be internalized by melanoma cells *in vitro* and in preliminary experiments *in vivo*[1].

Purpose - In this work we evaluate the *in vivo* distribution and localization of HFt-MSH-PEG nanovectors by using independent and complementary techniques such as confocal microscopy, MRI and immunohistochemistry to detect NP constructs endowed with suitable tracers (i.e., fluorophores or magnetic metals).

Methods - B16F10-derived melanomas and TS/A-derived breast adenocarcinomas were analyzed in C57BL/6 mice by MRI with 4.7 T Agilent system, before and after intracardiac or intravenous injection of 0.1 ml of magnetite-maghemite encapsulated NP solutions (0.5 mg). T2-weighted (TR/TE=2500/60 ms) spin echo images were acquired.

Results and Discussion- One day after injection of targeted HFt-MSH-PEG NPs in melanoma-bearing mice, clear MR contrast changes due to accumulation of NPs were observed at the tumor site as shown in Figure 1. The diffused intratumor accumulation of NPs was visible in the MR images as a strong darkened area extended throughout the tumor. In contrast, significantly reduced signals were detected following injection of untargeted HFt-NPs in melanoma bearing mice. Similar results were obtained two days after NP injection. HFt-MSH-PEG NPs accumulated to a significantly lower extent and with a different distribution in a diverse type of tumor (adenocarcinoma), which does not express α-MSH receptors.

Primary tumors were removed from the animals after MRI analyses and were analyzed by immunohistochemistry. A positive staining was present in melanoma tumor sections obtained from HFt-PEG NPs treated animals and in TS/A sections only in peri-tumoral vessels.

The results indicate that *in vivo* targeting of melanoma by HFt-MSH-PEG NPs is achieved not only by passively exploiting tumor-related features, such as increased angiogenesis, vascular permeability and recruitment of monocyte/macrophage cells, but it is significantly contributed by the ability of the NP constructs bearing α-MSH peptide to specifically recognize MC1R receptors expressed by melanoma.

Conclusions - The combined use of different and complementary techniques such as confocal microscopy, MRI and immunohistochemistry allowed to demonstrate that HFt-MSH-PEG NPs are able to target primary melanoma efficiently, selectively and with long lasting accumulation after systemic administration to melanoma-bearing mice. By using similar strategies it is possible to develop ferritin-based nanoplatform selective for specific receptors which are overexpressed in different pathologies.

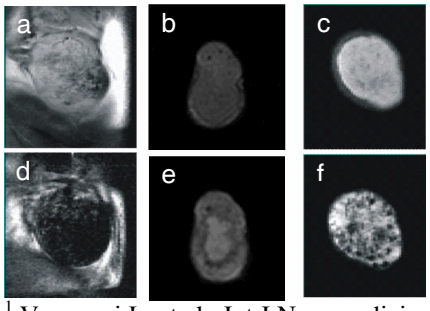

Fig. 1. *In vivo* T2-weighted MRI (section thickness of 0.6 mm) of tumor-bearing mice analyzed before and 24 h after intracardial administration of NPs. B16F10 melanoma (a and b) and TS/A adenocarcinoma (c) are shown before and d and f after injection of melanoma-targeting HFt-MSH-PEG NPs or untargeted HFt-PEG NPs (e). The accumulation of NPs appears as dark areas in the tumor section

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[1] Vannucci L. et al., Int J Nanomedicine 2012;7:1489-509