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

Giulia Carpinelli1, Rossella Canese1, Elisabetta Falvo2, Cristina Maria Failla1, Miriam Carbo1, Manuela Fornara3, Serena Cecchetti1, Lenka Rajsiglova1, Dmitry Stakheev1, Jiri Krizan3, Alberto Boffi12, Veronica Morea1, Luca Vannucci1, and Pierpaolo Ceci1

1Cell Biology and Neurosciences Dept, Istituto Superiore di Sanitá, Rome, Italy, 2Institute of Molecular Biology and Pathology, CNR – National Research Council of Italy, Rome, Italy, 3Molecular and Cell Biology Laboratory, IDI-IRCCS, Rome, Italy, 4Department of Biochemical Sciences “A. Rossi Fanelli”, University of Rome “Sapienza”, Rome, Italy, 5Institute of Microbiology, Academy of Sciences of the Czech Republic (ASCR), Prague, Czech Republic

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 (HFn) 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.

Purpose - In this work we evaluate the in vivo distribution and localization of HFn-PEG NPs 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-magemehite 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 HFn-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 HFn-NPs in melanoma bearing mice. Similar results were obtained two days after NP injection. HFn-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 HFn-PEG NPs treated animals and in TS/A sections only in peri-tumoral vessels.

The results indicate that in vivo targeting of melanoma by HFn-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 HFn-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 HFn-MSH-PEG NPs or untargeted HFn-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