

Proceedings Article

Preliminary results: Imaging of in situ labeled tumor-associated macrophages with Magnetic Particle Imaging

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Abstract

Tumor-associated macrophages (TAMs) are thought to be protumoral, enhancing and contributing to cancer progression. The presence of TAMs has been correlated with the metastasis of tumor cells and the inhibition of the antitumoral immune responses mediated by T cells. In this brief paper, we used Magnetic Particle Imaging (MPI) to detect passively targeted TAMs homing to a breast cancer model. TAMs were passively targeted using PEG-coated Magnetic Nanoparticles (PEG-MNPs). Escalating doses of PEG-MNPs were tested to evaluate TAM uptake at different time points. An MPI signal was detected in liver and tumor for all the groups except at the lowest dose. A novel quantitation workflow has also been proposed to calculate the quantity of iron inside the different tissues.

I Introduction

Magnetic Particle Imaging (MPI) is a promising tool for tracking cells tagged with Magnetic Nanoparticles (MNPs). Of great interest is tracking the behavior of cells associated with the immune system. One cell type, Tumor Associated Macrophages (TAMs), may act as a potential biomarker for both cancer detection and prognosis. In this study, we tag TAMs in situ with MNPs, and track their location using MPI co-registered with X-ray/CT.

I.I Tumor-Associated Macrophages

Immune cells (e.g. leukocytes), together with fibroblasts and endothelial cells, form the tumor microenvironment. These immune cells interact with tumor cells to influence tumor growth. One type of immune cell, macrophages, are part of the mononuclear phagocyte system that is

responsible for the clearance for foreign matter from the body. Consequently, nanoparticles that interact with macrophages will be recognized and internalized [1]. When a macrophage is recruited to a tumor site by a tumor, it is known as a Tumor Associated Macrophage, or TAM. TAMs can promote proliferation, promote metastasis, and stimulate tumor angiogenesis while inhibiting the antitumor immune response mediated by T cells [2]. Due to their role, TAMs are being recognized as potential biomarkers for diagnosis and prognosis of cancer. In this study, we explore a non-invasive, in vivo imaging approach using MPI for evaluating TAM recruitment to a mouse breast tumor.

I.II Magnetic Particle Imaging

MPI is an emerging imaging modality that exploits time-varying magnetic fields to directly detect Magnetic

Nanoparticles (MNPs) [3]. MPI has unique capabilities when imaging MNPs. Specifically, MPI enables linear MNP quantitation with excellent dynamic range, positive contrast, deep tissue depth penetration capability, no use of ionization radiation, and no background signal. In contrast, Magnetic Resonance Imaging (MRI) detects MNPs as difficult-to-quantitate T2* signal dropouts. Of note, MPI does not see anatomy, and so we often co-register the technology with an anatomic imaging modality such as MRI and X-ray/CT. In recent years, MPI has been reported for the tracking of superparamagnetic iron-oxide (SPIO) MNPs in stem cells, for imaging of brain injuries and xenografted tumors in animals [4], [5], [6]. Here we propose to use MPI co-registered with X-ray/CT to evaluate TAM recruitment in a mouse model of mammary carcinoma.

II Material and methods

II.I Animal Procedures

All animal procedures were conducted according to the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee at Stanford University. Mouse 4T1 mammary carcinoma were grown in the 4th pair (inguinal) mammary fat pad of 6-8 weeks old female Balb/c mice. The tumors became palpable 6-8 days after inoculation. A single dose of tracer (1, 3, 6, 12, 24 mg/kg) of Synomag-D[®] PEG (Micromod, Germany) was intravenously injected ~7 days after implantation of the tumor (N = 5 mice for each dose). MPI images were acquired 8, 11, 14 and 17 days after tumor implantation. Mice were sacrificed on day 17.

II.II Imaging and histology

Mice were imaged longitudinally using the Momentum[™] MPI system (Magnetic Insight, Inc. Alameda, CA, USA). Animals were anesthetized with isoflurane during the duration of their scans. Projection images were taken with a field of view (FOV) of 4 x 4 x 12 cm³. Tomographic acquisition was taken using 35 projections, with each projection taking ~10 seconds, for a total scan time of 40 minutes including pauses for system temperature and image reconstruction. Mice were imaged with an X-ray/CT (Trifoil Imaging CT120, Northridge, CA, USA) under isoflurane anesthesia at occasional timepoints during the study. Following sacrifice, the liver, spleen and tumor were dissected and collected. Tumors were sectioned and stained with Hematoxylin and Eosin and Prussian Blue. The stained sections were scanned, visualized and documented with a Nanozoomer (Hamamatsu, Japan). The remaining tissue was analyzed using inductive coupled plasma (ICP) spectroscopy.

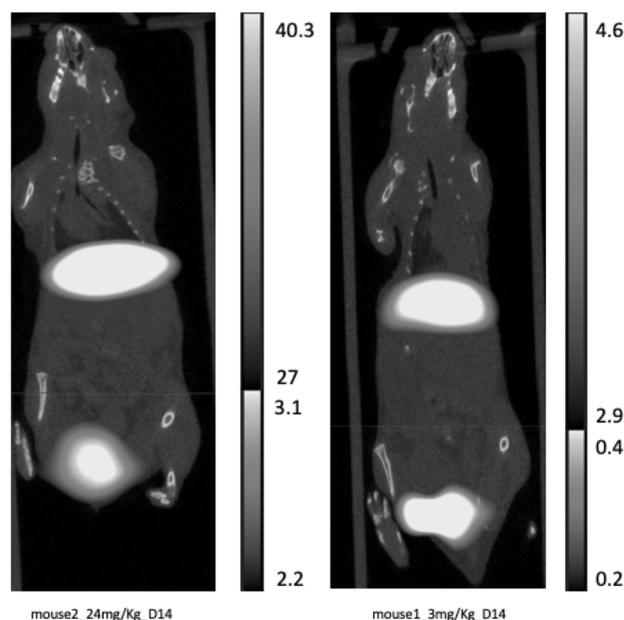


Figure 1: Two different mice at day 14 post implantation, two doses of the SynomagPEG, 24 mg/kg and 3 mg/kg. White arrows indicates the location of the tumor.

II.III Data Analysis

MPI and CT were co-registered using VivoQuant (Invicro, MA, USA). For MPI quantification, the MPI signal is defined as the sum of pixel values within the Region of Interest (ROI) normalized by the ROI's volume. The uptake of Synomag-D[®] PEG in tumors and in livers respect to the dose injected via tail vein has been calculated normalizing the MPI signal collected from the tissue with the MPI signal collected from the tracer. The signal from the tracer was evaluated by a calibration curve obtained by images of a series of dilution which correspond to the dose adopted in the study.

III Results and discussion

In this study we demonstrated MPI imaging of in situ labeled tumor associated macrophages. In a typical imaging result (Figure 1) escalating doses of Synomag-D[®] PEG showed consistent labeling of the tumor. The MPI tumor signal increased with the escalating dose. Signal was detected at all levels except the lowest dose (1 mg/kg). Increases in dose correlated with concentrations of iron in the tumor and liver.

From our results, we determined that, for this tumor model, the MPI signal can be detected as early as 8 days post implantation, just after the tumor becomes palpable. Tumor and liver signals peaked around day 11-14, and then gradually declined due to the clearance of the MNPs by physiological pathways.

Prussian Blue staining confirmed iron presence in the tumors of injected mice. Iron staining patterns were different from control mice. These results must be confirmed by ICP Spectroscopy.

IV Conclusions

In vivo dose studies are a useful way to evaluate the quantitation capability of MPI. Based on the MPI workflow for iron quantification, we concluded that the method is quantitative and reliable. Our next steps are to validate iron with ICP of ex vivo samples.

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Author's Statement

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competing financial interest(s): Drs. Gang Ren, Patrick Goodwill and James Mansfield are employees of Magnetic Insight. Informed consent: Informed consent has been obtained from all individuals included in this study.

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