Supporting informations

1) Experimental details:

Preparation of Mn-reconstituted apoferritin: The iron-free horse spleen apoferritin (Sigma-Aldrich) was reconstituted in the presence of $MnCl_2$ solution (Fluka, Mn Standard) at pH = 9.0, under air. To avoid the fast oxidation of the Mn^{II} ion, the apoferritin and Mn^{II} were added into a N_2-saturated AMPSO (N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid) (Fluka, ≥ 99.0 %) solution. The protein and the Mn(II) solution were added to give a 1×10^{-6} and 3×10^{-3} M concentration (3000 Mn^{II}/apoferritin), respectively. After 1 week reaction time, the samples were concentrated using Vivaspin centrifugal concentrators (50000 MWCO), treated 1 hour with EDTA (Ethylenediaminetetraacetic acid, Fluka, ≥ 97%) in 1 to 1 metal to ligand ratio to eliminate MnOOH particles and the bound Mn^{II} ions from the outer surface of the protein shell. Mn(III)OOH-Apoferritin particles were then purified by gel filtration (superdex G25 column, Amersham). At the end of this process the concentration of the protein (Bradford method, BSA standard) and the metal was measured. The final Mn concentration was determined by T_1 measurements of water protons of the mineralized complex solution (at 20 MHz, in 6M HCl at 120 °C for 15')

Proton Nuclear Magnetic Relaxation Dispersion (NMRD) $1/T_1$ profiles: Data were measured at 25 °C over a continuum of magnetic field strengths from 0.00024 to 0.47 T (corresponding to 0.01-20 MHz proton Larmor Frequency) on a Stelar field-cycling relaxometer (Stelar, Mede, Italy), under complete computer control with an absolute uncertainty of 1%. Data points from 0.47 T (20 MHz) to 1.7 T (70 MHz) were collected on a Spinmaster spectrometer (Stelar, Mede, Italy) working at variable field.

Cell Lines. Mouse melanoma (B16-F10) cell lines were purchased from the American Type Culture Corporation. Melanogenic B16-$F10_m$ cells were obtained by growing cells in standard DMEM (Lonza) medium supplemented with sodium bicarbonate, and glutamine. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. Non-melanogenic B16-$F10_{non\text{-}m}$ cells were obtained by growing B16-F10 in RPMI (Lonza) containing sodium bicarbonate and glutamine. The cells were incubated at 37°C in a humidified atmosphere of 10% CO_2. Both media were supplemented with 10% (v/v) FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin.

Uptake Experiments. For the in vitro uptake experiments, about 3 x 10^5, B16-F10 were seeded in 6 cm diameter dishes. 24 hours after the cells were incubated for 4 hours at 37°C in the presence of Mn(III)OOH-Apo particles at 0.4 mM Mn concentration. At the end of the incubation, cells were washed three times with 10 ml ice-cold PBS, detached with trypsin/EDTA, and transferred into glass capillaries for MRI analysis (see below).

Experimental Mice and Induction of Transplantable Tumours. Adult C57BL/6 mice were maintained in specific pathogen free conditions at both the animal facilities of the Department of Genetics, Biology and Biochemistry, Molecular Biotechnology Center Turin University, Italy, and at Charles River Laboratories (Calco, Italy). Handling and all manipulations were carried out in accordance with European Community guidelines, and all the experiments were approved by the Ethical Committee of the University of Turin. B16-F10 cells were cultured as described above and tumours were generated by subcutaneous injection in the mouse flank of 1x10^6 cells in 0.2 ml PBS. One week after B16 cell injection, mice developed solid tumours of 20-40 mm^3 volume.

MRI: "In vitro" cells MR images were acquired on a Bruker Avance300 spectrometer (7 T) equipped with a Micro 2.5 microimaging probe (Bruker BioSpin, Ettlingen, Germany). Glass capillaries containing about 2x10^6 cells were placed in an agar phantom and MR imaging was performed by using a standard T_1-weighted multislice spin-echo sequence (TR/TE/NEX=200/3.3/8, FOV=1.2 cm, NEX=number of exitations; FOV=field of view). The T_1 relaxation times were calculated using a standard saturation recovery spin echo.

"In vivo" MR images were acquired at 1 T with an Aspect M2-High Performance MRI System (Aspect Magnet Technologies Ltd, Netanya, Israel) consisting of a NdFeB magnet, equipped with a 35 mm solenoid Tx/Tr coil of inner diameter 35 mm. This system is equipped with fast gradient coils (gradient strength, 450 mT m−1 at 60 A; ramp time, 250 μs at 160 V) with a field homogeneity of 0.2–0.5 gauss. The animals were anesthesised before MRI examination by injecting tiletamine/zolazepam (20 mgkg_1; Zoletil 100, Virbac, Milan, Italy) and xylazine (5 mg/kg; Rompun, Bayer, Milan, Italy). Mn(III)OOH-Apo was intratumor administered through a direct injection. MR images were acquired before and from 15 to 180 minutes after contrast administration by using a T_1-weighted protocol (TR/TE/NEX=250:8:10, FOV= 3.5 cm). The mean signal intensity (SI) values were calculated in the regions of interest (ROI) manually drawn on the whole tumour and on the muscle. The mean measured SI was normalised by using a standard solution of Gd in 1% HNO3 . The mean SI enhancement (%) of target tissues (TT) was calculated according to the following equation: SI % Enhancement = ((mean SI (TT) post contrast - mean SI (TT) pre contrast)/ mean SI (TT) pre contrast) x 100

2) Ferritin uptake

In order to assess whether the two B16 cell clones express the same number of ferritin receptors, B16-F10$_m$ and B16-F10$_{non-m}$ were incubated in the presence of increasing amounts of native ferritin. Protein uptake has been evaluated by measuring the internalized iron using ICP-MS technique. No significantly difference in iron uptake has been evidenced thus confirming that the relaxivity increase measured on melanogenic B16 is not the consequence of a different expression of ferritin receptors.