Supporting Information

Magnetic resonance imaging of mesenchymal stem cell – migration towards glioblastoma using nano-calcium phosphate contrast agent

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Experimental section

Cells and culture conditions

Rat mesenchymal stem cells (MSCs) were isolated from the bone marrow of 3-4 weeks old Wistar rats by following the protocol described in our previous study.[27] All animal procedures were approved by the Institutional Animal Ethics Committee (Amrita Institute of Medical Sciences and Research Centre) and were performed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. The isolated cells were cultured under standard conditions (5% CO_2, 37^0C) in IMDM basal medium supplemented with 10% MSC-specific FBS (Gibco-Life technologies, India) and 1000 IU/mL penicillin and 1000 µg/mL streptomycin, and cultured guidelines. MSCs of passage 3-5 were used for all the experiments in this study.

Rat C6-glioma cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 1000 IU/ml penicillin and 1000 µg/ml streptomycin (all constituents from Gibco-Life technologies, India) under standard cultural conditions (5% CO_2, 37^0C).

Synthesis of nCP:Fe nanoparticle and labelling of MSCs

nCP:Fe nanoparticles were synthesized through a wet chemical approach as per the protocol discussed in our previous study.[23,26] The nanoparticle was synthesized under sterile conditions using endotoxin free water (HyClone, Fischer Scientific, USA) for all the *in vitro* and *in vivo* experiments. The hydrodynamic size and zeta potential of nCP:Fe was analysed using Nano ZS, Zetasizer-Nanoseries

(Malvern,UK). Further, the particle size and morphology was characterized using SEM-JSM-6490LA (JEOL, Japan).

For magnetic labelling, MSCs were plated at a density of 7500 cells/cm^2 in IMDM complete growth media and incubated overnight for cell attachment. The medium was then replaced with opti-MEM medium (Gibco-Life technologies, India) containing 100 µg/mL nCP:Fe and incubated for 6hrs. We have optimized this labelling condition in our previous study, since provided a labelling efficiency of ~87% (~22.34 pg Fe/cell) and detectable T2 contrast for MSCs in MRI, without impairing its viability and cellular functions.[26] After incubation, opti-MEM medium containing nanoparticles was aspirated and the cells were replenished with IMDM complete medium for another 6 hrs. After labelling, the cells were washed with PBS and then taken for further experiments. For analysing the cell labelling, Prussian-blue staining of the cells was performed as discussed previously and examined under light microscope (Olympus, BX5).[26]

In vitro co-culture of nCP:Fe labelled MSCs with rat C6 glioma cells

In order to study the anti-tumor effect of labelled MSCs on rat C6-glioma cells *in vitro*, both the cells were co-cultured and monitored for 18 hrs live under confocal microscope. Rat C6 glioma cells were stained using Carboxyflourescein succinimidyl ester (CFSE) (Invitrogen, USA), by incubating them in DMEM basal media containing 5µM CFSE for 10 min. After washing with PBS, 1×10^5 C6 cells seeded onto glass bottom culture disc and incubated overnight for cell attachment in DMEM complete media. Then, 1×10^5 labelled MSCs (labelled at optimized conditions) were added into the culture disc and co-cultured under standard culture condition. The interactions of co-cultured cells were monitored under laser scanning confocal microscopy (Leica TCS SP5 II, Germany) in live for 18 hrs and images were captured at every 3 mins.

Animal study

All the animal experiments were approved by the Institutional Animal Ethics Committee (Amrita Institute of Medical Sciences and Research Centre) and were performed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. The orthotopic C6- rat glioma model was established using previously reported protocol.[32] For the glioma model establishment, male Wistar rats (8-10 weeks old) were anesthetized by intra-peritoneal injection of ketamine/xylazine combination. The heads of the recipient rats were secured in a stereotactic frame (ASI Instruments, USA) and an incision of length 1.5 cm was made in the skin above the skull. A burr hole was prepared 1.0 mm posterior to the bregma and 1.0 mm to the right of the sagittal suture using a micro-drill. Then $1x10^5$ rat C6-glioma cells in 5µl PBS was slowly injected (rate of 1µl/min) at 2mm depth below the brain surface, using a syringe (Hamilton, NV, USA). The syringe was left *in situ* for 5 min prior to withdrawal. The intracranial tumor growth was monitored

using MRI and the establishment of glioma model was confirmed by six days, post-inoculation of tumor cells.

For *in vivo* MRI tracking study, $1x10^5$ nCP:Fe labelled MSCs (in 5µl PBS) were stereotaxically injected into the left lateral ventricle of rat glioma model, at day 7 post-inoculation (The injection coordinates were relative to bregma: A-P: -1.0 mm; L-M: -1.8 mm (left); D-V: +3.5 mm). *In vivo* MRI was performed immediately after MSC administration (Day 0) and on Day 5, Day 8 and Day 14 post cell injection.

In vivo MRI

After implantation of labelled MSCs, *in vivo* MRI was performed using 7T preclinical MRI scanner (Bruker-Biospec, USA). Turbo RARE T2-weighted images and T2*W images were acquired to observe the tropism of MSCs. The TURBO RARE sequence parameters were: TR = 3000 msec; TE = 44 msec; flip-angle = 180º; FOV = 3.50 cm, MTX = 256. The T2* weighted images were acquired at TR = 232 msec; TE = 20 msec; flip-angle = 15º. T2 values at different regions of interest (ROI) were calculated from the MSME T2 map.

Histological analysis

All animals were euthanized, 16 days after the stem cell implantation, by an overdose of Xylazine/Ketamine (1:4).The brains were harvested, fixed in 10% formalin and embedded in paraffin wax. Serial coronal sections of 5 µm thickness were cut through the area of cell injection using a cryostat (Leica Microsystems GmbH, Germany). Prussian-blue staining (stains for Fe^{3+}) and Alizarin-red staining (stains for Ca^{2+}) were performed as previously described, to detect the localization, migration and fate of implanted nCP:Fe labelled MSCs.[26]

(As the histological sections in Figure 4 were incidentally flipped 180º horizontally during the tissue mounting step, the MR images included in the respective figure were rotated 180º horizontally for comparing with the histological images).