

Dental pulp mesenchymal stem/stromal cells labeled with iron sucrose release exosomes and cells applied intra-nasally migrate to intracerebral glioblastoma

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We report on a simple iron oxide (Venofer) labeling procedure of dental pulp mesenchymal stem cells (DP-MSCs) and DP-MSCs transduced with yeast cytosinedeaminase::uracilphosphoribosyltransferase (yCD::UPRT-DP-MSCs). Venofer is a drug approved for intravenous application to treat iron deficiency anemia in patients. Venofer labeling did not affect DP-MSCs or yCD::UPRT-DP-MSCs viability and growth kinetics. Electron microscopy of labeled cells showed internalized Venofer nanoparticles in endosomes. MRI relativity measurement of Venofer labeled DP-MSCs in a phantom arrangement revealed that 100 cells per 0.1 ml were still detectable. DP-MSCs or yCD::UPRT-DP-MSCs and the corresponding Venofer labeled cells release exosomes into conditional medium (CM). CM from yCD::UPRT-DP-MSCs in the presence of a prodrug 5-fluorocytosine caused tumor cell death in a dose dependent manner. Iron labeled DP-MSCs or yCD::UPRT-DP-MSCs sustained their tumor tropism *in vivo*; intra-nasally applied cells migrated and specifically engrafted orthotopic glioblastoma xenografts in rats.

Key words: dental pulp MSCs, iron labeling, yCD::UPRT-exosomes, intranasal application, migration to intracerebral glioblastoma

Mesenchymal (stromal) stem cells (MSCs) can be readily isolated from human tissues like bone marrow, adipose, umbilical cord, dental pulp and others. MSCs represent novel promising therapeutic tools of emerging regenerative medicine. MSCs of different origin are tested in large number of clinical trials for treatment of diseases, which do not have at present adequate therapy. As of July 2016, the public clinical trials database showed 627 clinical trials using MSCs for a very wide range of therapeutic applications [1]. It has been increasingly observed that the transplanted MSCs did not necessarily engraft and differentiate at the site of injury but might exert their therapeutic effects through paracrine manner inducing endogenous reparatory processes. It has been recognized that MSCs released rich secretome together with extracellular exosomes that might be responsible at least to some degree for transfer of regulatory gene products needed for reparatory processes induction [2].

The therapeutic potential of MSCs-derived extracellular vesicles was recently comprehensively reviewed [3].

Dental pulp derived mesenchymal stem cells (DP-MSCs) are known for the high proliferative potential for self-renewal, plasticity and multipotential capabilities. Dental pulp is made of ecto-mesenchymal elements, containing neural crest-derived cells. DP-MSCs are intensively studied because of their neural characteristics being considered as cells for the peripheral neural regeneration [4]. Our comparison of DP-MSCs with bone marrow and adipose tissues derived MSCs revealed differences in the expression of pluripotent stem cells genes that reflected ecto-mesenchymal origin of DP-MSCs [5].

MSCs engineered to express therapeutic gene products are becoming more promising in cancer therapy for their ability to selectively target tumor cells. Their administration in animal models resulted in successful inhibition of growth of variety

of tumors, regardless of tumor kind [6]. Prodrug gene therapy for cancer therapy mediated by mesenchymal stem/stromal cells engineered to express yeast cytosinedeaminase:uracil phosphoribosyltransferase (yCD::UPRT) is at present the most efficient experimental gene therapeutic system for cancer [7]. The ability of yCD::UPRT-expressing adipose tissue derived mesenchymal stem cells (AT-MSCs) to convert the relatively non-toxic 5-fluorocytosine (5-FC) into the highly toxic anti-tumor drug 5-fluorouracil (5-FU) along with their ability to home to tumor sites and micrometastases, makes these cells designated therapeutic stem cells (ThSCs) an unique tool to convert prodrugs to cytotoxic drugs directly within the tumor mass thus avoiding systemic toxicity. Prodrug administration not only eliminates tumor cells, but consequently kills the more resistant ThSCs as well [8-10]. Furthermore, we have shown that human AT-MSCs or bone marrow derived mesenchymal stem cells (BM-MSCs) transduced with suicide gene have the ability to track and engraft into glioblastoma with positive therapeutic outcome [11, 12].

Tumor tracking ability of mesenchymal and neural stem cells to brain was proved in the experiments with MSCs labeled with superparamagnetic iron oxide nanoparticles [13, 14]. Tumor tropism of AT-MSCs genetically modified with suicide gene was sustained as we demonstrated it with labeling them with poly(L-lysine)-modified superparamagnetic iron oxide nanoparticles [11].

In this study, we explored, by *in vitro* and *in vivo* investigations, a safe and effective protocol for Venofer labeling of DP-MSCs and DP-MSCs transduced with suicide gene. Labeling procedure is using three clinically approved drugs: Venofer, heparin and protamine sulphate that could facilitate the rapid translation of this technique to clinical trials. We examined exosomes released from DP-MSCs stably transduced with yCD::UPRT-fused gene and labeled with Venofer for their ability to kill tumor cells. The broader additional objective of the current study was to develop a safe, non-invasive and effective strategy to monitor tumors and metastases by MRI and to examine glioblastoma tropism of the iron oxide labeled DP-MSCs intranasally applied.

Materials and methods

DP-MSCs culture isolation and maintenance. All donors of tissue specimens used for isolation and propagation of MSCs were informed about the nature of the study and pro-

vided their written informed consent. Dental pulp stem cells (DP-MSCs) were isolated from dental pulp tissue fragments adhered to plastic tissue culture dishes [5]. For expansion the cells were seeded at 4000 cells/cm² to plastic dishes (Corning Life Sciences) and grown with medium exchange every 2-3 days. Adherent cells were split after reaching confluence with 0.05% trypsin/EDTA (Difco). DP-MSCs used for the experiments were up to fifth passage or less. Cell cultures were grown in a complete culture medium DMEM low glucose (1 g/L) supplemented with 4% human platelet extract (PE), antibiotic antimycotic solution (Gibco Life Technologies) and incubated at 37 °C in humidified atmosphere with 5% CO₂. The cells were frozen in 1 ml aliquots in liquid nitrogen suspended at 10×10⁶ cells/ml in medium containing 10% dimethylsulfoxide and 10% human serum albumin.

Preparation of DP-MSC expressing yCD::UPRT gene. DP-MSCs with yCD::UPRT gene (therapeutic stem cells) were prepared with the same procedure as described previously for the adipose tissue derived MSCs [8]. Briefly, subconfluent cultures of DP-MSCs were transduced thrice in 3 consecutive days with virus-containing medium from GP+envAM12/pyCD::UPRT cells supplemented with 5 µg/ml protamine sulfate. Transduced cells were selected for resistance to G-418 (0.4 mg/ml).

The therapeutic stem cells were expanded in low glucose (1 g/l) DMEM supplemented with 4% PE. Rat glioblastoma C6 cells were cultured in high glucose DMEM supplemented with 5% FCS and an antibiotic-antimycotic mix (Life Technologies) in a humidified atmosphere and 5% CO₂ at 37 °C.

Labeling of mesenchymal stem cells with Venofer. Labeling procedure used resulted from small modifications of the labeling approach described by others [13, 14, 23]. The variations of three concentrations of Venofer (200, 400, 1000 µg/ml) with protamine sulphate (10 µg/ml) and heparin (2 IU/ml) were tested to optimize the labeling. The combinations are presented in Table 1. At first, heparin and protamine sulphate were mixed together to form a stable complex. Then final labeling complex was created by adding Venofer to already prepared stable complex in PE-free culture media at room temperature. The labeling complex was added to MSCs plated to dishes 24 hours ahead and incubated (4 hours, 37°C, 5% CO₂). The culture medium was supplemented with 4% of PE as a growth supplement and the cells were incubated overnight (37 °C, 5% CO₂). After 24 hours, MSCs were washed first with DMEM only, then with DMEM containing heparin (10 U/ml) and finally PE free medium was added for 24 hours to obtain CM. Venofer labeled DP-MSCs for animal applications were trypsinized, centrifuged, and resuspended in PBS for intranasal applications.

Assessment of cell viability and growth rate by IncuCyte live cell monitoring system. Cell growth was monitored by “real time *in vitro* micro-imaging” using the IncuCyte ZOOM™ Kinetic Imaging System. The IncuCyte system allows for the hourly monitoring of cell growth by determining the confluence of cells and displaying morphologic changes associated with treatment.

Table 1. Labeling schemes of DP-MSCs with Venofer

Venoferr µg/ml	Protamine sulphate µg/ml	Heparin IU/ml	Corresponding picture
200	10	0	1
200	10	2	2
400	10	0	3
400	10	2	4
1000	0	0	5

Differentiation of DP-MSCs in vitro. The ability of MSCs to differentiate to adipocytes, osteoblasts and chondrocytes was evaluated using Human Mesenchymal Stem Cell Functional Identification Kit (R&D SYSTEMS Minneapolis, MN 55413). Protein concentration was measured by Pierce™ BCA Protein Assay Kit (Thermo SCIENTIFIC) according to the manufacturer's instructions.

Prussian blue staining for Venofer nanoparticle-labeled DP-MSCs and glioblastoma slices. Prussian blue staining was performed using the Accustain iron stain kit (Sigma-Aldrich, St. Louis, MO, <http://www.sigmaaldrich.com>) according to the manufacturer's protocol. Venofer-labeled MSCs were washed with PBS to remove any free Venofer nanoparticles. Smears of cells on glass slides or cells in Petri dishes were fixed for 10 minutes using 4% paraformaldehyde (Sigma-Aldrich). Samples were then washed three times in deionized water and incubated with Pearls' reagent (4% potassium ferrocyanide/12% HCl, 1:1 vol/vol) for 10 minutes at room temperature. Samples were observed by an inverted optical microscope. For each MSCs, the comparative labeling efficiency of each concentration of Venofer (expressed in μg of Fe) was assessed visually. The similar labeling protocol (40 minutes under agitation) was used to stain brain sections obtained from rats. Glioblastoma sections were dehydrated through graded alcohols and mounted with Eukitt (O. Kindler GmbH & Co).

Electron microscopy analysis. The MSCs labeled with complex of Venofer nanoparticles, were replaced in Venofer-free growth medium, the samples were harvested, washed with PBS and centrifuged ($180 \times g$, 15 min). The pellet was fixed using 4% PFA+ 2% glutaraldehyde in 50 mM cacodylate buffer for 15 minutes at RT. Samples were washed three times with PBS and postfixed in 2% osmium tetroxide for 90 min on ice. After further washing with water the cells were stained overnight in an aqueous solution of uranyl acetate (0.5%), dehydrated through graded alcohols and embedded in epoxy resin (EPON; Miller-Stephenson Chemical Company). Ultrathin sections were analyzed with a transmission electron microscope (JEOL, Japan).

MRI and relaxometry studies on Venofer-labeled DP-MSCs. For phantom relaxometry studies, 500 μL of cell suspension (5×10^5 Venofer-labeled or unlabeled control cells in PBS) were mixed to an equal volume of gelatin (8% w/w) in plastic dishes (area: 2 cm^2), to obtain cell suspension dispersion. In order to obtain T2 relaxometry (R2), spinecho-multiecho sequence (TR 2.500, thickness 1 mm) with 8 echo times ranging between 50 and 400 milliseconds (ms) was acquired and reconstructions of T2 maps were obtained by interpolating the signal curve for different echo times. Sample was prepared in the Petri dish of 38 mm in diameter. The dish was filled with the 2% agarose in a layer of about 2 mm. During agarose cooling phase small cavities were made into the agarose gel using bottom part of a small Eppendorf tube. These cavities served as a nest for Venofer-labeled DP-MSCs and were subsequently filled with the suspension

of Venofer-labeled DP-MSCs and covered by second 2mm thick agarose layer. The 7Tesla whole body Siemens scanner (Siemens Healthcare, Erlangen, Germany) was used for measurements. In order to increase image resolution, microgradient insert was placed into the center of the whole body scanner. Microgradient insert is capable to provide 750 mT/m magnetic field gradients, which allows to perform MR experiment with image resolution of $180 \mu\text{m} \times 180 \mu\text{m}$ and 2 mm slice thickness.

Concentration of exosomes by ultracentrifugation and gene expression analysis. Cell conditional medium freed from cell debris by sequential centrifugation was passed through $0.22 \mu\text{M}$ syringe filter and centrifuged at $150.000 \times g$ for 14 hours. Sediment was washed with PBS and resuspended by intensive Vortexing. Gene analysis was performed by standard PCR technique using primers previously described (8). Total RNA was isolated by TRIzol reagent (Gibco). For PCR Phire Hot Start II DNA polymerase (Thermo Fisher Scientific) was used. RNA was reverse transcribed with SensiFAST cDNA Synthesis Kit (Bioline. Electrophoretic separation was performed in 4% MetaPhor agarose gel (Lonza) in TAE buffer. GeneRuler 50 bp DNA Ladder (Thermo Fisher Scientific) served as a size comparison tool.

Animal experiments. Adult male CD[®] IGS rats purchased from Charles River were used for in vivo experiments in accordance with institutional guidelines under the approved protocols. In all experiments the rats were anaesthetized with an intramuscular injection of a mixture containing ketamine (Narkamon 5% – 1.2 ml/kg bw) and xylazine (Rometar 2% – 0.4 ml/kg bw) before the surgical procedures. A dose of 5×10^5 C6 cells injected by a stereotaxic technique was chosen for all experiments to guarantee quick onset of tumor growth. Animals were inspected every day for their behavior and body weights. Animals with excessive weight loss and uncoordinated behavior were euthanized and their brains were analyzed histopathologically.

Stereotaxic intracerebral implantation of glioblastoma cells. Freshly prepared C6 cell suspensions in PBS was injected with a stereotaxic apparatus (David-Kopf Instruments). The selected stereotaxic coordinates for C6 cells were chosen according to Paxinos and Watson²³ and were as follows: anteroposterior: 0.0mm; medio-lateral: 3.0mm; vertical: -6.0mm. Cell suspension was slowly injected in $5 \mu\text{L}$ volume of PBS over a five-minute period using a Hamilton syringe. The needle was left in place for 2 minutes and then slowly elevated for 3 minutes.

Statistical analysis. Statistical comparisons of groups were performed using the Student's t-test. Statistical significance: *** P value was ≤ 0.001 .

Results

Characterization of Venofer nanoparticles. Measurements of Venofer suspension in PBS revealed that Venofer nanoparticles are of round morphology. They have a char-

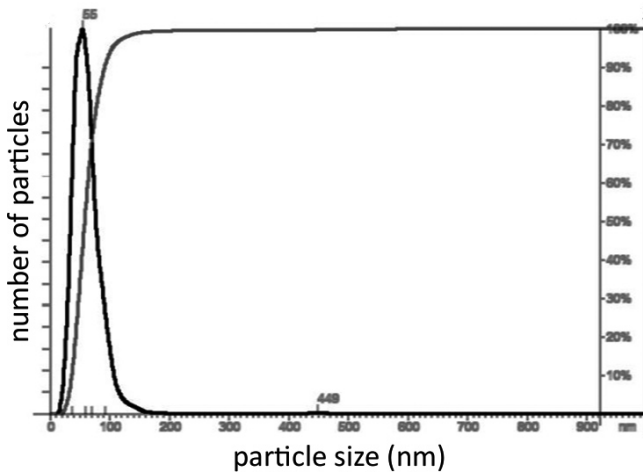


Figure 1. Characterization of Venofer nanoparticles by Nanosight.

acter of highly homogenous nanoparticles with mean peak diameter of 65 nM. The nanoparticles concentration of the commercial drug measured by Nanosight was 8.75×10^{13} particles/ml (Figure 1).

Labeling of DP-MSCs with Venofer. The labeling was performed as described in Materials and Methods section. Several combinations of Venofer, with protamine sulphate and heparin were tested as listed in Table 1. Labeling intensity was Venofer dose dependent. The highest Venofer concentration (1000 $\mu\text{g/ml}$) was only slightly toxic to DP-MSCs. The cells labeled with 400 $\mu\text{g/ml}$ Venofer in combination with protamine sulphate and/or heparin lead to optimally labeled cells with a viability

reaching 98%. Venofer-labeled DP-MSCs morphologically did not differ from their parental cells. Figure 2 shows the morphology of Venofer-labeled DP-MSCs stained for Fe by Prussian blue. The labeled cells can be kept by trypsinization for at least 10 passages without any signs of senescence, but the number of Venofer nanoparticles in the cells diminished with increasing number of passages.

Characterization of Venofer-labeled DP-MSCs by differentiation and by cell surface markers. To assess multipotency, Venofer-labeled MSCs and unlabeled control cells were plated in differentiation media and the differentiation capability was compared to MSCs isolated from adipose tissue and bone marrow (Figure 3). It was found that DP-MSCs as well as the Venofer-labeled cells can be induced to osteogenic and chondrogenic differentiation lineages but not to adipogenic lineage. Under the same conditions BM-MSCs and AT-MSCs can be induced to all three differentiation lineages required for MSCs characterization *in vitro*. Interestingly, while BM-MSCs and AT-MSCs showed a gradual loss of osteogenic differentiation potential with increasing number of passages *in vitro*, DP-MSCs on the other hand had an increasing osteogenic potential. DP-MSCs and $\gamma\text{CD}::\text{UPRT-DP-MSCs}$ were positive for CD44, CD73, CD90 and CD105 markers and negative for CD45 and CD34 similarly as we described previously for AT-MSCs [8]. The Venofer labeling did not change this behavior.

Intracellular iron localization measurement. Analysis of ultrathin sections of Venofer-labeled DP-MSCs by transmission electron microscopy revealed that Fe-nanoparticles are localized in cell cytoplasm only (Figure 4).

Cell viability of Venofer-labeled DP-MSCs and $\gamma\text{CD}::\text{UPRT-DP-MSCs}$. Figure 5 shows growth curves of Venofer la-

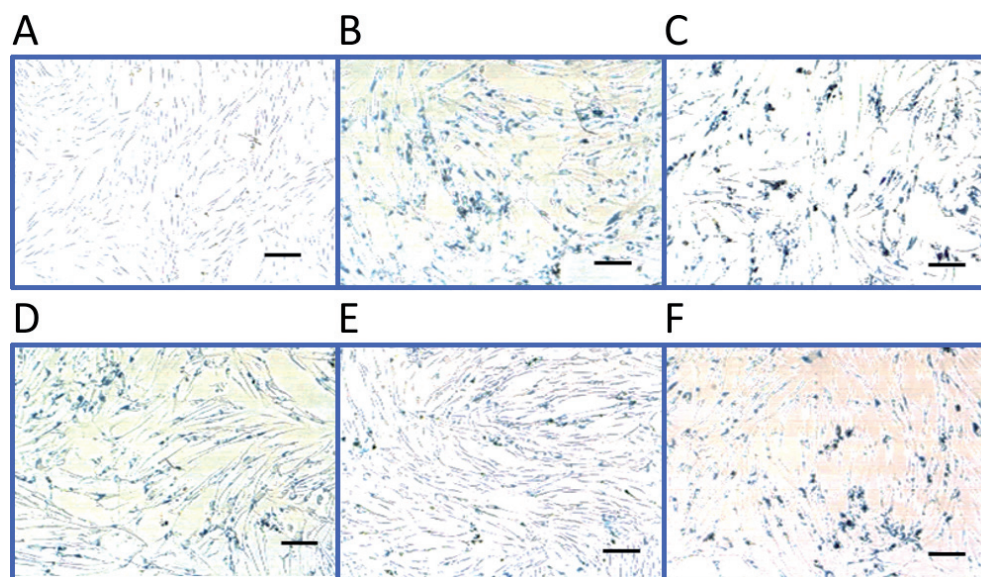


Figure 2. Morphology of Venofer-labeled DP-MSCs with various combinations of labeling procedure. Cells were stained by Prussian blue. (A); Control cells; (B); Venofer 200 $\mu\text{g/ml}$, protamine sulphate 10 $\mu\text{g/ml}$; (C); Venofer 200 $\mu\text{g/ml}$, protamine sulphate 10 $\mu\text{g/ml}$, heparin 0.4 IU/ml; (D); Venofer 400 $\mu\text{g/ml}$, protamine sulphate 10 $\mu\text{g/ml}$; (E); Venofer 400 $\mu\text{g/ml}$, protamine sulphate 10 $\mu\text{g/ml}$, heparin 0.4 IU/ml; (F); Venofer 1000 $\mu\text{g/ml}$.

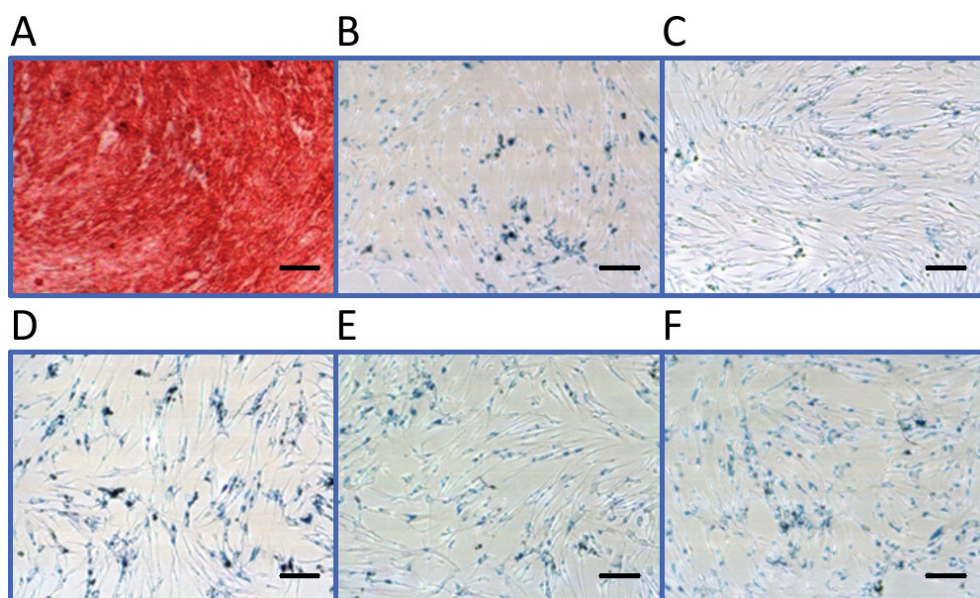


Figure 3. Differentiation of Venofer-labeled DP-MSCs stained for Fe. Pictures of DP-MSCs/Fe induced to osteogenic and adipogenic differentiation and corresponding cells, both stained by Prussian blue. (A); DP-MSC osteogenic differentiation (B); DP-MSCs (C); DP-MSC adipogenic differentiation (D); DP-MSC (Fe) osteogenic differentiation (E); DP-MSC (Fe) osteogenic differentiation stained for Fe (F); DP-MSC (Fe) stained for Fe.

beled DP-MSCs compared with the corresponding naive cells. A combination of heparin, protamine sulphate, and Venofer did not affect cell viability and growth kinetics of both types of cells significantly. The growth of the Venofer labeled cells

was a little bit slower compared to the same naive cells. The difference in time the cells reached the saturation density was 24 hours during 5 days incubation period (Figure 5).

DP-MSCs, yCD::UPRT-DP-MSCs and Venofer labeled cells release exosomes. Recently we and others demonstrated that human MSCs release exosomes, possessing stimulating or inhibiting tumor cell growth activity depending on the MSC tissue origin and type of the tumor cells [2, 7]. We proved that DP-MSCs or yCD::UPRT-DP-MSCs and the corresponding

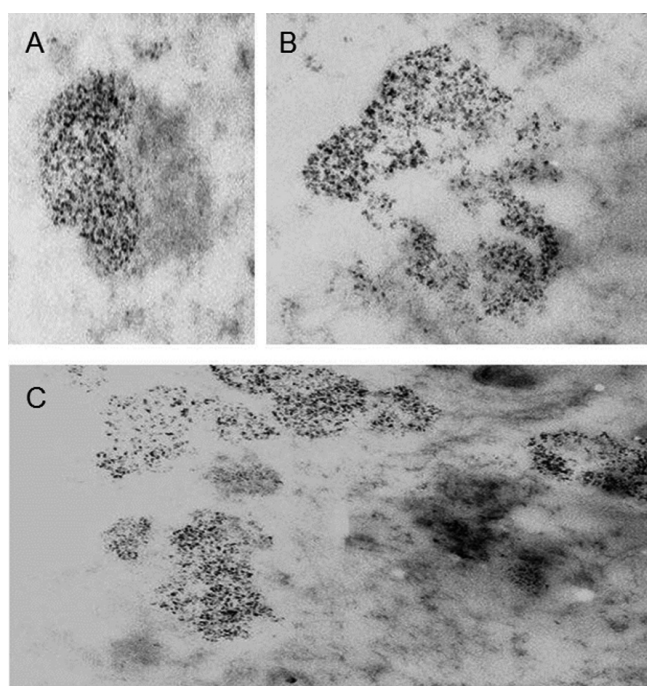


Figure 4. Pictures of ultrathin sections of the Venofer-labeled DP-MSCs obtained by the transmission electron microscopy (A, B, C).

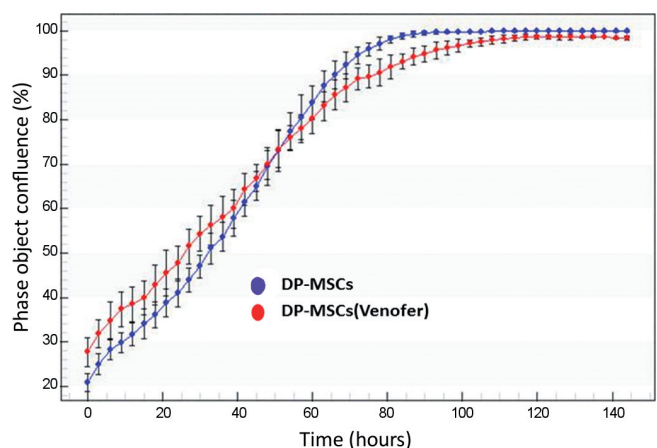


Figure 5. Growth curves of Venofer labeled DP-MSCs and corresponding naive cells monitored by real time *in vitro* imaging in undisturbed cultures using the Incucyte system. The cell density was followed by image scanning of the culture every 2 hours during 6 days. For each scan the standard deviation was calculated.

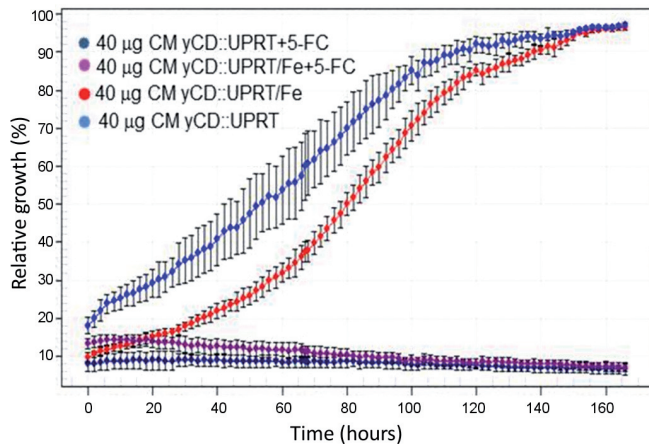


Figure 6. Growth curves of PC3 cells treated with CM from yCD::UPRT-DP-MSCs in presence and/or absence of 5-FC. PC3 cells (3×10^3) were plated in wells of the 96 well plate. Next day, 40 µg CM protein was added to growth medium in each well either with prodrug 5-FC or without 5-FC. The course of growth/inhibition was monitored by the Incucyte system under the same conditions as in figure 5.

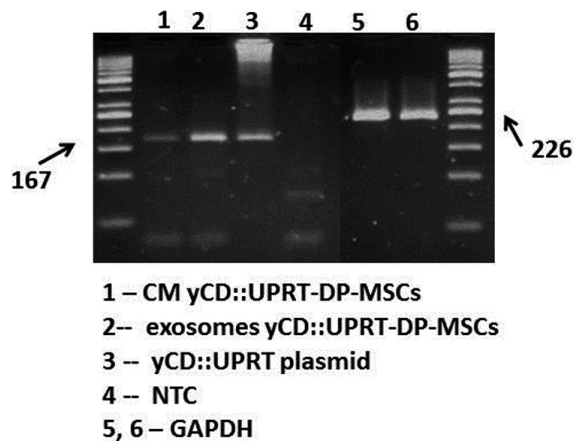


Figure 7. Detection of yCD::UPRT mRNA by PCR in exosomes released from yCD::UPRT-DP-MSCs cells.

Venofer labeled cells release exosomes in the conditional medium (CM).

CM with exosomes from yCD::UPRT-DP-MSCs and corresponding Venofer-labeled cells internalized tumor cells and in the presence of 5-FC caused their death in a dose dependent manner (Figure 6).

MSC-yCD-UPRT cells release exosomes with coding mRNA. In order to see what was responsible for tumor cell killing activity in the CM, we concentrated exosomes by ultracentrifugation and inspected the sediment for presence of yCD::UPRT m-RNA. We isolated total RNA, treated with DNase I, reverse transcribed and analyzed by PCR by agarose gel electrophoresis. As shown in Figure 7, we detected the yCD::UPRT mRNA in the exosome's cargo. Therefore exo-

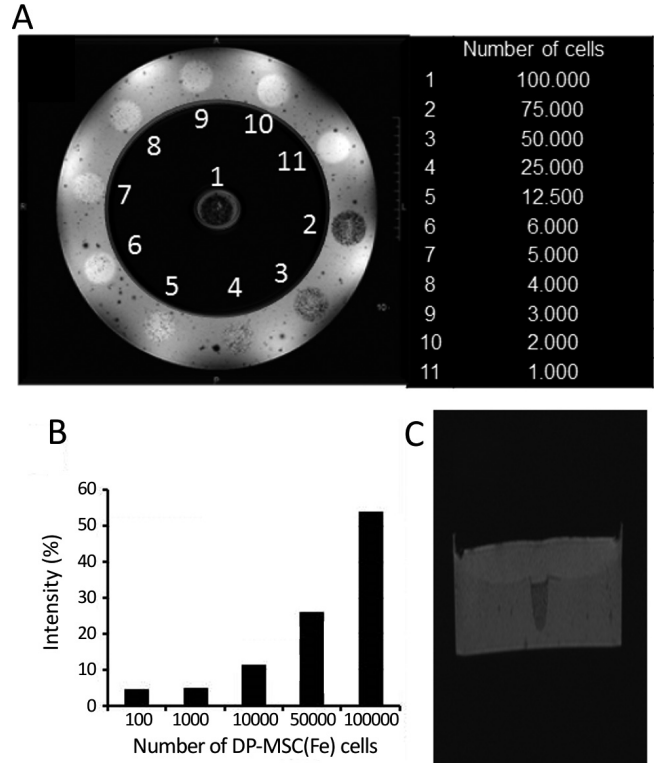


Figure 8. Magnetic resonance imaging and *in vitro* MRI relativity measurement. (A); Picture of phantom with different number of Venofer labeled DP-MSCs; (B); Graph of relationship between number of Fe-labeled DP-MSCs (DP-MSCs/Fe) and color intensity evaluated by ImageJ software; (C); MRI image in a transversal orientation shows bottom and top agarose layers and central cavity (cavity number 1 in picture A) filled with the suspension of DP-MSCs/Fe.

somes internalized in tumor cells acted through translation of yCD::UPRT mRNA to enzyme converting 5-FC to 5-FU.

Magnetic resonance imaging and *in vitro* MRI relativity measurement. In order to see what the minimal number of Venofer labeled cells could be visualized by MRI machine (3Tesla and 7Tesla whole body human scanner equipped with microgradient insert), we tested it in a phantom arrangement. MRI relativity measurement of Venofer labeled DP-MSCs revealed that 100 cells per 0.1 ml are still detectable (Figure 8).

Intranasal administration of Venofer-labeled human DP-MSCs to intracerebral rat glioblastoma C6. In order to see whether DP-MSCs could penetrate and be tracked in the brain after intranasal administration we used rats bearing glioblastoma C6 cells stereotaxically implanted into brain. A dose of 5×10^5 C6 cells caused death in all animals in 15-30 days. Fourteen days after tumor cells administration, Venofer-labeled DP-MSCs (6×10^6) were applied in the nasal cavity of both control and tumor bearing rats. The brains of rats with glioblastoma were removed at different hours after intranasal administration and examined by histological staining for iron labeled cells. Figure 9 shows the presence of Prussian blue

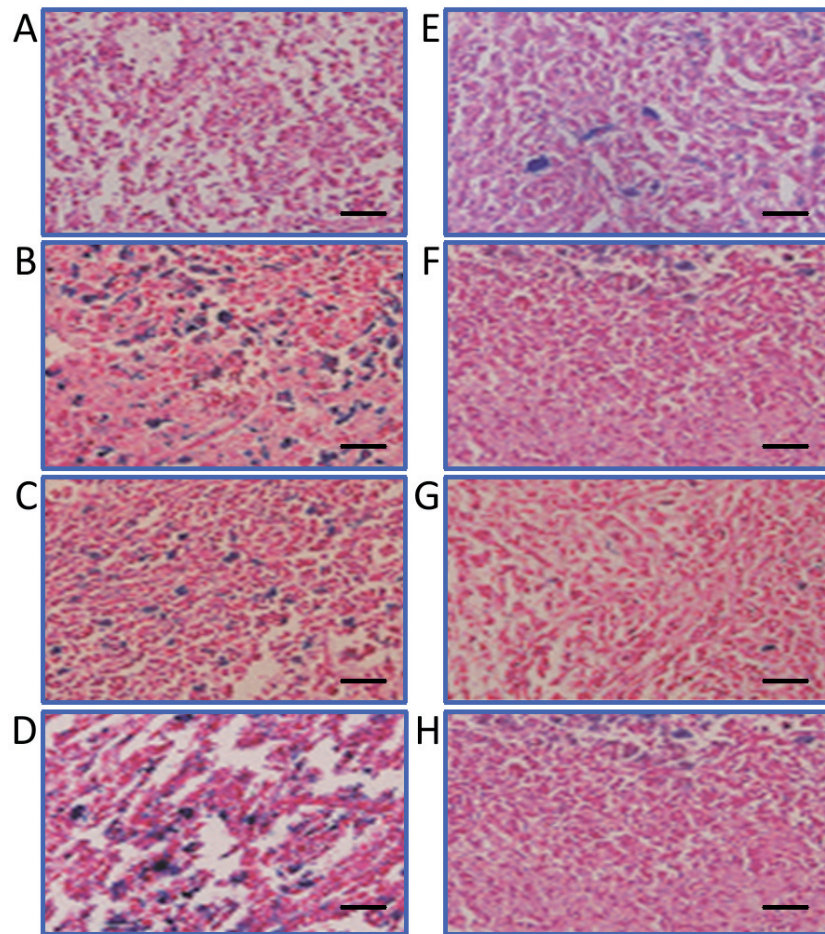


Figure 9. Migration of DP-MSC/Fe applied intranasal to intracerebral glioblastoma. Pictures of histological slices of rat glioblastoma stained for Fe. Brains were obtained at different time after intranasal application of Venofer labeled DP-MSCs. (A); Control, (B); 1 hour, (C); 2 hours, (D); 72 hours, (E); 120 hours, (F); 168 hours, (G); 168 hours, (H); 240 hours. The scale bars in pictures are 50 µm.

stained cells in glioblastoma slices up to 10 days after intranasal administration. None stained cells were detected in control, non-tumor bearing animals, or PBS treated animals. Obtained data show that DP-MSCs labeled with Venofer are capable of rapid penetration into the brain of rats bearing glioblastoma after intranasal inoculation thus confirming tumor tracking capability of MSCs generally.

Discussion

MSCs possess the ability to migrate to the site of injury and secrete a variety of factors capable of a number of functions inducing and supporting regenerative processes in the damaged tissue. Rich MSCs's secretome together with released exosomes participate on the paracrine cross-talk between cells leading to biological consequences [15]. The exosomes being able to bring into the cell their cargo composed from biologically active compounds like micro RNA, m-RNA, mitochondrial DNA, etc. play very likely a crucial role in

the induction of endogenous reparatory process leading, for instance, to induction of angiogenesis, protection from apoptotic cell death and in a concord with biologically active proteins of the secretome to a modulation of the immune system. The idea of trans-differentiation of injected MSCs to the required cell type at the defect site does not have sufficient experimental support.

DP-MSCs reflecting their cranial neural crest origin, slightly differ from bone marrow or the adipose tissue MSCs [5]. Our observation of the ability of DP-MSCs, unlike AT-MSCs, to support induction of osteogenic differentiation despite the large number of passages *in vitro* is in a good agreement with our earlier finding of higher expression of E-cadherin and lower expression of Snail gene, molecular paths of the reparative functions where EMT plays an important role. DP-MSCs behavior obviously contributed to the repair of alveolar bone defect in all patients in clinical trial finished recently [16].

Neurotropic property of DP-MSCs favors them for treatment of large number of neural diseases in the regenerative

medicine [17]. Neurotropic properties of dental pulp MSCs certainly might further facilitate the glioma migration ability of MSCs to brain malignancies [18, 19]. We found that intranasal application of DP-MSCs lead to engrafted Venofer-labeled DP-MSCs in intracranial rat glioblastoma. In addition, human DP-MSCs genetically modified with yeast uracilphosphoribosyltransferase release exosomes able to invade tumor cells and kill them in presence of prodrug 5-FC, contributing thus to the high therapeutic efficacy of the CDy-AT-MSCs/5-FC system [12]. Our results are in agreement with a previous report showing penetration MSCs in the brain after intranasal application in rats. Intranasal delivery of stem cells seems to be a promising noninvasive route to deliver neural stem/progenitor cells to the brain in attempts to treat ischemic brain damage or to target intracerebral glioma [20].

Tumor tropism of Fe labeled MSCs can be used for MRI tracking. In phantom model we found that 100 Venofer labeled MSCs per 0.1 mL are detectable by MRI. J. T. Rosenberg et al. [21] have shown that transplantation of the human MSCs labeled with Fe showed excellent retention of contrast in stroke-induced rats proving their stability for long-term MRI detection. Very recently iron sucrose labeled human MSCs were evaluated for cell therapeutic applications after transplantation into lapine intervertebral discs [22]. The results indicate that iron sucrose can be used as a cell tracer with a stable histological detection potential [23]. Furthermore, recently it was shown that the therapeutic regenerative potential of MSCs, being realized mainly through paracrine fashion, can be potentiated by iron nanoparticles. Iron oxide nanoparticle-mediated development of cellular gap junction crosstalk to improve MSCs therapeutic efficacy for myocardial infarction [24]. In summary, we report broad therapeutic and diagnostic usefulness of iron oxide labeled DP-MSCs and suicide gene transduced DP-MSCs. In addition exosomes released from these cells represent promising biomedical products that could substitute cells in the future.

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