Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Dextran-coated iron oxide nanoparticles turn protumor mesenchymal stem cells (MSCs) into antitumor MSCs

Tsai-Hua Chung, Chia-Chu Hsieh, Jong-Kai Hsiao, Szu-Chun Hsu, Ming Yao, and Dong-Ming Huang

Preparation and characterization of dextran-coated iron oxide nanoparticles (dex-IO NPs)

All chemicals are analytical grade without further purification. Iron (II) chloride tetrahydrate, Iron (III) chloride hexahydrate, and dextran (Mw ≈35,000–40,000) were purchased from Sigma Aldrich.

The dextran-coated iron oxide nanoparticles were prepared by a chemical co-precipitation method. Firstly, dextran (4 g) was dissolved in 5 ml of deionized water. Ferrite solution (5 mL), containing molar ratio of 3:2 Fe^{3+} and Fe^{2+}, was mixed well with an equal volume of prepared dextran solution. The mixture was heated up to 60 ℃ and ammonium hydroxide (0.1 mole, 10 mL) was added under stirring and reacted for 1 h. The mixture was cooled at room temperature, washed with methanol, and centrifuged at 3,000 rpm for 10 min to remove non-coated and aggregated dextran in the suspension.

Transmission electron microscope (TEM) images were conducted on a JEOL 100CX microscope with a CeB6 filament and an accelerating voltage of 100 kV. The samples for TEM analysis were prepared by dropping the dilute suspensions onto the carbon-coated copper grids and letting the solvent evaporate (Fig. S1A). The particle size and surface charge were measured at 25 ℃ using a Nano ZS90 laser particle analyzer (Malcern Instrument, UK) (Fig. S1B & 1C).

The surface structure of the samples was characterized by Fourier Transform Infrared Spectroscopy (FTIR). Measurements were performed with pressed pellets that were made by using KBr powder as diluent. The FTIR spectrum was collected between the wave numbers from 500 to 4000 cm^{-1} (Figure S1D). The data demonstrated the synthesis of dex-IO NPs.

Cell viability and proliferation assay

In vitro acute cytotoxicity and cell proliferation experiments were assessed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay. After incubation with dex-IO NPs suspension in serum-free medium for 1 h, cells were incubated with fresh serum-free medium containing 0.5 mg mL^{-1} MTT for 1 h at 37 ℃ for cytotoxicity assay. For proliferation assay, the cells after treatment with

dex-IO NPs for 1 h were allowed to grow in regular growth medium for 24 h, followed by incubation with MTT reagent. The dark blue formazan dye generated by the live cells was proportional to the number of live cells; and the absorbance at 570 nm was measured using a microplate reader. Cell numbers were determined from a standard plot of known cell numbers vs. the corresponding absorbance density (Fig. S2).

Preparation and characterization of dextran-coated Au nanoparticles (dex-Au NPs)

Dextran (12 g) was dissolved in 160 mL of deionized water. The dextran solution was heated up to 120 ℃; then 216 μL of hydrogen tetrachloroaurate (III) hydrate solution (Au) (Sigma Aldrich) was added. The mixture was boiled for 20 min until the color of the mixture turned deep violet. At the end of this reaction, the dex-Au NPs were recovered through Amicon filters at 0.45 μm and 0.22 μm and washed three times and dissolved in deionized water. The TEM samples for analysis were prepared by dropping the dilute suspensions onto the carbon-coated copper grids and letting the solvent evaporate (Fig. S3A). The particle size and surface charge were measured at 25 ℃ using a Nano ZS90 laser particle analyzer (Malcern Instrument, UK) (Fig. S3B &3C).

Preparation and characterization of citrate-coated IO nanoparticles (cit-IO NPs)

Iron (III) chloride hexahydrate (5 mmole) and iron (II) chloride hexahydrate (2 mmole) were dissolved in 10 mL of 0.4 N HCl each. Both ferrite solutions were mixed together, and then 60 ml of 12.5% tetramethylammonium hydroxide (TMAOH) was added under stirring and reacted for 4 h at room temperature. Then the mixture was heated to 80 ℃ and reacted for 20 min. The mixture (Fe_3O_4 core) was recovered through a 100k filter and dissolved in 0.01 N HNO_3. The Fe_3O_4 core was furthermore heated to 90℃ for 40 min until the color of mixture turned brown. Then the mixture, called Fe_3O_4 core/γ-Fe_2O_3 core, was cooled and recovered through a 100k filter and dissolved in 50 ml of deionized water.

In order to synthesize cit-IO NPs, 5.16 g of sodium citrate was dissolved in 250 mL of deionized water and the pH was adjusted to 6.5~7 by citric acid. Then the Fe_3O_4 core/γ-Fe_2O_3 core was added to the prepared citrate solution and the reaction was sonicated at 50 ℃ for 3 h. After completion of this reaction, the cit-IO NPs were recovered through a 100k filter and washed until the supernatant became clear and colorless. Finally, these nanoparticles were dissolved in deionized water. The TEM samples for analysis were prepared by dropping the dilute suspensions onto the carbon-coated copper grids and letting the solvent evaporate (Fig. S4A).

The particle size and surface charge were measured at 25 ℃ using a Nano ZS90 laser particle analyzer (Malcern Instrument, UK) (Fig. S4B & 4C).

In vitro migration of MSCs

Studies on chemotactic migration of MSCs were performed using the Costar Transwell chamber system (24-well; Costar) with membrane filters with a pore size of 8 μm. Samples, each containing 4×10^4 cells in 200 μL of growth medium, were added to the upper compartments (top chambers or inserts) and incubated overnight. HT-29, B16F10, PC-3, and MCF7 cancer cells at 2×10^5 cells 600 μL^{-1} of 10% FBS-containing DMEM per chamber were added to the lower compartments (bottom chambers) and incubated overnight. The cells in the upper compartments were then labeled with dex-IO NPs (300 μg mL^{-1}) in serum-free medium for 1 h at 37 ℃. After labeling, the upper compartments were washed once by PBS and then transferred to the bottom chambers. The migration chambers were incubated for 1 d at 37 ℃ in a humidified air atmosphere with 5% CO_2. After incubation, cells on the top surface of the filters of the upper compartments were wiped off with cotton swabs. Cells that had migrated toward the lower surface of the filters were counted after staining with 0.5% crystal violet (Sigma). Triplicates of each sample were counted. Each migration experiment was performed in triplicate. The migration rate was expressed as the percentage variation of migrated cell numbers with respect to the corresponding control as 100%.

As shown in Fig. 1A, dex-IO NPs and dex-Au NPs but not cit-IO NPs could promote the tumor tropism of our hMSCs (*aT*-MSCs) toward tested tumor cells; however, all dex-IO NPs, dex-Au NPs, and cit-IO NPs could induce the tumor tropism of BM-500 cells (*pT*-MSCs) toward various tumor cells (Fig. S5), suggesting that distinct molecular mechanisms for dex-IO NP-regulated tropism receptors and tumor tropism are MSCs-dependent.

Western blot analysis

MSCs (6×10^5 cells) were incubated with regular cultured medium in 100 mm dishes. The MSCs were treated with dex-IO NPs (300 μg mL) (*) in serum-free medium for 1 h at 37 ℃. After treatment cells were rinsed with ice-cold 1× PBS and were lysed by the addition of lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1% Igepal CA-630, 10 mM $MgCl_2$, 1 mM EDTA, 2% glycerol, 1 μM phenylmethylsulfonyl fluoride, 1 μg mL^{-1} leupeptin, and 10 μg mL^{-1} aprotinin) for 1 h at 4 ℃. The suspensions were centrifuged at 13,000 rpm for 30 min at 4 ℃. The protein concentration of the supernatant was assessed by the Bio-Rad protein assay kit.

Proteins were separated by electrophoresis in a gradient polyacrylamide gel and

transferred to a polyvinylidene difluoride membrane. Then membranes were incubated at room temperature in 0.1% Tween 20 with TBS plus 5% bovine serum albumin (BSA) for 1 h. The membranes were incubated with mouse anti-EGFR antibodies (ADI-CSA-330; dilution 1:1000, Enzo), mouse anti-CXCR4 antibodies (sc53534; dilution 1:100, Santa Cruz), or rabbit anti-integrin α4 antibodies (cs8440; dilution 1:1000, Cell signaling) in TBST containing 5% BSA at 4℃, and then washed three times in TBST, for 10 min each time. After washing, HRP-conjugated anti-rabbit (cs7074; dilution 1:5000, Cell signaling) or anti-mouse (cs7076, dilution 1:5000, Cell signaling) antibodies were incubated with membranes for 1 h at room temperature. After washing, the membranes were developed using the Immobilon Western chemiluminescent HRP substrate kit (Millipore). The original X-films were provided in Fig. S6.

Table S1 Summary of characterization of various nanoparticles.

	Core diameter (nm)	Hydrodynamic size (nm)	Zeta potential (mV)
dex-IO NPs	3–8	120–150	-5.5
dex-Au NPs	30–40	80–83	-27.3
cit-Io NPs	5–10	28–40	-59.0

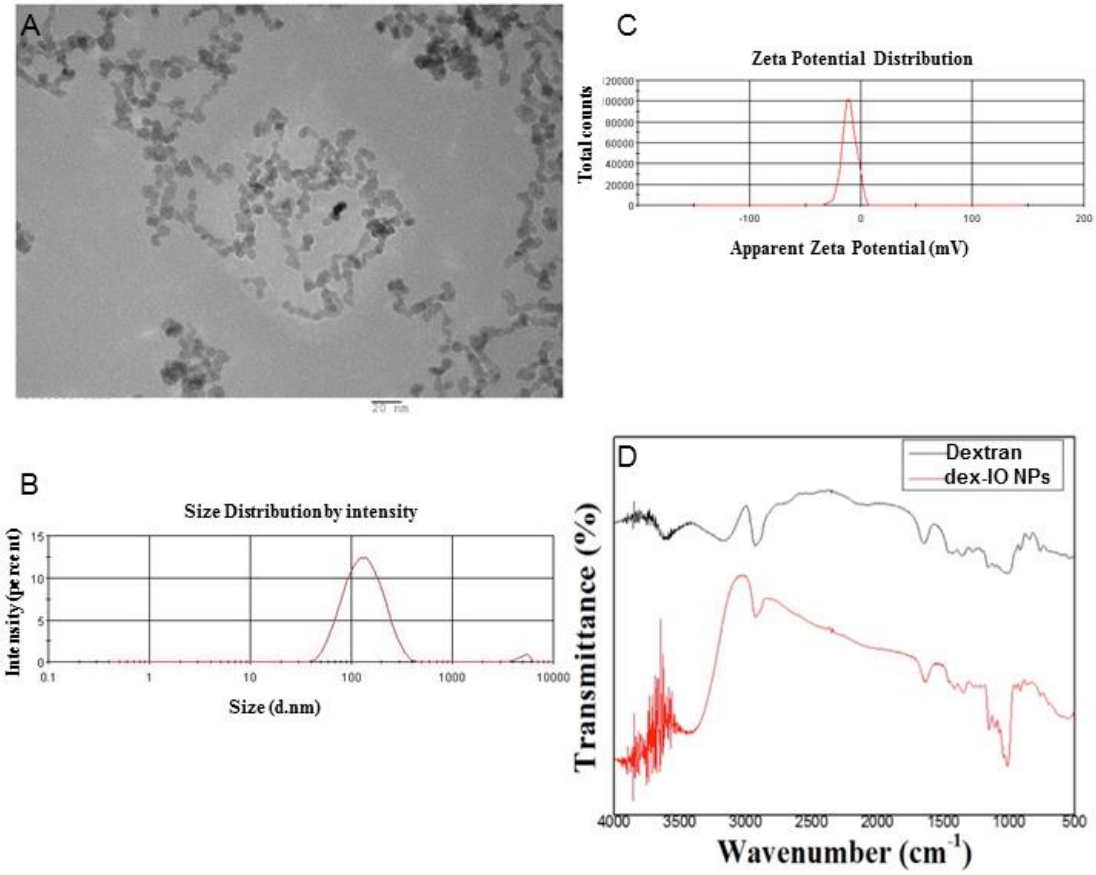

Fig. S1 Characterization of dex-IO NPs. (A) TEM image was obtained on a JEOL 100CX microscope. (B) Hydrodynamic diameter was measured by employing a Nano ZS90 laser particle analyzer. (C) Zeta potential distribution was measured by employing a Nano ZS90 laser particle analyzer. (D) Surface structure of the samples was characterized by FTIR.

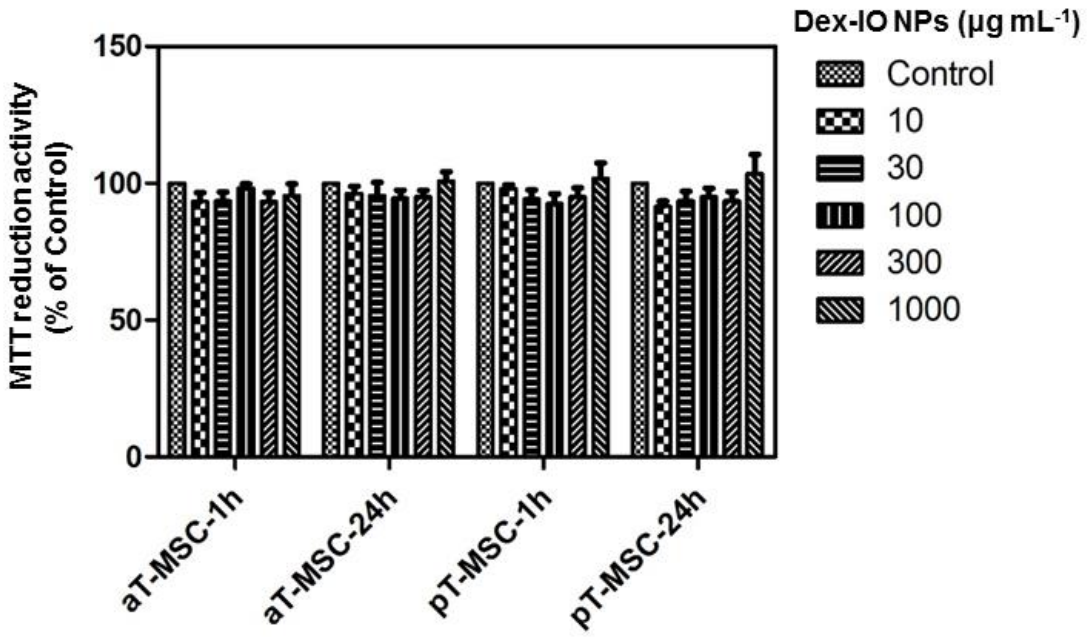

Fig. S2 Effect of dex-IO NPs on cytotoxicity and cell proliferation in MSCs. After treatment with 0–1000 μg mL^{-1} of dex-IO NPs for 1 h, MSCs were either processed immediately with MTT incubation for acute cytotoxicity determination or followed by 24-hour incubation in cell culture medium for cell proliferation by MTT reduction.

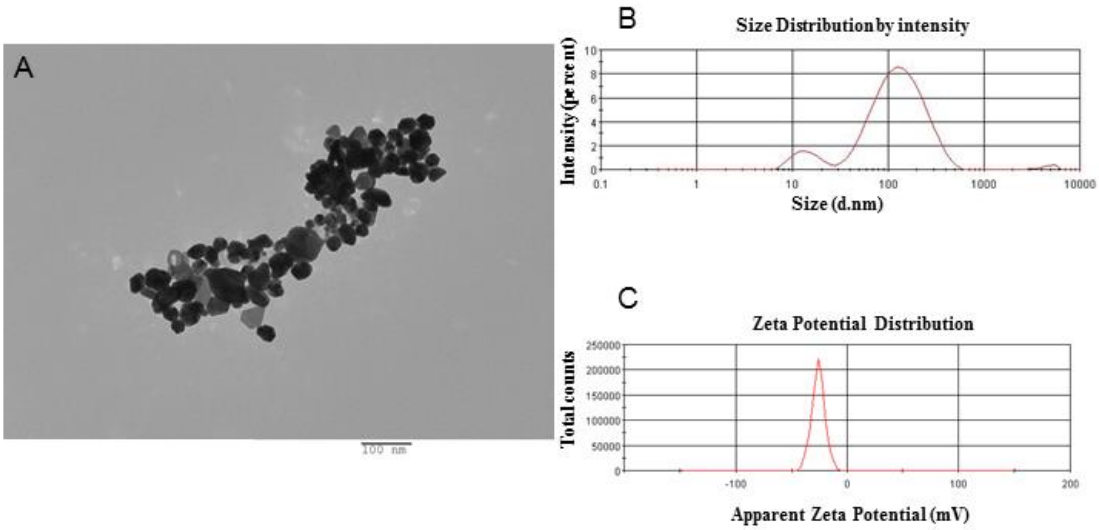

Fig. S3 Characterization of dex-Au NPs. (A) TEM image was obtained on a JEOL 100CX microscope. (B) Hydrodynamic diameter was measured by employing a Nano ZS90 laser particle analyzer. (C) Zeta potential distribution was measured by employing a Nano ZS90 laser particle analyzer.

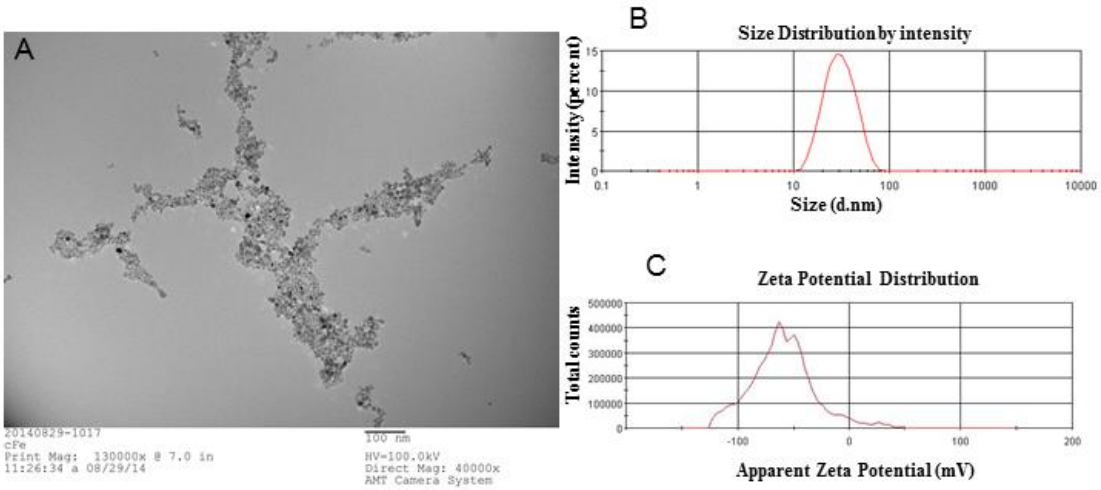

Fig. S4 Characterization of cit-IO NPs. (A) TEM image was obtained on a JEOL 100CX microscope. (B) Hydrodynamic diameter was measured by employing a Nano ZS90 laser particle analyzer. (C) Zeta potential distribution was measured by employing a Nano ZS90 laser particle analyzer.

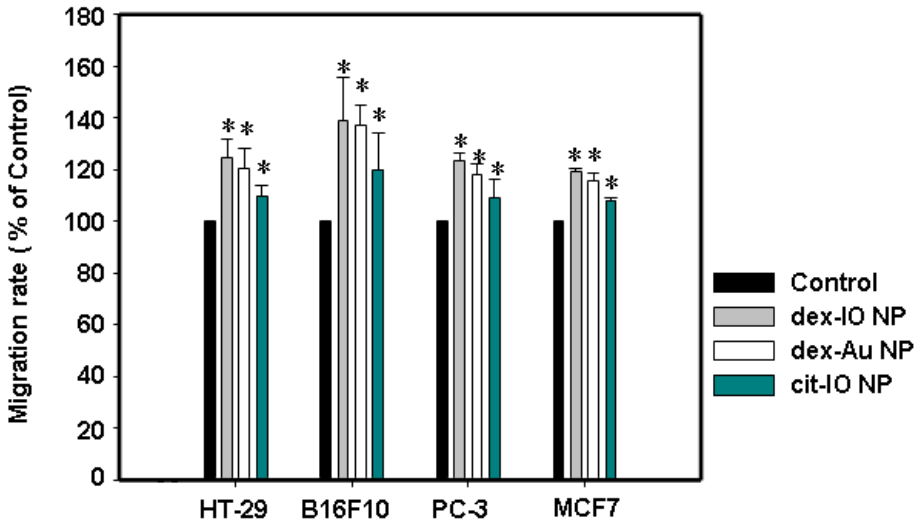

Fig. S5 The impacts of dex-IO NPs, dex-Au NPs and cit-IO NPs on BM-500 cells' *in vitro* tumor tropism. The migration activities of BM-500 cells (Control), dex-IO NP-labeled BN-500 cells (dex-IO NP), dex-Au NP-labeled BM-500 cells (dex-Au NP) and cit-IO NP-labeled BM-500 cells (cit-IO NP) toward HT-29, B16F10, PC-3, and MCF7 cells. The migration activities are expressed as mean ± standard error of three determinations (each in triplicate) as compared with Control. (*$P < 0.05$)

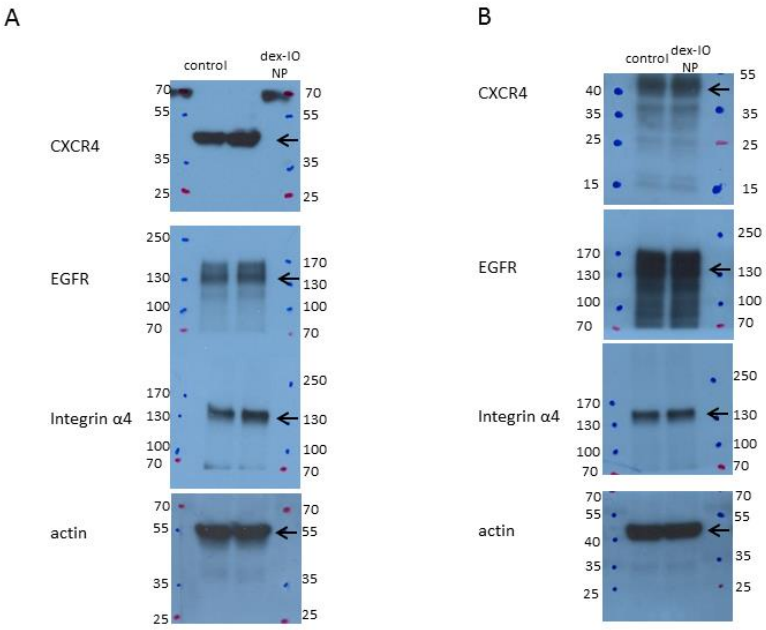

Fig. S6 The expression profiles of CXCR4, EGFR, and integrin α4 proteins in unlabeled *pT*-MSCs (Control) and dex-IO NP-labeled *pT*-MSCs (dex-IO). After treatment cells were either immediately processed for Western blot (A) or grown in regular growth medium for 24 h followed by Western blot (B). Molecular weight markers are indicated.