**Supplementary Material and Methods**

***Conditioned media collection***

To collect soluble factors released by hAMSCs and GSCs, 5×105 cells per well were seeded in 6-well plates and cultured in their corresponding media (Fig. 1C). Culture media was conditioned for 48 hours, collected and passed through a 0.45 µm filter (Corning). This conditioned media (CM) was stored at -80oC and thawed prior to use. Any remaining CM was stored at 4oC and utilized within a week of thawing.

***Quantification of secreted BMP4 from hAMSCs-BMP4***

To investigate how much BMP4 was secreted from transduced hAMSCs, Western blot was performed using conditioned media from hAMSCs-Vector and hAMSCs-BMP4 (1 million cells, cultured for 3 days) while various amounts of BMP4 were loaded as standard for quantification.

***In vitro MTS assay***

To determine the effects of cell-secreted factors on the proliferative capacity of hAMSCs and GSCs, MTS assay (CellTiter 96 AQueous One Solution Proliferation Assay, Promega) were used. hAMSCs or GSCs (1000 or 3000 cells/well, respectively) were seeded in 96-well plates and cultured in control media or CM. To determine the effect of BMP4 on cell proliferation, BMP4 (PeproTech, AF-120-05ET, 100 ng/ml) was added to control media for 2 weeks. Cell proliferation was analyzed every 3 days in triplicate for each experimental condition.

***In vitro EdU assay***

To determine the percentage of cell proliferation, EdU assay was used. hAMSCs, BMP4-treated hAMSCs (50 ng/ml for 24 hours or 48 hours), hAMSCs-Vector and hAMSCs-BMP4 were incubated with a fluorescent-labeled EdU reagent (Click-iT® EdU Flow Cytometry Assay Kits, Invitrogen) for 24 hours. EdU incorporation was detected via flow cytometry. For hAMSCs and GSCs co-culture experiments (Fig. 1G), td-tomato-hAMSCs and GSCs or hAMSCs and GFP-GSCs were co-cultured together for 5 or 13 days before performing flow cytometry. Flow cytometry data was analyzed using Kaluza (Beckman Coulter).

***Differentiation assay for hAMSCs***

To determine the effects of GSC-CM and BMP4 on the differentiation capacity of hAMSCs, cells were seeded into 24 wells plates (42,000 cells/well for adipogenic differentiation, 8,400 cells/well for osteogenic differentiation), or cultured as pellets (2.5×105 cells/tube for chondrogenic differentiation). For positive controls (differentiated cells), the cells were then exposed to differentiation conditions using adipogenic, osteogenic, or chondrogenic supplements as specified by the manufacturer (R&D, CCM007-008, and CCM011). For negative controls (undifferentiated cells), MesenPRO complete media without differentiation supplements was used. Groups were established as follows: hAMSCs, hAMSCs in GSC-CM, BMP4-treated hAMSCs (100 ng/ml), hAMSCs-Vector, and hAMSCs-BMP4. Cell lineage was evaluated using Oil Red O (Sigma, 00625), Alizarin Red S (Sigma, AB5533), and Masson’s Trichrome staining for adipocytes, osteocytes, and chondrocytes, respectively.

***Immunofluorescence staining***

To determine the effects of hAMSCs and BMP4 on the capacity of GSCs to differentiate, 2×104 GSCs were seeded on 24-well plates (with glass slides pre-coated with poly-l-ornithine (Sigma, P4957) and laminin). The following groups were established: undifferentiated group (negative control, in stem cell media); differentiated group (positive control, in stem cell media with 10% FBS); and experimental groups in hAMSC-CM, hAMSC-BMP4-CM, or stem cells media with BMP4 (100 ng/ml) for 2 weeks. The cells were fixed with 4% PFA, blocked with 10% normal goat/donkey serum, and immunostained for neuronal (Tuj1, Covance, MMS-435P) and glial (GFAP, Millipore, MAB3402) markers. Alexa-labeled secondary antibodies were used for visualization and DAPI was used to count cell nuclei. Images were visualized and recorded with an inverted fluorescence microscope (AxioObserver Z1, Zeiss) connected to a digital camera. The number of Tuj1+/DAPI and GFAP+/DAPI cells was counted from 6–9 random fields by blinded observers.

***Quantitative real-time RT-PCR***

Total RNA was isolated from samples with Trizol reagents (Invitrogen) and cleaned with RNase-free DNase treatment (Promega). mRNA was reverse transcribed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). Quantitative real-time PCR was performed with an ABI PRISM 7300 detection system (Applied Biosystems) following the comparative Ct method (2-ΔΔCt) using GAPDH expression as an internal control. The primer pairs used are listed below:

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| --- | --- | --- |
| **Gene** | **Forward Primer (5’-3’)** | **Reverse Primer (5’-3’)** |
| *GAPDH* | TGTGAACCATGAGAAGTATGAC | ATGAGTCCTTCCACGATACC |
| *CEBPA* | TCACCGCTCCAATGCCTACTG | CCTGCTCCCCTCCTTCTCTCAT |
| *LPL* | GTCAGAGCCAAAAGAAGCAGCAA | GGGTTTCACTCTCAGTCCCAGAA |
| *SOX9* | GCATGAGCGAGGTGCACTC | CAAGGCTGACCTGAAGCGAGA |
| *ALPL* | ACCACCACGAGAGTGAACCA | CGTTGTCTGAGTACCAGTCCC |
| *SPP1* | GACACATATGATGGCCGAGGTGAT | GGTGATGTCCTCGTCTGTAGCAT |
| *vimentin* | CGGAAAGTGGAATCCTTGCA | CACATCGATCTGGACATGCTGT |
| *ACTA2* | ATCGTCCACCGCAAATGC | AAGGAACTGGAGGCGCTG |

***In vitro transwell migration assay***

Cell migration was evaluated using Boyden chamber transwell chambers (Corning, #3422). To quantify hAMSC tropism to GSCs *in vitro*, 2×104 hAMSCs or hAMSCs-BMP4 were cultured in 100 µl MesenPRO complete media and seeded in the top well of the chamber, while 600 µl GSC-CM with 2% FBS or MesenPRO complete media with 2% FBS (control) were placed in the bottom well. After a 24-hour incubation, cells on top of the membrane (settled due to gravity) were removed using cotton swabs, and the cells on the bottom were stained with the Diff-Quik stain set (Siemens). To investigate the effects of hAMSCs, hAMSCs-BMP4, and BMP4 on the migratory capacity of GSCs, GSCs (276 and 612) were pre-cultured in stem cell media (control), hAMSC-CM (0-48 or 0-72 hours), hAMSC-BMP4-CM (24 hours), or BMP4 (50 ng/ml or 100 ng/ml; 24 hours or 48 hours) before the assays were performed. The bottom of the transwell chamber contained stem cell media with 2% FBS. After staining, migrated cells were counted from 9 random fields (10x magnification) using light microscopy by blinded observers.

***In vitro nanopattern cell migration assay***

Migration speeds of GSCs were quantified using a nano-patterned surface, a directional migration assay using nano-ridges and grooves constructed of transparent poly urethane acrylate (PUA), and fabricated using UV-assisted capillary lithography, as previously reported by our group ([1](#_ENREF_1)). Nanopattern surfaces were coated with laminin (3 mg/cm2) and 45,000 GSCs were plated in stem cell media (control), hAMSC-CM, hAMSC-BMP4-CM, or BMP4 (50 ng/ml or 100 ng/ml) treated stem cell media. Cell migration was quantified using timelapse microscopy. Long-term observation was performed with a motorized inverted microscope (Olympus IX81). Phase-contrast and epi-fluorescent cell images were automatically recorded for 15 hours at 10-20 minutes intervals using the Slidebook 4.1 (Intelligent Imaging Innovations, Denver, CO). Cell speed was calculated based on tracking 50-100 cells per condition using customized semi-automated program developed with MATLAB (Natick, MA) by blinded observers. Every experiment has at least 3 independent repeats.

**References:**

1. Garzon-Muvdi T, Schiapparelli P, ap Rhys C, Guerrero-Cazares H, Smith C, Kim DH, et al. Regulation of brain tumor dispersal by NKCC1 through a novel role in focal adhesion regulation. PLoS biology. 2012;10:e1001320.