Stromal cells in Modic type 1 change bone marrow promote neurite outgrowth

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COI Disclosure:

Nothing to disclose

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1. Background

Vertebral bone marrow lesions known as Modic type 1 changes (MC1) (Fig. 1) are a major cause of unspecific lower back pain. Pain may relate to higher innervation of MC1 endplates (Fig. 2). Blocking neo-innervation in MC1 could be a promising treatment approach for MC1. However, the neurotrophic mechanisms in MC1 are unknown. Bone marrow stromal cells (BMSC) can produce neurotrophic factors and are dysregulated in MC1. The aim of the study was to identify if BMSC in MC1 support neo-innervation through release of neurotrophic factors.

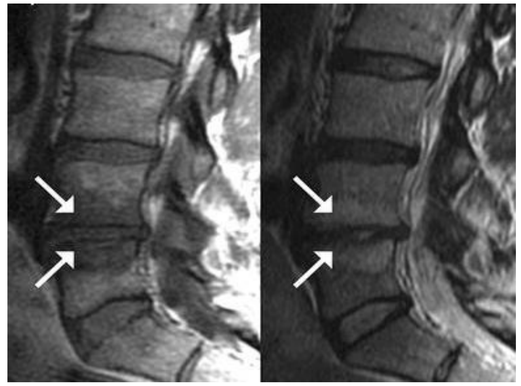

Fig. 1: *T1 weighted (left) and T2 weighted (right) lumbar MRI of MC1 lesion (arrows). T1 weighted MRI showing hypointense and T2 weighted MRI showing hyperintense signal intensity changes (figure adapted from Dudli et al., 2016).*

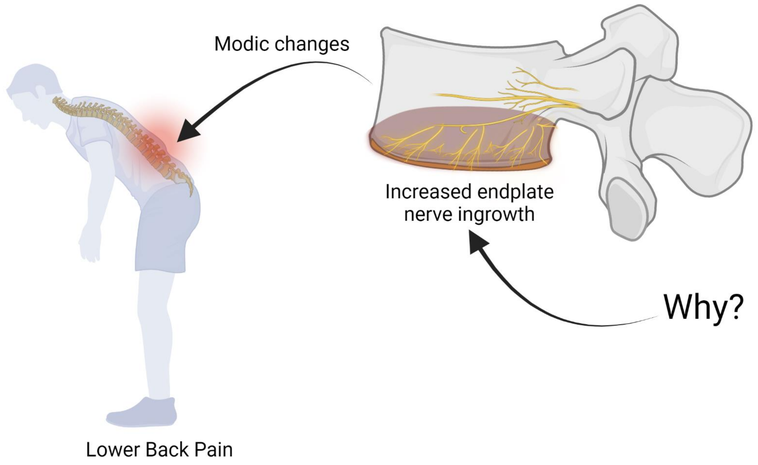

Fig. 2: Modic change bone marrow and endplates show increased nerve ingrowth.

Methods

BMSC were isolated through plastic adherence from vertebral bone marrow biopsies from a MC1 and an intra-patient control region of patients undergoing spinal fusion surgery (n=4+4). BMSC were co-cultured with the neuroblastoma cell line SH-SY5Y for 8 days. Neurite outgrowth from SH-SY5Y was quantified as a measure for neurotrophic activity.

Briefly, SH-SY5Y cells were pre-differentiated for 48h in B27 supplemented serum free media using retinoic acid (10µM). BMSC (passage 2 or 3) were seeded on cell culture inserts and SH-SY5Y cells in 6-well plates before being co-cultured for 8 days (Fig. 3). Neurite outgrowths of SH-SY5Y were analyzed on a widefield microscope and with the Image J Ridge Detection Plugin (Fig. 4). Fold-change to day 0 was calculated and compared between MC1 and intra-patient control using paired t-tests of log2 fold changes.

Thirty neurotrophic cytokines in the conditioned media were analyzed with C-Series Human Neuro Discovery Array C2. Images were analyzed using Protein Array Analyzer Plugin for ImageJ. Relative signal intensities between MC1 and control were compared with paired t-tests.

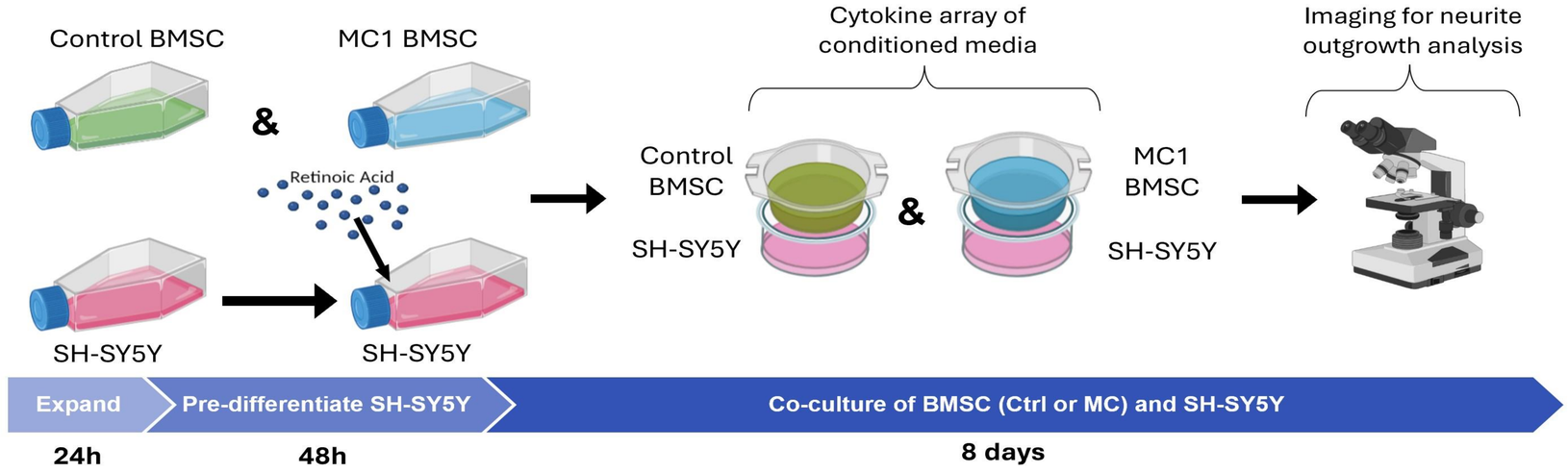

Fig. 3: *Experiment setup of BMSC and SH-SY5Y cells co-culture.*

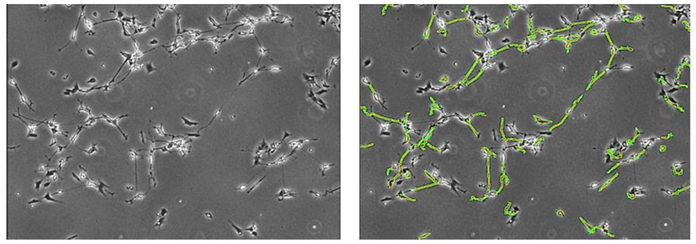

Fig. 4: *Original image showing differentiated SH-SY5Y cells (left) which are traced and quantified using ImageJ ridge detection plugin (right). 4x magnification.*

Results

Gene set enrichment analysis of MC1 vs. intra-patient control BMSC identified gene sets associated with BDNF signaling such as “BDNF TRKB signaling” (normalized enrichment score (NES) = 1.71, p<0.001) and “mBDNF and proBDNF regulation of GABA neurotransmission” (NES = 1.61, p<0.001) amongst the top enriched gene sets.

SH-SY5Y cells co-cultured with MC1 BMSC compared to intra-patient control showed significantly increased neurite outgrowth after 4 days (p = 0.045), 6 days (p = 0.027), and 8 days (p = 0.031) (Fig. 5).

Cytokine array analysis revealed significantly more mature brain-derived neurotrophic factor (mBDNF) (p = 0.021) and ciliary neurotrophic factor (CNTF) (p = 0.030) in supernatant of MC1 vs. control BMSC (Fig. 6).

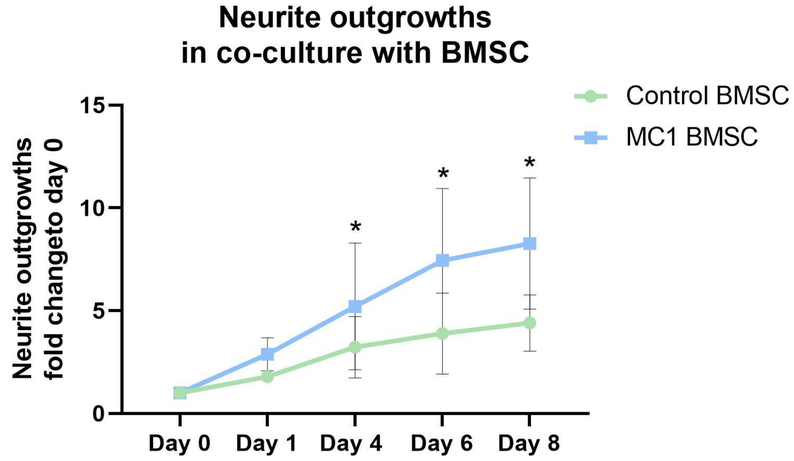

Fig. 5: *Effect of BMSC on neurite outgrowth of SH-SY5Y during 8 days in co-culture normalized to day 0 (n=4). * p < 0.05.*

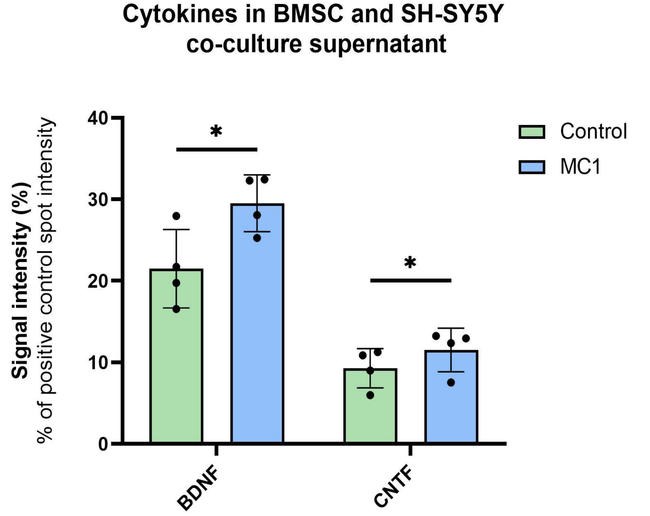

Fig. 6: *Cytokine array spot intensities of conditioned media from co-culture of BMSC and SH-SY5Y (n=4). Spot intensities calculated as percentage of positive control spot. * p < 0.05.*

Modic type 1 bone marrow stromal cells positively influence nerve growth

Conclusion

Neurotrophic activity of MC1 BMSC is increased. This might be mediated by BDNF and CNTF. Therefore, BDNF and CNTF may represent interesting novel treatment approaches for MC1 that directly target pain mechanisms.
Understanding the mechanisms behind increased nerve outgrowth may provide insights into pain generators in MC1 and suggest new treatment targets for MC1.

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