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STROMAL CELLS IN MODIC TYPE 1 CHANGE BONE MARROW PROMOTE NEURITE OUTGROWTH

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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[1]. 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[2]. The aim of the study was to identify if BMSC in MC1 support neo-innervation through release of neurotrophic factors.

2. 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. Neurite outgrowths of SH-SY5Y were analyzed on a widefield microscope and with the Image J Ridge Detection Plugin (Fig. 2). Average neurite length from three images was calculated. Fold-change to day 0 was calculated and compared between MC1 and intra-patient control using paired t-tests of log2 fold changes (Fig. 3).

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

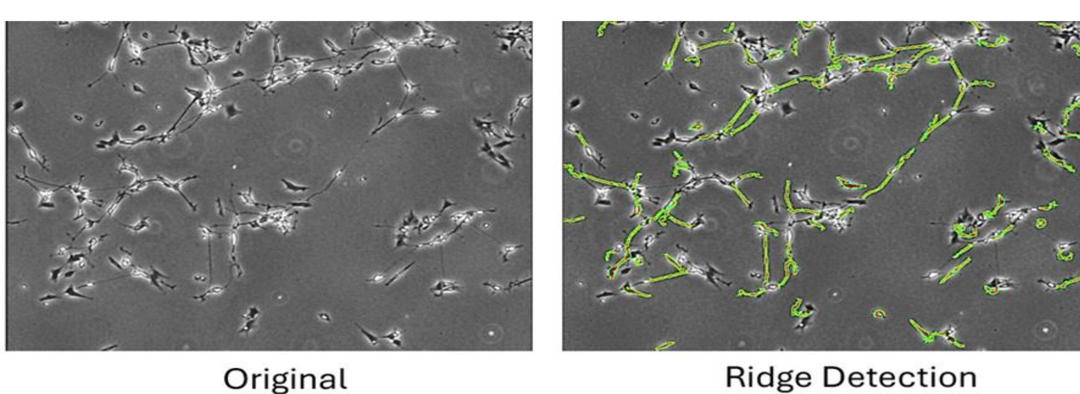

Fig. 2: *Original image showing differentiated SH-SY5Y cells (left) which are traced and quantified using ImageJ ridge detection plugin (right). 4x magnification.*

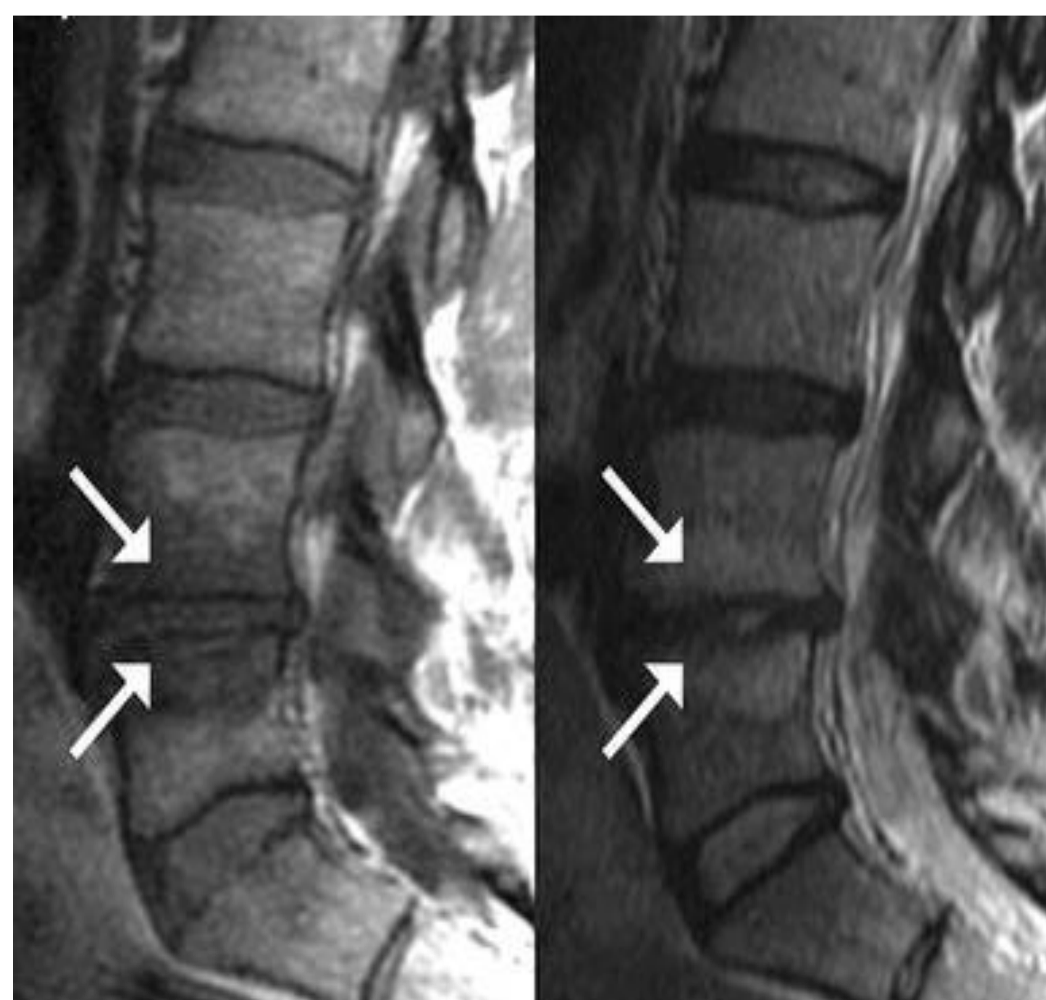

Fig. 1: *T1 weighted (left) and T2 weighted (right) lumbar MRI of MC1 lesion (arrows). T1 weighted MRI showing hypointense and T2 weighted MRI showing hyperintense signal intensity changes (figure adapted from Dudlli et al., 2016).*

3. Results

After four days of co-culturing MC1 BMSC with SH-SY5Y cells, neurite outgrowth was significantly increased compared to intra-patient control (5.211 ± 3.08 vs. 3.29 ± 1.50, p = 0.045) (Fig. 4). The same was seen on day 6 (7.45 ± 3.50 vs. 3.89 ± 1.97, p = 0.027) and day 8 (8.27 ± 3.19 vs. 4.40 ± 1.37, p = 0.031).

Cytokine array analysis revealed significantly more brain-derived neurotrophic factor (BDNF) (21.49 ± 4.81 vs. 29.53 ± 3.48, p = 0.021) and ciliary neurotrophic factor (CNTF) (9.28 ± 2.42 vs. 11.52 ± 2.68, p = 0.030) (Fig. 5) in MC1 BMSC conditioned media.

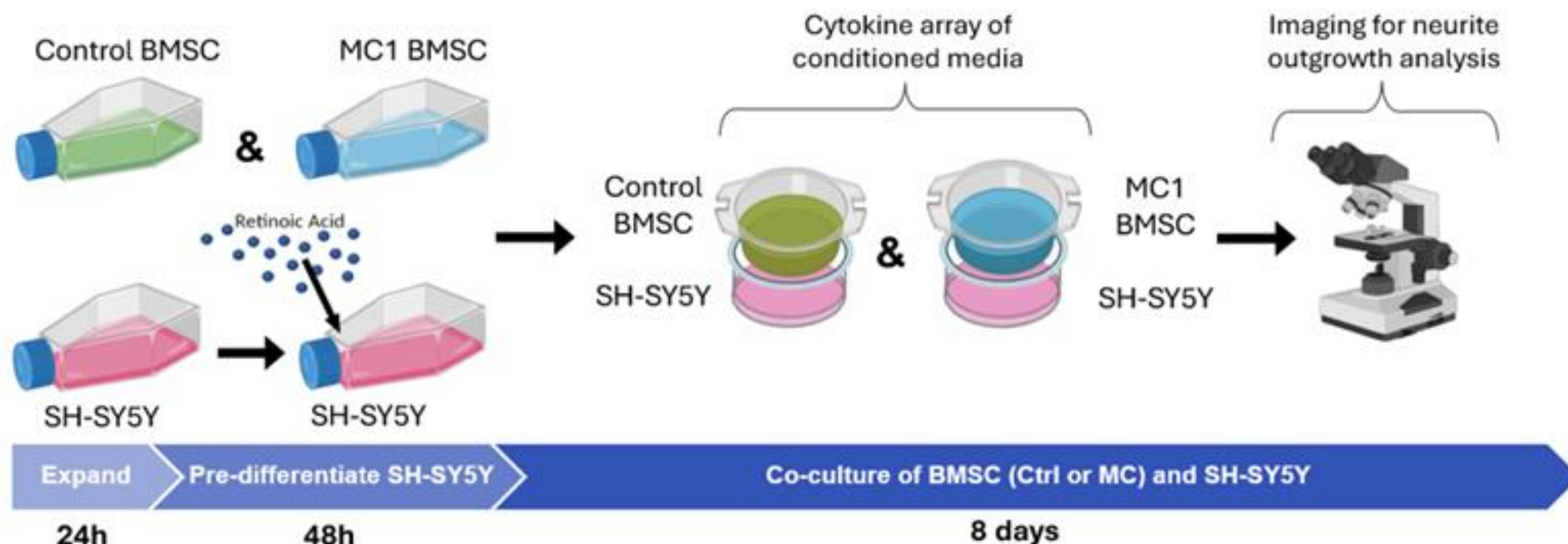

Fig. 3: *Experiment setup of BMSC and SH-SY5Y cells co-culture.*

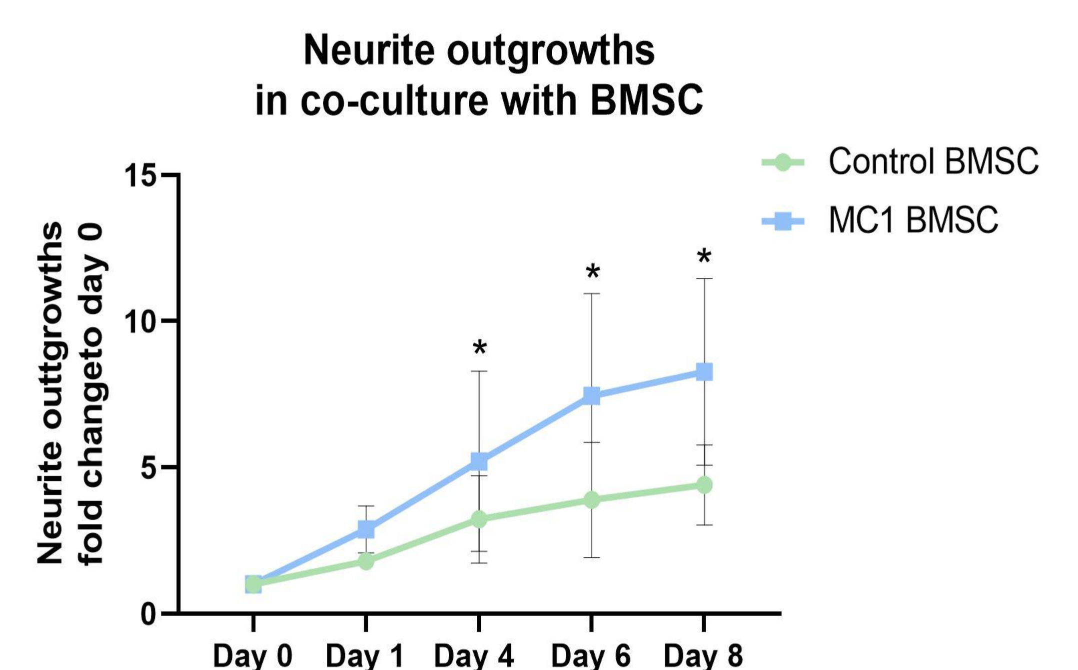

Fig. 4: *Effect of BMSC on neurite outgrowth of SH-SY5Y during 8 days in co-culture normalized to day 0 (n=4). * p < 0.05.*

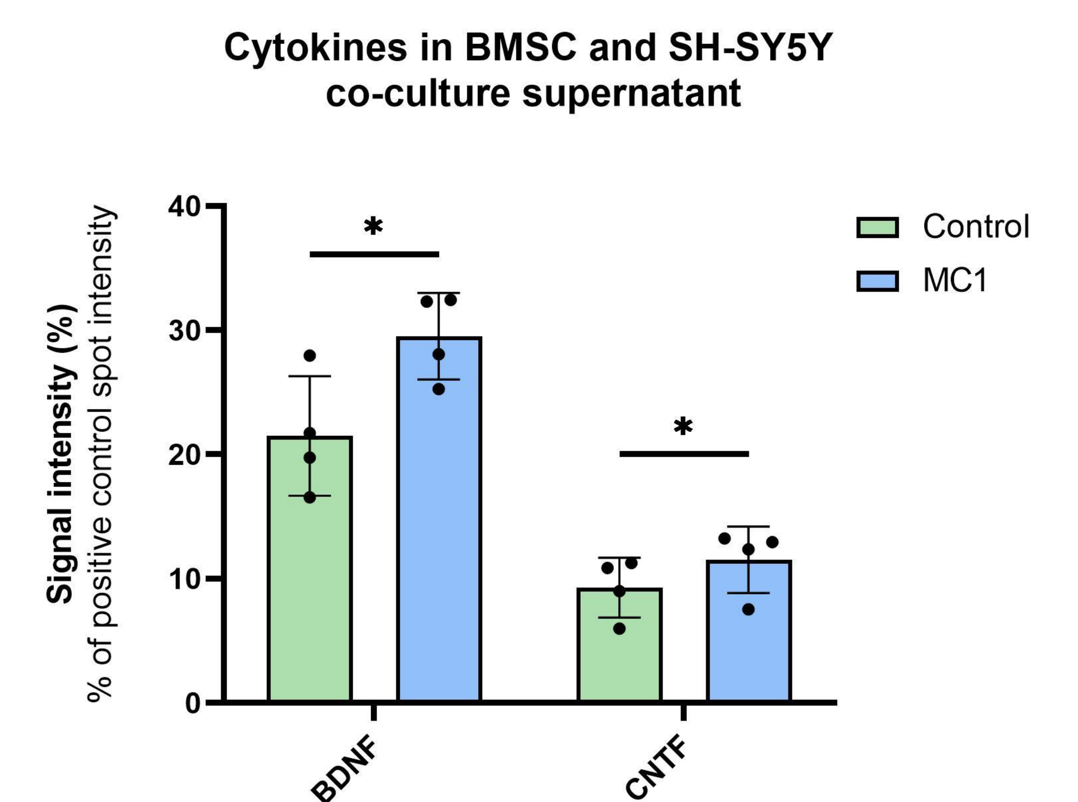

Fig. 5 *Cytokine array spot intensities of conditioned media from co-culture of BMSC and SH-SY5Y (n=4). Spot intensities calculated as percentage of positive control spot. * p < 0.05.*

4. Conclusion

BMSC from MC1 have an increased neurotrophic activity. Our data points towards a potential influence of BDNF and CNTF.

Understanding the mechanisms behind increased nerve outgrowth may provide insights into pain generators in MC1 and suggest new treatment targets for MC1.

References

1. Fields et al. Spine J. 2014,14(3):513-521
2. Heggli et al. Eur Cell Mater. 021,41:648-667.