# Bone marrow stromal cells in Modic type 1 changes promote neurite growth

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**INTRODUCTION**: The development of chronic lower back pain in patients with Modic changes, specifically Modic type 1 changes (MC1), is associated with abnormal neurite outgrowth in the vertebral body and the adjacent endplate which results in vertebral body endplate pain. Nerves within the bone marrow grow alongside blood vessels which are surrounded by bone marrow stromal cells (BMSCs). In MC1, BMSCs are dysregulated, specifically they are pro-fibrotic and perivascular MC1 BMSCs are increased in number. It is crucial to identify the drivers of the increased innervation of the vertebral body through the basivertebral nerve to understand LBP with MC1. The aim of this study was to determine if MC1 BMSCs enhance nerve growth.

**METHODS**: Neurotrophic gene expression in MC1 BMSC was investigated by (i) analyzing enriched gene sets in the existing RNA sequencing data set (ENA PRJEB39993) of MC1 BMSCs vs. intra-patient control vertebral BMSCs (n = 4 MC1 + 4 intra-patient control), and (ii) by specifically analyzing expression of neurotrophins with quantitative real-time polymerase chain reaction (qPCR) and their receptors in freshly isolated BMCS. For this, BMSC were isolated from bone marrow aspirates taken from a MC1 and an intra-patient control region of patients undergoing spinal fusion surgery. The capacity of BMSC to cause neurite growth was quantified by co-culturing BMSC from additional patients (n = 6 MC1 + 6 intra-patient control) with the neuroblastoma cell line SH-SY5Y for 8 days. Neurite outgrowth from SH-SY5Y was quantified as a measure for neurotrophic activity using microscopy. To identify the factors driving neurite growth, thirty neurotrophic cytokines were analyzed in the supernatant of the co-culture using C-Series Human Neuro Discovery Array C2. We then specifically investigated, if blocking BDNF signaling by antagonizing the BDNF receptor TrkB with 10µM ANA-12 can inhibit neurite growth using the co-culture system. Finally, bulk RNA sequencing was performed on co-cultured SH-SY5Y cells to further investigate the nerve growth mechanisms induced by MC1 BMSCs.

**RESULTS SECTION:**
Gene Set Enrichment Analysis (GSEA) compared MC1 BMSCs to control BMSCs and revealed several gene sets among the top hits to be strongly associated with brain-derived neurotrophic factor (BDNF) signaling. Notably, the gene set linked to BDNF tyrosine kinase receptor B (TRKB) signaling was found significantly enriched (normalized enrichment score = 1.71; FDR q-value = 0.20) (Figure 1A). A significant upregulation of BDNF receptor TRKB (p = 0.009, fold change = 2.20) as well as upregulation of nerve growth factor (NGF) (p = 0.055, fold change = 1.53) was detected in MC1 BMSCs with qPCR (Figure 1B).
SH-SY5Y cells sprouted significantly more when co-cultured with MC1 compared to when cultured with intra-patient control BMSCs. After 4 days of co-culture the sum of neurite outgrowth relative to day 0 was significantly greater in the MC1 co-culture (2.88 ± 0.80 vs. 1.79 ± 0.27, p = 0.028). This remained increased on day 6 (6.55 ± 3.64 vs. 3.44 ± 1.98, p = 0.027) and day 8 (7.67 ± 3.07 vs. 4.18 ± 1.29, p = 0.028) (Figure 2A).
Cytokine array analysis detected 17 out of 30 cytokines. Concentrations of BDNF (21.5 % ± 4.8 % vs. 29.5 % ± 3.5 %, p = 0.021, FDR q-value = 0.27) and of ciliary neurotrophic factor (CNTF) (9.3 % ± 2.4 % vs. 11.5 % ± 2.7 %, p = 0.030, FDR q-value = 0.272) were higher in conditioned media of MC1 vs. control BMSCs but not significantly after adjusting for multiple testing (Figure 2B). All other cytokines were not differentially abundant.
The control and MC1 co-cultured SH-SY5Y did not show any decrease in neurite outgrowth when TrkB was antagonized (Figure 2C), which indicates that multiple mechanisms are necessary to create the increased neurite outgrowth.
To determine if BDNF signaling or other mechanisms are responsible for the increased sprouting efficiency, the transcriptomic profile of 8-day co-cultured neuroblastoma cells was analyzed by bulk RNA sequencing. Only 13 differentially expressed genes were found (FDR < 0.25). GSEA analysis showed significant enrichment in the extracellular matrix (ECM) reformation related pathways such as activation of matrix metalloproteinases, degradation of ECM and collagen chain trimerization but no pathways were enriched that could be directly linked to factors secreted by MC1 BMSCs.

**DISCUSSION**: The study shows that MC1 BMSCs exhibit pro-neurotrophic activity and are important contributors to the increased vertebral body and endplate innervation in MC1. Although BDNF signaling in MC1 BMSC was increased and BDNF was found in higher concentrations in MC1 co-culture supernatant, antagonizing TrkB on SH-SY5Y did not diminish nerve growth in MC1 co-cultures. This indicates that the increased neurite growth is more probable the result from a combination of growth factors and cytokines produced or activated by MC1 BMSCs rather than solely mediated by BDNF. This functional finding accentuates that MC1 BMSCs provide strong pro-neurotrophic cues to nearby neurons through a multifactorial process.

**SIGNIFICANCE/CLINICAL RELEVANCE**: This finding underscores the significance of BMSCs dysfunction in MC1-related vertebrogenic low back pain and highlights BMSCs as a promising treatment target for addressing MC1.

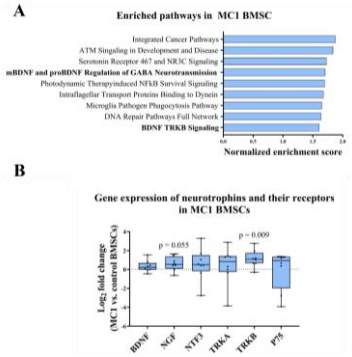


***Figure 1.*** *Gene expression of MC1 compared to intra-patient control BMSCs.* ***(A)*** *Gene set enrichment (GSEA) analysis of BMSCs bulk RNA sequencing indicates enrichment of BDNF signaling related pathways.* ***(B)*** *Gene expression of neurotrophic cytokines and respective receptors measured by qPCR (n = 6 MC1 + 6 intra-patient control).*

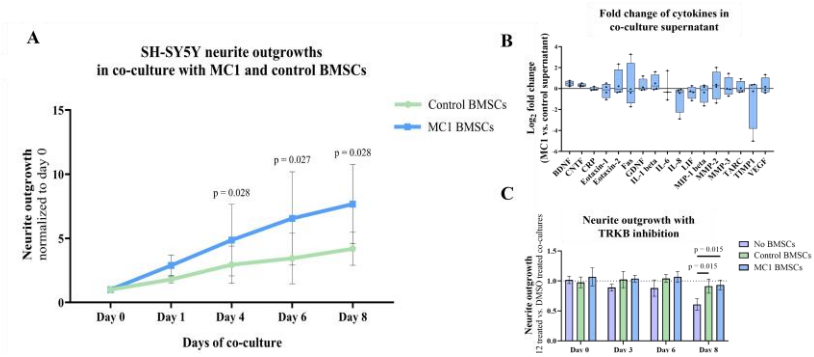


***Figure 2.*** *Co-culture of BMSCs with SH-SY5Y cells.* ***(A)*** *Total neurite length normalized to day 0 over 8 days. Data shown as fold change to day 0 of co-culture.* ***(B)*** *Cytokines in co-culture supernatant on day 8. Data are shown as $log_2$ fold change of MC1 co-culture supernatant compared to control ± standard error of mean.* ***(C)*** *Neurite outgrowth of SH-SY5Y with TrkB inhibitor ANA-12 in co-culture with either MC1 BMSCs, control BMSCs, or no BMSCs over 8 days. Data shown as fold change of each group to respective DMSO control ± standard deviation.*