Dysregulated Bone Marrow Stromal Cells in Modic Type 1 Changes

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Background: Modic type 1 changes (MC1) are fibrotic-inflammatory vertebral bone marrow lesions adjacent to degenerating discs. Patient with MC1 often develop low back pain [1]. In MC1, extra-cellular collagen is deposited, myelopoiesis is dysregulated, and bone is rapidly remodelled. These signs are of a chronic inflammation. The cellular mechanisms are unknown, yet bone marrow stromal cells (BMSC) are key regulators of myelopoiesis, can differentiate into collagen-producing cells, and modulate inflammation [2].

Objectives: To link BMSC phenotype and function to molecular changes in MC1.

Methods: From patients undergoing lumbar spondylodesis, bone marrow aspirates (n=5 MC1+5 controls) were taken through pedicle screw trajectory before screw insertion. Biopsies were fixed, dehydrated, and imaged with multiphoton fluorescence microscopy (MPE). Tissue auto-fluorescence and second-harmonics-generation microscopy (MPE) of the auto-fluorescence signal was performed in key areas of biopsies and single-photon-counting histograms were fitted with a triple-exponential decay function. BMSC were isolated from aspirates by plastic adherence and characterized (passage 2). Sequencing of ribosomal depleted RNA ( illumina Novaseq) with pathway analysis was performed, differentiation capacity was quantified histologically (Witcoxon test), duplication rate was measured with CellTrace™(t-test), and stem cell surface marker expression was quantified by flow cytometry (CD14, CD16, CD19, CD34, CD45, CD73, CD90, CD105, CD284 (t-test). BMSC were cultured in osteogenic (5μM DEX, 10nM rT3, 50μg/ml bFGF, 0.5mM L-ascorbic-2-phosphate) or adipogenic (1μM dexamethasone, 0.5mM 3-isobutyl-1-methylxanthine, 10mM insulin, 0.45mM indomethacin) medium and quantified with ALP activity and oil red-O staining.

Results: Biopsies: Collagen was qualitatively more abundant in MC1 than in Ctrl bone marrow, particularly in areas of adipocyte clusters and around adipocytes (Figure 1, arrows). FLIM was able to distinguish adipocytes (T<2.1-2.7ns), leukocytes (T=0.4-0.8ns), erythrocytes (T=0.2-0.4ns), and collagen (T<0.15ns) based on their different auto-fluorescent life-times (Figure 1, right).

BMSCs: By RNA sequencing 154 genes were differentially expressed between MC1 and Ctrl BMSCs (p<0.01; log2 ratio >0.5). Pathway analysis revealed significant alterations in processes important for ‘cell adhesion’ (p<9.3e-13) and ‘extracellular matrix organisation’ (p<1.8e-7). Aggrecan (fold change=0.25, p<1e-7) and osteopontin (fold change=5.26, p<1e-5) were the first and third most-differentially regulated genes, indicating a shift away from chondrogenic polarization towards osteogenic polarization. A shift in BMSC polarization was corroborated with differentiation assays: MC1 vs. Ctrl BMSCs had a reduced adipogenic (mean %sd: -33±13%, p=0.03) and chondrogenic (-31±25%, p=0.18) differentiation capacity (Figure 2). In addition, an increased duplication rate of MC1 vs. Ctrl BMSCs (29.3±1.7 vs. 26.2±1.0 hours, p<0.01) was observed, indicating a change in phenotype. There were no changes in the expression of surface markers.

Conclusion: These data suggest that MPE-FLIM is a prime technology to investigate fibrotic pathologies and it allows to morphologically study the importance of BMSCs in MC1. The BMSC/adipocyte axis seems to play a pivotal role in the fibrotic pathomechanism. Adipocytes have not been regarded as pathomechanically relevant yet and hence open novel targets for therapeutic approaches.

References:


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