Disclosure of Interests: Mayuko Hayashi: None declared, Ryoko Sakai Grant/research support from: Tokyo Women's Medical University (TWMU) has received unrestricted research grants for Division of Epidemiology and Pharmacoepidemiology of Rheumatic Diseases from Ayumi Pharmaceutical Co. Ltd., Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd., Nippon Kayaku Co. Ltd., Taisho Toyama Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma Corp., and with which TWMU paid the salary of R.S. RS has received a research grant from Bristol-Meyers Squibb. Eiichi Tanaka Speakers bureau: Abbvie, Asahi Kasei Pharma Co., Bristol Myers Squibb, Chugai Pharmaceutical, Daiichi Sankyo Co., Eisai Pharmaceutical, Janssen Pharmaceutical K.K., Nippon Kayaku, Pfizer, Takeda Pharmaceutical, Taisho Toyama Pharmaceutical Co., and UCB Pharma., Takefumi Furuya Speakers bureau: the Asahi Kasei Pharma Corporation, Bristol-Myers Squibb, Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Ono Pharmaceutical Co., Ltd., Pfizer Japan Inc., Takeda Pharmaceutical Co., Ltd., and UCB Japan Co. Ltd. Eisuke Inoue: None declared, Mai Abe: None declared, Mika Kawano: None declared, Eri Sugano: None declared, Naohiro Sugitani: None declared, Kumiko Saka: None declared, Moeko Ochiai: None declared, Yoko Shimizu: None declared, Rei Yamaguchi: None declared, Naoki Sugimoto: None declared, Katsunori Ikari: None declared, Atsuo Taniguchi: None declared, masayoshi harigai Grant/research support from: Tokyo Women's Medical University (TWMU) has received unrestricted research grants for Division of Epidemiology and Pharmacoepidemiology of Rheumatic Diseases from Ayumi Pharmaceutical Co. Ltd., Bristol Myers Squibb, Chugai Pharmaceutical Co. Ltd., Nippon Kayaku Co. Ltd., Taisho Toyama Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma Corp., and with which TWMU paid the salary of MH. MH has also received research grants from AbbVie Japan GK, Eisai Co. Ltd., Takeda Pharmaceutical Co., Ltd., and Teijin Pharma Ltd., Hisashi Yamanaka Grant/research support from: AbbVie, Eisai, Bristol-Meyers, Novartis, Behringer, Astellas, Kaken, Nippon-Shinyaku, Pfizer, UCB, Ayumi, Ono, Daiichi-Sankyo, Taisyo-Toyama, Takeda, Tanabe-Mitsubishi, Chugai, Teijin Pharma, Torii, YLbio, Speakers bureau: Bristol-Meyers, Astellas, Pfizer, Daiichi-Sankyo, Takeda, Tanabe-Mitsubishi, Chugai, Teijin Pharma, YLbio
DOI: 10.1136/annrheumdis-2019-eular.3859

WEDNESDAY, 12 JUNE 2019

Tackling chronic pain; fibromyalgia and back pain__

OP0096

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 remodeled. These are signs 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, adjacent level healthy bone marrow (Ctrl)) or biopsies (n=2 MC1+2 Ctrl) 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 (SHG) of collagen were recorded of full-length biopsies with up to 200 µm penetration depth and qualitatively evaluated. Fluorescence-life-time-imaging-microscopy (FLIM) 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 (Wilcoxon 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)).

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 ($p \leq 0.01$; $\log_2 \text{ratio} \geq 0.5$). Pathway analysis revealed significant alterations in processes important for 'cell adhesion' ($p < 9.3e-13$) and 'extracellular matrix organization' ($p < 1.8e-7$). Aggrecan (fold change=0.25, $p < 1e-7$) and osteopontin (fold change=5.26, $p < 1e-5$) were the first and third top-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 $\pm$ sd: -33 $\pm$ 13%, $p=0.03$) and chondrogenic (-31 $\pm$ 25%, $p=0.18$) differentiation capacity (Figure 2). In addition, an increased duplication rate of MC1 vs. Ctrl BMSCs (29.3 $\pm$ 1.7 vs. 26.2 $\pm$ 1.0 hours, $p=0.07$) was observed, also indicate 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 seem to play a pivotal role in the fibrotic pathomechanism. Adipocytes have not been regarded as pathomechanistically relevant yet and hence open novel targets for therapeutic approaches.

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Disclosure of Interests: Stefan Dudli: None declared, Dominik Haenni: None declared, Astrid Juengel: None declared, Michael Betz: None declared, Jose Spig: None declared, Florian Brunner: None declared, Mazda Farshad: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb, espeR-are foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders

DOI: 10.1136/annrheumdis-2019-eular.1051