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Purpose: Recent *in vitro* studies demonstrated a contribution of the sympathetic neurotransmitter norepinephrine (NE) to cellular processes involved in osteoarthritis (OA) pathogenesis. These effects were mediated by different adrenoceptor (AR) subtypes that are expressed in all joint tissues. Most *in vitro* data suggest that especially the β2-AR plays an important role during OA progression. However, at present, no study exists that investigated the role of the β2-AR in the knee joint in vivo. Therefore, we examined the contribution of the β2-AR to OA progression using β2-AR-deficient ($Adrb2^{-/-}$) mice.

Methods: We used 12 weeks old male wildtype (WT, C57BL/6J) and $Adrb2^{-/-}$ mice (C57BL/6J background). OA was induced by destabilization of the medial meniscus (DMM) or Sham surgery was performed. 8 weeks after surgery, the severity of cartilage degeneration as well as synovial inflammation were evaluated by histological scoring (OARSI and synovitis grading). Calcified cartilage (CC) thickness and subchondral bone parameters such as subchondral bone plate (SCBP) thickness and bone volume density (BV/TV) were analyzed 8 weeks post-surgery in the medial condyles using micro-computed tomography (μCT). In addition, BV/TV of the subarticular trabecular bone was examined. Activity of osteoclasts and osteoblasts in the subchondral bone was analyzed by histological staining of alkaline phosphatase (ALP) and Cathepsin-K (CatK). Moreover, body weight and serum leptin concentrations (ELISA) were evaluated.

Results: WT and $Adrb2^{-/-}$ mice developed comparable changes in cartilage degeneration and synovial inflammation after DMM surgery (mean OARSI score: WT DMM 2.78 ± 0.32, $Adrb2^{-/-}$ DMM 3.09 ± 0.55; mean synovitis score WT DMM 2.75 ± 0.25, $Adrb2^{-/-}$ 2.75 ± 0.25). The μCT analyses revealed no significant differences in bone parameters of sham animals between WT and $Adrb2^{-/-}$. In contrast, DMM-operated $Adrb2^{-/-}$ mice displayed significant elevated CC thickness (WT DMM 64.54 ± 2.07 μm, $Adrb2^{-/-}$ DMM 74.68 ± 2.79 μm; $p = 0.01$), significantly increased SCBP thickness (WT DMM 107.7 ± 3.1 μm, $Adrb2^{-/-}$ DMM 170.50 ± 11.84 μm; $p < 0.001$), as well as significantly increased BV/TV (WT DMM 0.563 ± 0.029, $Adrb2^{-/-}$ DMM 0.713 ± 0.024; $p = 0.006$) in the medial epiphysis. Further examination of the BV/TV in the subarticular trabecular bone exposed no differences between WT and $Adrb2^{-/-}$ (WT DMM 0.134 ± 0.011, $Adrb2^{-/-}$ DMM 0.123 ± 0.019). Analysis of osteoclast activity by ALP-staining showed no difference in ALP^{+}-area in subchondral bone between WT DMM and $Adrb2^{-/-}$ DMM (WT DMM 0.906 ± 0.148 %, $Adrb2^{-/-}$ DMM 1.212 ± 0.083 %). In contrast, when analyzing osteoclast activity by CatK-staining, a significant decrease was detected in $CatK^{+}$-area in $Adrb2^{-/-}$ DMM compared to WT DMM mice (WT DMM 2.316 ± 0.572 %, $Adrb2^{-/-}$ DMM 0.526 ± 0.068 %; $p = 0.002$). $Adrb2^{-/-}$ mice had a significantly higher body weight compared to WT mice, regardless whether DMM or Sham surgery was performed ($Adrb2^{-/-}$ DMM 33.54 ± 1.18 g, WT DMM 28.56 ± 0.28 g; $p < 0.001$; $Adrb2^{-/-}$ Sham 33.51 ± 1.54 g, WT Sham 28.79 ± 0.25 g; $p < 0.001$). The elevated body weight is due to an increase in body fat mass. Serum leptin concentrations were significantly elevated in $Adrb2^{-/-}$ mice compared to WT after DMM surgery (WT DMM 8.48 ± 2.45 ng/ml, $Adrb2^{-/-}$ DMM 85.39 ± 40.07 ng/ml; $p < 0.001$), but not in Sham animals (WT DMM 4.85 ± 1.8 ng/ml, $Adrb2^{-/-}$ DMM 10.28 ± 1.19 ng/ml).

Conclusions: This study demonstrated that β2-AR deficiency did not affect cartilage degeneration and synovial inflammation but contributed to OA progression by aggravating OA-related calcification at the interface of cartilage and subchondral bone as well as subchondral bone remodeling. The thickening of calcified cartilage and subchondral bone in $Adrb2^{-/-}$ DMM mice was on the one hand directly mediated by β2-AR-deficiency by increasing osteoblast and suppressing osteoclast activities. On the other hand, β2-AR deficiency led to an increased fat mass with elevated serum leptin concentration. Elevated leptin release caused by OA-associated inflammation aggravated the OA-related thickening of subchondral bone in the same way by increasing osteoblast and inhibiting osteoclast activities. Thus, subchondral bone changes in $Adrb2^{-/-}$ DMM mice are the result of a synergistic effect of β2-AR deficiency (direct and indirect) and OA induction. Therefore, targeting the β2-AR represents a novel treatment option, which might help to develop tissue-specific therapeutic drugs for the prevention of pathological subchondral bone remodeling in OA patients.

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CD38 DEFICIENCY PROTECTS MICE FROM AGE-RELATED SPONTANEOUS OA DEVELOPMENT

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Purpose: CD38 is recently shown to be the main NADase in mammalian tissue, which contributes substantially to degradation of cellular nicotinamide adenine dinucleotide (NAD^{+}), a key metabolite involved in cellular energy metabolism and adaptive responses of cells to bioenergetics and oxidative stress. CD38 expression and activity are increased during aging, which contributes to age-related decline of NAD^{+}. In our previous studies, we observed an age-related increase in expression of CD38 in human knee cartilage, and that increased CD38 expression in chondrocytes is associated with reduced NAD/NADH levels and increased catabolic responses to pro-inflammatory cytokine IL-1β. In addition, inhibition of CD38 attenuates chondrocyte catabolic responses to IL-1β and significantly inhibited OA development in mice in a post-traumatic OA model. Maintenance of intracellular NAD^{+} content is implicated to be critical for tissue homeostasis. Since NADase activity is almost absent in CD38 knockout (KO) mice, we determined the effect of CD38 deficiency on age-related spontaneous OA development in mice *in vivo*.

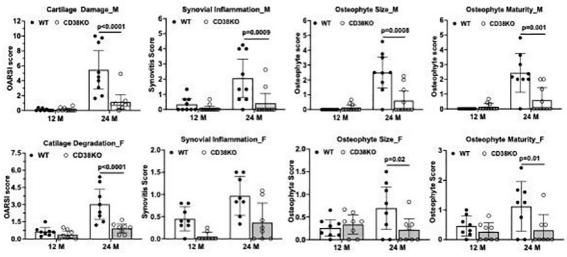
Methods: Both male or female CD38 KO and wild type (WT) mice at 12 and 24 months of age were used. Knee joints of these mice were collected, fixed, decalcified and embedded in coronal plane in paraffin, and sectioned for histological staining with safranin-O and fast green, as well as hematoxylin and eosin (H&E). The histological images were used to assess severity of cartilage damage of all four quadrants of the joint using the OARSI score system. The final OARSI scores were average sum scores of four quadrants. Synovitis scores were determined based on changes in synovial lining thickness and cellular density in the synovial stroma. Osteophyte formation was evaluated based on both size and maturity of osteophytes. Prism 9 GraphPad was used for statistical data analysis.

Results: Both male and female WT and CD38KO mice exhibited very little cartilage damage, synovitis, and osteophyte formation at 12 months of age. However, moderate cartilage damage was seen in both male and female WT mice at 24 months of age. Notably, the extent of cartilage damage was less in WT female with the mean OARSI score 2.6 compared to WT male mice (the mean OARSI score 5.47) at 24 months of age. Compared to WT mice, cartilage damage was significantly reduced with the mean OARSI scores 1.14 and 0.4 for male and female CD38 KO mice, respectively, suggesting that CD38 deficiency was chondroprotective (Table I and Figure 1). Synovitis and osteophyte size and maturity were also observed in both male and female WT mice, but they were milder in female compared to male WT mice with the mean scores of 2.07, 2.5 and 2.44 for male and 0.97, 0.69, 1.12 for female, respectively (Table I and Figure 1). These were significantly inhibited in both male and female CD38 KO male mice at 24 months of age (Table I and Figure 1). These results indicate that both male and female mice deficient in CD38 were protected from age-related spontaneous OA development.

Conclusions: Taken together with our previous finding that CD38 inhibitor apigenin limits post-traumatic OA development and associated pain in mice, inhibition of CD38 could be a novel therapeutic approach for OA prevention and treatment.

Table I		WT mean (95% CI)		CD38KO mean (95% CI)	
		12 M	24 M	12 M	24 M
OARSI	male	0.1 (0.02, 0.2)	5.47 (2.91, 8)	0.14 (0.03, 0.3)	1.14 (0.16, 2.1)
	female	0.46 (0.24, 0.67)	2.61 (1.44, 3.78	0.28 (0.03, .54)	0.4 (0.06, 0.87)
synovitis	male	0.34 (0.03, 0.7)	2.07 (0.8, 3.3)	0.08 (0.05, 0.22)	0.41 (0.23, 1.06)
	female	0.45 (0.22, 0.68)	0.97 (0.6, 1.34)	0.05 (0.03, 0.14)	0.37 (0.011, 0.74)
osteophyte-size	male	0	2.5 (1.45, 3.55)	0.14 (0.03, 0.32)	0.61 (0.04, 1.26)
	female	0.026 (0.08, 0.43)	0.69 (0.22, 1.17)	0.33 (0.13, 0.54)	0.22 (0.03, 0.46)
osteophyte-maturity	male	0	2.44 (1.4, 3.47)	0.14 (0.03, 0.32)	0.58 (0.03, 1.2)
	female	0.46 (0.17, 0.75)	1.12 (0.42, 1.82)	0.26 (0.01, 0.52)	0.31 (0.13, 0.76)

Figure 1. Both male and female mice deficient in CD38 were protected from age-related spontaneous OA development.



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DEFINING THE HIERARCHY OF FIBROBLASTS AND THEIR STEM CELLS IN THE ADULT SYNOVIAL JOINT AT SINGLE CELL RESOLUTION

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Purpose: The synovium, a specialised connective tissue, encapsulates synovial joints providing a barrier between the joint space and surrounding tissues. The healthy synovium consists of two layers, the sub-lining, composed of fibroblasts and other cell types, and the synovial lining that contains a specialised tissue-resident fibroblast known as the fibroblast-like synoviocyte (FLS). The FLS and sub-lining fibroblasts play a critical role in joint health and osteoarthritis. In the healthy joint, FLS produce essential joint lubricants such as hyaluronic acid and lubricin. In contrast, in osteoarthritis, synovial fibroblasts exhibit enhanced expression of inflammatory cytokines, chemokines, matrix metalloproteinases and other catabolic enzymes leading to cartilage breakdown. In the adult knee, FLS and sub-lining fibroblasts are derived, in part, from embryonic *Gdf5*-expressing joint progenitor cells, a subset of Pdgfrα-expressing fibroblasts, present in the joint interzone. These cells give rise to synovial joints during development forming joint tissues such as the synovium, cartilage, menisci and ligaments. Despite their critical roles, understanding of synovial fibroblast ontogeny, phenotypic diversity, molecular regulation of fate determination and renewal from adult stem/progenitor cells is limited.
Methods: Synovial fibroblasts were isolated from adult transgenic *Gdf5-Cre*;*Tom;Pdgfrα-H2BGFP* mouse knees, either healthy or 6 days after injury to the articular cartilage. Cells were sorted, by fluorescence-activated cell sorting, based on their ontogeny, and processed for single-cell RNA-sequencing using the 10x Genomics Chromium controller. Transcriptomic analysis was performed using the Seurat R package. RNA velocity was analysed using scVelo to infer differentiation pathways, and gene regulatory networks (regulons) were analysed to determine molecular regulation of fate determination using SCENIC. Cell cycle analysis was performed computationally to identify proliferating synovial fibroblast populations and confirmed by Ki67 immunofluorescence staining.
Results: In healthy joints, sub-lining fibroblasts were found to be of mixed ontogeny with partly overlapping phenotypes, while FLS descended from the *Gdf5*-expressing cells of the embryonic joint interzone. After cartilage injury, we identified an actively cycling, facultative *Gdf5*-lineage stem cell population that supplied new chondrocytes, immunoregulatory fibroblasts and FLS, the latter via transit-amplifying progenitors. Furthermore, we detected the appearance of injury-induced FLS and chondrocyte sub-populations that are not of *Gdf5*-lineage, demonstrating lineage plasticity during repair. Finally, we reveal the molecular regulation of the synovial fibroblast phenotype, with both mouse and human FLS exhibiting *Sox5* and *Creb5* regulon activity regardless of ontogeny or injury.
Conclusions: Our findings elucidate the functional hierarchies and differentiation trajectories of ontogenetically defined stromal cell populations in the knee, from adult stem cells to FLS and immunoregulatory fibroblasts, and provide novel insight into the molecular regulation that governs cell fate in the adult joint. These data advance our knowledge of the cell populations that maintain and repair the synovial joint in adult life.

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INHIBITION OF HISTONE DEMETHYLASES AS AN APPROACH TO RESTORE DEFICIENT DOT1L ACTIVITY IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA), the most common chronic joint disease worldwide, is characterized by progressive damage to the articular cartilage, increased joint-associated bone remodelling, and synovial inflammation. Current OA treatments are limited to pain relief, physiotherapy or replacement surgeries in severe cases, yet disease-modifying drugs are lacking. A genome-wide association study (GWAS) revealed a genetic association between polymorphisms in the *DOT1L* gene and OA. The *Disruptor of telomeric silencing 1-like (DOT1L)* gene encodes a unique histone methyltransferase that methylates Lysine 79 of Histone H3 (H3K79). We previously identified DOT1L as a key protector of cartilage homeostasis, using both human articular chondrocytes and different *Dot1l* genetic mouse models. Furthermore, we reported that DOT1L activity, indicated by the levels of H3K79 methylation (H3K79me), is reduced in OA as compared to non-OA cartilage. Therefore, maintaining H3K79me seems to be critical to preserve joint health and prevent the development or progression of OA. Here, we hypothesized that H3K79me could be restored or maintained by inhibiting demethylation at the H3K79 site, via targeting specific histone demethylases. There are two main families of histone demethylases: the lysine-specific demethylases (LSD) and Jumonji C (JmjC) demethylases. The LSD family contains two members: LSD1 and LSD2. The JmjC family is further classified into 6 subfamilies: from KDM2 to KDM7. In this study, we aimed to investigate which histone demethylases are responsible for H3K79 demethylation and whether their specific targeting can lead to protective effects in OA.
Methods: We determined the baseline mRNA expression of a panel of histone demethylases in primary human articular chondrocytes (hACs) from non-OA patients, and mapped their expression upon OA-mimicking stimuli (IL-1β and Wnt signalling activator CHIR99021), using real-time qPCR. To interrogate the role of JmjC demethylase family in H3K79me, human articular chondrocyte C28/I2 cells were treated with the JmjC pan inhibitor JIB-04. In parallel, the LSD family members LSD1 and LSD2 were pharmacologically inhibited using LSD1 inhibitor II or silenced using siRNA against LSD2, respectively. The role of the different JmjC subfamilies on H3K79me was studied using selective pharmacological inhibitors. We assessed the levels of H3K79me by Western blot and immunofluorescence analysis. Furthermore, we used a 3D micromass model of C28/I2 cells to evaluate changes in glycosaminoglycan content by Alcian blue staining upon histone demethylase targeting. siRNA silencing was used to dissect the individual role of selective JmjC histone demethylases on H3K79me in C28/I2 cells. This was followed by a translational assessment of the individual targeting in hACs from OA patients. In this setup, gene expression of healthy cartilage markers *Collagen2a1* (*COL2A1*) and *Aggrecan*, as well as of catabolic markers *MMP13* and *ADAMTS5*, was evaluated using real-time qPCR. Intra-articular injection of daminozide (inhibitor of KDM2/7 subfamily) was performed in an *in vivo* OA mouse model induced by destabilization of the medial meniscus (DMM), and histological analyses were performed.
Results: We found striking differences in the baseline expression of the different histone demethylases in hACs. Treatment with proinflammatory cytokine IL1-β resulted in an increase in *KDM6B* and *KDM7A* mRNA expression. Conversely, Wnt signalling activation by CHIR99021 led to a downregulation in most of histone demethylases' mRNA expression. Interestingly, pharmacological inhibition of the JmjC family using JIB-04 resulted in increased H3K79me levels in human articular chondrocytes. However, blockade of LSD family members did not lead to H3K79me changes. Inhibition of all JmjC demethylase subfamilies increased H3K79me levels, but only targeting of KDM2/7 and KDM6 subfamilies led to an increase in glycosaminoglycan content. Individual silencing of *KDM2B*, *KDM7A*, and *KDM6B* increased H3K79me. Interestingly, specific knockdown of *KDM7A* resulted in increased expression of chondrocyte healthy markers while reducing the expression of catabolic markers. Histological assessments after intra-articular injection of daminozide (inhibitor of the KDM2/7 subfamily) in DMM mouse model increased