Methods: Cybrids were developed using 143B.TK Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy (N) and OA donors. Glucose and FA metabolism were measured using D-[1-14C(U)]glucose and [1-14C] oleic acid respectively. Metabolic flexibility was evaluated by co-culturing with glucose and oleic acid acutely by using inhibitors against glucose and FA oxidation. 20µM UK5099 and 10µM etomoxir, respectively. Incorporation of FA into lipid droplet (LD) was evaluated by thin layer chromatography and LD were stained by LDS40 and analyzed by confocal microscopy and flow cytometry. Mitochondrial dynamics was measured by real-time PCR method. Percentage of mitochondrial Anion Superoxide (O$_2^-$) production was evaluated incubating cells with MitoSox® using Flow Cytometer. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: There were no changes in basal glucose metabolism between cybrids. N cybrids had higher acid-soluble metabolites, reflecting incomplete FA β-oxidation than OA cybrids. Comparing glucose and FA metabolism showed that both types of cybrids preferred to oxidize glucose. Co-culturing with glucose and Oleic acid, increased total cellular uptake and oxidation of glucose in N compared to basal condition (Figure-1) and in this condition the OA cybrids showed increased oxidative stress, inhibition of FA oxidation by etomoxir increased complete glucose oxidation of N cybrids but not in OA cybrids that had a preference to oxidize oleic acid compared to basal condition. Gene expression of mitochondrial 2 (MFN2) was higher in N than OA cybrids under inhibiting conditions. Complement data indicate that N cybrids are more metabolically flexible and have better adaptive response than OA. Cybrids presented different lipid distribution patterns. Lipid droplet (LD) formation increased in both groups incubated in presence of FA. Furthermore, N cybrids showed less LD formation than OA.

Conclusion: The results indicated that cybrids from OA patients had reduced metabolic flexibility compared to N cybrids. These results enhance our understanding of the mitochondrial metabolism in OA, suggesting a mitochondrial dysfunction and impairment of metabolic flexibility during the OA process.

**Figure 1.** Effect of oleic acid in glucose metabolism. A. Scheme of substrate oxidation protocol: cybrids were cultured for 48 h in DMEM-glucose and glucose metabolism was evaluated using D-[1-14C(U)]glucose. B. Effect of 100 µM oleic acid compared to 5.5 mM glucose (basal) on glucose metabolism in N and OA cybrids. Values are presented as mean ± SEM relative to basal. * P<0.05, unpaired t test. b versus basal (lb p<0.01, paired t test).

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addition to age and previous injury in target knee, the GG genotype (p=0.032) emerged as a potential risk factor for the RPOA when compared with non-rapid progressors (Table 2).

Table 2. Multinomial regression model comparing rapid vs. no-rapid progressors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>p-value</th>
<th>OR</th>
<th>CI: 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.000</td>
<td>1.064</td>
<td>1.041, 1.088</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>0.498</td>
<td>0.875</td>
<td>0.595, 1.287</td>
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<tr>
<td>BMI</td>
<td>0.096</td>
<td>1.034</td>
<td>0.994, 1.077</td>
</tr>
<tr>
<td>Contralateral OA (Yes)</td>
<td>0.792</td>
<td>1.052</td>
<td>0.719, 1.539</td>
</tr>
<tr>
<td>Previous Injury (Yes)</td>
<td>0.028</td>
<td>1.523</td>
<td>1.047, 2.216</td>
</tr>
<tr>
<td>WOMAC pain</td>
<td>0.032</td>
<td>1.055</td>
<td>0.992, 1.123</td>
</tr>
<tr>
<td>rs12107036 GG (Yes)</td>
<td>0.032</td>
<td>1.574</td>
<td>1.039, 2.382</td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: Odd Ratio; #: statistical significance declared at P ≤ 0.05

Conclusion: The G allele of the nuclear SNP rs12107036 of TP63 gen increases the risk of knee OA progression. Depending on the number of risk allele copies the level of progression varies, being the GG genotype a risk factor for the RPOA of the knee. The assignment of this nuclear polymorphism could be useful as complementary genetic biomarker for the early identification of this phenotype.

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THU0013 INTEGRATED ANALYSIS OF SYNOVIAL SINGLE CELL RNA SEQUENCING DATA DEEPENS THE CURRENT KNOWLEDGE OF SYNOVIAL PATHOLOGY IN ARTHRITIS

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Background: The heterogeneity of synovial tissues from patients with arthritis could contribute to the interpatient variability in disease course, prognosis and treatment response. Single-cell RNA sequencing (scRNA-seq) permits in-depth analysis of tissue heterogeneity, which could facilitate drug discovery and patient stratification for precision medicine.

Objectives: To construct a comprehensive landscape of synovial cell types and molecular pathways in arthritis by integrating our and published scRNA-seq data, generated across different scRNA-seq technologies [Smart-seq2, Drop-seq], cell preparation protocols [dissociated unsorted, sorted cells] and types of arthritis [differentiated (UA), rheumatoid arthritis, osteoarthritis].

Methods: Synovial tissues were obtained by ultrasound-guided biopsy from patients with UA [not fulfilling the classification criteria for a specific arthritis, n=3], Biopsies were disintegrated [enzymatic and mechanical disruption] and cell viability assessed with trypan blue. scRNA-seq libraries [2 per patient] were prepared with 10X Genomics Drop-Seq and sequenced on NovaSeq6000. Bioinformatics analysis of our and published [n=35] datasets was performed using Seurat protocol with correction for batch effects and filtering low-quality cells. Functional enrichment analysis of marker genes in clusters was done with STRING Protein-Protein networks. Synovitis was assessed with ultrasound and histology.

Results: Our tissue disintegration protocol resulted in good cell yield and viability (92%, 72%, 100%). The synovial cellular heterogeneity detected by scRNA-seq reflected the histological findings [Krenn score, pathotype]. These were supported with the ultrasound and clinically assessed disease activity. The integrated analysis of 41 datasets from 38 donors yielded 41845 scRNA-seq cell profiles, 50% contributed by our dataset. An independent analysis of our data and their integration with published data showed that different scRNA-seq methods and protocols can identify all the major synovial cell types and their activation states (Figure 1) with large heterogeneity between donors. We identified a previously undescribed synovial cell population, which was located near the fibroblast cluster, was negative for canonical cell markers, but highly enriched in cell division genes (80% of marker genes). These cells comprised a mixed population of CD34-, podoplanin (PDPN)high or PDPNhigh cells that were mostly negative for the sub-lining fibroblast marker THY. Furthermore, they appeared to be highly secretory (extracellular matrix components) and their gene expression profile was inclined towards cell migration, vascular development and insulin growth factor-dependent processes.

Figure 1. Heatmap with top 20 cluster gene markers, gene enrichment analysis and UMAP plot of synovial cell clusters.

Conclusion: By integrating synovial scRNA-seq data from 41845 cells, we identified a previously undescribed, highly proliferative and secretory synovial cell population in arthritis. We increased the number of known scRNA-seq synovial cell profiles in arthritis by two-fold and demonstrated the robustness of synovial scRNA-seq data outputs across different technologies and protocols. This broadens the current knowledge of synovial tissue heterogeneity and pathology in arthritis.

References:

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