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 LD540 and analyzed by confocal microscope and flow cytometry. Mitochondrial dynamics was measured by real-time PCR method. Percentage of mitochondrial Anion Superoxide (O₂⁻) 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 an increase in mitochondrial O₂⁻ production. 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 mitofusin-2 (MFN2) was higher in N than OA cybrids under inhibiting conditions. Combine these data indicate that N cybrids are more metabolically flexible and have better adaptative 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 mitochondria metabolism in OA, suggesting a mitochondrial dysfunction and impairment of metabolic flexibility during the OA process.

Figure-1

Figure -1. Effect of oleic acid in glucose metabolism. A. Scheme of substrate oxidation protocol: cybrids were cultured for 48 h in DMEM-glu/ole 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. * N versus OA cybrids (*p≤0.05, unpaired t test). b versus basal (bb p≤0.01, paired t test).

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THU0012

IMPACT OF RS12107036 POLYMORPHISM OF TP63 ON THE RISK OF RAPID PROGRESSIVE OSTEOARTHRITIS OF THE KNEE. DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Background: There is a need to identify patients with the rapid progressive phenotype of Osteoarthritis (RPOA) to include them in clinical trials and to implement prevention strategies. During the last years, nuclear single nucleotide polymorphisms (SNPs) were associated with susceptibility and progression of the disease, but not with the rapid progression phenotype.

Objectives: Analyze the influence of previously knee OA-associated nuclear SNPs on the risk of RPOA in patients of the OAI.

Methods: Caucasian patients from the OAI were selected and assigned into three different groups (N=252/group) based on the following criteria:

A.rapid progressors; baseline KL grade 0-1 in at least one knee and increase up to KL≥ 3 during a 48-month period; or baseline KL grade 2 in at least one knee and increase up to KL 4 or total knee replacement during the follow-up.

B.no-rapid progressors; baseline KL grade 0-1 in at least one knee and increase up to KL 2 during 48-month period; or baseline KL grade 2 in at least one knee and increase up to KL 3 during the follow-up.

C.no-progressors; KL grade 0-2 at baseline in at least one knee and bilaterally stable during 48-month period.

Groups were re-categorized into two groups: non-progressors and progressors (pooling A and B). Nuclear SNPs were previously assigned by mini-sequencing techniques. Preliminary chi-square analyses and binary and multinomial logistic regression models adjusted by gender, age, body mass index (BMI), contralateral OA, previous injury in target knee and WOMAC pain, were performed with IBM SPSS Statistics v24.

Results: We analyzed the effect of 7 SNPs that had been strongly associated with knee OA susceptibility in different GWAS studies: rs11177, rs4730250, rs11842874, rs12107036, rs8044769, rs10948172 and rs143383. Chi-square analyses only showed differences in the frequency distribution of rs12107036 between groups (p=0,028), being the GG genotype over-represented in the rapid progressors group and the AA genotype in the non-progressors group (Figure 1). The binary logistic regression showed that G allele was significantly over-represented in the (pooled) progressors group when compared with non-progressors (p=0,008) (Table 1). And the multinomial logistic regression showed that, in

Figure 1. Frequency distribution of the rs12107036 genotypes among the different progression groups

Table 1. Binary regression model comparing progressors pool vs. no-progress

Variables	p-value	OR	C.I. 95%	
			Min.	Max.
Age	0,217	1,012	0,993	1,030
Sex (Female)	0,000 [#]	2,049	1,478	2,842
BMI	0,000 [#]	1,085	1,044	1,127
Contralateral OA (Yes)	0,044 [#]	1,400	1,009	1,942
Previous Injury (Yes)	0,002 [#]	1,723	1,223	2,429
WOMAC pain	0,003 [#]	1,102	1,033	1,177
rs12107036 G (Yes)	0,008 [#]	1,682	1,148	2,463

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

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.

Variables	p-value	OR	C.I. 95%	
			Min.	Max.
Age	0,000 [#]	1,064	1,041	1,088
Sex (Female)	0,498	0,875	0,595	1,287
BMI	0,096	1,034	0,994	1,077
Contralateral OA (Yes)	0,792	1,052	0,719	1,539
Previous Injury (Yes)	0,028 [#]	1,523	1,047	2,216
WOMAC pain	0,091	1,055	0,992	1,123
rs12107036 GG (Yes)	0,032 [#]	1,574	1,039	2,382

CI: confidence interval; OR: Odd Ratio; #: statistical significance declared at $P \leq 0.05$

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 [undifferentiated (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] datasets¹⁻³ was performed using Seurat protocol⁴ 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, pathology]. 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 PDPN^{low} 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.

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