Chondrocyte protein co-synthesis network analysis links ECM mechanosensing to metabolic adaptation in osteoarthritis

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Objective: To systematically investigate the modular structure of the chondrocyte proteome organisation in healthy and osteoarthritic (OA) cartilage.

Design: We implemented a systems approach by making use of the statistical network concepts in Weighted Gene Co-expression Analysis to reconstruct the organisation of the core proteome network in chondrocytes obtained from OA patients and healthy individuals. Protein modules reflect groups of tightly co-ordinated changes in protein abundance across healthy and OA chondrocytes.

Results: The unbiased systems analysis identified extracellular matrix (ECM) mechanosensing and glycolysis as two modules that are most highly correlated with the disease. The ECM module was enriched in the OA genetic risk factors tenascin-C (TNC) and collagen 11A1 (COL11A1), as well as in cartilage oligomeric matrix protein (COMP), a biomarker associated with cartilage integrity. Mapping proteins that are unique to OA or healthy chondrocytes onto the core interactome of ECM-mechanosensing-glycolysis identified differences in metabolic and anti-inflammatory adaptation.

Conclusion: The interconnection between ECM remodeling and metabolism is indicative of the dynamic chondrocyte states and their significance in osteoarthritis.

Introduction

Knee osteoarthritis (OA) is the second most common musculoskeletal disorder affecting approximately 22.9% of individuals 40 years and over globally[1]. The disease is phenotypically heterogeneous with heterogeneity extending beyond histopathology to the predisposing risk factors (reviewed in[2]). The lack of effective disease modifying drugs and preventive tools highlights our incomplete understanding of the fundamental biological aspects of OA.

Large scale quantitative analyses of almost all molecular domains have aimed at elucidating the molecular mechanisms underlying the cause and progression of osteoarthritis [3-6]. Quantitative differences between the levels of gene transcription or proteins associated with specific phenotypes represents only the first step of the systematic understanding of human disease. In OA and healthy cartilage, the overlap between differentially expressed genes and differentially abundant proteins is limited to 15-16%, likely owed to the alternative post-transcriptional and post-translational control of protein levels [4,5] .

It is known that most disease phenotypes are the end-product of the interaction between proteins that mediate disease-associated processes. These biologically meaningful interactions are well modelled by so-called "network modules" which in the case of proteomes represent functionally coherent protein interactomes mediating the same biological processes[7]. Network theory concepts have been used to study large-scale datasets obtained from a variety of biological systems and have proven valuable for the dissection of biological mechanisms contributing to complex diseases [8,9].

Our previous quantitative proteomic analysis revealed abundance differences between healthy and OA chondrocytes in proteins enriched in metabolic and cytoskeletal processes, including those of the tricarboxylic acid (TCA) cycle and adhesion, cytoskeletal remodeling and cell-matrix interactions, suggesting a possible link between sensing of the ECM microenvironment and regulation of metabolism through ECM-cell focal adhesion and cytoskeletal dynamics [10]. Metabolic processes including lipid synthesis and glycolysis are regulated by changes in cytoskeleton dynamics induced by sensing of the ECM mechanical properties (reviewed in [11]). Rewiring chondrocyte metabolism in the context of OA has been extensively studied[12] and quantitative metabolomic profiling has revealed a link between chondrocyte metabolic adaptation and physiological compressive mechanical loads[13]. However, how chondrocytes' metabolism is regulated by the OA-altered ECM through mechanical cues caused by the combination of ECM degradation and the increase in cell-cell adhesion in the proliferative zone, remains elusive.

Here we revisited our previous proteomics dataset and implemented a systems modular approach to reconstruct in an unbiased way the organisation of the chondrocyte proteome and to parse the shared chondrocyte protein interactome in disease-associated modules. Construction of the chondrocyte protein network yielded modules of tightly covarying proteins which are associated with OA providing new insights into the connections between ECM mechanosensing and adaptive changes in metabolism, anti-inflammatory and antioxidant processes.

Methods

Patients and proteomic data

For the protein co-synthesis network analysis we used the common (shared OA-Normal) processed spectral count data obtained from our published study (n=937), which are reported in Suppl. Table 1 [10]. Articular cartilage was obtained from 10 osteoarthritic patients who had undergone total knee replacement surgery and 6 non-OA (termed healthy) adults. Peptides were obtained by 1D-SDS-PAGE and in-gel digestion and were analyzed using nano-Reverse Phase (RP) LC coupled to an LTQ-Orbitrap XL or an Orbitrap QE instrument. The Xcalibur 2.2 software (Thermo Scientific) was used for data acquisition. Protein abundance levels were determined by counting label-free spectra by implementing the methods in the Scaffold software, as previously described[10].

Data imputation and batch correction

Missing peptide counts and variation owing to systematic biases are inherent problems of shot-gun, label-free quantification [14]. We addressed these factors in our dataset by simulating the dataset and imputing the missing values with Multivariate Imputation By Chained Equations (MICE) [15] and by correcting for batch effects with RUVseq [16] package functions in R. We implemented the MICE algorithm with 10 imputations using a predictive mean matching model and a random number generator, seed=1 in log2 transformed data. To confirm the reliability of the 1st imputation we tested the correlation between the imputed and raw data (Pearson's $r=1$, $p<0.01$) (Fig.S1). We then upper-quantile normalized the simulated spectral counts and corrected unwanted variation i.e. not explained by the experimental design by implementing the RUVr function by factor analysis on deviance residuals after a running a regression of the counts on the covariate of interest (disease status). We chose to exclude an

outlier healthy sample (X152) following unsupervised hierarchical clustering because it mapped in the OA cluster (data not shown).

Weighted gene co-expression analysis (WGCNA)

WGCNA was performed with the tools in the WGCNA R package[17] on the simulated and batch corrected proteome data. We constructed a signed network of weighted correlations by calculating a pairwise correlation matrix and then raising the co-expression measure to a power β=30 to obtain the adjacency matrix. The power is a soft-threshold of the correlation matrix and its value is set to preserve the scale-free topology of the network (Fig S1). To identify "modules of co-synthesized proteins" in chondrocytes (hereafter "modules") we calculated the connection strength of each protein with all other proteins in the dataset and clustered the proteins based on their topological overlap. Briefly, the topological overlap matrix (TOM) was calculated (the strength of the correlation of 2 proteins with respect to all other proteins in the network) and the TOM dissimilarity measure (1-TOM) was used in hierarchical clustering with Ward's minimum variance method. We used the Dynamic Hybrid Tree Cut algorithm to cut the branches of the hierarchical clustering tree. Each branch represents a module, i.e. a group of highly correlated protein levels across healthy and OA samples. We set the minimum module size to 15 proteins. The expression profile of each module was then summarized by calculating the first principal component termed as module eigenprotein (MEP) with singular value decomposition of the scaled protein abundance. Modules whose MEs were highly correlated (r>0.8) were merged into one module. Meta-modules are defined as modules that form a single cluster in hierarchical clustering with the distance measure defined as 1-corrMEPs. The power of trait-module correlations tests was adjusted for multiple testing with the Benjamini-Hochberg method using R's base function. Disease significance is the averaged absolute correlation (r) between the MEP levels in a particular module with OA. Unsupervised hierarchical clustering was performed with Euclidean distances using Ward's minimum variance method.

Module annotation

Over-representation analysis was run for the pathways, biological processes ontology domains using the NIH NCATS Bioplanet 2019 database[18] and other functional (GO Biological Process) and disease databases (GWAS catalog 2019) in Enrichr[19]. Briefly, we mapped Uniprot IDs to gene symbols and we submitted the protein list to the Enrichr web service. Of the 10 top terms

we determined the most relevant by considering the redundancy of the enrichment and those with FDR<0.05.

Network and data visualization

Network visualization was performed in Cytoscape v.3.8.2. Known and predicted protein-protein interactions (PPIs) with combined score of high confidence (>0.7) were obtained from the STRING database[20]. We parsed the PPI network by implementing an edge-weighted spring embedded layout algorithm weighted by the String score into clusters of nodes that are highly attracted and at the same time repelled from other nodes or clusters of nodes. Briefly, the combined String score is computed by combining the probabilities from the different evidence sources, correcting for the probability of randomly observing an interaction. Data visualization was performed in R by using package built-in plotting functions and ggplot2 [21].

Results

Chondrocyte protein co-synthesis network construction in osteoarthritic and healthy cartilage

We have previously identified 937 proteins shared between OA and healthy cartilage proteome[10]. We now tested whether the shared chondrocyte proteome could be a classifier for osteoarthritic and healthy cartilage. We found that two proteome clusters classify samples by disease status and K/L score only and we therefore did not adjust for BMI, age or sex ~~K/L score~~ in subsequent analyses. Therefore, the shared proteome mediates biological processes that are altered in chondrocytes damaged by OA compared to healthy cells.

We then implemented WGCNA and parsed the shared proteome in 13 groups of strongly co-synthesized proteins termed modules (Fig. 1A, Fig. S1 and Table SII). Protein modules commonly include the functional protein components of a specific biological process or pathway [22]. We therefore tested which processes are represented by the module proteins and found that the 13 modules differ in the top enriched functional terms (Table SIII). Specifically, 4 out of the 13 modules were associated with metabolic processes including glycolysis (MEyellow), mitochondrial β-oxidation of unsaturated fatty acids (MEcyan), benzo(a)pyrene metabolism (MEtan) and amino acid metabolism (MEblue) (Table SIII). Metabolic changes in processes including fatty acid synthesis, amino acid synthesis, mitochondrial respiration and glycolysis in chondrocytes have been associated with impaired chondrocyte function and

disease progression [12]. MEGreenYellow is highly enriched in ECM-receptor interactions and includes proteins with high biological relevance to articular cartilage integrity and OA biology as COMP, COL11A1, COL6A2, TNC, fibronectin type I (Fn1) and hyaluronan and proteoglycan link protein 1 (HAPLN1). MEbrown is associated with clathrin-derived vesicle budding, a process that mediates exosome formation, which plays a role in articular cartilage integrity maintenance (reviewed in[23]). The pathway downstream of ERBB1- epidermal growth receptor (EGFR) signaling (MEblack) is essential for the maintenance of chondrocyte growth in the superficial zone of the articular cartilage[24]. The top term for MEgreen is the mu-calpain pathway and includes Rho GTPases RHOA, RAC1 and talin 1(TLN1).

Thus, WGCNA parsed the shared chondrocyte proteome in modules with biologically meaningful enrichment in osteoarthritis associated processes.

Chrondrocyte proteome modules associate with osteoarthritis

We hypothesized that the processes involved in impaired chondrocyte function and OA pathology would be organized in modules that would be strongly associated with the disease. To test this, we correlated the module protein synthesis levels to all traits (Fig. 1A) and found that 5 of the 13 modules were strongly and significantly correlated with OA (Benjamini-Hochberg, FDR<0.05) (Fig. 1A). To better understand the relationship between OA and the module protein synthesis levels, we calculated the module Disease Significance (DS) (see Methods) and found that MEblue, MEyellow, MEgreenyellow and MEgreen protein level changes are associated with OA (DS>0.5)(Fig. 1B). Furthermore, MEblue, MEyellow and MEgreenyellow form a meta-module (single branch in dendrogram, Fig.1A) representing the higher-order organization of the proteins in these 3 modules.

We then hypothesized that the most essential proteins for the maintenance of healthy cartilage would have tightly coordinated synthesis levels across samples and high disease significance. In the chondrocyte network these parameters are represented by high intra-modular connectivity and high DS (Fig. S3B,C). Our rationale is supported by evidence showing that proteins with high module interconnections are commonly essential to the processes they mediate[25] and are associated with disease[26]. The meta-module comprises protein components of ECM organization, integrin mediated focal adhesion, amino-acid and protein turnover as well as glycolysis and general metabolism (Fig. 1C). Therefore, essential proteins involved in glycolysis-gluconeogenesis are highly connected to key structural components of cartilage ECM organization and focal adhesion in the meta-module (Fig.1C,D). MEgreen comprises proteins involved in cytoskeleton dynamic changes in response to mechanical or ECM

remodeling stimuli, like the GTPase RAC1, the major cell surface adhesion molecule for hyaluronan CD44, as well as proteins involved in translation and osteoblast differentiation (integrin-αv-β3-osteopontin and sphingosine-1-phosphate S1P[27]) (Fig. 1C ,Tables SIII and SIV).

Meta-module protein levels display a strong negative correlation with OA highlighting the role of ECM integrity and metabolism in chondrocyte-cartilage homeostasis. As expected from the direction of the module-trait correlations, the abundance of the proteins in the meta-module was increased in healthy compared to OA chondrocytes (Fig.1D). On the other hand, protein levels in MEgreen are positively associated with OA and decrease in healthy compared to OA cells (Fig.1D).

Altogether, this analysis suggests that highly coordinated protein abundance changes in metabolic and ECM sensing processes are associated with healthy and OA chondrocytes.

Overlap of the altered interactome with an independent OA chondrogenesis proteome

We next sought to assess the degree of relevance between the chondrocyte proteome synthesis changes and the reported protein changes during chondrogenesis. We obtained the overlaps between our entire proteome (n=937) and the list of significantly up/down regulated proteins (n=43) reported in Rocha et al.[28]. Of the 43 significantly altered proteins, 37 overlapped with the chondrocyte proteome (86.04%) and of those, 20 are members of the OA-associated modules with the majority (12/20) mapping to MEyellow (Fig. S3). The direction of abundance level changes is concordant for 16 of the 20 overlapping proteins. The concordant group includes structural and matricellular cartilage ECM components (e.g. COL6A2, TNC), several glycolytic enzymes (e.g. GAPDH, TP1, LDHA) and cytoskeleton structural proteins (e.g. TPM4 and TLN1) (Fig.S3). Importantly, the proteins with discrepant changes are not essential members of the chondrocyte modules.

From this comparison we conclude that the 16 concordant overlapping proteins are central to the chondrocyte specific metabolic and cytoskeleton -ECM functions and that both glycolytic enzymes and ECM components are depleted in OA compared to healthy chondrocytes.

The chondrocyte protein interaction network links extracellular matrix remodeling to glucose metabolism

To assess the biological functional interactions between proteins in the meta-module and their functional inverse relationship to the proteins in the MEgreen module. (Fig.2A) we next tested the network for enrichment in protein- protein interactions in the STING database (PPI

enrichment , p< e-16). The parsed network yielded 3 prominent peripheral clusters with functional distinctions including cartilage-ECM-focal adhesion, glycolysis-TCA cycle, protein and amino-acid biosynthesis. The majority of essential proteins of the MEgreen module (abundant in OA chondrocytes) are located at the center of the network (18/30 proteins) including central components of integrin-focal adhesion (TLN1, RAC1, RHOA), cytoskeletal dynamics (DSTN, CCT2, CCT8, TUBA1B) and protein turnover (PSMD, EIF, RPL). We found that the top 20 proteins with the highest degree in the interactome, are important glycolytic enzymes including GAPDH, TPI1, ALDOA, PGM1, GPI, ENO1, PGK1, LDHA, PKM, LDHB, MDH1 (Fig. 2B). Top degree nodes include FN1, master actin tubulin folding chaperones CCT2 and CCT8 of the CCT/TriC complex, P4HB a multifunctional enzyme involved in the hydroxylation of the propyl residues in pro-collagen, the molecular motor DYNC1H1 and chaperones HSP90B1 and HSPA8.

Mapping the PPI network onto module-eigen proteins corroborated the existence of functional links between the processes of a core chondrocyte interactome which mediate the crosstalk between ECM integrity -mechanosensing with glycolysis through the cytoskeleton.

The unique interactomes of OA and healthy chondrocytes reveal differences in protective and adaptive processes

We next focused on the proteins which are unique to each condition (Tsolis et al. Supplementary Table 1[10]), we mapped them on the shared network and created a healthy (n=11 unique proteins) and an OA (n=131 unique proteins) PPI network (STRING score>0.7). Our rationale for the dichotomous mapping was that the protein level abundance changes in the shared proteome might reflect the deregulation of a core chondrocyte homeostatic ECM mechanosensing-metabolism interactome connected to unique OA or healthy proteins mediating processes that are specific to either cartilage damage or maintenance.

Integration of the unique proteins from healthy controls didn't affect significantly the structure or the topology of the core network because only four (SERPINA1, APOE, C3 and CRP) of the 11 proteins yielded highly confident interactions (Fig. 3A). GAPDH remains the node with the highest degree and Complement C3 is added in the top 20 nodes (Fig. 3B). Proteins with protective (SERPIN1A and APOE) or enhancing (C3, CRP) functions in inflammation interact with the core proteome. ApoE was recently shown to inhibit the Classical Complement Cascade in atherosclerosis and Alzheimer's disease by forming a complex with C1q which is activated in response to multiple stimuli such as oxidized lipids and oxidized-LDL[29]. SERPINA1 is an acute phase serine protease shown to be upregulated during chondrogenesis

from MSCs [30]and in response to IL-6 treatment of chondrocytes *in vitro*[31]. The integration of these proteins in the core homeostatic interactome suggests that APOE and SERPINA1 probably act as check points to the burden of low-grade chronic inflammation (CRP, C3) on cartilage integrity.

The network of proteins that are unique to OA comprises a total of 303 nodes expanding the core interactome by 129 proteins. Chaperonin CCT2 is the top node followed by beta-catenin (CTNNB1) (Fig. 4B). Two ribosomal proteins, Ribosomal Protein S4 X-Linked (RPS4X) and Ribosomal Protein Lateral Stalk Subunit P0 (RPLP0) were added to the top node list because their immediate interactors are uniquely identified in OA chondrocytes and comprise translation initiation and elongation factors and ribosomal subunits. Elevated levels of β-catenin are associated with cartilage degeneration and increased WNT signaling in mature chondrocytes and exacerbate hallmarks of the manifestation of OA such as chondrocyte hypertrophy and increased matrix metalloproteinase expression[32,33]. In the OA interactome CTNNB1 is linked to MMP14, which is secreted by invading osteoblasts in the cartilage hypertrophic zone. This interaction probably indicates that MMP14 signals to promote cartilage canal formation and secondary ossification center initiation[34].

Integration of phospholipid hydroperoxide glutathione peroxidase 4 (GPX4) connected the proteins involved in glutathione metabolism (GSTM2, GSTM3 and GSS) to the core interactome, which remained unconnected in the healthy network (Fig. 3A, Fig. 4A). GPX4 is a key enzyme that reduces phospholipid hyperoxides to alcohols and protects cells from ferroptosis[35], a process of cell death. A cell density induced adaptive mechanism rescues cells from the lipotoxic conditions by sequestering poly-unsaturated fatty acid (PUFA) TGAs in lipid droplets (LDs) protecting cells from ferroptosis[36]. Lysophosphatidylcholine acyltransferase 1 LPCAT1, one of the Lands cycle key enzymes, responsible for phospholipid remodeling at membranes, is exclusive to the OA interactome. LPCAT1 was found to localize to the phosphatidylcholine (PC) monolayer membrane of the neutral triacylglycerol (TGA) containing lipid droplet (LD) [37].

Mapping the OA and healthy PPI networks revealed differences in protective and adaptive mechanisms in cartilage homeostasis.

Discussion

Cell functions are maintained by the coordinated activity of functional modules driven in part by relatively synchronized protein abundance. Here we revisited our previous proteomics

dataset and implemented a systems approach using network concepts in Weighted Gene Co-expression Analysis to reconstruct the organisation of the chondrocyte proteome.

We demonstrate that the shared synchronously changing proteome is parsed into functional modules with well characterized significance to OA. As such, core structural components of the cartilage ECM are abundant in healthy chondrocytes but depleted in OA, reflecting the degradation and impaired homeostasis of the articular ECM.

Using a modular approach we showed that the depletion of cartilage ECM proteins is associated with a decrease in glycolytic enzymes and a parallel activation of the adhesion mediated cytoskeleton remodeling proteins (RhoA, RAC1) in chondrocytes obtained from late-stage knee OA patients compared to healthy donors. Indentation-type atomic force microscopy in biopsies from OA patients has revealed that the nano-stiffness of damaged cartilage is lower than the healthy non-degraded cartilage and that OA cartilage becomes softer due to progressive disintegration of the collagen meshwork[38]. The observed depletion of ECM proteins and the concurrent upregulation of CD44, a sensor of ECM integrity, indicate a shift towards a low ECM adhesion microenvironment in OA[39]. In low ECM adhesion conditions, integrin signaling is dampened and integrin-anchored actin filaments become shorter and fragmented[40]. We found that key structural components of integrin activation and cytoskeleton remodeling such as, TLN, vinculin (VCL), the Rho family GTPases RhoA and Rac1 as well as structural components of the F-actin stress fibers and focal adhesions, actinin-α isoform 1 (ACTN1,) cluster in the center of the core chondrocyte network (Fig. 2, A). ACTN1 is abundant in healthy chondrocytes and depleted in OA-derived ones, whereas F-actin depolymerizing destrin (DSTN) is abundant in OA chondrocytes (Fig.2, A), indicating increased actin remodeling in OA. Although the integrin activator and linker to actin filaments TLN is more abundant in OA, VCL which stabilizes the interaction between TLN and the actin cytoskeleton under high tension conditions, is depleted in OA chondrocytes. These interactions indicate an adaptation of the ECM-focal adhesion interface to low ECM forces.

It has been shown that glycolytic pathway components are among the most upregulated proteins during the early stages of chondrogenesis in OA and healthy hBMSCs, highlighting the central role of glycolysis in chondrogenic differentiation[28]. We found significant overlap between the 43 altered proteins with the majority of the proteins mapping to MEyellow, which is enriched for glycolysis/glucose metabolism proteins but also comprises key structural components of the cartilage ECM as COL2A1, COL6A1, COL6A2 and actin polymerization ACTN1, ACTN4) (Fig. 2A). Proteins mediating glycolysis and contributing to the formation

of cartilage ECM were found to be more abundant in healthy chondrocytes compared to OA, suggesting that the link between ECM-cytoskeleton and glucose metabolism might play a role in OA associated chondrocyte biology. Mechanosensing regulates glycolysis through cytoskeletal remodeling by actomyosin stress fiber disassembly[40]. Our findings are in line with a recently described mechanism, where soft ECM causes stress fiber disassembly and release of E3-ubiquitin ligase TRIM21, which targets phosphofructokinase (PFK) for degradation by the proteasome thus reducing glycolysis. Although our data cannot provide direct functional evidence for TRIM21-mediated PFK degradation, the concurrent depletion of structural ECM components and the decrease in glycolytic mediators indicate a connection between mechanosensing and glycolysis regulation in chondrocytes. Moreover, they suggest that perturbations in ECM proteins in OA cartilage could modulate glucose metabolism in chondrocytes.

Our analysis also showed that glucose-6-phosphate dehydrogenase (G6PD),-the first and rate limiting enzyme in the pentose phosphate pathway (PPP) that catalyzes the conversion of glucose-6-phospate to ribose-5'-phosphate, is over abundant in OA compared to healthy chondrocytes. In line with our findings, OA hBMSCs have increased levels of ribose-5'-phosphate compared to healthy hBMCs on the 2nd day post chondrogenesis induction (d2pc) indicating increased PPP flux[28]. Importantly, ribose-5'-phosphate was higher in the core of the OA hBMSC on day2pc micromass cultures. These results parallel the observations made in MCF-10A cells grown in 3D cultures where glucose uptake decreases in centrally located cells (ECM deprived/cell-cell adhesion stimulated). Furthermore, it has been demonstrated that reduced glycolytic flux increases ROS, which is reduced by PPP derived NADH that rescues ATP production and cell survival [41]. Importantly, the connection between low glycolytic flux and ROS production is corroborated by the *in viv*o inhibition of glycolysis with monosodium iodoacetate which induced ROS, auto-inflammation and enhanced OA progression in rats[43]. Taken together, our observations indicate that OA chondrocytes deprived of cell-ECM contacts rely on adaptive metabolic switches, such as the PPP NADH-driven antioxidant rescue, the alternative ATP production from FAO and alternative sources of acetyl-CoA import to the TCA to survive the low-glycolytic flux. The adaptive antioxidant responses might explain why superoxide dismutase levels are not increased in OA chondrocytes in response to excessive ROS production which is a hallmark of OA and why BNTA induction of SOD3 increases ECM biosynthesis and reduces inflammation in a mouse model[43-45].

In addition, chondrocyte lipid peroxidation and not ROS has been shown to induce cartilage matrix protein oxidation and degradation in OA but a connection with mechanosensing was

not established[46]. We found that GPX4 was only detected in the OA interactome suggesting that lipid peroxide scavenging activity is increased in OA. Recent studies in cancer cells brought forward new insights into the role of cell-cell contacts in enhancing escape from ferroptosis. In low cell density conditions, long-chain and highly unsaturated neutral TAGs become enriched while de novo lipogenesis and desaturation pathways decrease leading to increased PUFA uptake predisposing cells to ferroptosis[47].

Exclusive detection of APOE, C3, CRP and SERPINA1 only in healthy chondrocytes indicates that in healthy individuals the detrimental effects of chronic inflammation might be attenuated by an inflammation check-point mechanism mediated by an APOE-C1q complex similarly to atherosclerosis and Alzheimer's disease[29]. Both atherosclerosis and OA are a chronic inflammatory diseases and oxidized-LDL has detrimental effects in their progression[48] and CCC activation is elevated in OA compared to healthy synovial fluid and membranes[49].

Lack of strong or significant correlation between other modules and OA might be attributable to the dichotomous trait analysis (OA vs healthy) and the small sample size. We observed that changes in the level of protein abundance across the samples showed patterns indicative of donor subgroups (data not shown). Our sample size however does not allow testing for proteome-module based patient stratification. We anticipate that increasing the sample size and obtaining information on additional comorbidities will yield associations between modules and patient subgroups.

In conclusion, our unbiased systems proteomics approach recapitulates hallmarks of OA and provides new insights into the connection between cartilage ECM sensing and adaptive metabolic changes underlying OA. Further functional investigation of the dynamic crosstalk between the mechanical ECM property changes and the chondrocyte cellular state will increase our understanding of the molecular bridge underlying the adaptive cartilage changes that lead to OA. Importantly, it will expedite the elucidation of the cellular-microenvironment and systemic context where GWAS SNPs exert their effect as well as potential drug discovery.

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Author Contributions

A.D. and A.T. designed the study. A.D. analyzed the spectral imaging count data matrix, prepared the figures and interpreted the results. A.E and K.C.T performed the original proteomics analysis and produced the spectral count matrix. A.T., A.E., K.C.T., I.P., E.M. and C.B. interpreted the results. A.D. wrote the first draft of the paper. All authors read and edited the paper. A.T finalized the paper and provided funding for the study.

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The funding source has no role in the study design, collection, analysis and interpretation of data, nor in the writing of the manuscript and the decision to submit the manuscript for publication.

Conflict of Interest

The authors declare no conflicts of interest.

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Figure Legends

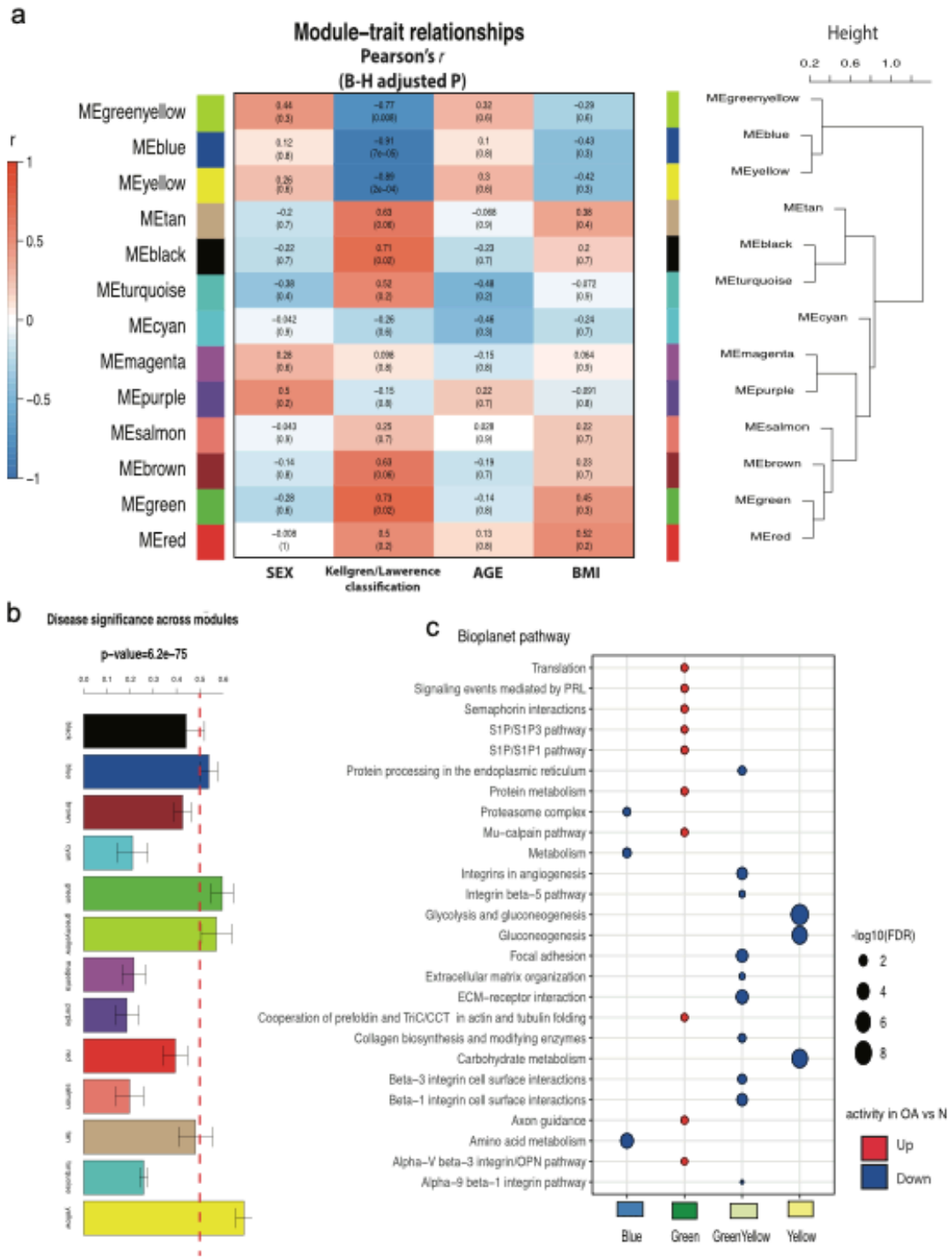

Figure 1. Modular structure of the shared OA and healthy chondrocyte proteome network

A. The heatmap illustrates trait – module protein associations represented by Pearson correlations and B-H adjusted P values. The dendrogram represents the hierarchical clustering of the module protein levels across the samples **B.** Histograms show the mean of the correlation

coefficient for the module proteins as a measure of the association between protein levels and osteoarthritis (OA) termed disease significance (DS). The red dashed line marks r=0.5. **C.** The top enriched terms for the modules with DS>0.5. Red: over abundant in OA vs healthy, Blue: depleted in OA vs healthy **D.** Scaled abundance (Z-scores) of the module essential proteins.

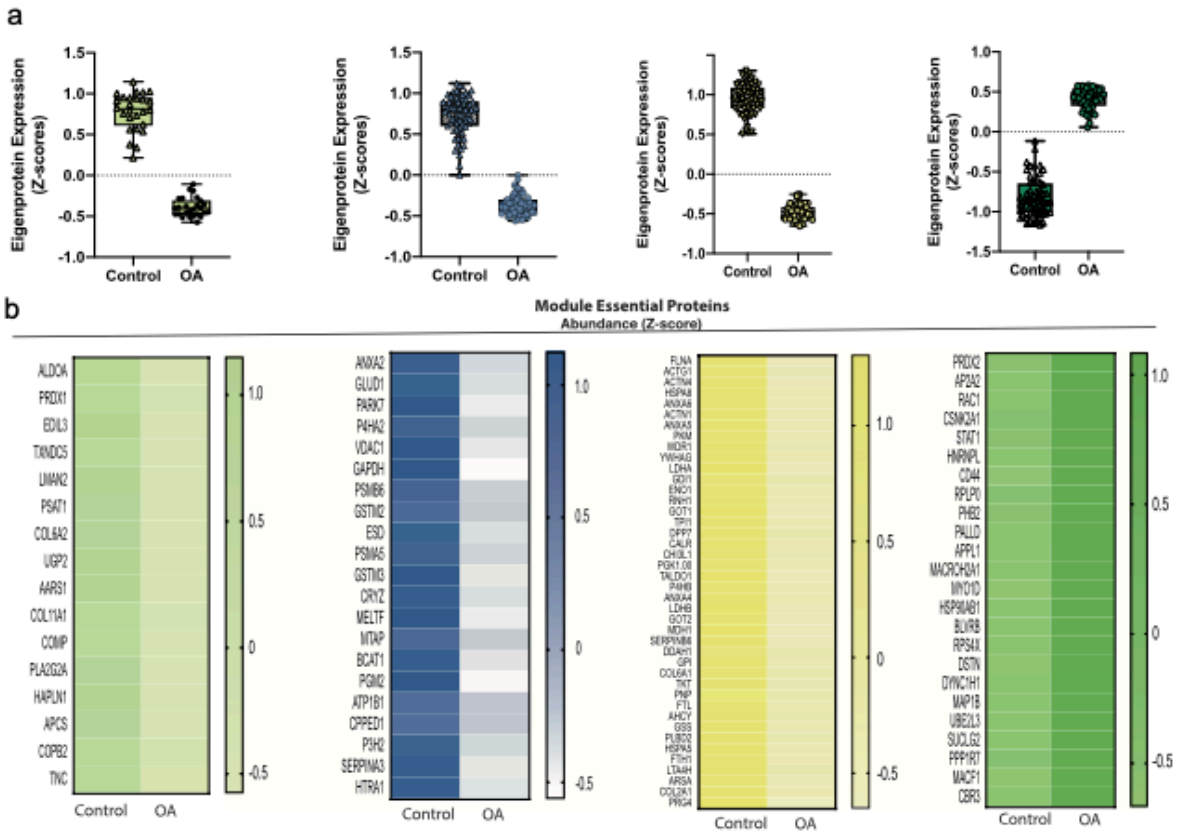

Figure 2. Protein-protein interaction network on the module-eigen proteins representing the core chondrocyte interactome

A. Spring weighted layout applied to PPIs obtained from STRING database (score>0.7, high confidence). The nodes represent module proteins and the color represents their module membership. Node border color represents abundance levels in OA vs healthy chondrocytes, red: abundant in OA vs healthy, black: depleted in OA vs healthy. Edges represent STRING scores. The colored areas represent distinct functional clusters. **B.** Distribution of the number of edges (degree) for the top 20 nodes.

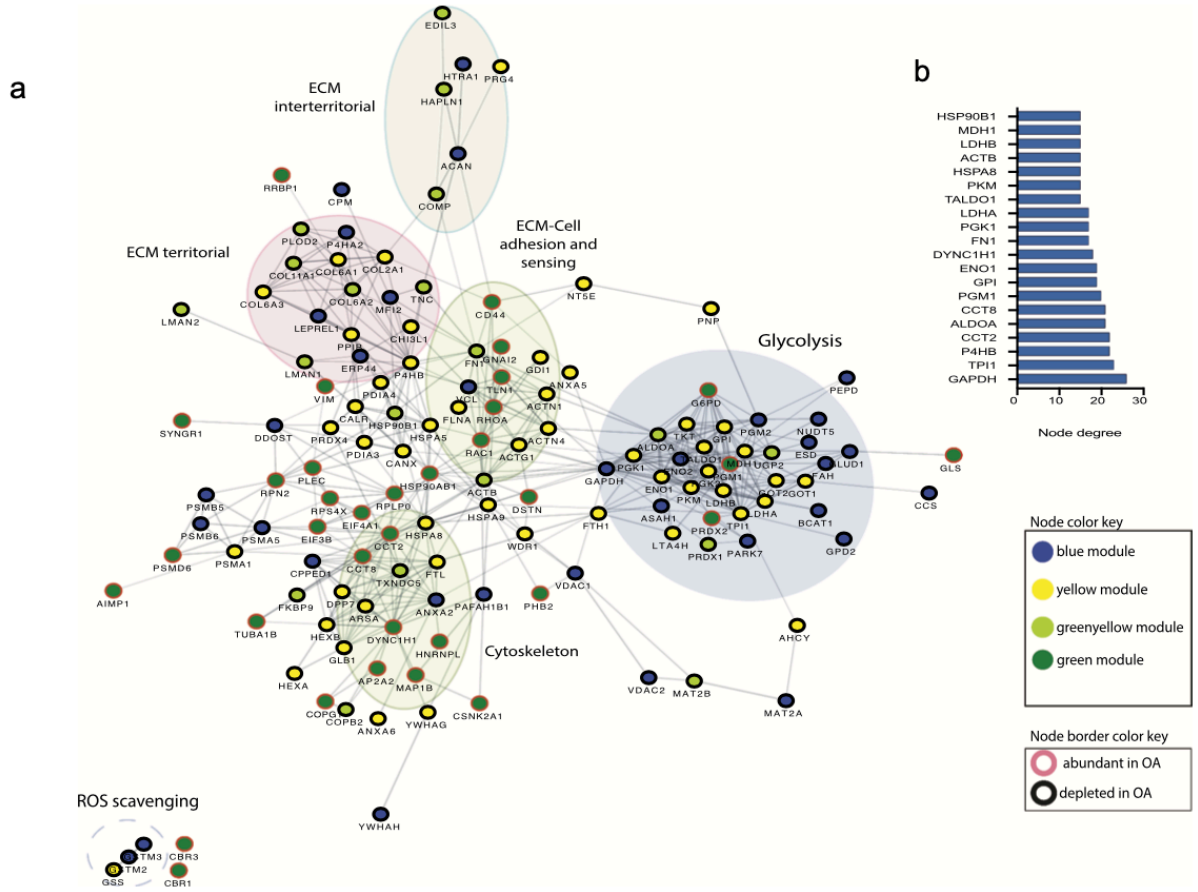

Figure 3. Integrated protein-protein interaction networks mapping core chondrocyte and healthy interactomes

A. Spring weighted layout applied to PPIs obtained from STRING database (score>0.7, high confidence). Nodes are proteins and edges represent STRING scores. The colored areas represent distinct functional clusters. **B.** Distribution of the number of edges (degree) for the top 20 nodes.

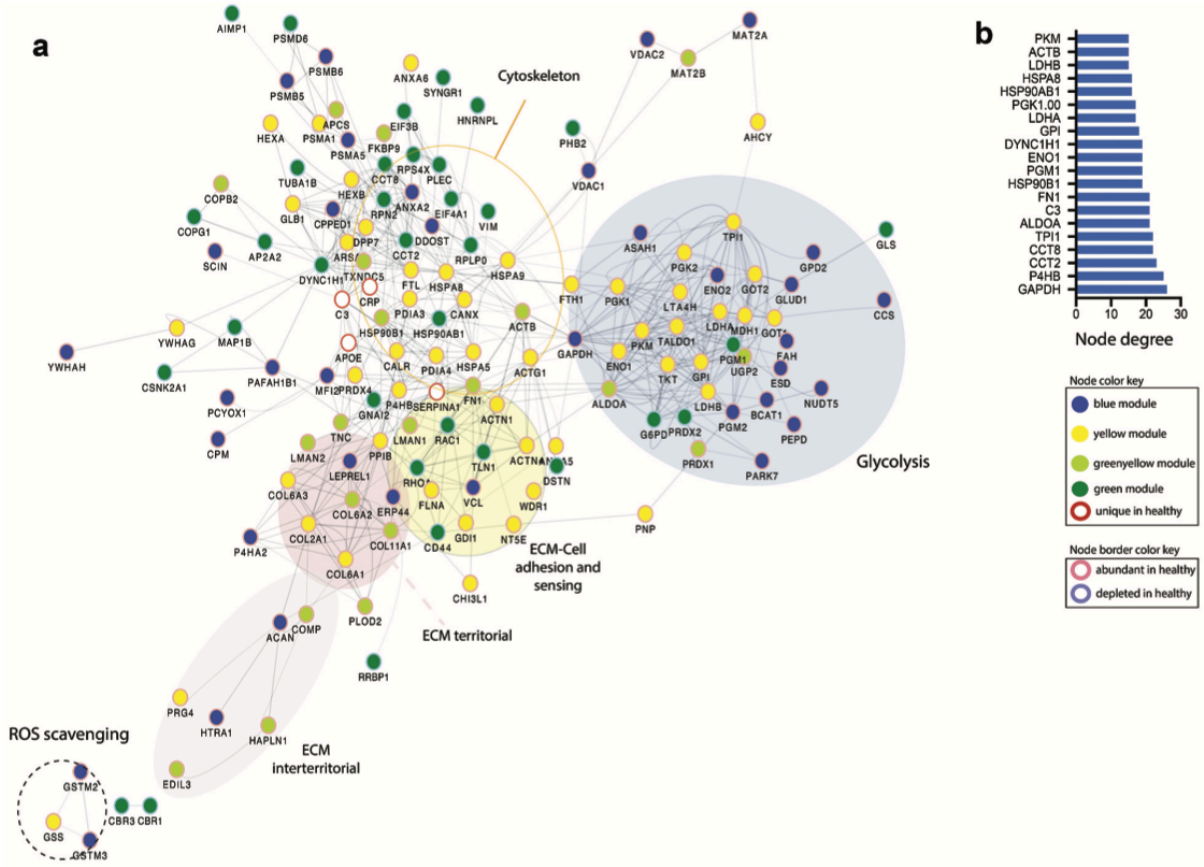

Figure 4. Integrated protein-protein interaction networks mapping core chondrocyte and healthy interactomes.

A. Spring weighted layout applied to PPIs obtained from STRING database (score>0.7, high confidence). Nodes are proteins and edges represent STRING scores. The colored areas represent distinct functional clusters. B. Distribution of the number of edges (degree) for the top 20 nodes.

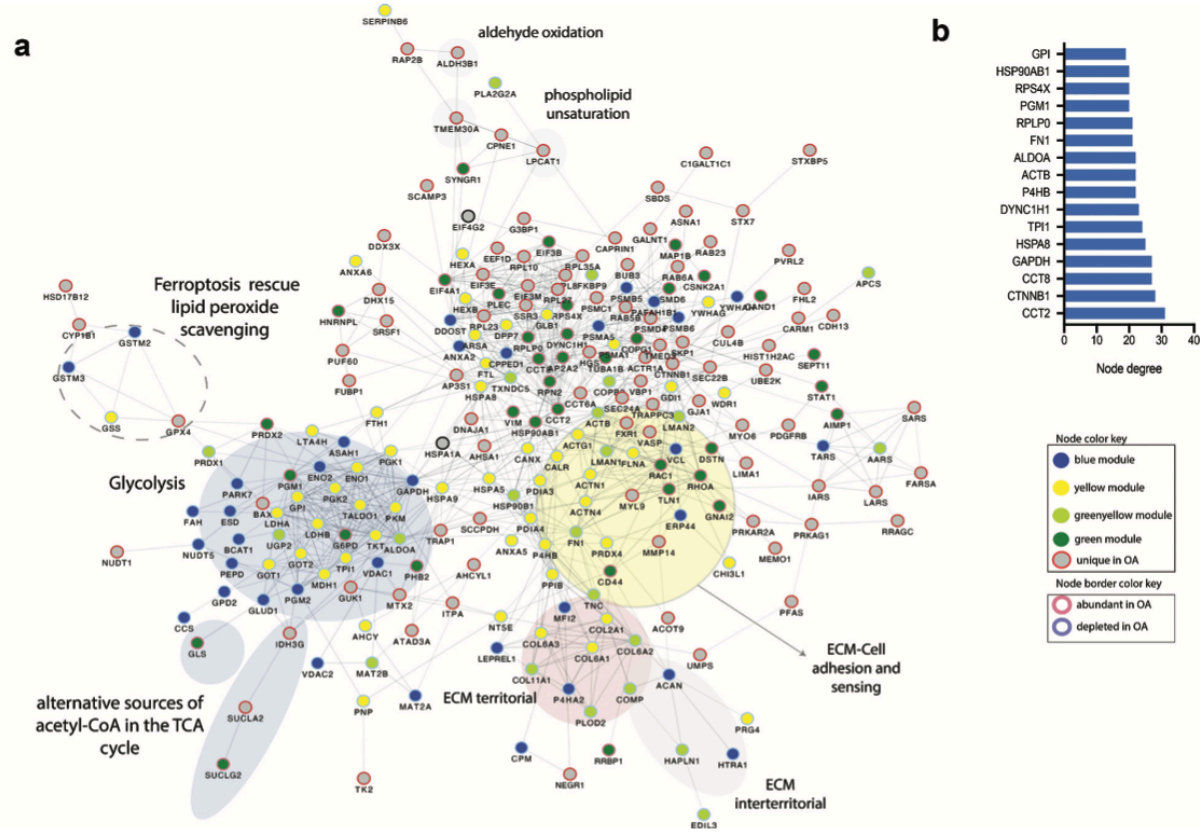

Figure 5. Integrated protein-protein interaction networks mapping core chondrocyte and OA interactomes

A. Spring weighted layout applied to PPIs obtained from STRING database (score>0.7, high confidence). Nodes are proteins and edges represent STRING scores. The colored areas represent distinct functional clusters. **B.** Distribution of the number of edges (degree) for the top 20 nodes.