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Defective chaperone-mediated autophagy is a hallmark of joint disease in patients with knee osteoarthritis

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SUMMARY

Objective: Defects in autophagy contribute to joint aging and Osteoarthritis (OA). Identifying specific autophagy types could be useful for developing novel treatments for OA.
Design: An autophagy-related gene array was performed in blood from non-OA and knee OA subjects from the Prospective Cohort of A Coruña (PROCOAC). The differential expression of candidate genes was confirmed in blood and knee cartilage and a regression analysis was performed adjusting for age and BMI. HSP90A, a chaperone mediated autophagy (CMA) marker was validated in human knee joint tissues, as well as, in mice with aging-related and surgically-induced OA. The consequences of *HSP90AA1* deficiency were evaluated on OA pathogenesis. Finally, the contribution of CMA to homeostasis was studied by assessing the capacity to restore proteostasis upon *ATG5*-mediated macroautophagy deficiency and genetic *HSP90AA1* overexpression.
Results: 16 autophagy-related genes were significantly down-regulated in blood from knee OA subjects. Validation studies showed that *HSP90AA1* was down-regulated in blood and human OA cartilage and correlated with risk incidence of OA. Moreover, HSP90A was reduced in human OA joints tissues and with aging and OA in mice. *HSP90AA1* knockdown was linked to defective macroautophagy, inflammation, oxidative stress, senescence and apoptosis. However, macroautophagy deficiency increased CMA, highlighting the CMA-macroautophagy crosstalk. Remarkably, CMA activation was sufficient to protect chondrocytes from damage.
Conclusions: We show that HSP90A is a key chaperone for chondrocyte homeostasis, while defective CMA contributes to joint damage. We propose that CMA deficiency is a relevant disease mechanism and could represent a therapeutic target for OA.

Introduction

Osteoarthritis (OA) is the most prevalent joint disease, and has a major impact on global health systems[1,2]. Alterations in the articular cartilage, subchondral bone, ligaments, meniscus and synovial membrane, ultimately leading to pain, and loss of function, resulting in joint failure[3]. However, the underlying molecular mechanisms of OA are still under study. Age-related diseases, including OA, are associated with the hallmarks of aging, such as loss of proteostasis. In fact, it is well established that a defect in autophagy is linked to the onset of age-related disease[4–8].

Autophagy is a major proteolytic pathway of eukaryotic cells contributing to maintain cellular homeostasis[6,9]. In physiologic conditions, autophagy plays a role in the renewal of intracellular components, by eliminating both damaged and/or non-functional organelles and proteins, and providing amino acids and essential constituents for the synthesis of new functional cytosolic components[10]. Autophagy occurs continuously in mammalian cells but

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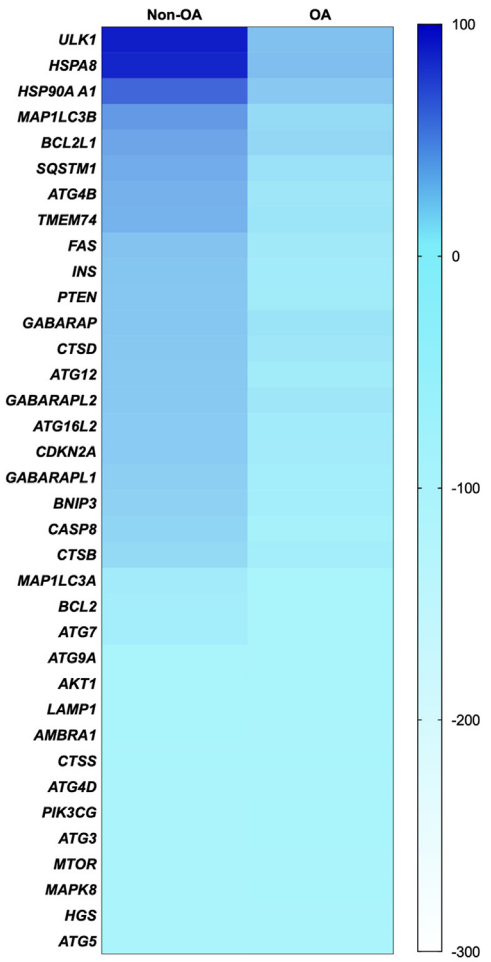

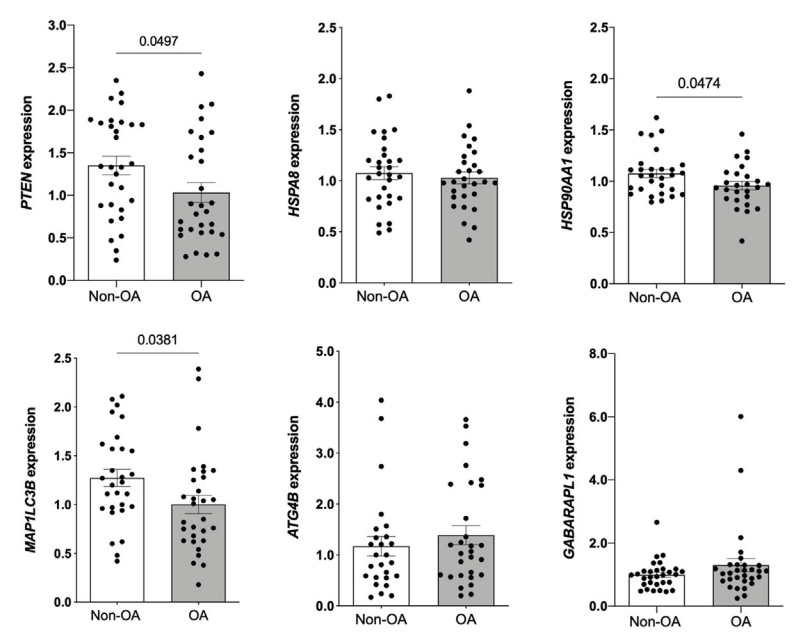

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	Adjusted OR	95% CI of OR	p-value
BMI	1,289	(1,096 - 1,516)	0,002
Age	1,538	(1,186 - 1,996)	0,001
PTEN (> 1,07 median)	4,435	(0,868 - 22,643)	0,073
	Estimation	**95% CI**	
AUC	0,673	(0,532 - 0,813)	

	Adjusted OR	95% CI of OR	p-value
BMI	1,278	(1,085 - 1,506)	0,003
Age	1,669	(1,244 - 2,239)	0,001
HSP90AA1 (> 0,98 median)	5,515	(0,937 - 32,469)	0,059
	Estimation	**95% CI**	
AUC	0,600	(0,456 - 0,744)	

	Adjusted OR	95% CI of OR	p-value
BMI	1,745	(1,258 - 2,418)	0,001
Age	1,290	(1,085 - 1, 533)	0,004
MAP1LC3B	0,095	(0,013 - 0,686)	0,020
	Estimation	**95% CI**	
AUC	0,949	(0,899 - 1,000)	

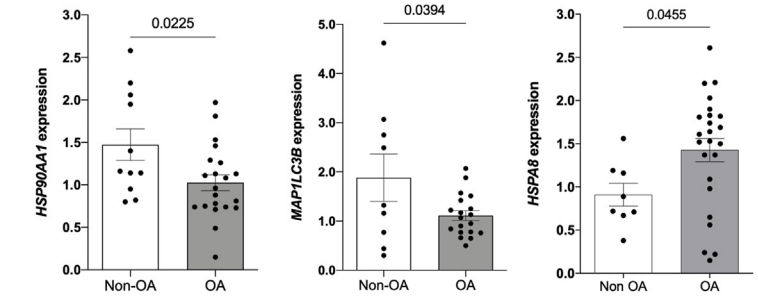

Fig. 1

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Target	Description	Regulation	*P*-value
PTEN	Phosphatase and tensin homolog	−2.78	0.000073
HSPA8	Heat shock protein family A (Hsp70) member 8	−2.75	0.000102
HSP90AA1	Heat shock protein 90 alpha family class A member 1	−2.45	0.000159
BNIP3	BCL2 interacting protein 3	−2.78	0.000178
BCL2	BCL2 apoptosis regulator	−2.23	0.000320
MAP1LC3B	Microtubule-associated protein 1 light chain 3 beta	−2.67	0.000545
ATG16L2	Autophagy related 16 like 2	−2.69	0.000591
ATG12	Autophagy related 12	−2.66	0.000603
ATG4B	Autophagy related 4 cysteine peptidase	−3.11	0.000744
SQSTM1	Sequestosome 1	−2.74	0.000823
GABARAPL1	GABA type A receptor-associated protein like 1	−2.79	0.001135
BCL2L1	BCL2-like 1	−2.14	0.001262
GABARAPL2	GABA type A receptor-associated protein like 2	−2.27	0.003412
CASP8	Caspase 8	−2.55	0.003774
GABARAP	GABA type A receptor-associated protein	−1.96	0.015348
MAP1LC3A	Microtubule-associated protein 1 light chain 3 alpha	−2.97	0.040994

Table I

Osteoarthritis and Cartilage

List of regulated genes in blood from non-OA and knee OA subjects (OA vs non-OA)

could be induced by cellular stress such as nutrient deprivation, reactive oxygen species or hypoxia[11], and plays a fundamental role in cellular survival[12]. At least three types of autophagy are recognized depending on how cellular constituents reach the lysosome for degradation: microautophagy, macroautophagy and chaperone mediated autophagy (CMA)[13].

Microautophagy, the least understood in mammals, is the mechanism by which cytosolic material is sequestered in bulk through invaginations in the lysosomal membrane and engulfed for degradation[13,14]. In macroautophagy, the most studied type of autophagy, the material is enclosed in a double-membrane vesicles called autophagosomes and then delivered to lysosomes for bulk degradation. Macroautophagy machinery depends on autophagy-related genes (ATG), that were first identified in yeast[15]. CMA, described so far only in mammalian cells, is critical for the selective degradation of approximately 40% of cytosolic proteins bearing a KFERQ-like motif[16]. These unique substrates are recognized by cytosolic chaperone heat shock cognate 71-kDa protein (hsc70 also known as HSPA8) that brings proteins for degradation to the lysosomal membrane and interacts with the receptor protein LAMP2A. LAMP2A is organized in a translocation complex, for which stabilization depends on heat shock protein 90A (HSP90A)[17]. Then, the unfolded protein is translocated into the lysosomal lumen for degradation. A functional decline of CMA with aging has been observed[16,18]. A reduction in LAMP2A expression, as well as dynamics and stability changes are responsible of the age-related decline of CMA activity[19,20]. CMA malfunction leads to the accumulation of abnormal proteins in the cytosol of cells and tissues in old organisms, impairing their ability to respond to stress[21] and likely results in age-related neurodegenerative disorders and cardiovascular diseases[22].

Articular cartilage is a post mitotic tissue with a very low cell turnover, therefore it is more susceptible to proteotoxicity by the accumulation of aggregated and toxic proteins[11]. Consequently, the maintenance of its biosynthetic activity is highly dependent on homeostatic mechanisms in physiological conditions. In fact, defective autophagy results in increased proteotoxicity contributing to the development of OA. In this regard, there is strong evidence that macroautophagy is defective in OA and its pharmacological activation prevents the progression of the disease[7,8,23,24]. However, the role of CMA in OA is still unknown.

Although macroautophagy and CMA have different regulatory mechanisms, they are functionally connected. Previous studies

Comparative analysis of autophagy gene expression patterns in blood and cartilage from non-OA and knee OA PROCOAC cohort patients. A. Heat map of modulated genes involved in the autophagy signaling pathway from non-OA ($n = 18$) and knee OA subjects ($n = 18$). B. *PTEN*, *HSPA8*, *HSP90AA1*, *MAP1LC3B*, *ATG4B* and *GABARAPL1* mRNA levels in blood from non-OA subjects (*PTEN*, $n = 30$; *HSPA8*, $n = 30$; *HSP90AA1*, $n = 29$; *MAP1LC3B*, $n = 29$; *ATG4B*, $n = 26$ and *GABARAPL1*, $n = 30$) and knee OA subjects (*PTEN*, $n = 29$; *HSPA8*, $n = 29$; *HSP90AA1*, $n = 26$; *MAP1LC3B*, $n = 30$; *ATG4B*, $n = 29$ and *GABARAPL1*, $n = 30$). Values are mean with 95% CI for *PTEN*: [−0.6367 to −0.00390]; *HSPA8*: [−0.2191 to 0.1]; *HSP90AA1*: [−0.2324 to −0.0013]; *MAP1LC3B*: [0.01549 to 0.5294]; *ATG4B*: [−0.3217 to −0.7]; *GABARAPL1*: [−0.1401 to 0.75] and n indicates the number of biologically independent human subjects. Two-tailed unpaired Student's *t*-test. C. Regression analysis of *PTEN*, *HSP90AA1* and *MAP1LC3B* expression with age and BMI. D. *HSP90AA1*, *MAP1LC3B* and *HSPA8* mRNA levels in cartilage from non-OA subjects (*HSP90AA1*, $n = 11$; *MAP1LC3B*, $n = 9$; HSPA8, $n = 8$) and OA subjects (*HSP90AA1*, $n = 21$; *MAP1LC3B*, $n = 19$; HSPA8, $n = 24$). Values are mean with 95% CI for *HSP90AA1*: [−0.8291 to −0.06780]; *MAP1LC3B*: [−1.499 to −0.04029], *HSPA8*: [0.01118 to 1.025], n indicates the number of biologically independent human subjects, two-tailed unpaired Student's *t*-test.

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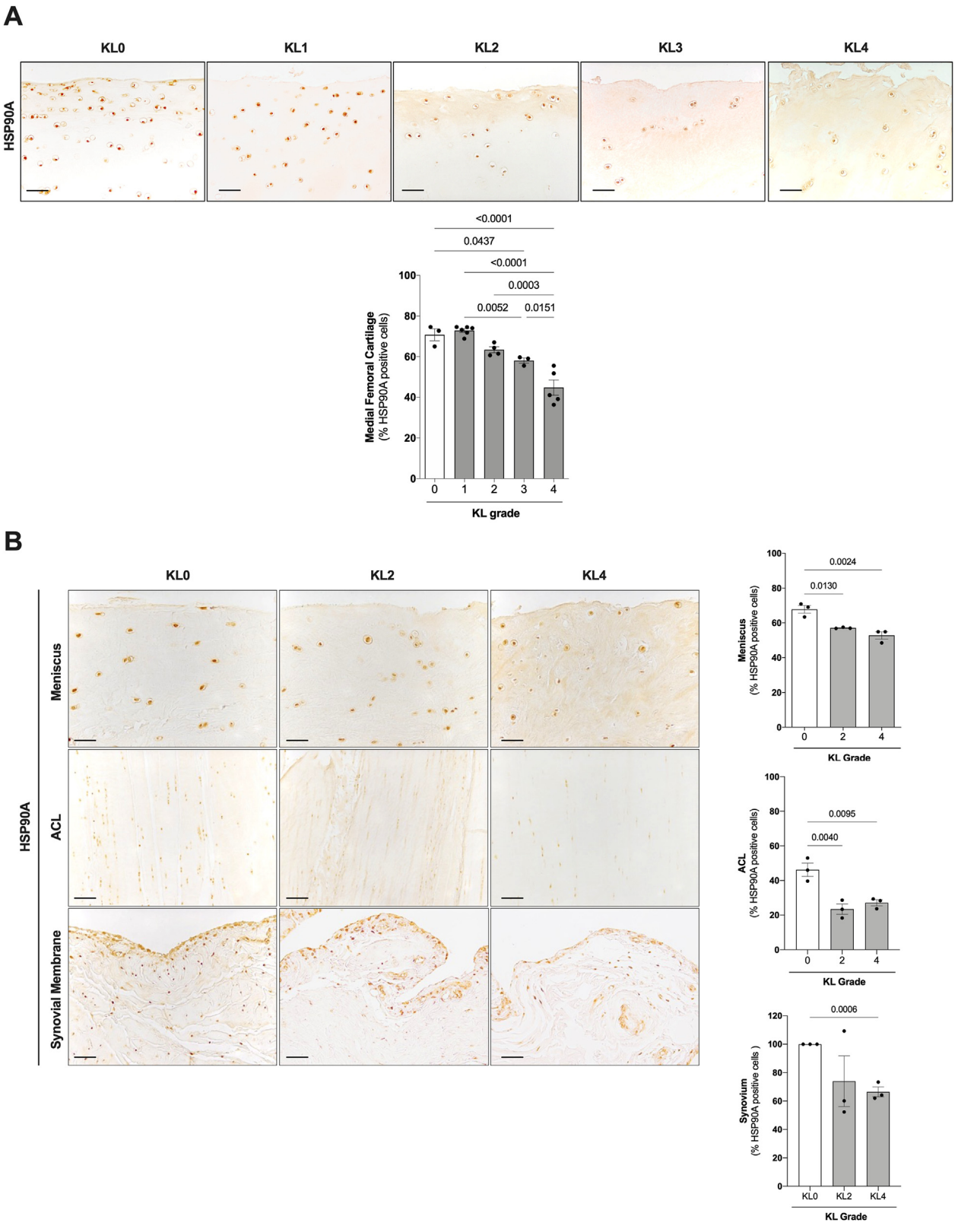

Fig. 2

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support the existence of a bidirectional crosstalk between macroautophagy and CMA[21,25], which is likely to maintain homeostasis. Defects on CMA and/or macroautophagy lead to altered proteostasis contributing to the development of age-related diseases[25], however, the key underlying mechanisms are not fully understood.

Here, we undertook a detailed investigation whether the relevant autophagy genes are differentially regulated in OA. We then took advantage of the availability of blood, primary chondrocytes and human cartilage tissues from a clinically well characterized cohort to investigate the role of CMA and the interplay with macroautophagy to elucidate the relevant mechanisms to maintain chondrocyte homeostasis and their contribution to OA pathophysiology.

Materials and methods

Information regarding chemicals and other reagents, sample consent statement, sample size justification, T/C28a2 human chondrocytes, cell culture conditions, autophagy gene expression array, RNA isolation from blood, cartilage and chondrocytes, reverse transcription and quantitative real time-polymerase chain reaction, flow cytometry and immunohistochemistry studies are included in the Supplementary material and methods.

Study participants

Non-OA and knee OA subjects included in this study belong to the PROspective Cohort of A Coruña (PROCOAC)[26]. A total of 60 subjects were included in the present study and divided in two groups (sample size justification is included in Supplementary material and methods). The non-OA group included females, older than the age of 50 years old (yo) (mean ± SD, Age: 60.13 ± 1.12) and body mass index (BMI): (mean ± SD, 24.85 ± 0.59), without a diagnosis of OA of the knee, hip, or erosive or inflammatory hand OA and with normal radiographic findings on the knee classified according to the Kellgren/Lawrence (K/L) scoring system[27] (K/L grade 0). The knee OA group were females, older than 50 yo (mean, ±SD; Age: 68.4 ± 1.11 and BMI: mean ± SD, 29.65 ± 0.55) without a diagnosis of OA of the hip, or erosive or inflammatory hand OA. In this cohort and with the inclusion criteria used, females have much higher prevalence of OA compared to male subjects[26]. The diagnosis of knee OA was based on clinical and radiographic evaluations according to ACR Criteria[28]. Only subjects with K/L grade 3 and 4 changes were included in the knee OA group. The distribution of clinical and demographic variables and the comparison between non-OA and knee OA subjects is shown in Supplementary Table 1.

Primary human chondrocyte isolation

Knee OA human cartilage was obtained from the femoral and tibial plateaus surfaces from nine donors (Age: mean ± SD = 79.11 ± 11.79 yo) who were undergoing knee replacement surgery. Briefly, cartilage slices were minced in small pieces and incubated at 37°C with trypsin (0.5 mg/ml) for 10 min. Then, cartilage was incubated overnight at 37°C with collagenase IV in DMEM with 5% FBS with shaking. The digest was centrifuged and chondrocytes were cultured in DMEM supplemented with 10% FBS and 1% P/S and incubated at 37°C in a humidified gas mixture containing 5% CO_2. Chondrocytes were used for experiments at confluence.

Human articular cartilage processing

Human OA cartilage was obtained from subjects undergoing knee replacement (Age: mean ± SD = 70.52 ± 6.65 yo, n = 21). Normal human cartilage was harvested at the time of autopsy from the femoral condyles and the tibia plateaus of subjects who had no history of joint disease (Age: mean ± SD = 66 ± 6.65 yo, n = 11). OA was diagnosed according to the American College of Rheumatology Diagnostic criteria for OA[28]. Scalpels were used to separate cartilage onto sections parallel to the subchondral bone. Then cartilage strips were cut in small pieces, frozen in liquid nitrogen and stored at −80°C until RNA extraction was performed.

Autophagy gene expression in blood from OA patients

The gene expression analysis was performed using a predesigned human autophagy PCR array (PrimePCR Pathway Plate, 96 well Autophagy, SAB Target List, H96) and then, results were analyzed using the PrimePCR analysis software. Information regarding the autophagy panel and the experimental procedure and analysis were included in Supplementary Table 2 and Supplementary material and methods.

Small interference RNA transfection (siRNA)

Primary human OA chondrocytes (n = 5 donors; Age: mean ± SD = 74.4 ± 13.08 yo) and T/C28a2 chondrocytes were transiently transfected with siRNA for *HSP90AA1*. Primary human OA chondrocytes from five donors (Age: mean ± SD = 72.2 ± 10.98 yo) were transiently transfected with siRNA for *ATG5*. Transfections were carried out using Lipofectamine RNA/iMAX transfection reagent according to manufacturer's instructions. Silencer Cy-labeled negative Ctrl N.1 siRNA was employed as control.

HSP90A is reduced in human knee joint tissues with OA severity. A. Healthy medial femoral cartilage (MFC, KL0) and OA (MFC, KL1-4) were analyzed by immunohistochemistry (IHC) for HSP90A, Scale bar, 10 μm. Quantitative analysis of HSP90A positive cells expressed as percentage of positive cells related to the total number of cells. Values are the mean with 95% CI KL0 vs KL1: [−12.81 to 8.708]; KL0 vs KL2: [−4.213 to 19.03]; KL0 vs KL3: [0.2843−25.14]; KL0 vs KL4: [14.92−37.15]; KL1 vs KL2: [−0.3595 to 19.29]; KL1 vs KL3: [4.002−25.53]; KL1 vs KL4: [18.87 −37.31]; KL2 vs KL3: [−6.324 to 16.92]; KL2 vs KL4: [8.417−28.84]; KL3 vs KL4: [2.212−24.44], KL0: n = 3; KL1: n = 6, KL2: n = 4, KL3: n = 3, KL4; n = 5 human donors; one-way ANOVA with Tukey's multiple comparison test. B. Healthy meniscus, ACL and synovial membrane (KL0) and OA meniscus, ACL and synovial membrane (KL2-4) were analyzed by IHC for HSP90A, Scale bar, 10 μm. Quantitative analysis of HSP90A positive cells. Values are the mean with 95% CI Meniscus: KL0 vs KL2: [−2.935 to 18.41]; KL0 vs KL4: [7.265−22.74]; KL2 vs KL4: [−3.408 to 12.07]; ACL: KL0 vs KL2: [9.813−35.75]; KL0 vs KL4: [6.146−32.08]; KL2 vs KL4: [−16.63 to 9.300]; synovial membrane: KL0 vs KL2: [−75.65 to 23.41]; KL0 vs KL4: [−43.15 to −23.95]; KL2 vs KL4: [−57.89 to 43.02], KL0: n = 3; KL2: n = 3, KL4: n = 3 human donors; one-way ANOVA with Tukey's multiple comparison test.

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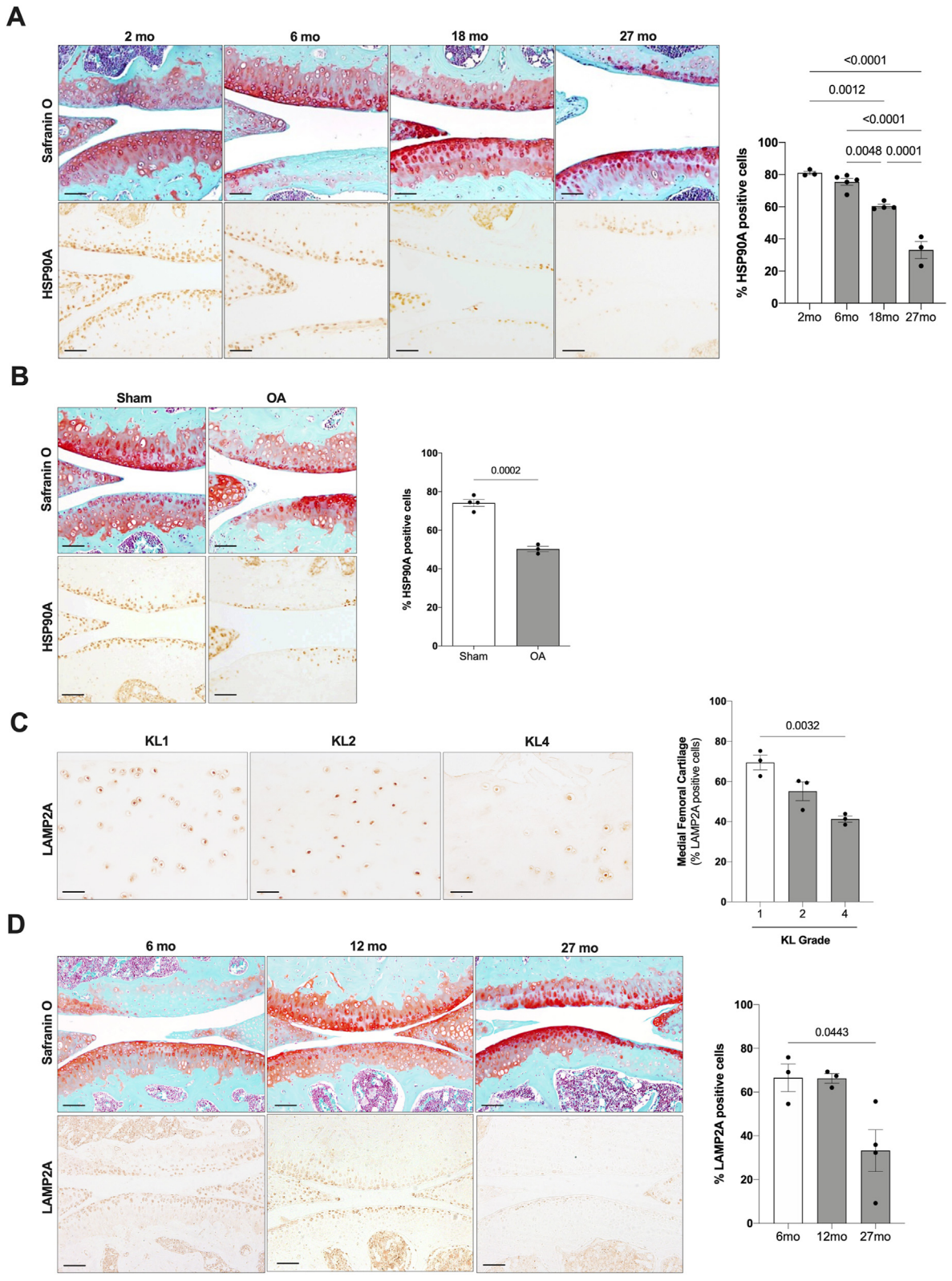

Fig. 3

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Protein isolation and Western blotting

Western blotting was performed with a chemiluminescence detection system. Cell lysates were obtained from primary OA human chondrocytes using 6 M urea/2% SDS and were sonicated at 4°C. Protein concentrations were determined with Pierce® BCA Protein Assay. Proteins were separated on 4–20% SDS-polyacrylamide gels and transferred to PVDF membranes, blocked 1 h with 5% dry milk or 5% bovine serum albumin in TBS-T and blotted with primary rabbit polyclonal antibodies specific for LC3 (1:1000), ATG5 (1:1000), prp-S6 (1:2000), p62 (1:1000), p16 (1:5000), p21 (1:1000) HSP90A (1:2000), LAMP2A (1:2000), GAPDH (1:1000), CASP3 (1:1000) mouse antibody tubulin (1:1000) were applied at 4°C overnight. Then, after washing with TBS-T, membranes were incubated with horseradish peroxidase (HRP)–conjugated donkey anti-rabbit IgG or sheep anti-mouse IgG (1:5.000) for 1 h at room temperature. Afterward, membranes were washed with TBS-T and protein bands were detected using a chemiluminescence substrate. The intensity of the bands was quantified by using ImageJ software. A comprehensive explanation of methods used for indirect measurement of autophagic activity by immunoblotting (i.e., LC3 lipidation, p62 turnover, RPS6 phosphorylation) was included in Supplementary material and methods section.

Histological analyses

Human joint tissue sections from non-OA and knee OA subjects with different KL grades (Age: KL0 = 28.3 ± 4.50, $n = 3$; KL2 = 77 ± 6.08, $n = 3$; KL4 = 62.3 ± 3.05, $n = 3$) were fixed in 10% zinc-buffered formalin (Z-fix) for 24 h. Mouse knee joints from 2, 6, 12, 18, 27 and 30 mo C57BL/6 male mice with spontaneous aging-related OA and from C57BL/6 male mice with OA induced by transection of the medial meniscotibial ligament and medial collateral ligament (MMTL + MCL, Sham and 10 weeks after surgery) were fixed in 10% Z-fix for 24 h and decalcified in TBD-2 for 48 h. Sections were stained with Safranin O-Fast green. Histological scoring of cartilage changes was performed using the Osteoarthritis Research International (OARSI) scoring system[29]. Paraffin sections from mouse models were provided from Dr. Martin Lotz at Scripps Research, La Jolla, CA, USA. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Scripps Research and in accordance with the ARRIVE (Animal Research: Reporting of In vivo Experiment Guidelines).

HSP90AA1 overexpression by DNA plasmid transfection

HSP90AA1 overexpression in primary human OA chondrocytes from five donors (Age: mean ± SD = 87.66 ± 6.80 yo) was carried out by performing transient transfection with pRP[Exp]-EGFP-CAG > h*HSP90AA1* [NM_001017963.3] (VB900123-7805yhw) DNA plasmid construct. Transfection was performed using FuGENE® following manufacturer's protocol. Empty DNA plasmid construct was employed as negative control.

Statistical analysis

A descriptive statistical analysis of the variables collected in the study was performed. To test for normal distribution of the data, we used the Kolmogorov–Smirnov test. The comparison of means between two groups was performed using the Student's *t*-test. Statistically significant differences between multiple comparisons were determined by analyzing variance (ANOVA) in conjunction with Tukey's multiple comparison test. The impact of the variables under study on the presence or absence of OA was determined using logistic regression models, adjusting for those variables that were significant in the univariate analysis or clinically relevant. The ability of the various parameters to predict OA was evaluated using ROC curves, providing the area under the curve (AUC) with its corresponding 95% confidence interval. *P* values less than 0.05 were considered significant. Data are presented as the mean with 95% confidence interval (CI). Data analysis and statistical inference was performed by using Prism 9 software (GraphPad Software, La Jolla, CA, USA).

Results

Specific autophagy markers are down-regulated in blood and knee cartilage from OA patients

To investigate the role of autophagy as a potential target for OA, a comparative analysis of autophagy-related genes was performed using a pre-designed gene expression array in blood from non-OA ($n = 18$) and knee OA subjects ($n = 18$) from the PROspective Cohort of A Coruña (PROCOAC). The baseline information of subjects included in this study is described in Supplementary Table 1. As shown in Fig. 1(A), a subset of 36 autophagy-related genes were modulated, however only 16 genes were significantly decreased in the knee OA group as compared to non-OA subjects (Table I). Interestingly, macroautophagy-related genes, including *PTEN*, *MAP1LC3B*, *ATG4B* and *GABARAP1* and chaperone-mediated autophagy (CMA) genes, such as *HSP90AA1* and *HSPA8*, were significantly down-regulated in knee OA subjects (Table I). Validation analysis of the significant modulated genes in subjects from the PROCOAC cohort ($n = 30$ non-OA and $n = 30$ knee OA) confirmed a significant down-regulation of PTEN, *HSP90AA1* and *MAP1LC3B* [Fig. 1(B)]. As age and BMI are significantly higher in the OA subjects (Supplementary Table 1), a regression analysis of *HSP90AA1,*

CMA is compromised with aging and OA. A. Knee joint sections from 2, 6, 18 and 30 mo mice stained with SafO and HSP90A. *Scale bar*, 10 μm. Quantitative analysis of HSP90A-positive cells. Values are the mean with 95% CI for HSP90A: 2 mo vs 6 mo [−5.552 to 16.72]; 2 mo vs 18 mo [8.986–32.28]; 2 mo vs 27 mo [35.51–60.41]; 6 mo vs 18 mo [4.817–25.28]; 6 mo vs 27 mo [31.24–53.51]; 18 mo vs 27 mo [15.68–38.97]; 2 mo: $n = 3$, 6 mo: $n = 5$, 18 mo: $n = 4$, 27 mo: $n = 3$, one-way ANOVA with Tukey's multiple comparison test. B. Knee joint sections from 6 mo mice subjected to surgical OA for 10 weeks stained with SafO and HSP90A. *Scale bar*, 10 μm. Quantification of HSP90A-positive cells. Values are the mean with 95% CI for HSP90A [−30.13 to −17.87], Sham: $n = 4$, OA: $n = 3$ mice, two-tailed unpaired Student's *t*-test. C. MFC sections (KL1-4) were stained for LAMP2A, Scale bar, 10 μm. Quantitative analysis of LAMP2A-positive cells. Values are the mean with 95% CI for HSP90A: KL1 vs KL2 [−1.037 to 29.70]; KL1 vs KL4 [12.83–43.56]; KL2 vs KL4 [−1.498 to 29.23], KL1,2,4, $n = 3$ human donors; one-way ANOVA with Tukey's multiple comparison test. D. Knee joint sections from 6, 12 and 27 mo mice stained with SafO and LAMP2A. Scale bar, 10 μm. Quantitative analysis of LAMP2A-positive cells. Values are the mean with 95% CI for HSP90A: 6 mo vs 12 mo [−33.18 to 33.77]; 6 mo vs 27 mo [1.939–64.57]; KL2 vs KL4 [1.645–64.28], 6 mo and 12 mo: $n = 3$, 27 mo: $n = 4$, one-way ANOVA with Tukey's multiple comparison test.

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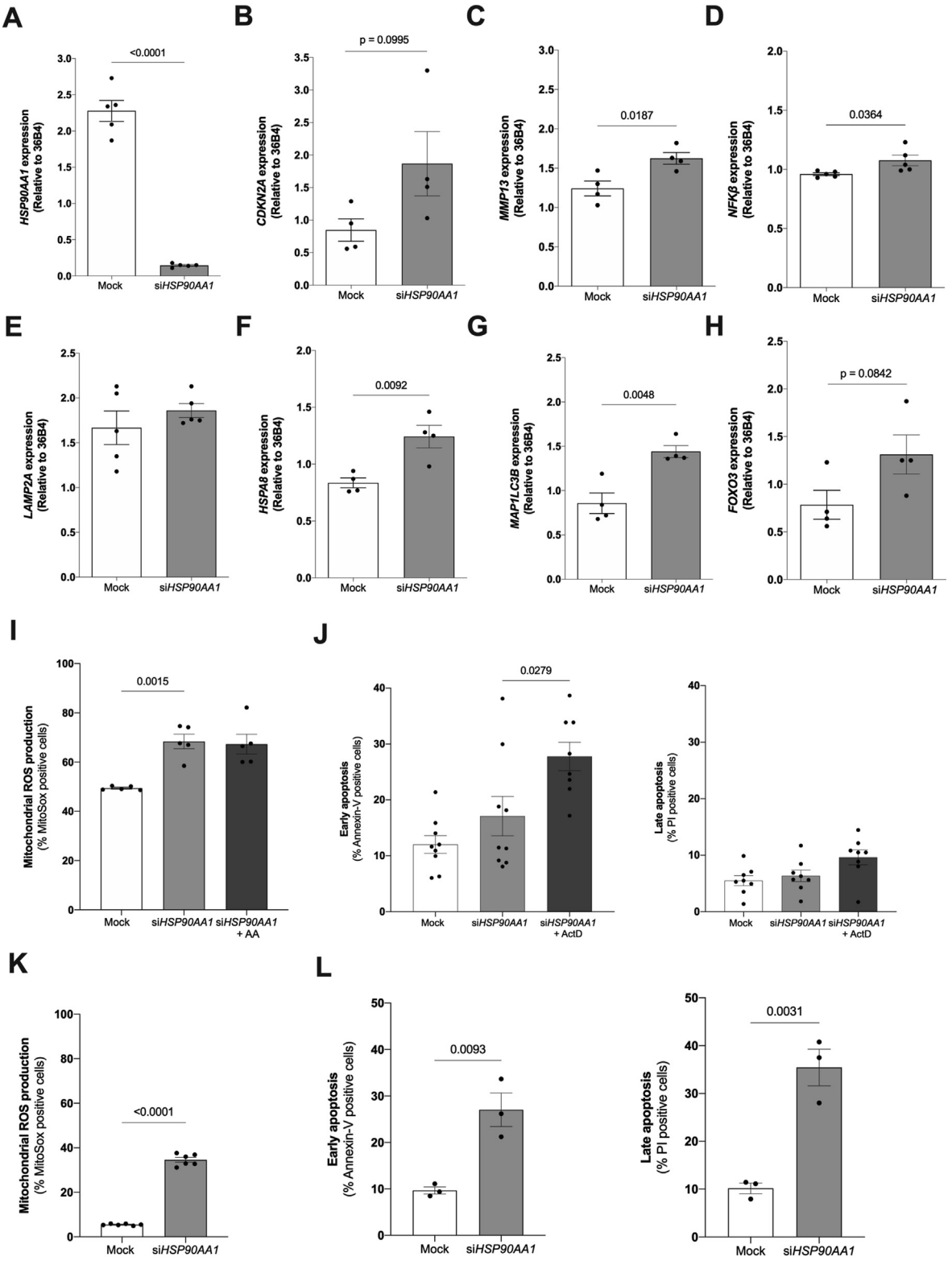

Fig. 4

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MAP1LC3B and PTEN was performed considering age and BMI as dependent variables. The results showed that increased BMI and age are strongly associated with risk incidence of OA as well as with decreased expression of *HSP90AA1* and *MAP1LC3B* in subjects with knee OA [Fig. 1(C)]. The candidate genes were then investigated by RT-qPCR in knee cartilage from non-OA and knee OA from a population of male and female subjects. We found that *HSP90AA1* and *MAP1LC3B* expression was significantly down-regulated in OA subjects [Fig. 1(D)]. These results suggest that defective autophagy signaling pathways could be a relevant pathology feature in patients with knee OA. Since macroautophagy is a well-known mechanism defective in OA[7,8] and the role of CMA in joint aging and OA remains to be determined, *HSP90AA1* was chosen for further studies. In fact, HSP90A has been revealed as an important modulator of selectivity during the autophagy mechanisms including macroautophagy and CMA outstanding its relevant contribution to cellular homeostasis[22,30]. To further explore the role of chaperones, *HSPA8* was investigated in cartilage from knee OA subjects. The results showed increased levels in OA. In addition, we performed an analysis of data separating samples by sex. The results showed no significance differences in both male subjects, while in females *MAP1LC3B* with OA was significantly reduced (Supplementary Fig. S1).

CMA is compromised in joint aging and osteoarthritis

The expression of HSP90A was investigated in human joint tissues, including medial femoral cartilage, meniscus, anterior cruciate ligament (ACL), and synovial membrane. Our results showed that HSP90A was progressively reduced with increased OA severity in all the joint tissues [Fig. 2(A) and (B)].

Changes in CMA during joint aging and OA were studied in preclinical mouse models. A significant reduction in HSP90A expression was found in spontaneous age-related OA [Fig. 3(A)]. In addition, surgically-induced OA showed a significant decrease in HSP90A expression at 10 weeks after surgery compared to sham surgery group [Fig. 3(B)]. Since HSP90A plays a key role in CMA conferring stability to LAMP2A multimerization, we investigated if the reduction in HSP90A expression was associated with a change in LAMP2A. The results showed a reduction in LAMP2A-positive chondrocytes in human OA cartilage [Fig. 3(C)] as well as in the preclinical mouse model of spontaneous age-related OA [Fig. 3(D)].

Taking together, the expression pattern of CMA markers were decreased with aging and OA, possibly contributing to altered chondrocyte homeostasis.

HSP90AA1 deficiency impairs chondrocyte homeostasis

Genetic knockdown of *HSP90AA1* in human OA chondrocytes [Fig. 4(A)] promoted expression of genes associated with senescence (p21), cartilage degradation (MMP13) and inflammation (*NFκB*/RELA) [Fig. 4(B)–(D)]. Moreover, homeostasis genes including *HSPA8* and *MAP1LC3B* were increased. This increase could indicate a compensatory response to damage [Fig. 4(E)–(H)]. In addition, *HSP90AA1* deficiency promotes oxidative stress [Fig. 4(I)] and cell death by apoptosis [Fig. 4(J)]. Remarkably, similar results were obtained in normal human T/C28a2 chondrocytes [Fig. 4(K), (L) and Supplementary Fig. S3]. The fact that absence of *HSP90AA1* have deleterious effects in normal chondrocytes compared to OA primary human chondrocytes strongly supports its key role in the maintenance of chondrocyte homeostasis.

Defective CMA compromises macroautophagy, induces senescence and leads to chondrocyte death by apoptosis

The overall activity of autophagy occurs due to communication between interconnected pathways such as via a bidirectional crosstalk between macroautophagy and CMA[25]. We investigated the underling regulatory mechanisms of *HSP90AA1* impairment and the interplay between CMA and macroautophagy in primary human OA chondrocytes. Transfected chondrocytes showed an approximately 50% of HSP90A protein knockdown at 72 h and 120 h post-transfection [Fig. 5(A)]. Moreover, LAMP2A protein was decreased at 120 h post-transfection, suggesting that CMA is compromised under *HSP90AA1* deficiency at late stages [Fig. 5(A)]. Interestingly, macroautophagy activation was suppressed under CMA deficiency due to a reduction of LC3II and p62 protein expression [Fig. 5(B)]. Moreover, defective CMA was correlated with chondrocyte senescence by increasing p21 expression and apoptosis by increasing CASP3 cleavage [Fig. 5(C) and (D)]. These results indicate that CMA plays an important role in the maintenance of chondrocyte homeostasis and its impairment compromises chondrocyte survival.

HSP90AA1 deficiency compromised chondrocyte homeostasis. A–H. Relative expression of *HSP90AA1*, *CDKN2A*, *MMP13*, *NFκB*, *LAMP2A*, *HSPA8*, *MAP1LC3B*, *FOXO3* in primary human OA chondrocytes subjected to *HSP90AA1* silencing. Values are the mean with 95% CI for *HSP90AA1:* [−2.466 to −1.798]; *CDKN2A:* [−0.2618 to 2.302]; *MMP13:* [0.08957–0.6754]; *NFκB:* [0.009424–0.2226]; *LAMP2A:* [−0.2752 to 0.6592]; *HSPA8:* [0.1434–0.6716]; *MAP1LC3B:* [0.2549–0.9101]; *FOXO3:* [−0.09673 to 1.152], $n = 5$ for HSP90AA1, *NFκB* and LAMP2A; $n = 4$ for C*DKN2A*, *MMP13, HSPA8, MAP1LC3B* and *FOXO3* human donors, two-tailed unpaired Student's *t*-test. I. Quantitative analysis of mitochondrial superoxide using MitoSox red dye in primary human OA chondrocytes subjected to *HSP90AA1* silencing. Values are the mean with 95% CI of si*HSP90AA1* vs Mock: [−29.86 to 8.088]; si*HSP90AA1* + ActD vs si*HSP90AA1*: [−9.780 to 11.90], $n = 5$ human donors, one-way ANOVA with Tukey's multiple comparison test. J. Quantitative analysis of early and late apoptosis by Annexin-V and propidium iodide (PI) staining in primary human OA chondrocytes subjected to *HSP90AA1* silencing. Values are the mean with 95% CI for early apoptosis: *HSP90AA1* vs Mock: [−14.41 to 4.252]; *HSP90AA1* + ActD vs *HSP90AA1*: [−20.28 to −1.045] and late apoptosis: *HSP90AA1* vs Mock: [−4.737 to 3.059]; *HSP90AA1* + ActD vs *HSP90AA1*: [−7.174 to 0.0219], $n = 9$ human donors for early apoptosis and $n = 8$ human donors for late apoptosis, one-way ANOVA with Tukey's multiple comparison test. Antimycin A (AA, 40 μg/ml) and Actinomycin D (ActD, 1 μg/ml) were employed as positive controls for oxidative stress and cell death by apoptosis, respectively. K. Quantitative analysis of mitochondrial superoxide using MitoSox red dye in T/C28a2 normal human chondrocytes subjected to *HSP90AA1* silencing. Values are the mean with 95% CI of si*HSP90AA1* vs Mock: [−26.69 to 31.48], $n = 6$ independent experiments, two-tailed unpaired Student's *t*-test. L. Quantitative analysis of early and late apoptosis by Annexin-V and PI staining in T/C28a2 normal human chondrocytes subjected to *HSP90AA1* silencing. Values are the mean with 95% CI of early apoptosis: si*HSP90AA1* vs Mock [7.114–27.60] and late apoptosis: si*HSP90AA1* vs Mock [14.24–36.32], $n = 3$ independent experiments, two-tailed unpaired Student's *t*-test.

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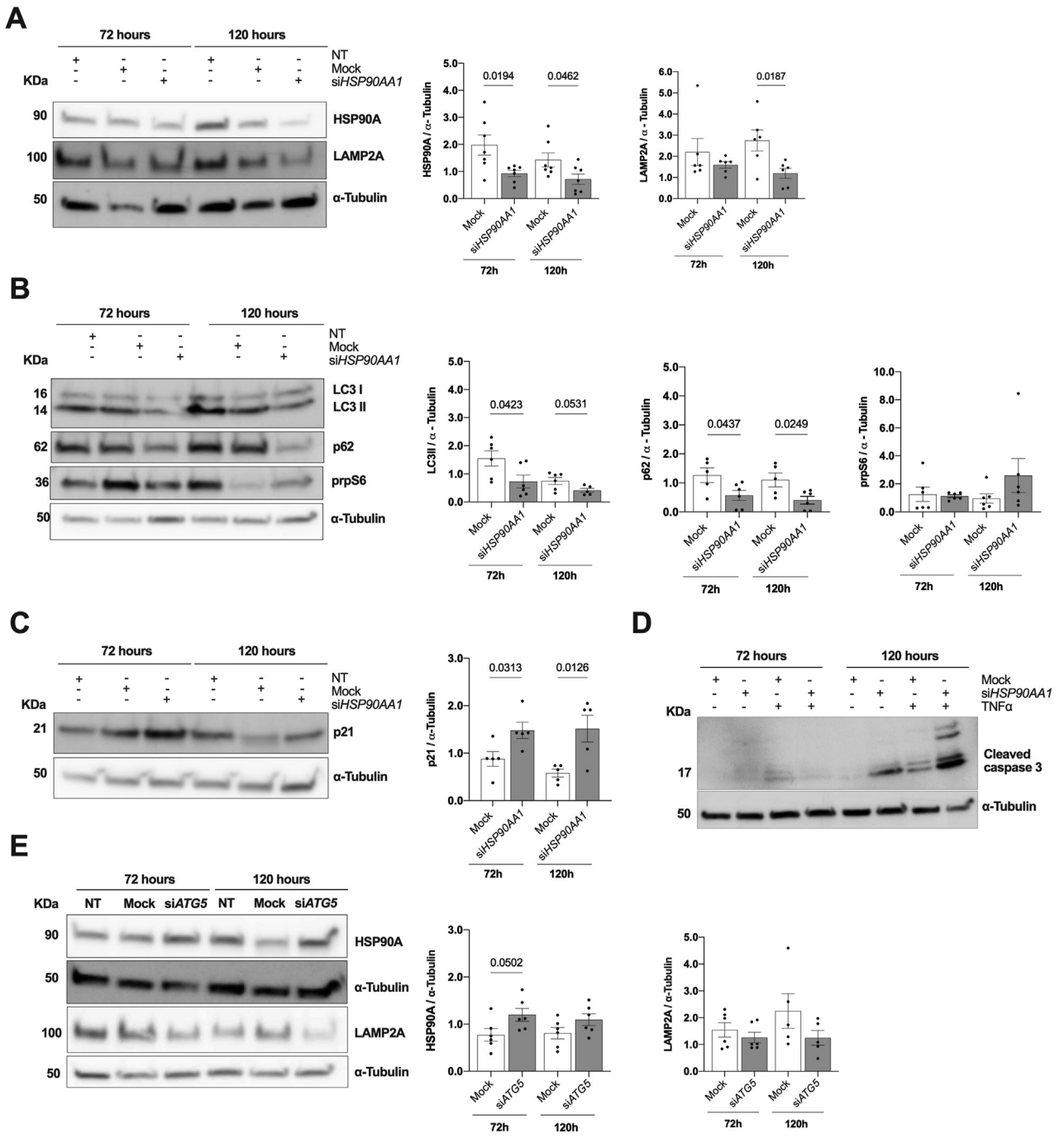

Fig. 5

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Next, we determined the capacity of CMA to restore proteostasis upon macroautophagy deficiency by *ATG5* knockdown in primary human OA chondrocytes. The results showed that silencing of *ATG5* induces macroautophagy impairment at 72 h and 120 h post-transfection [Supplementary Fig. S4(A)]. Moreover, this effect was correlated with an increase of p21 and p16 [Supplementary Fig. S4(B)]. Interestingly, protein levels of HSP90A were increased at 72 h under *ATG5* deficiency likely as a compensatory response to macroautophagy defect [Fig. 5(E)]. We propose that CMA activity increases in response to defective macroautophagy primarily in the early stages, however, the efficiency of this compensatory mechanism may decrease if damage persist and contributes to chondrocyte dysfunction.

HSP90A overexpression induces CMA and protects against chondrocyte damage

Pharmacological and genetic induction of macroautophagy protects against joint damage in OA[23,31]. However, little is known about the potential protective effect of CMA activation in human chondrocytes. Our results indicate that HSP90A plays an important role on chondrocyte homeostasis and low levels of CMA contributes to chondrocyte damage and OA pathogenesis. To determine the therapeutic role of CMA activation, *HSP90AA1* was overexpressed in primary human OA chondrocytes. The results showed a HSP90A increased at 48 and 72 h [Fig. 6(A)]. Besides, LC3II and p62 were increased [Fig. 6(B)], suggesting that HSP90A activation positively regulates macroautophagy. Importantly, HSP90A overexpression attenuates chondrocyte senescence by decreasing p21 and p16 expression [Fig. 6(C)]. These data suggest that CMA activation might be an interesting therapeutic candidate for the prevention of joint damage in OA.

Discussion

This study reports the first evidence that HSP90A is a key chaperone for chondrocyte homeostasis by regulating the crosstalk between CMA and macroautophagy, which are critical mechanisms for cellular homeostasis.

Aging-related diseases are linked to hallmarks of aging, such as loss of proteostasis[32]. Macroautophagy and CMA are key to prevent proteotoxicity by removing dysfunctional organelles and proteins[33]. Since the turnover rate is different among cell types and tissues, aged-related changes in macroautophagy and CMA are tissue-specific[5,32,33]. In this sense, non-dividing cells with low turnover, such as chondrocytes, are more susceptible to proteotoxicity. In fact, impaired macroautophagy precedes joint damage in OA[7,11], while restoring macroautophagy by pharmacological, genetic or nutritional modulation delays aging associated changes and extend lifespan[23,34,35].

Molecular chaperones are an integral part of the cellular proteostasis quality control and act as modulators of the selectivity of autophagy and proteasome[22]. During aging, the efficacy of CMA often declines, leading to the development of aging-related pathologies. However, the specific contribution of CMA to joint pathologies, including OA, has not yet been investigated. Here, we investigated blood samples from knee OA subjects and found decreased expression of chaperone-mediated CMA genes, such as *HSP90AA1* and *HSPA8*. Interestingly, *HSP90AA1* was significantly decreased in blood and cartilage from Knee OA subjects. Importantly, regression analysis revealed that reduced levels of *HSP90AA*1 are a risk factor for OA incidence, together with other relevant clinical factors, such as age and BMI.

HSP90A isoform is highly conserved and plays an important role in maintaining the stability of signaling proteins promoting cell survival and growth[36,37]. HSP90A is induced by stress in a tissue-specific manner[38]. In fact, HSP90A changes during aging are not uniform and vary across cell types[39]. Thus, HSP90A is upregulated in specific solid tumor subtypes[40], or in aged peripheral blood mononuclear cells[41]. Conversely, it is decreased in human brain aging[42]. In CMA, HSP90A, together with HSP70, are the key components for the assembly of LAMP2A translocation complex[43] and mediate the selectivity of CMA target proteins[39]. Degradation of specific proteins by CMA is a common feature in age-related diseases, while decreased stability and expression of LAMP2A during aging is associated with the decline in CMA activity[16,18,44].

Heat shock proteins affect the differentiation, maturation and metabolism in chondrocytes; however, little is known about their implication in OA. Genetic knockdown of *HSP90AA1* in human OA chondrocytes increase homeostasis genes, such as HSPA8 and MAP1LC3B, likely to compensate this chaperone defect. However, macroautophagy response was not sufficient to balance CMA deficiency, since LC3-II protein expression is reduced and mTOR activity is increased, indicating defective macroautophagy. Importantly, a reduction of p62 expression was observed upon CMA deficiency. In fact, decreased p62 expression does not always correlate with increased autophagic flux. Thus, decreased levels of p62/SQSTM1 in chaperone deficient cells can be related to macroautophagy blockage due to protein cleavage, together with other autophagy proteins, such as caspases or calpains[45]. In addition, we found increased expression of senescence markers in response to CMA deficiency. Several observations indicate that CMA activity is impaired during cellular senescence. Indeed, CMA failure is sufficient to induce cellular senescence in primary human fibroblasts as LAMP2A knockdown induces expression or p21/Cdkn1a and p16/Cdkn2a[46]. Therefore, we propose that apoptosis activation in *HSP90AA1* deficient chondrocytes may be driven by macroautophagy deficiency.

Defective CMA compromises macroautophagy and induces senescence and chondrocyte death by apoptosis. A. Western blot and densitometric analysis of HSP90A and LAMP2A expression at 72 and 120 h (h). Values are the mean with 95% CI for HSP90A, 72 h: [−1.898 to −0.2022] and 120 h: [−1.414 to −0.01396], $n = 7$ human donors; LAMP2A, 72 h: [−2.076 to 0.8560] and 120 h: [−2.783 to 0.3183], $n = 6$ human donors, two-tailed unpaired Student's t-test. B. Western blot and densitometric analysis of LC3, p62 and prpS6 expression at 72 and 120 h. Values are the mean with 95% CI for LC3, 72 h: [−1.608 to −0.03482] and 120 h: [−0.6793 to −0.005573]; p62, 72 h: [−1.368 to −0.02447] and 120 h: [−1.287 to −0.1106]; prbs6, 72 h: [−1.301 to 1.039] and 120 h: [−1.156 to 4.442], $n = 6$ human donors, two-tailed unpaired student's t-test. C. Western blot and densitometric analysis of p21 expression at 72 and 120 h. Values are the mean with 95% CI for p21, 72 h: [0.06939 to 1.136] and 120 h: [0.2624−1.612], $n = 5$ human donors, two-tailed unpaired Student's t-test. D. Western blot and densitometric analysis of CASP3 in T/C28a2 human chondrocytes at 72 and 120 h. E. Western blot and densitometric analysis of HSP90A and LAMP2A at 72 and 120 h. Values are the mean with 95% CI for HSP90A, 72 h: [−0.0004889 to 0.8512] and 120 h: [0.1046−0.6732], $n = 6$ human donors; LAMP2A, 72 h: [−1.028 to 0.4602] and 120 h: [−2.614 to 0.6219], $n = 5$ human donors, two-tailed unpaired Student's t-test. α-Tubulin was employed as a loading control. Non-transfected chondrocytes (NT) were employed as an internal control and were not used for quantitative analysis.

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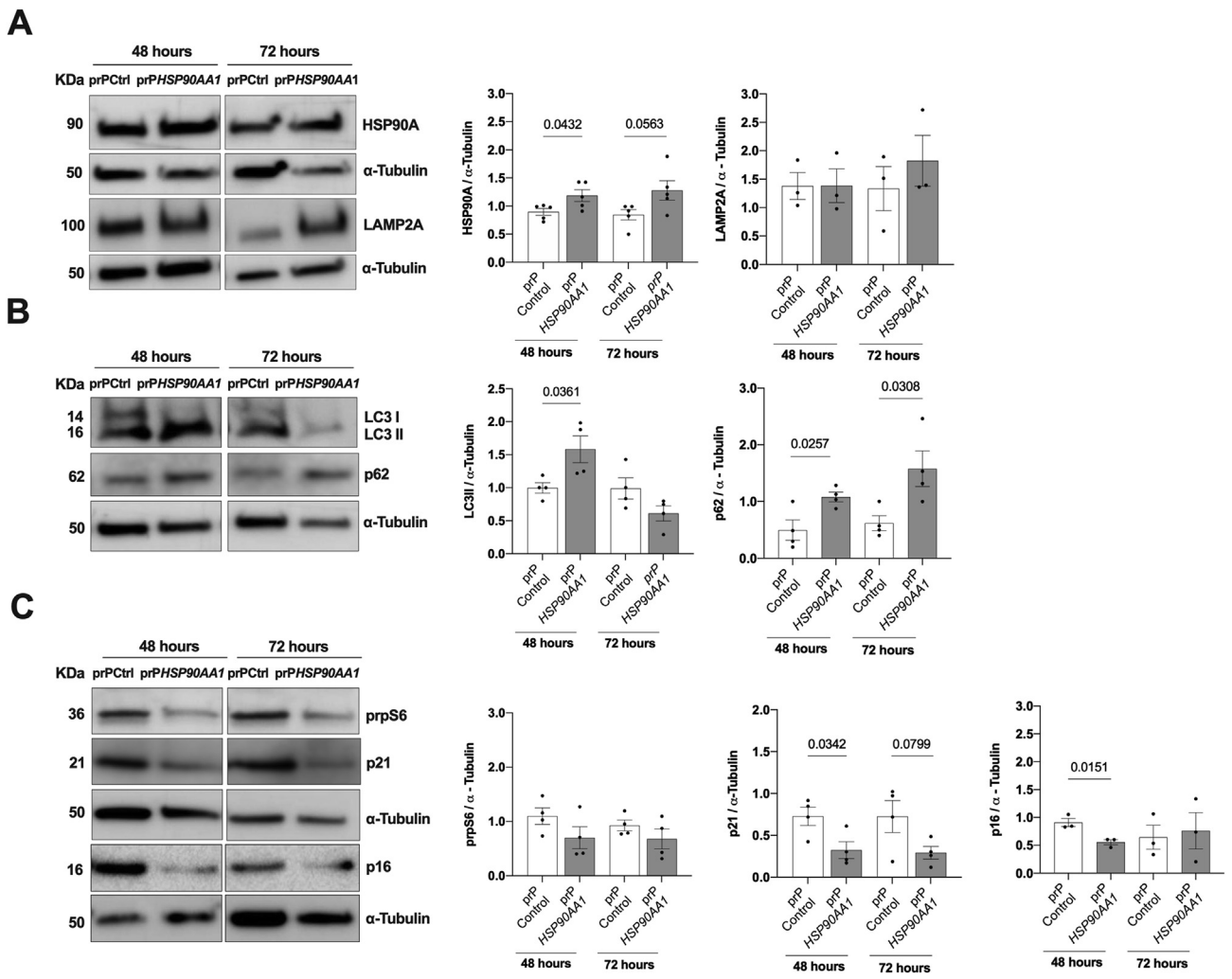

Fig. 6

Osteoarthritis and Cartilage

Genetic up-regulation of CMA reduces disease markers in human OA chondrocytes. A. Western blot and densitometric analysis of HSP90A and LAMP2A expression at 48 and 72 h (h). Values are the mean with 95% CI for HSP90A, 48 h: [0.01133−0.5700] and 72 h: [−0.01490 to 0.8848], $n = 5$ human donors; LAMP2A, 48 h: [−1.047 to 1.058] and 72 h: [−1.151 to 2.134], $n = 3$ human donors for LAMP2A, two-tailed unpaired Student's test. B. Western blot and densitometric analysis of LC3 and p62 expression at 48 and 72 h. Values are the mean with 95% CI for LC3, 48 h: [0.05292−1.120] and 72 h: [−0.8574 to 0.1027]; p62, 48 h: [0.09904−1.066] and 72 h: [0.1237−1.792], $n = 4$ human donors, two-tailed unpaired Student's test. C. Western blot and densitometric analysis of prpS6, p21 and p16 expression at 72 and 120 h. Values are the mean with 95% CI for prpS6, 48 h: [−1.020 to 0.2241] and 72 h: [−0.7585 to 0.2616]; p21, 48 h: [−0.7640 to 0.04171] and 72 h: [−0.9364 to 0.07025], p16, 48 h: [−0.5963 to −0.1133] and 72 h: [−0.9719 to 1.202], $n = 4$ human donors for prpS6 and p21 and $n = 3$ human donors for p16, two-tailed unpaired Student's test. α-Tubulin was employed as a loading control.

Our results are in agreement with the notion that imbalanced HSP90A contribute to the onset of aging pathologies[47,30] as pharmacological inhibition of HSP90 decreased LAMP2A and CMA activity[43]. Macroautophagy and CMA degradation systems are linked, and cells respond to changes in one pathway by regulating the other. However, this response is not always bidirectional and may be cell and/or tissue specific[48]. In brain and liver cells, a compensatory activation of macroautophagy upon CMA defect is observed[49,50]. Moreover, increased proteotoxicity due to blockage of CMA is alleviated by macroautophagy activation[51]. In OA chondrocytes lacking HSP90A, macroautophagy is not activated in response to CMA impairment. Consistent with our data, studies in mouse retina, neurons and hematopoietic stem cells show that CMA impairment does not induce macroautophagy activation[52–54].

To better characterize CMA function on chondrocyte homeostasis, we blocked macroautophagy by *ATG5* knockdown. Interestingly, we found HSP90A increased, however, expression of LAMP2A was not changed, suggesting that the compensatory response of CMA to macroautophagy impairment may not be permanently mediated by CMA activation. Consistent with our data, *ATG5* deficiency indicates that the CMA compensatory response to macroautophagy inhibition protects from oxidative-stress-mediated cell

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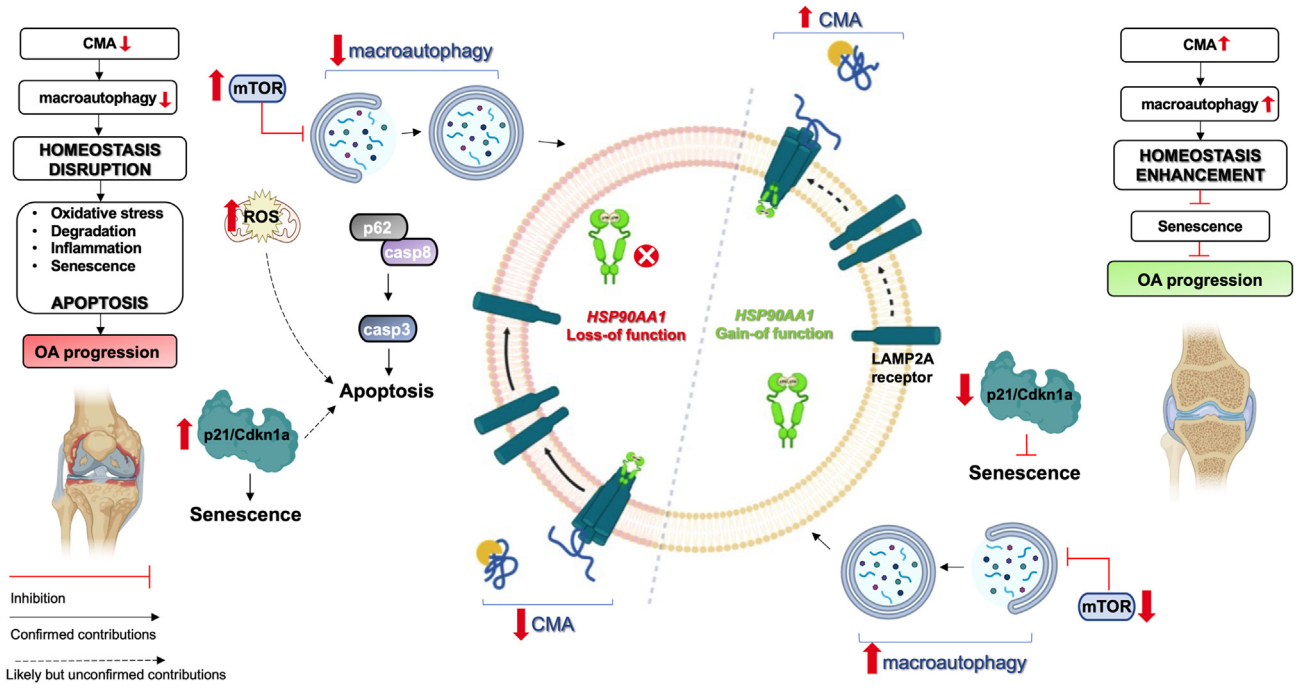

Fig. 7

Osteoarthritis and Cartilage

Schematic representation of the CMA regulation on chondrocyte homeostasis and its potential contribution to protect against joint damage.

death and contribute to preserve cone retinal cell viability[54]. Moreover, in embryonic fibroblasts from *ATG5* knockout mouse, CMA enhancement in response to macroautophagy failure was sufficient to prevent medanodine-induced oxidative stress and cell death[55].

To investigate the effect of CMA activation, *HSP90AA1* was overexpressed in primary human OA chondrocytes. The results showed that CMA activation increased macroautophagy and reduced mTOR signaling pathway as well as chondrocyte senescence. These results are consistent with previous observations, where genetic and pharmacological CMA enhancement ameliorate age and disease related changes on hematopoietic stem cells, Alzheimer disease and hepatic function[44,52,53].

Altogether, our results indicate that HSP90A may play a central role in chondrocyte homeostasis. Besides, these findings reveal its role as part of the crosstalk between macroautophagy and CMA. Additionally, increased deleterious markers as inflammation, cartilage degradation, cell senescence, as well as increased ROS production and cell death by apoptosis support that loss of HSP90A function may contribute to the onset of OA. Likewise, the reduction in senescence markers in OA chondrocytes overexpressing *HSP90AA1* suggest that CMA activation could be a promising target to develop novel therapeutics (Fig. 7). However, the limitations of this study include the challenge at detecting signals from other autophagy related genes (not included in the array) and a more detailed investigation of the mechanisms by which this chaperone interplays between lysosomal system and proteasome to balance chondrocyte homeostasis.

In conclusion, we describe for the first time that HSP90A is a biomarker of defective autophagy in joint aging and OA pathology. Genetic activation of CMA by overexpressing HSP90A restores proteostasis and protects chondrocytes from death. These findings highlight CMA as a promising therapeutic target to prevent disease progression in OA patients. Therefore, identifying molecules modulating CMA could facilitate the translation of this mechanism into novel therapies.

Author's contributions

All authors approved the final version to be published.

Concept and design: Pinto-Tasende, Blanco, Caramés.

Performed the experiments: Lorenzo-Gómez, Nogueira-Recalde, García-Domínguez, Caramés.

Data analysis and interpretation: Lorenzo-Gómez, Caramés.

Writing and review of Manuscript: Lorenzo-Gómez, Nogueira-Recalde, García-Domínguez, Oreiro, Lotz, Pinto-Tasende, Blanco and Caramés.

Declaration of competing interest

The authors of this papers have declared no potential conflicts of interest.

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Supplementary data

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References

1. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, *et al*. Osteoarthritis. Lancet 2015;386:376–87, https://doi.org/10.1016/S0140-6736(14)60802-3.
2. Hawker GA. Osteoarthritis is a serious disease. Clin Exp Rheumatol 2019;120:3–6. PMID: 31621562.
3. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheumatol 2012;64:1697–707, https://doi.org/10.1002/art.34453.
4. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27–42, https://doi.org/10.1016/j.cell.2007.12.018.
5. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194–217, https://doi.org/10.1016/j.cell.2013.05.039.
6. Vellai T, Takács-Vellai K, Sass M, Klionsky DJ. The regulation of aging: does autophagy underlie longevity? Trends Cell Biol 2009;19:487–94, https://doi.org/10.1016/j.tcb.2009.07.007.
7. Caramés B, Taniguchi N, Otsuki S, Blanco FJ, Lotz M. Autophagy is a protective mechanism in normal cartilage, and its aging-related loss is linked with cell death and osteoarthritis. Arthritis Rheumatol 2010;62:791–801, https://doi.org/10.1002/art.27305.
8. Caramés B, Olmer M, Kiosses WB, Lotz MK. The relationship of autophagy defects to cartilage damage during joint aging in a mouse model. Arthritis Rheumatol 2015;67:1568–76, https://doi.org/10.1002/art.39073.
9. Mizushima N, Klionsky DJ. Protein turnover via autophagy: implications for metabolism. Annu Rev Nutr 2007;27:19–40, https://doi.org/10.1146/annurev.nutr.27.061406.093749.
10. Meijer AJ, Codogno P. Regulation and role of autophagy in mammalian cells. Int J Biochem Cell Biol 2004;36:2445–62, https://doi.org/10.1016/j.biocel.2004.02.002.
11. Lotz MK, Caramés B. Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. Nat Rev Rheumatol 2011;7:579–87, https://doi.org/10.1038/nrrheum.2011.109.
12. Massey AC, Zhang C, Cuervo AM. Chaperone-mediated autophagy in aging and disease. Curr Top Dev Biol 2006;73:205–35, https://doi.org/10.1016/S0070-2153(05)73007-6.
13. Cuervo AM. Chaperone-mediated autophagy: Dice's "wild" idea about lysosomal selectivity. Nat Rev Mol Cell Biol 2011;12:535–41, https://doi.org/10.1038/nrm3150.
14. Tekirdag K, Cuervo AM. Chaperone-mediated autophagy and endosomal microautophagy: jointed by a chaperone. J Biol Chem 2018;293:5414–24, https://doi.org/10.1074/jbc.R117.818237.
15. Levine B, Kroemer G. SnapShot: macroautophagy. Cell 2008;132:162.e1–3, https://doi.org/10.1016/j.cell.2007.12.026.
16. Kaushik S, Cuervo AM. The coming of age of chaperone-mediated autophagy. Nat Rev Mol Cell Biol 2018;19:365–81, https://doi.org/10.1038/s41580-018-0001-6.
17. Cuervo AM. Chaperone-mediated autophagy: selectivity pays off. Trends Endocrinol Metab 2010;21:142–50, https://doi.org/10.1016/j.tem.2009.10.003.
18. Cuervo AM, Dice JF. Age-related decline in chaperone-mediated autophagy. J Biol Chem 2000;275:31505–13, https://doi.org/10.1074/jbc.M002102200.
19. Kaushik S, Massey AC, Cuervo AM. Lysosome membrane lipid microdomains: novel regulators of chaperone-mediated autophagy. EMBO J 2006;25:3921–33, https://doi.org/10.1038/sj.emboj.7601283.
20. Kiffin R, Kaushik S, Zeng M, Bandyopadhyay U, Zhang C, Massey AC, *et al*. Altered dynamics of the lysosomal receptor for chaperone-mediated autophagy with age. J Cell Sci 2007;120:782–91, https://doi.org/10.1242/jcs.001073.
21. Massey AC, Kaushik S, Sovak G, Kiffin R, Cuervo AM. Consequences of the selective blockage of chaperone-mediated autophagy. Proc Natl Acad Sci U S A 2006;103:5805–10, https://doi.org/10.1073/pnas.0507436103.
22. Kaushik S, Cuervo AM. Chaperones in autophagy. Pharmacol Res 2012;66:484–93, https://doi.org/10.1016/j.phrs.2012.10.002.
23. Caramés B, Hasegawa A, Taniguchi N, Miyaki S, Blanco FJ, Lotz M. Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. Ann Rheum Dis 2012;71:575–81, https://doi.org/10.1136/annrheumdis-2011-200557.
24. López de Figueroa P, Lotz MK, Blanco FJ, Caramés B. Autophagy activation and protection from mitochondrial dysfunction in human chondrocytes. Arthritis Rheumatol 2015;67:966–76, https://doi.org/10.1002/art.39025.
25. Kaushik S, Massey AC, Mizushima N, Cuervo AM. Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. Mol Biol Cell 2008;19:2179–92, https://doi.org/10.1091/mbc.e07-11-1155.
26. Oreiro-Villar N, Raga AC, Rego-Pérez I, Pértega S, Silva-Diaz M, Freire M, *et al*. Descripción de la cohorte PROCOAC (PROspective COhort of A Coruña): Cohorte prospectiva española para el estudio de la osteoartritis. Reumatol Clin 2020, https://doi.org/10.1016/j.reuma.2020.08.010. S1699-258X:30231-X.
27. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502, https://doi.org/10.1136/ard.16.4.494.
28. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al*. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheumatol 1986;29:1039–49, https://doi.org/10.1002/art.1780290816.
29. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthr Cartil 2010;18(Suppl 3):S17–23, https://doi.org/10.1016/j.joca.2010.05.025.
30. Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, *et al*. The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Investig 2007;117:648–58, https://doi.org/10.1172/JCI29715.

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31. Zhang Y, Vasheghani F, Li Y, Blati M, Simeone K, Fahmi H, *et al*. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. Ann Rheum Dis 2015;74:1432–40, https://doi.org/10.1136/annrheumdis-2013-204599.
32. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 2009;78:959–91, https://doi.org/10.1146/annurev.biochem.052308.114844.
33. Salminen A, Kaarniranta K. Regulation of the aging process by autophagy. Trends Mol Med 2009;15:217–24, https://doi.org/10.1016/j.molmed.2009.03.004.
34. Madeo F, Tavernarakis N, Kroemer G. Can autophagy promote longevity? Nat Cell Biol 2010;12:842–6, https://doi.org/10.1038/ncb0910-842.
35. Klionsky DJ, Petroni G, Amaravadi RK, Baehrecke EH, Ballabio A, Boya P, *et al*. Autophagy in major human diseases. EMBO J 2021;40, e108863, https://doi.org/10.15252/embj.2021108863.
36. Subbarao Sreedhar A, Kalmár É, Csermely P, Shen Y-F. Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 2004;562:11–5, https://doi.org/10.1016/s0014-5793(04)00229-7.
37. Zuehlke AD, Beebe K, Neckers L, Prince T. Regulation and function of the human HSP90AA1 gene. Gene 2015;570:8–16, https://doi.org/10.1016/j.gene.2015.06.018.
38. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 2010;10:537–49, https://doi.org/10.1038/nrc2887.
39. Shelton LB, Koren J, Blair LJ. Imbalances in the Hsp90 chaperone machinery: implications for tauopathies. Front Neurosci 2017;11:724, https://doi.org/10.3389/fnins.2017.00724.
40. Bohonowych J, Hance M, Nolan K, Defee M, Parsons C, Isaacs J. Extracellular Hsp90 mediates an NF-κB dependent inflammatory stromal program: implications for the prostate tumor microenvironment. Prostate 2014;74:395–407, https://doi.org/10.1002/pros.22761.
41. Njemini R, Lambert M, Demanet C, Kooijman R, Mets T. Basal and infection-induced levels of heat shock proteins in human aging. Biogerontology 2007;8:353–64, https://doi.org/10.1007/s10522-006-9078-y.
42. Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, *et al*. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep 2014;9:1135–50, https://doi.org/10.1016/j.celrep.2014.09.042.
43. Bandyopadhyay U, Kaushik S, Varticovski L, Cuervo AM. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. Mol Cell Biol 2008;28:5747–63, https://doi.org/10.1128/MCB.02070-07.
44. Zhang C, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. Nat Med 2008;14:959–65, https://doi.org/10.1038/nm.1851.
45. El-Khoury V, Pierson S, Szwarcbart E, Brons NHC, Roland O, Cherrier-De Wilde S, *et al*. Disruption of autophagy by the histone deacetylase inhibitor MGCD0103 and its therapeutic implication in B-cell chronic lymphocytic leukemia. Leukemia 2014;28:1636–46, https://doi.org/10.1038/leu.2014.19.
46. Kang HT, Lee KB, Kim SY, Choi HR, Park SC. Autophagy impairment induces premature senescence in primary human fibroblasts. PLoS One 2011;6, e23367, https://doi.org/10.1371/journal.pone.0023367.
47. Evans CG, Wisén S, Gestwicki JE. Heat shock proteins 70 and 90 inhibit early stages of amyloid β-(1-42) aggregation in vitro. J Biol Chem 2006;281:33182–91, https://doi.org/10.1074/jbc.M606192200.
48. Wu H, Chen S, Ammar AB, Xu J, Wu Q, Pan K, *et al*. Crosstalk between macroautophagy and chaperone-mediated autophagy: implications for the treatment of neurological diseases. Mol Neurobiol 2015;52:1284–96, https://doi.org/10.1007/s12035-014-8933-0.
49. Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L. Abberant α-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One 2009;4, e5515, https://doi.org/10.1371/journal.pone.0005515.
50. Cuervo AM, Stafanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science 2004;305:1292–5, https://doi.org/10.1126/science.1101738.
51. Cuervo AM, Wong E. Chaperone-mediated autophagy: roles in disease and aging. Cell Res 2014;24:92–104, https://doi.org/10.1038/cr.2013.153.
52. Bourdenx M, Martín-Segura A, Scrivo A, Rodriguez-Navarro JA, Kaushik S, Tasset I, *et al*. Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome. Cell 2021;184:2696–2714.e25, https://doi.org/10.1016/j.cell.2021.03.048.
53. Dong S, Wang Q, Kao YR, Diaz A, Tasset I, Kaushik S, *et al*. Chaperone-mediated autophagy sustains hematopoietic stem cell function. Nature 2021;591:117–23, https://doi.org/10.1038/s41586-020-03129-z.
54. Rodríguez-Muela N, Koga H, García-Ledo L, de la Villa P, de la Rosa EJ, Cuervo AM, *et al*. Balance between autophagic pathways preserves retinal homeostasis. Aging Cell 2013;12:478–88, https://doi.org/10.1111/acel.12072.
55. Wang Y, Singh R, Xiang Y, Czaja MJ. Macroautophagy and chaperone-mediated autophagy are required for hepatocyte resistance to oxidant stress. Hepatology 2010;52:266–77, https://doi.org/10.1002/hep.23645.

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