Expression pattern of Syndecan-1 and HSP-70 in hip tissue of patients with osteoarthritis

Julian Kaufman^a, Davor Caric^b, Katarina Vukojevic^{a,*}

^a Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Soltanska 2, 21000, Split, Croatia

^b Department of Orthopedic Surgery and Traumatology, KBC Split – Krizine, Soltanska 1, 21000, Split, Croatia

ARTICLE INFO

Keywords:

Osteoarthritis
Hip
Syndecan-1
HSP-70
Cartilage
Subchondral tissue

ABSTRACT

Objectives: We investigated the expression of Syndecan-1 and HSP-70 in synovial, chondral and subchondral tissue in the hips of patients with osteoarthritis (OA).

Methods: Total hip arthroplasty was performed on 6 patients and 3 controls. Patients were divided into two groups according to disease severity. The tissues were immunohistochemically analyzed.

Results: Cartilage and subchondral tissue in patients with severe OA contained abundant positive cells with a mean positive percentage of $94,51 \pm 5\%$ and $78,95 \pm 14\%$ for Syndecan-1 and $94,42 \pm 5\%$ and $75,67 \pm 17\%$ for HSP-70, respectively.

Conclusion: In conclusion, HSP-70 and Syndecan-1 might play a role in the onset and regulation of OA in the hip.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative disease of the joints in the human body.¹ It is the most common form of arthritis nowadays affecting 250 million people worldwide.² The most frequent risk factors are obesity, trauma, age and genetic predisposition. OA can be classified as primary (idiopathic, multifactorial and unknown cause) and secondary (following fractures, joint infections, joint dysplasias etc.).² Even though OA is not considered to be an inflammatory disease, it should be noted that there is local inflammation like synovitis.² However, it is not known whether the inflammatory reaction is induced by it or whether this is secondary to changes of OA.² Furthermore, cartilage itself is incapable of inducing pain or inflammation since there is lack of vasculature and innervation.² This means that pain must derive from other joint components, like synovium, joint capsule, ligaments, subchondral bone and muscles.²

The pathogenesis of OA is multifactorial and mainly based on pathologic changes in the articular cartilage, subchondral bone and synovium.^{2–4} One major cause is biomechanical stress from trauma, abnormal joint morphology and increased weight bearing on articular cartilage.^{2,3} This results in damage to the homeostatic balance of synthesis and degradation of joint tissue consequently leading to end stage OA.³ Repetitive shear stress in the joint from trauma or obesity has been affiliated with molecular and cellular changes, such as increased expression of pro-inflammatory mediators and increased

apoptotic activity.³ The area between subchondral bone and articular cartilage, called the osteochondral junction, has also been found to be an important component of OA.³

Syndecan-1 belongs to the family of Syndecans.⁵ They are trans-membrane cell surface proteoglycans that carry glycosaminoglycan (GAG) chains, such as heparan and chondroitin sulfate, which are covalently bound to the core protein.^{5,6} Syndecans and matrix metalloproteinases work together on keeping the normal ECM homeostasis in balance and may be implicated in modification of cellular activities such as proliferation and differentiation.⁶ Moreover, Syndecans are known to be coreceptors for cytokines and more importantly ECM receptors that cause cell adhesion with the matrix and other cells.^{5,6} Therefore, Syndecans may have a role in angiogenesis and migration of leukocytes which are essential constituents of disease development for synovitis.⁵ In fact, heparan sulfate chains specific for Syndecans also affect osteoblastogenesis and osteoclastogenesis which are both associated with OA.⁶

Focusing on Syndecan-1, it is expressed in many different developmental processes, such as tissue organization, like angiogenesis and wound healing, and limb bud & tooth development.^{5,6} Increased expression has also been detected in OA due to proliferation of chondrocytes.⁶ Previous research on mice has shown that in early OA maturing chondrocytes classically change their phenotype, similar to tissue repair, producing not only more type IIa procollagen but also transcription factor Sox9.⁶ However, this does not suffice as the

* Corresponding author. University of Split School of Medicine, Department of Anatomy, Histology and Embryology, Soltanska 2, 21000, Split, Croatia.

E-mail addresses: julian_kaufman@yahoo.de (J. Kaufman), caric.davor@gmail.com (D. Caric), katarina.vukojevic@mefst.hr (K. Vukojevic).

cartilagenous damage continues to endstage OA.⁶ This repair is very similar to that of wounds in which Syndecan-1 plays a crucial role.⁶

HSP-70 belongs to the family of Heat shock proteins.⁷ Its physiologic role is to protect the structural integrity and cellular proteins by inhibiting multiple pathways of apoptosis, such as Nitric Oxide induced caspase 3, the previously mentioned NF- κ B, JNK, apoptosome formation and mitochondrial cytochrome C release.^{7–10} Moreover, it protects the cells from oxidative stress which otherwise would result in apoptosis and necrosis.⁷

Furthermore, it has been researched in rats, when exposed to different severities of exercise, that little and intermediate workout increased HSP-70 expression actually preventing OA by promoting cell metabolism to provide enough sulfated glycosaminoglycan to the ECM in the joint.⁹ However, severe exercise resulted in OA despite increased HSP-70 potentially exceeding its capacity of protection.⁹ This may be due to increased expression of HSP-90 which was found to restrict the properties of HSP-70 and activate the NF- κ B which is a major factor in the pathogenesis of OA.⁹

The purpose of this study is to investigate the expression of Syndecan-1 and HSP-70 in synovial, chondral and subchondral tissue in the hip of human patients with osteoarthritis. Since OA in the knee has been researched frequently the interest was whether the same process with the same markers also occurs in the hip. Depending on the results, the expression could be the aim of a new pharmacologic agent targeting Syndecan-1 and HSP-70 to ameliorate the symptoms of osteoarthritis in the hip.

2. Materials and methods

2.1. Study population

This cross-sectional study was performed in the Department of Orthopedics and Traumatology of the University Hospital Split (KBC) and in the Department of Anatomy, Histology and Embryology of the University of Split School of Medicine. The tissue was collected in the period of 2017–2018. The experimental protocol was approved by The Ethics Committee of the University Hospital Center Split. All performed procedures were in compliance with the ethical standards of the institutional research committee and the 1964 Helsinki declaration.

Nine patients were included in this study. Six patients were diagnosed with severe osteoarthritis of the hip by an orthopedic surgeon on the basis of anamnesis, clinical examination and standard hip x-ray. The other three patients underwent total hip replacement for a femoral neck fracture and were used as control. Written informed consent from each subject was obtained prior to the procedure. Patients were included and excluded according to the American College of Rheumatology criteria for the diagnosis of hip osteoarthritis. They were scored according to the Harris Hip Score, Oxford Hip Score and the radiologic Kellgran Lawrence grading scale. In order to be put in the group of severe OA they had to reach a Kellgran Lawrence grade of at least 3, an Oxford Hip Score of maximally 27 out of 48 and/or a Harris Hip Score of maximally 70 out of 100.¹¹ The patients used as control all had Kellgran Lawrence grades of 0 or 1. The Oxford Hip Score and the Harris Hip Score were not able to be assessed in these patients with a femoral neck fracture since these two scores assess the range of motion.

2.2. Tissue collection

Total hip arthroplasty was performed on included patients (Implant: Pinnacle Acetabular Cup System and Corail Hip System by Depuy, Johnson and Johnson, USA).

Then, tissue from the weight bearing area of the pathologic femur adjacent to the most damaged but still cartilage containing zone was removed in a triangular manner with an oscillating saw (Trauma Reckon Sytem by Synthes, Switzerland).

2.3. Immunohistochemistry

The tissue samples were collected and fixed in 4% Paraformaldehyde (PFA) in Phosphate buffered saline (PBS) overnight and transferred to osteosoft solution for 6 months.

The tissue samples were then embedded in paraffin blocks and cut transversely (7 μ m thin sections) and mounted on slides. These were then deparaffinated in Xylol for three times 5 min.

Following this, they were rehydrated two times for 10 min in 100% ethanol, 5 min in 95% ethanol, 5 min in 70% ethanol and 5 min in distilled water. Next, the slides were heated in citrate buffer (pH 6.0) at 700 Watt in a microwave for 12 min. After they had cooled off at room temperature for 20 min the slides were rinsed with PBS for four times 5 min. Afterwards, they were dried and the tissue was encircled by a PAP pen. Block protein was then put on the tissue sections for 30 min. After the block protein was removed and the slides had dried the primary antibodies were applied on all of the sections except for one which was used as negative control. The two antibodies from Abcam (Cambridge, England) used were raised in mouse (ab34164-100) diluted at 1:100 targeting Syndecan-1 and in rabbit (ab31010) diluted at 1:500 targeting HSP-70. They were kept overnight covered by parafilm in a humidified dark chamber at room temperature.

The next day the slides were rinsed with PBS two times for 5 min. Afterwards, the secondary antibodies from Abcam (Cambridge, England) were applied for 1 h. The secondary antibodies for Syndecan-1 were raised in goat (ab6786) diluted at 1:400 appearing red on immunofluorescence and the ones for HSP-70 were raised in donkey (ab150073) diluted at 1:400 appearing green on immunofluorescence. The slides were then again rinsed with PBS twice each for 5 min before 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining was applied for 2 min. Following this, they were again rinsed with PBS twice for 5 min and dried. Lastly, they were mounted in Immu-mont and cover-slipped in order to observe and capture images of the sections using an Olympus (Tokyo, Japan) BX51 microscope equipped with an Olympus (Tokyo, Japan) DP71 digital camera.

All images were observed and saved with Cella Imaging Software for Life Sciences Microscopy from Olympus (Tokyo, Japan). The sections were analyzed focusing mainly on synovial, chondral and subchondral tissue. Ten non overlapping fields were taken using 40 $\times$ objective magnification, each field representing three images. One for Syndecan-1 expression, one for HSP-70 expression and one for nuclear expression with DAPI. These images were then further observed and merged using Adobe Photoshop (Adobe Systems, MI, USA). The cells staining positively for Syndecan-1 and HSP-70, respectively, and all the nuclei stained with DAPI were counted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

The number of positive and negative nuclei from all useable merged images for each patient was calculated and compared between groups (control and severe OA).

2.4. Statistical analysis

Data analysis was conducted with GraphPad Prism (GraphPad Software, La Jolla, CA, USA) and Mann-Whitney *t*-test was used for statistical analysis to examine the difference between control and severe OA in the cartilage, subchondral and synovial tissue.

3. Results

Immunohistochemical staining with Syndecan-1 and HSP-70 markers revealed positive expression of both markers (Fig. 1)

The following graphs (Fig. 2) demonstrate the amount of mean percentage of cell positivity in cartilage and subchondral tissue in the control patients with mild OA and patients with severe OA according to Harris Hip Score, Oxford Hip Score and Kellgran Lawrence grading. One can see that the cartilage and subchondral tissue in patients with severe

Fig. 1. HSP-70 positive cells were seen as green staining (arrows) and Syndecan-1 as red staining within different areas of the chondral and synovial tissue. Co-localization of HSP-70 and Syndecan-1 merged with DAPI nuclear stain are displayed in the far right column (arrowheads). Control patients (a) and patients with severe osteoarthritis (b). Scale bar for a is 17 μ m and for b 10 μ m. Legend: s-synovium; sc-subchondral tissue; c-cartilage; OA-severe osteoarthritis.

OA contained abundant positive cells with a mean positive percentage of $94,51 \pm 5\%$ and $78,95 \pm 14\%$ for Syndecan-1 and $94,42 \pm 5\%$ and $75,67 \pm 17\%$ for HSP-70, respectively. On the contrary, the control patients only had minimal positivity with a mean percentage of $55,76 \pm 8\%$ and $34,18 \pm 4\%$ for Syndecan-1 and $55,15 \pm 6\%$ and $33,67 \pm 5\%$ for HSP-70, respectively. Synovial tissue could not be identified enough for presentation, but followed a similar trend.

It was established that there was a statistical significance of both Syndecan-1 and HSP-70 with a $p < 0.05$ and 95% CI in cartilage and $p < 0.0001$ and 95% CI in subchondral tissue between control and severe OA.

4. Discussion

The results of immunohistochemical staining in this study show that there in fact is an increased expression of Syndecan-1 and HSP-70 in chondral, subchondral and synovial tissue of patients with severe OA in comparison with the control patients. Both markers were found abundantly in cartilage, subchondral and synovial tissue.

When interpreting the results one can say that there is an increase in percentage of positive cells due to damage of cartilage and a resulting proliferation of chondrocytes trying to repair the damage. Moreover, the inflammation of the synovium causes an increase in synovial lining cell thickness. Also, the accelerated turnover of subchondral bone as a reaction to structural damage is a cause for increased cell production. The reason for a higher number of cells in subchondral tissue compared to cartilage is probably the more developed vascularity and the resulting higher differentiation of cells in the subchondral tissue. Nevertheless, there is also an increased hyperplasticity and layering in the cartilage in the patients with severe OA, whereas normally the cartilage is lined with only one layer.

Most of these chondral, subchondral and synovial cells were found to coexpress Syndecan-1 and HSP-70. This can be explained by the fact that in the chondral, subchondral and synovial tissue Syndecan-1 becomes expressed by the proliferating cells that try to repair the damage. In this way, Syndecan-1 can trigger angiogenesis and attach to chemokines provoking a chemokinetic gradient similar to wound healing. In addition, the proliferating cells also produce HSP-70 to protect their structure and proteins since it inhibits apoptosis inducing pathways.

There is a lack of similar studies on this topic since most research papers focused on the knee. Nevertheless, results were all alike. When tested on knees in mice, Salminen-Mankonen et al. also proved an up-regulation of Syndecan-1 in OA of the knee.⁶ He attributed it to the body's attempt to repair the damage that had taken place in the joint.⁶ Furthermore, increased expression of Syndecan-1 in OA and decreased expression in the control group is in concordance with Patterson et al. who demonstrated a similar increase in heparan sulfate proteoglycans (HSPGs) in inflammatory diseases of the knee in his study in 2007.⁵

Focusing on HSP-70, results of other studies also correlate with our findings. Grossin et al. transduced chondrocytes with HSP-70 vectors and found out that these cells were not only more resistant to cell death

but also that overexpression of HSP-70 decreased the severity of OA in rats.¹² Furthermore, in 2018 Ngamurkos et al. came to the conclusion that HSP-70 levels in human knees were increased according to their disease severity and that it could be used as a biomarker for the prediction of OA severity.⁷ Nevertheless, HSP-70 overexpression alone might not be sufficient in protection of cartilage.⁹ According to Siebelt et al., HSP-90 might play a bigger role.⁹ They suggested that HSP-70 is less responsive to a higher load because HSP-90 restricts its action in increased biomechanical stress.⁹ When inhibiting HSP-90 in rats they realized that it not only improved subchondral bone thickness but also reduced macrophage activation.⁹ Further research is needed in order to find out the exact correlation between HSP-70 and HSP-90 in humans since this could be a possible therapeutic intervention to impede OA.

One of the limitations of our study was that we did not have another group without any OA to see a baseline and whether there is a constant rise in Syndecan-1 and HSP-70. Since most people have at least mild OA in their sixties and THR is usually not done before it is difficult to find a group without any OA. However, this was beyond the scope of this research. In addition, the sample size could be increased in the next studies as we only included 6 patients with severe OA and only 3 as controls. Moreover, since there wasn't enough synovial tissue identified it was not possible to present tangible results. However, the trend was similar. For future research it would be important to extract solely synovium.

In conclusion, the results of our study strongly suggest that HSP-70 and Syndecan-1 play an important role in the onset and regulation of OA in the hip and that they do not differ from results in previous research on the knee. They are part of the process that tries to heal or protect the chondral, subchondral and probably synovial tissue in the hip by causing angiogenesis, increasing inflammatory cells and preventing apoptosis. This means that pharmaceutical agents might help by increasing the expression of HSP-70. However, increasing HSP-70 alone might not work since HSP-90 will limit its activity in severe OA as mentioned previously. It would be important to create an agent that can enhance HSP-70 and block HSP-90 at the same time. On the contrary, overexpression of Syndecan-1 will increase inflammation and exacerbate symptoms. Consequently, a pharmaceutical drug blocking Syndecan-1 could decrease the inflammatory response and its associated symptoms.

Declarations

- Each author declares that there is no conflict of interest.
- This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Julian Kaufman: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization,

Fig. 2. Mean percentage of positive staining of Syndecan-1 and HSP-70 in cartilage (a), subchondral tissue (b) and in coexpression (c) in control and severe OA. Data were presented as mean $\pm$ SD.

Writing - original draft, Writing - review & editing. **Davor Caric:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Katarina Vukojevic:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources,

Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

References

1. Pereira D, Ramos E, Branco J. Osteoarthritis. *Acta Med Port.* 2015;28:99–106.
2. Mora JC, Przkora R, Cruz-Almeida Y. Knee Osteoarthritis: pathophysiology and

- current treatment modalities. *J Pain Res.* 2018;11:2189–2196 <https://doi.org/10.2147/JPR.S154002>.
3. Murphy NJ, Eyles JP, Hunter DJ. Hip osteoarthritis: etiopathogenesis and implications for management. *Adv Ther.* 2016;33:1921–1946 <https://doi.org/10.1007/s12325-016-0409-3>.
 4. Ostojic M, Soljic V, Vukojevic K, Dapic T. Immunohistochemical characterization of early and advanced knee osteoarthritis by NF- κ B and iNOS expression. *J Orthop Res.* 2017;35:1990–1997 <https://doi.org/10.1002/jor.23504>.
 5. Patterson AM, Cartwright A, David G, et al. Differential expression of syndecans and glypicans in chronically inflamed synovium. *Ann Rheum Dis.* 2007;67:592–601 <http://doi.org/10.1136/ard.2006.063875>.
 6. Salminen-Mankonen H, Säämänen AM, Jalkanen M, Vuorio E, Piriälä L. Syndecan-1 expression is upregulated in degenerating articular cartilage in a transgenic mouse model for osteoarthritis. *Scand J Rheumatol.* 2005;34:469–474 <https://doi.org/10.1080/03009740500304338>.
 7. Ngarmukos S, Scaramuzza S, Theerawattanapong N, Tanavalee A, Honsawek S. Circulating and synovial fluid heat shock protein 70 are correlated with severity in knee osteoarthritis. *Cartilage.* 2018;1 1947603518790075 <https://doi.org/10.1177/1947603518790075>.
 8. Takahashi K, Kubo T, Goomer RS, et al. Analysis of heat shock proteins and cytokines expressed during early stages of osteoarthritis in a mouse model. *Osteoarthr Cartil.* 1997;5:321–329.
 9. Siebelt M, Jahr H, Groen HC, et al. Hsp90 inhibition protects against biomechanically induced osteoarthritis in rats. *Arthritis Rheum.* 2013;65:2102–2112 <https://doi.org/10.1002/art.38000>.
 10. Kang EH, Kim DJ, Lee EY, Lee YJ, Lee EB, Song YW. Downregulation of heat shock protein 70 protects rheumatoid arthritis fibroblast-like synoviocytes from nitric oxide-induced apoptosis. *Arthritis Res Ther.* 2009;11:R130 <https://doi.org/10.1186/ar2797>.
 11. Nilsdotter A, Bremander A. Measures of hip function and symptoms: Harris hip score (HHS), hip disability and osteoarthritis outcome score (HOOS), Oxford hip score (OHS), lequesne index of severity for osteoarthritis of the hip (LISOH), and American academy of orthopedic surgeons (AAOS) hip and knee questionnaire. *Arthritis Care Res.* 2011;11:S200–S207 <https://doi.org/10.1002/acr.20549>.
 12. Grossin L, Cournil-Henrionnet C, Pinzano A, et al. Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis. *FASEB J.* 2006;20:65–75 <https://doi.org/10.1096/fj.04-2889com>.