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Review

Osteoarthritis year in review 2018: biology

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SUMMARY

This Year in Review highlights a selection of articles published between the 2017 and 2018 Osteoarthritis Research Society International (OARSI) World Congress meetings within the field of osteoarthritis biology, presented at OARSI 2018. Selected articles were obtained from a PubMed search covering cartilage, subchondral bone, inflammation, ageing, pain and animal models. Studies focused on biomechanics, biomarkers, genetics and epigenetics, imaging and clinical studies were excluded due to their coverage in other articles within the OARSI Year in Review series. Significant themes including the role of progenitor cells in cartilage homeostasis and repair, novel signalling mechanisms controlling chondrocyte phenotypic stability and the influence of disrupted or senescent chondrocytes were identified and are discussed in this review. Overarching conclusions derived from these study areas indicate that promising avenues of intervention are on the horizon, however further understanding is required in order to target therapeutic treatments to suitable patient subgroups and disease stages.

Introduction

The basis of this Year in Review of osteoarthritis biology is formed from a personal selection of articles found during Pubmed searches using terms including “osteoarthritis”, “cartilage”, “inflammation”, “subchondral bone” and “ageing” and published between the 2017 and 2018 Osteoarthritis Research Society International (OARSI) Congress meetings. Key research areas to be included were then derived from themes developed either by multiple research groups or by significant breakthroughs in understanding and were presented at the OARSI World Congress on April 29, 2018. This review does not intend to cover all of the commendable research published in the last year, however aims to focus the reader towards several key developments within our understanding of how cartilage is homeostatically regulated and of how it behaves during osteoarthritis or following trauma. Such emerging findings will be key in directing future research aiming to prevent early joint damage from progressing towards disease phenotypes requiring invasive and costly interventions.

As summarised in Fig. 1, themes emerging in the past year summarised in this review have localised around the understanding of factors involved in maintaining chondrocytes within a healthy and functional state. Aspects ranging from the ability of progenitor cells to respond to signals following injury in order to support and repair cartilage, through the tuning of an incredible range of factors produced and required by chondrocytes, to the undesirable effects of chondrocytes that have left their stable equilibrium state have undergone significant recent advances, leading to novel opportunities for OA therapeutic development.

The role of cartilage progenitor cells in joint homeostasis and repair

Progressing from huge developments in the use of transgenic murine models used in recent years to uncover the role of bone marrow mesenchymal stromal/stem cells (MSCs) carrying markers including Gremlin1, Leptin receptor and Nestin as skeletal progenitors[1–3], together with the recently demonstrated contribution of proteoglycan 4 (Prg4)$^{+}$ synovial lining cells to cartilage growth and repair[4–6], the potential nature of the long fabled ‘cartilage stem cells’ has been further traced to a growth/differentiation factor 5 (Gdf5)$^{+}$ population of synovial cells.

GDF5 has long been observed as a marker of the joint interzone during skeletal development. Indeed all adult joint structures, including the articular cartilage, synovium, menisci, ligaments and tendons are directly contributed to by GDF5^{+} lineage cells[7,8]. Working from the hypothesis that Gdf5-expressing joint interzone progeny form a defined cell population that may contribute to cartilage homeostasis and repair, Roelofs *et al.*[9] demonstrated the

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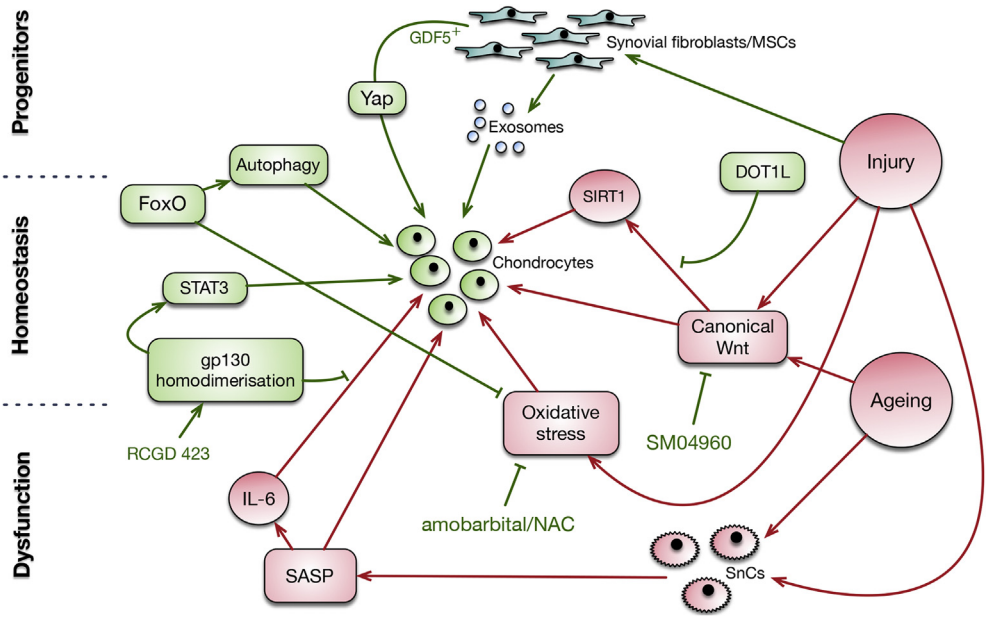

Fig. 1. A schematic summary of the findings of the Osteoarthritis biology studies featured within this 2017–2018 review. Features are stratified based on the developing core themes of progenitor cell function, chondrocyte homeostasis and mechanisms involving dysfunctional chondrocytes. Green lines indicate a supportive effect, while red lines indicate a detrimental effect. Pointed arrows indicate stimulation, whereas blocked lines indicate inhibition.

persistence of Gdf5-expressing cells within the adult synovium. Following mechanical injury to the articular cartilage, these cells were found to proliferate in a Yes-associated protein 1 (Yap1)-dependant manner, localising within a Nestin+ perivascular population and contributing to repair of the murine cartilage defect. When sorted and cultured *in vitro*, Gdf5-lineage MSCs were found to possess a high chondrogenic capacity with resistance to mineralisation and were able to directly contribute to tissue repair when injected *in vivo*.

The identification of this stem cell niche within the synovium adds significant weight towards the belief that synovial-derived MSCs could be used to repair joint surface defects in humans. It remains to be investigated whether other distinct progenitor populations co-exist, or whether dependencies and hierarchies between populations control responses to damage according to factors of timing, location, severity and co-morbidity. Importantly, the impact of OA and inflammation on progenitor subpopulation maintenance cannot be underestimated. Analysis of progenitor number and activity in normal and OA donors revealed the divergence of an additional subpopulation, characterised by increased cellular senescence[10]. Nonetheless the findings indicating that Yap1 is a key regulator of the synovial MSC injury response, expressed in the synovial lining layer of OA and intra-articular fracture patients, provide a lead towards the understanding of the injury response mechanisms required for translational realisation.

Further research has begun to demonstrate the potential of MSC derived exosomes as an option for harnessing the regenerative capacity of MSCs. While the mechanisms by which stem cell based therapies act remain unclear, direct effects of injected cells are believed to be limited, with recent studies suggesting that secreted mediators may instead reduce pain and inflammation via a paracrine response[11,12]. Extracellular vesicles (EVs) are a heterogeneous class of particles released from nearly all cell types, which have been shown to be involved in cell communication mechanisms[13]. Both exosomes and microparticles derived from MSCs were shown to exert protective effects within the collagenase-induced OA (CIOA) murine model with notable reductions observed in apoptosis and in macrophage activation, independently reproducing the protective effects of MSC injection[14].

In vitro, rat chondrocytes were shown to internalise exosomes from human embryonic stem cell derived MSCs, which led to significant increases in cell proliferation and migration, AKT and ERK phosphorylation[15]. These findings give mechanistic support to the previous results of Zhang *et al.*, where osteochondral defects in adult rats were injected intra-articularly with human MSC-derived exosomes, resulting in enhanced restoration of both cartilage and subchondral bone in comparison to PBS treated controls[16].

It has been demonstrated that exosomes can be modified in order to ensure their cargo can provide optimal chondrogenic effects[17]. Such properties enhance the possibility of EVs being harnessed for use within cell-free regenerative therapies. Whilst evidence of their penetrance into articular cartilage is convincing[18], their persistence and potency must be well examined in order to optimise clinical efficacy.

Signalling mechanisms involved in cartilage homeostasis

Articular chondrocytes, as the lone cell population within the articular cartilage, have long been held responsible for the maintenance of the anabolic and catabolic processes required to preserve the health and function of the joint. During OA, chondrocytes actively participate in disease development, both by losing their ability to maintain normal gene expression and matrix production and by contributing to matrix degradation, abnormal matrix production, undergoing increased proliferation and increased apoptosis. These features are generally classified as a hypertrophic differentiation of chondrocytes, which is a normal behaviour of chondrocytes during cartilage and subsequent endochondral bone development, but appears to be aberrantly reconstituted during OA. Understanding how the chondrocyte phenotype is modified via transcriptional networks, epigenetic regulation and via intracellular signalling pathways gives researchers a vital insight into how chondrocytes should be targeted therapeutically in OA patients.

During the last decade, autophagy has been recognised as a mechanism by which chondrocytes act to avoid uncontrolled and destructive cell death during conditions of physiological challenge[19]. Expression of autophagy related genes were seen to be down-regulated in OA chondrocytes, whilst the forced activation of autophagy using rapamycin was found to protect against experimental OA[20]. More recently, it has been demonstrated that the expression of FoxO trancription factors, the regulation of which are known to be disrupted in other age-related musculoskeletal diseases[21,22], are significantly reduced in both murine and human OA cartilage[23].

Matsuzaki *et al.*[24] demonstrated that the cartilage-specific deletion of FoxO transcription factors results in the spontaneous breakdown of articular cartilage, even in unchallenged mice at a relatively young age. FoxO deletion resulted in increased apoptosis and a decreased expression of genes required for autophagy and defence against cell stress. FoxO1 in particular was found to synergise with transforming growth factor β (TGFβ) in the upregulation of proteoglycan-4 (Prg4), which is known to play a key role in chondrocyte survival mechanisms[25,26]. These findings have identified FoxO transcription factors as vital homeostatic regulators in cartilage, required for autophagic chondrocyte repair and resistance to inflammation. Their reduced expression during ageing and in OA suggest that a therapeutic restoration of FoxO activity may enhance cartilage homeostasis during disease.

The role of inflammation in OA pathogenesis has been the subject of much debate with a general acceptance that while inflammatory mediators expressed within OA joints contribute to pathogenesis and to pain, controlled levels of activation of inflammatory pathways are required for both cartilage homeostasis and for tissue repair processes[27–30]. A wide range of studies investigating the pathogenic role of Interleukin-6 (IL-6) signalling in OA have demonstrated that IL-6 is expressed in OA patient serum[31], promotes mineralisation[32] and protease expression while decreasing cartilage matrix production[33]. Furthermore, IL-6 blockade in murine OA models was found to reduce joint destruction[33,34] leading to the testing of IL-6 receptor antagonists within OA clinical trials.

The downstream signalling pathway of IL-6 is typically complex, firstly in the sharing of a common co-receptor (gp130) between IL-6 family members (IL-6, oncostatin M (OSM), leukaemia inhibitory factor (LIF)). Activated intracellular pathways include MAPKs, AKT, JAK/STAT and nuclear factor kappa-light-chain-enhancer of activated B-cells (NFκB). Although inhibition of IL-6/STAT3 was found to be chondroprotective within the murine destabilisation of the medial meniscus (DMM) model[33], inhibition of this pathway has been shown to enhance chondrocyte hypertrophy during skeletal development[35] and was found to decrease Sox9 expression and lead to increased hypertrophic differentiation in chondrocytes[36], suggesting significant context-dependency for IL-6 signalling in cartilage. A novel strategy aimed at promoting the pro-regenerative capacity of IL-6 signalling was published by Shykan *et al.*[37], using modulation of the gp130 co-receptor as a means to prevent cartilage breakdown and to facilitate articular cartilage repair. The small-molecule compound RCGD 423 was discovered to promote the homodimerization of gp130, thereby reducing the availability of co-receptors for dimerization with IL-6 family receptors. This in turn reduced the activation of pro-inflammatory MAPK signalling and reduced structural damage in the rat DMM model, while also increasing signalling via STAT3 phosphorylation, leading to increases in articular cartilage regeneration within a rat osteochondral injury model. The development of compounds able to harness the homeostatic and regenerative capacities of inflammatory signalling pathways while avoiding the detrimental effects associated with pro-inflammatory signalling factors is a significant breakthrough for the translation of the ever increasing understanding of inflammation during OA towards potential therapies.

Wnt signalling is essential for joint development[38–40] and recent advances have provided the current understanding that tightly regulated and precisely restricted Wnt activity is required for cartilage and bone homeostasis and prevention of disease[40,41]. While both gain and loss of function studies investigating the role of β-catenin and canonical Wnt signalling were found to induce osteoarthritis-like changes in mice[42,43], findings suggest that a hyperactivation of canonical Wnt signalling leads to increased cartilage degradation. With this in mind, significant advances have recently been made towards the development of pharmaceutical Wnt inhibitors suitable for clinical use. Held *et al.*[44] demonstrated the chondrocyte penetration capacity *in vitro* of two small molecule inhibitors, stapled β-catenin binding domain of Axin (StAx-35R) and stapled peptide derived from the Bcl9 homology domain-2 (SAH-Bcl2). Both inhibitors block canonical Wnt signalling via direct interaction with β-catenin, disrupting transcriptional function, demonstrated by reduced β-catenin reporter activity. In this study, each inhibitor was found to prevent Wnt-induced hypertrophic differentiation of articular chondrocytes, alongside a significant upregulation of Sox9 gene expression.

Screening of a compound library for a capacity to suppress canonical Wnt activity led to the identification of SM04960 as a potential DMOAD. Deshmukh *et al.*[45] evaluated the effects of SM04960 on the chondrocyte phenotype and within the anterior cruciate ligament (ACL) transection plus partial medial menisectomy (ACLT+pMMx) model of OA in rats. Following in its role as a Wnt antagonist, it was observed to support chondrogenic differentiation of MSCs and additionally reduced the expression of matrix metalloproteinases and cartilage breakdown during OA. Most strikingly, pharmacokinetic analysis following a single intra-articular injection revealed that SM04960 was still detectable at therapeutic levels 180 days after treatment, without being quantifiably detected at plasma level. Penetration, accumulation and retention alongside low toxicity have long been idealised as properties for DMOADs, in order that the effect of signalling pathway modulation is limited to specific cellular targets. Whether the retention of SM04960 is attributable to interactions of the compound to the cartilage matrix remains to be investigated. The therapeutic regime of compound injection 1 week following ACLT+pMMx is difficult to accurately translate into OA patients suffering from longer term disease, with more investigation required to understand the specific timings and duration of Wnt inactivation required to address specific disease stages without affecting any Wnt-related repair mechanisms. Completion of phase I clinical trials have demonstrated tolerability in humans with SM04960 undetected in plasma following intra-articular injection. Phase II trial assessment is required to support suggestions of improvement in OA pain and joint function[46].

Canonical Wnt signalling was found to be regulated in cartilage via the histone methyltransferase Disruptor of telomeric silencing-1 (DOT1L)[47]. DOT1L methylates histone H3 lysine 79 (H3L79) specifically, acting as a positive regulator for gene transcription and of the cell cycle[48]. Associations between DOT1L polymorphisms and increased OA susceptibility have previously been demonstrated[49,50]. By investigating the effect of DOT1L disruption in chondrocytes, Monteagudo *et al.*[47] were able to demonstrate that DOT1L exerts a protective function in cartilage. *In vivo* inhibition of DOT1L using the specific inhibitor EPZ-5676 in mice led to a rapid breakdown of articular cartilage, which was associated with increased activation of the canonical Wnt pathway. In order to investigate the molecular mechanisms involved, the authors tested the hypothesis that DOT1L interacts with a repressor in order to restrict Wnt target gene expression. Systematic analysis of the inhibition of candidate interaction partners revealed that blockade of Sirtuin-1 (SIRT1) in EPZ-5676 treated cells eliminated the Wnt

pathway-induced upregulation of LEF1 and TCF1. DOT1L inhibition was found to increase the enzymatic activity of SIRT1 and silencing of downstream chromatin-binding factors reproduced the effects of SIRT1 blockade, indicating that DOT1L inactivation promotes the upregulation of Wnt target gene expression via SIRT1 promoted chromatin binding of transcriptional regulators. The clinical implications of targeting DOT1L in OA were demonstrated by the treatment of EPZ-5676-induced OA mice with a SIRT1 inhibitor, which reduced TCF1 expression and significantly improved the cartilage phenotype.

Although DOT1L exhibits a protective function in chondrocytes, no such effect occurred in osteoblasts, while both DOT1L and H3K79 methylation have previously been linked to increased Wnt activation[49,51]. Such observations support the notion that a finely tuned tissue-specific balance of Wnt signalling is required for cartilage health. The findings regarding DOT1L as a balancing factor controlling canonical Wnt activation support the suggestion that intrinsic mechanisms exist within cartilage homeostasis that allow for physiological function of morphological signalling pathways, while self-regulating against pathological activation[26,27].

Role of disrupted and senescent chondrocytes

Post-traumatic OA (PTOA) frequently develops in patients following significant joint injury, intra-articular fracture and meniscal or ligament damage. Disease development is often associated with a short-term inflammatory phase following initial injury, including intra-articular bleeding. Although surgical repair can often resolve the most acute symptoms, such joint injuries often trigger chronic inflammatory and remodelling processes typically leading to OA development[52]. Articular cartilage damaged by injury is often found to have high levels of chondrocyte cell death leading both from the initial trauma and from the spreading of cell loss via apoptotic mechanisms, alongside increased extracellular matrix degradation[53–55]. Chondrocytes in and around trauma sites have been demonstrated to initially respond to tissue damage via a rapid burst of mitochondrial activity, producing high levels of reactive oxygen species (ROS)[56]. Such cumulative oxidative damage resulting from mitochondrial dysfunction and exposure to oxidants is known to contribute to further chondrocyte death[57,58]. Coleman *et al.*[59] aimed to test whether the inhibition of mitochondrial function within the post-traumatic joint, exposed to a high influx of oxygen, blood and inflammatory factors, could be therapeutically targeted to reduce oxidative damage in order to reduce PTOA development. The study used either amobarbital or *N*-acetylcysteine (NAC) to inhibit the electron transport chain I within a minipig intra-articular fracture model, resulting in significant protection from PTOA analysed 6 months following injury. Notably, treatment did not impact upon early inflammatory responses, suggesting that the targeting of the oxidation pathway may not detrimentally affect pro-reparative roles of inflammation during fracture healing.

Senescent cells (SnCs) have previously been found within cartilage isolated from OA patients[60], while selective elimination of senescent cells from young mice undergoing ACL transection reduced cartilage erosion and decreased pain[61]. Indeed, transplantation of senescent cells obtained from ear cartilage injected into the knee joint of wild type mice induced pain and histological indications of OA-like cartilage degradation[62], providing further evidence of the disease exacerbating role of senescent cells[63–65].

Conclusion

This review aimed to summarise a number of emerging themes within the field of osteoarthritis biology research, covering work published between the 2017 and 2018 OARSI World Congress meetings. By no means an exhaustive summary of published articles of merit, the focus is on clear developing topics, primarily related to a central theme of chondrocyte phenotype.

Firstly, further development in the investigation of cartilage stem cell sources has identified a GDF5^{+} cell population within the synovium that is able to respond to articular cartilage injury through proliferation and subsequent repopulation of cartilage defects. Although this is not thought to be the only source of stem cells within the joint able to participate in cartilage repair, this work recognises the Yap-dependent mechanism via which stem cell populations may respond when required, therefore opening new possibilities for the development of therapies employing resident cell populations.

Secondly, significant findings regarding the maintenance of the healthy chondrocyte phenotype have increased our understanding of active intracellular signalling mechanisms and how pathological disruptions to their equilibria may be pharmaceutically controlled. Promising translational results have been produced within the Wnt signalling field, while further dissection of IL-6 inflammatory and FoxO-directed autophagic pathways have a clear potential to be developed further to treat cartilage pathology.

Finally, studies regarding the destructive effects of senescent and trauma-challenged cells have demonstrated that the elimination or metabolic silencing of malfunctioning chondrocytes has the potential to reduce the rate at which PTOA can develop. Translation of each potential therapeutic target identified within this review will depend on the detailed analysis of disease progression in different OA patient phenotypes in order to precisely target the disease stage that intervention will be most effective. Many examples included here affect signalling pathways that have multiple functions, both homeostatic and pathogenic. It is therefore essential that basic research continues to be directed towards the investigation of how intrinsic mechanisms and properties can be harnessed within OA treatment.

Author contributions

Dr. Sherwood performed the literature search, selected the themes, wrote and edited the work.

Conflict of interest

No direct financial or other conflicts of interest are related to this work.

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References

1. Worthley DL, Churchill M, Compton JT, Tailor Y, Rao M, Si Y, *et al.* Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell 2015, https://doi.org/10.1016/j.cell.2014.11.042.
2. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell 2014, https://doi.org/10.1016/j.stem.2014.06.008.
3. Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, MacArthur BD, Lira SA, *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010, https://doi.org/10.1038/nature09262.

4. Kozhemyakina E, Zhang M, Ionescu A, Ayturk UM, Ono M, Kobayashi A, *et al*. Identification of a *Prg4* -expressing articular cartilage progenitor cell population in mice. Arthritis Rheumatol 2015;67(5):1261–73, https://doi.org/10.1002/art.39030.
5. Li L, Newton PT, Bouderlique T, Sejnohova M, Zikmund T, Kozhemyakina E, *et al*. Superficial cells are self-renewing chondrocyte progenitors, which form the articular cartilage in juvenile mice. FASEB J 2017;31(3):1067–84, https://doi.org/10.1096/fj.201600918R.
6. Decker RS, Um H-B, Dyment NA, Cottingham N, Usami Y, Enomoto-Iwamoto M, *et al*. Cell origin, volume and arrangement are drivers of articular cartilage formation, morphogenesis and response to injury in mouse limbs. Dev Biol 2017;426(1):56–68, https://doi.org/10.1016/J.YDBIO.2017.04.006.
7. Rountree RB, Schoor M, Chen H, Marks ME, Harley V, Mishina Y, *et al*. BMP receptor signaling is required for postnatal maintenance of articular cartilage. PLoS Biol 2004, https://doi.org/10.1371/journal.pbio.0020355.
8. Koyama E, Shibukawa Y, Nagayama M, Sugito H, Young B, Yuasa T, *et al*. A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. Dev Biol 2008, https://doi.org/10.1016/j.ydbio.2008.01.012.
9. Roelofs AJ, Zupan J, Riemen AHK, Kania K, Ansboro S, White N, *et al*. Joint morphogenetic cells in the adult mammalian synovium. Nat Commun 2017;8:15040, https://doi.org/10.1038/ncomms15040.
10. Fellows CR, Williams R, Davies IR, Gohil K, Baird DM, Fairclough J, *et al*. Characterisation of a divergent progenitor cell sub-populations in human osteoarthritic cartilage: the role of telomere erosion and replicative senescence. Sci Rep 2017, https://doi.org/10.1038/srep41421.
11. Maumus M, Manferdini C, Toupet K, Peyrafitte JA, Ferreira R, Facchini A, *et al*. Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis. Stem Cell Res 2013, https://doi.org/10.1016/j.scr.2013.05.008.
12. Manferdini C, Maumus M, Gabusi E, Piacentini A, Filardo G, Peyrafitte JA, *et al*. Adipose-derived mesenchymal stem cells exert antiinflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E2. Arthritis Rheum 2013, https://doi.org/10.1002/art.37908.
13. van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 2012, https://doi.org/10.1124/pr.112.005983.
14. Cosenza S, Ruiz M, Toupet K, Jorgensen C, Noël D. Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis. Sci Rep 2017, https://doi.org/10.1038/s41598-017-15376-8.
15. Zhang S, Chuah SJ, Lai RC, Hui JHP, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. Biomaterials 2018, https://doi.org/10.1016/j.biomaterials.2017.11.028.
16. Zhang S, Chu WC, Lai RC, Lim SK, Hui JHP, Toh WS. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. Osteoarthritis Cartilage 2016;24:2135–40.
17. Tao S-C, Yuan T, Zhang Y-L, Yin W-J, Guo S-C, Zhang C-Q. Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. Theranostics 2017, https://doi.org/10.7150/thno.17133.
18. Headland SE, Jones HR, Norling LV, Kim A, Souza PR, Corsiero E, *et al*. Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. Sci Transl Med 2015, https://doi.org/10.1126/scitranslmed.aac5608.
19. 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 Rheum 2010, https://doi.org/10.1002/art.27305.
20. 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, https://doi.org/10.1136/annrheumdis-2011-200557.
21. Rached MT, Kode A, Xu L, Yoshikawa Y, Paik JH, DePinho RA, *et al*. FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. Cell Metabol 2010, https://doi.org/10.1016/j.cmet.2010.01.001.
22. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, *et al*. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 2004, https://doi.org/10.1016/S0092-8674(04)00400-3.
23. Akasaki Y, Hasegawa A, Saito M, Asahara H, Iwamoto Y, Lotz MK. Dysregulated FOXO transcription factors in articular cartilage in aging and osteoarthritis. Osteoarthritis Cartilage 2014;22:162–70.
24. Matsuzaki T, Alvarez-Garcia O, Mokuda S, Nagira K, Olmer M, Gamini R, *et al*. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. Sci Transl Med 2018, https://doi.org/10.1126/scitranslmed.aan0746.
25. Waller KA, Zhang LX, Elsaid KA, Fleming BC, Warman ML, Jay GD. Role of lubricin and boundary lubrication in the prevention of chondrocyte apoptosis. Proc Natl Acad Sci USA 2013;110:5852–7, https://doi.org/10.1073/pnas.1219289110.
26. Nalesso G, Thomas BL, Sherwood JC, Yu J, Addimanda O, Eldridge SE, *et al*. WNT16 antagonises excessive canonical WNT activation and protects cartilage in osteoarthritis. Ann Rheum Dis 2016, https://doi.org/10.1136/annrheumdis-2015-208577. annrheumdis-2015-208577.
27. Sherwood J, Bertrand J, Nalesso G, Poulet B, Pitsillides A, Brandolini L, *et al*. A homeostatic function of CXCR2 signalling in articular cartilage. Ann Rheum Dis 2014, https://doi.org/10.1136/annrheumdis-2014-205546.
28. Sherwood JC, Bertrand J, Eldridge SE, Dell'Accio F. Cellular and molecular mechanisms of cartilage damage and repair. Drug Discov Today 2014;19(8):1172–7, https://doi.org/10.1016/j.drudis.2014.05.014.
29. Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011;23(1531–6963 (Electronic)):471–8.
30. Malfait A-M, Miller RJ. Emerging targets for the management of osteoarthritis pain. Curr Osteoporos Rep 2016;14(6):260–8, https://doi.org/10.1007/s11914-016-0326-z.
31. Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FMK, *et al*. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford Study. Arthritis Rheum 2009, https://doi.org/10.1002/art.24598.
32. Nasi S, So A, Combes C, Daudon M, Busso N. Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis. Ann Rheum Dis 2016, https://doi.org/10.1136/annrheumdis-2015-207487.
33. Latourte A, Cherifi C, Maillet J, Ea HK, Bouaziz W, Funck-Brentano T, *et al*. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. Ann Rheum Dis 2017, https://doi.org/10.1136/annrheumdis-2016-209757.
34. Ryu JH, Yang S, Shin Y, Rhee J, Chun CH, Chun JS. Interleukin-6 plays an essential role in hypoxia-inducible factor 2??-induced experimental osteoarthritic cartilage destruction in mice. Arthritis Rheum 2011, https://doi.org/10.1002/art.30451.

35. Hall MD, Murray CA, Valdez MJ, Perantoni AO. Mesoderm-specific Stat3 deletion affects expression of Sox9 yielding Sox9-dependent phenotypes. PLoS Genet 2017, https://doi.org/10.1371/journal.pgen.1006610.
36. Kondo M, Yamaoka K, Sakata K, Sonomoto K, Lin L, Nakano K, *et al.* Contribution of the interleukin-6/STAT-3 signaling pathway to chondrogenic differentiation of human mesenchymal stem cells. Arthritis Rheumatol 2015, https://doi.org/10.1002/art.39036.
37. Shkhyan R, Van Handel B, Bogdanov J, Li S, Yu Y, Scheinberg M, *et al.* Drug-induced modulation of gp130 signalling prevents articular cartilage degeneration and promotes repair. Ann Rheum Dis 2018, https://doi.org/10.1136/annrheumdis-2017-212037.
38. Hartmann C, Tabin CJ. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. Cell 2001, https://doi.org/10.1016/S0092-8674(01)00222-7.
39. Guo X, Day TF, Jiang X, Garrett-Beal L, Topol L, Yang Y. Wnt/??-catenin signaling is sufficient and necessary for synovial joint formation. Genes Dev 2004, https://doi.org/10.1101/gad.1230704.
40. Usami Y, Gunawardena AT, Iwamoto M, Enomoto-Iwamoto M. Wnt signaling in cartilage development and diseases: lessons from animal studies. Lab Invest 2016, https://doi.org/10.1038/labinvest.2015.142.
41. Nalesso G, Sherwood J, Bertrand J, Pap T, Ramachandran M, De Bari C, *et al.* WNT-3A modulates articular chondrocyte phenotype by activating both canonical and noncanonical pathways. J Cell Biol 2011;193:551–64.
42. Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, *et al.* Activation of β-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult β-catenin conditional activation mice. J Bone Miner Res 2009, https://doi.org/10.1359/jbmr.080901.
43. Zhu M, Chen M, Zuscik M, Wu Q, Wang Y-J, Rosier RN, *et al.* Inhibition of beta-catenin signaling in articular chondrocytes results in articular cartilage destruction. Arthritis Rheum 2008, https://doi.org/10.1002/art.23614.
44. Held A, Glas A, Dietrich L, Bollmann M, Brandstädter K, Grossman TN, *et al.* Targeting β-catenin dependent Wnt signaling via peptidomimetic inhibitors in murine chondrocytes and OA cartilage. Osteoarthritis Cartilage 2018;26:818–23.
45. Deshmukh V, Hu H, Barroga C, Bossard C, KC S, Dellamary L, *et al.* A small molecule inhibitor of the Wnt pathway (SM04690) as a potential disease modifying agent for the treatment of osteoarthritis of the knee. Osteoarthritis Cartilage 2018;26:18–27.
46. Yazici Y, McAlindon TE, Fleischmann R, Gibofsky A, Lane NE, Kivitz AJ, *et al.* A novel Wnt pathway inhibitor, SM04690, for the treatment of moderate to severe osteoarthritis of the knee: results of a 24-week, randomized, controlled, phase 1 study. Osteoarthritis Cartilage 2017;25:1598–606.
47. Monteagudo S, Cornelis FMF, Aznar-Lopez C, Yibmantasiri P, Guns LA, Carmeliet P, *et al.* DOT1L safeguards cartilage homeostasis and protects against osteoarthritis. Nat Commun 2017, https://doi.org/10.1038/ncomms15889.
48. Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylationanh. Genes Dev 2011, https://doi.org/10.1101/gad.2057811.
49. Castano Betancourt MC, Cailotto F, Kerkhof HJ, Cornelis FMF, Doherty SA, Hart DJ, *et al.* Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis. Proc Natl Acad Sci USA 2012, https://doi.org/10.1073/pnas.1119899109.
50. Evangelou E, Valdes AM, Castano-Betancourt MC, Doherty M, Doherty S, Esko T, *et al.* The DOT1L rs12982744 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical significance in males. Ann Rheum Dis 2013, https://doi.org/10.1136/annrheumdis-2012-203182.
51. Mohan M, Herz HM, Takahashi YH, Lin C, Lai KC, Zhang Y, *et al.* Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). Genes Dev 2010, https://doi.org/10.1101/gad.1898410.
52. Lotz MK. New developments in osteoarthritis. Posttraumatic osteoarthritis: pathogenesis and pharmacological treatment options. Arthritis Res Ther 2010, https://doi.org/10.1186/ar3046.
53. D'Lima DD, Hashimoto S, Chen PC, Colwell CWJ, Lotz MK. Human chondrocyte apoptosis in response to mechanical injury. Osteoarthritis Cartilage 2001, https://doi.org/10.1053/joca.2001.0468.
54. Levine A, Burton-Wurster N, Chen CT, Lust G. Intercellular signalling as a cause of cell death in cyclically impacted cartilage explants. Osteoarthritis Cartilage 2001, https://doi.org/10.1053/joca.2001.0467.
55. Kim HT, Lo MY, Pillarisetty R. Chondrocyte apoptosis following intraarticular fracture in humans. Osteoarthritis Cartilage 2002, https://doi.org/10.1053/joca.2002.0828.
56. Wolff KJ, Ramakrishnan PS, Brouillette MJ, Journot BJ, McKinley TO, Buckwalter JA, *et al.* Mechanical stress and ATP synthesis are coupled by mitochondrial oxidants in articular cartilage. J Orthop Res 2013, https://doi.org/10.1002/jor.22223.
57. Martin JA, McCabe D, Walter M, Buckwalter JA, McKinley TO. N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. J Bone Jt Surg – Ser A. 2009, https://doi.org/10.2106/JBJS.H.00545.
58. Goodwin W, McCabe D, Sauter E, Reese E, Walter M, Buckwalter JA, *et al.* Rotenone prevents impact-induced chondrocyte death. J Orthop Res 2010, https://doi.org/10.1002/jor.21091.
59. Coleman MC, Goetz JE, Brouillette MJ, Seol D, Willey MC, Peterson EB, *et al.* Targeting mitochondrial responses to intra-articular fracture to prevent posttraumatic osteoarthritis. Sci Transl Med 2018, https://doi.org/10.1126/scitranslmed.aan5372.
60. Price JS, Waters JG, Darrah C, Pennington C, Edwards DR, Donell ST, *et al.* The role of chondrocyte senescence in osteoarthritis. Aging Cell 2002, https://doi.org/10.1046/j.1474-9728.2002.00008.x.
61. Hee Jeon O, Kim C, Laberge R-M, Demaria M, Rathod S, Vasserot AP, *et al.* Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med 2017, https://doi.org/10.1038/nm.4324.
62. Xu M, Bradley EW, Weivoda MM, Hwang SM, Pirtskhalava T, Decklever T, *et al.* Transplanted senescent cells induce an osteoarthritis-like condition in mice. J Gerontol A Biol Sci Med Sci 2017, https://doi.org/10.1093/gerona/glw154.
63. Van Deursen JM. The role of senescent cells in ageing. Nature 2014, https://doi.org/10.1038/nature13193.
64. Childs BG, Durik M, Baker DJ, Van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med 2015, https://doi.org/10.1038/nm.4000.
65. Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol Mech Dis 2010, https://doi.org/10.1146/annurev-pathol-121808-102144.