Editorial

Epigenetic changes regulate the fate and progression of osteoarthritis

Ramesh Bhonde[1], Kalpana Joshi[2], Anjan Kumar Das[3]

[1]Dr. D. Y. Patil University, Pimpri, Pune 411018, Maharashtra, India; [2]Department of Biotechnology, Sinhgad College of Engineering, Pune, Maharashtra, India; [3]Department of Surgery, Taylor's University School of Medicine, Sungai Buloh Hospital, Selangor, Malaysia

Correspondence to: Dr. Rameah Bhonde. Director (Research), Dr. D. Y. Patil University, Pimpri, Pune 411018, India. Email: ramesh.bhonde@dpu.edu.in.

Comment on: Alvarez-Garcia O, Fisch KM, Wineinger NE, *et al.* Increased DNA Methylation and Reduced Expression of Transcription Factors in Human Osteoarthritis Cartilage. Arthritis Rheumatol 2016;68:1876-86.

Received: 08 November 2016; Accepted: 28 November 2016; Published: 08 December 2016.

doi: 10.21037/aoj.2016.11.03

View this article at: http://dx.doi.org/10.21037/aoj.2016.11.03

The latest global burden of diseases study reviewed 187 countries and 27 regions. Their report confirms what was already suspected by many clinicians and epidemiologists. Osteoarthritis (OA) is one of the commonest causes of disease burden worldwide, ranking as high as 11th as a contributor to global disability. This burden is increasing exponentially; the years lived with disability (YLD) for hip and knee OA has increased from 10.5 million in 1990 to 17.1 million in 2010. The study also confirms that the disease is ubiquitous and spread all over the surveyed geographical regions and was most commonly seen in females over the age of 50, though no age was exempt (1). It was a pleasure reading an article entitled "Increased DNA Methylation and Reduced Expression of Transcription Factors in Human Osteoarthritis Cartilage", written by Oscar Alvarez-Garcia, which was published in *Arthritis & Rheumatology* in Aug. 2016 (2). The authors have shown that normal and OA knee articular cartilage can be distinguished by their DNA, methylation profiles. This raises an important issue of regulation of arthritic changes by epigenetic changes. Epigenetics is a complex process of chromatin and gene modulation through DNA methylation, histone deacetylation or microRNA, which might contribute to the inheritability of disease (3). OA is an age old disease of the old age characterized by degradation and calcification of articular cartilage, but the disease also affects the synovial tissue, ligaments as well as the bone; leading to chronic pain, limitation of joint movement and loss of physical function. The patient suffers from stiffness, joint pains, and joint swellings and may be incapacitated and unable to work.

Unfortunately, there is at present no specific molecule which can modify the disease natural history. Many molecules have been proposed and discarded as definitive treatment for OA and treatment is now mostly confined to analgesia, exercise and surgical joint modification including replacement. Much work has been carried out to elucidate the molecular processes which cause the disease in the hope of understanding mechanisms which would lead to the development of better methods of disease control. Studies have elucidated genetic, epigenetic, proteomic and other pathways which increase and modify the disease pathology (4). The pathogenesis of OA is multifactorial, with aging, obesity, and genetic susceptibility being the main risk factors (5). During OA development, articular chondrocytes (the only cell type in articular cartilage that is responsible for maintaining tissue homeostasis) undergo marked transcriptional and phenotypic changes that compromise their function and lead to cartilage degradation. However, genome-wide association studies have shown only a small genetic variance component for OA, and recent evidence has pointed to epigenetic regulation as a key driver of the transcriptional alterations observed in OA chondrocytes (6).

Arthritis & Rheumatology published an editorial on "Is it time for epigenetics in osteoarthritis?" (7), and made a call to the research community to increase efforts concerning the role played by epigenetics in the development of OA. It was put forth that the characterization and analysis of cartilage DNA methylomes not only offers potential therapeutic targets for the treatment of OA, but also

enables the design of a map of epigenetic marks that can help develop potential biomarkers for diagnosis, prognosis, drug response, chondrogenesis, or homeostasis from high-resolution screening technologies. In 2 years' time several articles proved association between epigenetics and OA phenotypes. Different stimuli (environmental factors), such as joint injury, oxidative stress, and inflammatory cues, aging, diet, metabolic (Mtb.) disorders, or both nuclear and mitochondrial genetics are prone to alter the DNA methylome in OA, leading to the development of different phenotypes of OA (8).

In recent years the epigenetic mechanisms associated with disease have garnered a lot of attention as evidence has been generated that epigenetic regulation is a key driver of transcriptional alterations observed in chondrocytes of OA patients (9). The term epigenetics was first introduced by Conrad Waddington in the early 1940s. The original definition was broad, but in recent times, epigenetics has been defined more narrowly as the "*the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.*" (10). To put it very simply, epigenetics allows information in the long DNA sequence to be turned into expressed characteristics. These expressions can alter in each cell or group of cells thus changes can take place in response to environmental influences.

The epigenetic modifications can have many clinical implications. Environmental influences can cause changes which are relevant in disease causation and progression.

The two principal elements which cause these epigenetic modifications are methylation and histone modifications. Basically the process of methylation involves addition of a methyl group to a cytosine base and in mammals this takes place mainly at CpG dinucleotides (4). There is a growing body of evidence which suggests that DNA methylation plays an important part in the pathophysiology of OA (11). It has been suggested that this may occur in two ways: some individuals with poor epigenetic profiles may be susceptible to OA or that age related changes in the epigenome may lead to cartilage damage. There is some evidence for either point of view and it is quite possible that both mechanisms are responsible for the epigenetic changes in OA.

The development of comparatively cheap methylation arrays has now made it possible to develop genome wide DNA methylation profiles of cartilage and to compare diseased with normal cartilage. Studies tend to be focused on cartilage as it is the principal pathological feature of the disease. In addition, it is composed of a single cell type (12). Roach *et al.* (11) first hypothesized that abnormal gene expressions of clonal OA chondrocytes is associated with heritable epigenetic alterations in the DNA methylation pattern. Their study provided the first direct evidence of an association between the loss of DNA methylation and abnormal expression of MMP 3, MMP 9, MMP 13 and ADAMTS 4 by OA chondrocytes. Several studies subsequently confirmed these findings. However all these studies were unable to demonstrate causality as longitudinal studies of the same cartilage samples were understandably not done.

The advent of high-throughput arrays for the analysis of DNA methylation has significantly boosted the amount of epigenetic data that has emerged in the past few years. OA has taken part in this enhanced activity and is in fact quite well placed to do so, for two principal reasons: the central tissue involved in the disease process, cartilage, is readily accessible via joint replacement surgery; and the cartilage contains only a single cell type, the chondrocyte, thus limiting the scope for confounding heterogeneity that can be encountered when studying the epigenetics of a multicellular tissue in which cell-specific effects are likely.

Five methylation array analyses of cartilage DNA have been published in the recent years, focused on knee and/or hip OA. A number of interesting observations have emerged. For example, knee and hip cartilages are strikingly different, with DNA methylation differences at a number of genes, including homeobox genes, which are key regulators of skeletogenesis. This is reminiscent of the joint-specific genetic effects touched on earlier, and suggests that cartilages from different parts of the skeleton are not only genetically but also epigenetically distinct. The DNA methylation studies have also revealed that genes that have previously been implicated in OA typically harbor CpG sites that are differentially methylated between OA and non-OA cartilage. This implies that the regulation of gene expression via DNA methylation is a major driver of the OA disease process (13).

Genome wide methylome studies have utilized more sophisticated arrays to analyze up to 485,000 sites. The results of these studies have been summarized by Reynard *et al.* (4).

DNA methylation contributes to the chronicity of OA and could be responsible for the limitation of current therapies. It may predict progression of the disease as well as response. Epigenetic treatments being used in oncology may prove beneficial also in OA (14).

Ayugenomics, a recently evolving novel method

combining the Ayurveda doctrine based constitution determination of study subjects with contemporary genome analysis tools, seem to hold promise for complex traits research efforts. A few groups in the country have already provided both preliminary and established evidence to show that personalized, predictive and protective health approach practiced in Ayurveda since 1500 BC can address some of the contemporary limitations and improve our understanding of biology of health and disease in molecular terms (15). Our recent study on DNA methylation changes in asthmatic individuals in response to Ayurvedic intervention using microarrays has shown encouraging results (16,17). Modalities from alternative and complementary medicine like Ayurveda are often pursued by many patients as well (18,19). Aqueous extracts of Withania somnifera root powder and fruit of Phyllanthus emblica have shown chondroprotective activities in an *in vitro* model of cartilage degradation with explant cultures of articular knee cartilage from OA patients (20,21). The possible mechanism of this unique activity of chondroprotection could be through epigenetic modifications or stem cell differentiation (22,23).

Mesenchymal stem cells (MSCs) have been proposed by many as an optimal regenerative cellular therapeutic for musculoskeletal regeneration, especially in the setting of degenerative pathology like OA (24,25). The translation of MSCs to clinical therapy for OA has been shown; however, signs of progress are evident and ongoing trials may show efficacy to indicate these products can serve as the disease-modifying therapy necessary to stem the tide of OA.

MSCs have been used in several clinical studies, clinical trials and have been shown to have improved symptoms and developed articular cartilage. Two of the most recent studies are summarized here. A single arm open label Spanish study from Barcelona (26) recruited 15 patients with grade II or III OA and injected round 40 million autologous MSCs into the joint. The patients were followed up for 6 months and the efficacy of the procedure was measured by the visual analogue scale for pain, algofunctional Health Assessment Questionnaire, quality of life (QoL) SF-36 questionnaire, Lequesne functional index and WOMAC score. The cartilage integrity was assessed by magnetic resonance imaging (MRI) and quantitative T2-mapping at 0, 6 and 12 months. There were mild side effects, but improvements were noted in all the criteria for efficacy and cartilage growth was also noted by MRI. Another study (27) also from Spain used a multicenter randomized design to compare two doses of cultured autologous MSCs with hyaluronic acid. In this study, similar results were obtained. MRI studies also showed cartilage growth. There are many similar studies on these lines (28). However the clinical improvements are difficult to explain. Earlier hypotheses of cartilage regeneration by the MSCs are not now generally accepted. However it remains to be seen whether autologous/allogeneic MSC therapy works via reversing the epigenetic changes occurring during progression of OA. Since the epigenetic changes are reversible it would be interesting to see whether interventional therapies for degenerative diseases like OA modify the DNA methylation pattern and facilitate cartilage regeneration.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Executive Editor-in-Chief, Dongquan Shi, MD, PhD (Department of Sports Medicine and Adult Reconstruction, Drum Tower Hospital, Medical School, Nanjing University, Nanjing, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aoj.2016.11.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Cross M, Smith E, Hoy D, et al. The global burden of hip

and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73:1323-30.
2. Alvarez-Garcia O, Fisch KM, Wineinger NE, et al. Increased DNA Methylation and Reduced Expression of Transcription Factors in Human Osteoarthritis Cartilage. Arthritis Rheumatol 2016;68:1876-86.
3. Gabay O, Clouse KA. Epigenetics of cartilage diseases. Joint Bone Spine 2016;83:491-4.
4. Reynard LN. Analysis of genetics and DNA methylation in osteoarthritis: What have we learnt about the disease? Semin Cell Dev Biol 2016. [Epub ahead of print].
5. Lotz M, Loeser RF. Effects of aging on articular cartilage homeostasis. Bone 2012;51:241-8.
6. Goldring MB, Marcu KB. Epigenomic and microRNA-mediated regulation in cartilage development, homeostasis, and osteoarthritis. Trends Mol Med 2012;18:109-18.
7. Blanco FJ, Rego-Pérez I. Editorial: Is it time for epigenetics in osteoarthritis? Arthritis Rheumatol 2014;66:2324-7.
8. Zhang M, Wang J. Epigenetics and Osteoarthritis. Genes Dis 2015;2:69-75.
9. Barter MJ, Young DA. Epigenetic mechanisms and non-coding RNAs in osteoarthritis. Curr Rheumatol Rep 2013;15:353.
10. Wu Ct, Morris JR. Genes, genetics, and epigenetics: a correspondence. Science 2001;293:1103-5.
11. Roach HI, Yamada N, Cheung KS, et al. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. Arthritis Rheum 2005;52:3110-24.
12. Le LT, Swingler TE, Clark IM. Review: the role of microRNAs in osteoarthritis and chondrogenesis. Arthritis Rheum 2013;65:1963-74.
13. Loughlin J. Genetic contribution to osteoarthritis development: current state of evidence. Curr Opin Rheumatol 2015;27:284-8.
14. Meda F, Folci M, Baccarelli A, et al. The epigenetics of autoimmunity. Cell Mol Immunol 2011;8:226-36.
15. Rotti H, Mallya S, Kabekkodu SP, et al. DNA methylation analysis of phenotype specific stratified Indian population. J Transl Med 2015;13:151.
16. Bhat S, Mallya S, Varghese VK, et al. DNA methylation detection at single base resolution using targeted next generation bisulfite sequencing and cross validation using capillary sequencing. Gene 2016;594:259-267.
17. Joshi KS, Nesari TM, Dedge AP, et al. Dosha phenotype specific Ayurveda intervention ameliorates asthma symptoms through cytokine modulations: Results of whole system clinical trial. J Ethnopharmacol 2016. [Epub ahead of print].
18. Ernst E. Complementary or alternative therapies for osteoarthritis. Nat Clin Pract Rheumatol 2006;2:74-80.
19. Chopra A, Saluja M, Tillu G, et al. Ayurvedic medicine offers a good alternative to glucosamine and celecoxib in the treatment of symptomatic knee osteoarthritis: a randomized, double-blind, controlled equivalence drug trial. Rheumatology (Oxford) 2013;52:1408-17.
20. Sumantran VN, Kulkarni A, Chandwaskar R, et al. Chondroprotective Potential of Fruit Extracts of Phyllanthus emblica in Osteoarthritis. Evid Based Complement Alternat Med 2008;5:329-35.
21. Sumantran VN, Kulkarni A, Boddul S, et al. Chondroprotective potential of root extracts of Withania somnifera in osteoarthritis. J Biosci 2007;32:299-307.
22. Warrier SR, Haridas N, Balasubramanian S, et al. A synthetic formulation, Dhanwantharam kashaya, delays senescence in stem cells. Cell Prolif 2013;46:283-90.
23. Joshi KS, Bhonde R. Insights from Ayurveda for translational stem cell research. J Ayurveda Integr Med 2014;5:4-10.
24. Barry F, Murphy M. Mesenchymal stem cells in joint disease and repair. Nat Rev Rheumatol 2013;9:584-94.
25. Nöth U, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheumatol 2008;4:371-80.
26. Soler R, Orozco L, Munar A, et al. Final results of a phase I-II trial using ex vivo expanded autologous Mesenchymal Stromal Cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. Knee 2016;23:647-54.
27. Lamo-Espinosa JM, Mora G, Blanco JF, et al. Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). J Transl Med 2016;14:246.
28. Gupta PK, Das AK, Chullikana A, et al. Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Res Ther 2012;3:25.

doi: 10.21037/aoj.2016.11.03
Cite this article as: Bhonde R, Joshi K, Das AK. Epigenetic changes regulate the fate and progression of osteoarthritis. Ann Joint 2016;1:28.

