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The Role of Micrornas in Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) represents one of the most common degenerative disorders around the globe, its prevalence having a significant impact in the general population. Its complexity relays not only on the great variety of factors that lead to its development, but also the heterogeneity of pathophysiological aspects that occur during its progression. In the last decade, a great deal of interest was drawn by microRNAs (miRNA), single-stranded non-coding molecules involved in gene regulation. Several detection methods have been developed, each having its own characteristics and targeting specific types of miRNAs. This work is an overview regarding the impact of miRNA in OA development, pathophysiology and underlines its importance as a biomarker and future therapeutic target.

Keywords: Osteoarthritis; Mirna; Cartilage; Epigenetics; Biomarker; Detection Methods; Gene Regulation

Osteoarthritis

Due to the increasing lifespan around the globe, musculoskeletal disorders like Osteoarthritis (OA) tend to have a higher prevalence comparing to the past decades. As of 2017, more than 300 million people had been diagnosed with osteoarthritis. Therefore, early diagnosis and treatment becomes a serious matter considering the impact in the quality of life for these patients [1]. Osteoarthritis is a musculoskeletal disorder characterized largely by pain, join disfunction, synovial effusion and swelling due to the degenerative destruction of the articular cartilage. Articular cartilage lacks the capacity of self-healing, due to the lack of vascularization [2]. During the degenerative process occurs an imbalance between anabolic factors and catabolic factors in the favor of the latter.

Several matrix-degrading enzymes alter the structure of the extracellular matrix (matrix metalloproteinases, disintegrin, etc.), targeting especially type-II collagen or aggrecans [3-5]. Among the risk factors we can mention aging, metabolic disorders, cartilage injury, obesity and mechanical stress exerted upon the cartilage [6]. These factors that trigger the disorder bring several small-scale alterations like genomic instability, epigenetic alterations, altered intercellular communication, telomere attrition, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, etc. [7].

MicroRNA

The discovery of the first microRNA (miRNA), lin-4, in 1993 opened a new door for studying molecular biology [8]. Nowadays, MiRNA is considered a target for both diagnosis and treatment of OA by regulating the endogenous miRNA [4]. MiRNA are tiny, single-stranded, noncoding RNA molecules that regulate gene expression, having an average length of 22 nucleotides [9,10]. In most cases, miRNAs interact with the 3′ UTR (UnTranslated Region) of target mRNAs, suppressing the expression of the genes. In other cases, miRNAs interact with other regions, such as the 5′ UTR, coding sequence, and gene promoters. Also, miRNAs are involved in activation of gene expression under certain conditions, regulating cell cycle, apoptosis or differentiation [8,11,12]. There are several types of endogenous RNA molecules, like transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), small

interfering RNA (siRNA) and microRNA (miRNA) [9]. MicroRNAs can be identified in several subcellular structures: mitochondria, endoplasmic reticulum, P-bodies, nucleus, and nucleolus. Also, miRNAs can be found outside the cells via exosomes and detected in plasma and other bodily fluids [13]. Several miRNAs were identified in OA pathology, having an abnormal expression. The most common miRNAs are miR-9, miR-27, miR-34a, miR-101, miR-107, miR-140, miR-146a, miR-558, and miR-602 [14].

Mirna Detection Methods

The first miRNA isolation and detection methods used phenol-chloroform associated with RNA precipitation [15]. Nowadays, as the technology improved, many techniques were developed. Some authors divide these techniques in 2 categories: traditional techniques and modern techniques [16]. The most common techniques used are qRT-PCR, in-situ hybridisation, RNAsequencing, microarray, northern blot. qRT-PCR. It allows the instant detection and the quantification of genetic products generated during the repetitive PCR cycles [17]. RT-qPCR is a combination of three steps: RNA to cDNA conversion using reverse-transcriptase (RT); PCR-based amplification of the cDNA; real-time detection and quantification of cDNA [18]. In-situ hybridisation. It can localize genetic material in a sample by hybridizing and labeling a complementary strand of RNA or DNA. Using the labeled strand, a certain sequence of nucleic acid can be identified. The detection can be performed using isotopic or nonisotopic methods [19,20]. RNA-sequencing. It can reveal the entire structure of a transcriptome using high-throughput sequencing methods.

It can also provide analysis for other aspects like gene expression, translatome, alternatively spliced genes, etc [21,22]. Microarray. It can analyze simultaneously the expression of thousands of genetic sequences in a single experiment. The sequences are arranged in a row-column array on a glass slide known as "chip", facilitating their identification [23,24]. Northern blot. No special equipment is necessary. It uses the following steps: an agarose gel electrophoresis is performed in order to separate RNA sample (separation is performed according to the size of the sequence); it is transferred to a nylon membrane (preserving the separation in the gel and keeping the same arrangement), fixed and labeled by marking it with an isotope. A wash is performed, removing the unnecessary marked probes. The analyze can be performed using autoradiography or other techniques [16,25]. Despite the differences between these techniques, the results depend firstly on the quality of the sample. Secondly, not all detection methods cand isolate the miRNAs equally. Different extraction techniques isolate different RNAs, depending on the length of the molecules, their concentration in the sample and the sequence differences between miRNAs [11,26]. As a downside, most of the detection methods require long processing time, laborious techniques, and provide many false-positive results. Thereby, at the moment there is no universal detection method for miRNAs [16].

Epigenetics and MiRNA

Epigenetics affects OA in two major ways. First, the development of joints and bones is regulated via epigenetic mechanisms. Any change in these processes modify the risk of developing OA at some point in life by changing the joint shape, the extracellular matrix composition and/or the responsiveness of joint cells to cytokines and growth factors. Second, epigenetics processes can be triggered by external factors, such as articular traumatic injuries or metabolic disorders [27] Inflammation. The inflammatory process induces early alterations in cartilage structures way before the appearance of radiographic signs in OA. Micro fissures in articular cartilage and ECM catabolic products have been discovered in the synovial fluid in the early stages of OA [28]. MiRNA expression is determined by proinflammatory cytokines that lead to activation of the target genes that induce OA progression. Also, miRNAs are linked to modulation of proinflammatory cytokine expression, such as TNF-α, IL-1β, IL-6 [2,12]. Also, there are high CRP serum levels that correlate with the histological alterations in the synovial inflammatory site.

Due to high activity of IL-1β and TNF-α, there is an overexpression of other critical inflammatory and chrondrolytic mediators, including MMP-1, MMP-9, MMP-13, NO, PGE2, and IL-6. IL-6 acts as B-cell and T-cell activator, but also as a regulator for the recruitments of other inflammatory cells. IL-8 has a synergic effect along IL-6, recruiting and activating neutrophils [28-30]. Proliferation. In normal synovial membrane, the thickness is about 1-3 cell layers and a low level of inflammatory cells in the synovial fluid. In OA, however, the synovial reaction consists in hyperplasia associated with a high degree of inflammatory cells, mainly macrophages, B and T cells. During the early stages of OA, due to the increased cellular activity at the site of the articular cartilage, chondrocytes tend to form clusters consisting of 50 or more cells. On the other hand, simultaneously with the chondrocyte proliferation, ECM tends to decrease, having a reduced level of glycosaminoglycans in its composition compared with a normal ECM, resulting in degradation of intraarticular homeostasis and opening a door for further destruction mechanisms.

During the late stages of OA, the cartilage structure consists of hypocellularity due to a high level of chondrocyte apoptosis and lacunar emptying [28,31]. IL-6 and IL-8 are the main proliferationinductors involved in OA. Recent experiments revealed that miR-373 acts as a downregulatory for expression of IL-6 and IL-8 by inhibiting a specific receptor called P2X7R. In OA, the

plasmatic level of miR-373 is lower when compared with the non-OA plasmatic level samples [32]. Also, the expression of MiR-27b and MMP-13 activity is inversely proportional, miR-27b acting as a negative regulator. Mir-488 inhibits MMP-13 via Zinc-transporter 8 (ZIP-8), thus enhancing both chondrocyte differentiation and cartilage development. In OA, its expression is strongly diminished [10,33]. ECM degradation. ECM of the cartilage is composed of proteoglycans such as aggrecan (the main component), decorin, lumican, and biglycan; adhesive glycoproteins (fibronectin); collagens, mainly type II and in smaller proportions type VI, IX and XI collagens. These glycosaminoglycans (GAGs) have an absorptive function, increasing water concentration into cartilage ECM, thus enhancing compression resistance.

Type II collagen provides cartilage mechanical resistance to tension due to the fibrillar structure. Proteinases released in the articular cartilage in OA play a crucial role in degradation of ECM by targeting mainly the aggrecans and the collagen, damaging the two main components of the ECM. The destruction of these structures is mediated mostly by collagenases (MMP-1, MMP-8, MMP-13) and aggrecanases (ADAMTs) mainly via IL-1β, TNFα [34-36]. There are several other factors that contribute ECM breakdown such as Gc-globulin, α1-microglobulin, and α2-macroglobulin, but also VEGF. Also, IL-1β acts as a downregulatory in production of type II and type IX collagen, thus inhibiting ECM production [28]. Several miRNAs play a role in cartilage protection by modulating ADAMTS-5 expression, resulting in preservation of extracellular matrix. One of the first "protective" miRNA discovered is miR-140 that contribute to articular cartilage and normal enchondral bone development. In OA, miR-140 is downregulated compared with normal cartilage [2,37,38]. Apoptosis. Inflammatory cells migrate to the site of the injury in order to initiate tissue repair. After the reparation process is finished and the cells completed their task, they are eliminated via programmed cell death or apoptosis in order to prevent excessive inflammation [31].

Predominant factors that regulate apoptosis include enzymes, genes and proteins such as p53, Fas receptor, BCl-2 and Bax, cytochrome C, caspases, protein kinases regulated via extracellular signaling. The first stage in the apoptotic process is represented by the genetic control that decides, according to a stimulus, if the apoptosis should be initiated. It is regulated by two genes, BCl-2 and p53. The second stage is represented by the morphological alterations of apoptosis regulated by caspases [39]. Chondrocyte apoptosis may be linked directly to the destruction of the ECM. Most cells attach to the neighboring cells or ECM, creating junctions and ensuring the necessary nutrient intake in order to sustain their activity. Disruption of ECM may affect chondrocyte survival, inducing premature apoptosis.

In vitro experiments using enzymatic treatment via high purity collagenase in order to cleave type II collagen proved that the degradation of collagen induced chondrocyte apoptosis [31]. IL-1β modifies the expression of collagen gene Col_2a_1, having a downregulation effect and also having an upregulation effect over inducible nitric oxide synthase (iNOS). This enzyme has a chondrolytic effect over the cartilage, promoting apoptosis in chondrocytes. Along IL-1β, miR-101 is also an important factor that promotes chondrocyte ECM degradation. MiR-34a can decrease the IL-1β-induced effects [10,40]. Cartilage injury initiated by mechanical pressure leads to a high expression of miR-146a, having an apoptotic effect in human chondrocytes by inhibiting of Smad4. Its expression decreases with the OA progression [31,41]. Autophagy. In order to maintain homeostasis, living organisms tend to trigger mechanisms involved in cellular turnover. Autophagy represents the process regulated by several autophagyrelated genes like Beclin-1 and Light Chain 3 (LC3) that dismantle damaged or unnecessary cells and their components prior to their removal [42].

The expression of these genes is directly proportional to the metabolic activity of the involved tissue [43]. Depending on the region of the cartilage, the expression of the autophagy-related proteins differs. Thus, in the superficial layer, cells display a higher expression of proteins like BECN1, ATG5, and MAP1LC3. The cells located in the deep layer have a lower expression of MAP1LC3. Also, their expression decreases with aging. Once the autophagic process is disrupted due to the reduction of these proteins, apoptotic activity increases [44]. In OA, miR-107 acts as an autophagy-inductor, having a protective effect on chondrocytes and reducing ECM degradation [33,45].

Potential Therapeutic Targets

Current management of OA involves pain-ameliorating medication and joint replacement surgery. None of these treatments adress to the underlying cause of OA. Due to this fact, there is a need for a Disease-Modifying-OsteoArthritis-Drug (DMOAD), a treatment that not only reduces the symptoms, but also interfere with the pathophysiology of OA, stopping the progression and at least prevent further joint degradation [46,47]. Currently, miRNAs represent a therapeutic target in the management of OA. So far, the clinical success of miRNA-based treatment is not satisfying. Due to the very unstable nature of miRNA, it has a short half-life, it is unstable *in vivo* and interferes with the disruption and saturation of endogenous RNA. Also, because of the lack of vascularization, miRNA hardly reaches chondrocytes in order to exert the expected effect. Therefore, various techniques must be developed to acquire a better miRNA stability and also a proper drug-delivery technique

[48]. There are early studies involving miR-140 treatment in rats where the miRNA was administered intraarticular via exosome. It was observed that the chondrocyte numbers and cartilage thickness were greater after the miR-140 treatment and a reduction in MMP-13 and ADAMTS-5 expression, thus reducing the ECM degradation and reducing OA progression [49,50].

Conclusion

MicroRNAs have a diversity of biological functions, being involved in a wide range of pathological processes. Also, they regulate gene expressions, activating or inhibiting gene expression. In the pathological mechanism of osteoarthritis, miRNAs have a regulatory role, so they are considered to be potential targets for diagnosis and treatment of OA. Present research directions are effectively promoting miRNA study, due to the increased potential of becoming diagnosis and treatment biomarkers of OA.

References

1. Kloppenburg M, Berenbaum F (2020) Osteoarthritis year in review 2019: epidemiology and therapy. Osteoarthritis and Cartilage 28: 242-248.
2. Endisha H, Jason Rockel, Igor Jurisica, Mohit Kapoor (2018) The complex landscape of microRNAs in articular cartilage: biology, pathology, and therapeutic targets. JCI Insight 3(17): e121630.
3. Yu C, Chen WP, Wang XH (2011) MicroRNA in Osteoarthritis. The Journal of International Medical Research 39: 1-9.
4. Nakasa T, Yoshihiko Nagata, Keiichiro Yamasaki, Mitsuo Ochi (2011) A mini review: microRNA in arthritis. Physiol Genomics 43: 566-570.
5. Martel Pelletier J, Boileau C, Pelletier JP (2008) Cartilage in normal and osteoarthritis conditions. Best Practice & Research Clinical Rheumatology 22(2): 351-384.
6. Mihanfar A, Zeinab Latifi, Hamid Reza Nejabati, Mohammad Nouri, Amir Fattahi, et al. (2020) Exosomal miRNAs in osteoarthritis. Mol Biol Rep 47(6): 4737-4748.
7. A Mobasheri, Csaba Matta, Róza Zákány, Giuseppe Musumeci (2015) Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis. Maturitas 80: 237-244.
8. O'Brien J (2018) Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol 9: 402.
9. MacFarlane LA, Murphy P (2010) MicroRNA Biogenesis, Function and Role in Cancer. Current Genomics 11(7): 537-561.
10. Nugent M (2016) MicroRNAs: exploring new horizons in osteoarthritis. Osteoarthritis and Cartilage 24(4): 573-580.
11. Brown R, Michael R Epis, Jessica L Horsham, Tasnuva D Kabir, Kirsty L Richardson, et al. (2018) Total RNA extraction from tissues for microRNA and target gene expression analysis: not all kits are created equal. BMC Biotechnology18(1): 16.
12. Jianwei He, Weiwei Cao, Inayat Azeem, Zengwu Shao (2020) Epigenetics of osteoarthritis: Histones and TGF-β1. Clinica Chimica Acta 510: 593-598.
13. Sondag G, Haqqi T (2016) The Role of MicroRNAs and Their Targets in Osteoarthritis. Curr Rheumatol Rep 18(8): 56.
14. Malemud C (2018) MicroRNAs and Osteoarthritis. Cells 7(8): 92.
15. Lu T, Rothenberg M (2018) MicroRNA. J Allergy Clin Immunol 141(4): 1202-1207.
16. Ye J, Mingcheng Xu, Xueke Tian, Sheng Cai, Su Zeng, et al. (2019) Research advances in the detection of miRNA. Journal of Pharmaceutical Analysis 9(4): 217-226.
17. Pestana EA (2009) Real-Time PCR The Basic Principles. In: Early, rapid and sensitive veterinary molecular diagnostics - real time PCR applications. Springer, Dordrecht.
18. Nolan T, Hands R, Bustin S (2006) Quantification of mRNA using real-time RT-PCR. Nat Protoc 1(3): 1559-1582.
19. Unger (2010) Chapter 7 - In Situ Hybridization: Principles and Applications. Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory, pp. 71-79.
20. Jensen E (2014) Technical Review: *In Situ* Hybridization. Anat Rec 297(8): 1349-1353.
21. Kukurba K, Montgomery S (2015) RNA Sequencing and Analysis. Cold Spring Harb Protoc (11): 955-969.
22. Stark R, Grzelak M, Hadfield J (2019) RNA sequencing: the teenage years. Nat Rev Genet 20: 631-656.
23. Liu CG, George Adrian Calin, Stefano Volinia, Carlo M Croce (2008) MicroRNA expression profiling using microarrays. Nat Protoc 3: 563-578.
24. Govindarajan R, Jeyapradha Duraiyan, Karunakaran Kaliyappan, Murugesan Palanisamy (2012) Microarray and its applications. J Pharm Bioall Sci 4: 310-312.
25. He S, Green R (2013) Northern Blotting. Methods in Enzimology 530: 75-87.
26. Krepelkova I, Robert Mikulik, Ondrej Slaby, Milan Bartos, Viktor Ruzicka, et al. (2019) Evaluation of miRNA detection methods for the analytical characteristics necessary for clinical utilization. BioTechniques 66(6): 277-284.
27. Rice S (2020) Interplay between genetics and epigenetics in osteoarthritis. Nature Reviews Rheumatology 16: 268-281.
28. Sokolove J, Lepus C (2013) Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. Ther Adv Musculoskel Dis 5(2): 77-94.
29. Chow YY, Chin KY (2020) The Role of Inflammation in the Pathogenesis of Osteoarthritis, Mediators of Inflammation Volume.
30. Wang Y, Shen S, Li, Z (2020) MIR-140-5p affects chondrocyte proliferation, apoptosis, and inflammation by targeting HMGB1 in osteoarthritis. Inflamm. Res 69(1): 63-73.
31. Hwang H, Kim H (2015) Chondrocyte Apoptosis in the Pathogenesis of Osteoarthritis. Int J Mol Sci 16(11): 26035-26054.
32. Zhang W (2018) miR-373 regulates inflammatory cytokine-mediated chondrocyte proliferation in osteoarthritis by targeting the P2X7 receptor. FEBS Open Bio 8(3): 325-331.
33. Portal Núñez S, Pedro Esbrit, María José Alcaraz, Raquel Largo (2015) Oxidative stress, autophagy, epigenetic changes and regulation by miRNAs as potential therapeutic targets in osteoarthritis. Biochem Pharmacol 108: 1-10.
34. Perez Garcia S, Isidoro González Álvaro, Francisco J Blanco, Yasmina Juarranz, Rosa P Gomariz, et al. (2020) Profile of Matrix-Remodeling Proteinases in Osteoarthritis: Impact of Fibronectin. Cells 9(1): 40.
35. Maldonado M, Nam J (2013) The Role of Changes in Extracellular Matrix of Cartilage in the Presence of Inflammation on the Pathology of Osteoarthritis. BioMed Research International Volume.

36. Bertrand J (2010) Molecular mechanisms of cartilage remodelling in osteoarthritis. The International Journal of Biochemistry & Cell Biology 42(10): 1594-1601.

37. Raman S, FitzGerald U, Murphy JM (2018) Interplay of Inflammatory Mediators with Epigenetics and Cartilage Modifications in Osteoarthritis. Front Bioeng Biotechnol 6: 22.

38. Gabay O, Clouse KA (2015) Epigenetics of cartilage diseases. Joint Bone Spine 83(5): 491-494.

39. Kunwar A, Kumar M, Singh S (2017) Pathological perspective of chondrocyte apoptosis in osteoarthritis. J Orthop Traumatol Rehabil 9: 1-95.

40. Yu XM, Hao Ye Meng, Xue Ling Yuan, Yu Wang, Quan Yi Guo, et al. (2015) MicroRNAs' Involvement in Osteoarthritis and the Prospects for Treatments. Evidence-Based Complementary and Alternative Medicine Volume.

41. Panagopoulos PK, Lambrou GI (2018) The Involvement of MicroRNAs in Osteoarthritis and Recent Developments: A Narrative Review. Mediterr J Rheumatol 29(2): 67-79.

42. YS Li (2016) Autophagy in osteoarthritis. Joint Bone Spine 83(2): 143-148.

43. Cheng NT, Li Feng Ma, Liang Zhang, Hao Miao Yu, Zhen Zhong Wang, et al. (2017) Role of autophagy in the progression of osteoarthritis: The autophagy inhibitor, 3-methyladenine, aggravates the severity of experimental osteoarthritis. International Journal of Molecular Medicine 39(5): 1224-1232.

44. Shapiro I (2014) Boning up on autophagy. Autophagy 10(1): 7-19.

45. Zhao X, Hongyan Li, Linlin Wang (2019) MicroRNA-107 regulates autophagy and apoptosis of osteoarthritis chondrocytes by targeting TRAF3. International Immunopharmacology 71: 181-187.

46. Grandi F, Bhutani N (2020) Epigenetic Therapies for Osteoarthritis. Trends in Pharmacological Sciences.

47. Song J, Dongkyun Kim, Jiyeon Han, ChurlHong Chun, Eun Jung Jin, et al. (2013) MicroRNA-181b regulates articular chondrocytes differentiation and cartilage integrity. Biochemical and Biophysical Research Communications 431(2): 210-214.

48. Oliviero A, Antonio Oliviero, Giovanna Della Porta, Giuseppe M Peretti, Nicola Maffulli (2019) MicroRNA in osteoarthritis: physiopathology, diagnosis and therapeutic challenge. British Medical Bulletin 00: 1-11.

49. Si HB, YN Chen, JQ Cheng, YR Lu, B Shen, et al. (2017) Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. Osteoarthritis and Cartilage 25(19): 1698-1707.

50. Liang Y, Xiao Xu, Xingfu Li, Jianyi Xiong, Biquan Li, et al. (2020) Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes toward a Cell-Free Osteoarthritis Therapy. ACS Applied Materials & Interfaces 12(33): 36938-36947.

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