Interplay between C-type lectin receptors and microRNAs in cellular homeostasis and immune response

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Abbreviations: CLRs, C-type lectin receptors; miRNA, microRNA; UTR, Untranslated region; CTLD, C-type lectin like domain; SP, Surfactant protein; MBL, Mannose-binding lectin; Reg proteins, Regenerating islet-derived family proteins; LOX-1, Lectin-like oxidized low-density lipoprotein receptor 1; MR, Mannose receptor; DC-SIGN, Dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin; Dectin 1, Dendritic cell associated C-type lectin-1; Clec5A, C-type lectin domain family 5, member A; KLRG1, Killer cell lectin-like receptor subfamily G member 1

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Conflict of Interest

The authors declare that there is no conflict of interest with regard to the topics discussed in this review.

Abstract

C-type lectin receptors (CLRs) belong to the family of pattern recognition receptors (PRRs). They have a critical role to play in the regulation of a range of physiological functions including development, respiration, angiogenesis, inflammation, and immunity. CLRs can recognize distinct and conserved exogenous pathogen-associated as well as endogenous damage-associated molecular patterns. These interactions set off downstream signaling cascades, leading to the production of inflammatory mediators, activation of effector immune cells as well as regulation of the developmental and physiological homeostasis. CLR signaling must be tightly controlled to circumvent the excessive inflammatory burden and to maintain the cellular homeostasis. Recently, MicroRNAs (miRNAs) have been shown to be important regulators of expression of CLRs and their downstream signaling. The delicate balance between miRNAs and CLRs seems crucial in almost all aspects of multicellular life. Any dysregulations in the miRNA-CLR axes may lead to tumorigenesis or inflammatory diseases. Here, we present an overview of the current understanding of the central role of miRNAs in the regulation of CLR expression, profoundly impacting upon homeostasis and immunity, and thus, development of therapeutics against immune disorders.

Introduction

The innate immune system imparts body's first line of defense against pathogenic challenges and endogenous stresses through evolving different families of germline encoded pattern recognition receptors (PRRs) which includes Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), and cytosolic DNA sensors [1,2]. Among all these families of PRRs, C-type lectin or C-type lectin receptors (CLRs), comprised of at least one characteristic C-type lectin like domain (CTLD), form a large heterogeneous group of transmembrane or soluble receptors, almost as diverse as the immunoglobulin 'superfamily' [3]. Based on the conserved motif present in CTLD, CLRs are either mannose or fucose-specific (characterized by the presence of Glu–Pro–Asn amino acid triplet or EPN motif) or galactose-specific (carry Gln–Pro–Asp amino acid triplet or QPD motif) [4,5]. Selectins represent another class of CLRs that recognize sialylated, fucosylated glycan structures with sialyl-Lewis^x (sLe^x) motif [6]. Initially, 'C-type lectins' were so named to highlight them as calcium-dependent carbohydrate-binding lectin, but later on, it was found that certain CLRs lack the coordinated calcium binding or carbohydrate specificity, and hence, C-type lectin-like receptors. CLRs can recognize a wider array of ligands, including carbohydrates as well as non-carbohydrates such as lipids, proteins, and inorganic salts [7]. CLRs are mostly expressed by myeloid and lymphoid cells, in addition to epithelial and endothelial cells [5]. After encountering distinct exogenous 'non-self' pathogen-associated molecular patterns (PAMPs), endogenous 'damaged self' ligands like damage associated molecular patterns (DAMPs) or endogenous 'altered self' ligands like tumour-associated molecular patterns (TAMPs), multivalent CLRs can modulate downstream immune signaling and several cellular response [8]. Due to their ability to recognize and internalize antigens, and initiate independent signal transmission, CLRs are considered as non-TLR member of the PRR family [9]. Additionally, some CLRs also collaborate with TLRs to drive downstream signaling pathways [10].

In vertebrates, more than 1000 proteins of CLR superfamily are categorized into 17 subgroups, according to their phylogeny and domain alignment [5] (**Fig. 1**). For example, soluble CLRs such as surfactant protein-A (SP-A) and surfactant protein-D (SP-D) (group III collectin family) act as opsonin proteins and contribute to host defense [9,10]. Aggrecan and versican (group I proteoglycan family), which are crucial components of the extra-cellular matrix (ECM), regulate inflammation [13]. Mannose-binding lectin (MBL) (group III collectin family) and regenerating islet-derived (Reg) family proteins (group VII free CTLDs) offer anti-microbial immunity [9]. Selectin family comprises of L-selectin (expressed on leukocytes), E-selectin (expressed on endothelial cells) and P-selectin (expressed on platelet and endothelial cells); the proteins are involved in adhesion, extravasation, and regulation of inflammation [6]. In addition, transmembrane CLRs trigger different intra-cellular signaling pathways that promote immune cells activation,

phagocytosis, apoptosis either by directly signaling through integral motifs in their cytoplasmic tails, or indirectly by using adaptor molecules like Fc receptor γ-chain (FcRγ) and DNAX-activating protein 10 (DAP10) [5,14]. There are CLRs bearing inhibitory motifs too, for example, killer cell lectin-like receptor subfamily G member 1 (KLRG1), to inhibit inflammatory signaling pathways induced by other immunoreceptors [5]. CLRs, like mannose receptor (MR) on human alveolar macrophage act in a dual mode, based on a temporal and spatial interplay of interacting signaling adaptor proteins. MR induce phagocytosis after recognizing *Mycobacterium tuberculosis* (M.tb) via interacting with growth factor receptor-bound protein-2 (Grb2) as well as inhibit phagolysosome formation via recruiting Src homology region-1 domain-containing phosphatase (SHP-1) which facilitates M.tb survival in human [15] **(Fig. 1)**. However, CLR signaling is a double-edged sword. While CLR-triggered innate immune signaling and subsequent enhancement of adaptive immune responses are crucial for clearing pathogens and maintaining homeostasis, inappropriate activation can cause autoimmune or inflammatory diseases as well as cancer. Thus, CLR expression and signaling need to be tightly regulated [16,17].

Involvement of microRNAs (miRNAs) in regulating TLR signaling has been intensely studied; however, how miRNA regulate CLRs has received great attention in recent years [7,18]. miRNAs are short non-coding RNA molecules comprising of 19 to 25 nucleotides, and regulate post-transcriptional and translational silencing of more than 30% of protein-coding genes [19]. The biogenesis of miRNAs involves nuclear as well as cytoplasmic processing events that are carried out by two double-stranded RNA-specific endoribonuclease III enzymes, Drosha and Dicer, respectively. After the final processing in the cytoplasm, pre-miRNA duplexes are loaded onto the RISC (RNA-induced silencing complex) together with an argonaute (AGO) protein, where one mature miRNA strand is selected to exert the biological functions. In addition to this canonical biogenesis pathway, noncanonical biogenesis pathways also exist for the production of functional miRNAs [20]. By RNA interference mechanism, miRNAs can bind to 3′ untranslated region (3′UTR) of target mRNAs that results in the mRNA cleavage or degradation, or translation repression [21]. Key roles of miRNAs have been explored in development, defense, apoptosis [22], tumorigenesis [23] and inflammation [24]. Thus, the biogenesis cascade and the function of miRNAs themselves need to be under tight temporal and spatial control, as their dysregulation often leads to several human diseases, including cancer. Multiple strategies are applied by the cell to regulate miRNA biogenesis at transcriptional (by RNA polymerase II-associated transcription factors) or post-transcriptional (by RNA-binding proteins) levels [20,25]. The details of the regulatory events are given in **Table 1A and 1B**.

Recent studies examining the roles of miRNAs in regulating immune cell functions revealed that few miRNAs co-regulate CLRs as well as other PRRs. For instance, miR-155 regulates TLRs (TLR4 and TLR3) as well as CLRs [Dendritic cell-specific intercellular adhesion

molecule-3 grabbing nonintegrin (DC-SIGN/CD209), M2 associated galactose C-type lectin (MGL-1), Reg3b]. Similarly, miR146 regulates TLR4 and MGL-1 expression. miR-let-7 modulates TLR4 and lectin-like oxidized low-density lipoprotein receptor (LOX-1) expression [7]. A range of studies have also addressed the idea that TLR signaling can alter miRNA expression like miR-155 and miR-146 can regulate the expression of TLR2, TLR3, TLR4, TLR9 expression [7,18]. It is, therefore, important to study the involvement of miRNA in CLR signaling cascade.

As novel therapeutic agents, miRNA mimics and miRNA inhibitors, currently in early clinical development, have shown promising results [26].

Here, we review the recent knowledge in the area of miRNA-mediated regulation of CLR expression and signaling that is central to mammalian homeostasis and host immunity [5,7,8,12].

miRNA-CLR axes in developmental and physiological homeostasis

From embryonic development to organogenesis, both soluble and transmembrane CLRs play pivotal roles. Like CLRs, miRNAs also contribute to regulation of several cellular functions.

SP-A and SP-D are referred to as lung collectins (collagen containing C-type lectins) for their immense contribution to the lung development and lung physiology [27]. Initially, these were found to be secreted in the lungs by alveolar type II epithelial cells (AECII) and Clara cells but extra-pulmonary existence of SP-A and SP-D is now well documented [28]. SP-A and SP-D act as potent dual role surveillance molecules that can act either as pro-inflammatory or anti-inflammatory molecules depending on the microenvironment [29]. A growing number of studies indicate expression of surfactant proteins is highly regulated by miRNAs. For example, decreased miRNA biogenesis by silencing Drosha enhanced the potential of human alveolar type II cells to secrete SP-A protein [30]. Furthermore, several *in-vitro* or *in vivo* studies revealed that miRNAs such as miR-200 family [31], miR-29 [32], miR-20a-5p [33], and miR-16 [34] positively regulate pulmonary surfactant proteins synthesis whereas miR-431-5p [30], miR-199a/214 cluster [36], miR-26a [37–39], miR-206 [40] were found to exert an inhibitory role in AECII differentiation and synthesis of surfactant proteins **(Table 2)**. Interestingly, it has been also reported that SP-A2 (SP-A has two variants: SP-A1 and SP-A2) regulates miRNome of alveolar macrophages (AM) in a sex-specific manner. Transgenic mice expressing human SP-A1 and SP-A2 exposed to ozone-induced oxidative stress (OxS) demonstrated that OxS induced male SP-A2 AM miRNome is related to inflammatory responses, regulation of ROS, and apoptosis which make the males less susceptible to oxidative stress than females [41].

Versican (VCAN), a chondroitin sulfate proteoglycan (CSPG), binds to hyaluronan and constitute 'provisional ECM' which is involved several aspects of tissue homeostasis via regulating cellular migration, proliferation, sorting and phenotype [42]. The miR-21 targets tissue inhibitors of metalloproteinases (TIMP) and causes increased expression of matrix metalloproteinases (MMPs), disintegrin and metalloproteinases with thrombospondin motifs (ADAMTS) in porcine cumulus cells. ADAMTSI-mediated cleaved versican expression during *in-vitro* cumulus-oocyte-complex maturation facilitates cumulus cell expansion and oocyte maturation via ECM remodeling [43]. Similarly, in mice, ectopic versican 3′UTR helps to maintain endogenous versican and β-catenin levels associated with dermal wound healing and fibroblast cell migration via engaging versican-associated miRNAs to bind with ectopic versican mRNAs [44].

Another secreted CLR proteoglycan aggrecan [alternative names: Aggrecan 1 (AGC1), Chondroitin sulfate proteoglycan core protein 1(CSPG1)], key ECM component of cartilaginous tissue [45], has similarly been found to be regulated by miRNAs. For instance, miR-140 [46], miR-92a [47], miR-141-3p [48] are involved in chondrogenesis via enhancing aggrecan expression. On other hand, miR-143-3p is associated with chondrogenic differentiation by inhibiting aggrecan in rat model [49]. Furthermore, Wu *et al*. unraveled the underlying molecular mechanisms of dextrose prolotherapy for osteoarthritis treatment. They have shown that glucose induces chondrogenesis in murine chondrocytic cell line, ATDC5, by enhancing aggrecan expression via activating PKCα-p38 pathway which eventually inhibit miR-141-3p [48].

Additionally, transmembrane CLRs, like regenerating islet-derived (Reg) proteins were initially identified in chronic calcific pancreatitis patients, but subsequent studies revealed the extra-pancreatic implications of Reg proteins in others pathologies such as diabetes, colon cancer, and hepatocellular cancer [50,51]. Reg proteins are associated with increased cellular proliferation, differentiation and inhibition of apoptosis so its regulation could be an alternative strategy for prevention of many diseases. However, the miR-7-mediated direct suppression of only murine *reg1,* but not the human *REG1A* and *REG1B,* exemplifies species-specific regulation of Reg proteins [52].

Moreover, lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1 or OLR1) is involved with adipocyte proliferation, triglyceride storage, lipid metabolism. LOX-1-miRNAs interaction in the context of atherosclerosis disease progression has been well studied [53]. However, how this interaction affects production of milk and beef quality traits in cattle was first illustrated by Li *et al*., stating that bta-miR-370 suppresses LOX-1, formation of cytoplasm lipid droplets, and thus, regulates lipid metabolism in bovine adipocytes [54].

Role of miRNA-CLR axes in regulating cancer

Initially, miRNAs were considered only as developmental regulators, but the concept has now substantially extended; dysregulated miRNAs contribute to the onset of many diseases, including cancer. Thus, making these molecules promising therapeutic candidates either in the form of miRNA mimics (for miRNAs acting as tumor suppressors), or as anti-miRs (for miRNAs acting as oncogenes) [50,51]. Furthermore, an increasing number of studies have stated that CLR dysregulation could be a result of the altered expression of miRNAs, or miRNA-mediated regulation, which can cause oncogenic transformation **(Table 3**). In this section, we discuss some prominent examples of CLRs-miRNAs axes regulating cancer.

Increased versican (VCAN) acts as a pro-tumorigenic factor. Its association with unfavorable prognosis and relapse of tumor has been examined in several types of malignancies. For instance, in epithelial ovarian cancer (EOC), high miR590 accelerated tumor cell proliferation, migration and invasion via enhancing VCAN expression. Forkhead box A2 (FOXA2) that serves as a negative regulator of VCAN was found to be suppressed by miR-590. Via inhibiting FOXA2 and de-repressing VCAN expression, miR-590 is negatively correlated with survival of EOC patients [23]. Similarly, miR-23b and miR-143 also target VCAN expression. IL-17A mediated downregulation of miR-23b in tongue squamous cell carcinoma cells (TSCC) [57] and TGF-β-mediated downregulation of miR-143 in osteosarcoma cells [58] resulted in enrichment of versican and was found to be associated with increased cell migration and invasion. Furthermore, miR-135a-5p acts as a thyroid carcinoma suppressor via targeting VCAN 3'UTR [59]. In addition, the diagnostic potential of miRNAs and CLRs has been reported for multiple diseases. For example, a positive trend of VCAN and a negative trend of VCAN-targeting miR-203 with the progression of disease suggests their potential as non-invasive diagnostic biomarkers with optimum specificity and sensitivity for testing multiple myeloma [60].

Similar to versican, oncogenic potential of RegIV has been investigated recently. In gastric cancer tissue, decreased miR-24 and increased RegIV expression than the non-tumor tissues suggested a potential anti-cancer role of miR24. Enhanced expression of miR-24 inhibited the sequential events of carcinogenesis via targeting RegIV 3′UTR [61]. Additionally, overexpression of miR-363 repressed GATA6 transcription factor, which in turn, inhibited colon cancer cell growth under adherent conditions (by blocking GATA6/REG4 pathway) and suppressed clonogenicity (by blocking GATA6/LGR5 pathway) [62].

Moreover, some miRNAs can also act as oncogenes (oncomiRs) via targeting tumor suppressor CLRs. In hepatocellular cancer (HCC), increased miR-942-3p is inversely correlated with the survival in HCC patients and enhanced cell proliferation and invasion *in*

vitro via directly targeting expression of apoptosis activator mannose-binding lectin 2 (MBL2) [63].

Interestingly, miRNAs regulate E-selectin-mediated trans-endothelial migration and metastasis dynamics in colon cancer cells either by inhibiting its transcriptional regulator, NF-κB (by miR-146a and miR-181b), or by directly targeting E-selectin mRNA (by miR-31) [64]. Similarly, hsa-miR-370 specifically targets sLex, the ligand of P-selectin, and represses metastatic potential of human colon adenocarcinoma cells via modulating P-selectin induced p38-phosphatidylinositol 3-kinase (PI3K) signaling [65].

In addition to soluble CLRs, involvement of transmembrane CLRs in cancer has also been examined. For instance, C-type lectin domain family 5, member A (CLEC5A), a key regulator of myeloid differentiation, is targeted by miR-125a [66]. Inoue *et al*. reported that additional sex combs-like 1 (ASXL1) mutation inhibits polycomb repressive complex 2 (PRC2) function and de-repress miR-125a and Homeobox A9 (HOXA9). HOXA9 is a homeotic transcription factor associated with normal hematopoiesis and acute leukemia. It is essential for anterior–posterior patterning during embryonic development; its expression is absent in most of the adult cells, with the exception of hematopoietic stem cells where HOXA9 is highly expressed, and its expression gradually decreases with further differentiation [67].) Thus, using murine BMT model (where, isolated bone marrow from donor mice, retrovirally infected with ASXL-MT, ASXL-WT or empty vector is transferred to recipient mice), they first demonstrated appearance of myelodysplastic symptoms in ASXL1-MT transplanted mice compared with mock-transplanted mice due to dysregulation of CLEC5A-miR-125 axis.

Furthermore, the role of killer cell lectin-like receptor subfamily G member 1 (KLRG1) expression on memory T cells in ovarian tumor, colitis and colon tumor microenvironment revealed that $KLRG1^+$ T cells impaired anti-tumor T cell immunity by downregulating miR-101 expression and subsequently elevating the expression of co-repressor C-terminal binding protein-2 (CtBP2). This in turn results in $KLRG1^+$ T senescence with a declined capacity of production of effector cytokines by T cells [IFN-γ, TNF-α and Granzyme B of CD8 T cells and IL-17 of CD4 T cells] and promote immunosuppression [68].

miRNA-CLR axes in immunomodulation

CLRs are the members of PRR family and as they can recognize a broad repertoire of PAMPs, most research attention has focused on their contribution to anti-microbial immunity. There are several reports on CLR-mediated regulation of immunological responses [69]. In this section, we highlight CLR-miRNA axes in the modulation of various functions of immune cells relevant for maintaining immune homeostasis.

miRNA-mediated regulation of the expression of CLRs by immune cells has a remarkable influence on the effector signal transduction as well as in the leukocyte polarization **(Fig. 2)**. For example, an altered level of miR-511-3p in human dendritic cells (DCs) is involved in regulation of DC functions and Th cell polarization through modulating expression of two transmembrane CLRs like mannose receptor (MR/CD206) and dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN/CD209) expressed on human DCs. On an interesting note, miR-511 is found to be embedded in human *MRC1* gene (which encodes MR) within intron 5. It is therefore reasonable to assume that miR-511 should coordinately be expressed with MRC1 mRNA [70]. However, miR511-3p knockdown via transfecting miR-511-3p inhibitors in DCs led to an increased expression of MR and DC-SIGN, highlighting the existence of discordant expression between MRC1 and miR-511-3p in human DC. Therefore, it is possible that additional pathways are involved; changes in PPARγ activity may contribute to increased MR level in miR-511-3p silenced DC [71]. Both these CLRs contribute to the anti-inflammatory DC phenotype defined by increased inteleukin-10 (IL-10) and programmed death-ligand 1 (PD-L1) and Th2 polarization via upregulating Th2 promoting cytokines IL-10 and IL-4 while inhibiting IL-17 [72]. In addition to this, another study in mice reported that, MALAT1, a long non-coding RNA (lncRNA) exerts a regulatory role in the induction of DC-SIGN expression that polarizes DC towards tolerogenic phenotype and promotes IL-10-mediated immune-suppressive environment, by acting as an miR-155 sponge, an RNA molecule with multiple miRNA-binding sites to saturate miRNA-mediated mRNA regulation. The same study also highlighted that *in vivo* adoptive transfer of MALAT1-enriched DCs induced immune tolerance by shaping miR-155/DC-SIGN axis in cardiac-allograft transplantation (where BALB/c recipient mice which had undergone heart transplantation from the donor C57BL/6 murine cardiac tissue) and autoimmune myocarditis (where BALB/c mice was immunized with α-myosin H-chain peptide) [73].

Moreover, therapeutic implication of miRNA-mediated macrophage repolarization in pneumonia has been reported. Enhancement of miR-155 and miR-146 shifted the balance from M1 macrophage towards M2 type via enhancing M2 associated galactose C-type lectin (MGL-1) expression *in vitro* [74]. In accordance with this study, M1-M2 balance in allergic inflammation has also been regulated via mannose receptor (MRC1/MR/CD206) through miR-511-3p in mice [75].

Furthermore, like myeloid cells, T cell activity is also regulated by miRNA-CLR axes. C-type lectin CD69 has an integral role in the development of thymic regulatory T cells through increasing the signal transducer and activator of transcription 5 (STAT5) phosphorylation and BIC/miR155 transcription in mice [76]. On the other hand, miR-126 knockdown in mice demonstrated significantly enhanced $CD69^{+}$ functional T cells as a result of the elevated insulin receptor substate-1 (IRS1) signaling [77].

Role of miRNA-CLR axes in inflammatory disorders

CLRs and miRNAs both are increasingly being recognized as a crucial controllers of inflammation [24,78]. In this section, we discuss the most recent understanding on CLR-miRNA axes in the regulation of different inflammatory disorders.

Allergy

Allergy is a common type I hypersensitivity disorder. Several C-type lectins, including MBL, dectin 1, dectin 2, DC-SIGN, mincle, MMR/CD206 and collectins, have been reported to play essential roles in the pathogenesis of allergic inflammation [5]. Recent data also suggest that miRNAs can regulate immune responses, immune cell polarization and tissue inflammation in allergic diseases. Thus, miRNAs are promising candidates for biomarker development in allergic inflammation [79,80].

Zhou *et al.* showed the implication of mannose receptor (MRC1/MR/CD206) and miR-511-3p in cockroach allergen-induced lung inflammation in a murine model. *MRC1*-deficient murine alveolar macrophages exhibited less allergen uptake by MRC1/MR/CD206 with a reduced level of miR-511-3p that resulted in M1-driven allergic inflammation. However, the presence of MRC1 caused enhanced miR-511-3p expression which exerted M2-driven anti-inflammatory responses [75]. Although miR-511 is embedded in *MRC1* gene in human and mice, MRC1 deficiency results in decreased miR-511 transcription in lung macrophage in mice. This is in contrast with the human data, where discordant expression of MRC1 and miR-511 is present in DCs [72]. These observations suggest that the role of CLR-miRNA axes might be cell- (i.e., macrophages and DCs) and species-specific (as in human and mice).

Osteoarthritis

Involvement of aggrecan in the pathogenesis of osteoarthritis (OA) is well studied. Excessive degradation of aggrecan, one of the crucial constituents of articular ECM, due to excessive matrix-degrading enzymes (MMPs, ADAMTS) [81], or pro-inflammatory mediators (IL-1β) [82], disrupted the metabolic homeostasis of articular chondrocyte ECM, the driving force for the development of OA lesions. The miRNAs are one of the most studied components for aggrecan regulation in chondrocytes **(Fig. 3)**. For example, few miRNAs [such as miR-483-3p [83], miR-30 [84], miR-206 [85], miR-216b [86], miR-448 [87] and miR-195 [88] that directly or indirectly target aggrecan, are upregulated in OA cartilages or chondrocytes. They serve as pro-OA factors and contribute to the progression of OA. On the other hand, miR-671 is downregulated in the plasma and cartilage tissue of OA patients when compared to healthy controls [89]. Moreover, treatment with miR-671 mimics prevented the progression of OA by increasing the aggrecan deposition in mice [89]. Similarly, miR-92a-3p level was also suppressed in human OA cartilage. miR-92a-3p positively contributes to chondrogenesis

and causes aggrecan hyperacetylation by suppressing histone deacetylases 2(HDAC2) [90]. Thus, miRNA-aggrecan axis has a promising therapeutic potential for OA prevention and treatment.

Intervertebral disc degeneration

Similar to OA, dysregulation of ECM homeostasis, with a subsequent matrix degradation due to depletion of ECM macromolecule collagen type II and aggrecan, contributed to the intervertebral disc degeneration (IDD). Intervertebral discs are made up of annulus fibrosus (AF) and nucleus pulposus (NP), cartilage and end plates [91]. 50% of the wet weight of NP is composed of aggrecan, which helps in tissue hydration [92]. Role of miRNAs in cell proliferation, apoptosis regulation, ECM metabolism, and autophagy regulation suggests that miRNAs may serve as major regulators of IDD **(Table 4)**. The miR-21 and miR-210 promoted MMP-mediated aggrecan depletion by suppressing autophagy via targeting PTEN/Akt/mTOR signaling pathway [93] and autophagy-related gene 7 (ATG7) [94]**,** respectively. Additionally, miR-665 [95], miR-3150-3p [96], and miR-494 [91] also contribute to ECM catabolism and IDD progression by targeting aggrecan. In contrast, miR155 provides the therapeutic advantage against IDD via targeting MMP-16 [92].

Atherosclerosis

Atherosclerosis is another chronic inflammatory disease of the cardio-vascular system, which typically begins with the deposition of cholesterol-laden atherosclerotic plaques in the artery walls [97]. Recently, the scavenger receptor LOX-1, a transmembrane CLR (Group V) expressed on endothelial cells, vascular smooth muscles cells (VSMCs) and macrophages, has gained considerable attention in the pathogenesis of atherosclerosis [98,99]. Accumulated oxLDL in the neointima is internalized by LOX-1, which triggers endothelial dysfunction, pro-inflammatory responses, proliferation and migration of VSMCs, and formation of foamy macrophages, which ultimately contribute to progression of atherosclerosis **(Fig. 4)**. Several miRNAs play a key role in regulating LOX-1 expression. For instance, miRNA-98 [100], miR-30c-1-3p [101], miR-let family [102–104], miR-590-5p [105,106] serve as the therapeutic candidates for treating atherosclerosis by inversely regulating LOX-1. On the other hand, increased expression of cardiac miR-221 is associated with the induction of LOX-1-mediated inflammation and ROS production in the mice exposed to multi-walled carbon nanotubes (MWCNT) and thus, promoted cardiotoxicity [107]. Ox-LDL enhances the formation of c-JUN and NF-κB complex, which in turn, bind to miR-146a promoter and induce its transcription. Activated miR-146a inhibited the macrophage functional activity and was associated with the inflammatory blockage and may be relevant in atherosclerosis treatment [108]. In addition to lipid plaques, arterial stiffness also acts as a key factor for development of atherosclerosis. It was found that miR-1185

influenced arterial stiffness by promoting VCAM-1 and E-selectin expression in primary human umbilical vein endothelial cells (pHUVECs) and human umbilical vein smooth cells (HUVSMCs) [109] .

Others inflammatory diseases

Involvement of CLRs in other inflammatory diseases including pulmonary hypertension, cardiac fibrosis, and gut inflammation has also been assessed. For example, hypoxia results in suppressed expression of miR-let-7g via upregulation of LOX-1 in the pulmonary artery smooth muscle cells of rats, which is associated with the pulmonary hypertension (PH). Upregulated LOX-1 negatively regulated the miR-let-7g via a feedback regulation involving the calpin-OCT-1-PKC axis [110]. Similarly, cardiac fibrosis is associated with the downregulation of miR-367-3p and an enhanced expression of CD69 [111]. Lippai *et al*. showed miRNA-CLR axes was also involved in the alcohol-mediated gut inflammation. Acute and chronic alcohol consumption impair the intestinal barrier integrity via enhanced production of endotoxin and inflammatory mediators by dysregulating miRNA155-Reg3b balance in mice. Chronic alcohol consumption results in an increased miR-155 expression and a decreased Reg3b. miR-155 knock-out mice exhibited less endotoxin-mediated gut inflammation, suggesting miR-155 deficiency helps in maintaining the gut barrier integrity [24]. Furthermore, a recent paper demonstrated that miR-126-3p/-5p effectively suppressed VCAM-1 and E-selectin expression which improved blood-brain barrier integrity after ischemic stroke in lentivirus-miR-126-3p/-5p transduced mice [112]. It was also found that miR-150 directly targeted nuclear factor-κB1 (NF-κB1), which in turn, downregulated E-selectin and other pro-inflammatory genes in LPS-treated HUVECs. Thus, miR-150 could be used as a biomarker for the diagnosis of sepsis [113].

Role of CLRs in modulating miRNA expression: Impact on anti-microbial immunity and immune homeostasis

Understanding a key role of CLRs in orchestrating host innate and adaptive immune responses against the microorganisms, based on *in vitro* and *in vivo* studies, laid a foundation for translational applications of CLRs. Interestingly, initiation of the CLR signaling cascade can drive regulation of miRNAs expression during infection and inflammation **(Table 5).**

CLRs act as immune boosters against the fungal infections [114,115]. For instance, dendritic cell-associated C-type lectin 1 (Dectin 1) can recognize cell wall β-glucan structure of *C. albicans*. Moreover, different morphological forms (yeast or hyphae) of Candida affect miRNAs expression that subsequently impacts upon the host immunity. Furthermore, increased expression of β-glucan in Candida hyphal form, sensed by dectin 1 on bone marrow-derived macrophages (BMDMs), triggers miR-155 response in a SYK-dependent signaling pathway in mice [116]. To avoid extravagant inflammatory response against the

Candida infection, dectin 1 also upregulates miR-146, which acts as a negative feedback regulator of dectin 1-mediated pro-inflammatory signaling by inhibiting the NF-κB pathway in human monocyte leukemic cell line, THP-1 [117]. Interestingly, the use of miRNA-CLR axes as a potential biomarker for pulmonary tuberculosis (PTB) was first demonstrated by Wang et al. in 2018. Screening urine proteomic profiles from healthy controls and PTB affected patients revealed the usefulness of a combination of miR625-3p, MBL-2 and 35-kDa fragment of inter-α-trypsin inhibitor H4 (ITIH4-35k) as urinary diagnostic biomarkers for PTB [118].

Activation of CLR signaling regulates miRNAs expression which ultimately affects the immune homeostasis. For example, CD69 plays a potential role in the development and homeostasis of regulatory T cells in mice. Activation of the CD69 signaling cascade promotes miR-155 transcription, which eventually promotes immunosuppressive thymic regulatory T cell development [76]. Similarly, $KLRG1^{+}$ memory T cells restricts the expression of miR-101 and promotes an immunosuppressive microenvironment [68].

Impact of polymorphisms on miRNA-CLR interaction

Functional polymorphisms in the 3'UTRs of several mRNAs have been reported to overlap with miRNA seed sequences, which eventually interfere with the miRNA binding with target mRNA, and alter post-transcriptional regulation of gene expression [119]. For instance, the 3'UTR of OLR1 gene (encoding LOX-1 protein) is found to be naturally mutated by rs1050286 single nucleotide polymorphism (SNP) and led to disruption of miR-24-mediated regulation of OLR1 expression. Furthermore, the suppression of OLR1 at both RNA and protein level in the heterozygous (A/G) HeLa, but not in the homozygous (A/A) HepG2 cell lines indicates rs1050286 SNP affected miR-24 ligation to *OLR1*, thus, limiting miR-24-mediated OLR-1 degradation in the presence of G allele [120]. Similarly, the heterozygous genotype (A/G) of the KLRG1 rs1805672 polymorphism was found to disrupt the miR-584-5p binding with 3′UTR of KLRG1, leading to a differential accumulation of KLRG1 mRNA, and progression of the autoimmune disease pemphigus foliaceus, a chronic blistering skin condition caused by IgG autoantibodies against keratinocyte adhesion molecules [121]. Moreover, the SNP, substituting methionine (Met) for a threonine (Thr) in the amino-terminal side of SP-D protein affects the oligomeric organization and functionality of the protein. Thr SP-D mice and Met SP-D mice subjected to OVA challenge revealed that inflammation severity is not significantly affected by this SNP but by the differential regulation of miR-21 and miR-155 expression, suggesting that polymorphism regulates miRNA-CLR interaction [122].

Concluding remarks

CLRs are extensively involved in a broad range of mammalian cellular processes and miRNAs are found to be one of the crucial regulators of CLR signaling. Also, there are some evidences of CLRs regulating miRNA levels. In this review, we have summarized the nature of miRNA-CLR interaction in maintaining developmental and physiological homeostasis, host immunity and their involvement in cancer, infection and inflammatory diseases. The next challenge is to explore the underlying complex mechanisms behind these integrated networks of interactions and, more importantly, to determine whether CLR-miRNA axes have a promising therapeutic implication for limiting cancer, infections, or inflammatory diseases. The development of miRNA-based therapeutics is still in their infancy. Recently, MesomiR-1 (a miR-16 mimic) (clinicaltrials.gov identifier NCT02369198), first candidate of human trials with newer "TargomiR" technology, has completed its phase-1 trial in malignant pleural mesothelioma and non-small cell lung cancer. It has shown an encouraging potential of TargomiR (delivery system made up of miRNA mimic, bacterially derived minicells, and antibody that targets a specific protein on target cells). In addition, Miravisen (anti-miR-122) (clinicaltrials.gov identifier NCT02508090) has completed its Phase 2 trial for hepatitis C virus infection with infrequent and mostly mild adverse events, long-lasting suppression of viral load, no effect on other miRNAs expression in plasma, and was well tolerated in patients [123,124]. Cobomarsen or MRG-106 (anti-miR-155) (clinicaltrials.gov identifier NCT03713320) has entered phase 2 trial for treating mycosis fungoides (a type of cutaneous T-cell lymphoma) with sustainable improvement in quality of life and lesion burden, no serious adverse events reported in the first trial, and no evidence of immunosuppression over the course of almost two years [125]. Therefore, miRNA therapeutics will arguably continue to evolve and need to be explored for CLR regulation in various disease conditions [26].

Author Contributions

KG prepared the first draft and incorporated the suggestions provided by UK and TM.

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References

1 Chen SN, Zou PF and Nie P (2017) Retinoic acid-inducible gene I (RIG-I)-like receptors (RLR s) in fish: current knowledge and future perspectives. *Immunology* **151**, 16–25.

2 Feerick CL and McKernan DP (2017) Understanding the regulation of pattern recognition receptors in inflammatory diseases–a 'Nod'in the right direction. *Immunology* **150**, 237–247.

3 Zelensky AN, Gready JE (2005) The C-type lectin-like domain superfamily. *FEBS J* **272**, 6179–6217.

4 García-Vallejo JJ. and Van Kooyk Y (2009) Endogenous ligands for C-type lectin receptors: The true regulators of immune homeostasis. *Immunol Rev* **230**, 22–37.

5 Brown GD, Willment JA and Whitehead L (2018) C-type lectins in immunity and homeostasis. *Nat Rev Immunol* **18**, 374–389.

6 Borsig L (2018) Selectins in cancer immunity. *Glycobiology* **28**, 648–655.

7 Bayraktar R and Calin GA (2019) The interaction between two worlds: MicroRNAs and Toll-like receptors. *Front Immunol* **10**, 1053.

8 Dambuza IM. and Brown GD (2015) C-type lectins in immunity: Recent developments. *Curr Opin Immunol* **32**, 21–27.

9 Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S and e Sousa CR (2006) Myeloid C-type lectins in innate immunity. *Nat Immunol* **7**, 1258–1265.

10 Willcocks S, Offord V, Seyfert H, Coffey TJ & Werling D (2013) Species-specific PAMP recognition by TLR2 and evidence for species-restricted interaction with Dectin-1. *J Leukoc Biol* **94**, 449–458.

11 Sorensen GL (2018) Surfactant protein D in respiratory and non-respiratory diseases. *Front Med* **5**, 18.

12 Wright JR (2005) Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* **5**, 58–68.

13 Wight T & Kang I, Evanko S et al. (2020) Versican—A Critical Extracellular Matrix Regulator of Immunity and Inflammation. *Front Immunol* **11**, 1–12.

14 Kerscher B, Willment JA and Brown GD (2013) The Dectin-2 family of C-type lectin-like receptors: An update. *Int Immunol* **25**, 271–277.

15 Rajaram MVS, Arnett E, Azad AK, Guirado E, Ni B, Gerberick AD, He LZ, Keler T, Thomas LJ, Lafuse WP & Schlesinger LS (2017) M. tuberculosis-Initiated Human Mannose Receptor Signaling Regulates Macrophage Recognition and Vesicle Trafficking by FcRγ-Chain, Grb2, and SHP-1. *Cell Rep* **21**, 126–140.

16 Zhao X, Guo Y, Jiang C et al. (2017) JNK1 negatively controls antifungal innate immunity by suppressing CD23 expression. *Nat Med* **23**, 337–346.

17 Zhu LL, Luo TM, Xu X et al. (2016) E3 ubiquitin ligase Cbl-b negatively regulates C-type lectin receptor-mediated antifungal innate immunity. *J Exp Med* **213**, 1555–1570.

18 O'neill LA, Sheedy FJ & McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* **11**, 163–175.

19 Cui SY, Wang R and Chen LB (2014) MicroRNA-145: A potent tumour suppressor that regulates multiple cellular pathways. *J Cell Mol Med* **18**, 1913–1926.

20 Ha M & Kim VN (2014) Regulation of microRNA biogenesis. *Nat Rev Mol cell Biol* **15**, 509–524.

21 Zhang Y and Li Y (2013) Regulation of innate receptor pathways by microRNAs. *Sci China Life Sci* **56**, 13–18.

22 Chandan K, Gupta M and Sarwat M (2020) Role of Host and Pathogen-Derived MicroRNAs in Immune Regulation During Infectious and Inflammatory Diseases. *Front Immunol* **10**, 3081.

23 Salem, M., O'Brien, J. A., Bernaudo S et al. (2018) MiR-590-3p promotes ovarian cancer growth and metastasis via a novel FOXA2-versican pathway. *Cancer Res* **78**, 4175–4190.

24 Lippai D, Bala S, Catalano D, Kodys K and Szabo G (2014) Micro-RNA-155 Deficiency Prevents Alcohol-Induced Serum Endotoxin Increase and Small Bowel Inflammation in Mice. *Alcohol Clin Exp Res* **38**, 2217–2224.

25 Michlewski G & Cáceres JF (2019) Post-transcriptional control of miRNA biogenesis. *Rna* **25**, 1–16.

26 Hanna J, Hossain GS and Kocerha J (2019) The potential for microRNA therapeutics and clinical research. *Front Genet* **10**, 478.

27 Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, Bernal AL, Reid KBM, Madan T & Chakraborty T (2006) Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol Immunol* **43**, 1293–1315.

28 Vieira F, Kung JW & Bhatti F (2017) Structure, genetics and function of the pulmonary associated surfactant proteins A and D: The extra-pulmonary role of these C type lectins. *Ann Anatomy-Anatomischer Anzeiger* **211**, 184–201.

29 Gardai SJ, Xiao Y-Q, Dickinson M, Nick JA, Voelker DR, Greene KE & Henson PM (2003) By binding SIRPα or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* **115**, 13–23.

30 Silveyra P, Chroneos ZC, DiAngelo SL et al. (2014) Knockdown of Drosha in human alveolar type II cells alters expression of SP-A in culture: a pilot study. *Exp Lung Res* **40**, 354–366.

31 Benlhabib H, Guo W, Pierce B.M. and Mendelson CR (2015) The miR-200 family and its targets regulate type II cell differentiation in human fetal lung. *J Biol Chem* **290**, 22409–22422.

32 Guo W, Benlhabib H and Mendelson CR (2016) The MicroRNA 29 Family Promotes Type II Cell Differentiation in Developing Lung. *Mol Cell Biol* **36**, 2141–2141.

33 Gong Y, Xu W, Chen Y et al. (2019) miR-20a-5p regulates pulmonary surfactant gene expression in alveolar type II cells. *J Cell Mol Med* **23**, 7664–7672.

34 Li Z, Jiang W, Wu G, Ju X, Wang Y and Liu W (2018) miR-16 inhibits hyperoxia-induced cell apoptosis in human alveolar epithelial cells. *Mol Med Rep* **17**, 5950–5957.

35 Li S, Sun Z, Chen T et al. (2019) The role of miR-431-5p in regulating pulmonary surfactant expression in vitro. *Cell Mol Biol Lett* **24**, 25.

36 Mishra R, Benlhabib H, Guo W, Cervantes CBL and Mendelson CR (2018) Developmental decline in the microRNA 199a (miR-199a)/miR-214 cluster in human fetal lung promotes type ii cell differentiation by upregulating key transcription factors. *Mol Cell Biol* **38**.

37 Zhang XQ, Zhang P, Yang Y, Qiu J, Kan Q, Liang HL, Zhou XY and Zhou XG (2014) Regulation of Pulmonary Surfactant Synthesis in Fetal Rat Type II Alveolar Epithelial Cells by MicroRNA-26a. *Pediatr Pulmonol* **49**, 863–872.

38 Sun YF, Kan Q, Yang Y et al. (2018) Knockout of microRNA-26a promotes lung development and pulmonary surfactant synthesis. *Mol Med Rep* **17**, 5988–5995.

39 Zhang YH, Wu LZ, Liang HL et al. (2017) Pulmonary surfactant synthesis in miRNA-26a-1/miRNA-26a-2 double knockout mice generated using the CRISPR/Cas9 system. *Am J Transl Res* **9**, 355.

40 Zhang H, Guo Y, Mishra A, Gou D, Chintagari NR and Liu L (2015) MicroRNA-206 regulates surfactant secretion by targeting VAMP-2. *FEBS Lett* **589**, 172–176.

41 Noutsios GT, Thorenoor N, Zhang X, Phelps DS, Umstead TM, Durrani F and Floros J (2017) SP-A2 contributes to miRNA-mediated sex differences in response to oxidative stress : anti-oxidant pathways are involved. *Biol Sex Differ* **8**, 37.

42 Wight TN (2017) Provisional matrix: A role for versican and hyaluronan. *Matrix Biol* **60**, 38–56.

43 Pan B and Li J (2018) MicroRNA-21 up-regulates metalloprotease by down-regulating TIMP3 during cumulus cell-oocyte complex in vitro maturation. *Mol Cell Endocrinol*

477, 29–38.

44 Yang W and Yee A (2014) Versican 3'-untranslated region (3'UTR) promotes dermal wound repair and fibroblast migration by regulating miRNA activity. *Biochim Biophys Acta (BBA)-Molecular Cell Res* **1843**, 1373–1385.

45 Hodax JK, Quintos JB, Gruppuso PA, Chen Q, Desai S & Jayasuriya CT (2019) Aggrecan is required for chondrocyte differentiation in ATDC5 chondroprogenitor cells. *PLoS One* **14**.

46 Karlsen TA, Jakobsen RB, Mikkelsen TS and Brinchmann JE (2014) MicroRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9 and ACAN. *Stem Cells Dev* **23**, 290–304.

47 Hou C, Zhang Z, Zhang Z et al. (2015) Presence and function of microRNA-92a in chondrogenic ATDC5 and adipose-derived mesenchymal stem cells. *Mol Med Rep* **12**, 4877–4886.

48 Wu TJ, Fong YC, Lin CY, Huang YL and Tang CH (2018) Glucose enhances aggrecan expression in chondrocytes via the PKCα/p38-miR141-3p signaling pathway. *J Cell Physiol* **233**, 6878–6887.

49 Tian J, Rui YJ, Xu YJ & Zhang SA (2018) MiR-143-3p regulates early cartilage differentiation of BMSCs and promotes cartilage damage repair through targeting BMPR2. *Eur Rev Med Pharmacol Sci* **22**, 8814–8821.

50 De Caro A, Lohse J & Sarles H (1979) Characterization of a protein isolated from pancreatic calculi of men suffering from chronic calcifying pancreatitis. *Biochem Biophys Res Commun* **87**, 1176–1182.

51 Chen Z, Downing S & Tzanakakis ES (2019) Four Decades After the Discovery of Regenerating Islet-Derived (Reg) Proteins: Current Understanding and Challenges. *Front cell Dev Biol* **7**, 235.

52 Downing S, Zhang F, Chen Z and Tzanakakis ES (2019) MicroRNA-7 directly targets reg1 in pancreatic cells. *Am J Physiol - Cell Physiol* **317**, C366–C374.

53 Kattoor AJ, Goel A & Mehta JL (2019) LOX-1: Regulation, Signaling and Its Role in Atherosclerosis. *Antioxidants* **8**, 218.

54 Li HF, Wang SH, Guo Y, Zhao HB, Li XY & Wang X (2017) Identification of the interaction between bta-miR-370 and OLR1 gene in bovine adipocyte. *Anim Genet* **48**, 455–458.

55 Rupaimoole R and Slack FJ (2017) MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* **16**, 203.

56 Rupaimoole R, Calin GA, Lopez-Berestein G and Sood AK (2016) miRNA deregulation

in cancer cells and the tumor microenvironment. *Cancer Discov* **6**, 235–246.

57 Wei T, Cong X, Wang XT et al (2017) Interleukin-17A promotes tongue squamous cell carcinoma metastasis through activating miR-23b/versican pathway. *Oncotarget* **8**, 6663–6680.

58 Li F, Li S and Cheng T (2014) TGF-β1 Promotes Osteosarcoma Cell Migration and Invasion Through the miR- 143-Versican Pathway. *Cell Physiol Biochem* **34**, 2169–2179.

59 Zhao X, Sun Z, Li H, Jiang F, Zhou J and Zhang L (2017) MiR-135a-5p modulates biological functions of thyroid carcinoma cells via targeting VCAN 3′-UTR. *Cancer Biomarkers* **20**, 207–216.

60 Gupta N, Kumar R, Seth T, Garg B, Sati HC and Sharma A (2019) Clinical significance of circulatory microRNA-203 in serum as novel potential diagnostic marker for multiple myeloma. *J Cancer Res Clin Oncol* **145**, 1601–1611.

61 Duan Y, Hu L, Liu B et al. (2014) Tumor suppressor miR-24 restrains gastric cancer progression by downregulating RegIV. *Mol Cancer* **13**, 127.

62 Kawasaki Y & Matsumura K, Miyamoto M et al. (2015) REG4 is a transcriptional target of GATA6 and is essential for colorectal tumorigenesis. *Sci Rep* **5**, 1–10.

63 Xu CY, Dong JF, Chen ZQ, Ding GS. and Fu ZR (2019) MiR-942-3p Promotes the Proliferation and Invasion of Hepatocellular Carcinoma Cells by Targeting MBL2. *Cancer Control* **26**, 1073274819846593.

64 Zhong L, Huot J & Simard MJ (2018) p38 activation induces production of miR-146a and miR-31 to repress E-selectin expression and inhibit transendothelial migration of colon cancer cells. *Sci Rep* **8**, 1–13.

65 Wei Y, Shao J, Wang Y, Shen H, Yu S, Zhang J & Yin L (2019) Hsa-miR-370 inhibited P-selectin-induced cell adhesion in human colon adenocarcinoma cells. *Mol Cell Biochem* **450**, 159–166.

66 Inoue D, Kitaura J, Togami K et al. (2013) Myelodysplastic syndromes are induced by histone methylation–altering ASXL1 mutations. *J Clin Invest* **123**, 4627–4640.

67 Lambert M, Alioui M, Jambon S, Depauw S, Seuningen I Van & David-Cordonnier M-H (2019) Direct and Indirect Targeting of HOXA9 Transcription Factor in Acute Myeloid Leukemia. *Cancers (Basel)* **11**, 837.

68 Li L, Wan S, Tao K, Wang G and Zhao E (2016) KLRG1 restricts memory T cell antitumor immunity. *Oncotarget* **7**, 61670–61678.

69 Bermejo-Jambrina M, Eder J, Helgers LC, Hertoghs N, Nijmeijer BM, Stunnenberg M and Geijtenbeek TB (2018) C-type lectin receptors in antiviral immunity and viral

escape. *Front Immunol* **9**, 590.

70 Heinsbroek SEM, Squadrito ML, Schilderink R, Hilbers FW, Verseijden C, Hofmann M, Helmke A, Boon L, Wildenberg ME & Roelofs JJTH (2016) miR-511-3p, embedded in the macrophage mannose receptor gene, contributes to intestinal inflammation. *Mucosal Immunol* **9**, 960–973.

71 Rajaram MVS, Brooks MN, Morris JD, Torrelles JB, Azad AK & Schlesinger LS (2010) Mycobacterium tuberculosis activates human macrophage peroxisome proliferator-activated receptor γ linking mannose receptor recognition to regulation of immune responses. *J Immunol* **185**, 929–942.

72 Awuah D, Alobaid M, Latif A, Salazar F, Emes RD and Ghaemmaghami AM (2019) The Cross-Talk between miR-511-3p and C-Type Lectin Receptors on Dendritic Cells Affects Dendritic Cell Function. *J Immunol* **203**, 48–157.

73 Wu J, Zhang H, Zheng Y et al. (2018) The Long Noncoding RNA MALAT1 Induces Tolerogenic Dendritic Cells and Regulatory T Cells via miR155 / Dendritic Cell-Specific Intercellular Adhesion Molecule-3 Grabbing Nonintegrin / IL10 Axis. *Front Immunol* **9**, 1847.

74 Yang Y, Wu, BQ, Wang YH et al. (2016) Regulatory effects of miR-155 and miR-146a on repolarization and inflammatory cytokine secretion in human alveolar macrophages in vitro. *Immunopharmacol Immunotoxicol* **38**, 502–509.

75 Zhou Y, Do DC, Ishmael FT et al. (2018) Mannose receptor modulates macrophage polarization and allergic inflammation through miR-511-3p. *J Allergy Clin Immunol* **141**, 350–364.

76 Sánchez-Díaz R, Blanco-Dominguez R, Lasarte S et al. (2017) Thymus-Derived Regulatory T Cell Development Is Regulated by C-Type Lectin-Mediated BIC/MicroRNA 155 Expression. *Mol Cell Biol* **37**, e00341-16.

77 Hu L, Xu H, Lu J et al. (2018) MicroRNA-126 Deficiency Affects the Development of Thymus CD4 + Single- Positive Cells through Elevating IRS-1. *Int Arch Allergy Immunol* **3**, 207–218.

78 Chiffoleau E (2018) C-Type Lectin-Like Receptors As emerging Orchestrators of Sterile inflammation Represent Potential Therapeutic Targets. *Front Immunol* **9**, 227.

79 Rebane A (2015) microRNA and Allergy. In *microRNA: Medical Evidence* pp. 331–352. Springer.

80 Szymczak I, Wieczfinska J & Pawliczak R (2016) Molecular background of miRNA role in asthma and COPD: an updated insight. *Biomed Res Int* **2016**.

81 Miller RE, Ishihara S, Tran PB, Golub SB, Last K, Miller RJ, Fosang AJ & Malfait A-M (2018) An aggrecan fragment drives osteoarthritis pain through Toll-like receptor 2. *JCI*

insight **3**.

82 Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier J-P & Fahmi H (2011) Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* **7**, 33.

83 Xu R, Li J, Wei B, Huo W and Wang L (2017) MicroRNA-483-5p modulates the expression of cartilage-related genes in human chondrocytes through down-regulating TGF-β1 expression. *Tohoku J Exp Med* **243**, 41–48.

84 Chang T, Xie J, Li H, Li D, Liu P and Hu Y (2016) MicroRNA-30a promotes extracellular matrix degradation in articular cartilage via downregulation of Sox9. *Cell Prolif* **49**, 207–218.

85 Ni Z, Shang X, Tang G, Niu L (2018) Expression of miR-206 in Human Knee Articular Chondrocytes and Effects of miR-206 on Proliferation and Apoptosis of Articular Chondrocytes. *Am J Med Sci* **355**, 240–246.

86 He J, Zhang J and Wang D (2017) Down-regulation of microRNA-216b inhibits IL-1β-induced chondrocyte injury by up-regulation of Smad3. *Biosci Rep* **37**.

87 Yang H, Wu D, Li H, Chen N and Shang Y (2018) Downregulation of microRNA-448 inhibits IL-1β-induced cartilage degradation in human chondrocytes via upregulation of matrilin-3. *Cell Mol Biol Lett* **23**, 7.

88 Wang Y, Yang T, Liu Y, Zhao W, Zhang Z, Lu M & Zhang W (2017) Decrease of miR-195 promotes chondrocytes proliferation and maintenance of chondrogenic phenotype via targeting FGF-18 pathway. *Int J Mol Sci* **18**, 1–18.

89 Zhang B, Sun M, Wang J et al. (2019) Pathology - Research and Practice MiR-671 ameliorates the progression of osteoarthritis in vitro and in vivo. *Pathol - Res Pract* **215**, 152423.

90 Mao G, Zhang Z, Huang Z, Chen W, Huang G, Meng F, Zhang Z & Kang Y (2017) MicroRNA-92a-3p regulates the expression of cartilage-specific genes by directly targeting histone deacetylase 2 in chondrogenesis and degradation. *Osteoarthr Cartil* **25**, 521–532.

91 Kang L, Yang C, Song Y et al. (2017) MicroRNA-494 promotes apoptosis and extracellular matrix degradation in degenerative human nucleus pulposus cells. *Oncotarget* **8**, 27868–27881.

92 Zhang WL, Chen YF, Meng HZ et al. (2017) Role of miR-155 in the regulation of MMP-16 expression in intervertebral disc degeneration. *J Orthop Res* **35**, 1323–1334.

93 Wang W, Yang W & Ouyang ZH et al. (2018) MiR-21 promotes ECM degradation through inhibiting autophagy via the PTEN/akt/mTOR signaling pathway in human degenerated NP cells. *Biomed Pharmacother* **99**, 725–734.

94 Wang C, Zhang ZZ, Yang W et al. (2017) MiR-210 facilitates ECM degradation by suppressing autophagy via silencing of ATG7 in human degenerated NP cells. *Biomed Pharmacother* **93**, 470–479.

95 Tan H, Zhao L, Song R, Liu Y and Wang L (2018) microRNA-665 promotes the proliferation and matrix degradation of nucleus pulposus through targeting GDF5 in intervertebral disc degeneration. *J Cell Biochem* **119**, 7218–7225.

96 Zhang B, Guo W, Sun C et al. (2018) Dysregulated miR-3150a-3p Promotes Lumbar Intervertebral Disc Degeneration by Targeting Aggrecan. *Cell Physiol Biochem* **45**, 2506–2515.

97 Schaftenaar F, Frodermann V, Kuiper J and Lutgens E (2016) Atherosclerosis: the interplay between lipids and immune cells. *Curr Opin Lipidol* **27**, 209–215.

98 Kattoor AJ, Goel A and Mehta JL (2019) LOX-1: Regulation, Signaling and Its Role in Atherosclerosis. *Antioxidants* **8**, 218.

99 Ulrich-Merzenich G and Zeitler H (2013) The lectin-like oxidized low-density lipoprotein receptor-1 as therapeutic target for atherosclerosis, inflammatory conditions and longevity. *Expert Opin Ther Targets* **17**, 905–919.

100 Dai Y, Wu X, Dai D, Li J and Mehta JL (2018) MicroRNA-98 regulates foam cell formation and lipid accumulation through repression of LOX-1. *Redox Biol* **16**, 255–262.

101 Li X, Feng S, Luo Y et al. (2018) Expression profiles of microRNAs in oxidized low-density. *Vitr Cell Dev Biol* **54**, 99–110.

102 Bao MH, Zhang YW, Lou XY, Cheng Y and Zhou HH (2014) Protective Effects of Let-7a and Let-7b on Oxidized Low- Density Lipoprotein Induced Endothelial Cell Injuries. *PLoS One* **9**, e106540.

103 Hsu PY, Lin WY, Lin RT and Juo SHH (2018) MicroRNA let-7g inhibits angiotensin II-induced endothelial senescence via the LOX-1-independent mechanism. *Int J Mol Med* **41**, 2243–2251.

104 Liu M, Tao G, Liu Q, Liu K and Yang X (2017) MicroRNA let-7g alleviates atherosclerosis via the targeting of LOX-1 in vitro and in vivo. *Int J Mol Med* **40**, 57–64.

105 Dai Y, Zhang Z, Cao Y, Mehta JL and Li J (2016) MiR-590-5p Inhibits Oxidized-LDL Induced Angiogenesis by Targeting LOX-1. *Sci Rep* **6**, 1–9.

106 Luo P, Zhang WF, Qian ZX et al. (2016) MiR-590-5p-meidated LOX-1 upregulation promotes Angiotensin II- induced endothelial cell apoptosis. *Biochem Biophys Res Commun* **471**, 402–408.

107 Davis G, Lucero J, Fellers C, McDonald JD and Lund AK (2018) The Effects of Subacute Inhaled Multi-Walled Carbon Nanotube Exposure on Signaling Pathways Associated with Cholesterol Transport and Inflammatory Markers in the Vasculature ofWild-Type Mice. *Toxicol Lett* **296**, 48–62.

108 Li Z, Wang S, Zhao W, Sun Z, Yan H and Zhu J (2016) Oxidized low-density lipoprotein upregulates microRNA-146a via JNK and NF-κB signaling. *Mol Med Rep* **13**, 1709–1716.

109 Deng H, Song Z, Xu H, Deng X, Zhang Q, Chen H, Wang Y, Qin Y & Li Y (2017) MicroRNA-1185 promotes arterial stiffness though modulating VCAM-1 and E-selectin expression. *Cell Physiol Biochem* **41**, 2183–2193.

110 Zhang WF, Zhu TT, Xiong YW, Xiong AZ, Ge XY, Hu CP and Zhang Z (2017) Negative feedback regulation between microRNA let-7g and LOX-1 mediated hypoxia-induced PASMCs proliferation. *Biochem Biophys Res Commun* **488**, 655–663.

111 Hu H, Li J, Zhang J (2018) Dysregulation of CD69 by overexpression of microRNA - 367 - 3p associated with post - myocardial infarction cardiac fibrosis. *Mol Med Rep* **18**, 3085–3092.

112 Pan J, Qu M, Li Y, Wang L, Zhang L, Wang Y, Tang Y, Tian H-L, Zhang Z & Yang G-Y (2020) MicroRNA-126-3p/-5p Overexpression Attenuates Blood-Brain Barrier Disruption in a Mouse Model of Middle Cerebral Artery Occlusion. *Stroke* **51**, 619–627.

113 Ma Y, Liu Y, Hou H, Yao Y & Meng H (2018) MiR-150 predicts survival in patients with sepsis and inhibits LPS-induced inflammatory factors and apoptosis by targeting NF-κB1 in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* **500**, 828–837.

114 Hou H, Guo Y, Chang Q, Luo T, Wu X and Zhao X (2017) C-type lectin receptor: old friend and new player. *Med Chem (Los Angeles)* **13**, 536–543.

115 Tang J, Lin G, Langdon WY, Tao L and Zhang J (2018) Regulation of C-type lectin receptor-mediated antifungal immunity. *Front Immunol* **9**, 123.

116 Agustinho DP, de Oliveira MA, Tavares AH et al. (2017) Dectin-1 is required for miR155 upregulation in murine macrophages in response to Candida albicans. *Virulence* **8**, 41–52.

117 Du L, Chen X, Duan Z, Liu C, Zeng R, Chen Q and Li M (2017) MiR-146a negatively regulates dectin-1-induced inflammatory responses. *Oncotarget* **8**, 37355.

118 Wang J, Zhu X, Xiong X et al. (2018) Identification of potential urine proteins and microRNA biomarkers for the diagnosis of pulmonary tuberculosis patients. *Emerg Microbes Infect* **7**, 63.

119 Saunders MA, Liang H and Li WH (2007) Human polymorphism at microRNAs and microRNA target sites. *Proc Natl Acad Sci* **104**, 3300–3305.

120 Morini E, Rizzacasa B & Pucci S et al. (2016) The human rs1050286 polymorphism alters LOX-1 expression through modifying miR-24 binding. *J Cell Mol Med* **20**, 181–187.

121 Cipolla GA, Park JK, de Oliveira LA et al. (2016) A 3′UTR polymorphism marks differential KLRG1 mRNA levels through disruption of a miR-584-5p binding site and associates with pemphigus foliaceus susceptibility. *Biochim Biophys Acta (BBA)- Gene Regul Mech* **1859**, 1306–1313.

122 Winkler C, Bahlmann O & Viereck J et al. (2014) Impact of a Met(11)Thr single nucleotide polymorphism of surfactant protein D on allergic airway inflammation in a murine asthma model. *Exp Lung Res* **40**, 154–163.

123 Lindow M & Kauppinen S (2012) Discovering the first microRNA-targeted drug.

124 Van der Ree MH, Van Der Meer AJ, Van Nuenen AC, De Bruijne J, Ottosen S, Janssen HL, Kootstra NA & Reesink HW (2016) Miravirsen dosing in chronic hepatitis C patients results in decreased micro RNA-122 levels without affecting other micro RNA s in plasma. *Aliment Pharmacol Ther* **43**, 102–113.

125 Witten L & Slack FJ (2020) miR-155 as a novel clinical target for hematological malignancies. *Carcinogenesis* **41**, 2–7.

126 Kawahara Y & Mieda-Sato A (2012) TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proc Natl Acad Sci* **109**, 3347–3352.

127 Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A, Gherzi R & Rosenfeld MG (2009) The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* **459**, 1010–1014.

128 Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S & Miyazono K (2009) Modulation of microRNA processing by p53. *Nature* **460**, 529–533.

129 Davis BN, Hilyard AC, Lagna G & Hata A (2008) SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* **454**, 56–61.

130 Guil S & Cáceres JF (2007) The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. *Nat Struct Mol Biol* **14**, 591–596.

131 Viswanathan SR, Daley GQ & Gregory RI (2008) Selective blockade of microRNA processing by Lin28. *Science (80-)* **320**, 97–100.

132 Heale BSE, Keegan LP, McGurk L, Michlewski G, Brindle J, Stanton CM, Caceres JF & O'connell MA (2009) Editing independent effects of ADARs on the miRNA/siRNA pathways. *EMBO J* **28**, 3145–3156.

133 Xhemalce B, Robson SC & Kouzarides T (2012) Human RNA methyltransferase BCDIN3D regulates microRNA processing. *Cell* **151**, 278–288.

134 Suzuki HI, Arase M, Matsuyama H, Choi YL, Ueno T, Mano H, Sugimoto K & Miyazono K (2011) MCPIP1 ribonuclease antagonizes dicer and terminates microRNA biogenesis through precursor microRNA degradation. *Mol Cell* **44**, 424–436.

135 Upton J-P, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, McManus MT, Ruggero D & Goga A (2012) IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. *Science (80-)* **338**, 818–822.

136 Kawahara Y, Zinshteyn B, Chendrimada TP, Shiekhattar R & Nishikura K (2007) RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer–TRBP complex. *EMBO Rep* **8**, 763–769.

Tables

Table 1A. Transcriptional Regulators of miRNA biogenesis

Regulator	miRNA biogenesis step	Effect	Target miRNAs
p53	miRNA transcription by RNA pol II	Transactivate	miR-34 cluster
MYC	miRNA transcription by RNA pol II	Transactivate	miR-17 cluster
Myoblast determination protein 1 (MYOD1)	miRNA transcription by RNA pol II	Transactivate	miR-1 cluster
MYC	miRNA transcription by RNA pol II	Suppress	miR-15a cluster
ZEB1 and ZEB2	miRNA transcription by RNA pol II	Suppress	miR-200 cluster

Table 1B. Post-transcriptional regulators (RNA-binding Proteins) of miRNA biogenesis

Regulator	miRNA biogenesis step	Effect	Mechanism	Target miRNAs	Study Model	Ref.
TDP-43	Nuclear processing event	Stabilization and increased processing	Interacts with Drosha	miR-132, miR-143, miR-558-3p, miR-574-3p	HeLa cells, HEK293T cells, Neuro2a cells	[126]
KSRP	Nuclear processing event	Positively regulates pri-miRNA processing	Interacts with Drosha	let-7, miR-21, miR-16	HeLa, U2OS, P19 or NIH-3T3 cells	[127]
P53	Nuclear processing event	Positively regulates pri-miRNA processing	Binds to p68 and promotes Drosha cleavage	miR-16-1, miR-143, miR-145	HCT116, WI-38, TIG-3, TOV21G cells	[128]
SMADs	Nuclear processing event	Positively regulates pri-miRNA processing	Binds to p68 and promotes Drosha cleavage	miR-21, miR-199a	C3H10T1/2, Cos7, MCF7, MDA-MB-468, and MDA-MB-231 cells	[129]
HNRNPA1	Nuclear processing event	Positively regulates pri-miRNA processing	Promotes Drosha cleavage by restructuring pri-miRNA.	miR-18a	HeLa cells	[130]
LIN28	Nuclear processing event	Negatively regulates pri-miRNA processing	Binds let-7 TL and blocks Drosha processing.	let-7	Murine P19 cells, human 293T cells	[131]

ADAR1, 2	Nuclear processing event	Negatively regulate pri-miRNA processing	Adenosine to inosine editing in stem interferes with Drosha cleavage.	miR-142	HEK 293T cells, Drosophila	[132]
KSRP	Cytoplasmic processing event	Positively regulates Dicer processing	Unknown	let-7, miR-16, miR-21,	HeLa, U2OS, P19 or NIH-3T3 cells	[127]
BCDIN3D	Cytoplasmic processing event	Negatively regulates Dicer processing	Methylates monophosphate ends of pre-miRNAs leading to inhibition of Dicer Processing	miR-145, miR-26b	Human MDA-MB-23, MCF7, and BJ+TERT cells	[133]
MCPIP1	Cytoplasmic processing event	Negatively regulates Dicer processing	Decreases RNA stability	miR-146a, miR-135b	HEK293T, Jurkat, HepG2 and THP-1 cells	[134]
Ser/Thr protein kinase/ endoribonuclease IRE1α	Cytoplasmic processing event	Inhibit Dicer processing	Unknown	miR-17, miR34a, miR-96, miR-125b	T-REx-293 cells	[135]
ADAR1	Cytoplasmic processing event	Inhibit Dicer processing	Adenosine to inosine editing in the stem leads to inhibition of Dicer cleavage	miR-151	ADAR2$^{-/-}$ mice, HeLa and HEK293T cells	[136]

TDP43, TAR DNA-binding protein 43; KSRP, KH-type splicing regulatory protein; SMADs, Mothers against decapentaplegic homolog; p53, Tumor protein P53; HNRNPA1, Heterogeneous nuclear ribonucleoprotein A1; LIN28, Protein lin-28 homolog; ADAR1,2, adenosine deaminase acting on RNA enzymes; BDCIN3D, BCDIN3 domain containing RNA methyltransferase; MCPIP1, MCP-induced protein 1

Table 2: Role of miRNAs on pulmonary surfactant proteins

miRNA	Target/Pathway	Study model	Function	Ref.
miR-20a-5p	Surfactant proteins (PTEN)	Blood sample of premature infants with and without RDS; rat fetal alveolar type II cells	Overexpression of miR-20a-5p promotes surfactant proteins expression via modulating PTEN expression	[33]
miR-431	Surfactant proteins (TGF- β /Smad4)	Human non-squamous lung carcinoma cell line A549	miR431 inhibit surfactant protein synthesis via targeting Smad4	[35]
miR-199a/miR-214	SP-A (NF-κB, COX-2, CREB1, and C/EBPβ)	Human Fetal Lung (HFL) explants and AECII	miR-199a/miR-214 expression increased AECII cell differentiation and SP-A expression	[36]
miR16	SP-A (TGF-β /Smad2 and JAK/STAT3)	Human AECII	Enhanced miR16 results in increased SP-A synthesis via downregulating TGF-β/Smad2 and JAK/STAT3 pathway in hyperoxia-induced AECII	[34]
miR-29	SP-A (TGF- β2/SMAD2)	Human and murine fetal lung epithelial tissue	Increased expression of miR29 family promotes type II differentiation and SP-A synthesis by repressing TGF-β and ZEB1/2 signaling	[32]
miR-200	Surfactant proteins (TGF- β /ZEB1/2)	Isolated AECII cells from mid-gestational HFL explants and A549 cell line	miR-200 family target TGF-β and ZEB1 which in turn enhance lung epithelial cell differentiation and PS synthesis	[31]
miR-206	Surfactant proteins (VAMP2)	HEK293A cells; A549 cells; rat AECII	miR-206 targets VAMP-2 protein and restrict VAMP-2 availability for surfactant proteins synthesis	[40]
miR26a	SP-B, SP-C (SMAD1)	Fetal rat AECII	Overexpression of miR-26a inhibited SP-B, SP-C synthesis in AECII by suppressing SMAD1	[37]
miR26a	Surfactant proteins	miR-26a-1/miR-26a-2 double knockout mouse	miR-26a knockout mice showed matured lung formation and increased PS synthesis	[38,39]

SP, Surfactant proteins; PTEN, Phosphatase and tensin homolog; RDS, Respiratory distress syndrome; HFL, Human fetal lung; TGF- β, Transforming growth factor-beta; VAMP2, Vesicle-associated membrane protein 2; AECII, Alveolar type-II epithelial cells; ZEB1/2, Zinc finger E-box-binding homeobox 1/2

Table 3: The regulatory effect of CLR-miRNA axes in different type of cancers

miRNA	Target CLR	Cancer type	Study model	Ref.
miR-125a	Clec5A	Myelodysplastic syndromes (MDS)	Mice BMT model; murine myeloid cell lines 32Dcl3; whole BM from healthy (control) and from MDS patients with/without ASXL1 mutation	[66]
miR-942-3p	MBL2	Hepatocellular cancer (HCC)	HCC patient data; HCC cell lines including, Huh7, SMMC-7721, HepG2, LO2, SK-hep-1 and Huh6	[63]
miR-590	Versican	Epithelial ovarian cancer (EOC)	Plasma and tissue samples from EOC patients; human EOC cell lines including SKOV3.ip1, ES-2 and HEY; CD-1 nude murine tumor xenograft model	[23]
miR-23b	Versican	Tongue squamous cell carcinoma (TSCC)	Blood and tumor tissue from TSCC patients; TSCC cell line SCC15	[57]
miR-143	Versican	Osteosarcoma	Human osteosarcoma tumor tissue; osteosarcoma cell line MG63 and U2OS	[58]
miR-135a-5p	Versican	Thyroid carcinoma	Human thyroid carcinoma tissue; human thyroid carcinoma cell lines including TPC1, FTC-133 and K1, SW579, and normal thyroid cell-line, HT-ori3	[59]
miR-203	Versican	Multiple myeloma (MM)	Peripheral blood and BM aspirate of MM patients and healthy individuals (control)	[60]
miR-24	RegIV	Gastric cancer (GC)	Tumor samples from GC patients; human GC cell lines including, SNU-1 SNU-16, NCI-N87, KATO III, BGC-823, MKN-28, MKN-45, SGC-7901and AGS and normal gastric epithelial cell line (GES-1)	[61]
miR-363	REG4	Colorectal cancer	Human colorectal tumor samples; colon cancer cell line HT29	[62]
miR-101	KLRG1	Tumor microenvironment	Human tissues samples of colon cancer, colitis, ovarian cancer, traumatic colon patients; Peripheral blood from healthy individuals	[68]
miR-146a, miR181b, miR-31	E-selectin	Colon cancer	HUVECs and human colon cancer cell line HT29	[64]
miR-370	P-selectin	Colon cancer	Colo 320 human colon adenocarcinoma cells	[65]

Clec5A, C-type lectin domain family 5, member A; BMT, Bone marrow (BM) transplant; ASXL1, additional sex combs-like 1; MBL2, Mannose-binding lectin 2; RegIV, Regenerating islet-derived (Reg) proteins-IV; KLRG1, Killer cell lectin-like receptor subfamily G member 1; HUVECs, Human umbilical vein endothelial cell

Table 4: List of aggrecan-associated miRNAs in the regulation of Intervertebral disc degeneration (IDD) pathogenesis

miRNAs	Targets	Effect on Aggrecan	Study models	Regulatory role	Ref.
miR-21	PTEN	-	NP cells of NP tissue samples from lumbar vertebral fracture (LVF) patients (Control) and IDD patient	IDD pathogenesis (+)	[93]
miR-155	MMP-16	+	NP cells of NP tissue samples IDD patients and normal NP samples (control), mouse annular puncture model	IDD pathogenesis (-)	[92]
miR-665	GDF5	-	NP cells of NP tissues from IDD patients or scoliosis(control)	IDD pathogenesis (+)	[95]
MiR-3150a-3p	Aggrecan (direct)	-	NP cells from human lumbar degenerative NP tissue specimens of IDD patients and fresh traumatic lumbar fracture (control)	IDD pathogenesis (+)	[96]
MiR-210	ATG7	-	NP cells of Lumber NP tissue samples from lumbar vertebral fracture (LVF) patients (Control) and IDD patient	IDD pathogenesis (+)	[94]
miR-494	Sox9	-	NP cells of NP tissue sample from IDD patients	IDD pathogenesis (+)	[91]

"-" Signifies negative regulation and "+" signifies positive regulation. PTEN, Phosphatase and tensin homolog; ATG7, Autophagy-related gene 7; NP, Nucleus pulposus; GDF5, Growth differentiation factor 5

Table 5: Role of CLRs in modulating miRNA expression

miRNA	Interacting CLR	Study model	Function	References
miR-155	Dectin 1	Murine BMDMs	Dectin 1 upregulates miR-155 in murine BMDMs exposed to hyphal form of *C. albicans*	[116]
miR-146a	Dectin 1	Human monocyte leukemia THP-1 cells	Increased expression of miR-146a following Dectin 1 stimulation negatively regulates Dectin 1 mediated inflammation	[117]
miR155	CD69	Murine thymus derived regulatory T cells	CD69 is required for thymic Treg homeostasis via promoting STAT5 phosphorylation through BIC/miR-155 expression and SOCS-1 downregulation	[76]
miR-101	KLRG1	Human tissues samples of colon cancer, colitis, ovarian cancer, traumatic colon patients	$KLRG1^+$ T cells results in T cell senescence and impaired T-cell anti-tumor immunity by downregulating miR-101	[68]

Dectin 1, Dendritic cell-associated C-type lectin-1; BMDMs, Bone marrow-derived macrophages; SOCS-1, Suppressor of cytokine signaling 1; KLRG1, Killer cell lectin-like receptor subfamily G member 1

Figure Legends

Figure 1: Soluble and transmembrane C-type lectin structures and intra-cellular signaling.

Graphical representation of the structure of major CLRs discussed in the review. Group distribution is as indicated by the Imperial College London C-type Lectins website (http://www.imperial.ac.uk/research/animallectins/ctld/classes/C-type1.html). CTLD and major protein domains for each CLR are represented and are annotated as per the UniProtKB database. Based on ITAM or ITIM motifs present in the CLRs, CLRs can trigger signaling cascade that broadly result in the activation (via ITAM) or inhibition (via ITIM) of cellular functions. Followed by the recognition of specific ligands, transmembrane CLRs can trigger activating intra-cellular signaling directly, through ITAM motif in their cytoplasmic tails (Dectin 1) or indirectly, through recruiting ITAM-containing signaling adaptors like DAP10 (CLEC5A) or FcRγ (MMR). ITAM facilitates involvement and activation of SYK-family kinases which subsequently leads to ROS production and induce activation of transcription factors (NF-κB, NFAT, AP-1) by CARD9-BCL10-MALT1. In the case of MMR, via FcRγ-SYK it recruits second messenger Grb2 which eventually activate Rac-1/Cdc42/PAK-1 complex and promote F-actin polymerization that is required for MR-mediated phagocytosis by human alveolar macrophages. But, after phagocytosis, phosphorylated MMR on phagosomal wall recruits SHP-1 and inhibit phagosome-lysosome formation. Furthermore, ITAM-independent signaling is mediated by DC-SIGN and Dectin 1. DC-SIGN associate with the adaptor LSP1 and can recruit the serine/threonine-protein kinase RAF1 signalosome which eventually induce NF-κB-mediated pro-inflammatory responses in a SYK-independent pathway. Particularly, effector responses resulting from RAF1 signalosome pathway depend on the nature of the carbohydrate ligands recognized, like binding of mannose specific ligand to DC-SIGN requires LarG (also known as arHGeF12) and RHOA proteins for activating RAF1 signalosome cascade [5]. Contrastingly, inhibitory receptors like KLRG1 contains ITIM motif which recruits and activates protein tyrosine phosphatases such as SHP-1 and SHP-2 to inhibit inflammatory signaling pathways induced by other immunoreceptors.

(DAP10, DNAX-activating protein 10, MBL, Mannose-binding lectin; SP-D, Surfactant protein-D; LOX-1, Lectin-like oxidized low-density lipoprotein receptor; Reg proteins, Regenerating islet-derived proteins; ITAM, Immunoreceptor tyrosine-based activation motifs; ITIM, Immune-receptor tyrosine-based inhibition motif; SYK, Spleen tyrosine kinase; CARD9, Caspase-recruitment domain protein 9; BCL-10, B cell lymphoma/leukemia 10; MALT1, Mucosa-associated lymphoid tissue lymphoma translocation protein 1; MMR, Macrophage mannose receptor; Grb2, Growth factor receptor-bound protein-2; LSP1, Lymphocyte-specific protein 1; LarG, Leukemia-associated rHO-GeF; SHP-1/SHP-2, Src homology region 1/2 domain-containing phosphatase; ROS, Reactive oxygen species; KLRG1, Killer cell lectin-like receptor subfamily G member 1; EGF, Epidermal growth factor; DC-SIGN, Dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin; Dectin 1, Dendritic cell associated, C-type lectin 1; Clec5A, C-type lectin domain

family 5, member A; GTP, Guanosine triphosphate; GDP; Guanosine diphosphate; PI3K, Phosphatidylinositol-3-kinase)

Figure 2: Graphical representation of the immune-modulatory impact of miRNA-CLR axes. miRNA-CLR interaction contributes to modulation of immune responses. For example, lungs of pneumonia affected patients are dominated with M1 macrophages whereas healthy lungs exhibit upregulation of M2 type. However, miR155 and miR146a could enhance M2-specific MGL-1 expression and shift the balance of pneumonia associated macrophages towards M2 **(A-i).**Uptake of allergens by MRC1 on macrophages leads to an increased expression of miR-511-3p, which in turn, targets inflammatory mediator, PTGDS, and can inhibit macrophage-driven lung inflammation in patients of allergic asthma **(A-ii)**. Tolerogenic DCs are characterized by an increased DC-SIGN surface expression, which is mediated by downregulation of miR-155 **(B)**. miR-511^{low}DC promotes Th2 polarization via increasing the MR and DC-SIGN expression on the DCs **(C-i)**. Downregulated CD69 results in the suppressed STAT5 phosphorylation and miR-155 transcription, which in turn, leads to an increased SOCS1, and ultimately, suppressed thymic Treg cell generation **(C-ii)**.

(M1, classically activated Macrophage; M2, alternatively activated macrophage; MGL-1, M2 associated galactose C-type lectin; MRC1, Mannose receptor; PTGDS, Prostaglandin D2 synthase; DC, Dendritic cells; STAT5, Signal transducer and activator of transcription 5; SOCS1, Suppressor of cytokine signaling 1;DC-SIGN, Dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin; Ptgds, Prostaglandin D synthase; Treg, Regulatory T cells)

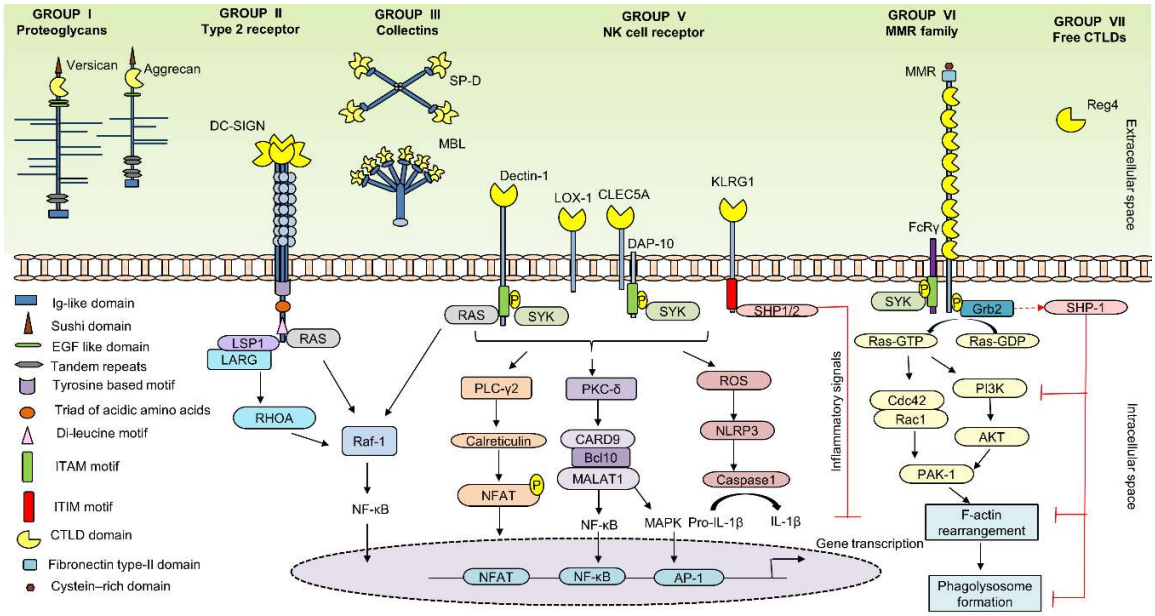
Figure 3: Key miRNAs and factors balancing osteoarthritis (OA) via influencing aggrecan.

The anabolic pathways in OA joints, associated with an increased aggrecan expression are indicated on the left panel, and catabolic pathways of OA joints, involving degradation of aggrecan are indicated on the right panel**.** miR-92a-3p and miR-671 help to maintain a healthy joint via upregulating the aggrecan synthesis. Different aggrecan driving factors like matrillin, TGF-β, Sox9, FGF18, Smad3 are targeted by several miRNAs and shift the balance towards ECM degradation leading to OA progression.

(ECM, Extracellular matrix; HDAC2, Histone deacetylase 2; MMP-13, Matrix metalloproteinase13; TGF-β1, Transforming growth factor-β1; FGF-18, Fibroblastic growth factor 18)

Figure 4: Key miRNAs involved in the LOX-1 mediated atherosclerosis.
The initial stage of atherosclerosis is associated with the deposition of ox-LDL in the arterial wall. LOX-1, expressed on vascular endothelial cells, vascular smooth muscle cells and macrophages binds to ox-LDL and triggers the downstream inflammatory signaling that results in the endothelial cell dysfunction (characterized by increased apoptosis, senescence, angiogenesis, inflammation and ROS generation), increased proliferation and migration of VSMCs along with the infiltration of macrophages into the sub-endothelial lining which ultimately get transformed into the lipid-laden foam cells. Together all these phenomena drive the lipid plaque formation in the arterial wall and block the blood flow. Several miRNAs block these phenomena and are potential strategies to inhibit atherosclerosis progression.

(VSMCs, Vascular smooth muscle cells; ROS, Reactive oxygen species; Ox-LDL, Oxidized low-density lipoprotein; LOX-1, Lectin-like oxidized low-density lipoprotein receptor)


GROUP I
Proteoglycans
GROUP II
Type 2 receptor
GROUP III
Collectins
GROUP V
NK cell receptor
GROUP VI
MMR family
GROUP VII
Free CTLDs
Versican
Aggrecan
DC-SIGN
SP-D
MBL
Dectin-1
LOX-1
CLEC5A
DAP-10
KLRG1
MMR
FcRγ
Reg4
Extracellular space
Ig-like domain
Sushi domain
EGF like domain
Tandem repeats
Tyrosine based motif
Triad of acidic amino acids
Di-leucine motif
ITAM motif
ITIM motif
CTLD domain
Fibronectin type-II domain
Cystein–rich domain
LSP1
LARG
RAS
RHOA
Raf-1
NF-κB
RAS
SYK
SYK
SHP1/2
PLC-γ2
Calreticulin
NFAT
PKC-δ
CARD9
Bcl10
MALAT1
NF-κB
MAPK
ROS
NLRP3
Caspase1
Pro-IL-1β
IL-1β
Inflammatory signals
Gene transcription
NFAT
NF-κB
AP-1
SYK
Grb2
SHP-1
Ras-GTP
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Cdc42
Rac1
PI3K
AKT
PAK-1
F-actin rearrangement
Phagolysosome formation
Intracellular space

Figure 1

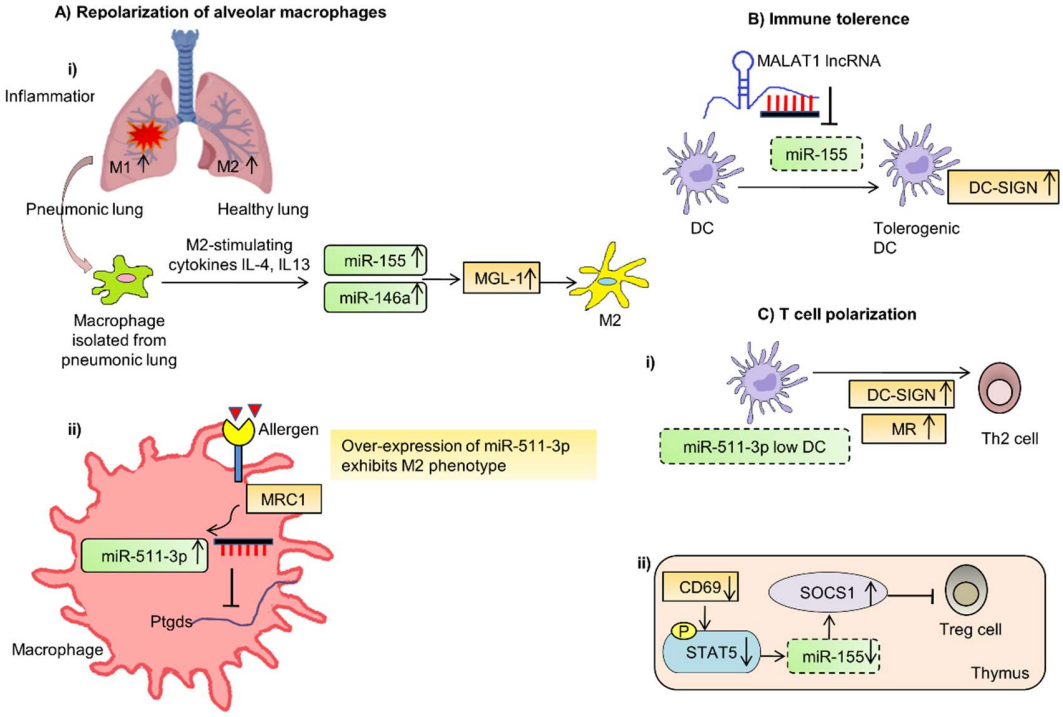

A) Repolarization of alveolar macrophages
i)
Inflammatior
M1
M2
Pneumonic lung
Healthy lung
Macrophage isolated from pneumonic lung
M2-stimulating cytokines IL-4, IL13
miR-155
miR-146a
MGL-1
M2
B) Immune tolerence
MALAT1 lncRNA
miR-155
DC
DC-SIGN
Tolerogenic DC
C) T cell polarization
i)
DC-SIGN
MR
Th2 cell
miR-511-3p low DC
ii)
Allergen
MRC1
miR-511-3p
Ptgds
Macrophage
Over-expression of miR-511-3p exhibits M2 phenotype
ii)
CD69
SOCS1
Treg cell
P
STAT5
miR-155
Thymus

Figure 2

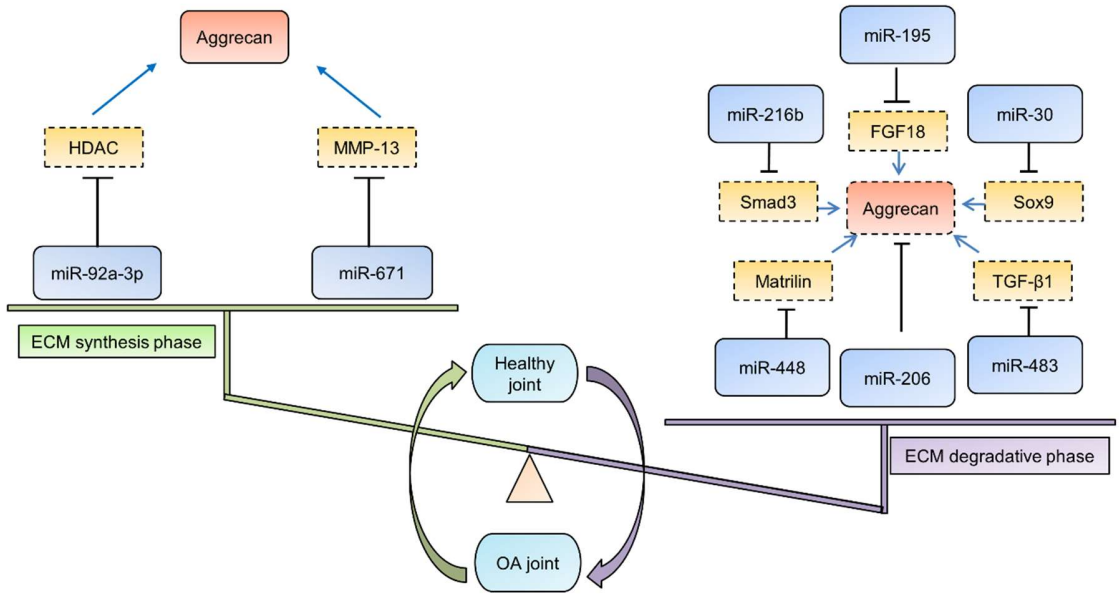

Aggrecan
HDAC
MMP-13
miR-92a-3p
miR-671
ECM synthesis phase
Healthy joint
OA joint
miR-195
miR-216b
FGF18
miR-30
Smad3
Aggrecan
Sox9
Matrilin
TGF-β1
miR-448
miR-206
miR-483
ECM degradative phase

Figure 3

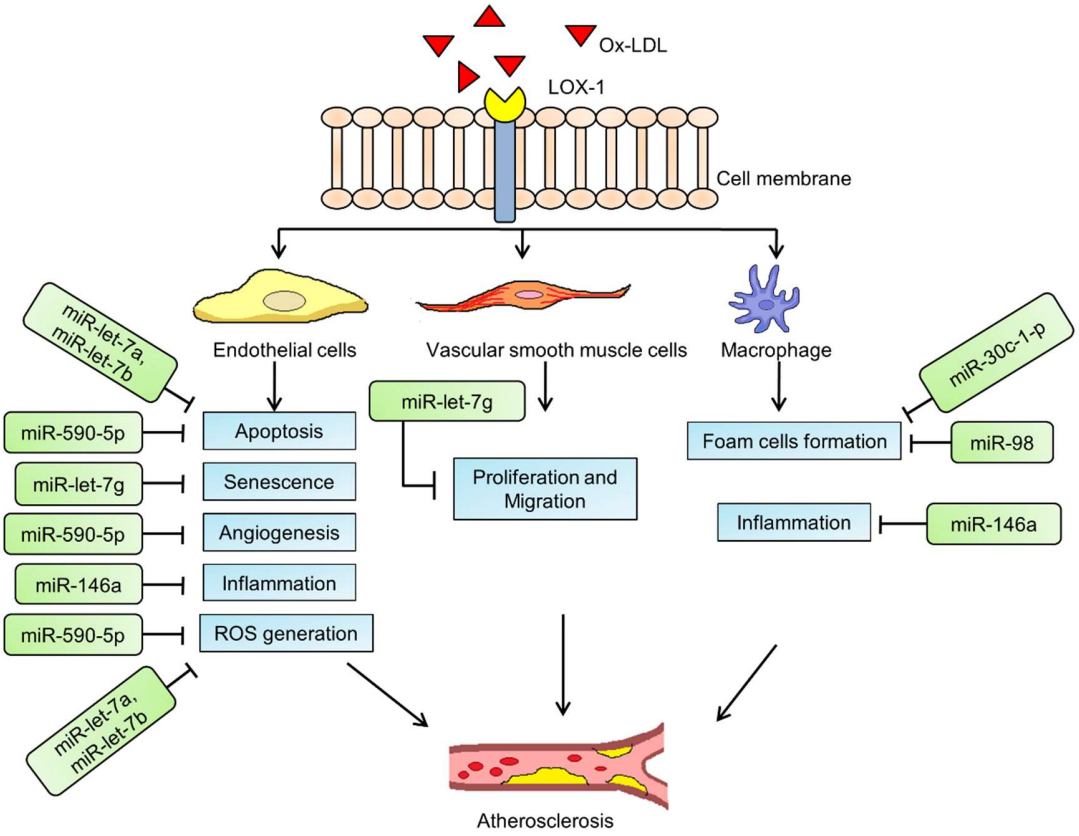
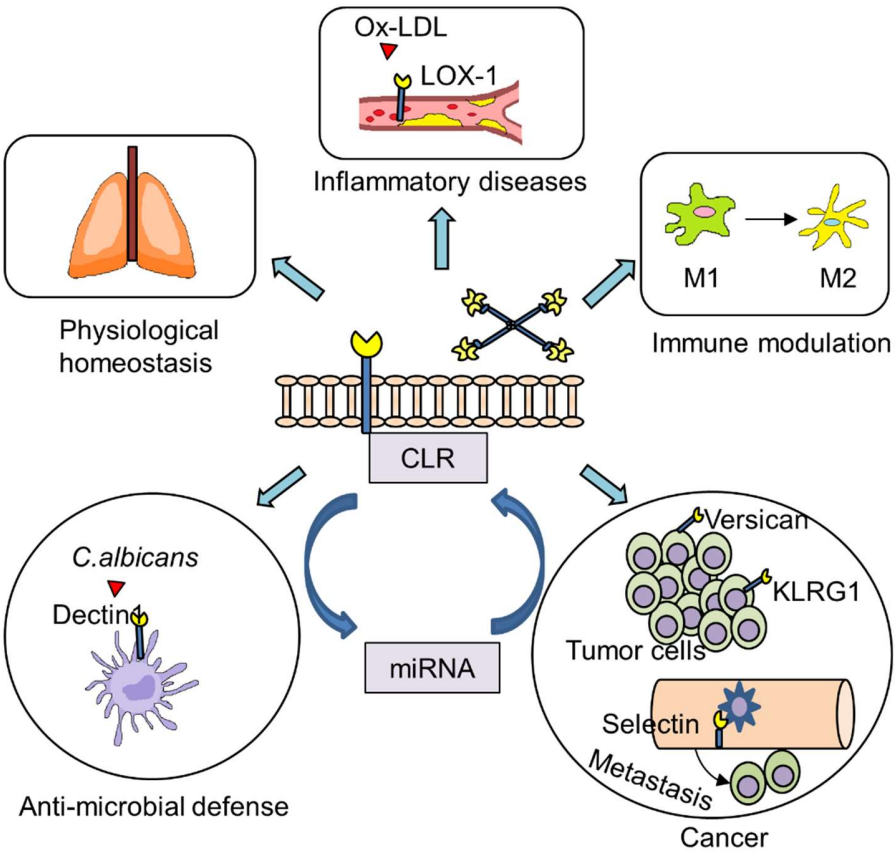

Ox-LDL
LOX-1
Cell membrane
Endothelial cells
Vascular smooth muscle cells
Macrophage
miR-let-7a, miR-let-7b
miR-590-5p
Apoptosis
miR-let-7g
Senescence
miR-590-5p
Angiogenesis
miR-146a
Inflammation
miR-590-5p
ROS generation
miR-let-7a, miR-let-7b
miR-let-7g
Proliferation and Migration
miR-30c-1-p
Foam cells formation
miR-98
Inflammation
miR-146a
Atherosclerosis

Figure 4



Graphical Abstract