Long non-coding RNAs (lncRNAs) and their transcriptional control of inflammatory responses

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Long non-coding RNAs (IncRNAs) have emerged as potential key regulators of the inflammatory response, particularly by modulating the transcriptional control of inflammatory genes. IncRNAs may act as an enhancer or suppressor to inflammatory transcription, function as scaffold molecules through interactions with RNA-binding proteins in chromatin remodeling complexes, and modulate dynamic and epigenetic control of inflammatory transcription in a gene-specific and time-dependent fashion. Here, we will review recent literature regarding the role of IncRNAs in transcriptional control of inflammatory responses. Better understanding of IncRNA regulation of inflammation will provide novel targets for the development of new therapeutic strategies.

Because of the potentially destructive nature of the inflammatory response, the expression of inflammation-related genes is finely regulated at multiple levels, including transcription, mRNA processing, translation, phosphorylation, and degradation. Transcription represents an essential, and often the most important, regulatory determinant of the inflammation process. Many signaling pathways and transcription factors are involved in the inflammatory response, including the transcription factor nuclear factor-kB (NF-κB), MAPK, and JAK/STAT pathways, together controlling a multitude of inflammatory response genes (1, 2). Upon activation, these pathways directly activate and induce the expression of a limited set of transcription factors that promote the transcription of inflammatory genes. Whereas the mechanisms of initial activation and subsequent nuclear translocation of associated transcription factors for these signaling pathways are well-characterized, how the transcription of inflammatory genes is finely controlled in the nucleus to ensure that each individual gene is transcribed at the "right" place at the "right" time remains elusive. Perhaps this can be best represented by the inflammatory transcription induced by NF-κB. The NF-κB signaling cascade can be activated by inflammatory signals, including LPS, TNF-α, IL-1β, and reactive oxygen species, among others; many Toll-like receptors (TLRs) also activate NF-κB (3–8). Prior to an activating signal, NF-κB dimers are sequestered in the cytoplasm and held inactive by association with 1κB proteins (9). Activating stimuli cause 1κB degradation (10, 11), and consequently, free NF-κB dimers translocate to the nucleus, bind to κB sites, and promote gene transcription (12). Common targets of NF-κB include inflammatory cytokines, chemokines, adhesion molecules, cytoprotective proteins, and proteins regulating cell differentiation, proliferation, and survival (13, 14). In addition to its initial cytoplasmic activation, both the recruitment of NF-κB to target genes in the nuclei and NF-κB-induced transcriptional events after recruitment are finely controlled to ensure proper transactivation of NF-κB target genes (15). Indeed, several waves of gene transcription have been demonstrated in macrophages following LPS stimulation. Broadly, these waves are categorized as transcription of early inflammatory genes (e.g. Tnfa, Cxcl2, Ptgs2/Cox2, and Il1b) and late inflammatory genes (e.g. Ccl5, Saa3, Ifib1, Il6, Il12b, Nos2/INOS, and Marco) (16–18). The underlying molecular mechanisms of this dynamic transcription are unclear, and the transcription of late inflammatory genes may require synthesis of additional molecules and chromatin remodeling triggered by NF-κB activation (19, 18).

Large-scale transcriptome studies have revealed that transcription of protein-coding genes is far outweighed by the production of non-coding RNAs (ncRNAs), including thousands of long ncRNAs (IncRNAs) (20, 21). Emerging evidence recognizes IncRNAs as key regulators of gene expression implicated in diverse cellular processes. Although IncRNAs have been identified in almost all immune cells, their function remains largely unknown (22, 23). However, recent studies demonstrate that IncRNAs can be induced in innate immune cells and may act as key regulators of the inflammatory response (22, 24–27). Increased understanding of how IncRNAs function in this manner may impact future therapies for inflammation-associated...
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The definition of an IncRNA is a non-protein-coding transcript greater than 200 nucleotides in length, arbitrarily separating them from shorter classes of ncRNAs, such as the microRNAs. Multiple subclasses of IncRNAs have been proposed based upon their genomic locations (Fig. 1) (33–35). Long intergenic ncRNAs (lincRNAs) are located between protein-coding genes, whereas others are located and transcribed from introns of coding regions and are termed intronic lncRNAs, with reports suggesting that these form the largest class of lncRNAs (33). Sense lncRNAs are found on the sense strand of protein-coding genes, and may contain exons from these genes. Alternatively, antisense IncRNAs are located on the antisense strand and include natural antisense transcripts. Enhancer RNAs (eRNAs) are produced from the bidirectional transcription of enhancer regions (34). Although functionally different from mRNAs, the biogenesis of IncRNAs and mRNAs is remarkably similar. The majority of IncRNAs are transcribed by RNA polymerase II from genomic loci sharing similar chromatin states, are 5’-capped, spliced, and polyadenylated (22, 35).

IncRNAs are versatile molecules that can interact physically with DNA, other RNA, and protein, either through nucleotide base pairing or via formation of structural domains generated by RNA folding. These properties endow IncRNAs with a versatile range of capabilities to modulate gene transcription that are only beginning to be appreciated. Despite the great number of IncRNAs identified and the wide variety of effector proteins with which they are able to interact, four common mechanistic themes have been proposed (36). The first mechanistic theme that an IncRNA may exert its function through is by acting as a signal molecule. The specificity of lncRNA expression in specific cell and tissue types, along with their induction by many diverse stimuli, imply that IncRNAs can function as molecular signals in response to unique stimuli, serving as an indicator of transcriptional activity (37). Presumably acting to negatively regulate gene transcription, IncRNAs may function as decoys, the second mechanistic theme. These act as a decoy by binding their targets, without producing any further effect, as a mechanism of competitive regulation between IncRNAs and other molecules trying to exert an effect on the same molecular targets (38). Functionally opposite of the decoy, IncRNAs may act as guides, the next mechanistic theme. These lncRNAs bind their target effector proteins and serve to direct the ribonucleoprotein complex to sequence-specific sites, resulting in either positive or negative regulation of gene transcription (39). The final mechanistic theme is scaffolding, by which lncRNAs serve to bring multiple effector proteins or subunits of a complex together in a temporospatial fashion, coordinating their activities (40). In addition, new mechanisms are being discovered, such as eRNAs, which have been hypothesized to remain bound to enhancer regions, functioning to “tether” interacting proteins to the enhancer region (Fig. 1) (43). These mechanisms described above are

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Figure 1. General classification of IncRNAs and their functional models in transcriptional control. A, classification of IncRNAs into five classes: exonic sense, intronic sense, antisense, bidirectional and intergenic, based upon their genomic locations and transcription (35). B, functional models of transcriptional control. IncRNAs may act as signals, decoys, guides or scaffolds to modulate gene expression at the transcriptional level (36).
not mutually exclusive, and many lncRNAs function by fulfilling more than one category (36).

Under normal circumstances, many lncRNAs are ubiquitously expressed across all human tissue types at a basal level and may be involved in maintaining cellular function (44). Similar to protein-coding inflammatory genes, many lncRNAs have been found to be targets of inflammatory pathways, and consequently, their expression profile is altered in various cell types during inflammation. Indeed, lncRNAs are differentially regulated in virus-infected cells (45) and in dendritic cells or macrophages following stimulation by ligands for TLR4 (LPS) and TLR2 (Pam3CSk4) (22, 26, 27). Through NF-κB signaling, lncRNA-Cox2, AS-IL1α, and PACER (p50-associated COX-2 extragenic RNA) are up-regulated in macrophages upon ligation of numerous TLRs, including TLR4 by LPS (26, 46, 47). Similarly, IL1β-eRNA, IL1β-RBT46, and Inc13 are induced in human monocytes and macrophages in response to LPS through NF-κB signaling (48, 49). NF-κB-interacting lncRNA (NKILA) is highly induced in response to IL-1β and TNF-α stimulation (50). Contrary to lncRNAs that are up-regulated in response to inflammation, lncRNA-EP5 (erythroid prosurvival) is down-regulated upon TLR ligation through an NF-κB-dependent mechanism (51). Therefore, these lncRNAs may represent important members of an inducible inflammatory response. Whereas some inflammatory-responsive lncRNAs may only serve as molecular signals, it becomes clear that many others possess regulatory functions for controlling inflammatory gene expression, adding a new avenue for the transcriptional control of inflammatory responses.

**lncRNAs as enhancers of the inflammatory response**

Many lncRNAs can enhance the inflammatory response through a variety of means, commonly by increasing the transcription of pro-inflammatory cytokines or other inflammatory target genes or by enhancing inflammatory signals, such as NF-κB signaling. THRIL (TNFα- and hnRNPL-related immunoregulatory lincRNA) is one of the many lncRNAs induced after TLR2 activation (52). Knockdown of THRIL suppresses the induction of TNF-α secretion, both after TLR2 activation and in unstimulated cells. Mechanistically, THRIL acts as a scaffold through interacting with heterogeneous nuclear ribonucleoprotein L (hnRNPL), and this complex binds to the TNF-α promoter to induce its transcription. Up-regulated after stimulation with LPS, IL-1β, or TNF-α, PACER has been shown to regulate the expression of a neighboring gene, PTGS2 (COX-2), a key inflammatory gene (46, 53, 54). PACER functions as a decoy by binding and sequestering NF-κB p50 homodimers, which lack transcription activation domains, away from the PTGS2 promoter. This sequestration promotes the formation and binding of NF-κB p50-p65 heterodimers to activate PTGS2 transcription. In response to *Listeria monocytogenes* infection and TLR1–4 activation, AS-IL-1α is highly up-regulated through NF-κB signaling (47). AS-IL-1α is required for the inducible expression of IL-1α. Knockdown of AS-IL-1α decreases acetylation of H3K9 and diminishes binding of RNA polymerase II to the transcription start site of IL-1α but not to control genes, indicating that AS-IL-1α specifically regulates the induction of IL-1α after TLR4 activation.

Moreover, as they are transcribed from enhancer regions, several eRNAs, such as IL1β-eRNA and IL1β-RBT46, are found to be induced via NF-κB in response to TLR4 activation by LPS and are predominantly localized to the nucleus. Knockdown of IL1β-eRNA and IL1β-RBT46 together in activated cells specifically reduces the mRNA and protein levels of IL1-β and CXCL8, complying with their functional archetype as eRNAs (43, 48, 49).

**lncRNAs as suppressors of the inflammatory response**

Other lncRNAs have been identified to suppress or limit the extent of the inflammatory response. They achieve this effect by limiting the transcription of pro-inflammatory cytokines or by interfering with inflammatory signal pathways, including NF-κB signaling. In this regard, lncRNA-EP5 is down-regulated after TLR4 ligation with LPS, despite being highly expressed in resting macrophages (51, 55). lncRNA-EP5 represses expression of immune response genes in resting macrophages, but after activation by microbial ligands such as LPS, this suppression is released, and gene transcription is induced. Accordingly, lncRNA-EP5 deficient mice produce higher systemic levels of inflammatory cytokines in response to LPS challenge and are more prone to LPS-induced lethality (51). Mechanistically, lncRNA-EP5 functions as a scaffold molecule and can interact with hnRNPL, which may partially explain how lncRNA-EP5 is able to maintain a repressive chromatin state at the transcription start sites of many immune response genes. Another lncRNA implicated in suppression of the inflammatory response is Lethe. Knockdown of Lethe enhances the expression of several NF-κB target genes upon TNF-α stimulation (27). Lethe binds directly to the NF-κB p65 homodimer and acts as a decoy, preventing binding at the aforementioned target genes. Accordingly, overexpression of Lethe decreases p65 binding at NF-κB target genes such as Il6, Sod2, Il8, and Nfkbia upon TNF-α stimulation. Similar to Lethe, NKILA has been found to restrain NF-κB-driven inflammation. NKILA can mask the phosphorylation motifs of 1κB to block 1κB degradation, and thus it prevents the translocation of NF-κB to the nucleus, representing a class of lncRNAs that are able to regulate gene expression via post-translational modification of signaling proteins (49). Interestingly, lncRNA-p21 has been found to sequester p65 mRNA and thus attenuates the translation of p65, resulting in inhibition of basal and TNF-α-stimulated NF-κB activity, as measured by phospho-p65 (56). Taken together, it appears that induction of the above lncRNAs during inflammation provides negative regulatory feedback to inflammatory responses.

**Curious case of lncRNA-Cox2**

Arguably the best studied lncRNA that functions to modulate the inflammatory response is lncRNA-Cox2, which has been found to act as both an enhancer and a suppressor of inflammation in a gene-specific manner (18, 22, 26, 57). After TLR4 activation and ensuing NF-κB signaling, lncRNA-Cox2 was induced greater than 1000-fold in bone marrow-derived dendritic cells over the course of the following 12 h (22). Analysis of the expression time course revealed that it is an early primary NF-κB response gene, similar to Cxcl2 (18, 57). Func-
tionally, silencing of lincRNA-Cox2 resulted in up-regulation of almost 800 genes and down-regulation of many other genes in non-stimulated cells (22, 57), suggesting a role for lincRNA-Cox2 in basal gene transcription. Many of these genes are inflammatory genes, including Ccl5, Cx3cl1, Ccrl, and IFN-stimulated genes, including Irf7, Oas1a, Oas1l, Oas2, Ifi204, and Isg15. Likewise, about 700 genes were found to be down-regulated, including Tlr1, Il6, and Il23. Mechanistically, it appears that induction of lincRNA-Cox2 does not affect the NF-κB signaling cascades or RNA degradation machinery (57).

Instead, lincRNA-Cox2 has been found to form a complex with heterogeneous ribonucleoprotein (hnRNP) A/B and hnRNP-A2/B1, which together function to modulate the transcription of immune response genes (22, 26).

A more detailed analysis revealed that lincRNA-Cox2 knockdown causes a general down-regulation of the late primary responsive genes in LPS-stimulated macrophages (57). Different from the transcription of the late secondary responsive genes, transcription of the late primary genes does not require new protein synthesis (17, 18). Promoter recruitment of the ATP-dependent SWI/SNF complex has been demonstrated in the transcription of late primary response genes following NF-κB activation (26, 58). The SWI/SNF complex is a nucleosome remodeling complex composed of several proteins encoded by the SWI and SNF genes (e.g., SWIs, Brg1, or Brm) (58, 59). The SWI/SNF complex has DNA-stimulated ATPase activity and can destabilize histone-DNA interactions in reconstituted nucleosomes in an ATP-dependent manner (58, 60). After induction, lincRNA-Cox2 is assembled into the SWI/SNF complex through its interaction with the RNA-binding protein component (i.e., MyBBP1A, MYB-binding protein 1a) in both macrophages and microglia in response to LPS stimulation. This resulting lincRNA-Cox2–SWI/SNF complex can modulate SWI/SNF-associated chromatin remodeling and, consequently, transcription of late primary response genes (57).

Another study has shown that lincRNA-Cox2 is induced in intestinal epithelial cells in response to TNF-α and that its knockdown with siRNA significantly enhanced expression of Il12b (a late secondary responsive gene) in response to TNF-α compared with control siRNA after stimulation (61). Functionally, lincRNA-Cox2 promotes the recruitment of the Mi-2 nucleosome remodeling and deacetylase (Mi-2–NuRD) repre-
sor complex to the promoter region of *Il12b* in intestinal epithelial cells in response to TNF-α/H9251 stimulation. Recruitment of the Mi-2–NuRD complex facilitated by lincRNA-Cox2 suppresses the transcription of the *Il12b* gene through epigenetic histone modifications. Increased acetylation of H3K9 and H3K27 and decreased H3K27 methylation at the *Il12b* promoter region are found in intestinal epithelial cells following TNF-α/H9251 stimulation. Knockdown of lincRNA-Cox2 attenuated the associated histone modifications in the *Il12b* promoter region induced by TNF-α (61).

This curious case of lincRNA-Cox2 indicates that lncRNAs can modulate inflammatory responses at every step of the regulatory network, including acting as an enhancer or suppressor to inflammatory transcription, functioning as scaffold molecules through their interactions with various RNA-binding proteins in chromatin remodeling complexes, and modulating dynamic and epigenetic control of gene transcription, in a gene-specific and time-dependent fashion (Fig. 2).

### lncRNAs in macrophage M1/M2 switch

Another method by which lncRNAs may regulate the inflammatory process is by modulating the polarization state of macrophages. Upon activation, resting macrophages undergo phenotypic polarization based upon their microenvironment and transition into one of two functionally different states: the classically activated macrophage (M1) or an alternatively activated macrophage (M2). The M1 state is characterized by the production and release of pro-inflammatory mediators and is induced upon IFN-γ stimulation (62). Conversely, the M2 state is induced in response to IL-4 and has been proposed to be anti-inflammatory, serving as an immunomodulator by participating in the resolution of inflammatory responses (63, 64). The regulatory mechanisms controlling the expression of the constellation of genes in macrophages responding to activating conditions are not fully defined.

A recent report indicates that lncRNAs are partially responsible for the coordinated changes in gene expression occurring during macrophage polarization (65). A detailed genome-wide transcriptome analysis revealed a differentiated lncRNA expression profile of both mRNAs and IncRNAs in M1 (IFN-γ + LPS) and M2 (IL-4) macrophages (human monocyte-derived macrophages). Scatter plots show the variation in lncRNA and mRNA expression levels between the M1 (IFN-γ + LPS) and M2 (IL-4) and non-stimulated macrophages, based on the work of Huang et al. (66). TCONS_00019715 is expressed at a high level in M1 macrophages versus a lower level in M2 macrophages. Overexpression or knockdown of TCONS_00019715 causes reciprocal macrophage switch (66).

**Figure 3. lncRNAs in M1 and M2 macrophage activation.** Upon activation, resting macrophages can be activated into one of two functionally different states: the classically activated macrophage (M1, by IFN-γ, or LPS) or an alternatively activated macrophage (M2, by IL-4 or IL-10). Genome-wide analysis reveals differentiated lncRNA expression profiles of both mRNAs and IncRNAs in M1 (IFN-γ + LPS) and M2 (IL-4) macrophages (human monocyte-derived macrophages). Scatter plots show the variation in lncRNA and mRNA expression levels between the M1 (IFN-γ + LPS) and M2 (IL-4) and non-stimulated macrophages, based on the work of Huang et al. (66). TCONS_00019715 is expressed at a high level in M1 macrophages versus a lower level in M2 macrophages. Overexpression or knockdown of TCONS_00019715 causes reciprocal macrophage switch (66).
macrophages than in M2 (IL-4) macrophages. TCONS_00019715 expression was decreased when M1 (IFN-γ + LPS) converted to M2 (IL-4) and increased when M2 (IL-4) converted to M1 (IFN-γ + LPS). Knockdown of TCONS_00019715 following the activation of THP-1 human mononuclear cells using IFN-γ and LPS diminished the expression of M1 (IFN-γ + LPS) markers and elevated the expression of M2 (IL-4) markers (66). Underlying mechanisms of the TCONS_00019715-mediated macrophage M1/M2 switch remain unclear. Therefore, a significantly altered lncRNA and mRNA expression profile occurs in macrophages exposed to different activating conditions. Dysregulation of some of these lncRNAs may play important roles in determining macrophage polarization (Fig. 3).

**lncRNAs and pathogenesis of inflammatory disease**

Because of the recently discovered functions of these many lncRNAs in the inflammatory response, it is not surprising that their dysregulation can lead to disease states. Although the underlying molecular mechanisms are still unclear, many lncRNAs have been implicated in the pathogenesis of various inflammatory diseases. THRIL has been found to be associated with Kawasaki disease, an inflammatory disease in children that can lead to coronary artery abnormalities and possible myocardial infarction (67). CXCL10, one of the genes found to be regulated by THRIL, is up-regulated in patients in the acute phase and has been identified as a possible biomarker of the disease (68). PACER is overexpressed in tissue samples from osteosarcoma when compared with control tissue and osteoblasts, and it enhances osteosarcoma cell proliferation and invasion (46). It is highly expressed in both knee and hip osteoarthritis chondrocytes when compared with non-osteoarthritic samples, suggesting a possible role in mediating inflammation-driven cartilage damage in osteoarthritis (54). Lethe is found to enhance the cell cycle of osteosarcoma cells when compared with non-osteoarthritic samples, suggesting a possible role in mediating inflammation-driven cartilage damage in osteoarthritis (54). Lethe is found to enhance the replication of the hepatitis C virus by suppressing interferon-stimulated gene expression after a type 1 interferon response (69). NIKLA is found to serve as a tumor suppressor through a negative feedback loop of NF-κB and inhibits breast cancer progression and metastasis (50). lincRNA-p21 has been found to be a key negative regulator of NF-κB signaling in rheumatoid arthritis, and its decreased levels in disease may play a role in the inflammatory state seen in arthritic tissues (56). Inc13 has been implicated in celiac disease, a chronic, immune-mediated intestinal disorder that is triggered by ingested gluten. Levels of Inc13 are significantly down-regulated in samples from patients afflicted with celiac disease compared with control, suggesting that its down-regulation may be a contributing factor to the underlying inflammation in this disease (49).

**Conclusions and perspectives**

It has been found that a set of lncRNAs is induced in response to inflammation and that it plays a role in the regulation of gene transcription during the inflammatory response. This fine regulation is important for production of functional immune responses, and numerous studies have found that dysregulation of lncRNAs is associated with an array of human diseases. Thus far, we have learned that lncRNAs are able to enhance or suppress the inflammatory response in a gene-specific and time-specific fashion. They accomplish this fine regulation by acting as signals, decoys, guides, or scaffolds. As research in this area moves forward, continuing to uncover the mechanisms by which lncRNAs function, new mechanisms in addition to those summarized here will likely be found. Increasing our understanding of precisely how lncRNAs function in the regulation of inflammation will provide novel targets for the development of new therapeutic strategies for many inflammatory diseases.

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