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REVIEW ARTICLE

Functional diversity of long non-coding RNAs in immune regulation

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Abstract Precise and dynamic regulation of gene expression is a key feature of immunity. In recent years, rapid advances in transcriptome profiling analysis have led to recognize long non-coding RNAs (lncRNAs) as an additional layer of gene regulation context. In the immune system, lncRNAs are found to be widely expressed in immune cells including monocytes, macrophages, dendritic cells (DC), neutrophils, T cells and B cells during their development, differentiation and activation. However, the functional importance of immune-related lncRNAs is just emerging to be characterized. In this review, we discuss the up-to-date knowledge of lncRNAs in immune regulation.

Introduction

The mammalian immune system orchestrates innate and adaptive immune responses that are a remarkable complex of biochemical processes regulated by various protein and lipid mediators such as pattern recognition receptors, cytokines, chemokines, hormones, growth factors, and prostaglandins. Recently, a growing body of evidence suggests that non-coding RNAs (ncRNAs) also play an important role in regulation of the immunity. ncRNAs are a group of RNA molecules that are transcribed from DNA but are not

Abbreviations: DC, dendritic cell; eRNA, enhancer RNA; lincRNA, long intergenic noncoding RNA; lncRNAs, long non-coding RNAs; LPS, lipopolysaccharide; miRNA, microRNA; ncRNA, non-coding RNAs; RNA-seq, RNA sequencing.

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translated into proteins. In general, regulatory ncRNAs are classified as short ncRNA including microRNA (miRNA) (22—23 nts) and piwi-interacting RNA (piRNA) (26—31 nts), medium ncRNA (50—200 nts), and long ncRNA (>200 nts). With the development of next generation sequencing technique (RNA-seq), the total amount of lncRNAs has been expanded to 92343 and 67628 in humans and mice respectively (NONCODE database 4.0). This observation suggests that ncRNA transcription might be more prevalent than previously estimated. The role of short regulatory ncRNA (such as miRNAs) in controlling immune responses is now being elucidated in details in recent years. By contrast, we are still far from understanding whether and how long noncoding RNAs (lncRNA) contribute to immune regulation although more than thousands of lncRNAs have been discovered so far.[1] The central roles of lncRNAs have been uncovered in diverse biological processes such as X chromosome inactivation (Xist) and genomic imprinting (H19). Recently, several novel findings suggest the link between regulatory lncRNAs and immunity. Thus, we reviewed the rapid progress in the field of lncRNAs and discussed various potential roles of regulatory lncRNAs in immune regulations in this review.

LncRNAs and their regulatory functions

LncRNAs are a large and diverse class of non-protein coding transcripts longer than 200 nucleotides. They are transcribed from pseudogenes or DNA sequences that resemble known genes but cannot themselves code for an active protein. In general, lncRNAs are transcribed by RNA polymerase II and thus capped, polyadenylated and spliced through similar processes that occur in mRNA biogenesis. Sequence comparison across species has suggested a relatively low degree of evolutionary conservation of lncRNA sequences. Many lncRNAs exhibit dynamic expression patterns in a cell type-, tissue-, developmental stage-, and context-specific manners. They have been demonstrated to participate in various aspects of biological and pathological processes, including X-chromosome inactivation, genomic imprinting, stem cell pluripotency, development, cancer progression and metastasis, as well as immune regulation.[2—4] Indeed, the concept that lncRNAs possess regulatory functions in maintaining cellular and tissue homeostasis has been recognized for years, but underlying molecular mechanisms remain poorly characterized. Up to date, recent advances pointed out that lncRNAs contain modular domains with binding capacity to proteins or nucleic acids via secondary structures or base pairing, which resulted in the interactions of RNA-protein, RNA-DNA, and RNA-RNA. Not surprisingly, depending on the subcellular locations of lncRNAs (cytoplasmic or nuclear) and their targets, lncRNAs can participate in regulation of genome activity through a variety of mechanisms.

One of classic examples for regulatory lncRNAs is *Xist*, a lincRNA located on X chromosome. Evidence shows that *Xist* plays a critical role in X-chromosome inactivation. It recruits polycomb repressive complex 2 (PRC2) to the silenced X chromosome and acts in *cis* to trigger X-linked gene silencing throughout development and adult life.[5] Another example is H19, the first well-studied imprinted lncRNA. The H19 was once thought to act as a *trans* regulator of the imprinted gene network in controlling growth.[6] Recently, H19 has been shown to harbor a miRNA-containing hairpin that serves as the template for miR-675.[7,8] In addition, H19 is revealed to play a regulatory role in controlling gene expression.[9] More recently, H19 is demonstrated to function as a molecular "sponge" for the let-7 family miRNAs, which in turn contributes to regulating expression of genes targeted by let-7.[10,11] Another well-characterized lncRNA is HOTAIR. This lncRNA is typically expressed on one chromosome and influences gene transcription occurred on another chromosome. HOTAIR has been proposed to function as a scaffold that physically associates and coordinates the distinct repressive histone modifying complexes to target loci.[2,12] Evidence shows that HOTAIR is involved in cancer metastasis.[13,14] Together, these findings strongly suggest that lncRNAs play crucial roles in diverse biological processes and disease pathogenesis.

Involvement of lncRNAs in immune response

The development and activation of immune cells rely on a highly integrated and dynamic gene expression programs which are regulated through complex transcriptional and post-transcriptional mechanisms. The roles of proteins (such as transcription factors) in the regulation of gene expression in the immune system have been fairly well studied. In contrast, the regulatory roles of non-coding RNAs in immune responses are still poorly elucidated.

Previously, a large number of studies demonstrated the link between lncRNAs and immune regulations such as immune responses and infectious diseases. For example, Guttman and colleagues reported that $CD11C^{+}$ bone-marrow-derived dendritic cells increase in expression of about 20 lincRNAs after being challenged by lipopolysaccharide (LPS), a specific agonist of the Toll-like receptor 4.[15] This is the first evidence to suggest that lncRNAs may play a potential role for in the innate immune regulation. Using microarray and RNA sequencing (RNA-seq), investigators have further assessed genome-wide differential lncRNA expression patterns associated with inflammation, infection, and differentiation of monocytes into macrophage and dendritic cells.[16—22] In addition to the innate immune responses, increasing evidence showed the role for lncRNAs in T cell development, differentiation and activation in the adaptive immune responses. Using custom microarrays, Pang et al provided the first view of lncRNAs expression profiles in mammalian $CD8^{+}$ T cells and uncovered hundreds of lncRNAs which are expressed in a lymphoid-specific manner and/or changed dynamically during lymphocyte differentiation or activation.[23] Recently, Hu et al identified 1524 lincRNA clusters in 42 T cell samples, from early T cell progenitors to terminally differentiated helper T cell subsets. Their analysis revealed highly dynamic and cell-specific expression patterns for lincRNAs during T cell differentiation.[24] Furthermore, Ranzani et al identified over 500 previously unknown lincRNAs and described lincRNA signatures in human lymphocytes.[25,26] Collectively, accumulating genome-wide datasets have suggested that lncRNAs emerge as a group of important

molecules that may dynamically regulate the immune system and control immunity.

Functional diversity of immune-related lncRNAs

The role of lncRNAs in the immune regulation is an emerging theme, but far from understood. It has been shown that various lncRNAs are present in immune cells including monocytes, macrophages, dendritic cells, neutrophils, T cells and B cells. The levels of lincRNA expression have been shown to be associated with development, differentiation and activation of immune cells.[1] With the increasing publications regarding to immune-related lncRNAs, it is worth highlighting the functional diversity of these lncRNAs. Currently, many of the reported immune-related lncRNAs are located close to, or overlapping of immune-responsible protein coding gene clusters, such as IL1β-RBT46,[27] lnc-IL7R,[28] and lincRNA-Ccr2-5′ AS.[24] These lncRNAs have been found to regulate their adjacent protein coding genes in *cis* or in *trans*-acting manners. Moreover, recent reports revealed that the regulatory functions of many immune-related lncRNAs are mainly involved in processes of RNA/protein binding or RNA/DNA base-pairing.[29] Given the vast number of interactions discovered, immune-related lncRNA can interact with transcription factors and signaling molecules (NF-κB, STAT3),[21,22,30] RNA binding proteins (hnRNP, HuR),[16,19,29] as well as chromatin remodeling components (PRC2, WDR5).[31,32] Nonetheless, further understanding of immune-related lncRNA functions and their underlying molecular mechanisms will undoubtedly expand our knowledge about how lncRNAs function in immune regulation. In this review, we focus on the current understanding of the intersection between immunology and lncRNA biology. We touch briefly on individual lncRNAs and summarize according to their functions in various cellular contexts, including transcription control, post-transcriptional regulation, organization of protein complex and regulation of protein activity, as well as host-pathogen interactions.

LncRNAs as regulators of transcriptional regulation of immunogene expression

Early discoveries supported a notion that lncRNA regulate transcription via chromatin modulations.[33] Additionally, several lncRNAs have been found to target directly or indirectly on specific transcription factors.[34] More recently, enhancer RNA (eRNA) as a specific type of lncRNAs displays enhancer-like activities to modulate target gene expression.[35] In the following, we highlighted several immune regulatory lncRNAs that modulate gene transcription through their unique mechanisms.

NeST/Tmevpg1

NeST(Nettoie Salmonella pas Theilers's), formally known as *Tmevpg1*, is a long noncoding RNA gene located downstream adjacent to the IFN-γ-encoding gene and transcribed in a convergent manner to the IFN-γ gene in both mice and humans.[36] NeST is present in $CD4^+$ T cells, $CD8^+$ T cells and natural killer cells.[31,37] Its expression has been found to be correlated with IFN-γ expression and is induced in response to the Th1-differentiation program by mechanisms dependent upon Stat4 and T-bet.[31,36,37] Mice over-expressing NeST show marked resistance to Salmonella pathogenesis but increased susceptibility to Theiler's virus persistence. Mechanistic analysis indicated that lncRNA NeST interacts with WDR5, a core subunit of the histone H3K4 methyltransferase complex, leading to alteration of H3 methylation at the IFN-γ locus, thereby epigenetically regulating IFN-γ expression.[31,37] Recently, a report showed that T-bet guides epigenetic remodeling of lncRNA *NeST* proximal and distal enhancers in developing and differentiated effector Th1 cells, which subsequently leads to recruitment of stimulus-inducible transcription factors, including NF-κB and Ets-1, to the locus to achieve Th1-lineage-specific expression of IFN-γ.[38] Thus, it appears that NeST regulates T cell function via multiple mechanisms. Collectively, these findings have broadened our knowledge on the role of lncRNAs in regulating the adaptive immune response in pathogen infections.

NRON

NRON is non-coding repressor of NFAT (Nuclear Factor of Activated T cells), first identified during a short hairpin RNA (shRNA) library screening against 512 evolutionarily conserved lncRNAs.[39] NFAT is a heavily phosphorylated transcription factor resided in the cytoplasm of resting cells. In response to calcium-dependent signals, NFAT is dephosphorylated and transported from the cytoplasm into the nucleus to activate expression of target genes such as IL-2. It has been found that heavily phosphorylated NFAT is located within a cytoplasmic RNA-protein complex that contains lncRNA NRON, a scaffold protein IQGAP1, and three NFAT inhibitory kinases CK1, GSK3, and DYRK.[40] Subsequent studies confirmed that knockdown of lncRNA NRON results in nuclear accumulation of NFAT,[39] suggesting that NRON functions as a transcription repressor by inhibiting nucleocytoplasmic shuttling of NFAT. Collectively, it appears that lncRNAs such as NRON can function as a transcription regulator for immune regulation.

Lnc-IL7R

Through microarray assay of human lncRNA in LPS-stimulated monocytic THP-1 cells, a recent study identified a novel lncRNA, namely, lnc-IL7R that is transcribed from the 3′UTR of IL-7R in the sense orientation.[28] The expression of lnc-IL7R is rapidly increased following LPS stimulation. The levels of lnc-IL7R are also elevated in LPS- or Pam3CSK4-stimulated human peripheral blood mononuclear cells. Lnc-IL7R has been shown to negatively regulate expression of IL-7R, IL-6, IL-8, E-selectin and VCAM-1. Furthermore, the study showed that lnc-IL7R knockdown diminished trimethylation of histone H3K27 at the proximal promoters of the inflammatory mediators, suggesting that lnc-IL7R epigenetically regulates inflammatory responses.[28]

IL1β-eRNA, IL1β-RBT46 and antisense transcript of IL1β

Recent studies identified multiple non-coding transcripts that are located close to the IL-1β gene, including antisense-transcript of IL-1β (anti-IL1β transcript), IL1β-eRNA, and IL1β-RBT46.[27,41] The anti-IL1β transcript and IL-

1β gene are in head-to-head positions. Moreover, the non-coding anti-IL-1β is transcribed from the 5′ upstream promoter sequence of the coding gene IL-1β.[41] In mouse macrophages, the expression of anti-IL1β transcript is dynamically regulated during LPS-induced macrophage activation.[41] The ectopic overexpression of anti-IL1β transcript significantly suppressed LPS-induced IL-1β expression in RAW264.7 cells.[41] The anti-IL1β transcript shows to modulate the chromatin structure surrounding IL-1β promoter by decreasing H3K4 trimethylation.[41] Together, antisense IL-1β seems to function as a natural antisense transcript of IL-β gene to regulate the homeostasis of IL-1β in cells.

Most recently, Ilott et al identified a large amount of long non-coding RNAs including 76 enhancer RNAs (eRNAs), 40 canonical lncRNAs, 65 antisense lncRNAs, and 35 regions of bidirectional transcription (RBT) in LPS-stimulated human monocytes.[27] Of particular interest, genomic region surrounding inflammatory cytokine IL-1β gene displays high transcriptional complexity. Situated within IL-1β locus, a downstream eRNA, namely, IL1β-eRNA and an upstream mRNA-flanking RBT called IL1β-RBT46 have been identified.[27] Further studies showed that both IL1β-eRNA and IL1β-RBT46 are predominantly localized in the nucleus of naive and LPS-stimulated cells. In addition, the expressions of IL1β-eRNA and IL1β-RBT46 are mediated by NF-κB, a classical proinflammatory transcription factor.[27] Interestingly, knockdown of IL1β-eRNA and IL1β-RBT46 selectively attenuates LPS-induced expression of proinflammatory mediators including IL-1β and CXCL8 through unknown mechanisms in human monocytes.[27] Given the genomic position of these eRNAs and lncRNAs, they are predicted to function as important regulators of the immune response, while the underlying mechanisms remain to be determined in futures.

Regulatory lncRNAs that modulate immune gene expression via influencing activity of transcription factors and other proteins

LncRNAs have been shown to physically interact with transcription factors, structural proteins, and RNA binding proteins (RBPs), which in turn contributes to regulate activity and function of these molecules.[29] In addition to regulation of a gene transcription, lncRNAs can also exert their role at the protein level.[16] They can function as scaffolds for protein complex and coordinate the gene expression at post-transcriptional level.[21] Here, we provide the following examples in regards to this notion in the immune system.

LincRNA-Cox2

LincRNA-Cox2 is located 51 kb upstream of the protein-coding gene for human cyclooxygenase 2 (COX2, also known as prostaglandin-endoperoxide synthase 2 or Ptgs2). The expression of LincRNA-Cox2 is markedly upregulated in macrophages and dendritic cells challenged by microbial pathogens and various TLR ligands such as LPS, Pam3CSK4, and R848 in MyD88 and NF-κB dependent manner.[15,16] Silencing of lincRNA-Cox2 does not alter Cox2 (Ptgs2) expression, but causes increase in expression of several immune responsible genes in resting macrophages, including chemokines (Ccl5, Cl3cl1), chemokine receptors (Ccrl), and IFN-stimulated genes (Irf7, Oas1a, Oas1l, Oas2, Ifi204 and Isg15). Interesting, Carpenter et al recently showed that lincRNA-Cox2 is required for the induction of other immune-related genes, such as IL-6, Tlr1, and IL-23a in bone marrow-derived macrophages by Pam3CSK4 treatment.[16] Thus, it appears that lincRNA-Cox2 plays a role in either activation or repression of expression of immune-regulatory genes in macrophages. Previously, lincRNA-Cox2 is found to repress transcription of target genes through its interactions with heterogeneous nuclear ribonucleoprotein (hnRNP) A/B and A2/B1.[16] RNA binding protein family of hnRNPs are multifunctional proteins which play a pivot role in the processing of precursor mRNA and regulating gene expression.[42] While the interaction between lincRNA-Cox2 and hnRNPs may contribute to regulation of gene expression, the precise mechanism of lincRNA-Cox2 mediated gene activation is still unclear. With availability of lincRNA-Cox2 knockout mouse,[43] future studies are needed to clarify the *in vivo* functions and mechanisms of lincRNA-Cox2 in the immune responses.

PACER

PACER (p50-associated Cox2 extragenic RNA) is another well-known lncRNA that resides within COX2 genomic locus in humans. It is located directly upstream of the Cox2 transcriptional start site and expressed in the antisense direction. The PACER homolog in mice has also been identified as Cox2-divergent (*Ptgs2os*). In mouse embryonic fibroblasts, the expression of lncRNA Cox2-divergent is highly induced by proinflammatory cytokines (TNFα and IL-1β) and various TLR agonists such as Pam3CK4, HKLM, Poly(I:C) and LPS. Interestingly, Cox2-divergent shows similar upregulated expression patterns upon the cytokine/TLR agonist stimulations in $RelA^{-/-}$ MEFs as compare to wild type MEFs, suggesting lncRNA Cox2-divergent is not directly regulated by NF-κB component RelA.[21] Furthermore, Krawczyk and Emerson reported that lncRNA Cox2-divergent homolog PACER are expressed in primary human mammary epithelial cells (HMECs) and in human monocytes that are undergoing macrophage differentiation induced by PMA. They further revealed that PACER is involved in regulation of COX-2 gene expression.[30] Interestingly, PACER is recently found to physically interact with NF-κB p50 and sequester the transcription factor binding to promoters of target genes such as COX2, suggesting that it is involved in regulation of NF-κB signaling.[30] Meanwhile, this event facilitates the recruitment of histone acetyltransferase p300 and assembly of RNA polymerase II pre-initiation complex at COX2 promoter. PACER expression is induced by chromatin boundary/insulator factor CCCTC-binding factor (CTCF), which in turn establishes a permissive chromatin environment in the upstream region of COX2.[30] Taken together, these studies suggest that PACER lncRNA is engaged in multiple processes related to regulation of immunogene expression.

Lethe

lncRNA Lethe is a Rps15a pseudogene (Rps15a-ps4). Lethe was first identified as a functional pseudogene through genome wide sequencing of TNFα-stimulated mouse

embryonic fibroblast cells.[21] Lethe levels are markedly increased in response to stimulation with proinflammatory cytokines such as TNFα and IL-1β, and glucocorticoid receptor agonists such as dexamethasone, but the expression of Lethe is not responsive to TLR agonist challenges.[21] Lethe has recently been localized in chromatin. It is expected that Lethe can function as a negative regulator of NF-κB by physically binding to RelA (p65), resulting in the inhibition of RelA binding capacity at the target gene promoters, thus regulates the NF-κB target gene expressions, such as IL-6, IL-8 and SOD2.[21] Therefore, Lethe serves as a decoy lncRNA and is a negative feedback inhibitor of NF-κB signaling in inflammation.

Lnc-DC

Dendritic cells (DCs) are antigen-presenting cells which function as messengers linking the innate immune system to the adaptive immune system. A recent genome wide screening has uncovered a cohort of lncRNAs which are differentially expressed during development of human DCs. Of particular interest is lnc-DC which has been revealed to be dramatically induced during DC differentiation.[22] The transcription factor PU.1 is shown to control lnc-DC transcription through binding to promotor of lnc-DC gene, suggesting of a mechanistic insight into regulation of lnc-DC expression. Meanwhile, H3K4me3 and H3K27ac are found to activate histone modifications on lnc-DC loci. Thus the accessible chromatin structure may facilitate exclusive expression of lnc-DC in human DCs.[22] Functionally, lnc-DC is required for the differentiation of monocytes into DCs both *in vitro* and *in vivo*. Knockdown of lnc-DC is revealed to disrupt expression of many DC function-related genes, subsequently results in impairment of immune regulations such as antigen uptake, induction of allogenic $CD4^+$ T cell proliferation, and cytokine production.[22] In addition, lnc-DC directly binds to signal transducer and activator of transcription 3 (STAT3), and subsequently maintains phosphorylated STAT3 in its active form through preventing dephosphorylation of Tyr705 by SHP-1.[22] This observation supports the notion that lncRNAs are able to control T cell differentiation through interacting with other signaling molecules in the cell.

THRIL

THRIL (TNFα and heterogeneous nuclear ribonucleoprotein L related immunoregulatory lincRNA) is recently discovered through a custom microarray of the activation of the innate immune response in THP1 monocyte cells. It has been shown that THRIL expression is correlated with inflammation in Kawasaki disease.[19] Using differentiated human macrophage-like THP1 cell model, Li et al identified a panel of differentially expressed lncRNAs associated activation of the cells by Pam3CSK4, a TLR2 ligand.[19] Among them, THRIL is significantly downregulated in response to the stimulation. Furthermore, THRIL is shown to mediate the effect of Pam3CSK4 on induction of expression of TNFα, IL-6, IL-8, CXCL10, CCL1 and CSF1, suggesting its role in immune regulation.[19] In addition, THRIL is found to specifically interact with heterogeneous nuclear ribonucleoprotein L (hnRNPL). The resultant of THRIL-hnRNPL complex can bind to TNFα promoter and regulate its transcription in both basal and Pam3CSK4-stimulated conditions. Interestingly, the expression of THRIL can be inhibited by TNFα.[19] Therefore, THRIL is a novel negative feedback regulator for termination of TNFα expression in inflammation. The involvement of THRIL in TNFα expression highlights the significance of lncRNA in the regulation of immune-related gene expression.[44]

LncRNA as a regulator of host-pathogen interaction

The immune system plays an important role in defensing infections from microbial pathogens. Recently, a class of host-encoded lncRNAs such as NEAT1 and NRAV has been identified to play a functional role in controlling the host immune responses upon microbial infection. On the other hand, some microbial species can produce lncRNAs that play pivot roles in pathogen life cycles as well as affecting host-pathogen interactions. Of note, the lncRNA-mediated regulation of host-pathogen interactions during microbial infection has also emerged. Here, we highlighted the following lncRNAs in this category.

NEAT1

NEAT1 (nuclear paraspeckle assembly transcript 1 or nuclear enriched abundant transcript 1) was first identified as an inducible nuclear lncRNA in mouse brain infected with *Japanese encephalitis virus* or *Rabies virus*.[45] Later, it was found that NEAT1 can be dramatically induced in HIV-1 infected T cells as well as influenza virus and herpes simplex virus infected epithelial cells.[46,47] Moreover, treatment with TLR3 ligand poly I:C mimics the effect of viral infection on stimulation of lncRNA NEAT1 expression.[46] In addition, NEAT1 is shown to bind to SFPQ, a paraspeckle protein, and play an essential role in nuclear paraspeckle body formation. Recently, Imamura et al demonstrated that SFPQ silences IL-8 expression through binding to IL-8 promoter in normal physiological states.[46] In response to viral infection, induction of NEAT1 results in relocation of SFPQ from the IL-8 promoter to paraspeckles followed by triggering transcriptional activation of IL-8.[46] In addition, NEAT1 can regulate HIV-1 replication through affecting the nucleus-to-cytoplasm export of Rev-dependent instability element (INS) containing HIV-1 mRNA.[47] Taken together, lncRNA NEAT1 plays an important role in the innate immune response to viral infection.

NRAV

NRAV (negative regulator of antiviral) is recently discovered as a key regulator of antiviral innate immunity through a genome-wide lncRNA profiling in influenza virus A/WSN/33 (H1N1) infected human alveolar epithelial A549 cells.[48] The down-regulation of lncRNA NRAV is revealed to be associated with infections by numerous viruses including ssRNA virus such as influenza A virus (IAV) and Sendai virus (SeV), dsRNA virus such as Muscovy Duck Reovirus (MDRV), and DNA virus such as herpes simplex virus (HSV). Furthermore, NRAV is found to affect virus replication, production and virulence. On the other hand, lncRNA NRAV is involved in inhibiting the initial transcription of multiple interferon-stimulated genes (ISGs), such as IFITM3 and MxA, through epigenetically regulating histone modifications of these genes.[48] Together, lncRNA NRAV seems to play a role in

controlling ISG expression in normal conditions. Upon the virus infection, the reduction of NRAV could benefit the host innate immune response through accumulating antiviral proteins (such as ISGs), thus facilitates the virus clearance.

PAN

Recent studies revealed that microbial species can also express functional lncRNAs. One of well-characterized microbia-derived lncRNAs is PAN RNA (polyadenylated nuclear RNA).[49,50] The lncRNA PAN is encoded by Kaposi's sarcoma-associated herpesvirus (KSHV) genome. It is implicated in KSHV viral gene expression and replication.[51] PAN interacts with demethylases UTX and JMJD3 and recruits histone-modifying complexes to the KSHV genome, thus epigenetically regulates viral gene expression and promotes the switch from latent to lytic infection.[52–54] On the other hand, PAN RNA is involved in regulation of host immunity. The viral lncRNA PAN suppresses expression of host genes involved in the inflammatory and antiviral response, including IFNγ, IL-18, IFNα16, and RNase L.[54] A recent report showed that PAN can physically interact with polycomb group proteins, such as PRC2 and mediate repression of host cellular gene expression.[32] Taken together, PAN is a multifunctional viral lncRNA involved in regulation of both viral and host gene expression.

Regulatory lncRNAs that affect immune function via unknown mechanisms

With the expanding list of lncRNAs, growing evidence shows that lncRNAs contribute to various aspects of gene regulations at both transcriptional and post-transcriptional levels in the immune system. However, our knowledge on how immune modulatory lncRNAs function is still limited. Many lncRNAs display aberrant expression patterns during activation of immune responses and development of immune cells with unclear mechanisms. Recently, genome-wide RNA-seq profiling of mouse T cells identified 1524 lincRNA clusters from early T cell progenitors to terminally differentiated T helper subsets.[24] Among those lncRNAs, LincR-Ccr2-5′AS is found to regulate transcription of several chemokine receptor genes and required for T_H2 cell migration. However, knockdown of LincR-Ccr2-5′AS does not affect the epigenetic marks and chromatin accessibility of its target genes. It seems that the exact mechanism of action for LincR-Ccr2-5′AS is currently unknown.[24] The rapid increasing of genome-wide transcriptome dataset has uncovered a fast growing list of lncRNAs. Some of them have been verified as functional lncRNAs in immune regulation. Thus, there is an urgent need to investigate the underlying molecular mechanisms by which immunoregulatory lncRNAs function in futures.

LncRNA and immune-related diseases

Evidence has shown the linkage between lncRNAs and immune-related diseases such as autoimmune disorder sand infections. For example, it has been reported that lncRNAs H19 and HOTAIR are up-regulated in rheumatoid arthritis.[55,56] Genetic evidence suggests that genomic regions for numerous lncRNAs such as IGF2-AS and lncRNA MEG3 are associated with the susceptibility for diabetes.[57,58] In addition, a group of pathogen-derived long non-coding RNAs, namely, infectious lncRNAs are expected to be engaged in infection processes.[59] Furthermore, genome-wide association studies have revealed the linkage between various single nucleotide polymorphisms (SNPs) and autoimmune or immune-related diseases.[60] Among them, approximately 10% of disease-associated SNPs are mapped to genomic loci encoding lncRNAs.[61,62] Thus, lncRNAs are speculated to play a role in the etiology of immune-related diseases.[63] In recent years, rapid advances in the technology of next-generation sequencing and transcriptome analysis have provided a set of powerful tools for determining the association of lncRNAs to immune-related diseases. For instance, Hrdlickova et al recently revealed the lncRNA enrichments in several immune-related disorders including inflammatory bowel disease, celiac disease, juvenile idiopathic arthritis, primary biliary cirrhosis, psoriasis, primary sclerosing cholangitis and rheumatoid arthritis using RNA-seq technology.[64] Shi et al have found aberrant expression of a couple of candidate lncRNAs in patients with systemic lupus erythematosus via analysis of transcriptome profiles in peripheral blood mononuclear cells derived from the patients.[65] In addition, the association between abnormal expression of lncRNAs and inflammatory diseases such as obstructive pulmonary disease and inflammatory bowel disease has been further revealed using lncRNA microarray analysis.[66,67] While the comprehensive and valuable list of immune disorder-related lncRNAs is still growing, however, determining of the role of candidate lncRNAs in pathogenesis of immune-regulated diseases remains crucial. Elucidating molecular mechanisms underlying regulation of inflammation by lncRNAs will provide insights into current understanding of immune-related diseases and ultimately lead to novel therapeutic strategies.

Future perspectives

The emergence of lncRNAs as important regulators of gene expression has shed light on our understanding of the link between RNA world and immune regulation (Fig. 1). However, the research on roles of lncRNAs in immunity is still in its infancy. Evidence suggests that immune-regulatory lncRNAs can exert their functions through several mechanisms that have been discussed above. With a rapid research progress in study of mammalian transcriptome, future studies will undoubtedly uncover additional and novel insights into lncRNAs functions in immunity. Meanwhile, it is worth to mention the following three important research topics that need to be addressed in the field of immune-regulatory lncRNAs in futures.

First, the role of crosstalk between miRNAs and lncRNAs in immune regulation is a potential interest area in this field.[68] miRNAs are a group of important post-transcriptional gene expression regulators that play a critical role in various aspects of immune responses. Recently, a novel finding shows that both coding and long non-coding RNAs can co-regulate and communicate with each other

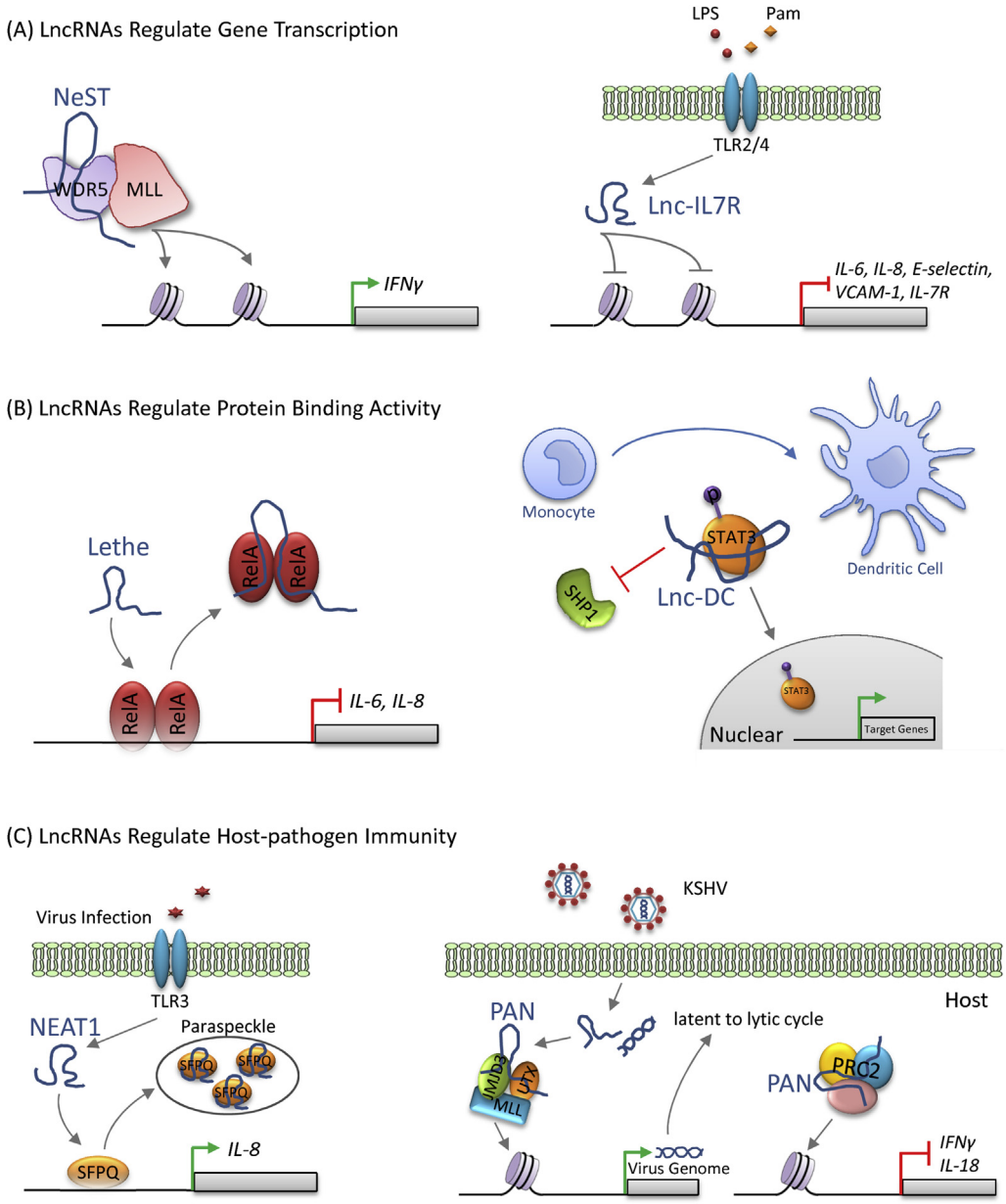

Fig. 1 Schematic diagram of distinctive mechanisms underlying lncRNA-mediated immune regulation. (A) LncRNAs are involved in transcriptional regulation of expression of cytokines and adhesion molecules. Left panel: LncRNA NeST epigenetically regulates IFN-γ expression via interaction with WDR5, a core subunit of the histone H3K4 methyltransferase complex.[31] Right panel: Pathogen-associated molecular pattern-induced Lnc-IL7R epigenetically regulates expression of inflammatory mediators and adhesion molecules via trimethylation of histone H3K27 at the proximal promoters of target genes.[28] (B) LncRNAs regulate cytokine expression and dendritic cell differentiation via binding to transcription factors. Left panel: Lethe physically interacts with RelA, which in turn prevents binding of RelA to promoters of NF-κB target gene such as IL-6 and IL-8.[21] Right panel: Lnc-DC is required for the differentiation of monocytes into DCs. Lnc-DC directly binds to STAT3 and maintains the phosphorylation of STAT3 through inhibiting its interaction with phosphatase SHP-1.[22] (C) LncRNAs regulate host-pathogen immunity. Left panel: In normal physiological state, SFPQ represses IL-8 expression through binding to IL-8 promoter. In response to viral infection, induction of NEAT1 results in relocation of SFPQ from the IL-8 promoter to paraspeckles and subsequently leads to transcriptional activation of IL-8.[46] Right panel: Infectious lncRNA PAN encoded by KSHV viral genome promotes switching of the virus from latent to lytic infection through recruitment of histone-modifying complexes to the KSHV genome.[53] PAN also physically interacts with polycomb group protein PRC2 and mediates repression of host inflammatory and antiviral gene expression.[32]

through competing the binding of shared miRNAs. In other words, lncRNAs can act as a molecular sponge for miRNAs and thus regulate the protein coding gene expression.[69] In addition, it has been suggested that lncRNAs can modulate the miRNA processing through base-pairing with primary miRNA, which in turn results in inhibiting the miRNA maturation.[70] The emerging roles of RNA—RNA crosstalk between diverse RNA species will lead to the new insights

of gene regulation network. The implication of this novel concept in regulation of immunity needs further investigations.[71]

Second, defining functions of immune-related lncRNAs *in vivo* using animal models is important for precisely understanding roles of lncRNAs in immune response.[1,2] However, lack of conservation of lncRNAs across species is a major hurdle for further elucidating roles of human lncRNAs in health and diseases using animal models. Thus, development of powerful bioinformatics tools is required for identification of the rapid evolving lncRNAs and their homologs across species. In addition, it is expected that new technologies for functional characterization of lncRNAs will advance our research in the field. Recently, a group of investigators developed novel technology to trace RNA molecules in living cells.[72] In addition, Paige et al used Spinach, an RNA-fluorophore complex which is a kind of RNA aptamers that bind to fluorophores of green fluorescent protein, to encode the fluorescent specific RNA molecules and image their localization in living cells.[73] It appears that this technology can be applied to advance our knowledge on live-imaging lncRNA *in vivo* in futures.

Last but not the least, it has been suggested that lncRNAs might be translated and coded for peptides or small proteins.[12,74] Therefore, it would be interesting to determine coding potentials for lncRNAs and roles of their coding peptides in immune regulation. Recent studies have shown that some lncRNAs can be bound by ribosomes, raising the possibility of coding potential of those ribosome-associated lncRNAs.[75] However, noncoding RNAs and 5′ UTRs of coding mRNA possess the similar ribosome occupancy patterns, indicating that ribosome occupancy alone is not sufficient to serve as a good indicator for testing the potential of lncRNA translation.[76] Indeed, a robust and sensitive method is needed to evaluate the lncRNA coding potential in futures.

Overall, the aberrant expression of lncRNAs has been revealed in inflammation, autoimmune and other immune-related diseases. LncRNAs are emerging as a group of important regulators for immune responses via multiple mechanisms. Undoubtedly, clarification of how lncRNAs influence diverse biological processes and further determination of relationships between regulatory lncRNAs and host-pathogen interactions has become excitingtopics in the field of immunology.

Conclusions

In this review, we discussed several examples from recent discoveries in regards to the functional diversity of lncRNAs in the immune system. With advances technology and methodology in genomic research, novel the discovery on lncRNAs are expected to impact our understanding on regulation of immunity, inflammation, infections. Steadily increasing evidence suggests that regulatory functions of lncRNAs have emerged as an additional layer for regulation of gene expression at both transcriptional and post-transcriptional levels. Future studies are needed to elucidate how lncRNAs regulate immune system. Given the rapid pace of lncRNA researches, additional novel mechanisms and concepts will emerge in the near future.

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Conflict of interest

All authors declare that they have no conflict of interests to disclose.

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