



The role of long noncoding RNAs in human T CD3+ cells

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ABSTRACT

Long noncoding RNAs (lncRNAs) are regulatory RNA molecules that are involved in various biological processes. In the immune system, the lncRNAs play important roles in development, differentiation, survival, cell fate determination, proliferation and activation of immune cells. Lymphocytes are the main players of the adaptive immunity and CD3+ T cells acts as a master regulator for the immune responses. These cells following activation by antigens and co-stimulatory signals are differentiated into various effector T cell subsets, including CD4 and CD8 T cells. These heterogeneous populations can be distinguished based on molecular surface markers and subsets of these markers can be used to denote various stages of T lymphocyte differentiation, notwithstanding the CD3+ T cells phenotypes are markedly influenced by lncRNAs. In the present review, we summarize recent research on the role of long noncoding RNAs in subtypes of CD4+ and CD8+ human T cells.

Noncoding RNAs (ncRNAs) are regulatory RNA molecules that do not code for proteins even though they affect and regulate a range of biologic processes. The ncRNAs can control several genes and are classified according to their size. They may be small, such as microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs), or long, such as long non-coding RNAs (lncRNAs) and long intergenic ncRNA (lincRNA)¹. Long noncoding RNAs (lncRNAs) are defined as greater than >200 nucleotides in length, and the most of those currently described are transcribed by RNA polymerase II, therefore they are polyadenylated, and harbor the N-terminal added of a methylated guanosine nucleotide (5'cap)².

The positioning of the lncRNAs genes in the genome is the parameter used to further classify these molecules, which can be divided into categories, such as: sense - lncRNAs that overlap one or more exons of a given transcript on the same strand; antisense - lncRNAs that overlap one or more exons of a transcript on the opposite strand; divergent - lncRNAs that initiate transcription on the opposite strand of a gene; bidirectional - when their expression and the expression of a neighboring coding transcript is initiated in close proximity; intronic - when they lay in an intron; intergenic - if they lie within a sequence between two genes^{3,4,5}.

The progress in the studies of transcriptomes allowed the discovery and characterization of several ncRNAs and deepened knowledge about the involvement of these molecules in various human biological pathways. These studies also showed that

ncRNAs regulate the gene expression at transcriptional, posttranscriptional and epigenetic levels³.

It is currently admitted that the differentiation of several lineages of the immune system are under regulation of lncRNAs⁶, such as monocytes, macrophages, dendritic cells, neutrophils, B and T lymphocytes⁷. Lymphocytes are the main regulators cells of the adaptive immunity and CD3⁺ T cells play a vast role in immune responses. These cells following activation by antigens and co-stimulatory signals are differentiated into various effector T cell subsets, including CD4 and CD8 T cells.

T cells play a central role in the regulation of the immune system and part of this function is performed by lncRNAs. In the thymus, each stage of differentiation of T cells is characterized by a unique pattern of expression of specific lncRNAs⁸. The identification of lncRNAs and their target genes that regulate T lymphocytes is important for the development of new therapies for autoimmune diseases, allergies, organ transplantation and cancer⁴. The purpose of this review is to describe the diversity of mechanisms used by lncRNAs to regulate T cell function and differentiation (**Table 1**).

NRON

The ncRNA repressor of NFAT is a lncRNA that inactivates the transcription factor NFAT under normal organism conditions. NFAT is a Ca²⁺ dependent transcription factor and controls the IL-2 production in activated T cells. In non-activated cells, NFAT is phosphorylated and inactive⁶. In response to increased intracellular Ca²⁺, the calcineurin enzyme dephosphorylates NFAT, allowing its translocation to the nucleus, where it will activate transcription of target genes, such as IL-2⁸. NRON is part of a ribonucleoprotein

complex that retains the NFAT factor in the cytoplasm in its phosphorylated form, preventing it from nuclear translocation. NRON is essential to maintain the structure of this protein complex, since in the absence of NRON the complex is not formed, and NFAT is dephosphorylated and routed to the nucleus where it performs its role as a transcription factor^{6,8,9}.

NEST

NeST, also known as IFNG-AS1 or Tmevpg1, is a lncRNA expressed in CD4⁺, CD8⁺ T and NK cells¹⁰. Expressed in the Th1 cell, this lncRNA is an antisense transcript in the locus encoding IFN γ , acting in *cis*, as an enhancer, to stimulate the expression of IFN γ ¹¹. Once NeST is involved in the regulation of Th1 differentiation, this lncRNA depends on the expression of T-bet and STAT4 transcription factors¹².

NeST binds to WDR5 subunit of the histone H3K4 methyltransferase complex altering by epigenetic mechanisms, methylation of the locus of the IFN γ gene, regulating its expression¹³.

Linc-MAF-4

Another lncRNA involved in T cell differentiation in Th1 lineage is MAF-4 (linc-MAF-4) long intergenic noncoding RNA. Linc-MAF-4 represses the expression of the MAF gene that in turn, encodes a transcription factor that promotes the expression of genes encoding specific cytokines of the Th2 line of CD4⁺ T lymphocytes¹⁴.

Linc-MAF-4 disrupts the differentiation of T cells into Th2 and promotes Th1 lineage differentiation⁶. Linc-MAF-4 and MAF genes lays close to each other in the

Table 1. lncRNAs involved in the functioning of CD3⁺ T cells.

lncRNAs	Target	Function	T cell model	References
NRON	NFAT	Inactivates the NFAT transcription factor	CD4 ⁺	6, 8, 9
NeST, IFNG-AS1 ou Tmevpg1	IFN γ	Regulates Th1 differentiation	Th1, CD8 ⁺	10 – 13
MAF-4 (linc-MAF-4)	MAF gene	Involved in T cell differentiation in Th1 lineage	Th1	6, 8, 14
Th2LCRR	Th2 cytokines	Regulates the Th2 cell polarization	Th2	8, 15
lincR-Ccr2-5' AS	Th2 cytokines	Controls the expression of genes related to the response of the Th2 subtype	Th2	11, 16
GATA3-AS1	?	Involved in allergic or asthmatic responses	Th2	8, 15, 17
RMRP	ROR γ t	Regulates the effector function of ROR γ t in several Th17 specific genes	Th17	6, 18, 19, 20
Flicr	FOXP3	Controls the regulatory T cells activity	Treg	21, 22
HULC	P18	Trigger the regulatory T cells differentiation in HBV related liver cirrhosis	Treg	23, 24
lnc-EGFR	EGFR receptor	Stimulates Treg differentiation, suppress TCD8 ⁺ and induces tumor growth in HCC patients	Treg	25
Linc-POU3F3	TGF- β	Regulates Treg distribution in gastric cancer	Treg	26
lncRNA-CD244	IFNG and TFN genes	Inhibits the expression of IFN- γ and TNF- α in tuberculosis	CD4 ⁺ , CD8 ⁺	8, 27, 28
lncRNA NTT	?	?	CD4 ⁺	11, 29
lincRNA-p21	NF- κ B	Negative regulator of NF- κ B activity	CD3 ⁺	8, 30

human chromosome 16, which facilitates the action of the linc-MAF-4 in *cis* to regulate gene expression⁶. Linc-MAF-4 associates with chromatin modifiers, such as LSD1 and EZH2, to facilitate the trimethylation of histone H3K27 markers in the MAF gene promoter, by forming a loop in the chromatin, and thereby silencing its expression in Th1 cells⁸.

Th2LCRR

The lincRNA Th2LCRR is co-expressed with the cytokines that characterize Th2 polarization: IL-4, IL-5, IL-13 and is transcribed as an antisense of the RAD50 gene¹⁵. This lincRNA binds to the WDR5 component of the histone H3K4 methyltransferase complex to maintain the epigenetics markers in the interleukins IL-4, IL-13, IL-15 promoters, and thereby regulate T cell polarization⁸.

lincR-Ccr2-5'

The lincR-Ccr2-5' AS is transcribed in the antisense direction of the Ccr2 gene and controls the expression of genes related to the response of the Th2 subtype¹¹. The knockout of this lincRNA negatively alters the expression of chemokine receptor genes such as Ccr1, 2, 3 and 5, which are located at the same genomic *loci* as the non-coding intergenic Ccr2-5'AS¹⁶.

GATA3-AS1

Another lincRNA characteristic of Th2 response, is the divergent lincRNA GATA3-AS1¹⁷. Transcribed in both human and mouse genome, GATA3-AS1 is involved in allergic or asthmatic responses in humans, having its expression induced by allergens¹⁵. The involvement of GATA3-AS1 in certain forms of breast cancer and acute lymphoblastic leukemia in children has also been studied⁸.

RMRP

T cells that differentiate into the Th17 subtype are involved in the pathogenesis of autoimmune diseases and play an important role in the defense against extracellular pathogens¹⁸. The transcription factor ROR γ t is responsible for inducing this differentiation pathway, along with the cytokines IL-6 and TGF- β . ROR γ t can be activated by the DEAD box RNA helicase DDX5, an important catalyst for unfolding of RNAs, and this association, regulates the transcription of genes involved in the Th17 response⁶.

The interaction of ROR γ t factor with DDX5 requires the RMRP lincRNA (RNA component of the Mitochondrial RNase complex) within the nucleus. This DDX5-RMRP complex is essential for regulating the effector function of ROR γ t in several Th17 specific genes. LincRNA acts in *trans* in the genes dependent on this transcription factor, with the aid of the DDX5 helicase¹⁹.

The discovery of this RMRP-DDX5 complex in the regulation of ROR γ t showed two important factors: the

complexity in Th17 cells and the possibility of other immune cells using the association of ncRNAs with RNA helicases to control gene expression²⁰.

Flicr

Recent studies have shown the presence of lincRNAs in regulatory T cells (Tregs). Flicr (FOXP3 long intergenic noncoding RNA) is a lincRNA that negatively regulates the expression of FOXP3 transcription factor in regulatory T cells of humans and mouse^{21,22}. Zemmour et al., suggests that the regulatory mechanism of lincRNA repression is related to changes in local accessibility of the FOXP3 gene in its conserved noncoding sequence 3 (CNS3) by controlling regulatory T cells activity, Flicr can trigger autoimmune diseases such as diabetes²¹. Since it is a newly discovered lincRNA, there is still scarce information concerning its functions and mechanisms of action.

HULC

Another lincRNA associated with regulatory T cells, is the lincRNA HULC (highly up-regulated in liver cancer). First described by Panzitt *et al.*, in hepatocellular carcinoma (HCC), this cytoplasmic lincRNA plays a role in the posttranscriptional modulation of gene expression in HCC cells²³. HULC was also studied in hepatitis B virus (HBV) related liver cirrhosis, an infection that can progress to the liver fibrosis stage, with an increase of CD4⁺ T cells, as described by ZHAO *et al.* These authors also suggested that the lincRNA HULC could trigger the regulatory T cells differentiation in HBV related liver cirrhosis. Also, HULC can down regulate the levels of the p18 protein that impairs the differentiation of regulatory T cells²⁴.

linc-EGFR

EGFR (epidermal growth factor receptor), a component of the receptor tyrosine kinase family, plays a role in the development of cancer, acting in the tumor microenvironment regulating the immune system cells²⁵. An EGF Receptor binding RNA, linc-EGFR, seems to block its ubiquitination, triggering a cascade of activation, differentiation or suppression of certain cells²⁵. Among T lymphocytes, the linc-EGFR was shown to be upregulated in regulatory T cells in HCC patients. Also, it can stimulate the differentiation of regulatory T cells, as well as to suppress the activity of cytotoxic T cells, stimulating tumor growth. JIANG et al., suggested that the interaction between linc-EGFR and regulatory T cells is a common pathway during the tumorigenesis process²⁵.

Linc-POU3F3

Linc-POU3F3 is a lincRNA found in T regulatory cells of gastric cancer patients. When it is upregulated, this lincRNA reorganizes T regulatory cells distribution, allowing gastric cancer cells proliferation. This mechanism occurs through recruitment of TGF- β and the activation of its signaling

pathway. These characteristics may pave the way for further exploration of lncRNAs function in immunotherapy of gastric cancer²⁶.

NTT

NTT (non-coding transcript in T CD4⁺ cells) was one of the first lncRNAs identified in immune cells, being expressed in activated human T CD4⁺ cells¹¹. Although its function has not yet been defined, it is known that there may be a link between NTT and the IFN- γ Receptor gene, since they share the same genomic *locus* and have similar expression profile during the activation of T lymphocytes^{11,27}.

LincRNA-p21

LincRNA-p21 was first described to be involved with the p53 protein in response to DNA damage, however, it is also known that these lincRNA has its reduced levels in inflammatory diseases such as rheumatoid arthritis (RA) and coronary artery disease, which depend on the T cells⁸. Spurlock et al. demonstrated the role of lincRNA-p21 as a negative regulator of NF- κ B activity in RA patients, where they also suggested that the expression of these lincRNA in RA can contribute to disease-dependent inflammation²⁸.

CD8⁺ associated lncRNA

In a study using custom microarray probes, Pang *et al.* unveiled the expression profile of various lncRNAs in mammalian T CD8⁺ lymphocytes, that have not yet been well characterized in the context of CD8 T cell immunity. Most of these molecules are lymphoid-specific and may undergo changes depending on events that occur within lymphocytes, such as differentiation or activation²⁹.

CD244 lncRNA

Some lncRNAs are not exclusive to T CD4⁺ cells, but their functions influence the development and activation of these cells, such as CD244 lncRNA. The CD244 costimulatory receptor regulates some functions of natural killer cells, is present in CD8⁺ T cells, and its persistent signaling is associated with some human viral infections such as Hepatitis B and C³⁰. In tuberculosis, this receptor is involved in inhibiting the expression of cytokines such as IFN- γ and TNF- α , by inducing the expression of the lncRNA-CD244, that regulates the repression of the IFNG and TFN genes by epigenetic mechanisms. This lncRNA may represent an important target for therapeutic intervention in tuberculosis^{8,30}.

Studies on the functions and mechanisms of action of lncRNAs are becoming trendy. Involved in several pathologies and related to various cellular subtypes, as was shown here, these molecules are being proposed as targets of therapies and diagnoses for diseases such as infections, autoimmune diseases, cancer⁸, asthma and severe allergic disease². However, questions such as how the lncRNAs interfere with the pathogen-host relationship,

or how they act in various cellular processes still need to be established⁹.

Conclusion

In this review we updated the involvement of several lncRNAs in CD3⁺ T cells, where they are involved in development, differentiation, activation and effector functions. As a relatively new subject, several lncRNAs, mechanisms of action and functions have not yet been elucidated. However, advances in bioinformatics studies and new experimental analysis technologies may facilitate the discovery of new lncRNAs and a deeper understanding of those that are already known. For the immune system, lncRNAs may be important therapeutic targets for the treatment of various diseases, such as autoimmunity, infectious disease and even cancer.

Conflict of interest

The authors declare no conflict of interest.

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