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The Significance of Long Non-Coding RNAs in Regulating Inflammatory Responses

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ABSTRACT

As part of the innate immune system, inflammation is a complex process that increases vascular permeability to eliminate pathogens and assist in the body's healing process. Recently, the field of immunogenetics has identified long non-coding RNAs (lncRNAs) Gastric Adenocarcinoma Associated Positive CD44 Regulator Long Intergenic Non-Coding RNA (GAPLINC) and long intergenic non-coding RNA-Cyclooxygenase 2 (lincRNA-Cox2), with roles to regulate inflammation through the NK-kB pathway, transcription of immune genes, and macrophage activity. More specifically, GAPLINC regulates the translational ability of NF-κB subunit p65 and the functions of macrophages such as phagocytosis, antigen uptake, differentiation, and migration. On the other hand, lincRNA-Cox2 regulates the expression of proximal gene PTGS2 through an eRNA mechanism and acts as a scaffold for synthesizing the SWI/SNF complex.

Introduction

The human body is constantly exposed to microorganisms including bacteria, viruses, fungi, and parasites. To protect itself, the body developed a complex defense system known as the immune system which can be broadly divided into two branches: the innate and adaptive immune system (Institute for Quality and Efficiency in Health Care, 2023). Both branches are necessary for identifying, targeting, and eliminating harmful pathogens and maintaining the body's tissue health and homeostasis.

The innate immune system is quick to react, executing the same response against all detected pathogens, thus referred to as the "general" or "nonspecific" immune response. Several key structures, such as the skin and mucosa, form a physical barrier and prevent pathogens from entering. Pathogens are recognized by structures on their surface, pathogen-associated molecular patterns (PAMPs), by immune cells using Pattern Recognition Receptors (PRRs) (Węgiel et al., 2015). Macrophages, one example of an immune cell known to recognize pathogens, are phagocytes that circulate through several tissues, patrolling for pathogens using toll-like receptors (TLRs) (Hoppstädter et al., 2019). Once activated, TLRs induce a pro-inflammatory signaling cascade resulting in the activation of the transcription factor nuclear factor-kappa B (NF-κB).

When the innate immune system fails to clear pathogens, the adaptive immune system targets the specific invader. Unlike the innate immune system, the adaptive immune system remembers past encounters with the same pathogens, contributing to the body's memory immune response (National Cancer Institute, 2011). The onset of the memory immune response is faster, and the response is more vigorous than the first immune response against the specific pathogen (Alberts, 2002). The adaptive immune system comprises of B and T lymphocytes, however, their specific roles are not discussed in this review.

Included among the plethora of innate immune responses, acute inflammation is a short-term response to tissue damage as a result of bacteria, physical trauma, toxins, and others (MedlinePlus, 2018). The primary purpose of an acute inflammatory response is to eliminate the stimuli and to facilitate the body's healing process. Initiated by releasing inflammatory mediators, blood vessels are increased in diameter, allowing for greater blood flow, vascular

permeability, and therefore, greater access of immune cells to the site of infection. Thus, common symptoms of inflammation are swelling, heat, and redness (David Zelman, 2020). Given its complexity, the inflammatory response demands precise regulation, highlighting the importance of understanding its key regulators. Recent research has revealed that long non-coding ribonucleic acids (lncRNAs) are among the many regulators of the inflammatory response.

LncRNAs stand as one of the most intriguing discoveries in modern genomics, challenging former beliefs on the roles of RNA and the central dogma of DNA transcribed into RNA which is translated into protein. Scientists have recently identified lncRNAs and long intergenic non-coding RNAs (lincRNAs) as regulators of the inflammatory response, adding them to a growing list of molecules and proteins known to play crucial roles in immune regulation (Vollmers et al., 2021). Scientists have reported through literature and databases that tens of thousands of lncRNAs exist (approximately 20,000 in humans), yet only a few have experimentally confirmed functions (Walther & Schulte, 2021). Currently, the exact roles of specific lncRNAs remain unclear, leaving much to explore in understanding their full impact on biological processes.

LncRNAs are defined as transcripts that are at least 200 nucleotides in length that do not translate into protein. These molecules are found across many species including plants, animals, yeast, prokaryotes, and viruses, and carry out several significant roles in transcription, splicing, translation, protein localization, cell structure integrity, imprinting, cell cycle, and apoptosis, among many others (Guennewig & Cooper, 2014). Within the scientific community, 'lncRNAs' is often used as an umbrella term to describe its various subcategories, including lincRNAs (Ransohoff et al., 2017). LincRNAs are RNA transcripts that are no more than 200 nucleotides in length and do not code for protein. Unlike other lncRNAs, lincRNAs are transcribed from regions of the genome that do not overlap with annotated protein-coding regions and instead reside in the intergenic regions. These transcripts are found to carry important roles in remodeling chromatin and genome architecture, RNA stabilization, and transcription regulation, including enhancer-associated activity in the context of cellular differentiation, development, and the immune response (Ransohoff et al., 2017).

This review will focus on the current research and understanding of the role of lncRNAs, particularly discussing the role of the two lncRNAs Gastric Adenocarcinoma Associated Positive CD44 Regulator Long Intergenic Non-Coding RNA (GAPLINC) and long intergenic non-coding RNA-Cyclooxygenase 2 (lincRNA-Cox2) and their role in regulating the inflammatory response. A total of four inflammation regulatory mechanisms are detailed here: macrophage migration, differentiation, and polarization, two different modulations of the NF-κB pathway, and enhancer RNA (eRNA) functions for nearby inflammatory genes.

Different Types of RNA and Their Functions

RNAs are single-stranded, complimentary copies of the parent DNA sequence formed during transcription. Although their functions are very diverse, they primarily facilitate protein synthesis via transcription and translation. Three RNAs are used during protein synthesis: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). During transcription, mRNA is created as a nucleotide sequence (transcript) of DNA and will be translated into the amino acid sequence of a protein (Brostrom & Brostrom, 2007). In the cytoplasm, mRNA will bind to ribosomes, a protein made of rRNA, converting the mRNA into proteins. This is known as the central dogma (Ostrander, 2022).

Some RNAs do not code for proteins, such as tRNA and rRNA, making them non-coding RNAs (ncRNAs). mRNAs strictly code for proteins and constitute much of our understanding of RNA to date, however, ncRNAs perform a wider variety of functions such as regulation of gene expression and cellular processes (Zhang et al., 2019). Their regulatory function can be executed at various levels through DNA, RNA, or protein interactions.

Functions of Long Non-Coding RNA

While the biological function of many lncRNAs remains unclear, lncRNA function is determined by silencing, deleting, or knocking out (KO), often using CRISPR interference (CRISPRi) or RNA interference (RNAi), to determine the effect of loss of function of specific genes in a cell culture or organism (Thermo Fisher Scientific, 2019). Some scientists can also choose to overexpress lncRNAs or express genes where they are not typically expressed, known as ectopic expression, so its effects can be observed. Although, these methods of over and ectopic expression are both less common among the scientific community (Walther & Schulte, 2021). LncRNAs, as we know it, are involved in cell differentiation and development, DNA repair, cellular signaling, and hormonal regulation, among the many other functions that have yet to be explored (Guennewig & Cooper, 2014).

As highlighted in this review, many lncRNAs enable gene expression through the activation of different pathways or serve as cis-regulatory eRNA. Below are just a few additional examples of gene regulation:

Epigenetic Regulation and Chromatin Modification

Genetic information, crucial for organism function, is precisely regulated and stored in deoxyribonucleic acid (DNA). With roughly two meters of DNA packed into each human cell, effective gene regulation is essential (Alberts et al., 2015). Gene expression at the epigenetic level involves modifications to the chromatin structure to make it more or less accessible to transcription proteins (MedlinePlus, 2021). One of the many mechanisms of epigenetic regulation of gene expression is chromatin modification, which is the change between an inactive, condensed state to an active, uncondensed state. Condensed chromatin called heterochromatin is not transcriptionally accessible since transcription factors and DNA binding proteins cannot access the site of transcription. On the other hand, transcription factors and other proteins can bind to DNA in uncondensed chromatin or euchromatin, allowing for transcription to occur. This action of condensation of chromatin is controlled by the addition of an acetyl group (acetylation), which neutralizes the positively charged lysine residues on the histones and causes the negatively charged DNA to relax. Removal of acetyl (deacetylation) on the histones results in condensed chromatin, decreasing gene expression (Li, 2002).

LncRNAs can also play roles in the epigenetic regulation of certain genes or chromosomes. Although a complex process, lncRNA X-inactive specific transcript (XIST) has a pivotal role in inactivating one of the two X chromosomes in female cells (Li et al., 2022). This is fundamental to ensure an even distribution of X-linked genes between males and females. Malfunctions in XIST expression may cause the X chromosome to remain active, which leads to protection from some diseases (cancers or COVID-19, for example) or impaired immune response (Li et al., 2022).

Transcriptional Gene Expression

Gene expression at the transcription level controls the formation of mRNA molecules which directly impacts how many mRNAs are translated into protein. One of many mechanisms of gene expression includes the use of enhancer RNA (eRNA) to enhance or repress transcription by interacting with enhancer regions of DNA, transcription factors, and other associated proteins (Sartorelli & Lauberth, 2020). These short non-coding (<2 kb) RNAs are transcribed from tissue-specific enhancer regions and show little evidence of undergoing any splicing or polyadenylation. Although eRNAs have been identified with epigenetic roles, they can also have cis and trans functions that recruit transcription factors to the promoter. It has been identified that eRNAs increase the occupancy of RNA Polymerase II at protein-coding sites, as well as interact with the Yin Yang 1 (YY1) protein to aid in recruiting transcriptional regulators at enhancers (Cooper, 2013).

Post-Transcriptional Regulation of Proteins

Post-transcriptional regulation occurs after mRNA is made into protein. HOTAIR, commonly studied for its role in the spread and progression of cancers, has a scaffold function that plays a role in the ubiquitin-proteasome pathway (UPP) (Yoon et al., 2013). The UPP degrades dysfunctional, misfolded, or unneeded proteins by labeling the protein with a chain of ubiquitin proteins. This polyubiquitinated protein will be recognized by the proteasome and degraded. HOTAIR can control the amount of protein after it has been translated.

Role of lncRNA GAPLINC in Macrophage Activity

GAPLINC is involved in the differentiation and polarization of phagocytic macrophages. Macrophages are specialized white blood cells involved in the detection and destruction of harmful organisms in the body, clearing cellular debris, and wound healing. Macrophages are derived from monocytes, a type of white blood cell that originates in the bone marrow. After maturing, monocytes will enter the bloodstream where they circulate and defend against pathogens. When pathogens enter the body's tissues, monocytes will differentiate into macrophages (Cleveland Clinic, 2021). Once differentiated, macrophages will begin to respond to inflammatory signals and migrate.

The primary function of macrophages is phagocytosis. These phagocytic cells engulf and digest pathogens, dead cells, and debris during the innate immune response or tissue destruction. Moreover, they can also have important roles in tissue repair. When exposed to inflammatory stimuli, macrophages secrete cytokines, which are proteins that regulate inflammation, such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and many others (Arango Duque & Descoteaux, 2014).

Macrophages are known to be plastic, meaning that they can adapt to various environmental signals through differentiation, migration, and polarization (Guilliams & Svedberg, 2021). These functions further highlight different aspects of their adaptability and functionality. Macrophage polarization is the process where macrophages adopt certain characteristics that are specific to the local microenvironment of the inflammatory site. Polarized macrophages can be broadly divided into two categories: Classically activated (M1) and alternatively activated (M2) macrophages, which differ in their cell surface markers expression and cytokines secretion (Yunna et al., 2020). In general, M1 macrophages are involved in pro-inflammatory and M2 macrophages in anti-inflammatory responses (Yunna et al., 2020). An important distinction to be made is that macrophage differentiation is a permanent process while polarization is reversible allowing macrophages to adapt to their environment (Yao et al., 2019).

The function of lncRNA GAPLINC has been demonstrated by Valverde et al. to control the differentiation and polarization of macrophages by observing a significant decrease of differentiation markers CDw93 and CD68 and M1 polarization marker HLA-DR+ in GAPLINC KO cells. Valverde et al. also observed that GAPLINC knockdown cells impair macrophage phagocytosis, uptake of soluble antigen Ovalbumin, and cytokine secretion (IL-1β, IL-6, and TNF-α). As for migration, it is found that GAPLINC knockout leads to impaired migration of GAPLINC-transinfected M2 macrophages, as observed from a wound-healing assay and the dysregulated expression of cytoskeleton signaling genes (Valverde et al., 2024).

Role of lncRNA GAPLINC in Gene-Regulatory NF-κB Pathway

Thus far, GAPLINC has primarily been studied in the context of cancer biology. Although information is limited, GAPLINC is expressed in colorectal (Luo et al., 2018), renal cell cancer (Wang et al., 2021), and LDL-induced endothelial cells (Tang et al., 2022) and is found to be predominately localized in the cytosol (Vollmers et al., 2021). Current research suggests that GAPLINC is involved in the regulation of important pro-inflammatory transcription factor NF-kB.

Nuclear factor kappa B (NF-κB) is a family of transcription factors that plays a central role in inflammatory responses by regulating the expression of a variety of genes involved in the immune responses (Liu et al., 2017). Members of this family, including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel, bind to the κB enhancer to regulate the transcription of target genes (Liu et al., 2017). Two pathways lead to the activation of NF-κB: canonical and noncanonical pathways, both of which are important for regulating the immune response (Liu et al., 2017). The canonical NF-κB pathway is triggered by various stimuli including proinflammatory cytokines (such as TNF and IL-1), and toll-like receptors (TLRs). This leads to the activation of p65 and the regulation of proinflammatory genes (Yu et al., 2020). The noncanonical pathway is activated by a variety of signaling molecules (namely LT β, CD40L, BAFF, and RANKL) which leads to the activation of the RelB/p52 complex and functions to regulate genes essential to lymph-organogenesis and B-cell activation (Sun, 2017).

Most members of the NF-κB family can homodimerize, meaning two identical proteins bind together through a variety of surface interactions to form a homodimer. Less commonly, NF-κB subunits can also heterodimerize, creating a heterodimer comprised of two different proteins (Tak & Firestein, 2001). The p65/p50 heterodimer mediates the transcription of inflammatory genes. When unstimulated, the p65/p50 heterodimer is held in the cytosol by the NF-κB inhibitor protein, IκBα. Upon activation, IκBα is phosphorylated, and the p65/p50 heterodimer is transported to the nucleus and activates target genes (Florio et al., 2022). The p65/p50 heterodimer is the most abundant form of NF-κB activated by LPS therefore studies have used it to investigate the functions of GAPLINC.

A study by Vollmers et al. concluded that GAPLINC affects the NF-κB pathway by regulating the translational ability of the p65 subunit of the p65/p50 heterodimer. Injected with the endotoxin lipopolysaccharide (LPS), a component of the outer cell wall membrane of gram-negative bacteria, induces septic shock (Vollmers et al., 2021). The transcription of inflammatory genes code for proteins protected mice from organ failure after LPS injection (Vollmers et al., 2021).

GAPLINC knockout (KO) cells produce more p65 proteins than the Wild Type (WT), indicating that GAPLINC downregulated the expression of p65 at a translational level. Additionally, it was found that more inflammatory genes are expressed in GAPLINC KO cells at baseline, when cells have not received any stimuli from pathogens, than after LPS stimulation (Vollmers et al., 2021). Together, these pieces of information suggest a role of GAPLINC in the regulation of inflammatory genes. As a result, GAPLINC KO mice have a higher survival rate in LPS shock since GAPLINC-KO cells will promote the translation of inflammatory genes (Vollmers et al., 2021).

The exact mechanism of how GAPLINC controls p65 is still unclear. A direct interaction between GAPLINC and p65 in the cytosol could not be found (Vollmers et al., 2021). Further understanding their interaction would provide a possible avenue for the development of therapeutics for chronic inflammation.

Critical Review of GAPLINC Papers

Currently, a vast majority of GAPLINC literature pertains strictly to its role in various types of cancer, including renal cell (Wang et al., 2021), gastric (Hu et al., 2014), and lung cancer (Gu et al., 2018). Together, these findings advance the understanding of GAPLINC and its performance in the formation of cancer, otherwise known as carcinogenesis or tumorigenesis.

Despite progress made towards the study of GAPLINC, significant gaps remain in our understanding of how this lncRNA affects the inflammatory response. Studies focusing on GAPLINC's role in NF-κB are limited, with only a single publication providing substantial evidence for their correlation. The mechanisms of how GAPLINC interacts with the NF-κB pathway are still not yet understood and call for more extensive research to confirm and expand upon these findings.

Likewise, limited studies exist regarding GAPLINC's role in macrophage polarization, differentiation, and migration. The study conducted by Valverde et al. is the only one that properly explores GAPLINC in the context of macrophage activity. Regardless, the findings of Valverde et al. are rather vague and do not contribute to the widening knowledge gap regarding the mechanisms of GAPLINC.

Role of lincRNA-Cox2 in Inflammation

Inflammation is commonly characterized by redness and swelling caused by a hormone-like lipid compound named prostaglandin (Cleveland Clinic, 2022). In the context of pharmacology, many doctors prescribe non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen to relieve pain and inflammation. NSAIDs inhibit the function of the enzyme cyclooxygenase (COX)-2, which thereby stops the release of prostaglandins and reduces the inflammatory response (Ghlichloo & Gerriets, 2023).

Located proximally to the lincRNA-Cox2 locus, the PTGS2 gene encodes the prostaglandin G/H synthase-2 enzyme, commonly known as COX-2. COX-2 is an inducible enzyme only expressed in response to inflammatory stimuli and works to convert arachidonic acid to prostaglandins, which is a lipid that marks sites of tissue damage or infection (PharmGKB, 2024).

Research conducted by Elling et al. focused on the in vivo functions of lincRNA-Cox2, identifying its cis-regulatory, eRNA-regulating prostaglandin biosynthesis (Elling et al., 2018). Others such as Hu et al. have recognized its trans-regulatory role in the modulation of NF-κB subunits, namely the switch/sucrose nonfermentable (SWI/SNF) complex (Hu et al., 2016).

Cis Function

Formed from the transcripts of enhancer regions, enhancer RNAs (eRNAs) are a class of relatively short (<2 kb) ncRNAs involved in transcriptional regulation (Kim et al., 2015). Although mechanisms of enhancer function are still yet to be understood, it is known that they directly regulate mRNA transcription through promoter-mediated transcription and facilitate promoter-enhancer interactions (Woolard & Chorley, 2019). Therefore, eRNAs help to modulate the expression of target genes in response to various signals.

Research conducted by Elling et al. using various models of lincRNA-Cox2 deficient mice concluded that lincRNA-Cox2 plays a significant cis function as a regulator of the PTGS2 pathway and the synthesis of prostaglandins. Elling et al. observed that the enhancer region of lincRNA-Cox2 does not fall within its two exons, meaning that the enhancer locus is upstream of lincRNA-Cox2. Their findings strongly suggest that the transcription of lincRNA-Cox2 functions to connect the upstream enhancer region with the PTGS2 locus to drive the expression of the COX-2 protein (Elling et al., 2018). Further experimentation concludes that PTGS2 is not necessary for lincRNA-Cox2 to be transcribed; the relationship is unidirectional where lincRNA-Cox2 is necessary for PTGS2 expression (Elling et al., 2018).

Trans Function

The SWI/SNF complex is a key regulator of chromatin remodeling, a method of gene expression that involves changing chromatin between a condensed, transcriptionally inactive accessible state to a transcriptionally accessible state. SWI/SNF binds to the nucleosome and facilitates the transformation, allowing transcription factors or other transcription proteins to access DNA and begin transcription (Tabassum & Parvez, 2021).

Hu et al. report that lincRNA-Cox2 acts as a scaffold, a framework for proteins to bind to, for assembling NF-κB subunits into the SWI/SNF complex, which would act to regulate transcription of late-primary inflammatory genes during LPS stimulation. The assembly of Rel A and p50 into the SWI/SNF complex requires lincRNA-Cox2. This lincRNA-Cox2/SWI/SNF complex would continue to modulate the expression of late-primary inflammatory response genes in macrophages (Hu et al., 2016). It is suspected that the loci of these late-primary response genes are located within a condensed area of DNA, meaning that they require chromatin remodeling to allow gene transcription to occur (Hu et al., 2016). Such proteins that would enable transcription are the NF-κB transcription factors.

In their experiment, Hu et al. also observed that lincRNA-Cox2 KO results in the trans-regulatory global inhibition of late-primary genes while overexpression of lincRNA-Cox2 results in late-primary genes becoming early response genes in response to LPS stimulation. Additionally, Hu et al. observed that the expression of lincRNA-Cox2 is controlled by NF-κB signaling. During LPS stimulation, NF-κB subunits p65 and p50 will bind to the potential promoter region of the lincRNA-Cox2 gene (Hu et al., 2016).

Critical Review of lincRNA-Cox2 Papers

In summary, it is known that lincRNA-Cox2 regulates inflammation through its cis-regulatory eRNA function, trans-regulatory NF-κB-modulating function, and the transcription of late-primary response genes. Collectively, these studies agree that the lincRNA-Cox2 knockout (KO) shows a significant reduction of the transcription of inflammatory genes. Comparatively to GAPLINC, lincRNA-Cox2 is well-researched in its functions in different immune stimulants including smoke-induced inflammation (Mays Mohammed Salih et al., 2023), acute lung injury (Elektra Kantzari Robinson et al., 2022), pulmonary arterial hypertension (Cheng et al., 2020), and many more.

Outside of inflammation, several publications exist discussing the functions of lincRNA-Cox2. For example, Xu et al. studied the effect of lincRNA-Cox2 on the apoptosis of macrophages, concluding that lincRNA-Cox2 increases the amount of apoptosis-inducing proteins, which further sheds light on the functions of lincRNA-Cox2 when infected with Bacillus Calmette-Guérin (Xu et al., 2021).

Conclusion

This review describes the current understanding of GAPLINC and lincRNA-Cox2 in regulating the inflammatory response. GAPLINC regulates p65 levels, thereby influencing the formation of the p65/p50 heterodimer which directly controls the expression of inflammatory genes. GAPLINC also regulates macrophage polarization, differentiation, and migration, all of which are key processes that control how macrophages respond to inflammatory stimuli. lincRNA-Cox2 functions as an eRNA to regulate the transcription of PTGS2, controlling the expression of the PTGS2/COX-2 protein and the intensity of the inflammatory response. LincRNA also acts as a scaffold in the formation of the NF-κB SWI/SNF complex, a subunit in the NF-KB pathway involved with chromatin remodification of immune and inflammatory genes.

While current research provided insights into their roles, there are still significant gaps in understanding the interactions between these lincRNA and other molecules involved in inflammation. Further research should focus on the interaction between p65 and GAPLINC. Expanding research in such areas will enhance our knowledge of lincRNAs and contribute to developing targeted immunogenetic therapy for inflammatory diseases.

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