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Review

Besides Pathology: Long Non-Coding RNA in Cell and Tissue Homeostasis

Amanda Salviano-Silva †, Sara Cristina Lobo-Alves †, Rodrigo Coutinho de Almeida, Danielle Malheiro and Maria Luiza Petzl-Erler *

Laboratory of Human Molecular Genetics, Department of Genetics, Universidade Federal do Paraná, Curitiba 81531-980, Caixa Postal 19071, Brazil; amandasalviano92@gmail.com (A.S.-S.); saralobo5@yahoo.com.br (S.C.L.-A.); rodrigocout@gmail.com (R.C.d.A.); mfdani@yahoo.com.br (D.M.)

* Correspondence: perler@ufpr.br or mlperler@gmail.com; Tel.: +55-41-3361-1746 or +55-41-3361-1724

† These joint-first authors contributed equally to this review.

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Abstract: A significant proportion of mammalian genomes corresponds to genes that transcribe long non-coding RNAs (lncRNAs). Throughout the last decade, the number of studies concerning the roles played by lncRNAs in different biological processes has increased considerably. This intense interest in lncRNAs has produced a major shift in our understanding of gene and genome regulation and structure. It became apparent that lncRNAs regulate gene expression through several mechanisms. These RNAs function as transcriptional or post-transcriptional regulators through binding to histone-modifying complexes, to DNA, to transcription factors and other DNA binding proteins, to RNA polymerase II, to mRNA, or through the modulation of microRNA or enzyme function. Often, the lncRNA transcription itself rather than the lncRNA product appears to be regulatory. In this review, we highlight studies identifying lncRNAs in the homeostasis of various cell and tissue types or demonstrating their effects in the expression of protein-coding or other non-coding RNA genes.

Keywords: long non-coding RNA; homeostasis; physiological regulatory mechanisms; gene expression; gene regulation; transcriptome

1. Introduction

Until a few years ago, non-coding RNAs (ncRNA) were an unexplored component of the transcriptome considered as transcriptional noise. The only well-characterized ncRNAs were the ribosomal RNAs (rRNA), transfer RNAs (tRNA), and small RNAs involved in the processing of primary transcripts, with the messenger RNAs (mRNA) being considered as the most numerous and diversified class of RNAs. However, technological advances, including large-scale sequencing and bioinformatics tools, revealed that ncRNAs are a major part of the total set of transcripts of the human genome [1,2]. These findings aroused interest in studying ncRNAs and, among them, long non-coding RNAs (lncRNAs).

Long non-coding RNAs are defined as a heterogeneous class of ncRNAs longer than 200 nucleotides [1]. These transcripts are located predominantly in the nucleus and secondarily in the cytoplasm, being mostly polyadenylated and transcribed by RNA polymerase II. Long non-coding RNA genes may present tissue- and cell-specific expression and differ from mRNA genes by having longer and fewer exons [3]. According to the genomic position relative to the nearest coding gene, lncRNA genes can be categorized as intergenic (lincRNAs), bidirectional (divergent), sense or overlapped (intronic or exonic), and antisense (intronic or NAT, natural antisense transcript) [1] (Figure 1A). According to regulatory mechanisms on the expression of target genes or on other molecules, lncRNAs can act as signals (e.g., Xist), decoys (e.g., Gas5, MALAT1), scaffolds (e.g., NEAT1), guides (e.g., HOTAIR,
Lin-p21) [4,5], competing endogenous RNAs (ceRNAs) (e.g., linc-MD1) [6], and others. Moreover, their action as regulators of enzyme function was recently described [7,8]. Long non-coding RNAs can interact with enzymes and modulate their activities, as exemplified by NBR2 that directly interacts with AMPK and promotes its kinase activity under energy stress [7] (Figure 1B).

Figure 1. Genomic location relative to protein-coding genes, and regulatory mechanisms of long non-coding RNAs (lncRNAs) in the nucleus, cytoplasm, and extracellular compartments. (A) Nomenclature of lncRNA genes (gold ellipses), according to their genomic location relative to the nearest coding gene (black ellipses) and/or to exons of coding genes (black rectangles). (B) lncRNAs regulatory mechanisms: (b1) lncRNA Xist, as a component of Barr body in females; (b2) acting as enhancers, inducing transcription in cis or in trans; (b3) a decoy to regulatory proteins, such as transcription factors and chromatin modifiers, blocking their binding to DNA; (b4) as molecular signals, activating or silencing gene expression through signaling to regulatory pathways; (b5) Guiding proteins (in general, chromatin modifiers) to specific target sites; (b6) as scaffolds, binding different proteins and forming ribonucleoprotein (RNP) complexes, which also affect gene expression; (b7) interacting with enzymes, such as kinases, regulating/enhancing their catalytic activity and altering their signalization; (b8) modulating alternative splicing of primary transcripts; (b9) as competing endogenous RNA (ceRNA), serving as a sponge for microRNAs (miRNAs), blocking their effect; (b10) targeting proteins, forming molecular complexes which can block or induce functional effects, or even alter their location in the cell; (b11) targeting messenger RNAs (mRNAs), inhibiting their translation in ribosomes. In addition, lncRNAs can be (b12) transferred to other cells by extracellular vesicles (EVs), where they can produce effects; (b13) precursors of miRNAs and other regulatory small RNA. An lncRNA can act by multiple regulatory mechanisms, in both the nucleus and/or in the cytoplasm. The b12 itself is not exactly a regulatory feature, however, the release of these functional lncRNAs through EVs is a way of regulating genes, RNAs, or proteins in other tissues. ASE—alternatively spliced exon.
Many studies have demonstrated the involvement of lncRNAs in processes of prime relevance for homeostasis [5]. One of the first lncRNAs studied, even before the recognition of this class of transcripts, was the X inactivation-specific transcript (Xist), located in the X inactivation center region and involved with X chromosome inactivation in mammalian females [9,10]. Xist expression is inhibited in the active X chromosome by another lncRNA, antisense to Xist promoter, called Tsix [11]. Overall, lncRNAs are known to be involved in gene expression regulation—at the transcriptional and post-transcriptional levels, by epigenetic or other mechanisms, such as interfering with the recruitment of RNA polymerase II or inducing chromatin remodeling. Furthermore, they participate in genomic imprinting; in nuclear and cytoplasmic trafficking; in protein localization and activity; and in interaction with miRNAs, among other processes (reviewed in [12]). In addition, they can be further processed to small ncRNAs [13] or even encode functional micropeptides [14,15]. However, little is known about how these transcripts control gene expression.

Long non-coding RNAs are strictly regulated [16,17] and participate in or are products of many biological processes [18,19]. Mutations in the primary sequence of lncRNAs, as well as aberrant variations of their expression, have been associated with several disorders, pointing to their potential as disease biomarkers [20]. Therefore, lncRNAs have been largely studied in different tissues’ homeostasis and pathology to understand their physiological effects and the consequences of their deregulation in complex diseases.

We performed an extensive search of the literature for articles presenting data about lncRNAs involved in the homeostasis of different tissues and cell types. Some of the lncRNA play fundamental roles in various tissues, while others present a tissue-specific expression pattern. We present the information by cell or tissue type throughout this review.

2. Long non-coding RNAs: Expression Patterns in Tissues or Cell Types

Long non-coding RNAs are strictly regulated and many present cell-specific expression, substantiating their crucial role in physiological mechanisms [1,3,21]. In the following, we summarized what is currently known about lncRNA expression among cell development and differentiation, and in specific pathways (more details in Table S1).

2.1. Hematopoietic Cells

Ontogenesis of blood cells from hematopoietic stem cells (HSCs) occurs throughout the whole individual’s life and is highly controlled by transcription factors and non-coding RNA. Circulating blood, where most of these cells are found, is easy to acquire and to work with, being routinely used in molecular studies. Yet, some authors analyzed bone marrow and thymus to understand early stages of hematopoiesis and the development of the different cell lineages.

The lincRNA H19 (also known as imprinted maternally expressed transcript) is one of the most abundant and conserved mammalian noncoding transcripts, and a key regulator of differentiation of various cell and tissue types, as will also be seen in other sections of this review. The H19 lincRNA is a transcript of the H19/IGF2 genomic imprinted cluster. While H19 is transcribed from the maternally-inherited locus, the mRNA for IGF2 (insulin-like growth factor II) is transcribed from the paternally-inherited locus. During murine hematopoiesis, the growth-restricting lincRNA H19 was downregulated in HSCs before their proliferation and upregulated in long-term HSCs. H19 is localized downstream of Igf2 in the H19/Igf2 locus. Both genes are co-expressed and have an antagonic effect on cell proliferation during hematopoiesis [22]. H19 also inhibits HSC activation and proliferation, serving as a precursor of miR-675, a miRNA that targets the insulin-like growth factor 1 receptor (Igf1r) in placenta [23]. In another study, among the 323 novel lncRNAs identified in murine HSC RNA sequencing, 159 were HSC-enriched or likely HSC-specific (lncHSCs), in comparison with the transcriptome of differentiated B cells and granulocytes. Among these 159 lncRNAs, IncHSC-1 was identified as involved in myeloid differentiation, and IncHSC-2 as involved in HSC
self-renewal and T cell differentiation. In addition, IncHSC-2 is enriched with target sites for important hematopoietic-specific transcription factors, especially E2A [24].

In the following, we will highlight well-established IncRNAs involved in ontogeny and the homeostasis of circulating blood cells and their progenitors (Figure 2).

**Figure 2.** Long non-coding RNAs described in the physiology of mature and progenitor hematopoietic cells, derived from myeloid (left) and lymphoid (right) differentiation from a hematopoietic stem cell (HSC), in which the lncRNA H19 plays a central role. In the grey rectangles are listed the lncRNAs specifically or differentially expressed in each cell type (rectangles at the side of cells), or lncRNAs involved in the differentiation and maturation of these cells (upon the arrows).

### 2.1.1. Erythrocytes

During red blood cells development, various IncRNAs regulate erythroid gene expression. Although their specific functions are still not elucidated, some critical processes for the homeostasis of erythropoiesis involve pro- and anti-apoptotic pathways. Fas-antisense 1 (Fas-AS1, also known as Saf), an lncRNA antisense to Fas cell surface death receptor coding gene, was upregulated during the differentiation stage of erythropoiesis by the erythroid transcription factors GATA binding protein 1 (GATA1) and Kruppel-like factor 1 (KLF1), and negatively regulated by the nuclear factor kappa B (NF-κB). In erythroblasts derived from CD34+ hematopoietic stem cells, the overexpression of Fas-AS1 reduced the Fas surface levels and protected cells from death signals [25].

The depletion of 12 out of 13 lncRNAs selected as candidate modulators of erythropoiesis impaired erythrocyte maturation, suggesting that they play crucial roles during late stages of maturation [26]. One of these candidate RNAs is lncRNA-EPS, which promotes erythroid differentiation by preventing apoptosis [27].

The lincRNAs lincRNA-EC2, -E4, and -EC9 were found to be required for erythrocyte maturation in the foetal liver, but they were absent in the adult bone marrow. The enhancer-derived alncRNA-EC7 is required for the activation of the neighboring gene SLC4A1, which encodes BAND3, a crucial anion...
transporter of the erythrocyte membrane. The IncRNA \( \text{alncRNA-EC7} \) has an evolutionary conserved enhancer site, and there is evidence of chromatin looping between this site and the \( \text{SLC4A1} \) promoter proximal region, supporting a model whereby \( \text{alncRNA-EC7} \) modulates erythropoiesis. In erythroblasts, the expression of \( \text{elncRNA-EC3} \) is positively correlated with the expression of \( \text{KIF2A} \), which encodes a microtubule-associated kinesin motor protein, required for mitotic progression. Moreover, the IncRNA \( \text{deleted in lymphocytic leukemia 2 (DLEU2)} \) is expressed from distinct promoters of \( \text{shlncRNA-EC6} \) in erythroblasts, compared to other cell types, and represses the neighbor gene \( \text{SPRYD7} \). A deletion in \( \text{shlncRNA-EC6} \) is frequent in B cell malignancies, and \( \text{DLEU2} \) might have an important role in erythropoiesis [26].

The expression of several IncRNAs is restricted to specific stages of erythrocyte differentiation. Often, these IncRNAs are co-expressed with mRNAs and miRNAs in the same stages of cell differentiation. Among the IncRNAs with potential roles during hematopoiesis, \( \text{JHDM1D-AS1}, \text{AC087294.2}, \text{RP11-66D17.5} \) were frequently associated with genes of the erythrocyte mature network, while \( \text{RP11-326C3.2} \), localized between \( \text{NLRP6} \) and \( \text{ATHL1} \) coding-genes, as well as the co-expressed \( \text{ATHL1} \) both increased during erythrocyte maturation. Moreover, as observed in in silico analysis, the IncRNA \( \text{TOPORS-AS1} \) could act as a ceRNA for three microRNAs involved in the inhibition of erythrocyte development (miR-532-3p, miR-191-5p, and hsa-miR-652-3p) [28]. In a similar previous study, co-expression was found between an IncRNA (not identified by the authors) and miR-4732, a key modulator of RNA during human erythropoiesis and abundant in erythrocytes within the erythroid-enriched miR-144/451 locus, a cluster upregulated by GATA1 during erythrocyte development. This ~250-bp IncRNA overlaps with miR-4732 and is proximal to the miR-144/451 cluster. This cluster has been highly related to erythropoiesis, and all RNAs in this locus were highly expressed in mature erythrocytes, in comparison with RNAs from other genomic regions [29].

2.1.2. Other Myeloid Lineage Cells

Beyond the erythrocytes, the myeloid lineage includes neutrophils, eosinophils, monocytes and dendritic cells, among other important cells for the innate immunity.

The lifespan of short-lived myeloid cells (neutrophils, eosinophils, and Ly6Chi classical monocytes) was found in mice as regulated by the \text{myeloid RNA regulator of Bim-induced death (Morbid, also known as MIR4435-2HG)}, in response to pro-survival cytokines. The nuclear IncRNA \( \text{Morbid} \), specifically expressed in mature short-lived myeloid cells, neighbors and \text{cis}-regulates the pro-apoptotic gene \( \text{Bcl2l11} \) (Bim) by promoting an H3K27me3 modification at \( \text{Bim} \) promoter through interaction with the polycomb repressive complex 2 (PRC2, a histone modifying complex), repressing the apoptosis in these cells. \text{Morbid} deficiency resulted in a drastic reduction of these myeloid cells in blood and spleen, increasing susceptibility to bacterial infection and protecting against eosinophil-driven allergic lung inflammation. Moreover, \text{MORRBID} dysregulation in humans is associated with hypereosinophilic syndrome, a disorder characterized by severe eosinophilia. Collectively, the IncRNA \( \text{Morbid} \), in response to extracellular pro-survival signals, is crucial for the physiologic control of the lifespan of neutrophils, eosinophils, and classical monocytes [30].

Various IncRNAs were highly expressed in monocytes, including the well-known IncRNAs \text{growth arrest-specific 5 (GAS5)}, \text{CCAAT/enhancer binding protein \( \beta \) antisense (CEBPB-AS1)}, \text{linRNA P53-induced transcript (LINCPINT)}, \text{integrin \( \beta \) 2 complement component 3 receptor 3/4 subunit antisense (ITGB2-AS1)}, and \text{FYVE RhoGEF/PH domain containing 5 antisense (FGD5-AS1)} [31]. Several other annotated but still uncharacterized IncRNAs were also highly expressed in monocytes, such as \text{LINCO0678}, \text{LINCO0657}, \text{LINC00989}, and \text{LINC01420}, which might have a potential role in monocyte regulation. Moreover, novel IncRNAs, such as \text{LINC01420}, whose gene is located between the \text{UBQLN2} and \text{SPIN3} coding genes, and with a moderated expression in other hematopoietic cell types, were also detected in monocytes, apart from the IncRNAs \text{TCONS_00282615} and \text{TCONS_00008494} (also known as \text{inc-IGFBP7-5}), specific to these cells [31].
During the differentiation of monocytes to dendritic cells (DCs), important epigenetic modifications were found in the promoter region of HOXA transcript antisense RNA myeloid-specific 1 (HOTAIRM1), an lncRNA gene located between HOXA1 and HOXA2 genes. HOTAIRM1 was downregulated in differentiated DCs, and its silencing caused expression changes of several monocyte differentiation markers. Moreover, the upregulation of miR-3960 could downregulate both HOTAIRM1 and the DC differentiation repression gene HOXA1, inducing the differentiation into DCs. The results indicate competitive binding between HOTAIRM1 and miR-3960, regulating DC differentiation [32].

The lincRNA-COX2, neighbor of the prostaglandin-endoperoxide synthase 2 (Ptgs2/Cox2) gene in mice, is highly induced in innate immune response [18,33] and was first described in DCs after toll-like receptor 4 (TLR4) stimulation with lipopolysaccharide (LPS) [18]. Some years later, it was demonstrated that in macrophages stimulated with Pam3CSK4 (a TLR2 ligand), numerous immunological genes were induced, including 62 lncRNAs, of which lincRNA-COX2 was the most highly induced [33]. In the same study the authors observed that silencing lincRNA-COX2 did not alter COX2 expression, but induced 787 genes in unstimulated cells, including chemokines (such as Ccl5, Cx3cl1), chemokine receptors (such as Ccr1), and interferon-stimulated genes (such as Irf7). In stimulated cells, lincRNA-COX2 silencing attenuated the expression of 713 genes, including Trl1, interleukin (IL)-6, and IL-23a. It was also demonstrated in vitro that lincRNA-Cox2 interacts with the heterogeneous ribonucleoprotein (hnRNP) A/B and A2/B1, and knockdown of both hnRNPs repressed Ccl5 and Irf7 [33]. In contrast, lincRNA-Cox2 was seen to induce Ccl5 expression in LPS-stimulated immortalized macrophage cell lines, by recruitment of the chromatin remodeling complex SWI/SNF to the Ccl5 promoter. Furthermore, lincRNA-Cox2 is required in the innate response for the expression of NF-κB-regulated late-primary inflammatory response genes [34].

An lncRNA exclusive to DCs is lnc-DC, highly expressed from the human Wdnm1-like pseudogene at the moment of DC differentiation, driven by PU.1 transcription factor [35,36], being present in Lin- MHCII+CD11c+ conventional DCs, and absent or lowly expressed in CD14+ monocytes, B cells, and other hematopoietic cells. The lnc-DC binds to the signal transducer and activator of transcription 3 (STAT3) that regulates DC differentiation, protecting STAT3 from inactivation by the protein-tyrosine phosphatase non-receptor type 6 (PTPN6, or SHP1). Therefore, through promoting STAT3 signaling, lnc-DC leads to DC differentiation from monocytes to DCs, in vitro and in vivo, and to the DC capacity to activate T lymphocytes [35].

2.1.3. Lymphocytes

Lymphocytes are a class of leucocytes of prime importance for both the innate immunity (innate lymphoid cells—ILCs, in which natural killer—NK—cells are included) and acquired immunity (T and B cells).

Many novel lncRNAs were identified by global gene-expression profiling of human bone marrow and thymic progenitor cells, spanning the earliest stages of B and T lymphoid lineages commitment. These lncRNAs were predominantly intergenic, followed by antisense lncRNAs. The expression of a high proportion of the lncRNAs was positively correlated with that of neighboring protein-coding genes, including genes involved in hematopoiesis and the regulation of lymphocyte development and proliferation. In addition, the authors identified lncRNAs co-expressed with known protein-coding genes specific for different lymphocyte lineages, such as PAX5, EBF1, CD3, BCL11B, NOTCH1, RAG1, and RAG2, among many others. The results provide a resource for candidate lncRNA to be further investigated in functional studies in order to clarify their role in lymphopoiesis [37].

Moreover, NONHSAT040475 (also known as lnc-BRF1-24) is an lncRNA widely expressed in human peripheral blood leukocytes. Its expression was compared between T cells (CD3+), B cells (CD19+), and NK cells (CD56+). B cells were the lymphocyte subset with the highest expression of NONHSAT040475, in comparison with NK cells and granulocytes, which presented the lowest expression among the analyzed cell types [38].

The next sections will address the lncRNAs associated with specific lymphocyte types.
T Lymphocytes

T lymphocytes (or T cells), as one of the central cell types of the immune system, perform crucial roles in adaptive immune responses, which are under constant regulation to maintain body homeostasis. T lymphocytes are subdivided in partially overlapping subgroups based on their more prominent known functions. So, CD4\(^+\) T helper lymphocytes (Th1, Th2, Th17) are essential for the activation and regulation of B lymphocytes and cytotoxic T lymphocytes (CTL or Tc); CD8\(^+\) T cytotoxic lymphocytes eliminate virus-infected cells, tumor cells, or otherwise damaged cells; regulatory T lymphocytes (Treg) are essential for immunological peripheral tolerance; and memory T lymphocytes, in their turn, provide a quick response upon re-exposure to their specific antigen. In the first genome-wide characterization of IncRNAs expressed in mammalian (human and mouse) CD8\(^+\) T lymphocytes, over a thousand IncRNAs were identified, many of which presented stage- or tissue-specific expression, and were mapped close to or overlapped shorter functional RNA or protein-coding genes with quite characterized roles in CD8\(^+\) T cells [39]. Most IncRNAs expressed in CD8\(^+\) T cells presented signatures of evolutionary conservation, and their secondary structures and the characteristics of the promoters provided further evidence of functional relevance [39].

Long non-coding RNAs also affect transcription factors present in T cells, as the NFAT complex (nuclear factor of activated T cells). In resting cells, NFAT proteins are located in the cytoplasm, where they are heavily phosphorylated. An increase of intracellular Ca\(^{2+}\) concentration activates calcineurin which dephosphorylates the NFAT regulatory domain, ultimately resulting in the nuclear accumulation of NFAT and transcription of NFAT target genes. Phosphorylated NFAT1 is present with the lincRNA ncRNA repressor of the nuclear factor of activated T cells (NRON) and the multidomain scaffold protein IQGAP1 in a cytoplasmic RNA-protein complex that also contains three known NFAT inhibitory kinases. This supports the hypothesis that lincRNAs may function as scaffolds for transcriptional regulators in large RNA-protein complexes [40]. Long non-coding RNAs can influence Th1/Th2 differentiation by affecting the expression of critical cytokines. The murine lincRNA Tmevpg1 is required for the control of Theiler’s virus infection, and its human orthologue TMEVPG1 (also known as IFNG-AS1) contributes to interferon gamma expression. The expression of TMEVPG1 and IFNG correlate positively. This lincRNA is preferentially expressed in Th1 cells, depending on transcription factors STAT4 and TBET/T-bet, which drive the Th1 differentiation program [41].

High-throughput RNA sequencing was used to investigate lncRNA expression along the development and differentiation of T cell lineages. Forty-two subsets of thymocytes and peripheral T cells at multiple time points of differentiation were analyzed, and 1524 genomic regions expressing lincRNAs were identified. The effect of TMEVPG1 in Th1 lymphocyte differentiation (see above) was corroborated, but none of the numerous other lincRNAs had been previously described in T cells. Results revealed highly dynamic stage- and cell-specific expression patterns for lincRNAs during T cell differentiation. Key transcription factors, specific for the Th1 (T-bet and STAT4) and Th2 (GATA3 and STAT6) lineages, were largely responsible for the lineage-specific expression of T cell lincRNAs [42]. A more specific result was obtained for the lincRNA LincR-Ccr2-5′AS, which together with transcription factor GATA3 was a fundamental component of the chemokine signaling pathway in Th2 subset cells and important for the migration of these cells [42]. More recently, another 13 subsets of T and B cells were analyzed by RNA sequencing and over 500 previously unknown lincRNAs were identified, suggesting that lincRNAs might collaborate for the determination of lymphocyte identity and for the modulation of their functional plasticity [43]. Beyond that, the influence of LincMAf-4 (also known as MAFTRR) in T cell differentiation was partially clarified. LincMAf-4 is a chromatin-associated lincRNA specific to the Th1 subset of helper T cells, whose expression was inversely correlated with expression of MAF, a Th2-associated transcription factor. Linc-MAF-4 regulated MAF transcription through the recruitment of chromatin modifiers LSD1 and EZH2; the downregulation of linc-MAF-4 alters T cell differentiation toward the Th2 phenotype [44]. Furthermore, progenitor cells spanning the earliest stages of B lymphoid and T lymphoid specification were isolated from the bone marrow and thymus for RNA sequencing analysis. More than 3000 genes encoding yet unexplored IncRNAs were
described. Highly stage-specific lncRNA expression patterns characterized lymphoid commitment, and several lncRNAs mark the beginning of the divergence of B and T lineages [37]. In addition, more than 2000 lncRNAs were identified in human T cell cultures by RNA sequencing. Moreover, a cluster of antisense lncRNA transcribed from the RAD50 locus that regulate the expression of Th2 cytokines, IL-4, IL-5, and IL-13 was described [45]. Another Th2 specific lincRNA, GATA3-AS1, was analyzed in 27 human tissues and immune cell types, where it was largely undetectable or had very limited expression, supporting the Th2-specific expression pattern and indicating that it might serve as a marker of Th2 response [46]. Microarray analysis of CD4+ T cells at different stages also confirmed distinguishable lncRNA expression profiles and highlighted the importance of lncRNAs in CD4+ T cell development and activation [47]. Specific lncRNA genes involved in the early differentiation of Th1 and Th2 subsets were identified, and a positive trend in the expression of the lineage-specific lncRNAs and the neighboring protein-coding genes was observed [48].

Long non-coding RNA profiles of naive T cells and two subsets of memory CD4+ T cells (central and effector) were obtained by RNA sequencing, and a total of 57,000 novel lncRNAs were found in at least one cell subtype. Many lineage-specific lncRNAs were reported, most of them mapped in a non-random manner, positioned close to the lineage-specific protein-coding genes with which they are co-expressed. Furthermore, it has been demonstrated that lncRNAs mapped near to the IFNG locus are necessary for IFNG prompt expression, since the expression inhibition of lncRNAs IFNG-AS1 and IFNG-R-49 by siRNAs abolishes IFNG expression by effector memory T lymphocytes [49].

In view of the huge amount of data generated, especially by high-throughput sequencing technologies, and the recent progress in lncRNA identification, the role of lncRNAs in T cells development as well as in immune responses became evident. Several lncRNAs present a highly lineage-specific expression and help to control the differentiation and function of these important immune cells. Although there is much to explore about T cells and lncRNAs, the importance of these non-coding RNAs is already settled.

B Lymphocytes

B lymphocytes are responsible for the production of immunoglobulins, also known as antibodies, which recognize foreign antigens, and are thus essential for the so-called humoral immunity. Activated B cells can evolve to plasmocytes and memory cells. In a transcriptome analysis of 11 B cells subsets, several lncRNAs expressed along human B cell development and correlated with other RNAs were identified. Among the lncRNAs expressed during the early stage of B cell development, the transcripts MYB-AS1, SMAD-AS1, and LEF1-AS1 are antisense to genes of known early B cell transcription factors (MYB, SMAD1, and LEF1, respectively). Another transcript identified was the lincRNA CTC-436K13.6, located between the genes EBF1 (which encodes the transcription factor early B cell factor 1) and CLINT1. Several lncRNAs were also identified in proliferative stages and in the germinal center. In the germinal center, the lncRNAs LINC00487, LINC00877, and RP11-203B7.2 were found to be highly connected genes (hub genes). Furthermore, the gene for lincRNA, RP11-132N15.3, found to be predominantly expressed in centroblasts, is localized upstream of the BCL6 gene, the main germinal center regulator [50].

In another transcriptome analysis of B cell subtypes of mice, 4516 lncRNAs, were identified as differentially expressed during B cell development and activation, of which 702 were predicted to be associated with enhancers (eRNAs) and 192 with promoter regions (pRNAs). Among them, a strong positive correlation between the eRNA LNCGme02323 and the cluster lightpink4, which contains 31 protein-coding genes regulated in marginal zone B cells, was observed. Moreover, 784 lncRNAs of pro-B cells and 717 lncRNAs of mature B cells overlapped or were less than 1 kb downstream of binding sites for PAX5, a transcription factor required for B cell development. Evidence of regulation by PAX5 was found for 109 of these lncRNAs, indicating a role for these lncRNAs in B cell homeostasis [51].

Finally, it was recently demonstrated that during female B cell development, the inactivated X chromosome (Xi) loses the heterochromatin modifications and the co-localization within the lncRNA
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Xist, which are required to compensate between sexes for the dosage of X-linked gene products. The lack of Xist at the Xi occurs at the pro-B cell stage and persists in non-activated B cells, being restored to Xi during the activation of mature B cells. For restoring of Xist localization and chromatin modifications, the transcription factor YY1, involved in V(D)J recombination (variable, diversity and joining gene segments recombination) of B cells, is required. While it remains unknown why the Xi loses heterochromatic modifications and Xist RNA during female B cell development, the authors suggested that these features might enable rapid immune-related X-linked genes reactivation in response to infections and could also explain some of the immunological differences between the sexes [52].

**Natural Killer Cells**

Long non-coding RNAs were also seen to be involved in NK cell function and differentiation. In an IncRNA profiling analysis of different populations of NK cells, numerous novel IncRNAs were identified, including lnc-CD56, an IncRNA specific to NK cells. lnc-CD56 positively correlated with the expression of the classical NK cells marker CD56 in primary and some differentiated human NK cells. Moreover, lnc-CD56 is predicted to interact with the NK-transcription factors TBX21, IRF2, IKZF2, ELF4, and EOMES [53].

A novel killer cell immunoglobulin-like receptor (KIR) gene-body associated antisense transcript not expressed in mature NK cells was described in cells with stem cell properties. The promoter of this spliced IncRNA is at intron 2 of several KIR genes, and its sequence is complementary to the proximal promoter of the KIR genes and KIR-coding exons 1 and 2. Its promoter contains binding sites for MZF-1, a bi-functional transcription factor that activates transcription in cells of hematopoietic origin and represses transcription in most other cells. In support of a role of that IncRNA in the initial silencing of KIR genes, the differentiation of human embryonic stem cells into CD34-expressing hematopoietic progenitor cells was accompanied by the loss of this IncRNA [54].

Moreover, it was recently demonstrated that the RNA-demarcated regulatory region of id2 (Rroid) of mice is an IncRNA locus located ~220 kb from the id2 gene, a transcriptional regulator that inhibits the expression of T and B cell-specific genes and is essential for lineage commitment and maintenance of mature group 1 innate lymphoid cells (ILC1s). The IncRNA Rroid is highly and specifically expressed in ILC1s, where the NK cells are included. In ILC1s, the Rroid locus (not the IncRNA itself) directly interacts with the id2 promoter and, in response to interleukin 15 (IL-15), promotes chromatin accessibility and the deposition of the transcription factor STAT5 at the id2 promoter, thus acting as a cis-regulatory element of the id2 gene and promoting its expression in ILC1s [55].

### 2.2. Solid Tissues

Several IncRNAs were identified in healthy solid tissues and organs, exercising critical roles during their differentiation, metabolism, and physiological functions (Figure 3). These IncRNAs and the regulatory mechanisms that they play in specific solid tissues are reviewed in this section.
Figure 3. Main lncRNAs described as playing physiological roles (in function, homeostasis, and/or differentiation) in the following (clockwise direction): nervous, cardiac, pancreatic, intestinal, epidermal, germ, bone, adipose, hepatic, lung, and muscular (skeletal and smooth) tissues.

2.2.1. Epidermal Tissue

Epidermal epithelial tissue is a stratified surface epithelium providing a barrier to the external environment. Epithelial cells are closely interconnected (juxtaposed). The epidermis is the outermost layer of the skin and has a high turnover rate due to the continuous shedding of the outer layer of cornified cells. The importance of lncRNAs in epithelial cells has been demonstrated by diverse studies, especially with regard to gene expression regulation [56, 57]. One of the first examples is the natural antisense transcript (NAT) Zeb2-AS1, an lncRNA that is an important component of the epithelial-mesenchymal transition (EMT) [56]. Among the regulatory proteins required for EMT is the Zeb2 (zinc finger E-box binding homeobox 2) transcriptional repressor of E-cadherin. Zeb2-AS1 acts to induce the expression of the Zeb2/Sip1 (ZFHX1B) gene by promoting the maintenance of a large intron in the 5’ untranslated region (UTR) of the Zeb2 transcript. This region contains an internal ribosome entry site (IRES) necessary for Zeb2 expression, which in turn participates in the downregulation of E-cadherin mRNA and protein levels [56]. Furthermore, several lncRNAs have been identified as differentially expressed during keratinocyte (KCs) differentiation by non-coding RNA array analysis of epidermal equivalents grown in vitro [58], including AF005081, UC003af, BC020554, AK022798, upregulated at different times and differentiation stages. In a study of primary human keratinocytes, lncRNA expression was measured by microarray before and after the induction of differentiation. A total of 687 lncRNAs were detected, of which 104 were differentially expressed following differentiation [59]. The activating long ncRNA 1 (ncRNA-a1, also known as FALEC, focally amplified long non-coding RNA in epithelial cancer) was found to upregulate ECM1, a neighbor protein coding gene, and both the protein and the lncRNA, were induced after keratinocyte differentiation [59]. Other examples are two lncRNAs that control the balance between the progenitor and differentiated compartment of human epidermis: anti-differentiation non-coding RNA (ANCR, also known as DANCR),
which is strongly suppressed in differentiated cells, and terminal differentiation-induced ncRNA (TINCR), which is highly induced during epidermal differentiation [57,60,61]. Anti-differentiation non-coding RNA is required to enforce the undifferentiated cell state within epidermis [60], whereas TINCR promotes human epidermal differentiation by a post-transcriptional mechanism, mediated by interactions with staufen1 (STAU1) protein [61]. Moreover, ANCR and TINCR act as regulators of transcription factors MAF and MAFB, which are important for epidermal differentiation regulation [57]. Interestingly, an lncRNA-miRNA interaction has been reported as influencing keratinocyte differentiation. The lncRNA H19 can function as a competitive endogenous RNA for miR-130b-3p. This miRNA targets the desmoglein 1 (DSG1) transcript, resulting in decreased DSG1 expression and action, thus keratinocyte differentiation is inhibited. However, lncRNA H19 adds a regulation point in this differentiation process by acting as a miR-130b-3p sponge and preventing its activity; hence, H19 can promote keratinocyte differentiation [62]. In addition, lncRNAs, including the aforementioned H19, can play a role also in the regulation of dermal papilla (DP) cell function, influencing hair follicle development and regeneration. A comparison of lncRNAs differentially expressed in early- and late-passage DP cells revealed numerous differentially expressed lncRNAs. Three of these, RP11-766N7.3, H19, and HOXAIR, may participate in hair gene regulation by targeting the Wnt signaling pathway, which plays critical roles in skin development and homeostasis and is required for hair follicle initiation [63].

Recently, 111 lncRNAs were shown to be differentially expressed in response to the activation of Wnt/β-catenin signaling in mouse dermal fibroblasts. The Wnt signaling-induced lncRNA Wincr1 is highly expressed in primary dermal fibroblasts and may affect complex cellular behaviors including collective cell migration and collagen processing and contraction. Also, Wincr1 affects the expression of several genes, such as Col1a1 and Mmp10 [64].

2.2.2. Nervous Tissue

Many lncRNAs are specifically expressed in the brain. These transcripts have been considered essential pieces in the regulation of a broad spectrum of neuronal processes, including brain development, establishment and maintenance of neural cell types, synapse formation and function, stress responses, and age-associated changes [65]. Some lncRNAs have been implicated in brain development, such as highly accelerated region 1A (HAR1A), which is expressed specifically in Cajal–Retzius neurons in the developing human neocortex from 7 to 19 gestational weeks, a crucial period for cortical neuron specification and migration. HAR1A is co-expressed with reelin, which is of fundamental importance in specifying the six-layer structure of the human brain cortex [66]. Another example is the lncRNA DLX6 antisense RNA 1 (DLX6-AS1, or Evf2), which influences the establishment of murine regional forebrain organization. This lncRNA acts in ventral forebrain development, recruiting DLX and MECP2 transcription factors to DNA regulatory elements in the Dlx5/6 intergenic region and controlling Dlx5, Dlx6, and Gad1 expression through cis-acting mechanisms [67]. Moreover, Pinky (Pnky) is an lncRNA that influences murine neural stem cell (NSC) differentiation. Pnky interacts with the PTBP1 protein and regulates neurogenesis from NSCs in the embryonic and postnatal brain [68]. In the Catarrhini branch of primates, the lncRNA for neuronal development (LncND, also known as lnc-TMEM18) is highly expressed in the embryonic and postnatal brain. It controls the expression of the Notch signaling pathway, which may be necessary to maintain the stemness of outer sub-ventricular zone radial glia-like cells (oRG), by functioning as a miRNA sponge for the microRNA mir-143-3p. The evolutionary emergence of LncND its co-evolution with miRNA-143-3p may have contributed to the expansion of the cerebral cortex in primates [69].

Global RNA sequencing techniques have also been used to investigate lncRNA expression in brain development and function. Neurons derived from induced pluripotent stem cells (iPSCs) have been analyzed by RNA sequencing, and emphatic changes in lncRNA expression during transition from iPSCs to early differentiating neurons were detected. The HOXAIRM1 (HOXA transcript antisense RNA myeloid-specific1) lncRNA is expressed early in neurogenesis, being important for HOXA gene
regulation during myelopoiesis [70]. Besides, through a microarray approach, 934 IncRNAs were found to be differentially expressed when comparing human embryonic stem cells (hESCs) with neural progenitor cells (NPCs). Of these IncRNAs, four were subjected to knockdown that indicated that these IncRNAs are required for adequate neuronal differentiation [71]. Microarray assay was also used to explore the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into neural cells. After differentiation induction, 23 IncRNAs were upregulated or downregulated. Further, reverse transcription—quantitative polymerase chain reaction (RT-qPCR) was performed to validate H19, Esco2, Pcdhb18, and RGD1560277 differential expression, corroborating IncRNAs as key regulators of BMSC differentiation [72]. A transcriptome and splicing database was generated for eight different brain cell types from mouse cerebral cortex: neurons, astrocytes, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, microglia, endothelial cells, and pericytes [73]. The expression of 811 IncRNAs was detected and some of them were among the highest expressed transcripts in the mouse brain, with 12 placing among the 7% most highly expressed genes in the RNA dataset. Moreover, some IncRNAs were expressed in cell type-specific or enriched manners [73]. RNA sequencing was also used to profile IncRNA expression in mouse auditory forebrain during postnatal development at time points before and after the onset of hearing [74]. This study revealed that the IncRNA expression profile was distinct between primary auditory cortex and medial geniculate body and between different postnatal time point, indicating spatial and temporal specificity during the maturation of the auditory forebrain [74]. Recently, the human neocortex transcriptome was also analyzed at the single-cell level [75]: hundreds of cells from human neocortex at different stages of development were individually sequenced, revealing that many IncRNAs are abundantly expressed in individual cells and are cell type-specific. For example, LOC646329, an IncRNA that regulates cell proliferation, is enriched in single radial glia cells but is detected at low abundance in bulk tissues [75]. In addition, 155 differentially expressed IncRNAs have been reported in a neuronal cell line after stimulation by brain-derived neurotrophic factor (BDNF), a critical neurotrophin for neural development. Long non-coding RNAs with fold changes above 2 included the already known C6orf176 and MIAT; however, many differentially expressed IncRNAs are not currently understood [76]. For oligodendrocytes, a dynamic expression profile at different stages of development was established. Among oligodendrocyte-restricted IncRNAs, the evolutionary conserved chromatin-associated IncOL1 was functionally explored. Its overexpression promotes oligodendrocyte differentiation in the developing brain, whereas its knockdown inhibited the expression of myelin genes, causing defects in central nervous system (CNS) myelination [77].

Long non-coding RNA are also involved in the regulation of synaptic processes. The nuclear IncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as NEAT2 (nuclear-enriched abundant transcript 2) or NCRNA00047, is highly expressed in neurons and regulates synapse formation by modulating the expression of genes involved in synapse formation and/or maintenance [78]. Furthermore, the IncRNA maternally expressed gene 3 (Meg3) in mice is important for maintaining synaptic plasticity by modulating the phosphatase and tensin homolog (PTEN) / (phosphatidylinoside-3-kinase (PI3K) / (protein kinase B (AKT) signaling pathway. In addition, knockdown of Meg3 in primary cortical neurons prevents the glycine-induced increase of the GluA1 subunit of AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) in the cell membrane. Thus, Meg3 is important for modulating AMPAR expression, which mediate fast synaptic transmission in the central nervous system [79].

2.2.3. Muscular Tissue: Cardiac, Skeletal, and Smooth

There are three types of muscle: smooth, skeletal, and cardiac. Recently, IncRNAs have been reported as important regulators of myocyte gene expression, and/or have a role in contraction mechanisms, being involved in muscle development, differentiation, and homeostasis. The cardiac muscle comprises the heart, the first functional organ formed during embryo development. The multistep process involved in cardiac myocyte differentiation has been detailed elsewhere
(reviewed in [80]) and involves highly coordinated pathways of gene expression modulated by the products of protein-coding and non-coding genes (reviewed in [81]).

The murine heart-associated IncRNA Braveheart (Boht or AK143260) was one of the first IncRNAs associated with cardiac development. Boht is required for the cardiovascular lineage commitment of nascent mesoderm, through the activation of a core regulatory network of cardiovascular transcription factors, and possibly also mediating epigenetic regulation during cardiomyocyte differentiation by interaction with SUZ12, a component of polycomb-repressive complex 2 (PRC2) [82]. In another study using the mouse model, the mesoderm-specific foetal-lethal non-coding developmental regulatory RNA (Fendrr) was found to be an activity modulator of two histone-modifying complexes. Fendrr binds to the PRC2 and trithorax group/mixed lineage leukaemia (TrxG/MLL) complexes, regulating the epigenetic role of both through the attenuation of the expression of transcription factors that are important in lateral mesoderm differentiation. Thereby, Fendrr is essential for normal heart and body wall development [83].

The cardiac-enriched IncRNA Upperhand (Uph, also known as HAND2-AS1 or DEIN) shares a bidirectional promoter with the Hand2 gene, which encodes a transcription factor involved in heart development, and is precisely controlled by an upstream super-enhancer locus. Uph transcription in cis is required for the permissive epigenetic signature, GATA4 binding, and transcription through the enhancer. Therefore, the loss of Uph transcription abolishes Hand2 expression and development of the heart [84].

Transcriptome analysis of human cardiovascular progenitor cells (CPCs) revealed 570 IncRNAs possibly involved in cardiac differentiation. Among them, the cardiac mesoderm enhancer associated noncoding RNA (CARMEN, also known as CARMN or MIR143HG), was one of the most highly expressed. The CARMEN IncRNA is upstream of the cardiac mesoderm-specifying gene regulatory network and interacts with the PRC2 components SUZ12 and EZH2. Knockdown of the mouse orthologue (Carmen or AK087736) inhibited cardiac specification and differentiation of CPCs. Furthermore, CARMEN also plays a role in maintaining the cardiac identity of differentiated cardiomyocytes [85].

In a transcriptome analysis searching for ncRNAs differentially regulated during hESC differentiation to vascular endothelial cells, 75 novel IncRNAs whose sequence and functionality might be conserved across vertebrate species were identified. Among them, ALIEN (also known as LINC00261), TERMINATOR, and PUNISHER (also known as AGAP2-AS1) were functionally characterized in human and other vertebrates’ cardiovascular development. The lincRNA ALIEN was expressed in vascular progenitors, and was related to cardiovascular development before vascular or cardiac specialization, being strongly correlated with genes involved in mesoderm and cardiovascular commitment, such as GATA4, EOMES, MIXL1, and T (Brachyury). Two outcomes were observed after knockdown of ALIEN: (1) the downregulation of 503 genes involved in blood vessel development and angiogenesis; (2) the upregulation of 738 genes related to cell adhesion and extracellular matrix remodeling [86]. Moreover, the lincRNA TERMINATOR, was expressed in cardiovascular progenitors, and was positively correlated with genes related to cell cycle, DNA repair, and chromatin assembly, and negatively correlated with genes involved in proliferation, regulation, and cell death. TERMINATOR was also associated with pluripotency regulators POU5F1 (Oct4), SOX2, ZIC5, and REX1, and its knockdown resulted in the downregulation of 185 genes involved in cell–cell interactions and chromatin remodeling related to pluripotent maintenance. Finally, the antisense IncRNA PUNISHER, expressed in mature endothelial cells, was positively correlated with definitive vascular development genes, such as GATA2, and negatively correlated with genes involved in cell cycle regulation, DNA damage response, and chromatin modification. In addition, PUNISHER’s knockdown resulted in the decrease of histone H3 phosphorylation and the expression of 802 genes involved in cell division, as well as the increased expression of 831 genes involved in cell adhesion and extracellular interactions, altogether compromising endothelial cell function [86].

In another study in ESCs, 4145 multiexonic IncRNAs (2608 novel) with an enhancer activity were identified during differentiation to cardiomyocytes. One of them was the SMAD7-IncRNA, proximal to
the Smad7 gene, and highly expressed in cardiac fibroblasts. SMAD7-lncRNA knockdown reduced Smad7 gene expression, demonstrating the relationship between enhancer-derived lncRNAs and the expression of proximal target genes in cardiac fibroblasts [87].

In a transcriptome analysis of left and right ventricles of neonatal mouse heart, 196 of the 23,949 identified lncRNAs presented dynamic regulation in the cardiac maturation process. In addition, 5% of the 2262 lncRNAs located within 50 kb of protein coding genes were correlated with their neighboring genes’ expression (90.4% positively correlated) [88]. Furthermore, the impact of Ppp1r1b-lncRNA on the regulation of its neighboring partner gene Tcap was demonstrated. Tcap encodes the Titin Cap protein that anchors Titin filaments at the Z disk in cardiomyocyte sarcomere organization and myogenesis. Interestingly, the expression ratio of Ppp1r1b-lncRNA/Tcap differed between normal cardiac maturation and congenital heart defect phenotypes [88].

In heart metabolism, the first lncRNA identified under physiological conditions was the cardiac apoptosis-related lncRNA (CARL or AK017121). As a sponge lncRNA highly expressed in the heart, CARL binds miR-539 that negatively regulates PHB2. Thereby CARL maintains the expression of PHB2, suppressing the effects of anoxia, such as mitochondrial fission and cardiomyocyte apoptosis [89].

In other muscular tissues, myogenesis-associated lncRNA (lnc-mg) was recently identified in murine skeletal muscle development and during the skeletal differentiation of muscle stem cells (muSCs). Myogenesis-associated lncRNA is enriched in skeletal muscle, and its expression is gradually increased during myocyte differentiation. The authors demonstrated that lnc-mg overexpression promotes cell differentiation in vitro, and muscle hypertrophy in vivo. Accordingly, knockout of lnc-mg in mice promoted muscle atrophy and loss of muscular endurance. Myogenesis-associated lncRNA was shown to function as a ceRNA, acting as a sponge for miR-125b, a negative regulator of insulin-like growth factor 2 (Igf2). Thus, lnc-mg overexpression downregulates miR-125b, leading to Igf2 protein increase and enhancing skeletal myogenesis [90].

As already mentioned above, in the H19/Igf2 imprinted genomic locus is also localized H19 [91], a trans-regulatory lncRNA also involved in skeletal muscle differentiation and regeneration. H19 is highly expressed in the majority of embryonic and neonatal tissues, especially in skeletal muscles, and is induced by MyoD, the main regulator of muscle differentiation. The H19 first exon gives rise to miR-675-3p and miR-675-5p, two conserved miRNAs equally upregulated in skeletal muscle differentiation, which downregulate the DNA replication initiation factor Cdc6 and the anti-differentiation transcription factors Smad1 and Smad5. Thus, the miRNAs miR-675-3p and miR-675-5p, transcribed within H19, repress Cdc6, Smad1, and Smad5, mediating skeletal muscle differentiation and regeneration [88]. Furthermore, H19 may have a role in controlling the timing of muscle differentiation, as this lncRNA also acts as a sponge for miRNAs of the let-7 family, which are involved in muscle differentiation anticipation and in glucose metabolism in muscle [92].

Another myogenic lncRNA is the MyoD upstream noncoding (MUNC or DRR(eRNA)), located 5 kb upstream the MyoD transcription start site (TSS), and of which the 5′ end overlaps with the cis-acting distal regulatory region (DRR) of MyoD. MUNC lncRNA induces myoblast differentiation by promoting MyoD association with the DRR enhancer and with the Myogenin promoter, and also by increasing the expression of MyoD, Myogenin, and Myh3, as well as the expression of non MyoD-inducible genes [93]. In addition, next to the MyoD gene is LncMyoD, an lncRNA activated by MyoD during myogenesis and myoblast differentiation. LncMyoD binds to IGF2-mRNA-binding protein 2 (IMP2), inhibiting N-Ras and c-Myc mRNAs translation, and thus facilitating cell-cycle exit and promoting terminal differentiation [94].

In the regulation of muscle differentiation, the muscle-specific lncRNA linc-MD1 acts as a ceRNA in human and murine myoblasts, presenting a decoy activity for two different miRNAs, miR-133, and miR-135. These miRNAs regulate the expression of the transcription factors MAML1 and MEF2C, respectively, which activate the expression of muscle-specific genes. In this way, Linc-MD1 controls the timing of myoblast differentiation, and when it is overexpressed, the anticipation of the muscle differentiation program occurs [95].
Angiotensin II (AngII) is a hormone involved in vasoconstriction, cellular growth, and other processes. The transcriptome analysis of rat vascular smooth muscle cells (VSMCs) revealed five previously annotated lncRNAs and 24 novel lncRNAs regulated by AngII [96]. Among them, lnc-Ang362, an AngII–regulated lncRNA, was identified as the host transcript for miR-221 and miR-222, miRNAs involved in cell proliferation. Knockdown of lnc-Ang362 leads to the decrease of VSMC proliferation [96]. The smooth muscle and endothelial cell-enriched migration/differentiation-associated lncRNA (SENCR) was seen in loss-of-function studies as related to decrease of myocardin expression and to the increase of pro-migratory genes expression, which indicates that SENC R is a repressor of smooth muscle cell migration and possibly stabilizes the smooth muscle cell contractile phenotype [97].

Finally, it has been discovered that some annotated lncRNAs also contain open reading frames (ORFs) that encode functional peptides, as the case of the muscle-specific lncRNA-derived micropeptide called dwarf open reading frame (DWORF). DWORF has 34 aminoacids, is localized in the sarcoplasmatic reticulum, and enhances the activity of the Ca\(^{2+}\) adenosine triphosphatase SERCA calcium pump, enhancing muscle contractility [14]. In this process, SERCA inhibitors are displaced, including the myoregulin (MLN) [15], another micropeptide encoded by a skeletal muscle-specific putative lncRNA, which has the opposite effect as DWORF, inhibiting SERCA calcium pump and reducing muscle performance [15].

### 2.2.4. Adipose and Bone Tissues

Adipose tissue is composed predominantly of adipocytes, cells specialized in storing energy as fat. White fat (subcutaneous and visceral) is the largest energy reservoir in the body, while brown (muscle-derived) and beige (or brite) fat (white-derived) are critical for thermogenesis, energy homeostasis, and metabolism. Besides these primary functions, adipose tissue also acts as thermal insulation and protection against mechanical shocks. The prevalence of obesity has led to a surge of interest in understanding the mechanisms underlying adipocyte development, as well as the process of differentiation of the pre-adipocyte precursor cells into adipocytes. There is strong evidence indicating that lncRNAs participate in adipogenesis regulation in physiological and pathological contexts.

Global expression patterns of lncRNA have been explored in adipocytes, and 175 lncRNAs were identified as specifically regulated during adipogenesis, a substantial fraction of which are under the control of the same key transcription factors that activate and repress coding genes during adipocyte differentiation [98].

During adipogenesis, most of the identified lncRNAs are involved in and/or bind the peroxisome proliferator-activated receptors (PPARs), a nuclear hormone receptor superfamily which regulates metabolic functions and energy homeostasis. The lncRNA steroid receptor RNA activator (SRA) has been observed as an adipogenesis promoter, acting in preadipocyte differentiation by associating with PPARc, a master transcriptional regulator of adipogenesis [99]. NEAT1 is another lncRNA upregulated in adipogenesis, regulating PPAR\(\gamma\)2 splicing [100]. NEAT1 expression is enhanced by miR-140, a regulator of developmental pathways and stem cell differentiation, leading to adipogenic phenotype [101]. Finally, lnc-U90926 decreases 3T3-L1 adipocyte differentiation in mice, via inhibiting the transactivation of PPAR\(\gamma\)2 or PPAR\(\gamma\) [102].

PU.1 (also known as SPI1) is a transcription factor involved in myelo-lymphoid differentiation and expressed in the adipocyte lineage. In mice, PU.1 antisense lncRNA (PU.1 AS) was shown to promote adipogenesis through preventing PU.1 mRNA translation via binding to PU.1 mRNA to form a PU.1 mRNA/PU.1 AS lncRNA duplex in preadipocytes [103].

The lncRNA HOTAIR was found expressed in gluteal, but not in abdominal adipose tissue. The ectopic HOTAIR expression in abdominal preadipocytes increased the proportion of differentiated cells and the expression of important adipogenic genes, such as PPAR\(\gamma\) and lipoprotein lipase (LPL, the main enzyme involved with lipid storage in adipocytes). These data indicate that HOTAIR expression may underline the heterogeneity of human fat distribution and might be involved in key processes of adipocyte differentiation [104].
Besides that, it has been demonstrated that adipocyte differentiation in brown fat is also regulated by lncRNAs, as nuclear brown fat lncRNA 1 (Blncl) forms a ribonucleoprotein complex with transcription factor EBF2, promoting brown and beige adipocyte differentiation [105]. This process is enhanced by the heterogeneous nuclear ribonucleoprotein U (hnRNPU or hnRNP-U) assembly, which physically interacts both with Blncl and EBF2, inducing the ribonucleoprotein complex Blncl/EBF2 formation and transcriptional activation of thermogenic genes in brown adipocytes [106].

Brown adipose tissue–enriched lncRNA 1 (lnc-BATE1) was identified in a murine transcriptome analysis as an lncRNA required for the development and maintenance of brown adipocytes. Lnc-BATE1 sustains the activation of brown adipose related genes, represses white adipose related genes, and interacts with hnRNP-U during brown adipogenesis [107]. A similar lncRNA, lncBATE10 (A530050N04Rik), is also necessary for the expression of brown adipose tissue genes in brown adipocyte differentiation and during the “browning” of white adipocytes. As an effect of the cAMP signaling pathway, lncBATE10 plays its role by promoting the expression of the transcriptional coactivator Pgc1α, an inducer of mitochondrial biogenesis. Moreover, lncBATE10 decoys the Pgc1α repressor Celf1, inhibiting their interaction and preventing Pgc1α inhibition [108].

GM13133 is an lncRNA enriched in murine brown adipocytes and induced by cold exposure and β3-adrenergic agonist and cAMP stimulation. When upregulated, GM13133 inhibits white adipocyte differentiation and may increase the expression of brown adipose-related genes as well as mitochondrial biogenesis, possibly leading to the conversion of white to brown adipocytes [109]. In addition, 704 upregulated and 896 downregulated lncRNAs were identified in brown adipose tissue when compared with skeletal muscle, again indicating an important role for lncRNAs in adipocyte differentiation, and in transdifferentiation between brown adipose tissue and skeletal muscle, both of which have abundant mitochondria and high energy consumption capacity [110].

Adipose tissues have been also studied in osteogenic processes. During human adipose stem cell (hASCs) differentiation, the expression of the tumor suppressor lncRNA MEG3 was increased during osteogenesis and decreased during adipogenesis. Accordingly, MEG3 knockdown blocked the osteogenic differentiation, promoting the adipogenic differentiation of these hASCs. Moreover, the adipogenic and osteogenic balance promoted by MEG3 during hASC differentiation might be modulated by miR-140-5p, a miRNA negatively correlated with MEG3 in both processes [111].

Osteogenic differentiation is supressed by cytokine-induced inflammation. Downregulation of the lncRNA myocardial infarction-associated transcript (MIAT, also known as Gomafu) leads to the osteogenic differentiation of hASCs and reverses the negative effects of inflammation. Treatment with tumor necrosis factor increases MIAT expression, inhibiting hASC osteoinduction [112]. The lncRNA MIR31HG (also known as LncHIFCAR or hsa-lnc-31) was not only found to be involved in the osteogenic differentiation of hASCs, but also in the regulation of the inflammation. The interaction between MIR31HG and NF-κB pathways may attenuate the osteogenic differentiation of hASCs, leading to an inflammation-mediated inhibition of bone formation [113].

As mentioned above, the lncRNA H19 is a key regulator for the differentiation of various cell and tissue types, and gives rise to miR-675, which is partially responsible for the pro-osteogenic effect of H19 by downregulating the transforming growth factor-β1 (TGF-β1), inhibiting Smad3 phosphorylation in addition to downregulating histone deacetylase HDAC 4/5, reducing the recruitment of histone deacetylases (HDACs) to the Runx-related transcription factor 2 (Runx2)-binding DNA sequence [114].

Bone morphogenetic protein 9 (BMP9, or GDF2) is one of the most osteoinductive factors. The well-coordinated expression of lncRNA H19 may be essential in the BMP9-induced osteogenic differentiation of mesenchymal stem cells (MSCs). H19 is highly expressed at the early stage of the BMP9 stimulation of MSCs, followed by a rapid decrease and gradual return to the basal level. Possibly, H19 functions as a key mediator of BMP9 signaling by modulating miRNAs that target Notch receptors and/or ligands [115].

The RNA profiling of human bone marrow mesenchymal stem cells (hBMSCs, or hMSCs) revealed 433 upregulated and 232 downregulated lncRNAs during osteogenic differentiation.
The lncRNA ENST00000502125.2 (also known as NR2F2-AS1) was further examined and the results pointed to its role as a negative regulator of osteogenic differentiation, since its downregulation promoted the osteogenic differentiation of hBMSCs [116]. Global RNA profiling during hBMSC osteogenic differentiation found 785 lncRNAs to be upregulated and 623 lncRNAs downregulated along osteogenic differentiation. Among the differentially expressed lncRNAs were XR_111050, NR_024031, FR374455, FR401275, FR406817, and FR148647. One of them, lncRNA XR_111050, could enhance the osteogenic differentiation potential of MSCs [117].

Furthermore, the lncRNA urothelial cancer-associated 1 (UCA1) was found to be expressed in human normal chondrocytes and induced chondrocytic differentiation in a murine chondrocyte precursor [118]. Still during osteogenic differentiation, the lncRNA differentiation antagonizing non-protein coding RNA (DANCR, also known as the aforementioned ANCR) was decreased in human bone marrow-derived mesenchymal stem cells (hBMSCs). When silenced, the proliferation of hBMSCs and osteogenic differentiation is significantly enhanced, and vice versa. This negative control of osteogenic differentiation and proliferation by DANCR is achieved through the inactivation of the p38 MAPK pathway, and DANCR is suggested by the authors as a potential therapeutic target [119].

2.2.5. Hepatic Tissue

The liver is the largest internal organ of the body, as well as an exocrine and endocrine gland. Like adipose tissue, the liver is responsible, among other important functions, for adipogenesis and lipid metabolism. The lncRNAs involved in liver homeostasis are shown in this section.

During liver development, the lncRNA H19 was found in a chromatin immunoprecipitation sequencing (ChIP-seq) analysis to be the most highly expressed lncRNA. H19 blocks the interaction between actin and the heterogeneous hnRNP-U, affecting the Wnt/β-catenin signaling pathway and the proliferation of foetal liver cells [120].

In liver metabolism, the NAT lncRNA APOA1-AS negatively regulates apolipoprotein A1 (APOA1) gene expression by epigenetic mechanisms. It was demonstrated, both in vitro and in vivo, that knockdown of APOA1-AS alters certain histone methylation patterns through the recruitment of histone-modifying enzymes, increasing the expression of the closely linked APOA1, APOA4, and APOC3 genes [121]. The process of cholesterol efflux and the inhibition of cholesterol biosynthesis in the liver is promoted by the ligand activation of liver X receptors (LXRs) and mediated by the lncRNA LeXis. LeXis can be induced by pharmacologic LXR activation and Western diet feeding, and interacts with ribonucleoprotein RALY, required for cholesterologenic gene expression, thus affecting hepatic and plasmatic cholesterol levels in mice [122].

Liver-specific triglyceride regulator (lncLSTR) regulates plasmatic triglycerides (TG) in mice. The knockdown of lncLSTR leads to the downregulation of Cyp8b1, altering the ratio of the most abundant bile acids, muricholic acid and cholic acid. This alteration results in the upregulation of apoC2 expression and lipoprotein lipase (LPL) activity, increasing farnesoid X receptor (FXR) activity (an important bile acid receptor) and plasmatic TG clearance. However, the increased expression of apoC2 in primary hepatocytes did not lead to the same effects of TG regulation, indicating the presence of other hepatic factors involved in plasmatic TG clearance [123].

A novel sense-overlapping lncRNA named lnc-HC was identified in rats, and homologues exist in mice and humans. It overlaps the predicted 3′UTR region of the coding gene Slc25a15 (mitochondrial carnitine transporter). Long non-coding RNA derived from hepatocytes (lnc-HC) is a negative regulator of cholesterol metabolism and is upregulated in hepatocytes through C/ERBB when exposed to high cholesterol. This lncRNA physically interacts with heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1), forming the lnc-HC/hnRNPA2B1 complex, which targets the mRNA of Cyp7a1 and Abca1, decreasing their protein levels and, consequently, downregulating bile acid biosynthesis and cholesterol export [124].

Among the 359 tissue-specific lncRNAs expressed in metabolic homeostasis and found in the transcriptome analysis of murine major metabolic organs, the liver metabolically sensitive lncRNA
Gm16551 was identified. The findings support a model in which SREBP1c, the master transcription factor of lipogenesis, induces Gm16551 to activate an lncRNA-mediated negative feedback loop to regulate SREBP1c activity in the liver. The Gm16551 knockdown led to the upregulation of key lipogenic genes and to high circulating triglyceride levels [125].

The benefits of green tea in cholesterol metabolism, due to the high levels of catechins—mainly epigallocatechin-3-gallate (EGCG)—are well-known, but the molecular mechanisms underlying this effect are incompletely understood. Microarray analysis of RNA expressed in cultured human hepatocytes (HepG2) revealed 15 genes related to cholesterol metabolism and 285 lncRNAs whose levels were altered by EGCG treatment. Among them, the lncRNA AT102202 mapped within the gene of its potential target, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). HMGCR is the rate-regulating enzyme in the cholesterol biosynthetic pathway. EGCG treatment enhanced AT102202 expression and decreased the expression of HMGCR. The downregulation of HMGCR expression leads to LDL receptor upregulation and cholesterol uptake by hepatocytes [126].

In liver regeneration, it was found in vitro that the lncRNA MALAT1 (or NEAT2), upregulated in this process by p53, stimulates cell cycle progression from G1 to S phase and inhibits apoptosis, leading to hepatocyte proliferation. MALAT1 also activated the Wnt/β-catenin pathway and promoted cyclinD1 expression. These data indicate the critical role of MALAT1 in liver regeneration, and its potential therapeutic benefit in liver failure and transplantation [127].

2.2.6. Pancreatic Tissue

The pancreas is both an endocrine and an exocrine gland, producing digestive enzymes delivered to the duodenum through the pancreatic duct, and endocrine hormones secreted into the blood, including insulin and glucagon produced, respectively, by β and α cells in the islets of Langerhans. IncRNAs have been studied in pancreatic β cells, due to the importance of these cells for glucose homeostasis and clinical relevance in certain disorders such as diabetes.

More than 1100 IncRNAs were identified in a transcriptome analysis of human pancreatic islets, of which various were specific to β cells and located in genomic regions close or antisense to coding genes involved in the development, expression, and function of β cells, such as HNF1A, PDX1, PAX6, ISL1, FOXA2, NEUROD1, GATA6, and MAFB. Among them, the expression of the lincRNA HI-LNC25, which shares a regulatory domain with the islet cell maturation regulator MAFB, was positively correlated with the mRNA levels of GLIS3, an important transcription factor for insulin biosynthesis. Other IncRNAs were upregulated by glucose exposure, such as HI-LNC78, HI-LNC80, and the murine lncRNA Mi-Linc80 [128]. HI-LNC15 (also named β-cell long intergenic noncoding RNA 1, blinc1) was further investigated [129]. It is a conserved lncRNA whose gene is located upstream to the islet homeobox transcription factor gene NKX2.2, and it is specific to embryonic pancreas and adult islets, especially β cells. blinc1 knockdown led to insulin resistance in adult mice and, in embryonic pancreas, to a 50% reduction in β cells of perinatal mice. Moreover, blinc1 deficiency dysregulated islet transcription factors, such as Nkx2.2, Pax6, Mafb, and Neurog3, and several genes involved in the specification of endocrine progenitors and in β cell maturation and function [129].

Furthermore, human β cell-specific IncRNAs and transcription factors were found to regulate a common transcriptional network in β cells. These IncRNAs were involved in the regulation of enhancer clusters. Among them, the lncRNA HI-LNC71, named by the authors as Pdx1 locus upstream transcript (PLUTO), is transcribed from a promoter located upstream from the key β cell transcription factor gene PDX1. PLUTO controls the transcription of PDX1 and, thereby, modulates the PDX1-dependent transcriptional program. The regulation of PDX1 by PLUTO is associated with its ability to promote three-dimensional (3D) interactions between the PDX1 promoter and its upstream enhancer cluster, which is contained within the body of the PLUTO gene [130].

It was shown in a mouse model that the expression levels of several transcription factors, including Pdx1 and the β cell-specific transcription factor Mafa, were reduced after knockdown of IncRNA Gas5 (growth arrest-specific transcript 5), or of the mouse maternal expressed gene 3 (Meg3), or also of the
taurine upregulated gene 1 (TUG1). These IncRNAs are highly expressed in pancreatic islets and their expression is upregulated at higher glucose concentrations and in diabetic mouse models. In addition, Gas5 downregulation induced cell cycle G1/G0 arrest, blocking β cell proliferation, while Meg3 or TUG1 suppression increased the rate of β cell apoptosis in vitro. Taken together, the IncRNAs Gas5, Meg3, and TUG1 might be involved β cell identity and function by regulating transcription factor levels, thereby influencing insulin synthesis and secretion [131–133].

In pregnancy, prolactin signaling stimulates β cell proliferation to increase the cell mass in response to greater insulin resistance. During the pregnancy stage corresponding to the peak of β cell proliferation in the mouse (14.5 days), six IncRNAs were found to be differentially expressed in pancreatic islets. Among them, the IncRNA Gm16308 (Lnc03) was prolactin-dependent, islet-enriched, and dynamically expressed at different pregnancy stages. Lnc03 is regulated by Stat5-dependent components of the prolactin activation pathway. Its expression peaked at gestational day 14.5, but was low before pregnancy and post-partum. Interestingly, the lnc03 human orthologue was not found. Therefore, lnc03 may belong to the set of non-conserved IncRNA and the human IncRNA functionally corresponding to lnc03 still remains unknown [134].

2.2.7. Intestinal Tissue

In intestinal mucosa, the epithelial cells form a single-cell layer that constitutes the largest and most important barrier protecting the intestinal lumen against intraluminal toxins, antigens, and enteric flora. The epithelium maintains its selective barrier function through the formation of complex protein–protein networks that mechanically link adjacent cells and seal the intercellular space.

Among the few studies investigating IncRNAs in intestinal tissue, it was demonstrated that the IncRNA SPRY4-IT1 increases the stability and translation of the mRNA encoding tight junction (TJ) proteins claudin-1, claudin-3, occludin, and JAM-1. Accordingly, the silencing of SPRY4-IT1 repressed the expression of these TJ proteins in the intestinal epithelial barrier. The direct interaction between SPRY4-IT1 and TJ mRNAs is enhanced by the RNA-binding protein HuR. Patients with some intestinal diseases presented lower levels of SPRY4-IT1, suggesting that decreased SPRY4-IT1 and the resulting lower expression of TJ proteins contribute to the pathogenesis of gut barrier dysfunction in patients [135]. Also regulating homeostasis of the intestinal epithelial barrier, the IncRNA H19 may act as a ceRNA, competing with the miR-874, a microRNA which suppresses the expression of the aquaporin-3 gene (AQP3) by binding to the 3′UTR of its mRNA. AQP3 downregulation opens the TJ complex, leading to dysfunction of the intestinal barrier. H19 competes with miR-874, reducing its levels and maintaining normal levels of AQP3, therefore playing a protective role in the intestinal barrier [136]. However, the overexpression of H19 and the consequent increase of miR-675 inhibited the synthesis of the TJ protein ZO-1 and the adherens junction protein E-cadherin, leading to intestinal epithelial barrier dysfunction. Epithelial barrier normal function was restored by an increase of the expression of HuR (also known as ELAVL1) in these cells [137].

A genome-wide transcriptome analysis of murine intestinal epithelial cells showed that tumor necrosis factor alpha (TNF-α) stimulation up- and downregulated several lincRNAs genes. Among them, lincRNA-Cox2 was upregulated in an early response to TNF-α, through the NF-kB signaling pathway activation. lincRNA-Cox2 knockdown altered the gene expression profile in response to TNF-α stimulation. It was found that lincRNA-Cox2 regulates the transcription of the proinflammatory cytokine interleukin 12b gene (Il12b) through epigenetic mechanisms, indicating a role of this lincRNA in the inflammatory responses of the intestinal epithelia [138].

2.2.8. Lung Tissue

The lungs are the primary organs of the respiratory system. Their major function is gas exchange between the air and the blood. They supply oxygen to be distributed to tissues and expel carbon dioxide that has been created throughout the body. Studies reporting the roles of IncRNAs in lung development and homeostasis are still limited.
A total of 363 lncRNAs have been identified in developing and adult lung by an RNA sequencing approach. Spatial correlation between the lncRNAs loci and transcription factor loci across the genome was reported. In addition, their expression pattern correlates with that of the neighboring genes, suggesting the involvement of these lncRNAs in the regulation of the transcription factor genes. In fact, a feedback loop has been identified within the NANC1-Nkx2.1 gene duplex that is critical for buffering the expression of the transcription factor Nkx2.1, maintaining lung epithelial cell identity during development and postnatal tissue homeostasis: while Nkx2.1 directly inhibits the expression of the lncRNA NANC1 (Nkx2.1-associated noncoding intergenic RNA, also called LL18), NANC1 acts in cis to promote Nkx2.1 transcription [139,140].

Moreover, a novel lncRNA RP11-380D23.2 has been reported to drive distal-proximal lung morphogenesis by regulating its nearest gene PITX2, which encodes a transcription factor crucial for maintaining the left-right symmetry during lung development. The lncRNA RP11-380D23.2 modulates PITX2 via physical interaction with PARP1, a nuclear protein that functions as a chromatin modulator. The RP11-380D23.2–PARP1 interaction orchestrates lung development by regulating the timely activation of the Wnt cascade that is important for lung morphogenesis [141].

2.3. Gametogenesis and Germ Cells

Gametogenesis is a highly regulated complex cellular differentiation process. The accurate development of oocytes and sperm is among the most fundamental processes for the success of sexual reproduction. Similar to other reproductive traits, many features of gametogenesis and especially of spermatogenesis are under strong selective pressure to change. However, some fundamental features of gametogenesis are evolutionary conserved. Moreover, among all tissues, the transcriptome of the testis displays the highest diversity and specificity.

The lncRNA/messenger RNA gene pair AK124742 and PSMD6 presents elevated expression in human cumulus cells, the cluster of cells that surrounds the oocyte, and are correlated with oocyte maturation, fertilization, embryo quality, and clinical pregnancy outcome [142]. Furthermore, a miRNA-lncRNA analysis indicated 41 lncRNAs able to interact with oocyte microRNAs and potentially involved in the regulation of folliculogenesis [143]. In a study that investigated the role of lncRNAs in mediating the effects of β-crystallin B2 (CRYBB2) in ovary development in mice, RNA microarray profiling showed 157 differentially expressed lncRNAs and 1085 differentially expressed mRNAs between ovary tissues from wild type and CRYBB2 knockout mice. In CRYBB2 knockout mice, the expression of lncRNA A-30-P01019163 was downregulated, as well as its likely downstream target gene P2rx7, affecting ovarian development [144].

Concerning mammalian spermatogenesis, the analysis of the Gc1-Spg cell line, derived from mouse spermatogonial cells, demonstrated that the lncRNA meiotic recombination hot spot locus (mrhl) negatively regulates the Wnt signaling pathway (implicated in the regulation of mammalian spermatogenesis) through Ddx5/p68 RNA helicase [145]. Furthermore, genes at 37 loci (referred to as GRPAM) also showed altered expression upon mrhl downregulation. The p68 helicase interacted with mrhl in chromatin at these loci, suggesting a role for p68-mediated mrhl RNA occupancy in regulating GRPAM expression [146]. Moreover, a functional study reported that the lncRNA AK015322 promotes the proliferation of the mouse spermatogonial stem cell line C18-4 in vitro by function as a decoy of miR-19b-3p. AK015322 antagonizes the function of this microRNA, thus indirectly upregulating its endogenous target transcriptional factor Ets-variant 5 (ETV5), which is a pivotal gene for spermatogonial stem cell self-renewal [147].

Long non-coding RNA profiling in mature mouse cells led to the identification of 20,907 known and 4088 novel lncRNAs. Compared to round spermatids, 1794 lncRNAs were upregulated and 165 downregulated, and 14,259 potential target coding-genes of differently expressed lncRNAs were suggested [148].

Purified cells at key stages of murine male spermatogenesis were submitted to RNA sequencing. The dynamic expression pattern of lncRNAs during the progression of spermatogenesis was reported,
where 1630 lncRNAs differently expressed in spermatogonia versus pachytene and 1534 lncRNAs
differently expressed in pachytene versus spermatids were identified. To examine the possible role
of one lncRNA, a mouse knockout of the X-linked lncRNA testis-specific long noncoding RNA 1 (Tslrn1)
was generated. These mice had normal fertility but expressive reduction in sperm count [149].

2.4. Extracellular Vesicles

Extracellular vesicles (EVs) play an important role in intercellular communication through the
transference of their cargo between cells. They are sub-micron membrane vesicles released to the
extracellular environment by all cell types. According to their biogenesis, membrane, size, and
composition, EVs can be classified into exosomes, apoptotic bodies, microvesicles, and ectosomes,
among others (reviewed in [150,151]). EVs have been found in body fluids, including blood and urine.
They are composed of a lipid bilayer from the plasma membrane and contain a variety of proteins,
nucleic acids, lipids, and metabolites from donor cells, transferring biomolecular information between
tissues and mediating diverse biological functions. Accordingly, RNA transcripts contained in the
EVs also may be transferred to the target cells and present functional impact [152,153]. The number
of studies concerning lncRNAs in EVs is steadily increasing. Most aim at finding potential disease
markers or at understanding pathological mechanisms of human diseases. Yet some of the findings
are useful also for the comprehension of physiological processes.

Interestingly, lncRNAs with low expression in cells may be enriched in EVs, such as lincRNA-p21,
HOTAIR, and ncRNA-CCND1 [154,155]. In fact, in a study comparing normal cell lines with a prostate
cancer cell line, the population of exosomal lncRNAs differed between both cell lines. Furthermore,
some lncRNAs enriched in EVs present complementary motifs to some microRNAs, such as members
of the let-7 family, among others, suggesting that these lncRNAs can act as sponges for EV microRNAs,
and to distinct RNA-binding proteins, such as ELAVL1 (HuR), which may play a role in the exosomal
transport of lncRNAs [155]. Another functional study provided evidence that specific lncRNAs
are preferentially packaged into exosomes, and some of these exosomal lncRNAs (such as Exo2,
Exo4, and RMRP) can modulate protein functions and cell viability by direct interactions with
L-lactate dehydrogenase B (LDHB), high-mobility group protein 17 (HMG-17), and CSF2RB, inside the
recipient [156].

In a total transcriptome profiling of plasma-derived EVs of five healthy women, thousands of
different transcripts were found. Among these, up to 220 lncRNA were detected. The lncRNAs
RN7SL1, RPPH1, VTRNA2-1, RMRP, RNU11, and MALAT1 were the most highly expressed [157].
To our knowledge, this is the first study characterizing EV-lncRNA exclusively in healthy individuals.
Thus, the EV-lncRNA cargo remains largely unknown, and the need for more research to characterize
EVs contents and biological effects becomes evident. Apart from the relevance of such studies for
learning more about extracellular lncRNAs (and other biomolecules) in physiological settings, there is
great interest in developing EVs analysis for “liquid biopsies”, which are minimally invasive assays
that will be of value in biology and medicine.

3. Conclusions

Although lncRNAs were overlooked for a long time, now their importance in various biological
processes has become evident. However, their description and especially their effects and functions
in homeostasis (and in disease) remain largely elusive. This is not surprising in view of the complex
interactions among the multiple players involved in every biological process, and the limitations
of the available biological materials and methods. These limitations certainly will be overcome by
the improvement of systems biology approaches in wet laboratories as well as bioinformatics, and
the development of more powerful integrative analysis methods. In this review, we provided a
broad overview of the information available in the literature regarding lncRNA identification and/or
effects in specific mammalian—and especially human—cell and tissue types, aiming at understanding
their effects in homeostasis. We limited our search to cells and tissues for which there is presently
growing interest among researchers. Future studies will expand the knowledge about IncRNAs in cell biology and physiological processes, contributing to a deeper understanding of their function in the context of the multiple interactions between IncRNAs, other ncRNAs, mRNAs, proteins, and small molecules. Furthermore, information about IncRNAs in homeostasis provides a framework for future studies of their functions in health and disease and in the development of novel disease markers and therapeutic targets.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2311-553X/4/1/3/s1, Table S1: IncRNAs described in the homeostasis of different cells and tissues.

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**References**

1. Harrow, J.; Frankish, A.; Gonzalez, J.M.; Tapanari, E.; Diekhans, M.; Kokocinski, F.; Aken, B.L.; Barrell, D.; Zadissa, A.; Searle, S.; et al. GENCODE: The reference human genome annotation for The ENCODE Project. *Genome Res.* 2012, 22, 1760–1774. [CrossRef] [PubMed]

2. Iyer, M.K.; Niknafs, Y.S.; Malik, R.; Singhal, U.; Sahu, A.; Hosono, Y.; Barrette, T.R.; Prensner, J.R.; Evans, J.R.; Zhao, S.; et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat. Genet.* 2015, 47, 199–208. [CrossRef] [PubMed]

3. Derrien, T.; Johnson, R.; Bussotti, G.; Tanzer, A.; Djebali, S.; Tilgner, H.; Guernec, G.; Martin, D.; Merkel, A.; Knowles, D.G.; et al. The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. *Genome Res.* 2012, 22, 1775–1789. [CrossRef] [PubMed]

4. Wang, K.C.; Chang, H.Y. Molecular mechanisms of long noncoding RNAs. *Mol. Cell* 2011, 43, 904–914. [CrossRef] [PubMed]

5. Moran, V.A.; Perera, R.J.; Khalil, A.M. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. *Nucleic Acids Res.* 2012, 40, 6391–6400. [CrossRef] [PubMed]

6. Rashid, F.; Shah, A.; Shan, G. Long Non-coding RNAs in the Cytoplasm. *Genom. Proteom. Bioinform.* 2016, 14, 73–80. [CrossRef] [PubMed]

7. Liu, X.; Xiao, Z.D.; Gan, B. An lncRNAs switch for AMPK activation. *Cell Cycle* 2016, 15, 1948–1949. [CrossRef] [PubMed]

8. Wang, P.; Xu, J.; Wang, Y.; Cao, X. An interferon-independent IncRNA promotes viral replication by modulating cellular metabolism. *Science* 2017, 358, 1051–1055. [CrossRef] [PubMed]

9. Brockdorff, N.; Ashworth, A.; Kay, G.F.; McCabe, V.M.; Norris, D.P.; Cooper, P.J.; Swift, S.; Rastan, S. The Product of the Mouse Xist Gene Is a 15 kb Inactive X-Specific Transcript Containing no Conserved ORF and Located in the Nucleus. *Cell* 1992, 71, 515–526. [CrossRef]

10. Brown, C.J.; Hendrich, B.D.; Rupert, J.L.; Lafrenière, R.G.; Xing, Y.; Lawrence, J.; Willard, H.F. The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. *Cell* 1992, 71, 527–542. [CrossRef]

11. Gayen, S.; Maclary, E.; Buttigieg, E.; Hinten, M.; Kalantry, S. A primary role for the Tsix lncRNA in maintaining random X-chromosome inactivation. *Cell Rep.* 2015, 11, 1251–1265. [CrossRef] [PubMed]

12. Quinn, J.J.; Chang, H.Y. Unique features of long non-coding RNA biogenesis and function. *Nat. Rev. Genet.* 2016, 17, 47–62. [CrossRef] [PubMed]

13. Jalali, S.; Jayaraj, G.G.; Scaria, V. Integrative transcriptome analysis suggest processing of a subset of long non-coding RNAs to small RNAs. *Biol. Direct* 2012, 7, 25. [CrossRef] [PubMed]

14. Nelson, B.R.; Makarewich, C.A.; Anderson, D.M.; Winders, B.R.; Troupes, C.D.; Wu, F.; Reese, A.L.; McAnally, J.R.; Chen, X.; Kavalali, E.T.; et al. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* 2016, 351, 271–275. [CrossRef] [PubMed]

15. Anderson, D.M.; Anderson, K.M.; Chang, C.L.; Makarewich, C.A.; Nelson, B.R.; McAnally, J.R.; Kasaragod, P.; Shelton, J.M.; Liou, J.; Bassel-Duby, R.; et al. A Micropeptide Encoded by a Putative long noncoding RNA Regulates Muscle Performance. *Cell* 2015, 160, 595–606. [CrossRef] [PubMed]
16. Dinger, M.E.; Amaral, P.P.; Mercer, T.R.; Pang, K.C.; Bruce, S.J.; Gardiner, B.B.; Askarian-Amiri, M.E.; Ru, K.; Soldà, G.; Simons, C.; et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Res.* 2008, 18, 1433–1445. [CrossRef] [PubMed]

17. Mercer, T.R.; Dinger, M.E.; Sunkin, S.M.; Mehler, M.F.; Mattick, J.S. Specific expression of long noncoding RNAs in the mouse brain. *Proc. Natl. Acad. Sci. USA* 2008, 105, 716–721. [CrossRef] [PubMed]

18. Guttman, M.; Amit, I.; Garber, M.; French, C.; Lin, M.F.; Feldser, D.; Huarte, M.; Zak, O.; Carey, B.W.; Cassady, J.P.; et al. Chromatin signature reveals over a thousand well conserved large non-coding RNAs in mammals. *Nature* 2009, 458, 223–227. [CrossRef] [PubMed]

19. Wilusz, J.E.; Sunwoo, H.; Spector, D.L. Long noncoding RNAs: Functional surprises from the RNA world. *Genes Dev.* 2009, 23, 1494–1504. [CrossRef] [PubMed]

20. Maurano, M.T.; Humbert, R.; Rynees, E.; Thurman, R.E.; Haugen, E.; Wang, H.; Reynolds, A.P.; Sandstrom, R.; Qu, H.; Brody, J.; et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012, 337, 1190–1195. [CrossRef] [PubMed]

21. Hrdlickova, B.; Kumar, V.; Kanduri, K.; Zherochkova, D.V.; Tripathi, S.; Karjalainen, J.; Lund, R.J.; Li, Y.; Ullah, U.; Modderman, R.; et al. Expression profiles of long non-coding RNAs located in autoimmune disease-associated regions reveal immune cell-type specificity. *Genome Med.* 2014, 6, 88. [CrossRef] [PubMed]

22. Venkatraman, A.; He, X.C.; Thorvaldsen, J.L.; Sugimura, R.; Perry, J.M.; Tao, F.; Zhao, M.; Christenson, M.K.; Sanchez, R.; Yu, J.Y.; et al. Maternal imprinting at the H19-Igf2 locus maintains adult haematopoietic stem cell quiescence. *Nature* 2013, 500, 343–349. [CrossRef] [PubMed]

23. Keniry, A.; Osley, D.; Monnier, P.; Kyba, M.; Dandolo, L.; Smits, G.; Reik, W. The H19 lincRNA is a developmental reservoir of miR-675 which suppresses growth and IgfIr. *Nat. Cell Biol.* 2012, 14, 659–665. [CrossRef] [PubMed]

24. Luo, M.; Jeong, M.; Sun, D.; Park, H.J.; Rodriguez, B.A.; Xia, Z.; Yang, L.; Zhang, X.; Sheng, K.; Darlington, G.J.; et al. Long non-coding RNAs control hematopoietic stem cell function. *Cell Stem Cell* 2015, 16, 426–438. [CrossRef] [PubMed]

25. Villamizar, O.; Chambers, C.B.; Mo, Y.Y.; Torry, D.S.; Hofstrand, R.; Riberdy, J.M.; Persons, D.A.; Wilber, A. Fas-antisense long noncoding RNA is differentially expressed during maturation of human erythrocytes and confers resistance to Fas-mediated cell death. *Blood Cells Mol. Dis.* 2016, 58, 57–66. [CrossRef] [PubMed]

26. Alvarez-Dominguez, J.R.; Hu, W.; Yuan, B.; Shi, J.; Park, S.S.; Gromatzky, A.A.; van Oudenaarden, A.; Lodish, H.F. Global discovery of erythroid long noncoding RNAs reveals novel regulators of red cell maturation. *Blood* 2014, 123, 570–581. [CrossRef] [PubMed]

27. Hu, W.; Yuan, B.; Flygare, J.; Lodish, H.F. Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. *Genes Dev.* 2011, 25, 2573–2578. [CrossRef] [PubMed]

28. Ding, N.; Xi, J.; Li, Y.; Xie, X.; Shi, J.; Zhang, Z.; Li, Y.; Fang, F.; Wang, S.; Yue, W.; et al. Global transcriptome analysis for identification of interactions between coding and noncoding RNAs during human erythroid differentiation. *Front. Med.* 2016, 10, 297–310. [CrossRef] [PubMed]

29. Doss, J.F.; Corcoran, D.L.; Jima, D.D.; Telen, M.J.; Dave, S.S.; Chi, J.T. A comprehensive joint analysis of the long and short RNA transcriptomes of human erythrocytes. *BMC Genom.* 2016, 17, 1307–1315. [CrossRef] [PubMed]

30. Miska, E.A.; & Ebert, B.S. Genomic islands of gene expression in mammals. *Nature* 2008, 105, 716–721. [CrossRef] [PubMed]
through modulating SWI/SNF-mediated chromatin remodeling. *J. Immunol*. 2016, 196, 2799–2808. [CrossRef] [PubMed]

35. Wang, P.; Xue, Y.; Han, Y.; Lin, L.; Wu, C.; Xu, S.; Jiang, Z.; Xu, J.; Liu, Q.; Cao, X. The STAT3-Binding Long Noncoding RNA An Inc-DC Controls Human Dendritic Cell Differentiation. *Science* 2014, 344, 310. [CrossRef] [PubMed]

36. Dijkstra, J.M.; Ballingall, K.T. Non-human Inc-DC orthologs encode Wdnm1-like protein. *F1000Research* 2014, 3, 160. [CrossRef] [PubMed]

37. Casero, D.; Sandoval, S.; Seet, C.S.; Scholes, J.; Zhu, Y.; Ha, V.L.; Luong, A.; Parekh, C.; Crooks, G.M. Long non-coding RNA profiling of human lymphoid progenitor cells reveals transcriptional divergence of B cell and T cell lineages. *Nat. Immunol.* 2015, 16, 1282–1291. [CrossRef] [PubMed]

38. Wang, F.Y.; Luo, L.; Zhao, S.S.; Wei, H.P.; Wang, L.; Gu, Z.Y.; Guan, L.X.; Yang, N.; Gao, Z.; Wang, L.L.; et al. Expression Profile of lncRNA NONHSAT040475 in Peripheral Blood Leukocytes of Healthy Persons. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2016, 24, 1196–1200. [CrossRef] [PubMed]

39. Pang, K.C.; Dinger, M.E.; Mercer, T.R.; Malquori, L.; Grimmond, S.M.; Chen, W.; Mattick, J.S. Genome-Wide Identification of Long Noncoding RNAs in CD8+ T Cells. *J. Immunol.* 2009, 182, 7738–7748. [CrossRef] [PubMed]

40. Sharma, S.; Findlaya, G.M.; Bandukwala, H.S.; Oberdoerffer, S.; Bausta, B.; Lib, Z.; Schmidt, V.; Hogana, P.G.; Sacksb, D.B.; Raoa, A. Dephosphorylation of the nuclear factor of activated T cells (NFAT) transcription factor is regulated by an RNA-protein scaffold complex. *Proc. Natl. Acad. Sci. USA* 2011, 108, 11381–11386. [CrossRef] [PubMed]

41. Collier, S.P.; Collins, P.L.; Williams, C.L.; Boothby, M.R.; Aune, T.M. Cutting Edge: Influence of Tmevpg1, a Long Intergenic Noncoding RNA, on the Expression of Ifng by Th1 Cells. *J. Immunol.* 2012, 189, 2084–2088. [CrossRef] [PubMed]

42. Hu, G.; Tang, Q.; Sharma, S.; Yu, F.; Escobar, T.M.; Muljo, S.A.; Zhu, J.; Zhao, K. Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat. Imunol.* 2013, 14, 1190–1198. [CrossRef] [PubMed]

43. Bonnal, R.J.P.; Ranzani, V.; Arrigoni, A.; Curti, S.; Panzeri, I.; Gruarin, P.; Abrignani, S.; Rossetti, G.; Pagani, M. De novo transcriptome profiling of highly purified human lymphocytes primary cells. *Sci. Data* 2015, 2, 150051. [CrossRef] [PubMed]

44. Ranzani, V.; Rossetti, G.; Panzeri, I.; Arrigoni, A.; Bonnal, R.J.P.; Curti, S.; Gruarin, P.; Provasi, E.; Sugliano, E.; Marconi, M.; et al. The long intergenic noncoding RNA landscape of human lymphocytes highlights the regulation of T cell differentiation by linc-MAF-4. *Nat. Immunol.* 2015, 16, 318–325. [CrossRef] [PubMed]

45. Spurlock, C.F., III; Tossberg, J.T.; Guo, Y.; Collier, S.P.; Crooke, P.H.; Aune, T.M. Expression and functions of long noncoding RNAs during human T helper cell differentiation. *Nat. Commun.* 2015, 6, 6932. [CrossRef] [PubMed]

46. Zhang, H.; Nestor, C.E.; Zhao, S.; Lentini, A.; Bohle, B.; Benson, M.; Wang, H. Profiling of human CD41+ T-cell subsets identifies the TH2-specific noncoding RNA GATA3-AS1. *J. Allergy Clin. Immunol.* 2013, 132, 1005–1008. [CrossRef] [PubMed]

47. Xia, F.; Dong, F.; Yang, Y.; Huang, A.; Chen, S.; Sun, D.; Xiong, S.; Zhang, J. Dynamic Transcription of Long Non-Coding RNA Genes during CD4+ T Cell Development and Activation. *PLoS ONE* 2014, 9, e101588. [CrossRef] [PubMed]

48. Kanduri, K.; Tripathi, S.; Larjo, A.; Mannerström, H.; Ullah, U.; Lund, R.; Hawkins, R.D.; Ren, B.; Lähdesmäki, H.; Lahesmaa, R. Identification of global regulators of T-helper cell lineage specification. *Genome Med.* 2015, 7, 122. [CrossRef] [PubMed]

49. Spurlock, C.F., III; Shaginurova, G.; Tossberg, J.T.; Hester, J.D.; Chapman, N.; Guo, Y.; Crooke, P.S., III; Aune, T.M. Profiles of Long Noncoding RNAs in Human Naive and Memory T Cells. *J. Immunol.* 2017, 199, 547–558. [CrossRef] [PubMed]

50. Petri, A.; Dybkær, K.; Bogsted, M.; Thrue, C.A.; Hagedorn, P.H.; Schmitz, A.; Bedker, J.S.; Johansen, H.E.; Kauppinen, S. Long Noncoding RNA Expression during Human B-Cell Development. *PLoS ONE* 2015, 10, e0138236. [CrossRef] [PubMed]

51. Brazão, T.F.; Johnson, J.S.; Müller, J.; Heger, A.; Ponting, C.P.; Tybulewicz, V.L.J. Long non-coding RNAs in B cell development and activation. *Blood* 2016, 128, e10–e19. [CrossRef] [PubMed]
52. Syrett, C.M.; Sindhava, V.; Hodawadekar, S.; Myles, A.; Liang, G.; Zhang, Y.; Nandi, S.; Cancro, M.; Atchison, M.; Anguera, M.C. Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells. *PLoS Genet.* 2017, 13, e1007050. [CrossRef] [PubMed]

53. Zhang, R.; Ni, F.; Fu, B.; Wu, Y.; Sun, R.; Tian, Z.; Wei, H. A long noncoding RNA positively regulates CD56 in human natural killer cells. *Oncotarget* 2016, 7, 72546–72558. [CrossRef] [PubMed]

54. Wright, P.W.; Huehn, A.; Cichocki, F.; Li, H.; Sharma, N.; Dang, H.; Lenvik, T.R.; Woll, P.; Kaufman, D.; Miller, J.S.; et al. Identification of a KIR antisense IncRNA expressed by progenitor cells. *Genes Immun.* 2013, 14, 427–433. [CrossRef] [PubMed]

55. Mowel, W.K.; McCright, S.J.; Kotzin, J.J.; Collet, M.A.; Uyar, A.; Chen, X.; DeLaney, A.; Spencer, S.P.; Virtue, A.T.; Yang, E.; et al. Group 1 innate lymphoid cell lineage identity is determined by a cis-regulatory element marked by a long non-coding RNA. *Immunity* 2017, 47, 435–449. [CrossRef] [PubMed]

56. Beltran, M.; Puig, I.; Peña, C.; García, J.M.; Álvarez, A.B.; Peña, R.; Bonilla, F.; Herreros, A.G. A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial-mesenchymal transition. *Genes Dev.* 2008, 22, 756–769. [CrossRef] [PubMed]

57. Lopez-Pajares, V.; Qu, K.; Zhang, J.; Webster, D.E.; Barajas, B.C.; Siprashvili, Z.; Zarnegar, B.J.; Boxer, I.D.; Rios, E.J.; Tao, S.; et al. An IncRNA-MAF: MAFB Transcription Factor Network Regulates Epidermal Differentiation. *Dev. Cell* 2015, 32, 693–706. [CrossRef] [PubMed]

58. Mazar, J.; Sinha, S.; Dinger, M.E.; Mattick, J.S.; Perera, R.J. Protein-coding and non-coding gene expression analysis in differentiating human keratinocytes using a three-dimensional epidermal equivalent. *Mol. Genet. Genom.* 2010, 284, 1–9. [CrossRef] [PubMed]

59. Ørom, U.A.; Derrien, T.; Guigo, R.; Shiekhattar, R. Long Noncoding RNAs as Enhancers of Gene Expression. *Cold Spring Harb. Symp. Quant. Biol.* 2010, 75, 325–331. [CrossRef] [PubMed]

60. Kretz, M.; Webster, D.E.; Flockhart, R.J.; Lee, C.S.; Zehnder, A.; Lopez-Pajares, V.; Qu, K.; Zheng, G.X.Y.; Chow, J.; Kim, G.E.; et al. Suppression of progenitor differentiation requires the long noncoding RNA ANCR. *Genes Dev.* 2012, 26, 338–343. [CrossRef] [PubMed]

61. Kretz, M.; Siprashvili, Z.; Chu, C.; Webster, D.E.; Zehnder, A.; Qu, K.; Lee, C.S.; Flockhart, R.J.; Groff, A.F.; Chow, J.; et al. Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature* 2013, 493, 231–235. [CrossRef] [PubMed]

62. Li, C.X.; Li, H.G.; Huang, L.T.; Kong, Y.W.; Chen, F.Y.; Liang, J.Y.; Yu, H.; Yao, Z.R. H19 IncRNA regulates keratinocyte differentiation by targeting miR-130b-3p. *Cell Death Dis.* 2017, 8. [CrossRef] [PubMed]

63. Lin, C.M.; Liu, Y.; Huang, K.; Chen, X.; Cai, B.; Li, H.; Yuan, Y.; Zhang, H.; Li, Y. Long noncoding RNA expression in dermal papilla cells contributes to hairy gene regulation. *Biochem. Biophys. Res. Commun.* 2014, 453, 508–514. [CrossRef] [PubMed]

64. Mullin, N.K.; Mallipeddi, N.V.; Hamburg-Shields, E.; Ibarra, B.; Khalil, A.M.; Atit, R.P. Wnt/β-catenin Signaling Pathway Regulates Specific IncRNAs That Impact Dermal Fibroblasts and Skin Fibrosis. *Front. Genet.* 2017, 8, 183. [CrossRef] [PubMed]

65. Qureshi, I.A.; Mehler, M.F. Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. *Nat. Rev. Neurosci.* 2012, 13, 528–541. [CrossRef] [PubMed]

66. Pollard, K.S.; Salama, S.R.; Lambert, N.; Lambot, M.A.; Coppen, S.; Pedersen, J.S.; Katzman, S.; King, B.; Onedera, C.; Siepel, A.; et al. An RNA gene expressed during cortical development evolved rapidly in humans. *Nature* 2006, 443, 167–172. [CrossRef] [PubMed]

67. Bond, A.M.; VanGompel, M.J.W.; Sametsky, E.A.; Clark, M.F.; Savage, J.C.; Disterhoft, J.F.; Kohtz, J.D. Balanced gene regulation by an embryonic brain ncRNA is critical for adult hippocampal GABA circuitry. *Nat. Neurosci.* 2009, 12, 1020–1027. [CrossRef] [PubMed]

68. Ramos, A.D.; Andersen, R.E.; Liu, S.J.; Nowakowski, T.J.; Hong, S.J.; Gertz, C.; Salinas, R.D.; Zarabi, H.; Kriegstein, A.; Lim, D.A. The long noncoding RNA Pnky regulates neuronal differentiation of embryonic and postnatal neural stem cells. *Cell Stem Cell* 2015, 16, 439–447. [CrossRef] [PubMed]

69. Rani, N.; Nowakowski, T.J.; Zhou, H.; Godshalk, S.E.; Lisi, V.; Kriegstein, A.R.; Kosik, K.S. A Primate IncRNA Mediates Notch Signaling during Neuronal Development by Sequestering miRNA. *Neuron* 2016, 90, 1174–1188. [CrossRef] [PubMed]
70. Lin, M.; Pedrosa, E.; Shah, A.; Hrabovsky, A.; Maqbool, S.; Zheng, D.; Lachman, H.M. RNA-Seq of Human Neurons Derived from iPS Cells Reveals Candidate Long Non-Coding RNAs Involved in Neurogenesis and Neuropsychiatric Disorders. *PLoS ONE* 2011, 6, e23356. [CrossRef] [PubMed]

71. Ng, S.Y.; Johnson, R.; Stanton, L.W. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. *EMBO J.* 2012, 31, 522–533. [CrossRef] [PubMed]

72. Wu, A.M.; Ni, W.F.; Huang, Z.Y.; Li, Q.L.; Wu, J.B.; Xu, H.Z.; Yin, L.H. Analysis of differentially expressed lncRNAs in differentiation of bone marrow stem cells into neural cells. *J. Neur. Sci.* 2015, 35, 160–167. [CrossRef] [PubMed]

73. Zhang, Y.; Chen, K.; Sloan, S.A.; Bennett, M.L.; Scholze, A.R.; O’Keefe, S.; Phatnani, H.P.; Guarnieri, P.; Caneda, C.; Rudericsh, N.; et al. An RNA-sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. *J. Neurosci.* 2014, 34, 11929–11947. [CrossRef] [PubMed]

74. Guo, Y.; Zhang, P.; Sheng, Q.; Zhao, S.; Hackett, T.A. lncRNA expression in the auditory forebrain during postnatal development. *Gene* 2016, 593, 201–216. [CrossRef] [PubMed]

75. Liu, S.J.; Nowakowsk, T.J.; Pollen, A.A.; Lui, J.H.; Horibeck, M.A.; Attenello, F.J.; He, D.; Weissman, J.S.; Kriegstein, A.R.; Diaz, A.A.; et al. Single-cell analysis of long non-coding RNAs in the developing human neocortex. *Genome Biol.* 2016, 17, 67. [CrossRef] [PubMed]

76. Aliperti, V.; Donizetti, A. Long Non-coding RNA in Neurons: New Players in Early Response to BDNF Stimulation. *Front. Mol. Neurosci.* 2016, 9, 15. [CrossRef] [PubMed]

77. He, D.; Wang, J.; Lu, Y.; Deng, Y.; Zhao, C.; Xu, L.; Chen, Y.; Hu, Y.C.; Zhou, W.; Lu, Q.R. lncRNA Functional Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb. Perspect. Biol.* 2013, 5. [CrossRef] [PubMed]

78. Bernard, D.; Prasanth, K.V.; Tripathi, V.; Colasse, S.; Nakamura, T.; Xuan, Z.; Zhang, M.Q.; Sedel, F.; Jourdren, L.; Couplier, F.; et al. A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. *EMBO J.* 2010, 29, 3082–3093. [CrossRef] [PubMed]

79. Tan, M.C.; Widagdo, J.; Chau, Y.Q.; Zhu, T.; Wong, J.J.; Cheung, A.; Anggono, V. The Activity-Induced Long Non-Coding RNA Meg3 Modulates AMPA Receptor Surface Expression in Primary Cortical Neurons. *Front. Cell. Neurosci.* 2017, 11, 124. [CrossRef] [PubMed]

80. Bruneau, B.G. Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb. Perspect. Biol.* 2013, 5. [CrossRef] [PubMed]

81. Ounzain, S.; Burdet, F.; Iberson, M.; Pedrazzini, T. Discovery and functional characterization of cardiovascular long noncoding RNAs. *J. Mol. Cell. Cardiol.* 2015, 89, 17–26. [CrossRef] [PubMed]

82. Klattenhoff, C.A.; Scheuermann, J.C.; Surface, L.E.; Bradley, R.K.; Fields, P.A.; Steinhauser, M.L.; Ding, H.; Butty, V.L.; Torrey, L.; Haas, S.; et al. Bravehearth, a Long Noncoding RNA Required for Cardiovascular Lineage Commitment. *Cell* 2013, 152, 570–583. [CrossRef] [PubMed]

83. Grote, P.; Wittler, L.; Hendrix, D.; Koch, F.; Währisch, S.; Beisaw, A.; Macura, K.; Bläss, G.; Kellis, M.; Werber, M.; et al. The tissue-specific IncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev. Cell* 2013, 24, 206–214. [CrossRef] [PubMed]

84. Anderson, K.M.; Anderson, D.M.; McAnally, J.R.; Shelton, J.M.; Bassel-Duby, R.; Olson, E.N. Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. *Nature* 2016, 539, 433–436. [CrossRef] [PubMed]

85. Ounzain, S.; Micheletti, R.; Arnan, C.; Plaisance, I.; Cecchi, D.; Schrøn, B.; Reverter, F.; Alexanian, M.; Gonzales, C.; Ng, S.Y.; et al. CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. *J. Mol. Cell. Cardiol.* 2015, 89, 98–112. [CrossRef] [PubMed]

86. Kurian, L.; Aguirre, A.; Sancho-Martinez, I.; Benner, C.; Hishida, T.; Nguyen, T.B.; Reddy, P.; Nivet, E.; Krause, M.N.; Nelles, D.A.; et al. Identification of Novel Long Noncoding RNAs Underlying Vertebrate Cardiovascular Development. *Circulation* 2015, 131, 1278–1290. [CrossRef] [PubMed]

87. Ounzain, S.; Pezzuto, I.; Micheletti, R.; Burdet, F.; Sheta, R.; Nemir, M.; Gonzales, C.; Sarre, A.; Alexanian, M.; Blow, M.J.; et al. Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. *J. Mol. Cell. Cardiol.* 2014, 76, 55–70. [CrossRef] [PubMed]
88. Touma, M.; Kang, X.; Zhao, Y.; Cass, A.A.; Gao, F.; Biniwale, R.; Coppola, G.; Xiao, X.; Reemtsen, B.; Wang, Y. Decoding the Long Noncoding RNA During Cardiac Maturation: A Roadmap for Functional Discovery. Circ. Cardiovasc. Genet. 2016, 9, 395–407. [CrossRef] [PubMed]

89. Wang, K.; Long, B.; Zhou, L.-Y.; Liu, F.; Zhou, Q.Y.; Liu, C.-Y.; Fan, Y.Y.; Li, P.F. CARL LncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. Nat. Commun. 2014, 5, 3596. [CrossRef] [PubMed]

90. Zhu, M.; Liu, J.; Xiao, J.; Yang, L.; Cai, M.; Shen, H.; Chen, X.; Ma, Y.; Hu, S.; Wang, Z.; et al. Lnc-mg is a long non-coding RNA that promotes myogenesis. Nat. Commun. 2017, 8, 14718. [CrossRef] [PubMed]

91. Kallen, A.N.; Zhou, X.B.; Xu, J.; Qiao, C.; Ma, J.; Yan, L.; Lu, L.; Liu, C.; Yi, J.S.; Zhang, H.; et al. The imprinted H19 IncRNA antagonizes let-7 microRNAs. Mol. Cell 2013, 52, 101–112. [CrossRef] [PubMed]

92. Dey, B.K.; Pfeifer, K.; Dutta, A. The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev. 2014, 28, 491–501. [CrossRef] [PubMed]

93. Mueller, A.C.; Cicheviwicz, M.A.; Dey, B.K.; Layer, R.; Reon, B.J.; Gaganj, R.; Dutta, A. MuINC, a long noncoding RNA that facilitates the function of MyoD in skeletal myogenesis. Mol. Cell. Biol. 2015, 35, 498–513. [CrossRef] [PubMed]

94. Gong, C.; Li, Z.; Ramanujan, K.; Clay, I.; Zhang, Y.; Lemire-Brachat, S.; Glass, D.J. A long non-coding RNA, LncMyoD, regulates skeletal muscle differentiation by blocking IMP2-mediated mRNA translation. Dev. Cell 2015, 34, 181–191. [CrossRef] [PubMed]

95. Cesana, M.; Cacciarelli, D.; Legnini, I.; Santini, T.; Sthandier, O.; Chinappi, M.; Tramontano, A.; Bozzoni, I. A Long Noncoding RNA Controls Muscle Differentiation by Functioning as a Competing Endogenous RNA. Cell 2011, 147, 358–369. [CrossRef] [PubMed]

96. Leung, A.; Trac, C.; Jin, W.; Lanting, L.; Akbany, A.; Sætrom, P.; Schones, D.E.; Natarajan, R. Novel Long Noncoding RNAs Are Regulated by Angiotensin II in Vascular Smooth Muscle Cells. Circ. Res. 2013, 113, 266–278. [CrossRef] [PubMed]

97. Bell, R.D.; Long, X.; Lin, M.; Bergmann, J.H.; Nanda, V.; Cowan, S.L.; Zhou, Q.; Han, Y.; Spector, D.L.; Zheng, D.; et al. Identification and Initial Functional Characterization of a Human Vascular Cell-Enriched Long Noncoding RNA. Arterioscler. Thromb. Vasc. Biol. 2014, 34, 1249–1259. [CrossRef] [PubMed]

98. Sun, L.; Goff, L.A.; Trapnell, C.; Alexander, R.; Lo, K.A.; Hacisuleyman, E.; Sauvageau, M.; Tazon-Vega, B.; Kelley, D.R.; Hendrickson, D.G.; et al. Long noncoding RNAs regulate adipogenesis. Proc. Natl. Acad. Sci. USA 2013, 110, 3387–3392. [CrossRef] [PubMed]

99. Xu, B.; Gerin, I.; Miao, H.; Vu-Phan, D.; Johnson, C.N.; Xu, R.; Chen, X.W.; Cawthorn, W.P.; MacDougald, O.A.; Koenig, R.J. Multiple Roles for the Non-Coding RNA SRA in Regulation of Adipogenesis and Insulin Sensitivity. PLoS ONE 2010, 5, e14199. [CrossRef] [PubMed]

100. Cooper, D.R.; Carter, G.; Li, P.; Patel, R.; Watson, J.E.; Patel, N.A. Long Non-Coding RNA NEAT1 Associates with SRp40 to Temporally Regulate PPARγ2 Splicing during Adipogenesis in 3T3-L1 Cells. Genes 2014, 5, 1050–1063. [CrossRef] [PubMed]

101. Gernapudi, R.; Wolfson, B.; Zhang, Y.; Yao, Y.; Yang, P.; Asahara, H.; Zhou, Q. MicroRNA 140 promotes expression of long noncoding RNA NEAT1 in adipogenesis. Mol. Cell. Biol. 2016, 36, 30–38. [CrossRef] [PubMed]

102. Chen, J.; Liu, Y.; Lu, S.; Yin, L.; Zong, C.; Cui, S.; Qin, D.; Yang, Y.; Guan, Q.; Li, X.; et al. The role and possible mechanism of IncRNA U90926 in modulating 3T3-L1 preadipocyte differentiation. Int. J. Obes. 2016, 41, 299–308. [CrossRef] [PubMed]

103. Pang, W.J.; Lin, L.-G.; Xiong, Y.; Wei, N.; Wang, Y.; Shen, Q.-W.; Yang, G.-S. Knockdown of PU.1 AS IncRNA Inhibits Adipogenesis through Enhancing PU.1 mRNA Translation. J. Cell. Biochem. 2013, 114, 2500–2512. [CrossRef] [PubMed]

104. Divoux, A.; Karamerriou, K.; Xie, H.; Weimin, G.; Perera, R.J.; Fried, S.K.; Smith, S.R. Identification of a novel IncRNA in gluteal adipose tissue and evidence for its positive effect on preadipocyte differentiation. Obesity (Silver Spring) 2014, 22, 1781–1785. [CrossRef] [PubMed]

105. Zhao, X.-Y.; Li, S.; Wang, G.-X.; Yu, Q.; Lin, J.D. A Long Noncoding RNA Transcriptional Regulatory Circuit Drives Thermogenic Adipocyte Differentiation. Mol. Cell 2014, 55, 372–382. [CrossRef] [PubMed]

106. Lin, M.; Zhao, X.-Y.; Li, S.; Yang, G.; Lin, J.D. Conserved function of the long noncoding RNA Blnc1 in brown adipocyte differentiation. Mol. Metab. 2017, 6, 101–110. [CrossRef]
107. Alvarez-Dominguez, J.R.; Bai, Z.; Xu, D.; Yuan, B.; Lo, K.A.; Yoon, M.J. De novo reconstruction of adipose tissue transcriptomes reveals long non-coding RNA regulators of brown adipocyte development. *Cell Metab.* 2015, 21, 764–776. [CrossRef] [PubMed]

108. Bai, Z.; Chai, X.-R.; Yoon, M.J.; Kim, H.-J.; LO, K.A.; Zhang, Z.-C.; Xu, D.; Siang, D.T.C.; Walet, A.C.E.; Xu, S.H.; et al. Dynamic transcriptome changes during adipose tissue energy expenditure reveal critical roles for long noncoding RNA regulators. *PLoS Biol.* 2017, 15, e2002176. [CrossRef] [PubMed]

109. You, L.; Zhou, Y.; Cui, X.; Wang, X.; Sun, Y.; Gao, Y.; Wang, X.; Wen, J.; Xie, K.; Tang, R.; et al. GM13133 is a negative regulator in mouse white adipocytes differentiation and drives the characteristics of brown adipocytes. *J. Cell. Physiol.* 2017, 233, 313–324. [CrossRef] [PubMed]

110. Zhang, J.; Cui, X.; Shen, Y.; Pang, L.; Zhang, A.; Fu, Z.; Chen, J.; Guo, X.; Gan, W.; Ji, C. Distinct expression profiles of LncRNAs between brown adipose tissue and skeletal muscle. *Biochem. Biophys. Res. Commun.* 2014, 443, 1028–1034. [CrossRef] [PubMed]

111. Li, Z.; Jin, C.; Chen, S.; Zheng, Y.; Huang, Y.; Jia, L.; Ge, W.; Zhou, Y. Long non-coding RNA MEG3 inhibits adipogenesis and promotes osteogenesis of human adipose-derived mesenchymal stem cells via miR-140-5p. *Mol. Cell. Biochem.* 2017, 433, 51. [CrossRef] [PubMed]

112. Jin, C.; Jia, L.; Huang, Y.; Zheng, Y.; Du, N.; Liu, Y.; Zhou, Y. Inhibition of IncRNA MIR31HG Promotes Osteogenic Differentiation of Human Adipose-Derived Stem Cells. *Stem Cells* 2016, 34, 2707–2720. [CrossRef] [PubMed]

113. Jin, C.; Zheng, Y.; Huang, Y.; Liu, Y.; Jia, L.; Zhou, Y. Long non-coding RNA MEG3 inhibits adipogenesis of human adipose-derived stem cells. *Cell Biol. Int.* 2016, 40, 999–1–9. [CrossRef]

114. Huang, Y.; Zheng, Y.; Jia, L.; Li, W. Long Noncoding RNA H19 Promotes Osteoblast Differentiation via TGF-β1/Smad3/HDAC Signaling Pathway by Deriving miR-675. *Stem Cells* 2015, 33, 3481–3492. [CrossRef] [PubMed]

115. Liao, J.; Yu, X.; Hu, X.; Fan, J.; Wang, J.; Zhang, Z.; Zhao, C.; Zeng, Z.; Shu, Y.; Zhang, R.; et al. LncRNA H19 mediates BMP9-induced osteogenic differentiation mesenchymal stem cells (MSCs) through Notch sinaling. *Oncotarget* 2017, 8, 53581–53601. [CrossRef]

116. Qiu, X.; Jia, B.; Sun, X.; Hu, W.; Chu, H.; Xu, S.; Zhao, J. The Critical Role of Long Noncoding RNA in Osteogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells. *Biomed. Res. Int.* 2017. [CrossRef] [PubMed]

117. Zhang, J.; Cui, X.; Shen, Y.; Pang, L.; Zhang, A.; Fu, Z.; Chen, J.; Guo, X.; Gan, W.; Ma, J.; et al. Long noncoding RNA H19 inhibits the proliferation of fetal liver cells and the Wnt signaling pathway. *Cell Biol. Int.* 2016, 40, 222–230. [CrossRef] [PubMed]

118. Zhang, J.; Tao, Z.; Wang, Y. Long non-coding RNA DANCR regulates the proliferation and osteogenic differentiation of human bone-derived marrow mesenchymal stem cells via the p38 MAPK pathway. *Int. J. Mol. Med.* 2018, 41, 213–219. [CrossRef] [PubMed]

119. Wang, S.; Wu, X.; Liu, Y.; Yuan, J.; Yang, F.; Huang, F.; Meng, Q.; Zhou, C.; Liu, F.; Ma, J.; et al. Long noncoding RNA H19 inhibits the proliferation of fetal liver cells and the Wnt signaling pathway. *FEBS Lett.* 2016, 590, 559–570. [CrossRef] [PubMed]

120. Halley, P.; Kadakkuzha, B.M.; Faghhi, M.A.; Magistri, M.; Zeiter, Z.; Khorkova, O.; Coito, C.; Hsiao, J.; Lawrence, M.; Wahlestedt, C. Regulation of apolipoprotein gene cluster by a long non coding RNA. *Cell Rep.* 2014, 6, 222–230. [CrossRef] [PubMed]

121. Sallam, T.; Jones, M.C.; Gilliland, T.; Zhang, L.; Wu, X.; Eskin, A.; Sandhu, J.; Casero, D.; Vallim, T.A.; Hong, C.; et al. Feedback modulation of cholesterol metabolism by LeXis, a lipid-responsive non-coding RNA. *Nature* 2016, 534, 124–128. [CrossRef] [PubMed]

122. Li, P.; Ruan, X.; Yang, L.; Kiesewetter, K.; Zhao, Y.; Luo, H.; Chen, Y.; Gucek, M.; Zhu, J.; Cao, H. A liver-enriched long non coding RNA, IncLSTR, regulates systemic lipid metabolism in mice. *Cell Metab.* 2015, 21, 455–467. [CrossRef] [PubMed]

123. Lan, X.; Yan, J.; Ren, J.; Zhong, B.; Li, J.; Li, Y.; Liu, L.; Yi, J.; Sun, Q.; Yang, X.; et al. A Novel Long Noncoding RNA Lnc-HC Binds hnRNPA2B1 to Regulate Expressions of Cyp7a1 and Abca1 in Hepatocytic Cholesterol Metabolism. *Hepatology* 2016, 64, 58–72. [CrossRef] [PubMed]
Yang, L.; Li, P.; Yang, W.; Ruan, X.; Kiesewetter, K.; Zhu, J.; Cao, H. Integrative Transcriptome Analyses of Metabolic Responses in Mice Define Pivotal lncRNA Metabolic Regulators. *Cell Metab*. 2016, 24, 627–639. [CrossRef] [PubMed]

Liu, G.; Zheng, X.; Xu, Y.; Lu, J.; Chen, J.; Huang, X. Long Non-coding RNAs Expression Profile in HepG2 Cells Reveals the Potential Role of Long Non-coding RNAs in the Cholesterol Metabolism. *Chin. Med. J.* 2015, 128, 91–97. [CrossRef] [PubMed]

Li, C.; Chang, L.; Chen, Z.; Liu, Z.; Wang, Y.; Ye, Q. The role of IncRNA MALAT1 in the regulation of hepatocyte proliferation during liver regeneration. *Int. J. Mol. Med.* 2017, 39, 347–356. [CrossRef] [PubMed]

Morán, I.; Akerman, I.; van de Bunt, M.; Xie, R.; Benazra, M.; Nammo, T.; Arnes, L.; Nakic, N.; García-Hurtado, J.; Rodríguez-Seguí, S.; et al. Human β Cell Transcriptome Analysis Uncovers lncRNAs That Are Tissue-Specific, Dynamically Regulated, and Abnormally Expressed in Type 2 Diabetes. *Cell Metab.* 2012, 16, 435–448. [CrossRef] [PubMed]

Arnes, L.; Akerman, I.; Balderes, D.A.; Ferrer, J.; Sussel, L. βlinc1 encodes a long noncoding RNA that regulates islet β-cell formation and function. *Genes Dev.* 2016, 30, 502–507. [CrossRef] [PubMed]

Akerman, I.; Tu, Z.; Beucher, A.; Rolando, D.M.Y.; Sauty-Colace, C.; Benazra, M.; Nakic, N.; Yang, J.; Wang, H.; Pasquali, L.; et al. Human Pancreatic β Cell lncRNAs Control Cell-Specific Regulatory Networks. *Cell Metab.* 2017, 25, 400–411. [CrossRef] [PubMed]

Jin, F.; Wang, N.; Zhu, Y.; You, L.; Wang, L.; De, W.; Tang, W. Downregulation of Long Noncoding RNA Gas5 Affects Cell Cycle and Insulin Secretion in Mouse Pancreatic β Cells. *Cell. Physiol. Biochem.* 2017, 43, 2062–2073. [CrossRef] [PubMed]

You, L.; Wang, N.; Yin, D.; Wang, L.; Jin, F.; Zhu, Y.; Yuan, Q.; De, W. Downregulation of long noncoding RNA meg3 affects insulin synthesis and secretion in mouse pancreatic beta cells. *J. Cell. Physiol.* 2016, 231, 852–862. [CrossRef] [PubMed]

Yin, D.D.; Zhang, E.B.; You, L.H.; Wang, N.; Wang, L.T.; Jin, F.Y.; Zhu, Y.N.; Cao, L.H.; Yuan, Q.X.; De, W.; et al. Downregulation of IncRNA TUG1 affects apoptosis and insulin secretion in mouse pancreatic β cells. *Cell. Physiol. Biochem.* 2015, 35, 1892–1904. [CrossRef] [PubMed]

Sisino, G.; Zhou, A.-X.; Dahr, N.; Sabirsh, A.; Soundararapandian, M.M.; Perera, R.; Larsson-Lekholm, E.; Magnone, M.C.; Althage, M.; Tyrberg, B. Long noncoding RNAs are dynamically regulated during β-cell mass expansion in mouse pregnancy and control β-cell proliferation in vitro. *PLoS ONE* 2017, 12, e0182371. [CrossRef] [PubMed]

Xiao, L.; Rao, J.N.; Cao, S.; Liu, L.; Chung, H.K.; Zhang, Y.; Zhang, J.; Liu, Y.; Gorospe, M.; Wang, J.Y. Long Noncoding RNA SPRY4-IT1 Regulates Intestinal Epithelial Barrier Function by Modulating the Expression Levels of Tight Junction Proteins. *Mol. Biol. Cell.* 2016, 27, 617–626. [CrossRef] [PubMed]

Su, Z.; Zhi, X.; Zhang, Q.; Yang, L.; Xu, H.; Xu, Z. LncRNA H19 functions as a competing endogenous RNA to regulate AQP3 expression by sponging miR-874 in the intestinal barrier. *FEBS Lett.* 2016, 590, 1354–1364. [CrossRef] [PubMed]

Zou, T.; Jaladanki, S.K.; Liu, L.; Xiao, L.; Chung, H.K.; Wang, J.Y.; Xu, Y.; Gorospe, M.; Wang, J.Y. H19 Long Noncoding RNA Regulates Intestinal Epithelial Barrier Function via MicroRNA 675 by Interacting with RNA-Binding Protein HuR. *Mol. Cell. Biol.* 2016, 36, 1332–1341. [CrossRef] [PubMed]

Tong, Q.; Gong, A.Y.; Zhang, X.; Lin, C.; Ma, S.; Chen, J.; Hu, G.; Chen, X.-M. LincRNA-Cox2 modulates TNF-α-induced transcription of Il12b gene in intestinal epithelial cells through regulation of Mi-2/ NuRD-mediated epigenetic histone modifications. *FASEB J.* 2016, 30, 1187–1197. [CrossRef] [PubMed]

Herriges, M.J.; Swarr, D.T.; Morley, M.P.; Rathi, K.S.; Peng, T.; Stewart, K.M.; Morrisey, E.E. Long noncoding RNAs are spatially correlated with transcription factors and regulate lung development. *Genes Dev.* 2014, 28, 1363–1379. [CrossRef] [PubMed]

Herriges, M.J.; Tischfield, D.J.; Cui, Z.; Morley, M.P.; Han, Y.; Babu, A.; Li, S.; Lu, M.; Cendan, I.; García, B.A.; et al. The NANCl-Nkx2.1 gene duplex buffers Nkx2.1 expression to maintain lung development and homeostasis. *Genes Dev.* 2017, 31, 889–903. [CrossRef] [PubMed]

Banerjee, P.; Surendran, H.; Bharti, K.; Morishita, K.; Varshney, A.; Pal, R. Long noncoding RNA RP11-380D23.2 drives distal-proximal patterning of the lung by regulating PITX2 expression. *Stem Cells* 2017. [CrossRef] [PubMed]
142. Li, J.; Cao, Y.; Xu, X.; Xiang, H.; Zhang, Z.; Chen, B.; Hao, Y.; Wei, Z.; Zhou, P.; Chen, D. Increased New lncRNA–mRNA Gene Pair Levels in Human Cumulus Cells Correlate With Oocyte Maturation and Embryo Development. Reprod. Sci. 2015, 22, 1008–1014. [CrossRef] [PubMed]

143. Battaglia, R.; Vento, M.E.; Borzi, P.; Ragusa, M.; Barbagallo, D.; Arena, D.; Purrello, M.; Di Pietro, C. Non-coding RNAs in the Ovarian Follicle. Front. Genet. 2017, 8, 57. [CrossRef] [PubMed]

144. Gao, Q.; Ren, Q.; Chen, M.; Niu, Z.; Tao, H.; Jia, Y.; Zhang, J.; Li, W. Long non-coding RNAs regulate effects of β-crystallin B2 on mouse ovary development. Mol. Med. Rep. 2016, 14, 4223–4231. [CrossRef] [PubMed]

145. Arun, G.; Akhade, V.S.; Donakonda, S.; Rao, M.R.S. mrhl RNA, a Long Noncoding RNA, Negatively Regulates Wnt Signaling through Its Protein Partner Ddx5/p68 in Mouse Spermatogonial Cells. Mol. Cell. Biol. 2012, 32, 3140–3152. [CrossRef] [PubMed]

146. Akhade, V.S.; Dighe, S.N.; Kataruka, S.; Rao, M.R.S. Mechanism of Wnt signaling induced down regulation of mrhl long non-coding RNA in mouse spermatogonial cells. Nucleic Acids Res. 2016, 44, 387–401. [CrossRef] [PubMed]

147. Hu, K.; Zhang, J.; Liang, M. LncRNA AK015322 promotes proliferation of spermatogonial stem cell C18-4 by acting as a decoy for microRNA-19b-3p. In Vitro Cell. Dev. Biol. Anim. 2016, 53, 277–284. [CrossRef] [PubMed]

148. Zhang, X.; Gao, F.; Fu, J.; Zhang, P.; Wang, Y.; Zeng, X. Systematic identification and characterization of long non-coding RNAs in mouse mature sperm. PloS ONE 2017, 12, e0173402. [CrossRef] [PubMed]

149. Wichman, L.; Saigopal, S.; Breindel, C.; Valerio, D.M.; McCarrey, J.R.; Hodges, C.A.; Khalil, A.M. Dynamic expression of long non-coding RNAs reveals their potential roles in spermatogenesis and fertility. Biol. Reprod. 2017, 97, 313–323. [CrossRef] [PubMed]

150. Hristov, M.; Erl, W.; Linder, S.; Weber, P.C. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in vitro. Blood 2004, 104, 2761–2766. [CrossRef] [PubMed]

151. Yañez-Mó, M.; Siljander, P.R.; Andreu, Z.; Zavec, A.B.; Borras, F.E.; Buzas, E.I.; Buzas, K.; Casal, E.; Cappello, F.; Carvalho, J.; et al. Biological properties of extracellular vesicles and their physiological functions. J. Extracell. Vesicles 2015, 4, 27066. [CrossRef] [PubMed]

152. Van der Grein, S.G.; Nolte, E.N. “Small talk” in the innate immune system via RNA-containing extracellular vesicles. Front. Immunol. 2014, 5, 542. [CrossRef] [PubMed]

153. Vader, P.; Breakefield, X.O.; Wood, M.J.A. Extracellular vesicles: Emerging targets for cancer therapy. Trends Mol. Med. 2014, 20, 385–393. [CrossRef] [PubMed]

154. Gezer, U.; Özgur, E.; Cetinkaya, M.; Isin, M.; Dalay, N. Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes. Cell. Biol. Int. 2014, 38, 1076–1079. [CrossRef] [PubMed]

155. Ahadi, A.; Brennan, S.; Kennedy, P.J.; Hutvagner, G.; Tran, N. Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes. Sci. Rep. 2016, 6. [CrossRef] [PubMed]

156. Hewson, C.; Capraro, D.; Burdach, J.; Whitaker, N.; Morris, K.V. Extracellular vesicle associated long non-coding RNAs functionally enhance cell viability. Non-Coding RNA Res. 2016, 1, 3–11. [CrossRef] [PubMed]

157. Amorim, M.G.; Valieris, R.; Drummond, R.D.; Pizzi, M.P.; Freitas, V.M.; Sinigaglia-Coimbra, R.; Calin, G.A.; Pasqualini, R.; Arap, W.; Silva, I.T.; et al. A total transcriptome profiling method for plasma-derived extracellular vesicles: Applications for liquid biopsies. Sci. Rep. 2017, 7. [CrossRef] [PubMed]

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