Long non-coding RNAs during normal erythropoiesis

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Abstract

The transcription of essentially the entire eukaryotic genome produces a huge amount of non-coding RNAs. Among them, long non-coding RNAs (lncRNAs) consist of a significant portion that widely exists across mammal genome, generating from high-throughput transcriptomic studies in the last decade. Although the functions of most lncRNAs remain to be further investigated, many of them have already been shown to play critical roles during normal development and disease conditions. Increasing evidence indicates that lncRNAs involve in versatile biological processes during erythroid proliferation and differentiation, including erythroid cell survival, heme metabolism, globin switching and regulation, erythroid enucleation, etc, via cis- or trans-mediated molecular mechanisms. In this review, we focus on recent advances regarding the functions and mechanisms of lncRNAs in normal erythropoiesis.

Keywords: Erythropoiesis, Globin regulation, Heme biosynthesis, Long non-coding RNAs

1. INTRODUCTION

Red blood cells (RBCs) enable to transport oxygen to all tissues over the body, which are generated in the bone marrow via a multistep process named erythropoiesis. During erythropoiesis, the earliest committed erythroid progenitors are burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E). Then the CFU-E progenitors divide 3 to 5 times over 2 to 3 days as they differentiate and undergo many substantial changes, including a decrease in cell size, chromatin condensation, and hemoglobinization, leading up to enucleation and expulsion of extra organelles, ultimately turning into mature RBCs.1

Recently, with the advances of high throughput RNA-sequencing technology, it has been increasingly appreciated that there are substantial RNAs without protein-coding potential in the cells, termed non-coding RNAs (ncRNAs). Albeit >60% of the mammalian genome (~2/3) is capable to be transcribed, protein-coding genes account for < 2% of these transcribed transcripts in the entire mammal genome.2 Such huge amounts of non-coding transcripts could be mainly classified as miRNAs, small interfering RNAs, housekeeping RNAs, and long non-coding RNAs (lncRNAs).3

LncRNAs are transcripts > 200 nucleotides in length that do not code for proteins. Based on their genomic location and their neighboring protein-coding gene(s), lncRNAs have been further classified into six subgroups: intergenic lncRNAs (lincRNAs), intronic overlapping lncRNAs (i-lncRNAs), antisense lncRNAs (alsncRNAs), enhancer lncRNAs (elncRNAs), sRNA-host lncRNAs (shlncRNAs), and Pseudogene lncRNAs (plncRNAs).4,5 Compared with protein-coding genes, lncRNAs, in general, process the characteristics such as relatively shorter transcript length with 2 to 3 exons, poor conservation across multiple species, lower expression abundance as well as developmentally spatiotemporal specificity.6,7 Mechanistically, lncRNAs could regulate its targets through diverse molecular mechanisms.8 First, lncRNAs can act as a ribonucleoprotein scaffold to modulate nuclear architecture and regulate gene expression.8 Secondly, LncRNAs also can be co-expressed with their neighbor protein-coding genes and regulate their expression.10 Thirdly, they can be involved in post-transcriptional RNA modifications, include splicing, editing, etc.11 In addition, a small subset of lncRNAs participate in signal transduction and thus control diverse biological processes.12 Since very few lncRNAs have been identified during erythroid disorders, in this review, we solely focus on the very recent advances of the functions and mechanisms of lncRNAs during physiological (normal) rather than pathological erythropoiesis.

1.1. LincEPS and LncRNA-Saf in erythroid cell survival

It has been well documented that lncRNAs serve as vital regulatory elements governing diverse cell differentiation processes, including erythropoiesis. Hu et al.13 first explored lncRNA landscape during mouse erythropoiesis utilizing RNA-seq technology from purified mouse fetal liver erythroid progenitors BFU-Es, CFU-Es, and terminal differentiated Ter119+ erythroid cells. They identified > 400 putative lncRNAs that are expressed...
The human b-globin locus comprises five coding genes, including the embryonic-specific ε (HBE1), fetal-restricted Gγ and Aγ (HBG2 and HBG1), and adult expressed δ and β (HBD and HBB) globin genes, which are sequentially activated during development. The intergenic region between the Aγ- and δ-globin genes contains a pseudogene (HBBP1) and a noncoding gene (BGLT3). BGLT3 is shown to specifically regulate fetal γ-globin expression.18 In erythroid cells BGLT3 co-transcribes with gamma-globin and primarily localizes in the nucleus. After combination of the CRISPR/Cas9 mediated whole gene body deletion, transcription start site deletion as well as antisense oligonucleotides (ASOs) mediated knockdown strategies, Ivaldi et al unraveled that BGLT3 locus and transcript have distinct functions. BGLT3 gene locus is exclusively involving in transcriptionally activation of fetal gamma globin genes, but not other types of globin genes, via chromatin looping. Whereas BGLT3 transcripts are dispensable for fetal gamma-globin/BGLT3 looping, it instead interacts with the Mediator complex, such as MED12 on chromatin to facilitate fetal γ-globin transcriptional assembly (Fig. 1 F). Since elevated fetal hemoglobin levels are capable to alleviate the syndrome of sickle cell disease or β-thalassemia in patients, HBBP1 and BGLT3 genes seem are two promising therapeutic targets for genome editing for those diseases.

1.4. UCA1 and heme biosynthesis
Shi et al has performed RNA sequencing (RNA-seq) at distinct differentiation stages derived from mobilized adult peripheral blood CD34+ cells and comprehensively identified 5326 potential long non-coding RNAs.19 After profiling the dynamically expressed lncRNAs during erythropoiesis, Liu and coworkers identified that lncRNA UCA1 regulates heme metabolism in...
Figure 1. Erythropoiesis and possible modes of action of lncRNAs involved in erythropoiesis. (A) Erythropoiesis. The earliest committed erythroid progenitors are burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E). During the terminal erythroid differentiation, the CFU-E progenitors divide 3 to 5 times and undergo many substantial changes, including a decrease in cell size, chromatin condensation, and hemoglobinization, leading up to enucleation and expulsion of extra organelles, ultimately turning into mature red blood cells. Here, Pro-E presents proerythroblast; baso-E presents basophilic erythroblast; Pol-E presents polychromatic erythroblast; Ortho-E presents orthochromatic erythroblast; Reti presents reticulocyte; RBCs presents red blood cells. (B) LincRNA-EPS, interacting with the RNA-binding protein HNRNPL, binds to the promoter of Pycard to repress its transcription, executing a potent anti-apoptotic activity. (C) LncRNA Saf promotes erythroid cell survival via inhibiting the Fas-mediated cell death signals, which is transcriptionally upregulated by GATA1 and KLF1 while downregulated by NF-κB. (D) AlncRNA-EC7 induces erythroid maturation by enhancing Band 3 expression. Here, alncRNA-EC7 acts as a protein scaffold to carry on its enhancer function by binding to the chromatin attachment factor HFPNPL, the nuclear lamina component LMA and other unknown transcription coactivator(s). (E) ShlncRNA-EC6 promotes erythroid enucleation via regulating Rac1 and its downstream target PIP5K. (F) (left) cis-regulatory mechanism: BGLT3 gene locus transcriptionally activates fetal γ-globin genes via facilitating chromatin looping between LCR and γ-globin promoters. (right) Tran-regulatory mechanism: albeit BGLT3 transcripts are dispensable for γ-globin/BGLT3 looping, it instead interacts with the Mediator complex, such as MED12 on chromatin to aid γ-globin transcriptional assembly. Here, LCR refers locus control region. (G) lncRNA UCA1 interacts with RNA binding protein PTBP1 to confer ALAS2 mRNA stability, which in turn regulates heme metabolism.
human erythroid cells (Table 1). UCA1 expression peaks in proerythroblasts. Consistent with its expression pattern, diminished UCA1 expression blocked the erythroid differentiation at the proerythroblast stage due to the insufficient heme biosynthesis. Mechanistic analysis suggests that UCA1 interacts with RNA binding protein PTBP1 to confer ALAS2 mRNA stability, which is the rate limiting enzyme of heme biosynthesis (Fig. 1 G). This study revealed a new layer of lncRNA-dependent, post-transcriptional regulation of ALAS2 mRNA, which might shed more lights on the heme disorders with currently unknown reasons.

1.5. Species-, tissue-, and cellular-specificity of LncRNA

Despite a subset of lncRNAs are highly conserved, the majority of lncRNAs seem to be species- or lineage-specific. Paralkar and coworkers used RNA-seq to investigate tissue- and specie-specific lncRNAs during mouse and human erythropoiesis. In analyzing transcripts of erythroblasts, megakaryocytes, and megakaryocyte-erythroid precursors, they identified 1109 potential lncRNAs expressed in mouse, while only 594 in humans. More importantly, they uncovered ~15% of mouse lncRNAs are detected in humans or vice versa, reflecting dramatic species-specificity. In summary, these lncRNAs might help to shape the species- and lineage-specific phenotypes.

1.6. Conclusions and future perspectives

The rapid development of high throughput transcriptome profiling techniques greatly aids the identification of lncRNAs, which has been exponentially increased for the past decade. Compared with the number of identified lncRNAs in erythroid development and differentiation, the lncRNAs with functional annotation are relatively few. Still, these studies raise interesting questions.

1. It is largely uncertain that the great number of lncRNAs identified during erythropoiesis is exclusively erythroid lineage specific or not. For example, although lncRNA UCA1 abundantly and dynamically expressed in erythroid cells, it is also highly expressed in spleen and heart. Since lncRNAs identified in prior studies were predominantly limited in hematopoietic cell lineages, a broader comparison across multiple tissues can answer this question soon.

2. It is intriguing to know whether the lncRNAs share the similar expression pattern possibly conduct resembling roles; given the majority of identified lncRNAs dynamically expressed during erythroid differentiation, it is yet to be known that the same lncRNA play distinct function at different differentiation stage; and, although an increasing evidence indicates that lncRNAs play crucial roles during malignant hematopoiesis, the functions of lncRNAs in erythroid diseases have less been demonstrated. In future, it is of importance to continually address the function of these potential regulators to answer these questions. Mechanistically, nuclear localized lncRNAs are more frequently targeting genomic regions to modulate gene expression by recruiting chromatin modifying proteins, whereas the cytoplasmic lncRNAs are more likely to interact with RNA-binding proteins to stabilize mRNA or participate in the translational regulation. Thus, combined biochemical and cell biology approaches are promising strategies to integrate the underlying molecular mechanisms of lncRNAs during erythroid development and differentiation, which will also provide valuable insights in various erythroid disorders.

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