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RNA splicing during terminal erythropoiesis

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Abstract

Purpose of review—Erythroid progenitors must accurately and efficiently splice thousands of pre-mRNAs as the cells undergo extensive changes in gene expression and cellular remodeling during terminal erythropoiesis. Alternative splicing choices are governed by interactions between RNA binding proteins (RBPs) and cis-regulatory binding motifs in the RNA. This review will focus on recent studies that define the genome-wide scope of splicing in erythroblasts and discuss what is known about its regulation.

Recent findings—RNA-seq analysis of highly purified erythroblast populations has revealed an extensive program of alternative splicing of both exons and introns. During normal erythropoiesis, stage-specific splicing transitions alter the structure and abundance of protein isoforms required for optimized red cell production. Mutation or deficiency of splicing regulators underlies hematopoietic disease in myelodysplasia syndrome (MDS) patients via disrupting the splicing program.

Summary—Erythroid progenitors execute an elaborate alternative splicing program that modulates gene expression post-transcriptionally, ultimately regulating the structure and function of the proteome in a differentiation stage-specific manner during terminal erythropoiesis. This program helps drive differentiation and ensures synthesis of the proper protein isoforms required to produce mechanically stable red cells. Mutation or deficiency of key splicing regulatory proteins disrupts the splicing program to cause disease.

Keywords

Erythropoiesis; alternative splicing; splicing regulation; nonsense-mediated decay

Introduction

Most human genes produce multiple transcripts through alternative splicing of their pre-mRNAs, resulting in enormously diverse transcriptome potential. Regulation of splicing in tissue-specific and developmental patterns plays a major role in establishing the specialized phenotype of differentiated cells. The erythroid lineage is no exception, as it executes a robust alternative splicing program that governs usage of both exons and retained introns

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Conflicts of interest

The author has no conflicts of interest.

during terminal erythropoiesis [1,2**,3,4**]. Erythroid differentiation-associated alternative splicing switches can functionally impact cell function, e.g., establishment of a mechanically stable red cell membrane requires up-regulation of splicing efficiency for exon 16 in the protein 4.1 (EPB41) gene. This splicing switch promotes interactions among protein 4.1, spectrin, and actin that stabilize the membrane [5].

Programmed RNA processing networks are governed by combinatorial interactions of multiple RNA binding proteins (RBPs) with cis-regulatory sequences in pre-mRNA, located in alternative exons and flanking intron sequences. Together these interactions promote or inhibit assembly of a functional spliceosome, which catalyzes excision of the intron and re-ligation of flanking exons during each splicing event. Key regulatory elements are often evolutionarily conserved, but prediction of alternative splicing remains difficult due to the degeneracy of RNA sequence motifs recognized by most RBPs. Nevertheless, our understanding of the ‘splicing code’ that governs tissue-specific splicing continues to improve [6].

The importance of accurate splicing is underscored by the increasing number of human diseases attributed to aberrant RNA processing. Defective splicing can arise from mutations in splice sites or nearby cis-regulatory sequences, thereby disrupting expression of individual erythroid genes. Indeed, splicing mutations are relatively common and have been described in more than 200 human diseases [7]. Broader network-wide disturbances in splicing arise when the regulators themselves are aberrantly expressed, resulting in loss of activity or change in binding properties [8*]. This review will discuss splicing-associated changes in the transcriptome during normal terminal erythropoiesis, factors that regulate the program, and disturbance of the splicing program associated with erythroid disease.

RNA processing [ensures] synthesis of appropriate erythroid protein isoforms

The RNA processing machinery in erythroid progenitors acts on the pool of pre-mRNAs presented by the transcriptional machinery, in order to execute an alternative splicing program designed to engineer synthesis of protein isoforms meeting the physiological needs of differentiating erythroblasts. The protein 4.1R gene (EPB41) mentioned above is a prime example; the gene encodes diverse protein isoforms in a tissue-specific manner through judicious regulation of multiple alternative splicing choices [9]. In differentiating erythroid progenitors, the splicing machinery skips several exons that are primarily utilized in non-erythroid cells, while ensuring correct alternative splice site choices at exons 2 and 16 that are required to generate 80kD protein isoforms with high affinity for spectrin and actin [10–12]. The mechanisms responsible for splicing of these two exons have been studied extensively [10,13–21].

RNA processing also shape other genes’ output of erythroid isoform ensembles that are distinct from nonerythroid counterparts, presumably optimizing red cell maturation. Use of tissue-specific alternative first exons facilitates expression of longer erythroid isoforms of ankyrin (ANK1) and band 3 (SLC4A1) compared with shorter isoforms in muscle and kidney, respectively, that are transcribed from internal promoters [22,23]. Conversely,

erythroid beta spectrin is shorter at the C-terminus than its muscle counterpart due to differential polyadenylation and splice site usage that prevents expression of 3' coding exons [24]. Very recently, selection of an alternative polyadenylation site in intron 17 was reported to generate 3' truncated isoforms of protein 4.1R mRNA [25], but the impact of this splicing variant on erythroid biology is unknown. Even the relatively simple GATA1 gene can be subject to a domain-altering splicing event: e.g., alternative splicing of exon 2 can control expression of a transcription activation domain. In mouse fetal liver erythroblasts, up-regulation of specific NDEL1 isoform is critical for enucleation in late erythroblasts [1]. Alternative splicing can generate alternative isoforms of acyl-CoA synthetase ACSL6 with differential substrate specificities presumably required to generate and repair many lipid species in erythroid membranes[26].

Erythroblasts execute an extensive stage-specific program of alternative splicing, affecting both exons and introns, during terminal erythropoiesis

Genome-wide studies of erythroid alternative splicing have been facilitated by RNA-seq analysis of defined cell populations [1,3,27]. An excellent model system is a series of RNA-seq libraries prepared from FACS-purified proerythroblasts (proE), early basophilic erythroblasts (e-basoE), late basophilic erythroblasts (l-basoE), polychromatophilic erythroblasts (polyE) and orthochromatophilic erythroblasts (orthoE), the last stage before enucleation [28]. Analysis of the data revealed an extensive alternative splicing program analogous to that found in other nucleated cell types. Alternative splicing is enriched for genes functioning in RNA processing, cell cycle regulation, organelle organization, and chromatin structure and function [3], suggesting that multiple isoforms of important erythroid proteins are expressed in these cells. Dynamic regulation of splicing transitions during terminal erythropoiesis can drive changes in protein isoform expression to add new functionalities advantageous for late erythroblasts and / or red cells. In the protein 4.1R gene, the switch from predominant E16 skipping in early cells to inclusion in late erythroblasts (Figure 1A) can be visualized as at the genome browser by a substantial increase in RNA-seq reads mapped to E16 peak (Figure 1B). Calculated PSI (percent exon inclusion) values during terminal erythropoiesis (Figure 1C) depicts this progressive increase in E16 splicing as erythroblasts mature. In contrast to this “productive” splicing transition, other splicing transitions introduce premature termination codons (PTCs) that mark transcripts for rapid degradation via nonsense-mediated decay (NMD). An example of alternative splicing coupled to NMD (AS-NMD) is shown in Figure 2 for the SNRNP70 splicing factor gene. The PTC exon is mostly skipped in early erythroblasts, but inclusion is up-regulated in mature cells leading to increased NMD [3]. AS-NMD can thereby function as a post-transcriptional mechanism to down-regulate gene expression [3].

Erythroid gene expression is also regulated by intron retention (IR), in which a transcript is polyadenylated and spliced at most introns, but remains unspliced at one or more introns [2**,4**]. Hundreds of retained introns are dynamically regulated during terminal erythropoiesis, exhibiting low IR in early progenitors but much higher IR – sometimes reaching 20–50% -- in late erythroblasts. This IR class is enriched in RNA processing genes, especially splicing factors including SF3B1 and SNRNP70, but it also includes other

important genes such as alpha spectrin (SPTA1). Figure 3 summarizes the regulation of SF3B1 intron 4 during terminal erythropoiesis. The relevant gene region is shown in Fig. 3A, while RNA-seq mapping data reveals elevated intron retention in late erythroblasts (Figure 3B), as does the PIR (percent intron retention) profile during terminal erythropoiesis (Figure 3C). In contrast to SF3B1, other genes contain introns that maintain high but stable IR level across erythroblast populations [4**]. Prominent in group are the mitochondrial iron importers mitoferrin1 and mitoferrin2 (SLC25A37 and SLC25A28) that are critical for heme biosynthesis. Many IR transcripts are retained in the nucleus where they are resistant to NMD but not translated into protein, contributing to a discordance between transcript and protein abundance in some genes [29*]. The function of IR is not well understood. It may represent a post-transcriptional limit to gene expression, or it could provide a reservoir for new mRNA production in response to appropriate signals.

Regulation of the erythroid splicing program

Master regulator(s) of the erythroid splicing program are not well defined as yet. Among splicing factors known to drive developmental splicing transitions in non-erythroid cells, the zinc finger RBPs known as MBNL1 and MBNL2 are leading candidates for performing this role in erythroblasts. Depending on the location of binding, MBNL proteins can regulate splicing, polyadenylation, RNA stability, and RNA localization in several systems [30–36]. Deficits in MBNL function also have disease relevance. Patients with the triplet repeat disease myotonic dystrophy, in which expansion of CUG occurs in DMPK mRNA, exhibit many splicing defects due to titration of MBNL activity away from pre-mRNA substrates [37]. In the erythroid system, MBNL binding motifs are enriched near exons that undergo developmental splicing transitions [1]; moreover, MBNL1 knockdown in mouse fetal liver alters splicing of transcripts such as Epb4.1 and Ndel1 and inhibits erythroblast enucleation [1]. Together these results suggest an important role for Mbnl1 during erythroid terminal differentiation.

Much work remains to be done in this area to identify positive and negative regulators of erythroid splicing events and splicing transitions that are not attributable to regulation by MBNL proteins. A few highly expressed RBPs with demonstrated ability to regulate of erythroid splicing event(s) will be mentioned here. PCBP1 and 2 (also known as α CP, or hnRNP E proteins) increase the stability of alpha globin via binding to C-rich sequences in the 3' UTR [38,39]. Unexpectedly, knockdown in K562 cells revealed that they also regulate alternative splicing events, especially for exons with C-rich regions in the upstream pyrimidine tract [40**]. RBM38 is an RBP that is dramatically up-regulated in late erythroblasts and is a known regulator of EBP41 splicing [41]; additional functions are suggested by genetic linkage studies showing that the RBM38 gene is near a GWAS marker for a red cell phenotype (MCV) [42,43*]. HNRNP A1 is highly expressed in early erythroblasts but down-regulated in more mature cells; it negatively regulates the EPB41 E16 splicing switch [14]. Analogous to the role of related HNRNP proteins in suppressing differentiated splicing events in other cell types [44,45], A1 could be a general repressor of erythroblast splicing transitions. Finally, little is known about upstream signaling pathways that might activate splicing factors expression or function; in that regard, activation of a MAPK p38 signaling pathway can promote E16 splicing [21].

Cis-acting mutations that disrupt splicing are a common cause of erythroid disease

Point mutations within exons or their flanking introns can disturb splicing of numerous erythroid transcripts. Splicing mutants often manifest as low expression alleles due to exon skipping or alterations of splice site choice that change translational reading frame, introduce premature termination codons (PTCs), and activate NMD. Such mutations are a common cause of beta thalassemia as well as red cell membrane disorders including hereditary pyropoikilocytosis (HPP), hereditary elliptocytosis (HE), and hereditary spherocytosis (HS) marked by deficiency of protein 4.1R [46], alpha spectrin (ref. [47] and references within) and band 3 genes [48]. A representative example is shown in Figure 4 for the spectrin α^{LEPRA} mutant, in which a splice site mutation at exon 37 leads to exon skipping, a translational frameshift, and NMD [49]. Unfortunately, intronic mutations can be difficult to discern by exome or transcriptome sequencing, since the effect of SNPs on splicing is not easy to predict, the mutations can reside deeper within the introns, or the affected transcript may be rapidly degraded.

Interestingly, nonsense mutations in tissue-specific alternative exons can sensitize selected transcript isoforms to NMD, leading to tissue-specific protein deficiency. Deletion of a single nucleotide in E16 of the protein 4.1 gene introduces a PTC that activates NMD, causing a protein 4.1R-deficient hereditary elliptocytosis [50]. However, 4.1R transcripts in lymphoid cells naturally skip E16, escape NMD, and express normal amounts of lymphoid 4.1R mRNA.

Disturbing the isoform balance among alternatively spliced transcripts can also cause erythroid disease. In the GATA1 gene, partial skipping of exon 2 occurs naturally to generate transcripts encoding a shortened protein isoform termed GATA1s. However, 3' splice site mutations that virtually eliminate exon 2 splicing are found in patients with Diamond-Blackfan Anemia (DBA) or dyserythropoietic anemia with megakaryocyte dysplasia [51,52*], in whom the deficiency of full length GATA1 compromises erythropoiesis. Altered splicing of mitoferrin-1 transcripts has been found in rare erythropoietic protoporphyria (EPP) patients that do not have the typical defects in ferrochelatase (FECH) [53]. These patients show elevated splicing at a cryptic 5' splice site in intron 2 that introduces a PTC predicted to cause NMD. Interestingly, the aberrant isoform is expressed at a low level in normal erythroblasts, and but over-expressed for unknown reasons in patients.

Mutations or deficiencies in core splicing machinery can cause erythroid disease

Mutations that alter expression of basic RNA processing machinery cause widespread defects in splicing that underlie many human genetic diseases (reviewed in [8*,54]). MDS is a bone marrow disorder often caused by missense mutations in the genes encoding splicing factors SF3B1, U2AF1, or SRSF2 [55**, 56**,57–59**]. The mutated proteins exhibit relatively modest changes in RNA binding specificity that, however, are sufficient to induce aberrant 3' splice site choice in many of the patients' transcripts [56**,60]. In the case of

SF3B1 mutants, altered branchpoint recognition [55**,61*], due in part to unmasking of alternative branchpoints that are normally sequestered by RNA secondary structure [62*], can promote aberrant splicing. Interestingly, many of the reported aberrant splices represent increased selection of splice sites that are used at low levels in normal cells.

An important goal of MDS studies is to identify how altered splicing disturbs erythroid biology. Spliceosomal mutations could exert their effects by perturbing the expression of many genes, or only a few key genes essential for red cell physiology. One attractive candidate is the mitochondrial transporter encoded by ABCB7, which is itself mutated in some patients with X-linked sideroblastic anemia with ataxia [63,64]. Expression of ABCB7 is reduced in SF3B1-mutant MDS patients, apparently due to splicing at an abnormal 3' splice site that alters translational reading frame, introduces a PTC, and activates NMD [64]. This mis-splicing event may contribute to iron accumulation in ring sideroblasts. Other genes with plausible functional involvement have also been proposed (reviewed in [65*], but their relative importance in disease is still unknown. Understanding key aberrant events could inform strategies for correction of individual splicing events by use of antisense oligonucleotide technology (reviewed in [66]). Alternatively, it may be possible to differentially target mutant cells by virtue of their increased sensitivity, relative to normal cells, to agents that inhibit SF3B1 activity [55**,67**].

Better understanding of MDS pathology is advancing through studies of patient transcriptomes, as well as analysis of cell line and animal models with engineered defects in the relevant RBPs. Continuing challenges for the field include deciphering mechanisms by which the mutations alter splicing, identifying specific aberrant transcripts responsible for developmental defects in patients' cells, and understanding why mutations in core splicing machinery have surprisingly tissue-restricted and/or gene-specific effects on splicing.

Conclusion

Differentiating erythroblasts elaborate a robust alternative splicing program that stage-specifically remodels the transcriptome. Most splicing transitions are temporally coordinated with extraordinary cellular remodeling that culminates in enucleation. Splicing transitions can also down-regulate expression via NMD-mediated degradation of transcripts containing PTCs. Aberrant splicing of individual transcripts, or networks of transcripts regulated by a common splicing factor, underlies numerous erythroid diseases. Understanding regulation of erythroid splicing may provide insights required for therapy of erythroid splicing diseases.

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Key points

An extensive program of differentiation stage-specific alternative splicing events is expressed during terminal erythropoiesis.

Alternative splicing of coding exons can introduce new functionality, while splicing of PTC-containing exons can post-transcriptionally down-regulate production of translatable mRNA.

Mutations that disrupt splice site choice or compromise expression of splicing regulators can cause erythroid disease.

Better understanding splicing mechanisms should lead to therapy for splicing diseases.

Fig. 1. Dynamic regulation of alternative exon splicing during terminal erythropoiesis
 A. Model of the exon 12–18 region of the EPB41. Early erythroblasts predominantly skip E16, while late erythroblast include E16. Tissue-specific exons mainly expressed in non-erythroid tissues are depicted as gray boxes. B. RNA-seq profiles for EPB41 in proerythroblasts and late erythroblasts C. Plot shows that PSI values for E16 increase substantially during terminal erythropoiesis.

Fig. 2. Splicing of PTC exons can post-transcriptionally regulate mRNA production

Stage-specific splicing of a naturally occurring PTC in SNRNP70 exon 8 is low in proerythroblasts, so that the majority of transcript represents translatable mRNA. A splicing transition diverts an increasing proportion of transcript into an NMD-sensitive isoform in mature cells.

Fig. 3. Dynamic regulation of intron retention during terminal erythropoiesis

A. Model shows the exon 2–6 region of the SF3B1 gene. The red stop sign indicates an in-frame PTC that would preclude translation of transcripts with intron retention. B. RNA-seq profiles for SF3B1 show efficient splicing (low retention) of intron 4 in early cells, but much higher retention in late erythroblasts. C. Plot of PIR values for intron 4 during terminal erythropoiesis shows substantially increased retention in mature cells.

Fig. 4. Mutations that introduce aberrant PTCs are responsible for low expression of erythroid transcripts

In alpha spectrin α^{LEPRA} , a splice acceptor site mutation at exon 37, indicated by the arrow, causes exon skipping in transcripts of the mutant allele. The resulting frameshifted transcript encounters an 'unnatural' PTC in exon 38, indicated by the "stop" sign, and therefore is subject to NMD.