Editorial

Stem Cell INVESTIGATION

Role of alternative splicing in hematopoietic stem cells during development

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With the advent and increasing resolution of genome-wide sequencing modalities, alternative splicing (AS) has become an emerging area of interest in normal development and disease (1). Splicing of mRNA transcripts results in the production of a multitude of alternative isoforms from a single genomic locus, dramatically enhancing the diversity of the transcriptome and proteome (2). AS may affect RNA nuclear export and transcript stability, thereby regulating transcript levels. Distinct splicing isoforms may produce functionally equivalent, divergent, or inactive mature proteins (3).

Recently, attention towards characterizing the impact of splicing events during hematopoiesis has intensified, driven in part by the discovery of recurrent splicing factor mutations in myelodysplastic syndromes (MDS), clonal disorders of the hematopoietic stem cell (HSC) (4). The finding that mutations in one of 4 splicing factors occur in nearly 50% of patients has resulted in numerous studies characterizing the roles of AS events during both normal hematopoiesis and disease. By mapping whole-transcriptome splicing in murine HSCs from several high-quality datasets, Goldstein *et al.* found that the majority (248/322) of HSC-specific genes, including *HoxA9*, *Meis1*, *Prdm16*, and *Hlf* are expressed as multiple isoforms with similar or divergent functions (5). Previously, Bowman *et al.* had identified an abundance of unspliced transcripts mapping to DNA binding and RNA processing factors in HSCs, regulated upon HSC activation (6). Similarly, Wong *et al.* identified a program of widespread intron retention and consequent non-sense mediated decay (NMD) of these transcripts during granulocytic differentiation as a rapid mechanism of post-transcriptional regulation that was conserved between mice and humans (7). Chen *et al.* compiled a comprehensive "atlas" of transcriptional diversity during human hematopoiesis by sequencing isolated human HSCs and their progeny, mapping gene expression, AS, and novel splice site utilization throughout the hematopoietic hierarchy (8). Of the approximately 2,000 genes with differential splice site selection between cell types as many as sixty percent were predicted to produce functional protein changes or result in premature stop codons that would slate the transcript for NMD (8). Komeno *et al.* demonstrated that AS of *Runx1* affecting exon 6 resulting in a *RUNX1a* ortholog unique to human, determines the size of the HSC compartment (9). In MDS and several other cancers AS is co-opted as evident by recurrent splicing factor mutations (4,10-14). While full understanding about how these mutations cause disease is still unclear, key splice events contributing to the disease have been identified. As an example, point mutations in the serine-arginine rich splicing factor 2 (*SRSF2)*, result in inclusion of a poison exon in *EZH2* thereby mimicking pathogenic loss of function mutations in *EZH2*, mutually exclusive with *SRSF2* mutations (15).

A recent publication by Cesana *et al.* from the Daley laboratory reports an interesting link between splicing and post-transcriptional regulation in the developmental adaptation of HSC function (16). During ontogeny HSCs lose their proliferative potential and alter their lineage output to adjust to the organism's needs. The investigators isolated early HSCs ($CD34^+$ $CD38^-$ $CD90^+$ $CD45RA^-$) and

$CD34^+$ $CD38^+$ progenitors from fetal livers (FL), cord blood (CB) and bone marrow (BM) and performed deep RNA-sequencing (RNA-seq) and miRNA profiling. Numerous transcripts were differentially expressed, originally thought of as "universal" markers of stemness, with only a small subset uniformly expressed across HSC populations. Among the uniformly expressed transcripts, a few showed differential regulation of their isoforms between FL, CB, and BM HSCs. *HMGA2* caught the investigators' eye. *HMGA2* is expressed in two specific isoforms, a full-length (*HMGA2-L*) and a shorter isoform (*HMGA2-S*). While *HMGA2-L* predominates in FL HSCs, *HMGA2-S* is more abundant in CB HSCs. Interestingly, differential splicing results in selection of an alternative and much shorter 3' UTR in *HMGA2-S* than in *HMGA2-L*. Upon further scrutiny it is evident that the *HMGA2-S* 3' UTR lacks most conserved miRNA binding sites, especially for the *let*-7 family of miRNAs. Functional relevance is experimentally confirmed: while the *HMGA2-L* isoform is repressed by the *let*-7 miRNA family, the *HMGA2-S* isoform is unaffected. Incidentally, *HMGA2-S* preference co-occurs with down-regulation of *LIN28B* in CB HSC. *LIN28B* is well known for its importance in stem cell maintenance and its role in inhibiting *let*-7 biogenesis (17,18). This suggests, that by escaping *let*-7 mediated suppression, *HMGA2-S* may maintain a stem cell program in CB HSCs despite down-regulation of *LIN28B* and up-regulation of *let*-7 miRNA family members. Indeed, *HMGA2-S* and *HMGA2-L* are functionally equivalent in driving HSC stemness. Frequently, alternative 3' UTR choices are achieved through alternative poly-adenylation through selection of proximal or distal polyadenylation sites within the 3' UTR sequence [reviewed in (19)]. In the case of *HMGA2*, the alternative 3' UTR is the result of AS of the otherwise skipped alternative terminal exon 4. To determine how this AS event may be achieved, the authors screened for potential splicing regulators and experimentally identified CLK3 kinase as the most likely candidate. CLK3 belongs to the CDC-like kinases, which regulate RNA binding protein and splicing factor activity via their phosphorylation (20). In a next step therefore, the investigators identified global splicing changes induced by knockdown of *CLK3* and computationally identified RNA binding proteins potentially regulated by CLK3 through extraction of most common binding motifs in differentially spliced exons. This data pointed towards SRSF1 as the CLK3 phosphorylation target responsible for *HMGA2* differential splicing. The *HMGA2-L* but not the *HMGA2-S* isoform contains SRSF1 binding motifs and the *HMGA2-L* alternative exons are indeed regulated by SRSF1. Interestingly, the two *HMGA2* isoforms are functionally indistinguishable. The CLK3-SRSF1 mediated AS solely seems to serve preservation of *HMGA2* expression and maintenance of a *HMGA2*-orchestrated HSC-specific program in an increasingly *let*-7 dominated cellular context. Interestingly, adult $CD34^+$ hematopoietic stem and progenitor cells but not FL or CB HSCs regain proliferative and repopulating potential upon overexpression of *HMGA2-S* or *CLK3*, suggesting that the CLK3-SRSF1-HMGA2 axis may contribute to the developmentally defined HSC-specific program inherent to FL and CB. In this context, it is of particular interest that SRSF1 is overexpressed in breast cancer (21,22) highlighting the close link between stemness and cancer.

Several questions remain. It would be of particular interest whether the CLK3-SRSF1-HMGA2 axis entirely explains the FL and CB stem cell phenotype and if therapeutic modulation of this axis could thus reverse the aging stem cell phenotype. While CLK3 and SRSF1 emerged as the investigators' top targets, it is well known that CLKs can phosphorylate more than one SR protein and that SR proteins cooperate and compete with each other and other splicing factors, such as members of the HNRNP family [reviewed in (23)]. It will thus be of importance to understand to what extent the overall cellular context modifies the CLK3-SRSF1-HMGA2 axis.

Understanding the broader role of the CLK3-SRSF1 module in HSC function and ontogeny may open up novel therapeutic avenues. With the recent FDA approval of the first therapeutic splicing regulator Nusinersen in spinal muscular atrophy (24), transient modulation of the SR protein mediated splicing program in HSC may be feasible and allow HSC expansion *in vitro* for the purpose of stem cell transplantation or other therapeutic needs.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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