Trends in Cancer

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Spotlight

Exons of Leukemia Suppressor Genes: Creative Assembly Required

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Alternative splicing (AS) has many important roles in the pathogenesis of leukemia. Recent papers suggest that one of its key aspects is exclusion of 3′-terminal exons in favor of premature termination using intronic polyadenylation signals. This process generates leukemia suppressor isoforms with truncated C termini and acting in loss-of-function or dominant-negative manners.

The advent of next-generation sequencing approaches to analyze cancer genomes at the single-nucleotide resolution has revolutionized our understanding of tumor pathobiology and led to the identification of cancer drivers recurrently affected by gain-of-function (oncogenes) and loss-of-function (tumor-suppressor genes, or TSGs) mutations. While in many adult solid cancers the mutational load is high (hundreds of genetic events per sample), hematologic malignancies (e.g., pediatric and adult leukemias) are genetically quiet, often with no more than one known oncogenic driver per sample and no evidence of TSG inactivation at the genomic level. This extreme parsimony creates a conundrum: how does a preleukemic cell manage to effect so much malignant change with so little genetic diversity? One possible answer is that its TSGs could be silenced by epigenetic events, for example DNA or histone methylation. Yet these mechanisms are not prevalent in common lymphoid malignancies, such as chronic lymphocytic (CLL) and B cell acute lymphoblastoid (B-ALL) leukemias. Additionally, epigenetic mechanisms typically affect large swaths of the genome well beyond individual TSGs, making establishing the causation a challenge. Over the past decade, two related but distinct post-transcriptional mechanisms of exon selection [AS and alternative polyadenylation (APA)] have emerged, which act on target transcripts with pinpoint accuracy. They affect both coding and noncoding exons and result in greater transcriptome and proteome diversity, which frequently confers growth advantage upon leukemic cells (Figure 1).

AS is a well-recognized mRNA maturation mechanism, which gained particular prominence in the leukemia field following the discovery in 2011 of driver mutations in genes encoding key spliceosome components (e.g., *SF3B1* and *U2AF1*) and nuclear proteins bound to exonic splicing enhancers (*SRSF2*) (reviewed in [1]). Such mutations have profound implication for leukemogenesis. *SRSF2* mutants, for example, were recently shown to promote inclusion of the stop codon-containing 'poison' exon in the EZH2 mRNA and to repress the frame-preserving exon of the BCOR mRNA [2]. This predictably results in nonsense-mediated decay (NMD), a quality-control mechanism eliminating nontranslatable transcripts [3]. Of note, both EZH2 and BCOR have prominent roles in hematologic malignancies, and restoring *EZH2* expression partially rescues hematopoiesis in *SRSF2*-mutated cells [2].

In other types of leukemia, such as B-ALL, splicing factor (SF) genes are rarely mutated. Yet, recent data demonstrated that, even in B-ALL, there is systemic deregulation of splicing affecting thousands of genes, some more consistently that others [4]. One of the most consistent changes affects 3′-untranslated region (3′UTR) selection by the transcript encoding the key SF hnRNPA1, making it an NMD substrate and reducing its mRNA levels. Also deregulated in B-ALL was *SRSF3*, which has a 'poison' exon of its own and is closely related to aforementioned *SRSF2*. Collectively, dysregulated hnRNPA1 and other SFs cause aberrant splicing of dozen of oncogenes and TSGs, including *DICER1*, *TP53*, and *NT5C2*, with frequencies far exceeding those of somatic mutations and copy number alterations [4].

AS is not limited to exons comprising open reading frames. It also affects 5′ and 3′UTRs, sometimes with drastic consequences, as exemplified by APA. APA can occur either in a splicing-independent form [by utilizing multiple poly(A) signals (PAS) in typically long 3′-terminal exons] [5] or in a splicing-dependent form, where mutually exclusive 3′-terminal exons can be found in otherwise identical mRNA species. This latter phenomenon is known as intronic polyadenylation (IPA). While the use of intronic PAS near transcription start sites would cause abortive transcription, PAS further downstream could yield stable proteins that nevertheless lack C-terminal domains and possibly have opposing or dominant-negative functions. There are individual examples of functional C-terminal isoforms, most famously the developmentally regulated AS and IPA of the immunoglobulin μ heavy chain transcript, which encodes the membrane-bound IgM in B cells but secreted IgM in plasma cells. However, testing the functional significance of IPA on the whole-transcriptome scale became possible only recently, through gradual improvements in 3′-end sequencing.

During the summer of 2018, the Mayr and Leslie laboratories at the Memorial Sloan-Kettering Cancer Center demonstrated that truncation of proteins through IPA

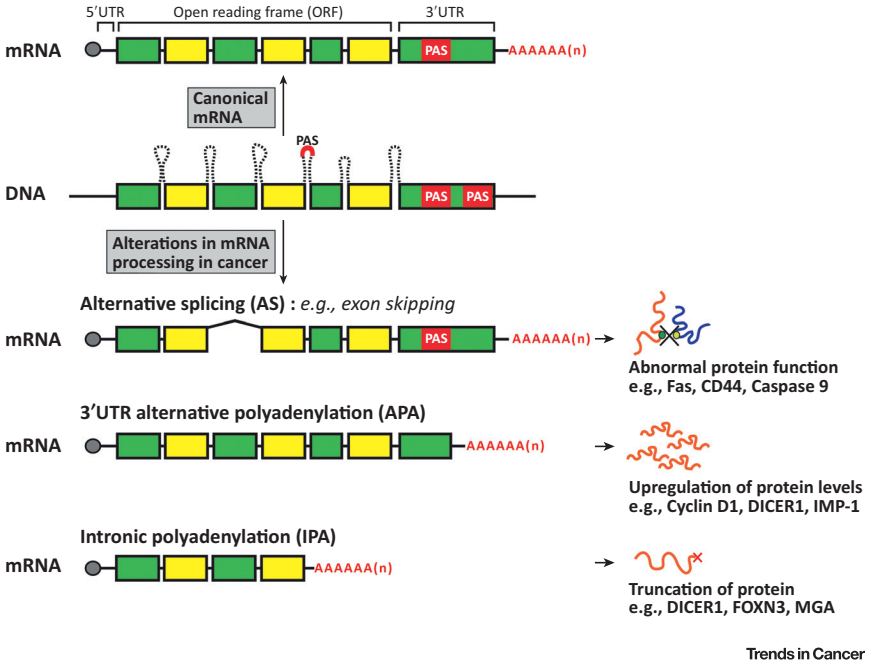

Figure 1. Noncanonical Usage of Exons and Polyadenylation Signals in Cancer. Alternative splicing (AS) and alternative polyadenylation (APA) are two key RNA-processing events that account for the diversity of cancer transcriptomes and proteomes. APA at multiple polyadenylation sites (PAS) within 3′-untranslated regions (UTRs) will affect the length of the 3′UTR and mRNA turnover, but not the protein-coding region. However, APA at cryptic PAS in introns can lead to truncated mRNA and protein isoforms. Green and yellow boxes indicate exons and dotted lines indicate introns. Gray circles denote 5′ caps of mature transcripts and 'AAAAA(n)' indicates 3′ poly(A) tails.

is prevalent in blood-derived immune cells [6]. The authors estimated that PASs are interspersed within the introns of 15–20% of genes, where they would compete with 5′ splicing sites (5′ss) for binding of various RNA-binding proteins. IPA was also tightly regulated throughout developmental stages (e.g., in germinal center B cells versus memory B cells and in plasma cells versus their malignant counterparts, multiple myelomas) [6]. The unique pattern of IPA in multiple myeloma suggested that this process contributes to the pathogenesis of blood cancers.

In an important follow-up paper, the same groups tested the hypothesis that, in addition to generating proteome diversity, IPA, similar to AS, can inactivate TSGs. Using as a model CLL versus normal CD5-positive B cells, Lee *et al*. discovered several truncated protein isoforms corresponding to known leukemia suppressors [7]. In fact, they observed a statistically significant enrichment for TSG transcripts among all alternatively terminated mRNAs. Of note, many IPA-mediated truncations occurred within domains also targeted by nonsense mutations in other patients with CLL. These events occurred in a mutually exclusive manner, suggesting that genetic and cotranscriptional inactivations were two means to the same end. Examples of dually affected TSGs were DICER1, the key enzyme in miRNA biogenesis, and MGA and FOXN3, transcriptional repressors of several leukemogenic programs. Another class of candidate TSGs was only affected by IPA in CLL but was known to suffer truncating mutations in solid tumors. Some of those 100+ genes are well characterized, but others we know little about. The case in point is *CHST11*, which encodes a carbohydrate sulfotransferase that modifies chondroitin on the surface of WNT ligand-expressing cells and prevents its diffusion and paracrine signaling. By contrast, dominant-negative *CHST11* IPA enabled WNT action on neighboring cells, which favored neoplastic transformation. Collectively, these observations suggest that AS in general, and IPA in particular, are the preferred ways to inactivate TSGs in leukemia.

Aside from the widespread deregulation of SFs in leukemia, what might the underlying molecular mechanisms be? Transcription and splicing are known to occur on similar timescales [8]. Therefore, as the authors point out, IPA signals within introns must compete with the alternate splicing machinery; and several *cis*-acting splicing elements, such as long introns and weak 5′ss, would favor IPA over splicing. Indeed, IPA genes were found to have longer introns, longer transcription units, and higher AT content [6]. Additionally, IPA could be affected by factors that alter the rate of transcription elongation by polymerase II, including DNA methylation and chromatin structure. One particularly relevant modification might be H3K36me3, which is written by the leukemia-suppressive SETD2 methyltransferase and preferentially marks expressed, slowly transcribed exons (reviewed in [9]). The effects of epigenetics do not appear to be limited to DNA modifiers: a recent study showed that diminished RNA methylation (namely, N6-methyl-adenosine) of the newly transcribed exon of the *MAGI3* gene enhances recognition of the PAS in the following intron, causing truncation of this protein in breast cancer cells [10].

In summary, there is emerging evidence that at least some splicing alterations in tumor-suppressor genes (including IPA) are functionally equivalent to deletions and/or known loss-of-function mutations.

This mechanism has profound implications for the entwined fields of precision medicine and targeted anticancer therapies. Across the world, centers for personalized diagnostics utilize next-generation sequencing and gene panels to detect mutations and copy number alterations in specific genes known to be drivers of a particular cancer type. For example, hematologic malignancy-sequencing panels yield clinically relevant and actionable insights into leukemia pathogenesis, progression, and therapeutic responses. Yet, this approach completely ignores RNA alterations, such as exon usage, and, therefore, is bound to miss important predictive and prognostic biomarkers. We envision that, over the next few years, follow-up studies will usher in the era of RNA-based diagnostics for liquid and solid tumors alike, in children as well as in adults. Additionally, interfering with splicing using RNA-based therapeutics and/or available small-molecule inhibitors could be used to reactivate dormant TSGs and reap significant therapeutic benefits.

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