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POINT-OF-VIEW

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The role of alternative splicing in cancer

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ABSTRACT

The functional capacity of cells is defined by the transcriptome. Many recent studies have identified variations in the transcriptome of tumors due to alternative splicing changes, as well as mutations in splicing factors and regulatory signals in most tumor types. Some of these alterations have been linked to tumor progression, metastasis, therapy resistance, and other oncogenic processes. Here, we describe the different mechanisms that drive splicing changes in tumors and their impact in cancer. Motivated by the current evidence, we propose a model whereby a subset of the splicing patterns contributes to the definition of specific tumor phenotypes, and may hold potential for the development of novel clinical biomarkers and therapeutic approaches.

Introduction

Cancer arises from genetic and epigenetic alterations that interfere with essential mechanisms of the normal life cycle of cells such as DNA repair, replication control, and cell death.[1,2] For example, DNA mutations occurring at genes and regulatory sites may cause the activation or suppression of crucial functions that lead to uncontrolled proliferation. These alterations also impact the transcriptome, which in turn can induce and sustain multiple mechanisms related to the progression of the tumor. In fact, genetic and epigenetic alterations could impair RNA processing before their effect is even visible at protein level, thereby defining the functional capacity of the cells. Accordingly, identifying the alterations of the transcriptome becomes relevant to advance our understanding of tumor biology.

Among all the steps during gene expression, alternative splicing (AS) provides perhaps the largest potential for molecular diversity and controlled regulation in the cell.[3] Genes are transcribed into pre-mRNA molecules that require extensive processing. For most genes, this processing involves the removal of introns through the process of splicing. Multiple molecular complexes, composed of RNA-binding proteins (RBPs), structural RNAs, and other protein factors, bind to the pre-mRNA

at various locations (RNA-binding motifs) and mediate the splicing process. On the other hand, different mature RNA molecules can be produced from the same pre-mRNA through the mechanism of AS. AS takes place through the controlled changes in the expression and activity of the complexes acting on the regulatory sequences on the pre-mRNA or as a consequence of the alterations in these complexes and motifs. AS is therefore, a critical mechanism not only in normal physiological processes, but also in multiple pathologies, including cancer.[4]

Splicing alterations in cancer

Multiple AS changes have been described that essentially recapitulate cancer-associated phenotypes. A large body of work has been devoted to determine the different alterations that lead to these AS splicing changes observed in cancer. We describe below some of them (Fig. 1).

Expression changes in splicing regulators

Multiple splicing regulatory factors have been observed to trigger tumorigenic properties in cells when overexpressed or downregulated, and have been characterized as oncogenes or tumor-suppressors,

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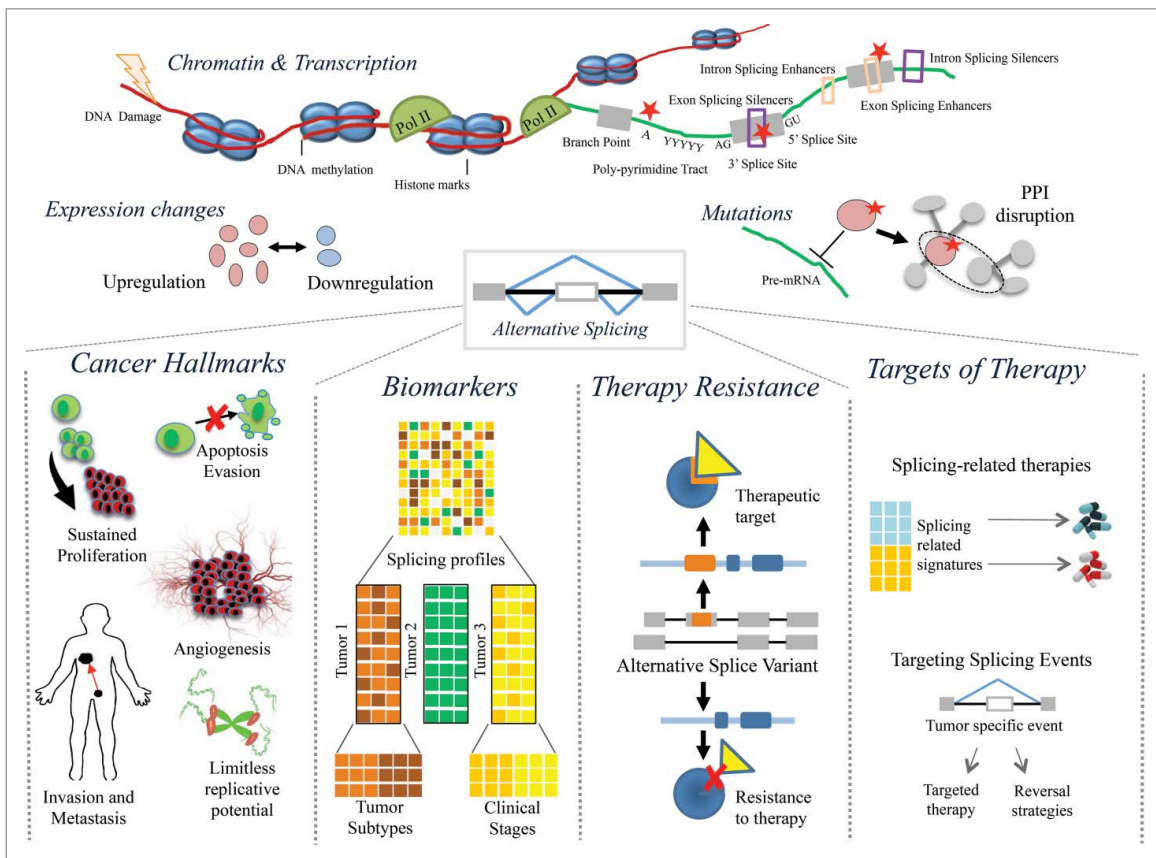
Figure 1. Alterations that lead to alternative splicing changes in cancer and their implication for the development of the disease and possible therapeutic strategies. Alterations include expression changes in splicing factors, mutations in splicing factors and splicing regulatory sequences, alterations in the transcription and chromatin state, and DNA damage. These alterations can lead to alternative splicing changes in tumors, which may recapitulate cancer hallmarks, like cell proliferation, disruption of apoptosis, cell motility and invasion, angiogenesis, and limitless replicative potential. Splicing patterns provide predictive signatures for tumor subtypes and clinical properties, and may be indicative of therapy resistance. Finally, some splicing changes are emerging as direct targets of therapy, and the splicing properties of a tumor, as well as the mutational status of splicing factors, can be informative for selection of specific therapies.

respectively, through the changes they induce in AS.[5,6] Some factors recapitulate this role across multiple tumor types, whereas others show a context-dependent expression pattern that may reflect the tissue of origin.[7] The expression alteration of splicing regulators may have different origins, like copy number alterations[7] or through changes in post-transcriptional modifications that are under the control of cell signaling pathways, which are frequently deregulated in tumors.[2] Additionally, multiple splicing factors are transcriptionally controlled by the oncogene *MYC*, which is frequently overexpressed in tumors and leads to multiple oncogenic splicing changes through the upregulation of splicing factors.[6,8] The expression changes in splicing factors is also linked to the metabolic transformations associated to tumors, often triggered by specific cellular microenvironments, which lead to AS changes in genes involved in metabolic processes.[9] The link between *MYC,* splicing, and cancer

has been further emphasized recently. Components of the spliceosome appear to be essential for the activity of *MYC* as oncogene, which underscores the central role of splicing in cancer.[10,11]

It has been further observed that gene expression alterations in cancer appear to recapitulate partially or extensively physiological pathways. For instance, breast tumors show a pattern in the expression of splicing factors and splicing events that resemble that of undifferentiated cells, including the downregulation of *MBNL1* and a splicing change in *NUMB*.[7] Similarly, AS analysis during metastatic colonization[12] shows extensive overlap with the changes that occur during epithelial-to-mesenchymal transition.[13] However, it is not yet clear whether such cellular programs are fully recapitulated or whether they co-exist with other alterations that appear in tumors, thereby providing tumor cells with a variety of molecular repertoires.

Mutations in splicing regulators

Access to the genome sequence from multiple tumors has uncovered recurrent mutations in core and auxiliary components of the spliceosome in various tumor types. They occur predominantly in hematological malignancies and often involve the factors *SF3B1, U2AF1, SRSF2,* and *ZRSR2* (reviewed in ref.[5]). Although generally at lower rate, splicing factors also appear mutated in solid tumors, including *SF3B1* in breast cancer and melanoma,[14,15] *U2AF1* and *RBM10* in non-small cell lung tumors,[16] and *HNRNPL* in colon tumors.[7] An analysis of genes coding for known and putative RBPs has shown that mutations in known and putative regulators of splicing is mostly limited to these cases in solid tumors.[7] Additionally, expression changes in splicing factors appear to produce more splicing changes in the events, compared with those related with mutations in splicing factors[7] or regulatory regions,[17,18] and both types of alterations do not seem to produce the same splicing changes. For instance, modulating the expression of *SF3B1* in cells does not recapitulate the changes observed when *SF3B1* is mutated.[19] The identification of the splicing changes related to mutations in splicing factors is instrumental to understand their relevance for cancer development and therapy and is currently an active area of research.[14,19-21]

Mutations on splicing regulatory sequences

Somatic mutations that disrupt splicing regulatory motifs can also be a source of splicing changes in cancer. For instance, mutations at the exon–intron boundaries have been associated with intron retention in tumor suppressors such as *TP53, ARID1A, PTEN, CHD1, MLL2,* and *PTCH1.*[22] Similarly, mutations on synonymous sites on coding exons appear enriched in oncogenes and have been proposed to disrupt the splicing of cancer drivers such as *ITK, ALK, IDH1,* and *BCL6.*[22] Since splicing regulatory sequences on exons span 4–6 nucleotides, hence possibly covering multiple codons, it is likely that mutations on non-synonymous sites also lead to splicing changes in cancer drivers.[23] Intronic mutations also appear to play a crucial role in cancer such as therapy resistance. For instance, a point mutation 51nt upstream of the 3′ splice-site of intron 8 of *BRAF* promotes a splice variant that confers resistance to Vemurafenib treatment.[24] However, in contrast to exonic mutations, not many recurrent intronic mutations have been described so far beyond the exon–intron boundaries, despite the fact that a significant fraction of the splicing regulation is controlled by intronic regulatory sequences, either through the branch-point and poly-pyrimidine tract sequences, or through intronic splicing enhancers and silencers.[25] This could be due to the fact that intronic regulatory motifs often present positional variability with respect to the exon–intron boundaries and are, therefore, less straightforward to identify. Although deep intronic mutations may be harder to characterize, they could also affect splicing. For instance, a considerable number of introns harbor distant branch-points located further than 50nt upstream of the 3′ splice-site,[26] and the structure of the RNA plays a role in its processing and may bring together distant regions.[27] By harnessing the power of characterizing the relevant intronic regulatory regions, we will be able to gain further insights into the disruption of splicing in cancer.

Chromatin and transcription dependent effects

Most of the mechanisms related to gene expression take place in a coordinated way that couples transcription with pre-mRNA processing. Co-transcriptional splicing seems to be quite prevalent and advantageous for the efficiency of splicing.[28] There is also plenty of evidence showing that splicing regulation depends on the coupling with the dynamics of RNA polymerase II (RNAPII). This is controlled by, among other elements, the activity of promoters and transcriptional enhancers, the chromatin state, and the recruitment of splicing factors by RNAPII or to the chromatin context.[28,29] Accordingly, alterations in cancer that affect transcription or chromatin may also impact splicing. For instance, the Histone methyltransferase *SETD2* appears frequently mutated in kidney tumors, which has been related to alterations in RNA processing and splicing.[30,31] It is thus conceivable that many of the splicing changes observed in tumors are direct or indirect effects due to global or local somatic alterations of transcription and chromatin.

DNA damage

DNA damage through the exposure to either radiation or toxic chemicals has been shown to induce AS in genes involved in cellular processes such as apoptosis, cell-cycle control, and DNA repair.[32,33] The splicing

response to DNA damage comes through various modulations such as the post-transcriptional modification of splicing factors that affect protein interactions with other splicing factors or with RNA. For example, dephosphorylation of SRSF10 through DNA damage prevents the interaction with hnRNP F/H, favoring a splicing change in *BCL2L1* to enhance the production of its pro-apoptotic isoform.[33] On the other hand, stable formation of ribonucleoprotein complexes prevents the appearance of RNA:DNA duplexes, which would otherwise promote mutations and genome instability.[32] For instance, the disruption of a complex of BRCA1 with RNA has been found to be related to defective DNA damage repair.[34] As the functional control of splicing emerges as essential for DNA damage repair, splicing-related alterations may contribute to a genome instability phenotype and to the accumulation of mutations.

Functional impact of the splicing alterations in cancer

The analyses of transcriptomes from multiple patient tumor samples have highlighted frequent splicing changes during tumor progression and metastasis transformation,[12,35] as well as in association to somatic alterations.[14,19,20] However, the functional impact of these AS changes and their significance in cancer is only starting to be elucidated.

Alternative splicing recapitulates hallmarks of cancer

Several AS events have been shown to recapitulate cancer-associated phenotypes. For instance, an exon inclusion change in *NUMB* has been shown to promote cell proliferation.[36] Similarly, an exon-skipping event in *MST1R* has been related to the acquisition of cell motility during cancer cell invasion.[37] Moreover, the modulation of these events can recapitulate the tumor phenotype or revert to a normal phenotype.[36,38] Therefore, understanding the general functional effects of AS potentially leads to the discovery of novel oncogenic mechanisms and therapeutic targets.

AS changes have been proposed to remodel the network of protein–protein interactions in a tissue-specific manner.[39,40] It is, therefore, possible that splicing changes in cancer also impact the network of protein–protein interactions, but in a disruptive, non-regulated way. In this direction, a recent study shows that an AS change in *NFE2L2* that occurs in various tumor types leads to the loss of a protein interaction with its negative regulator KEAP1, thereby providing an alternative way to activate the Nrf2 pathway.[41] This may in fact be a general mechanism whereby splicing alterations disrupt protein–protein interactions of cancer drivers and related pathways, providing other means to impact cell function that are equivalent to classical somatic mutations in drivers. Additionally, AS may also induce degradation of the transcripts through non-sense mediated decay,[42] a mechanism that was associated to somatic mutations on the splice-sites that induce intron-retention in tumor suppressors.[18]

Alternative splicing as biomarkers

Despite the abundance of splicing changes observed in tumors, only few cases have been characterized for their functional impact. It is possible that the majority of the splicing changes in tumors are passengers, merely reflecting upstream genetic mechanisms and the deregulation of splicing fidelity mechanisms. Yet, they may provide tale-tell signs of specific tumor characteristics. In this context, splicing changes have been shown to separate tumor types and subtypes[17] and have been related to tumor stage and patient survival,[35,43] so they have the potential to be used as biomarkers for specific clinical conditions. This could be relevant for cases for which a known prognostic marker is either not present in the sample or does not exist, as for pediatric tumors.[44]

Alternative splicing and therapy resistance

Alterations in AS also appear essential for understanding drug resistance.[21] For instance, a considerable proportion of patients that do not respond to targeted treatment against *BRAF* mutations express a *BRAF* isoform lacking exons 4–8, which encompass the RAS-binding domain.[45] Interestingly, small-molecule modulators of pre-mRNA splicing are capable of restoring the original *BRAF* splicing and reduce growth of therapy-resistant cells.[24] Similarly, AS also impacts immunotherapy in leukemia due to the disrupted activity of the splicing factor *SRSF3*.[46] These results highlight the importance of characterizing the transcriptome for therapy and suggest that specific splicing alterations may provide a selective advantage to tumors.

Alternative splicing as target of therapy

There is a growing interest to search for splicing-related alterations for which specific therapies could be developed. One of the strategies being tested at the moment consists in the synthetic design of antisense oligonucleotides (AONs) that target-specific splicing events. AONs are able to revert AS events to restore normal cellular phenotypes[36,38] and have reached already clinical trial stage for some splicing-related disorders.[50] Another promising strategy for cancer therapeutics is the use of small molecule compounds that modulate the activity of splicing factors.[21,51] These therapies have a wide range of effects depending on the tumor type or the mutational status of the targeted splicing factor. Thus, it becomes essential to know which patients may benefit from splicing-related therapies. One such possible class includes patients with overexpressed *MYC* in tumors, which are more dependent on the activity of the spliceosome.[10,11]

AS events are also emerging as direct actionable alterations for targeted therapies. This is the case of the skipping of *MET* exon 14 observed in some lung cancer patients, resulting in a deletion of the protein region that inhibits its kinase catalytic activity.[47] Importantly, the skipping of this exon is sufficient for *MET* activation and tumors that harbor the event respond to *MET*-targeted therapies.[48,49] Although this splicing change in *MET* has been explained so far as a result of somatic mutations on exon 14 or on its splice-sites, it is conceivable that the same splicing change could occur due to other mechanisms yet to be discovered. These results raise the interesting possibility that an AS event could be used as direct target of therapy. Thus, either as direct targets or as a means to characterize the tumor, the splicing properties may become fundamental to identify therapeutic vulnerabilities and potential resistance. This may be particularly relevant for tumors lacking somatic mutations in genes with known targeted therapy, as these patients cannot benefit from currently available therapies.

Combinatorial control of RNA splicing and possible implications for cancer

AS changes that characterize and contribute to the pathophysiology of cancer are triggered by alterations in a complex network of different mechanisms. These combinatorial effects have some interesting implications. Different alterations in tumors may in turn impact RNA processing and splicing in similar ways. For instance, mutations in RBM10 or downregulation of QKI lead to the same splicing change in NUMB that promotes cell proliferation.[36,52] This suggests that the splicing alterations observed in tumors may be indicative of a phenotypic advantage, and some may even phenocopy somatic mutations in cancer drivers to induce similar functional impacts. Accordingly, a subset of the splicing changes in cancer may play an important role in the neoplastic process independently of or in conjunction with the already characterized genetic alterations.

It is not clear yet whether a single splicing change may be sufficient to induce an oncogenic transformation in a normal tissue context, or even whether splicing events can be considered cancer drivers. It is possible that the splicing-related effects are additive, contributing to, and maintaining specific properties or favoring certain cellular environments that modulate the oncogenic impact of somatic mutations. Consistent with this, there is a relation between specific tumor microenvironments and AS.[53] Additionally, somatic mutations in splicing factors are generally heterozygous and appear to require a normal functional splicing machinery to exert their oncogenic function.[21,54] For example, the ratio of both mutant and wild-type U2AF1 splicing factor influences the splice-site selection in lung adenocarcinomas, questioning the functional significance of the mutant U2AF1 cells.[54] This suggests a context-dependent effect, by which somatic alterations may become relevant in the presence of certain splicing-related signatures. This is further supported by recent findings showing that tumors with overexpressed *MYC* are highly dependent on the splicing machinery for survival and may be more sensitive to splicing-related therapies.[10,11]

In conclusion, as selection on the tumor clones is exerted on the phenotype rather than on the genotype, we propose that the splicing patterns may define relevant molecular phenotypes in tumors, despite their genetic heterogeneity. The characterization of tumor transcriptomes – with respect to splicing – thus becomes essential to understand their clinical properties and to select appropriate therapeutic strategies.

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