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Review

Alternative splicing regulation: Implications in cancer diagnosis and treatment☆

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ABSTRACT

The accurate expression of the genetic information is regulated by processes like mRNA splicing, proposed after the discoveries of Phil Sharp and Richard Roberts, who demonstrated the existence of intronic sequences, present in almost every structural eukaryotic gene, which should be precisely removed. This intron removal is called "splicing", which generates different proteins from a single mRNA, with different or even antagonistic functions. We currently know that alternative splicing is the most important source of protein diversity, given that 70% of the human genes undergo splicing and that mutations causing defects in this process could originate up to 50% of genetic diseases, including cancer. When these defects occur in genes involved in cell adhesion, proliferation and cell cycle regulation, there is an impact on cancer progression, rising the opportunity to diagnose and treat some types of cancer according to a particular splicing profile.

Regulación del *splicing* alternativo: Implicaciones en el diagnóstico y tratamiento del cáncer

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RESUMEN

La expresión de la información genética es regulada por procesos como el *splicing* del ARN mensajero, mecanismo propuesto por Phil Sharp y Richard Roberts, quienes demostraron la existencia de secuencias intrónicas, las cuales interrumpen a la mayoría de los genes estructurales en eucariotes y deben ser removidas con gran precisión. Dicha remoción de intrones se denomina *splicing*, y permite generar variantes proteicas a partir de un solo gen, cada una con funciones diversas y, a menudo, antagónicas. Actualmente se sabe que el *splicing* es la principal fuente de diversidad proteica, ya que el 70% de los genes humanos lo sufren, y defectos en este proceso originan hasta el 50% de las enfermedades genéticas, incluido el cáncer. Cuando estos defectos se presentan en genes involucrados en adhesión, proliferación y ciclo celular, repercuten en la progresión de procesos cancerosos cuyo diagnóstico, tratamiento y prognosis puede determinarse en base a su perfil de *splicing*.

Introduction

The genes of eukaryotic organisms are frequently interrupted by non-coding sequences that must be eliminated during expression. These sequences receive the name of introns. Splicing is the process through which introns are removed to produce functional messenger RNA (mRNA) molecules by ligating the coding sequences or flanking exons. Alternative splicing occurs when some exons, introns or portions of them are differentially included to produce several mRNA molecules from the same immature precursor

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(pre-mRNA). Alternative splicing is employed by 75% of the human genes and constitutes the main source of protein diversity in eukaryotes.[1] Although it is estimated that between 15 and 50% of the human genetic diseases originate in some defect at splicing[2] level, the extraordinary contribution of alternative splicing in different biological and pathological processes in plants and mammals is only starting to be explained. As an example, it is well known that different alternative splicing episodes are responsible for determining sexual characters in *Drosophila*,[3] generating hormones with different biological activities or producing cytoplasmic or membrane proteins with antagonistic functions.[4] In this sense, there is increasing evidence regarding genes involved in different stages of cancer, whose alternative splicing is affected, with consequences in different processes such as cellular invasion and proliferation, resistance to apoptosis and susceptibility to different chemotherapeutic agents. In accordance with the information provided by the *Cancer Genome Project* of the Wellcome Trust Sanger Institute in the United Kingdom, 488 human genes possess mutations associated with some type of cancer. More relevantly, 63 of them present mutations that somehow affect their alternative splicing.

In the face of this approach, it is relevant to understand the mechanisms that regulate the splicing in different organisms, cellular types and environmental conditions. Even though until now the general principles determining the basic mechanism of splicing are known, the total number of regulatory proteins and the fine details leading to the production of a certain isoform in a particular context are still unknown.

General principles of alternative splicing

Before examining what happens during transcript maturation in different cancer cells, it is necessary to summarise some general concepts related to the splicing regulation process.

Every splicing episode enables the generation of different mRNA molecules, *i.e.*, different protein products from the same mould, increasing the genes coding capacity. This event is called alternative splicing and works as an on/off system of the genes expression for the production of specific proteins in certain type of tissue or in a determined development phase, which demands the fine regulation of splicing in space and time.

Splicing is regulated by several cis-acting elements (pre-mRNA sequence), as well as by trans-acting factors (nuclear proteins). Within the cis-elements, there are sequences that are essential and limit exon-intron bindings, which present characteristic consensus sequences. Moreover, there is an intronic region that precedes the exon start, known as *branch point sequence*, flanked by a region that is rich in pyrimidine residues. The degree of similarity for each one of these elements in a pre-mRNA, combined with their position, determines their relative "strength," since the more similar they are to the consensus reported and the best positioned they are, the easiest it will be for the splicing[5] machinery to recognise them. Moreover, if these splicing sites are altered by any mutation, some other neighbouring splicing sites that generate aberrant messengers might be activated, producing, in turn, non-functional proteins. There are also relatively short and conserved sequences, which may stimulate or inhibit the recognition of weak splicing sites. These are called splicing enhancers or silencers. Mutations in these sites may also alter the balance needed to generate functional messengers for the cell in question.

Spliceosome as catalytic centre

The aforementioned cis-elements must be recognised by *small nuclear ribonucleoprotein particles* (snRNP), which are the main splicing catalysts, and by some other protein factors that interact with the pre-mRNA, which are assembled forming an active complex known as spliceosome, where biochemical reactions occur that result in the removal of introns. The spliceosome constitutes one of the biggest and most complex cellular machineries. Although almost 100 splicing factors had been identified until 1999, technological innovations have enabled a better dissection of these complexes, and the number of proteins that form the spliceosome has practically doubled nowadays.[6]

The U1, U2, U4, U5 and U6 snRNPs interact with the different splicing sites and are responsible for the delimitation of exonic sequences as well as for the removal of introns. Besides the snRNPs, the main splicing regulatory factors are the SR proteins and the family of *heterogeneous ribonucleoprotein particles* (hnRNP) proteins, each one with specific blank sequences. Currently, it is well known that the differential expression as well as the changes in the nuclear concentration of such factors may be responsible for the modification of the decisions that occur during splicing depending on the cellular type or the differentiation level. Added to which, the expression of some splicing factors may be regulated, in turn, by alternative splicing episodes, while the functionality, localisation and activity of various factors may also be regulated by their phosphorylation status.

SR proteins constitute a family of splicing factors that is highly conserved in vertebrate, invertebrate and plants. These proteins present a protein interaction domain called SR due to its high content of arginine and serine residues, besides one or 2 binding sites for RNA. In general, it has been proposed that SR proteins activate splicing through binding to enhancing elements, recruiting the spliceosome towards the adjacent intron. Interestingly, the activity of SR proteins may also be modulated by phosphorylation and dephosphorylation cycles that affect protein folding, the RNA-binding ability and protein-protein interactions, with consequences in the spliceosome assembly and catalysis through the several phosphorylation sites that are located exactly in the SR domain.[7]

On the other hand, more than 20 members have been characterised inside the hnRNPs and they have been named alphabetically based on their size, from hnRNP A1 to hnRNP U.[8] These proteins are involved in a variety of biological processes and almost all of them have documented functions in splicing. The ability of some hnRNPs to interfere with snRNP or SR proteins binding has been described in several pre-mRNA. These observations have led to generalise the idea that hnRNP proteins act suppressing splicing. However, even though the initial evidence showed that hnRNP A1 suppresses splicing since it is capable of altering the selection of the splicing donor site and promoting the elimination of an alternative exon, it has currently been proposed that it also has the ability of facilitating the splicing of a long intron.[9]

To sum up, there are sequences in the pre-mRNA that function as binding sites for different nuclear proteins. The combination of the location and the availability of the sequences, together with the concentration and functionality of the factors involved, will favour the selection of a determined splicing site and, accordingly, the expression of a particular isoform.

Alterations in the alternative splicing of genes involved in cancer

In spite of the functional impact that some alternative splicing episodes have on the expression of genes related with cancer processes, the molecular mechanisms that regulate such selection have not been studied in depth.

The best documented examples of the role that splicing performs in the development of cancer processes are related to the alteration of the expression of oncogenes and tumour suppressors.