[RNA Biology 5:1, 1-3; January/February/March 2008]; ©2008 Landes Bioscience

Point of View

Alternative splicing and disease

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Abbreviations: SNP, single nucleotide polymorphisms; PTC, premature termination codon

Key words: splicing, alternative splicing, obesity, cancer, SNP

Splicing is a molecular mechanism, by which introns are removed from an mRNA precursor and exons are ligated to form a mature mRNA. Mutations that cause defects in the splicing mechanism are known to be responsible for many diseases, including cystic fibrosis and familial dysautonomia. If mutations that cause defects in splicing are responsible for such severe deleterious phenotypic differences, it is possible that mutations in splicing are also responsible for mildly deleterious phenotypic differences. Although deleterious mutations are rapidly eliminated from the population by purifying selection, the selection against mild deleterious effects is not as strong. Since mildly deleterious mutations have a chance of surviving natural selection, we might be mistakenly referring to these mutations as neutral variation between individuals. Splicing has also been shown to be seriously affected in cancer. Examination of cancerous tissues revealed alterations in expression levels of genes involved in mRNA processing and also a slight reduction in the level of exon skipping—the most common form of alternative splicing in humans. This implies that defects in genes involved in the regulation of splicing in cancerous tissues affect the delicate regulation of the inclusion level of alternatively skipped exons, shifting their mode of splicing back to constitutive. It may be that splicing silencers play a more prominent role in alternative splicing regulation than previously anticipated.

Introduction

The splicing process generates mature mRNA molecules through removal of introns from mRNA precursors and ligation of exons.[1] The splicing machinery recognizes exons and introns through use of multiple signals. Four main splice signals assist the splicing machinery in recognizing the proper exon-intron boundaries: the 5' and 3' splice sites, located at the upstream and downstream exon-intron junctions, respectively and the branch site and the polypyrimidine tract, both located upstream of the 3' splice site.[1-3] In metazoans, these four splice signals are not sufficient for the recognition of exons and introns by the splicing machinery; other exonic and intronic cis-acting regulatory sequences bind trans-acting factors and regulate splice-site selection in a combinatorial manner.[4,5] Sometimes, the boundaries of exons and introns are not sufficiently distinct, and hence sequence defined in one mRNA molecule as an exon might be identified as an intron in another mRNA molecule generated from the same gene. This process, termed alternative splicing, is a regulatory mechanism by which variations in the incorporation of coding and non-coding regions give rise to functionally different proteins that originate from the same genes.[1,6,7]

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Submitted: 03/11/08; Accepted: 03/22/08

Previously published online as an *RNA Biology* E-publication:
http://www.landesbioscience.com/journals/rnabiology/article/5944

Alternative Splicing and Phenotypic Differences

There are many visible phenotypic differences between individuals, from height to eye and hair color. These differences are attributed to large insertions and deletions of DNA, termed copy-number variants, and to single nucleotide polymorphisms (SNPs), in which a point mutation affects a single nucleotide.[8] SNPs may affect nucleotides in the coding regions of genes and consequently alter the translated proteins, and may hence be responsible for phenotypic differences. Therefore, it has long been postulated that SNPs, through slight changes in the coding sequence, account for many of the phenotypic difference between individuals. A significant portion of known SNPs appear to be silent mutations, which do not change the coded proteins, and were usually referred as neutral variation. However, as exons and introns are crowded with splicing regulatory sequences, such silent SNPs might disrupt these sequences and affect splicing (which is also true for non-silent mutations).[9-11] Rather than creating small variations in the amino-acid composition of proteins, alteration of the splicing process may result in the exclusion or inclusion of long coding regions, and hence may have the potential to gravely affect the translated proteins and consequently the visible phenotype. A recent deep-sequencing project compiled a large dataset of SNPs found in 757 individuals with extreme body mass index (BMI) values.[12] This dataset consisted of 496 SNPs in the coding region of genes related to obesity or body-weight related pathways; 189 of these SNPs where found to be silent. Using bioinformatic and experimental analyses, however, it was shown that many of these SNPs affect splicing regulatory motifs and consequently affect the splicing patterns of their harboring genes, suggesting that body weight, like eye color, has a strong genetic background.[13]

These results suggest that, while mutations that affect the splicing process constitute at least 14% of disease-causing mutations,[14-18]

they can also be responsible for "normal" phenotypic differences between individuals. In fact, while drastic shifts in the splicing pattern are plausibly deleterious, or even lethal, subtle changes in the splicing pattern are likely to be non-fatal, but will still affect the corresponding genes. We, therefore, suggest that many of the "neutral" variations between individuals are, in fact, not neutral at all; rather these variations are responsible for at least some of the common phenotypic differences between individuals. Just as the imperfection of DNA replication is a fundamental prerequisite for evolution by natural selection, we believe that the imperfection of the splicing mechanism allows for subtle changes in the recognition of coding and non-coding regions and facilitates evolution of higher eukaryotes. Current SNP databases contain millions of SNPs;[19] however, most of these SNPs were found by looking at a relatively small number of individuals. Since mildly deleterious SNPs are expected to be selected against to some extent, many might have been overlooked. More deep-sequencing efforts should uncover many additional SNPs. Analysis of this data will determine to what extent splicing variations affect the phenotype. We expect that the recently uncovered link between splicing and body mass is only the tip of the iceberg.

Alternative Splicing and Cancer

The connection between cancer and changes in levels of alternative splice forms has been well established. Over the years, experimental and computational studies revealed several examples of specific splice variants that are detectable only in cancerous tissues.[20-22] These studies raised the notion that alternative splicing is more common in cancerous tissues than in normal ones, supposedly the result of the splicing machinery going berserk. However, in a recent analysis, the prevalence of alternative splicing was found to be slightly higher in normal tissues than in tumor tissues.[23] Specifically, this difference was attributed to a reduction in the prevalence of exon skipping events—the most common form of alternative splicing in humans[24,25]—in cancerous compared to normal tissues, whereas the other main types of alternative splicing (alternative 5' and 3' splice site selection and intron retention) were slightly elevated in cancerous compared to normal tissues. It is important to bear in mind that the term cancer refers to many different diseases that originated from different lineages of cells via different molecular pathways. It may be that in some cancer types, alternative splicing does go berserk, but this is likely the exception rather than the norm.

The results revealed that in general alternative splicing is indeed less prevalent in cancerous than normal tissues, but that some genes exhibit unique alternative splice variants in cancerous tissues that are not detected in normal ones. Many of these variations are probably deleterious, as a result of introducing a premature termination codon (PTC). Interestingly, this group of genes was found to be enriched in genes involved in splicing and mRNA processing. This supports the recent finding published by the Brenner and Ares labs, indicating that SR protein genes, which are involved in regulation of alternative splicing, contain alternatively spliced PTC-containing "poison" exons that mark the transcript for degradation by the nonsense-mediated mRNA decay mechanism.[26,27] In further analyses, splicing factors were shown to not only be altered in sequence in cancerous tissues, but to also exhibit different expression levels and to contribute and even be directly involved in transformation.[28,29]

But why is there a reduction in the level of exon skipping in cancer? It was recently shown that many alternatively skipped exons originate from constitutively spliced exons.[30] There are two other known origins for alternatively skipped exons: exonization of intronic sequences and exon shuffling (reviewed in ref. 31). We anticipate that a transition from constitutive to alternative splicing is the main evolutionary origin of alternatively skipped exons, but this remains to be proven. These alternatively skipped exons usually exhibit high inclusion levels, presumably to ensure that the evolutionary conserved form (exon inclusion) is the major mRNA product generated from the gene.[30-33] One of the molecular mechanisms leading to the transition from constitutive to alternative splicing was suggested to be relaxation of splice site selection; this was found to be coupled with fixation of cis-acting splicing regulatory motifs. In an example from exon 5 of the SLC35B3 gene, cis-acting splicing regulatory motifs were present before the transition from constitutive to exon skipping, as is evident in orthologous exons that are constitutively spliced. However, these motifs were not essential for the constitutive mode of splicing. Examination of this exon revealed that four exonic splicing regulatory elements regulated inclusion level following the transition to alternative splicing. Two elements were found to act as enhancers, whereas the other two acted as suppressors. Surprisingly, the two suppressors were putative binding sites for the SF2 and SC35 proteins, which are commonly viewed as splicing enhancer proteins; their suppression activity is likely to be related to the relative position along the exon and to other combinatorial effects.[5,34] These results suggest that exonic splicing suppression is much more common in the regulation of alternative splicing of the exon skipping type, especially in exons that originated from previously constitutive exons. Thus, in cancer, when splicing regulatory proteins are affected, the splicing mode of such exons presumably shifts back to a constitutive state.

Acknowledgements

This work was supported by a grant from the Israel Science Foundation (1449/04 and 40/05), MOP Germany-Israel, GIF, DIP, the chief scientist of the Health Ministry; ICA through the Ber-Lehmsdorf Memorial Fund and EURASNET to GA. EK is a fellow of the Clore Scholars Programme. AG is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities.

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