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Human disease-causing mutations affecting RNA splicing and NMD

Abstract

Mutations at the coding sequences of genes are primarily predicted to affect encoded protein by altering amino acids. However, mutations can also affect encoded protein by altering pre-mRNA splicing, which has been arisen as a potential pathomechanism in many human diseases. Skipping of an exon can generate a protein isoform lacking an essential domain or signal. In contrast, inclusion of a non-functional exon can affect the structural integrity or stability of the encoded protein isoform. Besides these, alternative splicing can also give rise to a premature termination codon (PTC), which can generate a truncated protein with abnormal function, or may elicit nonsense-mediated mRNA decay (NMD) pathway, followed by mRNA degradation. Therefore, aberrant splicing can exploit the mRNA surveillance pathway for detrimental consequences to cells. This review focuses different underlying mechanistic bases of human genetic mutations associated with aberrant splicing and NMD. Understanding the molecular basis of the disease can be extrapolated to treat genetic mutation for therapeutic intervention by manipulating splicing misregulation and/or NMD-regulatory pathway.

Keywords: mutation, splicing, premature termination codon, *cis*-elements; *trans*-factors, nonsense-mediated MRNA decay

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Abbreviations: NMD, nonsense-mediated mrna decay; ISE, intronic splicing enhancer; ESE, exonic splicing enhancer; ISS, intronic splicing silencer; ESS, exonic splicing silencer; PTC, premature termination codon; ASC, adenosquamous carcinoma; NAS, nonsense-associated altered splicing; NASRE, nonsense-associated altered splicing of a remote exon; ASO, antisense oligonucleotide; TRIDs, translational read-through-inducing drugs

Introduction

Pre-mRNA splicing is an intricate regulation of gene expression in higher eukaryotes. This is carried out in the nucleus by a macromolecular complex called spliceosome, which is composed of RNA and proteins. Recognition of intron/exon boundary is directed by essential splicing regulatory *cis*-elements close to either end of an intron, including the 5' splice site, the branch point (BP), the poly pyrimidine tract (PPT), and the 3' splice site. Differential pre-mRNA splicing is regulated by *cis*-elements comprised of exonic/intronic splicing enhancers/silencers (ESEs, ISEs, ESSs, and ISSs) and *trans*-acting protein factors (*trans*-factors). The fidelity of splicing is indispensible to support cellular processes, and it requires multiple quality-control mechanisms, which comprise substantial interactions among components of the transcription, pre-mRNA processing, mRNA transport, and translation machineries.[1] mRNA surveillance pathways can prevent generation of aberrant mRNAs harboring PTC, or destroy them through NMD to circumvent deleterious effects to cells. Therefore, precise coordination of pre-mRNA splicing and NMD is essential to maintain proper gene expression to support cellular integrity. Genetic mutations disrupting this coordination between pre-mRNA splicing and NMD, can be deleterious to cells provoking pathological consequences.[2–7]

Discussion

Genetic mutations can generate a PTC and elicit NMD in several ways: directly by generating a nonsense codon; by disrupting a splicing *cis*-element, which generates a PTC due to altered splicing; or indirectly by altering the function of a *trans*-factor, which subsequently generates PTC in the targeted gene(s) due to misregulated splicing. About 30% of mutations linked to human disease introduce a PTC either by creating a nonsense codon or causing a frame shift.[8,9] The consequences of nonsense mutations have been well documented previously.[10,11] About 15 to 20% of human genetic diseases are caused by mutations that disrupt the splicing *cis*-elements.[12] As for example, a mutation (eIVS9-1G>C) reported in a patient suffering in congenital myasthenic syndrome (CMS), which affect the 3' splice site of *CHRNE* intron 9 causes retention of intron 9, and subsequently generates a PTC in exon 10 in the final transcript.[13] Another mutation reported in *CHRNE* is eE154X, which activates a cryptic 3' splice site in exon 6, results a PTC at the boundary of exons 6 and 7.[13] In contrast to splice site mutation, eEF157V reported in *CHRNE,* disrupts an ESE, and promotes skipping of exon 6, which subsequently results a PTC in exon 7.[13] In contrast to *cis*-acting defects, *trans*-acting defects can potentially exert a more detrimental consequence, because several target genes can be affected due to the defect in a single *trans*-factor. The affected *trans*-factors can be either an essential constituent of splicing/NMD-machinery or an auxiliary factor modulating alternative splicing or NMD. As for example, in late 2011, frequent mutations in genes encoding spliceosomal proteins were identified and subsequently revealed to be the most common group of mutations in myelodysplastic syndromes (MDSs), related myeloid malignancies, and clonal disorders of hematopoiesis.[14–18] The vast majority of these mutations is reported in one of three genes: SF3B1, U2AF1, or SRSF2. As these proteins have predominant role in RNA splicing, a number of studies have recently been accomplished to evaluate the mechanistic effects of these mutations on RNA splicing. Mutations in U2AF1[19–23] and SRSF2[24,25] alter RNA splicing in the targeted genes in a sequence specific manner, which is distinct from loss-of-function. SRSF2 mutations affecting the proline

95 (Pro95) residue drive recurrent mis-splicing of key hematopoietic regulators. This includes SRSF2 mutation-driven aberrant splicing of *EZH2* generating a PTC, which triggers nonsense-mediated decay.[24] This subsequently results in impaired hematopoietic differentiation.[24] On the other hand, mutations in SF3B1 appear to promote selection of cryptic 3' splice sites (SSs) in the targeted genes.[26] A number of cryptic 3' SSs induced in SF3B1 mutant cells result in frameshits, therefore potentially degraded by NMD.[26]

Another interesting example of *trans*-acting genetic defect is reported in pancreatic adenosquamous carcinoma (ASC), where frequent somatic mutations were identified in *UPF1* gene in ASC tumors.[27] *UPF1* encodes an RNA helicase protein, which is essential for NMD pathway. Mechanistic investigation revealed that these tumor-specific mutations alter RNA splicing of *UPF1* gene spanning important functional domains, predicted to compromise its catalytic effect in NMD, leading to an up regulation of NMD substrate mRNAs.[27] This includes up regulation of an aberrant transcript of *TP53* in ASC tumor, which retains intron 6 harboring an in-frame PTC.

Generation of a PTC can also affect the consequence of splicing. It often causes skipping of a PTC-harboring exon, termed as nonsense-associated altered splicing (NAS). For example, a nonsense mutation in exon 51 of *FBN1* gene, drives exon skipping, which is associated with Marfan syndrome.[28] NAS can be activated through different mechanisms.[10] One mechanism is the nuclear scanning, which ensures translation of mature nascent mRNA lacking a PTC. A nonsense mutation discontinuing the open reading frame can signal the splicing machinery to exclude the affected exon. This type of selective exon skipping enables retention of the residual function of a protein instead of complete removal of the coded protein by NMD. NAS can also be evident through an indirect mechanism, when a PTC is generated in an alternative exon, and subsequently the exon-included transcript is completely degraded by NMD, and the exon-skipped transcript becomes the only remaining transcript. Third, NAS can also be triggered when a nonsense mutation disrupts an ESE or compromises an essential splicing element in an RNA secondary structure. A mutation may also force skipping of a remote exon instead of the PTC-bearing exon. A 7-bp deletion in *CHRNE* exon 7 in a CMS patient, caused skipping of the preceding exon 6 carrying 101 nucleotides.[13] This phenomenon is termed as nonsense-associated altered splicing of a remote exon (NASRE). NASRE is likely to be under estimated, as remote exons are seldom scrutinized in human mutation investigations.

Conclusion

Analyses of genetic mutations in context of pre-mRNA splicing and NMD associated with human diseases not only allow us to understand the underlying maladies in pathological conditions, but also pave the way to gain insight into the regulation of gene expression under physiological conditions. These investigations have significant impacts in terms of diagnosis and treatment of genetic diseases. The accurate classification of genetic mutations, in respect to the molecular basis of gene inactivation, is essential for understanding structure–function relationships in the affected protein, to assess the phenotypic risk with familial disease predispositions and for conceiving challenges toward new therapies. Current progress in developing methods to correct genetic defects have been appreciated with considerable promise. As for example, development of antisense oligonucleotides (ASOs) to modify splicing of SMN2 was very promising for the treatment of spinomuscular atrophy.[29–31] In addition to splicing modulation, pharmacological suppression of PTCs by translational read-through strategy using translational read-through-inducing drugs (TRIDs) drew considerable attention as an alternative of gene therapy in recent past.[32] However, mRNAs targeted by pharmacological compounds that allow translational machinery to read through PTCs are often subject to NMD.[33] This observation reduced the therapeutic potential of TRIDs, and subsequently forward the attention to NMD suppression. However, suppression of NMD pathway by general inhibition of the NMD machinery may provoke detrimental consequences by causing global inhibition of NMD. An alternative approach is transcript-specific NMD inhibition. Recently, a promising approach has been reported which used a combination of gene-specific NMD inhibition using ASOs and TRIDs to effectively restore the expression of full-length protein from a nonsense-mutant allele.[34] To develop effective therapeutic strategies against genetic defects, more genetic investigations should be scrutinized to understand the molecular basis of genetic defects.

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Conflict of interest

Author declares that there is no conflict of interest.

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