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Nonsense-mediated mRNA decay among coagulation factor genes

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ABSTRACT

Objective(s): Haemostasis prevents blood loss following vascular injury. It depends on the unique concert of events involving platelets and specific blood proteins, known as coagulation factors. The clotting system requires precise regulation and coordinated reactions to maintain the integrity of the vasculature. Clotting insufficiency mostly occurs due to genetically inherited coagulation factor deficiencies such as hemophilia.

Materials and Methods: A relevant literature search of PubMed was performed using the keywords coagulation factors, Nonsense-mediated mRNA decay and premature translation termination codons. Search limitations included English language and human-based studies.

Results: Mutations that cause premature translation termination codons probably account for one-third of genetically inherited diseases. Transcripts bearing aberrant termination codons are selectively identified and eliminated by an evolutionarily conserved posttranscriptional pathway known as nonsense-mediated mRNA decay (NMD). There are many pieces of evidence of decay among coagulation factor genes. However, the hemophilia gene (F8) does not seem to be subjected to NMD. Since the F8 gene is located on the X-chromosome, a connection between X-linked traits and mRNA decay could be assumed.

Conclusion: Considering that not all genes go through decay, this review focuses on the basics of the mechanism in coagulation genes. It is interesting to determine whether this translation-coupled surveillance system represents a general rule for the genes encoding components of the same physiological cascade.

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Introduction

When a vessel is torn or injured, several mechanisms such as vascular spasm, platelet plug formation and blood clotting lead to permanent closure of vessels and bleeding stop (1, 2). Any contact of blood with damaged endothelial cells and exposed collagen converts prothrombin into thrombin by prothrombin activator (3, 4). Then in a tandem reaction manner, thrombin converts fibrinogen to fibrin, which traps platelets, blood cells, and plasma to form a clot (2, 3). Prothrombin activator is synthesized through two ways, however the two pathways continuously affect each other: 1) the extrinsic pathway starting from damage to the vessel wall and surrounding tissues and 2) the intrinsic pathway that begins inside the blood (5).

In the extrinsic pathway, injured tissue releases a complex called tissue factor (TF) (3, 6). The factor is formed by tissue membrane phospholipids plus lipoprotein complex. In the presence of calcium ions, TF along with coagulation factor VII (FVII) act as an enzyme, to convert factor X (FX) into activated factor X (Fxa) (7, 8). FXa is immediately combined with tissue phospholipids or other phospholipids released from platelets and along with Factor V (FV), form a complex called prothrombin activator. In the presence of calcium ions, within a few seconds, the prothrombin complex breaks into thrombin and coagulation process initiates (3, 5).

For the formation of prothrombin activator in the intrinsic pathway, vascular damage activates Factor XII (FXII) and releases platelet phospholipids (9, 10). When FXII is released, it turns into a proteolytic enzyme called FXIIa by changes in molecular shape (11). Platelet damage causes releasing of platelet phospholipids, as well (12). FXIIa as an enzyme activates Factor XI (FXI) and initiates the second step in the intrinsic pathway (9, 11, 13). This reaction also needs high molecular weight kininogen (HMWK). FXIa enzymatically affects Factor IX (FIX) toward its activation (14). Finally, FIXa with Factor VIIIa (FVIIIa) and platelet phospholipids activate FX (10). Subsequently, the steps of the intrinsic pathway are the same as the last step of the extrinsic pathway (4, 5).

Following blood vessel rupture, coagulation will

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be created simultaneously by both pathways. TF begins extrinsic pathway while FXII and platelets contact with tissue collagen initiates intrinsic pathway (12). There is one fundamental difference: the extrinsic pathway is completed quickly (within 15 sec) and the final clot is only limited by the released level of TF and values of circulating FX, FVII and FV. However, the intrinsic pathway is usually much slower and clotting takes 1 to 6 min (4, 15).

Materials and Methods

A relevant literature search of PubMed was performed using the keywords coagulation factors, nonsense-mediated mRNA decay, and premature translation termination codons. Search limitations included English language and human-based studies.

Results

Coagulation factors are a family of glycosylated plasma proteins that need to be activated to induce and enhance the coagulation process. Most of them are naturally found in tiny amounts in the blood stream. Congenital deficiency of the factors causes inherited bleeding disorders most of which are rare. Deficiency of coagulation factors can be quantitative or qualitative (16-18).

Hemophilia A (FVIII deficiency) is the most common severe inherited bleeding disorder (19, 20). The disease is created as a result of the F8 gene defect, which is located on the X chromosome. Thus, males are affected and females are carriers of the disease and usually do not have bleeding symptoms. Clinical signs of hemophilia A, vary according to the level of coagulation factor so that the patients are divided into severe, moderate and mild types (21).

In severe hemophilia A, FVIII levels are less than %1. These patients have frequent spontaneous bleeding in tissues of muscles and joints. Intracranial hemorrhage could be the leading cause of death. Moderate hemophilia A is characterized by FVIII levels between 1–5%. In this case, spontaneous hemorrhage episodes are less common and more caused by trauma. Mild hemophilia A is also specified with FVIII levels greater than 5%. The patients do not have spontaneous bleeding, except in the case of trauma, surgery, or tooth extraction (21-23).

Hemophilia B (FIX deficiency) is X-linked as well and has clinical signs similar to hemophilia A. The severity of clinical complications is associated with the level of factor deficiency so that the values below 5% are conducive to spontaneous bleeding (24-26).

FXI deficiency is a rare recessively inherited mild bleeding disorder representing increased hemorrhage tendency following trauma or surgery. In contrast to hemophilias A and B, in FXI deficiency also called hemophilia C, severity of the symptoms does not correlate with the factor levels. The human *F11* gene is located on chromosome 4 (27, 28).

The rarest coagulopathy is FXIII deficiency, inherited as an autosomal recessive trait. The factor stabilizes fibrin clots and is found in two forms; homodimers composing of subunits A, which are found only within the cell and heterodimers consisting of subunits A and B, present extracellularly in plasma. Genes encoding subunit A and subunit B are located on chromosome 6 and chromosome 1, respectively. FXIII deficiency is mostly due to mutations in the subunit A gene. Umbilical cord bleeding is common in neonates, accounting for nearly 80% of cases. Female sufferers experience menorrhagia and recurrent miscarriages (29, 30).

FVII is a vitamin K-dependent factor and activates internal and external pathways of coagulation in a complex with TF, as is mentioned earlier. FVII deficiency is typically transmitted in an autosomal recessive manner and the corresponding gene, *F7* is located on chromosome 13. Clinically, varying signs range in severity from lethal to mild or even asymptomatic forms (31, 32).

Clinical characteristics place FX deficiency among the most severe forms of the rare coagulation defects. Patients generally experience epistaxis, mucosal bleeding, hemarthrosis, hematomas and even CNS bleeding. A high-risk pregnancy is common among affected women. Human *F10* gene maps to the long arm of chromosome 13, approximately 2.8 kb downstream of the *F7* gene, and its mutation is inherited as an autosomal recessive allele (33, 34).

FV deficiency is commonly inherited in an autosomal recessive fashion due to reduced plasma levels of the factor. This reduction is caused by mutations in the *F5* gene located on 1q23. FV deficiency is generally mild and some people may be asymptomatic. Common characteristics of FV deficiency are epistaxis, bruising, and mucosal bleeding, however, some patients have experienced CNS bleeding. The severity of the bleeding manifestations correlates with the FV levels (35).

Von Willebrand Factor (VWF) is synthesized by endothelial cells and megakaryocytes and plays two major roles in hemostasis: first, it serves as a specific carrier of FVIII in circulation. Secondly, VWF high multimers act as a bridge between subendothelial tissues and platelet. In the presence of such strings, the platelets slow down, move away from the rapid flow of the blood and accumulate at the site of vascular damage (36-38).

Von Willebrand disease (VWD) is an autosomal bleeding disorder with a different mode of inheritance (39). Partial quantitative VWF defects lead to the VWD type1 that is associated with a variety of bleeding manifestations and typically inherited as an autosomal dominant disorder. However, qualitative abnormalities of VWF cause the VWD type2. Inheritance of this type of the disease is generally autosomal dominant, although some

cases are characterized by autosomal recessive transmission (40, 41). VWD Type3 is the rarest but the most serious form of the disease and is frequently associated with consanguinity (39). In type3, plasma VWF decreased to low quantity levels as well as FVIII due to its dependence on VWF (20). Human VWF is encoded by the *VWF* gene, which is identified on chromosome 12 (40).

Discussion

Despite the low prevalence of hemorrhagic diseases, a large number of mutations have been identified related to various disease types. By the same token, a remarkable portion of the genetic abnormalities is ended in null alleles arising by small insertions and deletions, nonsense and splice site mutations (39, 42).

Studies showed that mutations affect protein expression in many ways. Mutations of promoter or enhancer that play an eminent role in eukaryotic transcription, result in impaired gene transcription and mRNA expression level reduction (43).

Another impact of gene mutation on protein synthesis can be traced back to the cell protective mechanisms against translation errors in order to reduce abnormal proteins. By removing mRNA transcripts that contain premature translation termination codons (PTC), nonsense-mediated mRNA decay (NMD) prevents the biogenesis of defective or truncated polypeptides before completing the translation process (44).

Rapid degradation of mRNA containing PTC was first observed 30 years ago in saccharomyces cerevisiae. Since then, NMD has been found in many other eukaryotes including man. In most cases the translation of PTC-containing transcripts in the absence of NMD, lead to the accumulation of harmful truncated proteins in the cell. NMD regulates several disease-causative genes such as β-globin and dystrophin (45, 46). Although the major role of NMD is elimination of unusual transcripts, in recent years, the researchers have found that many physiological mRNAs, coding full-length proteins, are also substrates for NMD (47).

Among coagulation factor genes, NMD has been reported in FXIII. Arg661stop mutation on exon14 of *F13a* reduces gene expression of mutant allele by 10 to 30 times compared to wild-type allele (48).

The mechanism has also been described for FV deficiency. A 2-bp deletion in exon13 of *F5* gene that causes PTC in codon 900 was evaluated at the mRNA level. Following platelet mRNA isolation of heterozygous individuals, it became clear that the mutation containing cDNA much less expressed compared to wild-type transcript (49).

Common mutation, Glu117stop located on exon5 of *F11* gene has been reported in different populations with FXI deficiency. Homozygous mRNA analysis revealed no transcript production. Further evaluation of heterozygous mRNAs showed that only wild-type allele is expressed suggesting that the allele with PTC has undergone NMD (50).

One of the earliest evidence of NMD in coagulation disorders was described in 1991 by Nichols and colleagues on a family of VWD type3. They observed the lack of defective mRNA in carrier phenotype. Instead, by an increased expression, wild-type allele compensated for the reduction. The authors concluded that such a mechanism could explain the wide range severity of VWD type1 and type3 (51). It should be noted that another analysis on platelet mRNA bearing a PTC mutation revealed no mRNA level reduction in a VWD type3 family. Expression studies showed that the gene defect causes a truncated protein, which was retained in the transfected cells (52).

A research conducted in Italy studied NMD mechanism of VWF mutations in 3 unrelated patients who had at least one truncating mutation. PTC-introducing mutation in the first patient (intron 19) was c.2546 + 3G> C and in the second patient (intron 50) was c.8155 + 6T> C. The third was c.6182delT in a VWD type3 patient. Sequencing results showed that the c.8155 + 6T> C causes the deletion of exon 50 and the PTC in exon 51, which is located only 23 bp upstream of the last exon-exon junction. This positioning leads to escape from NMD. The two other mRNAs bearing PTC were selectively eliminated by NMD mechanism and only the expression of wild-type transcript was detected in heterozygous individuals. The results showed that the sensitivity of NMD depends on PTC location (53).

In this regard, investigations on two splice site mutations c.1109+2T>C and c.1534-3C>A and a nonsense mutation p.Q77X, all causing PTC, demonstrated an allele-specific reduction of the mRNA (54). Analysis of 7 different potential splice site mutations showed degradation of PTC-introducing alleles of either platelets or leukocytes mRNA. NMD was suggested as a general mechanism to prevent the biogenesis of VWF truncated proteins (55). We also studied c.7674-7675insC located on *VWF* exon 45 in a large consanguine type3 family. Quantitative analysis of RT-PCR products showed that the levels of mRNA bearing PTC have been dramatically reduced by decay mechanism (42).

However, all coagulation factor genes are not subject to NMD. One of those genes is *F8,* which escapes NMD through various mutations and codes transcript variants. The NMD resistance occurs not only following missense, silent, and splice site mutations but also, there is evidence of escaping nonsense mutations from NMD (53).

In 2003 David *et al*, found no support for the presence of NMD mechanism followed by nonsense mutations in hemophilia A. In this study, a series of mRNAs containing nonsense mutations were

obtained from peripheral blood lymphocytes. Studies revealed that in all cases the transcripts were reproduced (56). To investigate the basis of inhibitor creation in haemophilia patients, expression studies were carried out on 6 different nonsense mutations spread throughout the six FVIII domains. The results demonstrated that regardless of nonsense mutations, *F8* transcription occurred normally and truncated proteins were produced (57).

Recently, to elucidate the transcriptional effects of potential splice site mutations in haemophilia A, a comparison study of in-silico prediction and mRNA analysis was performed. They observed the expression of all defective transcripts and introduced mRNA study as a reliable approach to discover splicing alterations (58). The importance of mRNA study has already been described on different *F8* splice site mutations where they detected defective transcripts (59).

Taken together the absence of NMD in haemophilia can be evidence of a connection between X-linked traits and mRNA decay. It must be taken into account that the NMD is involved in mammalian X chromosome dosage compensation. However, by inhibiting the NMD process, Yin *et al* showed that autosomal genes are more likely to undergo NMD than X-linked genes. In their study, overall mRNA expression was increased in favor of autosomal genes indicating their susceptibility to the NMD (60).

Conclusion

These pieces of evidence support the view that the transcriptome analysis provides new insights into the real impact of NMD on human disease. The considerations suggest the need for mRNA analysis to augment the results of genomic DNA mutation detection.

Excluding the *F8*, NMD could be referred to as a general mechanism that prevents biogenesis of coagulation factor truncated proteins. In fact, there is substantive indication that the decay mechanism is skewed towards bleeding disorder genes. As this is a topic on which conflicting data were reported in the literature, more study should be performed to support these conclusions.

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