NMD in disease and potential therapies

Abstract

Traditionally, nonsense-mediated mRNA decay (NMD) is known as an mRNA quality control mechanism that prevents potentially toxic truncated proteins to be translated from premature termination codon (PTC)-containing transcripts. However, recent studies have shown that role of NMD extends beyond mRNA surveillance. Here, we review current understanding of various roles played by NMD in the context of normal tissue development and diseases including cancer. We first summarize the mechanism of NMD, and how it controls mRNA surveillance, and describe how NMD coupled with alternative splicing forms a regulatory feedback loop. We further survey how NMD affects disease outcome in the context of hereditary diseases as well as cancer. In summary, NMD is a mode of complex eukaryotic gene regulation that has broad implications in various biological contexts.

Keywords: nonsense-mediated mRNA decay, premature termination codon, exon junction complex, alternative splicing

Introduction

Mechanism of NMD

In the canonical model of NMD, transcripts that contain PTC >55nt upstream of an exon-exon junction are degraded through NMD.1 Biochemical and genetic studies have identified a protein complex called exon junction complex (EJC) as a key regulator of NMD. During the last step of pre-mRNA splicing, EJC is deposited ~24nt upstream of the exon-exon junction.2,4 EJC is composed of a tetrameric core (eIF4AIII, MAGOH/MAGOHB, Y14, and MLN51) and more than a dozen peripheral factors including NMD factors UPF2 and UPF3B.1,5,6 EJC forms a link between nuclear and cytoplasmic mRNA metabolism by recruiting various factors important for mRNA splicing, export, translation, and NMD.4 It is postulated that EJC’s remain bound to spliced mRNA until it is disassembled by translating ribosome during the first round of translation (also known as pioneer round of translation). As most termination codons are located in the last exon, any EJC remaining on the transcript after translation can be considered as aberrant. In the presence of a PTC, EJCs still bound downstream of the PTC will not be removed. UPF1, an ATPase-dependent RNA helicase and a major NMD factor, interacts with the eRF3 bound to the terminating ribosome and forms the SURF complex consisting of SMG1, UPF1, eRF1, and eRF3.1 Interaction between UPF2 and phosphorylated UPF1 remodels the SURF complex into decay-inducing complex (DECID). Subsequently, SMG1 kinase phosphorylates UPF1 and recruits NMD factors SMG6 and SMG5-SMG7 heterodimer.1 SMG6, an endonuclease, association with the NMD machinery is stabilized through its interaction with EJC through its EJC-binding motif (EBM), and this interaction is important for the SMG6-mediated degradation of NMD transcripts.7 SMG5-SMG7 recruits decapping enzyme and deadenylation enzyme which exposes mRNA to other RNA degradation enzymes.1 This model of NMD is referred to as “EJC-enhanced NMD”.8,9 “Faux 3’UTR” model of NMD is an alternative NMD mechanism that senses unusually long 3’ untranslated regions (UTR) instead of an EJC downstream of a PTC.2,9 In this model, UPF1 is proposed to bind to long 3’UTR independent of terminating ribosomes and senses its length.10 3’UTR-bound UPF1 is then postulated to interact with yet unclear mRNP to promote decay. This form of NMD was initially described in yeast,9 and also ascribed to NMD of transcripts during spermatogenesis.11 However, the precise detail of this mechanism is still under investigation.

Discussion

NMD regulates normal physiological gene expression

Approximately 10% of all human genes are regulated by NMD.12 Upstream open reading frames (uORF), unusually long 3’UTRs, regulated splicing events which introduce a PTC, and normal stop codons upstream of an intron are features which target an mRNA for NMD.12 As such, various NMD factors have been shown to be essential for embryonic and post-embryonic development in mice. UPF2, SMG1, UPF3A, and SMG9 knockout mice are all embryonic lethal.14 In case of UPF1 and SMG6 knockout mice, the mutations are lethal before implantation or around implantation.15,16 Knockout of UPF3B is not embryonic lethal, but cause severe neurological defect in humans.14

NMD factors are also important for tissue-specific developmental programs. UPF2 is important for spermatogenesis,11 and long 3’UTR-triggered NMD pathway dependent on TDRD6 promotes proper chromatoid body development during spermatogenesis.17 While Upf3b promotes NMD, Upf3a was suggested to suppress NMD.11 In mice, Upf3a is expressed exclusively in mice tests.11 Conditional knockout of Upf3a in meiotic germ cells results in reduced spermatogenesis and down regulation of NMD transcripts.12 NMD factors are also important for neuronal physiology and development. During neuronal differentiation, precise temporal control of UPF1 expression and activity is critical for proper neuronal development.19 In neuronal cells, mIR-128 expression suppressed UPF1 expression, but high NMD activity is also associated with low miR-129 expression.19 In the negative feedback between UPF1 and miR-128, high NMD activity and low NMD activity in developing neurons corresponded to undifferentiated or differentiated states, respectively.19 Commisural axon guidance during spinal cord development requires careful balance in expressions of ROBO3 isoforms.20 ROBO3.2 mRNA contains a PTC, thus an NMD target. ROBO3.2 mRNA is normally degraded during the commissural neuron development by NMD.20 Conditional knockout of UPF2 from spinal commissural neurons prevents proper axon guidance.20
Stress management is also an important part of NMD. Eukaryotic cells are under various types of stress such as endoplasmic reticulum (ER) stress, hypoxia, osmotic stress, and pathogen-induced stress. Under ER stress, a stress pathway called unfolded protein response (UPR) is activated. The UPR is mediated by three major branches of UPR pathways characterized by the activities of inositol requiring transmembrane kinase/endonuclease1 (IRE1), protein kinase RNA (PKR)-like ER Kinase (PERK), activating transcription factor 6 (ATF6). NMD pathway helps to fine tune activity of these sensors by regulating mRNA levels of UPR pathway components. Also, UPR pathway can suppress NMD pathway by reducing levels of NMD pathway genes post-transcriptionally.

Mammalian cells utilize alternative splicing to increase proteomic diversity by generating multiple mRNA isoforms from a single gene. Alternative splicing events, which introduce PTCs, can target those mRNA for NMD. Thus, alternative splicing coupled NMD (AS-NMD) can be used as an additional mechanism to fine-tune gene expression. Many splicing factors use AS-NMD to maintain homeostasis of their expression. SR proteins and hnRNP proteins have been shown to undergo extensive utilization of AS-NMD as a feedback mechanism. Expression of genes important for stem cell function can be regulated by NMD when coupled with alternative splicing. For example, poly pyrimidine tract-binding protein (PTB) represses neuronal homologue pNPTB by inducing its exon 10 skipping to prevent differentiation of a neuronal cell line. In contrast, nSR100, a neuron-specific SR protein, promotes pNPTB exon 10 inclusion to increase pNPTB expression.

RNA viruses have evolved to take advantage of cellular mRNA processing machinery and eukaryotes have developed mechanisms to target the viruses. Multi-cistronic genome of viruses harbor features that could be targeted for NMD such as upstream open reading frame (uORF) and long 3'UTR. Not surprisingly, in plants, NMD can target viral genomes, and in mammalian cells UPF1, SMG5, and SMG7 have been shown to be suppressive for replication of certain viruses. Viruses therefore have developed strategies to evade the NMD pathway. NMD components can be sequestered in Human T-lymphotropic Virus Type 1 (HTLV-1); Rous Sarcoma Virus (RSV) RNA stability element (RSE) that are present downstream of the first ORF helps RSV to evade NMD by preventing UPF1 binding. Intriguingly, human immunodeficiency virus (HIV) has been shown to utilize UPF1 for infectivity and viral RNA export into the cytoplasm. While this mechanism may involve NMD-independent role of UPF1, the usage of NMD pathway component for its survival and proliferation is interesting.

NMD in hereditary diseases

Nonsense mutations comprise about 20% of all known pathogenic genetic mutations within the coding region. Among these, some nonsense mutations can result in mutant alleles that are insensitive to NMD (NMD-insensitive) leads to dominant-negative phenotype. For example, nonsense mutant allele of SOX10 that is sensitive to NMD can cause Waardenburg syndrome due to the loss of SOX10 function. However, NMD-insensitive nonsense mutation in SOX10 that occurs downstream of the penultimate exon generates toxic SOX10 protein that results in a severe neurological disease characterized by peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome and Hirschsprung disease (PCWH). NMD-sensitive nonsense mutations can result in recessive diseases. Cystic fibrosis (CF) is caused by loss-of-function mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR-W1282X mutation causes a severe form of CF when combined with another loss-of-function mutation. The truncated CFTR-W1282X protein retains partial function, but due to NMD, CF patients with the mutation express little to no expression of functional CFTR. IDUA-W402X mutation causes a severe form of Hunter’s syndrome due to low levels of functional IDUA protein characterized by accumulation of dermatan and heparan sulfate within the lysosomes.

Aminoglycoside antibiotics, such as gentamicin or G418, and PTC124 are read-through compounds (RTC) that can promote generation of functional protein by suppressing translational termination at the PTC. Gentamicin can induce read-through in vitro for CFTR-W1282X and IDUA-W402X mRNA. However, the clinical trial of gentamicin for CF caused by was limited by NMD of CFTR-W1282X mRNA. Also, inhibition of NMD in combination with RTC treatment improved the nonsense suppression in vivo. Suppression of NMD can be a potential therapeutic strategy for diseases caused by NMD-sensitive nonsense mutations. One approach is to use small molecules to inhibit the activity of NMD factors. SMG8 is part of the SMG1-kinase complex, and pharmacological inhibition effectively inhibited NMD. Anti-sense-mediated knockdown of Upf3b is able to inhibit NMD. In a humanized mouse model of hemophilia B harboring hFIX-R29X mutation, knocking down Upf3b using an antisense oligonucleotides (ASO) conjugated to triantennary N-acetyl galactosamine (Gal-Nac) partially restored FIX activity. However, global inhibition of NMD may potentially detrimental. In a more targeted approach, ASOs that inhibit NMD by preventing EJC binding combined with RTC can restore full-length protein from a nonsense allele in a gene-specific manner. Further development of these approaches may help to alleviate the symptoms of a wide range of genetic diseases caused by PTC.

Impact of NMD in cancer

In various tumors, many tumor suppressor genes contain PTC and are targets for NMD. Based on a gene expression array-based assay called gene identification by NMD inhibition (GINI), various NMD transcripts have been found. These genes include nonsense mutations in well-known tumor suppressors such as ATM, CHK2, BRCA1, TP53, RB, and BRCA1/2. Microsatellite instability in colon cancer is an important hallmark in a subset of colon cancers, which results in various oncogenic mutations. A study identified a large number of NMD-sensitive mutations in microsatellite-unstable colon cancer cell lines, and these included several genes that can drive oncogenesis. In other cancer contexts, mRNAs that contain upstream of penultimate exons are NMD-insensitive. NMD-insensitive nonsense mutants of ATM, CHK2, BRCA1, TP53, and WT1 that produce truncated, dominant-negative proteins have been reported. AS-NMD can contribute to oncogenesis as well. Tumor associated changes in pre-mRNA splicing due to alterations in the core splicing factors have been reported. Mutations in the core splicing machinery components (reported by Yoshida et al. 2011) have also been well-characterized in hematological malignancies. Mutations in multiple splicing factors including SF3B1, SRSF2, U2AF1, and ZRS2 have been found in up to 60% of myelodysplastic syndrome (MDS) patients who have an increased risk of developing acute myeloid leukemia. SF3B1 mutations are associated with abnormal cryptic 3' splice site selection; SRSF2 mutations lead to altered exon inclusion patterns due to change in binding motif; U2AF1 can lead to abnormal 3' splice site selection. Such changes can lead to many aberrant splicing events that introduce PTCs in mRNAs. For example, in a mouse model of MDS, Srsf2 P95H mutation has been shown to...
promote inclusion of a poison exon in Ezh2, leading to its degradation by NMD and contribute to the pathogenesis of MDS.62,63

Aberrant UPF1 expression or activity can affect tumor progression. Mutations in the key NMD factor UPF1 was commonly found in pancreatic adenocarcinoma (ASC).64 These mutations were concentrated in the helicase domain and the SQ domain of UPF1. Helicase domain is essential for activation of NMD; SQ domain phosphorylation by SMG1 is important for activation of NMD.65 Although the impact of these mutations still remain to be elucidated, concentration of potentially inactivating mutations on UPF1 gene suggests that NMD may contribute to the tumorigenesis in ASC.65 Inflammatory myoblastic tumors (IMT) harbor UPF1 mutations which affect its alternative splicing.66 The mutations caused reduced NMD efficiency in these tumors and increased expression of chemokine genes as well as NF-κB induction that contributes to inflammatory response characteristic of IMTs.67 In hepatocellular carcinoma, UPF1 promoter hypermethylation that results in reduced UPF1 expression can lead to up-regulation of Smad7 and tumor progression.68 In lung adenocarcinoma, the tumor UPF1 expression is lower than in adjacent normal tissue. The decreased NMD efficiency resulting from lower UPF1 expression leads to higher TGF-β signaling, promoting epithelial-mesenchymal transition.69

In certain types of cancers, NMD inhibition could raise vulnerability for cancers. Some NMD-sensitive transcripts are pro-apoptotic genes. Growth arrest and DNA damage 45 (GADD45) is an NMD controlled gene that can induce apoptosis by activating mitogen-activated protein kinase (MAPK) signaling.70 Growth arrest-specific 5 (GAS5) is another NMD-controlled pro-apoptotic gene that can cause cell-cycle arrest and apoptosis.71,72 Also, NMD inhibition can suppress tumorigenesis in microsatellite-unstable colon cancer with high NMD factor levels.73 Large number of in-del mutations in cancer can increase the abundance of neo-antigens that are highly immunogenic.74 Indeed, tumor infiltrating lymphocytes were higher in tumors with more nonsense mutations.75 These in-del mutations can shift the reading frame and generate PTC. NMD could suppress expression of neo-antigens generated from PTC-containing transcripts in cancer cells. Thus, suppressing NMD to increase the level of novel cancer antigens could potentially benefit anti-cancer immunotherapy.76 As blanket inhibition of NMD could be toxic to various tissues, targeted NMD inhibition method may be a more feasible strategy.

Conclusion

The roles of NMD in normal physiology and disease settings are quite complex. On one hand, NMD is a necessary regulatory mechanism of gene expression that is essential for life. On the other hand, NMD inhibition could be beneficial in certain disease contexts. Especially in cancer, NMD suppression by UPF1 depletion can lead to opposite consequences for tumor survival depending on its type. These observations suggest that NMD pathway intersects with diverse biological processes. Deeper understanding of NMD beyond its role in mRNA surveillance could lead to discoveries that impact our health and diseases.

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Conflict of interest

The authors declare that there is no conflict of interest.

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