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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

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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.[6] It is postulated that EJCs 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).[1] 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 consisted 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] '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.[9] In this model, UPF1 is proposed to bind to long 3'UTR independent of terminating ribosomes and senses their lengths.[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.[13] 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] Knocking out 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.[17] In mice, *Upf3a* is expressed exclusively in mice testis.[18] Conditional knockout of *Upf3a* in meiotic germ cells results in reduced spermatogenesis and down regulation of NMD transcripts.[17] 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] Commissural 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.[21] 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).[21] NMD pathway helps to fine tune activity of these sensors by regulating mRNA levels of UPR pathway components.[23,24] Also, UPR pathway can suppress NMD pathway by reducing levels of NMD pathway genes post-transcriptionally.[24–26]

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, alterative 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, polypyrimidine tract-binding protein (PTB) represses neuronal homologue nPTB by inducing its exon 10 skipping to prevent differentiation of a neuronal cell line.[27,28] In contrast, nSR100, a neuron-specific SR protein, promotes nPTB exon 10 inclusion to increase nPTB expression.[27,28]

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.[29–31] 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.[32,33] Intriguingly, human immunodeficiency virus (HIV) has been shown to utilize UPF1 for infectivity and viral RNA export into the cytoplasm.[34] 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.[35] Among these, some nonsense mutations can result in mutant alleles that are insensitive to NMD (NMD-insensitive) leads to dominant-negative phenotype.[36] 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).[36] 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.[37] 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.[38–40] *IDUA-W402X* mutation causes a severe form of Hurler's syndrome due to low levels of functional IDUA protein characterized by accumulation of dermatan and heparan sulfate within the lysosomes.[41]

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.[42] Gentamicin can induce read-through *in vitro* for *CFTR-W1282X* and *IDUA-W402X* mRNA.[43] However, the clinical trial of gentamicin for CF caused by was limited by NMD of *CFTR-W1282X* mRNA.[40–44] Also, inhibition of NMD in combination with RTC treatment improved the nonsense suppression *in vivo*.[45] 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.[46] Antisense-mediated knockdown of *Upf3b* is able to inhibit NMD.[47] 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.[47] 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.[48] 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.[49] These genes include nonsense mutations in well-known tumor suppressors such as *WT1*,*TP53, RB,* and *BRCA1/2*.[50–54] 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.[49] 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.[53,55–58]

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.[59] 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 *ZRSR2* have been found in up to 60% of myelodysplastic syndrome (MDS) patients who have an increased risk of developing acute myeloid leukemia.[60] *SF3B1* mutations are associated with abnormal cryptic 3' splice site selection;[61] *SRSF2* mutations lead to altered exon inclusion patterns due to change in binding motif;[62] *U2AF1* can lead to abnormal 3' splice site selection.[63] 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.[62,64]

Aberrant UPF1 expression or activity can affect tumor progression. Mutations in the key NMD factor *UPF1* was commonly found in pancreatic adenosquamous carcinoma (ASC).[65] 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.[66] 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.[67] 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 neoantigens 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.

References

1. Popp MW, Maquat LE. The dharma of nonsense-mediated mRNA decay in mammalian cells. *Mol Cells*. 2014;37(1):1–8.

2. Saulière J, Murigneux V, Wang Z, et al. CLIP-seq of eIF4AIII reveals transcriptome-wide mapping of the human exon junction complex. *Nat Struct Mol Biol*. 2012;19(11):1124–1131.

3. Singh G, Kucukural A, Cenik C, et al. The Cellular EJC Interactome Reveals Higher Order mRNP Structure and an EJC-SR Protein Nexus. *Cell*. 2012;151(4):750–764.

4. Hauer C, Sieber J, Schwarzl T, et al. Exon Junction Complexes Show a Distributional Bias toward Alternatively Spliced mRNAs and against mRNAs Coding for Ribosomal Proteins. *Cell Rep*. 2016;16(6):1588–1603.

5. Chamieh H, Ballut L, Bonneau F, et al. NMD factors UPF2 and UPF3 bridge UPF1 to the exon junction complex and stimulate its RNA helicase activity. *Nat Struct Mol Biol*. 2008;15(1):85–93.

6. Le Hir H, Saulière J, Wang Z. The exon junction complex as a node of post-transcriptional networks. *Nat Rev Mol Cell Biol*. 2016;17(1):41–54.

7. Kashima I, Jonas S, Jayachandran U. et al. SMG6 interacts with the exon junction complex via two conserved EJC-binding motifs (EBMs) required for nonsense-mediated mRNA decay. *Genes Dev*. 24(21):2440–2450.

8. Metze S, Herzog VA, Ruepp MD, et al. Comparison of EJC-enhanced and EJC-independent NMD in human cells reveals two partially redundant degradation pathways. *RNA*. 2013;19(10):1432–1448.

9. Amrani N, Ganesan R, Kervestin S, et al. A faux 3'-UTR promotes aberrant termination and triggers nonsense-mediated mRNA decay. *Nature*. 2004;432(7013):112–118.

10. He F, Jacobson A. Nonsense-Mediated mRNA Decay: Degradation of Defective Transcripts Is Only Part of the Story. *Annu Rev Genet*. 2015;49(1):339–366.

11. Bao J, Vitting-Seerup K, Waage J, et al. UPF2-Dependent Nonsense-Mediated mRNA Decay Pathway Is Essential for Spermatogenesis by Selectively Eliminating Longer 3'UTR Transcripts. *PLOS Genet*. 2016;12(5):e1005863.

12. Mendell JT, Sharifi NA, Meyers JL, et al. Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and mutes genomic noise. *Nat Genet*. 2004;36(10):1073–1078.

13. Popp MW, Maquat LE. Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu Rev Genet*. 2013;47:139–65.

14. Nasif S, Contu L, Mühlemann O. Beyond quality control: The role of nonsense-mediated mRNA decay (NMD) in regulating gene expression. *Semin Cell Dev Biol*. 2017;75:78–87.

15. Medghalchi SM, Frischmeyer PA, Mendell JT, et al. Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. *Hum Mol Genet*. 2001;10(2):99–105.

16. Li T, Shi Y, Wang P, et al. Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. *EMBO J*. 2015;34(12):1630–1647.

17. Fanourgakis G, Lesche M, Akpinar M, et al. Chromatoid Body Protein TDRD6 Supports Long 3' UTR Triggered Nonsense Mediated mRNA Decay. *PLOS Genet*. 2016;12(5):e1005857.

18. Shum EY, Jones SH, Shao A, et al. The Antagonistic Gene Paralogs

Upf3a and Upf3b Article The Antagonistic Gene Paralogs Upf3a and Upf3b Govern Nonsense-Mediated RNA Decay. *Cell*. 2016;165(2):1–14.

19. Lou CH, Shao A, Shum EY, et al. Posttranscriptional Control of the Stem Cell and Neurogenic Programs by the Nonsense-Mediated RNA Decay Pathway. *Cell Rep*. 2014;6(4):748–764.

20. Colak D, Ji SJ, Porse BT, et al. Regulation of Axon Guidance by Compartmentalized Nonsense-Mediated mRNA Decay. *Cell*. 153(6):1252–1265.

21. Hetz C, Martinon F, Rodriguez D, et al. The Unfolded Protein Response: Integrating Stress Signals Through the Stress Sensor IRE1 . *Physiol Rev*. 2011;91(4):1219–1243.

22. Gardner LB. Hypoxic Inhibition of Nonsense-Mediated RNA Decay Regulates Gene Expression and the Integrated Stress Response. *Mol Cell Biol*. 2008;28(11):3729–3741.

23. Karam R, Lou CH, Kroeger H, et al. The unfolded protein response is shaped by the NMD pathway. *EMBO Rep*. 2015;16(5):599–609.

24. Wang D, Zavadil J, Martin L, et al. Inhibition of Nonsense-Mediated RNA Decay by the Tumor Microenvironment Promotes Tumorigenesis. *Mol Cell Biol*. 2011;31(17):3670–3680.

25. Usuki F, Fujimura M, Yamashita A. Endoplasmic reticulum stress preconditioning attenuates methylmercury-induced cellular damage by inducing favorable stress responses. *Sci Rep*. 2013;3:1–10.

26. Li Z, Vuong JK, Zhang M, et al. Inhibition of nonsense-mediated RNA decay by ER stress. *RNA*. 2017;23(3):378–394.

27. Calarco JA, Superina S, O'Hanlon D, et al. Regulation of vertebrate nervous system alternative splicing and development by an SR-related protein. *Cell*. 2009;138(5):898–910.

28. Grabowski P. Alternative splicing takes shape during neuronal development. *Curr Opin Genet Dev*. 2011;21(4):388–394.

29. Garcia D, Garcia S, Voinnet O. Nonsense-mediated decay serves as a general viral restriction mechanism in plants. *Cell Host Microbe*. 2014;16(3):391–402.

30. Balistreri G, Bognanni C, Mühlemann O. Virus Escape and Manipulation of Cellular Nonsense-Mediated mRNA Decay. *Viruses*. 2017;9(1):24.

31. Balistreri G, Horvath P, Schweingruber C, et al. The host nonsense-mediated mRNA decay pathway restricts mammalian RNA virus replication. *Cell Host Microbe*. 2014;16(3):403–411.

32. Nakano K, Ando T, Yamagishi M, et al. Viral interference with host mRNA surveillance, the nonsense-mediated mRNA decay (NMD) pathway, through a new function of HTLV-1 Rex: implications for retroviral replication. *Microbes Infect*. 2013;15(6):491–505.

33. Weil JE, Beemon KL. A 3' UTR sequence stabilizes termination codons in the unspliced RNA of Rous sarcoma virus. *RNA*. 2006;12(1):102–110.

34. Ajamian L, Abel K, Rao S, et al. HIV-1 Recruits UPF1 but Excludes UPF2 to Promote Nucleocytoplasmic Export of the Genomic RNA. *Biomolecules*. 2015;5(4):2808–2839.

35. Mort M, Ivanov D, Cooper DN, et al. A meta-analysis of nonsense mutations causing human genetic disease. *Hum Mutat*. 2008;29(8):1037–1047.

36. Miller JN, Pearce DA. Nonsense-mediated decay in genetic disease: Friend or foe? *Mutat Res - Rev Mutat Res*. 2014;762:52–64.

37. Shoshani T, Augarten A, Gazit E, et al. Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi

38. Rowe SM, Varga K, Rab A, et al. Restoration of W1282X CFTR Activity by Enhanced Expression. *Am J Respir Cell Mol Biol*. 2007;37(3):347–356.

39. Haggie PM, Phuan PW, Tan JA, et al. Correctors and Potentiators Rescue Function of the Truncated W1282X-CFTR Translation Product. *J Biol Chem*. 2016;292(3):771–785.

40. Clancy JP, Rowe SM, Bebok Z, et al. No detectable improvements in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. *Am J Respir Cell Mol Biol*. 2007;37(1):57–66.

41. Keeling KM, Brooks DA, Hopwood JJ, et al. Gentamicin-mediated suppression of Hurler syndrome stop mutations restores a low level of α-l-iduronidase activity and reduces lysosomal glycosaminoglycan accumulation. *Hum Mol Genet*. 2001;10(3):291–299.

42. Dabrowski M, Bukowy-Bieryllo Z, Zietkiewicz E. Advances in therapeutic use of a drug-stimulated translational readthrough of premature termination codons. *Mol Med*. 2018;24(1):25.

43. Kerem E. Pharmacologic therapy for stop mutations: how much CFTR activity is enough? *Curr Opin Pulm Med*. 2004;10(6):547–552.

44. Linde L, Boelz S, Nissim-Rafinia M, et al. Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. *J Clin Invest*. 2007;117(3):683–692.

45. Keeling KM, Wang D, Dai Y, et al. Attenuation of nonsense-mediated mRNA decay enhances in vivo nonsense suppression. *PLoS One*. 2013;8(4):e60478.

46. Usuki F, Yamashita A, Shiraishi T, et al. Inhibition of SMG-8, a subunit of SMG-1 kinase, ameliorates nonsense-mediated mRNA decay-exacerbated mutant phenotypes without cytotoxicity. *Proc Natl Acad Sci*. 2013;110(37):15037–15042.

47. Huang L, Low A, Damle SS, et al. Antisense suppression of the nonsense mediated decay factor Upf3b as a potential treatment for diseases caused by nonsense mutations. *Genome Biol*. 2018;19(1):1–16.

48. Nomakuchi TT, Rigo F, Aznarez I, et al. Antisense oligonucleotide-directed inhibition of nonsense-mediated mRNA decay. *Nat Biotech*. 2016;34(2):164–166.

49. Gardner LB. Nonsense-mediated RNA decay regulation by cellular stress: implications for tumorigenesis. *Mol Cancer Res*. 2010;8(3):295–308.

50. Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, et al. The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. *Hum Mol Genet*. 2002;11(23):2805–2814.

51. Ware MD, DeSilva D, Sinilnikova OM, et al. Does nonsense-mediated mRNA decay explain the ovarian cancer cluster region of the BRCA2 gene? *Oncogene*. 2005;25:323–328.

52. Pinyol M, Bea S, Plà L, et al. Inactivation of RB1 in mantle-cell lymphoma detected by nonsense-mediated mRNA decay pathway inhibition and microarray analysis. *Blood*. 2007;109(12):5422–5429.

53. Anczuków O, Ware MD, Buisson M, et al. Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins? *Hum Mutat*. 2008;29(1):65–73.

54. Reddy JC, Morris JC, Wang J, et al. WT1-mediated Transcriptional Activation Is Inhibited by Dominant Negative Mutant Proteins. *J Biol Chem*. 1995;270(18):10878–10884.

55. Fan S, Yuan R, Ma YX, et al. Mutant BRCA1 genes antagonize

37. Shoshani T, Augarten A, Gazit E, et al. Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. *Am J Hum Genet*. 1992;50(1):222–228.

phenotype of wild-type BRCA1. *Oncogene*. 2001;20:8215.

56. Cardinali M, Kratochvil FJ, Ensley JF, et al. Functional characterization in vivo of mutant p53 molecules derived from squamous cell carcinomas of the head and neck. *Mol Carcinog*. 19998;18(2):78–88.

57. Englert C, Vidal M, Maheswaran S, et al. Truncated WT1 mutants alter the subnuclear localization of the wild-type protein. *Proc Natl Acad Sci*. 1995;92(26):11960–11964.

58. Du L, Lai CH, Concannon P, et al. Rapid screen for truncating ATM mutations by PTT-ELISA. *Mutat Res*. 2008;640(1–2):139–144.

59. Anczuków O, Krainer AR. Splicing-factor alterations in cancers. *RNA*. 2016;22(9):1285–1301.

60. Kennedy JA, Ebert BL. Clinical implications of Genetic mutations in Myelodysplastic syndrome. *J Clin Oncol*. 2017;35(9):968–974.

61. DeBoever C, Ghia EM, Shepard PJ, et al. Transcriptome Sequencing Reveals Potential Mechanism of Cryptic 3' Splice Site Selection in SF3B1-mutated Cancers. *PLOS Comput Biol*. 2015;11(3):e1004105.

62. Kim E, Ilagan JO, Liang Y, et al. SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. *Cancer Cell*. 2015;27(5):617–630.

63. Ilagan JO, Ramakrishnan A, Hayes B, et al. U2AF1 mutations alter splice site recognition in hematological malignancies. *Genome Res*. 2015;25(1):14–26.

64. Reimer KA, Neugebauer KM. Blood Relatives: Splicing Mechanisms underlying Erythropoiesis in Health and Disease. *F1000Research*. 2018;7(0):1–11.

65. Liu C, Karam R, Zhou Y, et al. The UPF1 RNA surveillance gene is commonly mutated in pancreatic adenosquamous carcinoma. *Nat Med*. 2014;20(6):596–599.

66. Okada-Katsuhata Y, Yamashita A, Kutsuzawa K, et al. N-and C-terminal Upf1 phosphorylations create binding platforms for SMG-6 and SMG-5:SMG-7 during NMD. *Nucleic Acids Res*. 2012;40(3):1251–1266.

67. Lu JW, Plank TD, Su F, et al. The nonsense-mediated RNA decay pathway is disrupted in inflammatory myofibroblastic tumors. *J Clin Invest*. 2016;126(8):3058–3062.

68. Chang L, Li C, Guo T, et al. The human RNA surveillance factor UPF1 regulates tumorigenesis by targeting Smad7 in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2016;35(1):1–12.

69. Cao L, Qi L, Zhang L, et al. Human nonsense-mediated RNA decay regulates EMT by targeting the TGF-ß signaling pathway in lung adenocarcinoma. *Cancer Lett*. 2017;403:246–259.

70. Nelson JO, Moore KA, Chapin A, et al. Degradation of Gadd45 mRNA by nonsense-mediated decay is essential for viability. *Elife*. 2016;5:1–13.

71. Tani H, Torimura M, Akimitsu N. The RNA Degradation Pathway Regulates the Function of GAS5 a Non-Coding RNA in Mammalian Cells. *PLoS One*. 2013;8(1):1–9.

72. Mourtada-Maarabouni M, Williams GT. Growth arrest on inhibition of nonsense-mediated decay is mediated by noncoding RNA GAS5. *Biomed Res Int*. 2013:358015.

73. Bokhari A, Jonchere V, Lagrange A, et al. Targeting nonsense-mediated mRNA decay in colorectal cancers with microsatellite instability. *Oncogenesis*. 2018;7(9):70.

74. Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol*. 2017;18(8):1009–1021.

75. Tougeron D, Fauquembergue E, Rouquette A, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. *Mod Pathol*. 2009;22(9):1186–1195.

76. Gilboa E. Expression of new antigens on tumor cells by inhibiting nonsense-mediated mRNA decay. Immunol Res. 2013;57(1–3):44–51.