Single-Cell Biology
ISSN: 2168-9431

Lejeune, Single Cell Biol 2016, 5:2
DOI: 10.4172/2168-9431.1000136

Perspective Open Access

Triple Effect of Nonsense-Mediated mRNA Decay Inhibition as a Therapeutic Approach for Cancer

Fabrice Lejeune[1,2,3]*

[1]University of Lille, UMR8161-M3T-Mechanisms of Tumorigenesis and Target Therapies, F-59000 Lille, France
[2]CNRS, UMR 8161, F-59000 Lille, France
[3]Institut Pasteur de Lille, F-59000 Lille, France

Abstract

Cancer is a complex pathology involving different genes and cellular pathways. A combination of treatments with different targets is thus required to eliminate cancer cells. This review deals with a new potential therapeutic approach: inhibiting a single RNA degradation pathway so as to inhibit tumorigenesis by acting on different cellular processes and on the expression of various genes. Also discussed is the re-expression of various genes: tumor suppressor genes harboring a nonsense mutation, NMD-silenced oncogenes, and genes inducing a specific anticancer immune response. Lastly, putative limitations of cancer treatment by NMD inhibition are addressed.

Keywords: Cancer; Nonsense-mediated mRNA decay; Premature termination codon; Tumor suppressor; Immunotherapy

Introduction

Cells have developed quality control mechanisms to ensure that gene expression is accurately performed. One of them, called nonsense-mediated mRNA decay (NMD), targets for fast decay mRNAs harboring a premature termination codon (PTC) due, for example, to a nonsense mutation [1-7]. NMD prevents the synthesis of truncated proteins that are inactive or potentially deleterious. Unfortunately, it also results in degradation of nonsense-mutation-harboring mRNAs encoding truncated proteins that should retain partial or total wild-type activity. Nonsense mutations are responsible for about 10% of genetic disease cases, including some forms of cancer [8]. Inhibiting NMD has become an attractive therapeutic strategy for some cases of genetic disease and might also be a suitable therapeutic approach for cancer [7]. Over the last decade, several molecules have been identified as NMD inhibitors [9-13]. These inhibitors could be interesting as anticancer agents, as they might affect tumorigenesis in three different ways, detailed below.

Restoring the Capacity of a Cell to Enter Apoptosis

Cancer can be initiated by different events and notably by mutations impairing the expression of tumor suppressor genes. Among these mutations, nonsense mutations have been found in variable proportion according to the tumor suppressor gene and/or cancer type. For instance, nonsense mutations represent about 7% of the mutations affecting the TP53 gene when about 41.5% of those affect the adenomatous polyposis coli (APC) gene [14]. These mutations impairing the functions of tumor suppressor genes favor tumorigenesis by inactivating cell proliferation gatekeepers and apoptotic cell death inducers, which makes them cancer driver mutations [15]. In addition, the absence of the function linked to one tumor suppressor gene can be responsible for forms of cancer that resist chemotherapy, since the cancer cells lose some of their ability to initiate apoptosis.

A possible anticancer strategy involving NMD inhibition would be to target a nonsense mutation in a tumor suppressor gene and thus restore expression of the mutant tumor suppressor gene. The NMD inhibitor could be used alone or in combination with a PTC-read through approach, in order to synthesize, respectively, either a functional truncated protein or a full-length protein. PTC-read through allows translation of the complete original open reading frame despite the presence of a PTC, through introduction of an amino acid, instead of termination, when the ribosome reaches the PTC. PTC-read through can be activated by members of the aminoglycoside family and other, unrelated molecules [16-18]. Restored expression of the tumor suppressor gene should either make the cell sensitive to chemotherapy or trigger apoptosis directly as a result of accumulation of deleterious mutations and/or impairment of cellular processes. NMD inhibitors and read through-activating molecules have already been identified and have demonstrated their capacity to rescue the expression of nonsense mutation containing p53 mRNA in different cell lines [12,13,19].

Expressing PTC-containing mRNAs with Apoptotic Activity

Cancer cells are known to accumulate mutations [20,21], some of which interfere with splicing or cause a frame shift, for example. These mutations can lead to the presence of a PTC in the open reading frame and hence to activation of NMD, so as to prevent the synthesis of truncated proteins. If NMD is inhibited, the truncated proteins are synthesized and some of them could exert a deleterious action. Cell death might even be enhanced, since apoptosis causes NMD inhibition through caspase cleavage of the central NMD factors UPF1 and UPF2 [22,23] and the resulting cleavage fragments can in turn induce apoptosis. This leads to an amplification loop likely to prevent leaving the cell death pathway.

Inducing an Anticancer Immune Response

As described above, cancer cells accumulate mutations [20,21] and notably PTCs through frame shift mutations and splicing interferences. When a PTC is present, inhibition of NMD results in the synthesis of a truncated protein with a modified C-terminal part. For instance, any intron retention due to a mutation at a splice site generates a peptide

***Corresponding author:** Fabrice Lejeune, University of Lille, UMR8161-M3T-Mechanisms of Tumorigenesis and Target Therapies, F-59000 Lille, France, Tel: +33320434343; E-mail: fabrice.lejeune@inserm.fr

Received March 25, 2016; **Accepted** April 27, 2016; **Published** April 29, 2016

Citation: Lejeune F (2016) Triple Effect of Nonsense-Mediated mRNA Decay Inhibition as a Therapeutic Approach for Cancer. Single Cell Biol 5: 136. doi:10.4172/2168-9431.1000136

sequence encoded by the intron sequence up to the first stop codon. As a normal cellular process, a fraction of all proteins are degraded to small peptides to be presented at the cell surface to immune cells. The C-terminal part of a mutant protein presented at the cell surface should be recognized as a non-self-antigen and should thus activate an immune response targeting cells producing the mutant protein, i.e. cancer cells [24]. Inhibiting NMD could thus be an attractive way to elicit a specific immune response against cancer cells.

Putative Limitations of NMD Inhibition for Cancer Treatment

A major challenge in establishing a therapeutic approach is to predict possible side effects. Often molecules bind to several different targets. Multiple-target affinity is now well known and used increasingly to repurpose old drugs for new applications. In most cases, however, it leads to side effects that are difficult to anticipate and generally show up in long-term *in vivo* studies or clinical trials. When the molecular mechanism targeted by the therapy has been studied in detail, as has NMD for more than 30 years, some particular problems can be anticipated. For instance, inhibition of NMD through down regulation of NMD factors is reported to activate autophagy [25]. As autophagy is necessary for tumor growth, one might expect NMD inhibition to be ineffective as a cancer treatment. Yet some of the methods used to inhibit NMD, such as the use of various chemicals, do not appear to trigger autophagy (Jia and Lejeune, unpublished data).

It is also reported NMD inhibition can favor cell survival by activating the endoplasmic reticulum (ER) stress response. In particular, it stabilizes natural NMD substrates such as ATF4 mRNA, encoding a stress responsive transcription factor [26] and IRE1 α– mRNA, encoding a component of the unfolded protein response pathway [27]. Yet in cells harboring a nonsense mutation in the tumor suppressor gene TP53, p53 rescue and cell death have been found to result from combined NMD inhibition and read through activation. Hence, an anticancer effect appears obtainable even in the presence of ER stress [13]. In addition, some molecules are reported to stabilize PTC-containing mRNAs without affecting the expression of natural NMD substrates, probably because of low NMD inhibition efficiency [11,12].

Conclusion

Inhibition of NMD in cancer cells can restore expression of PTC-containing tumor suppressor genes, allow the synthesis of harmful truncated proteins in cancer cells (which show an increased rate of mutation), and cause induction of an anticancer immune response (Figure 1). As all three effects can occur simultaneously, inhibiting NMD could be an effective way to impair tumorigenesis by stimulating cell processes such as an immune response, apoptosis, or another type of cell death. Molecules inhibiting NMD have been identified and used on cultured cells, with encouraging results [9-13,22,23,28,29] (Table 1). The effectiveness of this approach needs to be demonstrated *in vivo* with a view to moving it into clinical trials and ultimately proposing it as a new form of cancer therapy. As this form of cancer therapy has never been attempted, its side effects are not known and one can only speculate about them. The method used to induce NMD inhibition should be crucial to preventing the activation of cell defenses such as autophagy or the ER stress response, and investigators seeking to develop this therapeutic approach will have to make sure that these cell defense pathways are not activated. It will be necessary to weigh carefully the risks and benefits for the patients. Although investigators have been exploring inhibition of NMD as a basis for cancer therapy for only a few years, the promising results obtained on cultured cells justify current attempts to develop it further.

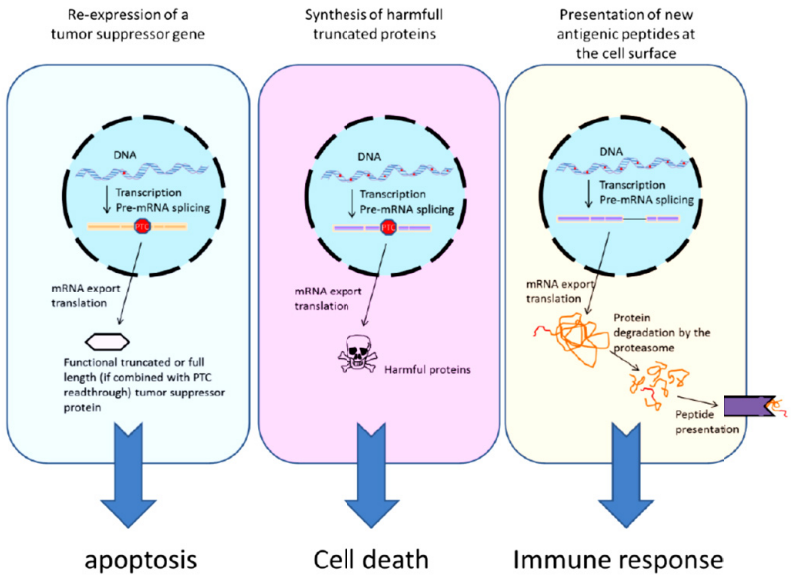
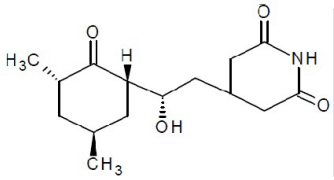
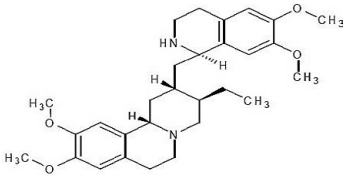
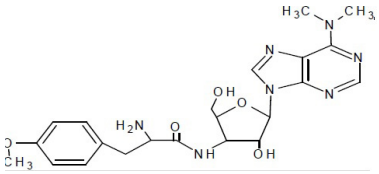
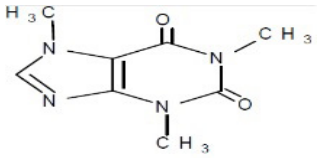
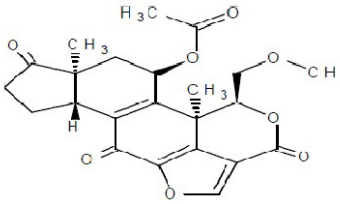
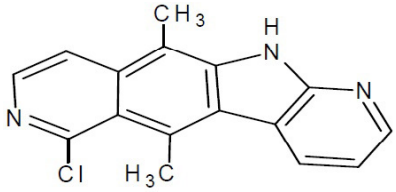
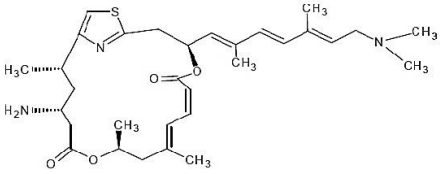
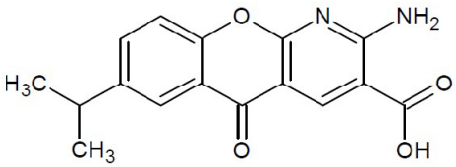
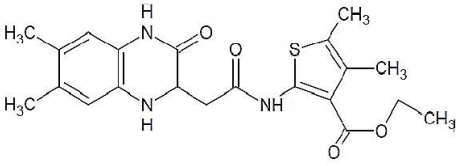


Figure 1: The triple effect of NMD inhibition on cancer cells. Left panel: a cancer cell harbors a nonsense mutation in a tumor suppressor gene (small red star in DNA molecule). The inhibition of NMD allows the translation of the truncated tumor suppressor protein or if the NMD inhibition occurs in combination with a PTC-read through approach, of a full length protein. The expression of the tumor suppressor gene provides sensitivity to apoptosis. Middle panel: Cancer cells accumulate mutations (small red stars in the DNA molecule) leading to the introduction of PTCs. Inhibition of NMD allows the synthesis of the truncated proteins encoded by PTC-containing mRNAs. Some of these proteins are expected to have harmful activity leading to the cell death. Right panel: Cancer cells accumulate mutations (small red stars in the DNA molecule) generating the synthesis of proteins with modified C-terminal end (red line) due to an intron retention introducing a PTC, for instance. A fraction of these proteins are degraded into small peptides that are presented at the cell surface to the immune cells. The abnormal C-terminal end of the proteins is then recognized as non-self-antigens and will elicit an immune response against these cancer cells.

Compound name	Structure	Reference
Cyclohexidine		[28]
Emetine		[28]
Puromycine		[28]
Caffeine		[9]
Wortmannin		[9]
NMDI 1		[11]
Pateamine A		[10]
Amlexanox		[12]
NMDI 14		[13]

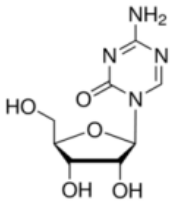
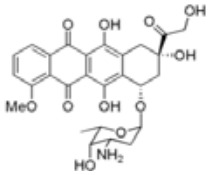
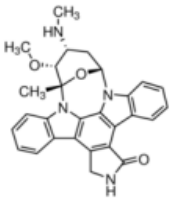
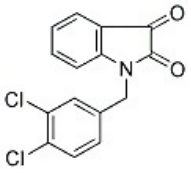
5-azacytidine		[29]
Doxorubicin		[23]
Staurosporine		[22]
Apoptosis Activator 2		[22]

Table 1: Current reported inhibitors of NMD.

References

1. Kervestin S, Jacobson A (2012) NMD: a multifaceted response to premature translational termination. Nat Rev Mol Cell Biol 13: 700-712.
2. Hug N, Longman D, Cáceres JF (2016) Mechanism and regulation of the nonsense-mediated decay pathway. Nucleic Acids Res 44: 1483-1495.
3. Popp MW, Maquat LE (2014) The dharma of nonsense-mediated mRNA decay in mammalian cells. Mol Cells 37: 1-8.
4. Schweingruber C, Rufener SC, Zünd D, Yamashita A, Mühlemann O (2013) Nonsense-mediated mRNA decay-mechanisms of substrate mRNA recognition and degradation in mammalian cells. Biochim Biophys Acta 1829: 612-623.
5. Karam R, Wengrod J, Gardner LB, Wilkinson MF (2013) Regulation of nonsense-mediated mRNA decay: implications for physiology and disease. Biochim Biophys Acta 1829: 624-633.
6. Bhuvanagiri M, Schlitter AM, Hentze MW, Kulozik AE (2010) NMD: RNA biology meets human genetic medicine. Biochem J 430: 365-377.
7. Benhabiles H, Jia J, Lejeune F (2016) Nonsense mutation correction in human diseases: an approach for targeted medicine. Elsevier 1-192.
8. Mort M, Ivanov D, Cooper DN, Chuzhanova NA (2008) A meta-analysis of nonsense mutations causing human genetic disease. Hum Mutat 29: 1037-1047.
9. Usuki F, Yamashita A, Higuchi I, Ohnishi T, Shiraishi T, et al. (2004) Inhibition of nonsense-mediated mRNA decay rescues the phenotype in Ullrich's disease. Ann Neurol 55: 740-744.
10. Dang Y, Low WK, Xu J, Gehring NH, Dietz HC, et al. (2009) Inhibition of nonsense-mediated mRNA decay by the natural product pateamine A through eukaryotic initiation factor 4AIII. J Biol Chem 284: 23613-23621.
11. Durand S, Cougot N, Mahuteau-Betzer F, Nguyen CH, Grierson DS, et al. (2007) Inhibition of nonsense-mediated mRNA decay (NMD) by a new chemical molecule reveals the dynamic of NMD factors in P-bodies. J Cell Biol 178: 1145-1160.
12. Gonzalez-Hilarion S, Beghyn T, Jia J, Debreuck N, Berte G, et al. (2012) Rescue of nonsense mutations by amlexanox in human cells. Orphanet J Rare Dis 7: 58.
13. Martin L, Grigoryan A, Wang D, Wang J, Breda L, et al. (2014) Identification and characterization of small molecules that inhibit nonsense-mediated RNA decay and suppress nonsense p53 mutations. Cancer Res 74: 3104-3113.
14. Cosmic v76. Catalogue of Somatic Mutations in cancer. Accessed on 03 may 2016.
15. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, et al. (2013) Cancer genome landscapes. Science 339: 1546-1558.
16. Du L, Damoiseaux R, Nahas S, Gao K, Hu H, et al. (2009) Nonaminoglycoside compounds induce readthrough of nonsense mutations. J Exp Med 206: 2285-2297.
17. Keeling KM, Du M, Bedwell DM (2006) Therapies of Nonsense-Associated Diseases. Nonsense-mediated mRNA Decay - Landes Bioscience 121-136.
18. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, et al. (2007) PTC124 targets genetic disorders caused by nonsense mutations. Nature 447: 87-91.
19. Floquet C, Deforges J, Rousset JP, Bidou L (2011) Rescue of non-sense mutated p53 tumor suppressor gene by aminoglycosides. Nucleic Acids Res 39: 3350-3362.
20. Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, et al. (2010) The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 467: 1109-1113.
21. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, et al. (2010) A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 463: 191-196.
22. Jia J, Furlan A, Gonzalez-Hilarion S, Leroy C, Gruenert DC, et al. (2015) Caspases shutdown nonsense-mediated mRNA decay during apoptosis. Cell Death Differ 22: 1754-1763.
23. Popp MW, Maquat LE (2015) Attenuation of nonsense-mediated mRNA decay facilitates the response to chemotherapeutics. Nat Commun 6: 6632.
24. Pastor F, Kolonias D, Giangrande PH, Gilboa E (2010) Induction of tumour

immunity by targeted inhibition of nonsense-mediated mRNA decay. Nature 465: 227-230.

25. Wengrod J, Martin L, Wang D, Frischmeyer-Guerrerio P, Dietz HC, et al. (2013) Inhibition of nonsense-mediated RNA decay activates autophagy. Mol Cell Biol 33: 2128-2135.

26. Wang D, Zavadil J, Martin L, Parisi F, Friedman E, et al. (2011) Inhibition of nonsense-mediated RNA decay by the tumor microenvironment promotes tumorigenesis. Mol Cell Biol 31: 3670-3680.

27. Karam R, Lou CH, Kroeger H, Huang L, Lin JH, et al. (2015) The unfolded protein response is shaped by the NMD pathway. EMBO Rep 16: 599-609.

28. Carter MS, Doskow J, Morris P, Li S, Nhim RP, et al. (1995) A regulatory mechanism that detects premature nonsense codons in T-cell receptor transcripts in vivo is reversed by protein synthesis inhibitors in vitro. J Biol Chem 270: 28995-9003.

29. Bhuvanagiri M, Lewis J, Putzker K, Becker JP, Leicht S, et al. (2014) 5-azacytidine inhibits nonsense-mediated decay in a MYC-dependent fashion. EMBO Mol Med 6: 1593-1609.