无义介导的mRNA降解在神经系统发育和神经疾病中的作用及机制研究进展

沈细亚[1] 宣丽娜[1] 杜雷雷[1] 李唐亮[2] 黄智慧[1]* 徐星星[1]*

([1]温州医科大学基础医学院, 神经科学研究所, 温州 325035; [2]杭州师范大学医学院衰老研究所, 杭州 311121)

摘要 无义介导的mRNA降解(nonsense-mediated mRNA decay, NMD)是真核生物细胞中广泛存在、高度保守的mRNA质量监控系统, 可识别并降解含有提前终止密码子、长3′非翻译区及上游开放阅读框等的mRNA, 以避免异常蛋白质的产生和累积。最近的研究表明, NMD能调节正常基因转录的稳定性, 在神经发生、突触可塑性中发挥重要作用, 且其功能异常与神经疾病相关。该文对NMD在神经系统发育和神经疾病中的作用及机制进行了综述, 以期为神经疾病的治疗提供理论基础。

关键词 无义介导的mRNA降解; 神经发生; 神经系统疾病; 神经干细胞

The Progress in the Roles and Mechanisms of Nonsense Mediated mRNA Decay in the Development of the Nervous System and Neurological Diseases

Shen Xiya[1], Xuan Lina[1], Du Leilei[1], Li Tangliang[2], Huang Zhihui[1]*, Xu Xingxing[1]*

([1]*Institute of Neuroscience, Basic Medical College, Wenzhou Medical University, Wenzhou 325035, China*;
[2]*Institute of Aging Research, School of Medicine, Hangzhou Normal University, Hangzhou 311121, China*)

Abstract Nonsense-mediated mRNA decay (NMD) is a common, highly conserved mRNA quality monitoring system in eukaryotic cells, which prevents production and accumulation of abnormal proteins by recognizing and degrading mRNAs containing premature termination codons, long 3′ untranslated regions and upstream open reading frame. Recent studies show that NMD can regulate the stability of normal gene transcription, plays an important role in neurogenesis, synaptic plasticity, and dysfunction of NMD involves in neurological diseases of the nervous system. This article reviews the roles and mechanisms of NMD in the development of the nervous system and neurological diseases, in order to provide a theoretical basis for the treatment of neurological diseases.

Keywords NMD; neurogenesis; neurological diseases; neural stem cells

无义介导的mRNA降解(nonsense-mediated mRNA decay, NMD)是一种广泛存在于真核生物中的mRNA质量监控系统[1-3]。在真核细胞中, 无义突变、移码突变或选择性剪接会导致提前终止密码子(premature termination codons, PTCs)的出现, 具有PTCs的mRNA可被NMD所识别并降解, 从而缩短mRNA

收稿日期: 2018-06-03 接受日期: 2018-07-11
国家自然科学基金(批准号: 31671071)和浙江省自然科学基金(批准号: LR18C090001)资助的课题
*通讯作者。Tel: 0577-86699117, E-mail: hzhzju021@163.com; Tel: 0577-88689796, E-mail: xuxingxing627@163.com
Received: June 3, 2018 Accepted: July 11, 2018
This work was supported by the National Natural Science Foundation of China (Grant No.31671071) and the Natural Science Foundation of Zhejiang Province (Grant No.LR18C090001)
*Corresponding authors. Tel: +86-577-86699117, E-mail: hzhzju021@163.com; Tel: +86-577-88689796, E-mail: xuxingxing627@163.com
网络出版时间: 2018-09-29 14:00:24 URL: http://kns.cnki.net/kcms/detail/31.2035.Q.20180929.1400.010.html

的半衰期[4-5]。自从在酿酒酵母和秀丽隐杆线虫基因筛选中发现第一个NMD的基因后, 大多数真核生物中的一些NMD相关基因相继被鉴定出来[6-7]。通过一系列的生物化学及结构分析的方法, NMD降解靶mRNA的相关因子和作用机制已被广泛研究, 然而NMD的具体生物学功能仍不清楚。通过分析人类基因突变数据库发现, 约12%的单核苷酸突变会导致含有PTC的mRNA的产生[8], 一些突变与人类疾病密切相关, 如地中海贫血和Duchenne型肌营养不良症等[9]。最近研究发现, NMD参与人类神经系统的发育过程, 如神经干细胞的分化、突触的形成、轴突导向等, 且NMD异常会引发许多神经疾病[10]。本综述中, 我们对NMD在神经系统发育和神经疾病中的作用及机制进行了重点阐述, 以期为与NMD功能异常相关的神经疾病的治疗提供理论基础。

1 NMD的作用机制

1.1 NMD的因子和组成

在哺乳动物中, NMD包括关键的磷酸肌醇3-激酶(PI3K)复合物(SMG1、SMG8和SMG9)、UPF蛋白质(UPF1、UPF2、UPF3A和UPF3B)、真核释放因子(eRF1和eRF3)、外显子连接复合物(exon junction complex, EJC)成员(eIF4A3、RBM8A、MAGOH和MLN51)和SMG蛋白(SMG5、SMG6和SMG7)。这些NMD因子共同作用, 触发mRNA的降解[2,11-14]。表1总结了这些NMD因子及其在mRNA降解中的主要作用。

1.2 NMD的作用过程

NMD通过识别mRNA中的特征性结构, 如PTC、上游开放阅读框(upstream open reading frame, uORF)和长3′非翻译区(3′ untranslated region, 3′UTR)[11]等来降解mRNA。在本综述中, 我们介绍NMD识别并降解含有PTC的mRNA的经典过程(图1)。实际上, 对于同一NMD底物的降解, 存在多种旁路。如对含有PTC的mRNA的降解, 除上述经典途径外, 还有独立于UPF2、UPF3B或EJC的NMD旁路[15-16], 且存在mRNA内切途径和外切途径冗余[17]。

1.3 NMD的自身调节

NMD因子的mRNA也具有3′UTR或uORF, 因此, NMD因子自身也可以作为NMD的靶标。研究发现, 在哺乳动物中敲减NMD因子, 会导致一些其他NMD因子如UPF1、UPF2、UPF3B、SMG1、SMG5、SMG6

表1 NMD因子的生物学作用

Table 1 Biological roles of NMD factors

NMD因子 NMD factors	分子量(kDa) Molecular weight (kDa)	作用结构 Structure	依赖NMD作用 Functions in NMD
SMG1	410	Protein kinase	Phosphorylates the N- and C-terminus of UPF1
UPF1	123	RNA helicase, ATPase	Direct RNA binding; helicase activity; (phosphorylated) N- and C-terminus are binding platforms for SMG5-7, PNRC2 and decapping factors
UPF2	148	Adaptor molecule	Regulates UPF1 helicase activity; stimulates SMG1 kinase activity; establishes a link between UPF1 and UPF3
UPF3A	55	Adaptor molecule	Establishes a link between UPF1-UPF2 and the EJC; EJC-independent function is unknown
UPF3B	56	Adaptor molecule	Establishes a link between UPF1-UPF2 and the EJC; EJC-independent function is unknown, functionally dominant over UPF3A
SMG5	114	14-3-3 like domain, PIN like domain	Forms a complex with SMG7; recruits PP2A for UPF1 dephosphorylation; provides additional binding affinity to phosphorylated UPF1
SMG6	160	14-3-3 like domain, PIN like domain	Binds phosphorylated as well as nonphosphorylated UPF1; executes endonucleolytic cleavage of the target mRNA
SMG7	122	14-3-3 like domain	Forms a complex with SMG5; required for SMG5-7 binding, and phosphorylates UPF1; recruits POP2 for mRNA deadenylation
SMG8	110	Subunit of SMG1	Regulates SMG1 kinase activity; leads to inactivation of SMG1 by inducing conformational changes
SMG9	58	Subunit of SMG1	Regulation of SMG1 kinase activity; required for SMG1 complex formation

中国细胞生物学学报

和SMG7的mRNA水平显著增加[18-19]。这表明: (1)NMD因子的转录本也是NMD的靶标; (2)NMD存在反馈调控机制[18-20]。在其他物种中, 一些编码NMD因子的mRNA, 如拟南芥中的SMG7和UPF3以及果蝇中的SMG5, 也被确定为是NMD靶标, 表明NMD的自身调节机制在进化上是比较保守的[21-22]。

2 NMD在神经发育和疾病中的作用及机制

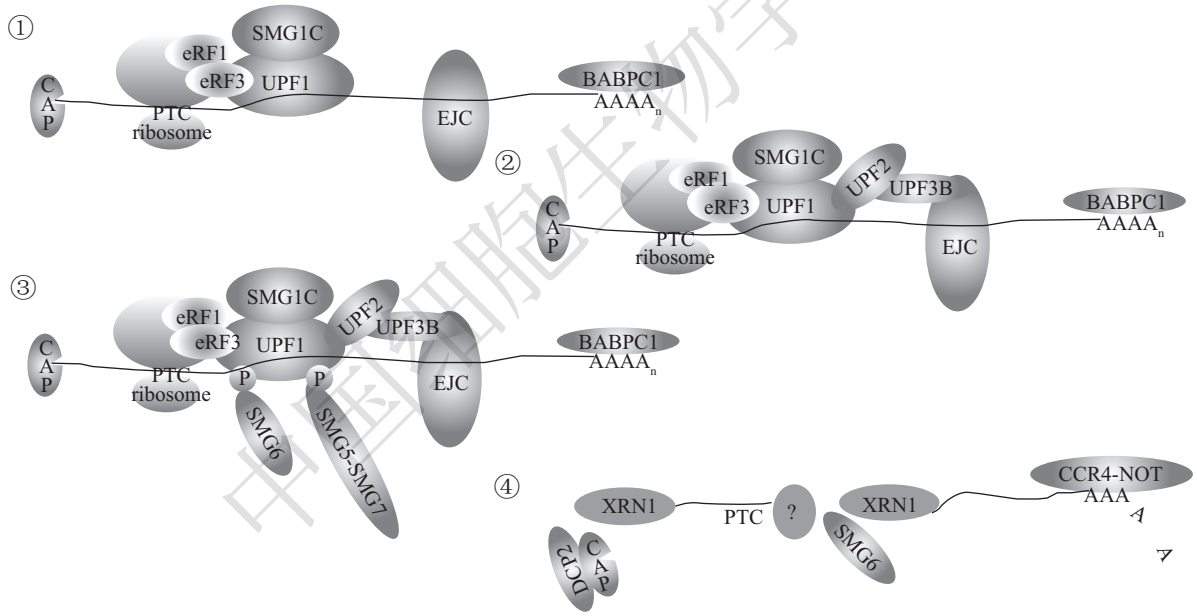
2.1 NMD在神经发生中的作用

神经干细胞(neural stem cells, NSCs)是一种能自我更新的多能干细胞, 在胚胎发育过程中形成动物神经系统的神经元和神经胶质细胞。神经干细胞分化为神经元的过程称为神经发生。神经发生包括神经干细胞的增殖、分化、成熟及凋亡。哺乳动物神经干细胞起源于神经上皮组织[34], 在哺乳动物脑部发育的早期, NSCs在脑室区的新皮层增殖, 并分化成不同类型的功能性神经元, 形成大脑[35]。

NMD是真核生物细胞中广泛存在的mRNA质量监控系统, 在神经发生中发挥重要作用。那么, NMD是如何参与神经发生呢?

2.1.1 NMD直接介导的作用 有研究表明, 神经细胞的分化需要NMD因子的下调[36-37]。UPF1、UPF2、SMG1和SMG6的mRNA转录物水平在小鼠NSCs和人类神经祖细胞(human nerve progenitor cells, hNPCs)的分化过程中降低。在大鼠NSCs分化过程中, UPF1和UPF3B表达下调, 分化至第7天到第8天时, 其表达量急剧降至未分化时的10%~20%。在NSCs中敲减UPF1或UPF3B可刺激大鼠NSCs分化并使神经元数目增多[36-37], 且NMD活性下降(用NMD抑制剂处理)的NSCs也出现类似现象, 表明NMD可以抑制NSCs的分化, 促进自我更新。

NMD在神经分化中的作用机制是什么? miR-



①核糖体在翻译过程中识别出PTC, 翻译被异常终止, 从而促进UPF1和eRF3-eRF1的相互作用[20,23]。通过招募SMG1C(SMG8、SMG9和SMG1形成SMG1C复合物), 形成SURF(SMG1C-UPF1-eRF1-eRF3)复合物[24-26]。②UPF2和UPF3B作为桥梁连接SURF与EJC, 共同形成降解诱导复合物(decay-inducing complex, DECID)[27]。③DECID的形成促进UPF1的磷酸化[28], SMG6和SMG5-SMG7复合物通过14-3-3样结构域与UPF1的磷酸化位点结合, 促使核糖体、eRF3-eRF1及UPF1解离[29-30]。④SMG6的PIN结构域的核酸内切酶活性对PTC附近的mRNA片段进行剪切[31-32]。SMG5-SMG7复合物招募DCP2、XRN1和CCR4-NOT, 分别参与mRNA的脱帽、5′→3′核酸外切和脱腺苷过程, 并以某种方式招募未知的5′→3′核酸外切酶(以“?”表示)。

① Translation is abnormally terminated when the ribosome recognizes PTC, which promotes the interaction between UPF1 and eRF3-eRF1[20,23]. The SURF (SMG1C-UPF1-eRF1-eRF3) complex forms by recruiting SMG1C (SMG8, SMG9 and SMG1 form the SMG1C complex)[24-26]. ② UPF2 and UPF3B act as a bridge connecting SURF and EJC, result in the formation of a decay-inducing complex (DECID)[27]. ③ The formation of DECID promotes the phosphorylation of UPF1[28]. SMG6 and the SMG5-SMG7 complex bind to the phosphorylation site of UPF1 through the 14-3-3-like domain, promoting the dissociation of ribosomes, eukaryotic release factors, and UPF1 from the target[29-30]. ④ The endonuclease activity of the PIN domain of SMG6 cleaves the mRNA segment nearby the PTC[31-32]. The SMG5-SMG7 complex recruits DCP2, XRN1 and CCR4-NOT, mediating mRNA decapping, 5′→3′ exonucleolytic cleavage and deadenylation. Unknown 5′→3′ exonuclease is attracted in some way (indicated by “?”).

图1 经典NMD的作用机制(根据参考文献[33]修改)

Fig.1 Molecular mechanism of classical NMD (modified from reference [33])

中国细胞生物学学报

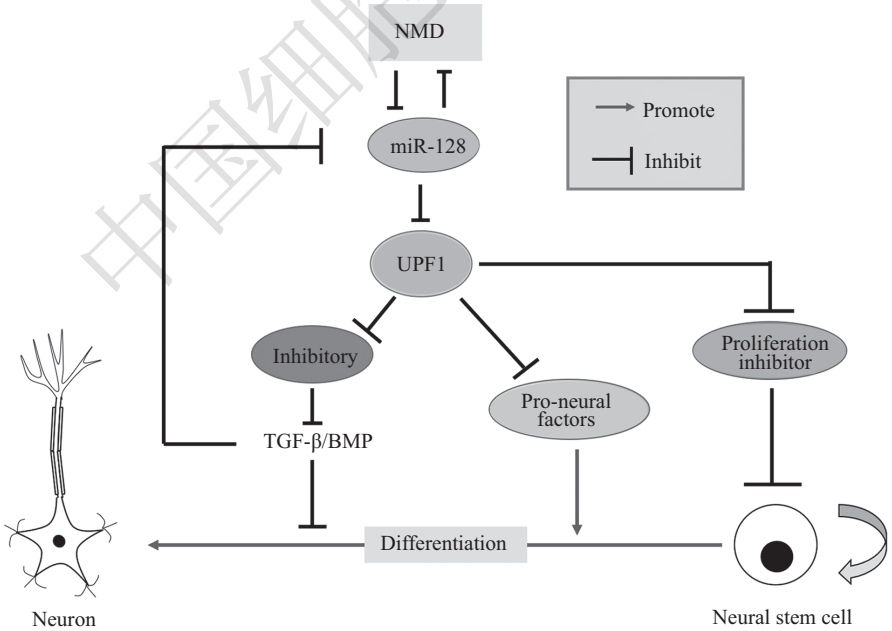
128是NMD的底物之一, 在体内外神经分化的过程中, 其表达量显著增加, 提示NMD可通过miR-128负向调控神经元的分化。此外, miR-128可以直接抑制UPF1的表达[36,38], 而UPF1表达的下调会抑制NMD活性, 说明NMD和miR-128彼此相互抑制, 调控神经干细胞的分化状态。TGF-β/BMP信号通路可以抑制神经分化。研究发现, UPF1表达的下调抑制NMD活性, 选择性地使TGF-β/BMP信号通路的一些抑制因子(SMADs)、促神经因子(SMAD7、Ascl1)和增殖抑制因子(p21、p27、MAPK6)的表达上升, 抑制神经干细胞增殖, 促进其分化[36]。而TGF-β/BMP信号通路又会抑制miR-128的表达水平, 形成调控环路(图2)。以上证据表明, 细胞处于未分化状态时, 具有高NMD活性, 而处于分化状态时, 具有低NMD活性。

然而, 也有一部分研究表明, NMD促进神经细胞的分化。*UPF3B*突变的小鼠NSCs分化存在缺陷[39]。UPF3B的缺失导致小鼠NSCs和人类神经祖细胞分化能力减弱, 而自我更新能力增加[37,40]。用氨来占诺(Amlexanox)或增加*UPF3B*错义突变的方法抑制NMD活性, 可以抑制NSCs向成熟神经元的分化[37]。

此外, 在原代培养的神经元细胞中单独转染人类的UPF1(human UPF1, hUPF1)或UPF2(human UPF2, hUPF2)都可以显著保护哺乳动物神经元免受TDP43相关毒害, 而共转染hUPF1和hUPF2后, 其保护作用更显著, 表明NMD机制可以发挥神经保护作用[41]。

综上所述, NMD在神经发生和神经保护中发挥重要作用, 但在NSCs的分化和自我更新中的作用目前仍有争议。原因之一可能是, 细胞NMD过程中NMD因子的mRNA和蛋白质表达水平可能并不能完全真实反映NMD的活化程度。另外, 在NSCs分化的不同阶段, NMD可能通过降解特定的RNA靶基因, 行使不同的功能。

2.1.2 NMD组件EJC介导的作用 NMD组件EJC (exon junction complex)的成员, 如RBM8A、Magoh和eIF4A3也在神经发生中具有重要作用[42-44]。RBM8A在新生皮层神经发生开始时表达水平上升, 促进NSCs的增殖, 防止NSCs过早分化为神经元[43]。另外, RBM8A对于皮层NPCs的增殖和分化至关重要, RBM8A过表达刺激胚胎NPCs增殖和抑制神经元分化。相反, 敲除*RBM8A*使得新皮层的NPCs增殖减少并促进早期神经元分化。此外, 过表达的RBM8A保



NMD和miR-128彼此相互抑制, miR-128也可以直接抑制UPF1, 而UPF1表达的下调影响NMD活性, 选择性地使一些TGF-β/BMP的抑制因子、促神经因子、增殖抑制因子的表达上升。TGF-β/BMP通路可以抑制神经分化, 增殖抑制因子的上调则抑制神经干细胞的自我更新。

NMD and miR-128 inhibit each other, miR-128 can also directly inhibit UPF1, while down-regulation of UPF1 influences NMD activity, selectively elevates the expression level of some TGF-β/BMP inhibitors, neurotrophic factors, and proliferation inhibitors. TGF-β/BMP pathway can inhibit neural differentiation, and the upregulation of proliferation inhibitors inhibit neural stem cell self-renewal.

图2 NMD在神经发生中的作用及分子机制(根据参考文献[36]修改)

Fig.2 The roles and the molecular mechanisms of NMD in neurogenesis (modified from reference [36])

中国细胞生物学学报

持皮质NPCs在一个增殖状态[43]。Magoh杂合突变小鼠NSCs在新生皮层发育的早期阶段比较倾向于分化而非自我更新, 而在新生皮层组织内Tbr2阳性的中间神经元及成熟神经元减少, 进一步研究发现, 这主要是由于杂合突变导致神经元的凋亡增加[44]。eIF4A3杂合突变小鼠表型类似RBM8A和Magoh杂合突变小鼠, 三者均表现为小头畸形[42]。进一步的证据表明, 这三种突变体均表现出RNA结合蛋白的剪接缺陷, 提示EJC依赖性的RNA调节网络可以微调基因的表达[42]。而下游基因*p53*的敲除显著拯救了所有三个EJC突变体引起的小头畸形, 这表明*p53*激活是EJC损伤后神经发育发病的主要节点, EJC相关组件杂合突变小鼠可以通过*p53*依赖性细胞凋亡途径促进神经发生[42]。然而, 由于未检测EJC相关组件杂合突变小鼠脑中的NMD活性[42], 因此, NMD与这些神经系统发病机制之间的联系并不十分清楚。

2.2 NMD在轴突导向、突触形成和突触传递中的作用及机制

最近的研究表明, NMD参与脊髓神经元的轴突导向[45]。Robo3受体的两种可变剪接亚型——Robo3.1和Robo3.2, 在脊髓神经元轴突导向的过程中发挥重要功能[46]。Robo3.2的内含子中含有PTC位点, 可能是NMD的潜在靶标[47]。神经元生长锥中富含UPF1、UPF2和SMG1蛋白, 选择性敲除UPF2时, Robo3.2 mRNA和蛋白水平增加, 引发异常的轴突导向[45]。此外, 在NMD功能受损时, Robo家族的其他转录物如Slit2、Epha4和Robo1水平也表现出上调, 进一步表明NMD在轴突导向中发挥作用[38,40]。

NMD在突触形成中也发挥重要作用。SMG1的缺失会影响神经骨骼肌接头(neuromuscular junction, NMJ)结构的完整性, 导致终板面积减小以及分支和突触数量减少, 但这些缺陷表型可以被SMG1的过表达有效逆转, 这表明NMD参与突触结构的维持[48]。在兴奋性神经元中特异性敲除NMD的必需因子UPF2, 影响了突触后致密斑中的重要蛋白PSD-95第18个外显子的剪接, 导致PSD-95的表达下降, 也会影响突触的形成和发育[49]。此外, 在原代培养的海马神经元中, UPF3B-NMD的活性降低会导致神经元的轴突和树突的生长发生细微的变化[40]。

NMD同时在突触传递中扮演重要的角色。通过记录NMJ的兴奋性突触后电流, 发现SMG1杂合突变体电流幅度降低约50%, 表明SMG1功能减弱会降低突触传递的效率。在高频刺激下, SMG1的缺失会导致突触囊泡回收受损, 使得突触传递效率下降[48]。NMD因子UPF2和SMG6的甲基磺酸乙酯(ethyl methanesulfonate, EMS)诱导突变同样降低突触囊泡循环和突触传递[48]。也有研究发现, EJC组件的核心因子eIF4AIII可以调节突触强度和神经元中蛋白质的表达[50]。Arc mRNA是NMD的靶标, 敲减eIF4AIII可增加神经元中Arc mRNA和蛋白质的表达, 使α-氨基-3-羟基-5-甲基-4-异恶唑丙酸受体(AMPA)受体的表面表达增加, 微小兴奋性突触后电位(mEPSC)幅度增加[50], 突触传递增强。此外, NMD因子UPF3B在神经元中具有丰富的表达, 同时也存在于树突棘上, 提示UPF3B可能在调节突触中mRNA的表达和降解中发挥功能[51]。

2.3 NMD在神经疾病中的作用和机制

NMD异常与多种神经疾病的发生有关, NMD功能失调是这些神经疾病的常见的分子机制。

2.3.1 NMD的关键因子在神经疾病发生中的作用

UPF3B是第一个被发现的与人类神经发育障碍相关的NMD因子。X染色体上*UPF3B*基因的突变导致人类X连锁智力障碍、自闭症和精神分裂症等的发生[37,52-53]。对人的转录组分析表明, 在携带UPF3B功能丧失性突变的智力障碍患者中, 约5%的转录组水平受到影响[54]。*UPF3B*的突变扰乱了NMD的一条旁路, 进而导致各种形式的智力障碍[51-52,55]。同时, 智力障碍表型越严重的患者, UPF3A蛋白的表达水平越低。这可能是由于在智力障碍患者中, UPF3A可以部分代偿UPF3B功能的缺失。所以, UPF3A表达水平越低, 代偿程度越低, 智力障碍表型越严重[54]。另外, 在智力障碍患者中, *UPF2*、*UPF3A*、*SMG5*、*SMG6*、*SMG7*、*SMG8*、*SMG9*、*RBM8A*、*eIF4A3*和*RNPS1*等NMD相关基因发生删除和/或复制突变的概率显著高于对照组, 进一步体现了NMD在学习和记忆中的重要性[54]。有研究报道, 人类常染色体隐性多重先天性异常综合征很可能与*SMG9*的功能丧失性突变有关。在小鼠中敲除*SMG9*, 可以观察到类似的多重先天性异常综合征的主要特征。所以, SMG9是人类和小鼠神经系统正常发育所必需的[56]。

另外, NMD关键因子的表达对癫痫的发作也有一定的影响。Claire等[58]的实验表明, 癫痫持续状态的小鼠海马中, UPF1及磷酸化的UPF1(p-UPF1)蛋

表2 NMD因子在神经系统中的生物学作用及相关的人类神经疾病

Table 2 The biological effects of NMD factors in the nervous system and related human neurological disorder

NMD因子 NMD factors	神经系统中的生物学作用 Biological function in the nervous system	相关人类神经疾病 Human neurological disorder
SMG1	Maintains synapse architecture and synaptic vesicle cycle efficacy[48]	Not reported
UPF1	Neurogenesis; neuronal activity[72]	Not reported
UPF2	Axon guidance; neurite outgrowth[45]	Intellectualdisability[72]
UPF3A	Unknown	Intellectual disability[72]
UPF3B	Differentiation; neurite outgrowth[51]	Intellectual disability; autism; schizophrenia[37,51-53]
SMG5	Unknown	Not reported
SMG6	Unknown	Epilepsy[58]
SMG7	Unknown	Not reported
SMG8	Unknown	Not reported
SMG9	Unknown	A multiple congenital anomaly syndrome[56]
eIF4A3	Neuronal activity[42]	Richieri-costa-pereira syndrome; Intellectual disability
RBM8A	Neurogenesis; neuronal activity[43]	TAR syndrome; intellectual disability[61]; altered brain size autism; seizures[73-74]
MAGOH	Neurogenesis[44]	Not reported

白水平升高。在颞叶癫痫患者的海马及难治性颞叶癫痫患者切除的海马中, UPF1的表达水平也显著升高。在脑室内注射NMD抑制剂NMDI14抑制p-UPF1和NMD活性, 发现小鼠单次癫痫发作的持续时间并未发生明显改变, 然而总的自发性癫痫发作次数、总癫痫发作次数和每日癫痫发作率均减少[57]。此外, NMD因子SMG6具有抑制癫痫的功能。对颞叶癫痫(temporal lobe epilepsy, TLE)患者的单核苷酸多态性分析显示, rs4523957处的*GC*突变成*GG*后, SMG6启动子的活性下降了22%, 且*GG*基因型携带者的发作频率和耐药发生率显著降低。进一步研究发现, 在大鼠癫痫模型中, SMG6的表达上升[58]。

2.3.2 NMD的EJC组件在神经疾病发生中的作用 RBM8A是EJC组件的核心成分, *RBM8A*杂合突变小鼠在新生皮质发育过程中表现出小头畸形和皮层的减少[43]。有研究发现, *RBM8A*是TAR(thrombocytopenia with absent radius)综合征的致病基因, TAR综合征主要表现为肢体缺陷和血液的紊乱, 并与神经发育障碍的发病率增加有关[59]。同时, RBM8A可以影响小鼠的情绪情感活动[60]。RBM8A在小鼠齿状回中的过表达会导致异常的社交活动、焦虑样行为及抑郁行为[60]。另有研究报道, 超过50%的发育性常染色体隐性遗传疾病(Richieri-Costa-Pereira)患者存在学习和语言障碍, 这可能是由EJC的另一核心成员*eIF4A3*的5′UTR内重复序列的扩增造成[61]。大鼠新环境探索实验表明, 海马神经元树突中的eIF4A3通过调节*Arc* mRNA的水平, 参与空间学习记忆过程[50,62]。这些证据均提示, eIF4A3与可能与发育性常染色体隐性遗传疾病有关[61]。

2.3.3 NMD的底物的表达异常在神经系统疾病发生中的作用 大约三分之一的人类遗传疾病是由过早的PTC引起的[63]。一些在癫痫中发挥重要功能的离子通道蛋白(如GABA), 发生突变后产生PTC位点, 从而成为NMD的靶标, 导致绝大部分突变mRNA发生降解, 少量未被NMD降解的mRNA翻译成错误的蛋白, 并在内质网的质控作用下降解, 这是最终导致先天性全身性癫痫(idiopathic generalized epilepsies, IGE)的重要原因[64-66]。另外, ATP-结合盒家族成员ABCA7原有的PTC位点发生突变后, 会导致PTC位点的缺失, 产生大量异常的mRNA, 使得NMD无法对其进行完全有效的降解, 这可能是导致晚发型阿尔茨海默病(Alzheimer′s disease, AD)的重要原因[67]。NMD可以维持选择性剪切后产生的各种异构体的平衡, 而选择性剪切异构体的不平衡可能是多发性硬化症的重要病因之一, 表明NMD可能与多发性硬化相关[68]。*SNRPB*基因由于选择性剪切, 会使PTC

中国细胞生物学学报

位点处于外显子的不同位置, 导致不同的*SNRPB*剪切变体具有不同的表达水平。*SNRPB*的选择性剪接异常与脑部–下颌骨综合征有关。而NMD可以调控*SNRPB*的选择性剪接, 表明NMD可能与脑部–下颌骨综合征有关[69-70]。研究表明, DNA/RNA结合蛋白基因FUS/TLS的外显子7的剪接具有自我调节的特性, NMD可以影响这一自动调节过程。在肌萎缩侧索硬化(amyotrophic lateral sclerosis, ALS)患者中, FUS的自动调节可以直接加剧致病性FUS蛋白的积累, 这间接表明, NMD可能在ALS中发挥一定功能[71]。

3 展望

NMD是真核生物中广泛存在的、高度保守的转录后调节机制, 参与神经发生、突触形成及神经系统发育和疾病等多种生理和病理过程, 并在其中发挥着重要的作用。然而, 目前仍存在一些问题有待解决。(1)NMD因子在神经系统的不同发育阶段的分子靶标尚不清楚。(2)不同的NMD因子对神经干细胞的分化和增殖有不尽相同的影响。(3)EJC组件在基因表达调节中起着非依赖NMD的作用[75-77], 然而EJC组件在神经发生障碍的具体作用机制尚不清楚。(4)NMD在整个神经系统的发育和疾病中发挥重要功能, 然而到目前为止, NMD在具有重要功能的神经胶质细胞中的作用尚无研究。

NMD在临床上有着巨大的应用前景。在mRNA翻译过程中, PTCs的识别将提前终止翻译过程, 从而产生功能异常的截短蛋白。因此, 正常蛋白质表达的减少被认为是无义突变引发人类遗传疾病的主要原因。允许终止密码子通读的药物, 例如庆大霉素, 可以在原有的PTC位点处诱导插入氨基酸, 从而翻译出完整正常的蛋白[78]。据此, 我们可以对疾病模型中人体细胞的缺陷进行改善。在遗传性椭圆细胞增多症和Ullrich型先天性肌营养不良疾病中, 抑制NMD可以有效增加功能性截短蛋白的表达并改善该致病细胞系的细胞缺陷[78-80]。而使用允许终止密码子通读的药物和NMD抑制剂联合治疗策略, 在NMD功能异常导致的人类疾病中显示出很好的效果和巨大前景[81-83]。然而, NMD抑制剂在疾病治疗中应谨慎使用, 因为敲除或敲减NMD因子的动物模型表明, NMD活性下降对细胞和组织会产生强毒性。此外, 不同NMD因子缺失会在人体内引起严重的中枢神经系统和免疫系统疾病[84]。另一种治疗NMD相关疾病的策略是利用反义寡核苷酸, 以基因特异性的方式抑制NMD。到目前为止, 虽然特异性抑制NMD基因的治疗价值有待商榷, 但是它仍在治疗无义突变导致的相关疾病中具有巨大的潜力。

参考文献 (References)

1 Frischmeyer PA, Dietz HC. Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet 1999; 8(10): 1893-900.

2 Lykke-Andersen S, Jensen TH. Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. Nature reviews. Mol Cell Biol 2015; 16(11): 665-77.

3 Fatscher T, Boehm V, Gehring NH. Mechanism, factors, and physiological role of nonsense-mediated mRNA decay. Cell Mol Life Sci 2015; 72(23): 4523-44.

4 Losson R, Lacroute F. Interference of nonsense mutations with eukaryotic messenger RNA stability. Pro Natl Acad Sci USA 1979; 76(10): 5134-7.

5 Maquat LE, Kinniburgh AJ, Rachmilewitz EA, Ross J. Unstable beta-globin mRNA in mRNA-deficient beta o thalassemia. Cell 1981; 27(3 Pt 2): 543-53.

6 Leeds P, Peltz SW, Jacobson A, Culbertson MR. The product of the yeast UPF1 gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev 1991; 5(12A): 2303-14.

7 Pulak R, Anderson P. mRNA surveillance by the *Caenorhabditis elegans* smg genes. Genes Dev 1993; 7(10): 1885-97.

8 Krawczak M, Ball EV, Fenton I, Stenson PD, Abeysinghe S, Thomas N, *et al*. Human gene mutation database-a biomedical information and research resource. Hum Mutat 2000; 15(1): 45-51.

9 Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. Eur J Hum Genet 2006; 14(10): 1074-81.

10 Han X, Wei Y, Wang H, Wang F, Ju Z, Li T. Nonsense-mediated mRNA decay: a 'nonsense' pathway makes sense in stem cell biology. Nucleic Acids Res 2018; 46(3): 1038-51.

11 Schweingruber C, Rufener SC, Zund D, Yamashita A, Muhlemann O. Nonsense-mediated mRNA decay-mechanisms of substrate mRNA recognition and degradation in mammalian cells. Biochim Biophys Acta 2013; 1829(6/7): 612-23.

12 He F, Jacobson A. Nonsense-mediated mRNA decay: degradation of defective transcripts is only part of the story. Annu Rev Genet 2015; 49: 339-66.

13 Karousis ED, Nasif S, Muhlemann O. Nonsense-mediated mRNA decay: novel mechanistic insights and biological impact. Wiley Interdiscip Rev RNA 2016; 7(5): 661-82.

14 Boehm V, Gehring NH. Exon junction complexes: supervising the gene expression assembly line. Trends Genet 2016; 32(11): 724-35.

15 Ivanov PV, Gehring NH, Kunz JB, Hentze MW, Kulozik AE. Interactions between UPF1, eRFs, PABP and the exon junction complex suggest an integrated model for mammalian NMD pathways. EMBO J 2008; 27(5): 736-47.

16 Gehring NH, Kunz JB, Neu-Yilik G, Breit S, Viegas MH, Hentze MW, *et al*. Exon-junction complex components specify distinct

中国细胞生物学学报

routes of nonsense-mediated mRNA decay with differential cofactor requirements. Mol Cell 2005; 20(1): 65-75.
17 Schmidt SA, Foley PL, Jeong DH, Rymarquis LA, Doyle F, Tenenbaum SA, *et al*. Identification of SMG6 cleavage sites and a preferred RNA cleavage motif by global analysis of endogenous NMD targets in human cells. Nucleic Acids Res 2015; 43(1): 309-23.
18 Yepiskoposyan H, Aeschimann F, Nilsson D, Okoniewski M, Muhlemann O. Autoregulation of the nonsense-mediated mRNA decay pathway in human cells. RNA 2011; 17: 2108-18.
19 Huang L, Lou CH, Chan W, Shum EY, Shao A, Stone E, *et al*. RNA homeostasis governed by cell type-specific and branched feedback loops acting on NMD. Mol Cell 2011; 43(6): 950-61.
20 Singh G, Rebbapragada I, Lykke-Andersen J. A competition between stimulators and antagonists of Upf complex recruitment governs human nonsense-mediated mRNA decay. PLoS Biol 2008; 6(4): e111.
21 Kerenyi Z, Merai Z, Hiripi L, Benkovics A, Gyula P, Lacomme C, *et al*. Inter-kingdom conservation of mechanism of nonsense-mediated mRNA decay. EMBO J 2008; 27(11): 1585-95.
22 Saul H, Elharrar E, Gaash R, Eliaz D, Valenci M, Akua T, *et al*. The upstream open reading frame of the Arabidopsis AtMHX gene has a strong impact on transcript accumulation through the nonsense-mediated mRNA decay pathway. Plant J 2009; 60(6): 1031-42.
23 Fatscher T, Boehm V, Gehring NH. Mechanism, factors, and physiological role of nonsense-mediated mRNA decay. Cell Mol Life Sci 2015; 72(23): 4523-44.
24 Yamashita A, Izumi N, Kashima I, Ohnishi T, Saari B, Katsuhata Y, *et al*. SMG-8 and SMG-9, two novel subunits of the SMG-1 complex, regulate remodeling of the mRNA surveillance complex during nonsense-mediated mRNA decay. Gene Dev 2009; 23(9): 1091-105.
25 Li L, Lingaraju M, Basquin C, Basquin J, Conti E. Structure of a SMG8-SMG9 complex identifies a G-domain heterodimer in the NMD effector proteins. RNA 2017; 23(7): 1028-34.
26 Causier B, Li Z, De Smet R, Lloyd JPB, Van de Peer Y, Davies B. Conservation of nonsense-mediated mRNA decay complex components throughout eukaryotic evolution. Sci Rep 2017; 7(1): 16692.
27 Bono F, Gehring NH. Assembly, disassembly and recycling: the dynamics of exon junction complexes. RNA Biol 2011; 8(1): 24-9.
28 Chamieh H, Ballut L, Bonneau F, Le Hir H. 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.
29 Okada-Katsuhata Y, Yamashita A, Kutsuzawa K, Izumi N, Hirahara F, Ohno S. 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): 1251-66.
30 Yamashita A. Role of SMG-1-mediated Upf1 phosphorylation in mammalian nonsense-mediated mRNA decay. Genes Cells 2013; 18(3): 161-75.
31 Eberle AB, Lykke-Andersen S, Muhlemann O, Jensen TH. SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells. Nat Struct Mol Biol 2009; 16(1): 49-55.
32 Ottens F, Boehm V, Sibley CR, Ule J, Gehring NH. Transcript-specific characteristics determine the contribution of endo- and exonucleolytic decay pathways during the degradation of nonsense-mediated decay substrates. RNA 2017; 23(8): 1224-36.
33 Hug N, Longman D, Caceres JF. Mechanism and regulation of the nonsense-mediated decay pathway. Nucleic Acids Res 2016; 44(4): 1483-95.
34 Namba T, Huttner WB. Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex. Wiley Interdiscip Rev Dev Biol 2017; doi: 10.1002/wdev.256.
35 Rakic P. Evolution of the neocortex: a perspective from developmental biology. Nat Rev Neurosci 2009; 10(10): 724-35.
36 Lou CH, Shao A, Shum EY, Espinoza JL, Huang L, Karam R, *et al*. Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. Cell Rep 2014; 6(4): 748-64.
37 Alrahbeni T, Sartor F, Anderson J, Miedzybrodzka Z, McCaig C, Muller B. Full UPF3B function is critical for neuronal differentiation of neural stem cells. Mol Brain 2015; 8: 33.
38 Bruno IG, Karam R, Huang L, Bhardwaj A, Lou CH, Shum EY, *et al*. Identification of a microRNA that activates gene expression by repressing nonsense-mediated RNA decay. Mol Cell 2011; 42(4): 500-10.
39 Huang L, Shum EY, Jones SH, Lou CH, Dumdie J, Kim H, *et al*. A Upf3b-mutant mouse model with behavioral and neurogenesis defects. Mol Psychiatry 2017; doi: 10.1038/mp.2017.173.
40 Jolly LA, Homan CC, Jacob R, Barry S, Gecz J. The UPF3B gene, implicated in intellectual disability, autism, ADHD and childhood onset schizophrenia regulates neural progenitor cell behaviour and neuronal outgrowth. Hum Mol Genet 2013; 22(23): 4673-87.
41 Barmada SJ, Ju S, Arjun A, Batarse A, Archbold HC, Peisach D, *et al*. Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. Proc Natl Acad Sci USA 2015; 112(25): 7821-6.
42 Mao H, McMahon JJ, Tsai YH. Haploinsufficiency for core exon junction complex components disrupts embryonic neurogenesis and causes p53-mediated microcephaly. PLoS Genet 2016; 12(9): e1006282.
43 Mao H, Pilaz LJ. Rbm8a haploinsufficiency disrupts embryonic cortical development resulting in microcephaly. J Neurosci 2015; 35(18): 7003-18.
44 Silver DL, Watkins-Chow DE, Schreck KC, Pierfelice TJ, Larson DM, Burnetti AJ, *et al*. The exon junction complex component Magoh controls brain size by regulating neural stem cell division. Nat Neurosci 2010; 13(5): 551-8.
45 Colak D, Ji SJ, Porse BT, Jaffrey SR. Regulation of axon guidance by compartmentalized nonsense-mediated mRNA decay. Cell 2013; 153(6): 1252-65.
46 Chen Z, Gore BB, Long H, Ma L, Tessier-Lavigne M. Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. Neuron 2008; 58(3): 325-32.
47 Black DL, Zipursky SL. To cross or not to cross: alternatively spliced forms of the Robo3 receptor regulate discrete steps in axonal midline crossing. Neuron 2008; 58(3): 297-8.
48 Long AA, Mahapatra CT, Woodruff EA 3rd, Rohrbough J, Leung HT, Shino S, *et al*. The nonsense-mediated decay pathway maintains synapse architecture and synaptic vesicle cycle efficacy. J

中国细胞生物学学报

Cell Sci 2010; 123(Pt 19): 3303-15.
49 Zheng S, Gray EE, Chawla G, Porse BT, O'Dell TJ, Black DL. PSD-95 is post-transcriptionally repressed during early neural development by PTBP1 and PTBP2. Nat Neurosci 2012; 15(3): 381-8, S1.
50 Giorgi C, Yeo GW, Stone ME, Katz DB, Burge C, Turrigiano G, *et al*. The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. Cell 2007; 130(1): 179-91.
51 Laumonnier F, Shoubridge C, Antar C, Nguyen LS, Van Esch H, Kleefstra T, *et al*. Mutations of the UPF3B gene, which encodes a protein widely expressed in neurons, are associated with nonspecific mental retardation with or without autism. Mol Psychiatry 2010; 15(7): 767-76.
52 Tarpey PS, Raymond FL, Nguyen LS, Rodriguez J, Hackett A, Vandeleur L, *et al*. Mutations in UPF3B, a member of the nonsense-mediated mRNA decay complex, cause syndromic and nonsyndromic mental retardation. Nat Genet 2007; 39(9): 1127-33.
53 Addington AM, Gauthier J, Piton A, Hamdan FF, Raymond A, Gogtay N, *et al*. A novel frameshift mutation in UPF3B identified in brothers affected with childhood onset schizophrenia and autism spectrum disorders. Mol Psychiatry 2011; 16(3): 238-9.
54 Nguyen LS, Jolly L, Shoubridge C, Chan WK, Huang L, Laumonnier F, *et al*. Transcriptome profiling of UPF3B/NMD-deficient lymphoblastoid cells from patients with various forms of intellectual disability. Mol Psychiatry 2012; 17(11): 1103-15.
55 Lynch SA, Nguyen LS, Ng LY, Waldron M, McDonald D, Gecz J. Broadening the phenotype associated with mutations in UPF3B: two further cases with renal dysplasia and variable developmental delay. Euro J Med Genet 2012; 55(8/9): 476-9.
56 Shaheen R, Anazi S, Ben-Omran T, Seidahmed MZ, Caddle LB, Palmer K, *et al*. Mutations in SMG9, encoding an essential component of nonsense-mediated decay machinery, cause a multiple congenital anomaly syndrome in humans and mice. Am J Hum Genet 2016; 98(4): 643-52.
57 Mooney CM, Jimenez-Mateos EM, Engel T, Mooney C, Diviney M, Veno MT, *et al*. RNA sequencing of synaptic and cytoplasmic Upf1-bound transcripts supports contribution of nonsense-mediated decay to epileptogenesis. Sci Rep 2017; 7: 41517.
58 Tao H, Zhou X, Xie Q, Ma Z, Sun F, Cui L, *et al*. SRR intronic variation inhibits expression of its neighbouring SMG6 gene and protects against temporal lobe epilepsy. J Cell Mol Med 2018; 22(3): 1883-93.
59 Albers CA, Newbury-Ecob R, Ouwehand WH, Ghevaert C. New insights into the genetic basis of TAR (thrombocytopenia-absent radii) syndrome. Curr Opin Genet Dev 2013; 23(3): 316-23.
60 Alachkar A, Jiang D, Harrison M, Zhou Y, Chen G, Mao Y. An EJC factor RBM8a regulates anxiety behaviors. Curr Mol Med 2013; 13(6): 887-99.
61 Favaro FP, Alvizi L, Zechi-Ceide RM, Bertola D, Felix TM, de Souza J, *et al*. A noncoding expansion in EIF4A3 causes Richieri-Costa-Pereira syndrome, a craniofacial disorder associated with limb defects. Am J Hum Genet 2014; 94(1): 120-8.
62 Barker-Haliski ML, Pastuzyn ED, Keefe KA. Expression of the core exon-junction complex factor eukaryotic initiation factor 4A3 is increased during spatial exploration and striatally-mediated learning. Neuroscience 2012; 226: 51-61.
63 Linde L, Kerem B. Introducing sense into nonsense in treatments of human genetic diseases. Trends Genet 2008; 24(11): 552-63.
64 Eom T, Zhang C, Wang H, Lay K, Fak J, Noebels JL, *et al*. NOVA-dependent regulation of cryptic NMD exons controls synaptic protein levels after seizure. eLife 2013; 2: e00178.
65 Huang X, Tian M, Hernandez CC, Hu N, Macdonald RL. The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. Neurobiol Dis 2012; 48(1): 115-23.
66 Kang JQ, Shen W, Macdonald RL. Two molecular pathways (NMD and ERAD) contribute to a genetic epilepsy associated with the GABA(A) receptor GABRA1 PTC mutation, 975delC, S326fs328X. J Neurosci 2009; 29(9): 2833-44.
67 De Roeck A, Van den Bossche T, van der Zee J, Verheijen J, De Coster W, Van Dongen J, *et al*. Deleterious ABCA7 mutations and transcript rescue mechanisms in early onset Alzheimer's disease. Acta Neuropathol 2017; 134(3): 475-87.
68 Cardamone G, Paraboschi EM, Rimoldi V, Duga S, Solda G, Asselta R. The characterization of GSDMB splicing and backsplicing profiles identifies novel isoforms and a circular RNA that are dysregulated in multiple sclerosis. Int J Mol Sci 2017; doi: 10.3390/ijms18112310.
69 Bacrot S, Doyard M, Huber C, Alibeu O, Feldhahn N, Lehalle D, *et al*. Mutations in SNRPB, encoding components of the core splicing machinery, cause cerebro-costo-mandibular syndrome. Hum Mutat 2015; 36(2): 187-90.
70 Lynch DC, Revil T, Schwartzentruber J, Bhoj EJ, Innes AM, Lamont RE, *et al*. Disrupted auto-regulation of the spliceosomal gene SNRPB causes cerebro-costo-mandibular syndrome. Nat Commun 2014; 5: 4483.
71 Zhou Y, Liu S, Liu G, Ozturk A, Hicks GG. ALS-associated FUS mutations result in compromised FUS alternative splicing and autoregulation. PLoS Genet 2013; 9(10): e1003895.
72 Nguyen LS, Kim HG, Rosenfeld JA, Shen Y, Gusella JF, Lacassie Y, *et al*. Contribution of copy number variants involving nonsense-mediated mRNA decay pathway genes to neuro-developmental disorders. Hum Mol Genet 2013; 22(9): 1816-25.
73 Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smethurst PA, *et al*. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. Nat Genet 2012; 44(4): 435-9, s1-2.
74 Klopocki E, Schulze H, Strauss G, Ott CE, Hall J, Trotier F, *et al*. Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. Am J Hum Genet 2007; 80(2): 232-40.
75 Choudhury SR, Singh AK, McLeod T, Blanchette M, Jang B, Badenhorst P, *et al*. Exon junction complex proteins bind nascent transcripts independently of pre-mRNA splicing in *Drosophila* melanogaster. Elife 2016; doi: 10.7554/eLife.19881.
76 Le Hir H, Sauliere J, Wang Z. The exon junction complex as a node of post-transcriptional networks. Nature reviews. Mol Cell Biol 2016; 17(1): 41-54.
77 Woodward LA, Mabin JW, Gangras P, Singh G. The exon junction complex: a lifelong guardian of mRNA fate. Wiley Interdiscip Rev RNA 2017; doi: 10.1002/wrna.1411.
78 Keeling KM. Nonsense suppression as an approach to treat lysosomal storage diseases. Diseases 2016; doi: 10.3390/dis-

中国细胞生物学学报

eases4040032.

79 Usuki F, Yamashita A, Higuchi I, Ohnishi T, Shiraishi T, Osame M, *et al*. Inhibition of nonsense-mediated mRNA decay rescues the phenotype in Ullrich's disease. Ann Neurol 2004; 55(5): 740-4.

80 Moriniere M, Delhommeau F, Calender A, Ribeiro L, Delaunay J, Baklouti F. Nonsense-mediated mRNA decay (NMD) blockage promotes nonsense mRNA stabilization in protein 4.1R deficient cells carrying the 4.1R Coimbra variant of hereditary elliptocytosis. Blood Cells Mol Dis 2010; 45(4): 284-8.

81 Usuki F, Yamashita A, Kashima I, Higuchi I, Osame M, Ohno S. Specific inhibition of nonsense-mediated mRNA decay components, SMG-1 or Upf1, rescues the phenotype of Ullrich disease fibroblasts. Mol Therapy 2006; 14(3): 351-60.

82 Bhuvanagiri M, Lewis J, Putzker K, Becker JP, Leicht S, Krijgsveld J, *et al*. 5-azacytidine inhibits nonsense-mediated decay in a MYC-dependent fashion. EMBO Mol Med 2014; 6(12): 1593-609.

83 Shao A, Wilkinson MF. Suppressing nonsense—a surprising function for 5-azacytidine. EMBO Mol Med 2014; 6(12): 1518-20.

84 Sartor F, Anderson J, McCaig C, Miedzybrodzka Z, Muller B. Mutation of genes controlling mRNA metabolism and protein synthesis predisposes to neurodevelopmental disorders. Biochem Soc Trans 2015; 43(6): 1259-65.

中国细胞生物学学报