Internal Ribosome Entry Site-Dependent Translation Dysregulation-Related Diseases

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Internal ribosome entry site (IRES)-mediated translation is an alternative mechanism of translation initiation, known for maintaining protein synthesis when canonical translation is impaired. During a stress response, it contributes to cell reprogramming and adaptation to the new environment.

IRES trans-acting factors | antisense oligonucleotides | IRES-based multicistronic vectors

1. Introduction

Internal ribosome entry site (IRES)-dependent translation in humans, either of mono- or polycistronic transcripts, is associated with many diseases. Cancer is, by far, the most well-characterised set of conditions affected by IRES or ITAF dysregulation. However, it is widely appreciated that dysregulation of IRES-mediated translation is also associated with other pathologies.

2. Neurodegenerative Diseases

2.1. Spinocerebellar Ataxia Type 6 (SCA6)

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant inherited neurodegenerative disease, presenting an incidence of about 5/100,000 persons. SCA6 is a late-onset progressive disease, in which the patients present progressive cerebellar ataxia and atrophy, and simultaneous selective Purkinje cell degeneration typically developed from the age of 40 [1]. SCA6 is caused by the polyQ expansion in α1ACT, which is translated through an IRES upstream of the second cistron of the CACNA1A gene. It was demonstrated that the elimination of the CACNA1A IRES sequence led to the abolition of the expression of the SCA6-associated α1ACT ($\alpha1ACT_{SCA6}$) protein. Mutated mice with the extended α1ACT presented a considerable reduction of the molecular layer thickness and a 50% loss of Purkinje cell dendritic tree density, which correspond to pathological features of SCA6 [1]. Since total silencing of CACNA1A gene expression would be lethal, selective elimination of α1ACT expression could be a safer therapeutic attempt for SCA6 [2]. For example, a CACNA1A IRES-targeted therapeutic method, using the expression of specific miRNAs, could offer a better approach for treating SCA6 [2][3]. The miRNA specifically interacts with CACNA1A IRES through the predicted binding site and inhibits α1ACT IRES-driven translation, without impairing α1A expression and CACNA1A mRNA expression [1]. Results have shown that the treatment promotes the protection of the Purkinje cells from degenerative changes, by inhibiting the degeneration caused by CACNA1A IRES-driven $\alpha1ACT_{SCA6}$ [1]. Additionally, the mice also exhibited an improvement in gait instability in all four limbs and avoid weaving movement, performing significantly better. In conclusion, these studies proved that the directed RNA-based therapy to selectively prevent α1ACT IRES-mediated expression could be used to treat SCA6 [1]. In the future, the use of ASOs and RNAi approaches would also be promising strategies to target and modulate α1ACT IRES-mediated expression [4].

2.2. Fragile X Syndrome

Fragile X syndrome (FXS) causes intellectual disability and autism. It is the most common hereditary neurological condition and is a consequence of the lack of fragile X mental retardation protein (FMRP) [5]. FMRP is an RNA-binding protein present in the brain, responsible for controlling the translation of several neuronal mRNAs and synaptic functions and structures [6]. While healthy individuals present about 30 repeats of CGG in the 5′ UTR of the fragile X mental retardation 1 (fmr1) gene, patients with FXS have over 200 repeats of CGG, which promotes the sequence hypermethylation. This causes the transcriptional inhibition of the fmr1 gene and consequent absence of FMRP, promoting an impairment of synaptic responses [7]. It was proven that fmr1 translation uses both cap-dependent and IRES-mediated mechanisms, as it contains an IRES in its 5′ UTR. Also, it was shown that fmr1 IRES-mediated translation occurs with the involvement of the hnRNPQ as ITAF [7]. However, little is known about the effect of this mechanism on FXS development. In neuron development, the axonal growth cone of a neuron travels large distances to connect to the dendritic spine of the next neuron, depending on axonal guidance cues like semaphorins to direct the appropriate connection. In semaphorin 3A (Sema3A) treatment, a neuronal repellent that induces growth cone collapse, hnRNPQ synthesis in primary hippocampal neurons increases, which, in turn, when up-regulated, leads to Sema3A-induced growth cone collapse and consequent FMRP synthesis. Depletion of fmr1 expression by siRNA, under treatment with Sema3A, prevented axonal growth cone collapse, which is also attenuated by reducing hnRNPQ expression. It was demonstrated that hnRNPQ over-expression restores IRES-mediated fmr1 translation activity in hnRNPQ knockout cells, thus increasing FMRP expression. Thus, hnRNPQ acts as an ITAF that activates IRES-mediated fmr1 translation, contributing to restoring FMRP levels, and, simultaneously, participates in Sema3A-induced axonal growth cone collapse [8]. Considering this dual effect, the role of fmr1 IRES-mediated translation and its ITAF regulation on both conditions and associated pathologies is crucial in the development of novel specific therapeutic approaches [7]. Also, there have already been described several mRNA targets of FMRP, which could be used to develop new therapies, taking into account that the expression of FMRP is modulated by m^6A modifications that can disrupt its binding to the respective targets [9].

2.3. Alzheimer’s Disease

Alzheimer’s disease (AD), a neurodegenerative disorder, is the most frequent type of dementia in the elderly. Autosomal dominant inherited forms of AD correspond to no more than 5% of the cases, the remaining of sporadic origin. The disease is characterised by the accumulation of extracellular amyloid-β (Aβ) plaques, which consist mostly of Aβ peptide precursor protein (APP), and an increase in the aggregation of tau in neurofibrils within neurons [10]. APP is a type I membrane protein encoded by the APP gene, which presents more than 25 pathogenic mutations, all causing an autosomal dominant form of AD, hence being strongly linked to the pathogenesis of AD. Data have shown that APP over-expression leads to an increase in full-length and truncated p53 (p53 and p44, respectively) expression levels in the brain tissue [11]. This leads to cognitive decline and synaptic and memory defects [11]. By analysing the levels of p44 in mice brains, it was observed a consistent and statistically significant increase in the levels of p44 when APP was over-expressed. However, no change in p44 levels was observed when APP was lacking, suggesting that APP induction of p44 expression is not required for p44 baseline levels [11]. Amyloid precursor protein intracellular domain (AICD), the cytosolic tail of APP, binds to the p53 IRES and regulates the translation of p44. When APP is over-expressed, mice rapidly develop AD-like neuropathology, indicating a possible link between ageing and AD [11]. Also, transgenic mice over-expressing AICD developed some of the features that characterise AD, such as abnormal activation or phosphorylation of tau kinases, synaptic deficits, and higher neuronal susceptibility to exogenous stress [11]. Reports have shown that patients with late-onset AD express increased levels of p44 [11][12]. Several proteins have been proven to be ITAFs of p53 and to regulate the translation of p53 isoforms, as is the case of nucleolin and PTBP1. While nucleolin seems to produce a negative

effect on p53 translation and decrease it in an age-dependent manner in the brain, AICD appears to be a positive factor [11]. So, there is a connection between AICD and p44 that may be involved in AD and other age-related tauopathies. Indeed, the role of p44 in longevity and cognitive-related events is complex requiring further studies. Further research has shown that m^6A levels are also decreased in AD brains, as a consequence of significantly reduced expression of METTL3 [13]. A natural product and small-molecule inhibitor of fat mass and obesity-associated protein (FTO) demethylase, rhein, can partially rescue this scenario, therefore having a promising therapeutic use [13]. There have been described several circRNAs and long non-coding RNAs dysregulated in AD patients [14], but their relationship with IRES-mediated translation initiation is yet to be clearly understood. However, they have the potential to become promising therapeutic targets to address such diseases [15].

2.4. Parkinson's Disease

Under hypoxia, the major transcription factor hypoxia-inducible factor (HIF)-1α binds to hypoxia-responsive elements (HREs) in the promoter to up-regulate HRE-containing genes [16]. This event regulates several cellular processes, like glucose metabolism, biosynthetic pathways, cellular metabolism reprogramming and cell viability [17][18]. There are numerous lines of evidence linking HIF-1α to Parkinson's disease (PD) [19]. PTEN-induced putative kinase-1 (PINK1) is a serine/threonine kinase that has several distinct functions on the mitochondria and cytosol, promotes cell survival, and activates the HIF-1α pathway. PINK1 mutations that contribute to protein instability or decreased kinase activity are linked to autosomal recessive Parkinson's disease. In the absence of wild-type PINK1, HIF-1α protein induction under hypoxia is reduced and cells present bioenergetic and mitochondrial unbalances, which are present in both sporadic and genetic forms of PD [19]. It seems that PINK1 exhibits protective effects against various oxidative stresses and facilitates stress response, due to the activation of 4E-BP1 and consequent up-regulation of IRES-dependent translation. On the contrary, in PINK1 deficiency, over-expressed 4E-BP1 fails to up-regulate IRES-dependent translational activity significantly. It was shown in some experiments that 4E-BP1 over-expression rescued the PINK1 deficient phenotype [19]. Additionally, HIF-1α and its targets are required to preserve dopaminergic neuron integrity, which might explain how HIF-1α loss can promote neurodegeneration in PD. The connection between translation and PD was also strengthened by a study that linked mutations in eIF4G1 with a familial case of PD and by the findings that 4E-BP1 may be a leucine-rich repeat kinase 2 (LRRK2) target, which is another protein associated with PD [19]. In conclusion, PINK1 is an important regulator of translation during stress response and an activator of the HIF-1α pathway, promoting the maintenance of energy metabolism and cell survival. It is yet to discover the role of HIF-1α and protein translation in PD pathogenesis, which will lead to better development of neuroprotective strategies. On the other hand, since alterations in the gene encoding α-synuclein (aSyn) protein can cause or increase the risk of developing PD, Cole et al. tested ASOs targeting the corresponding mRNA and observed inhibition in the protein synthesis that reverted the phenotype in rodent pre-formed fibril models of PD [20]. This supports the further use of strategies to correct the expression of mutated PINK1 ITAF and, thus, restore its protective effect.

2.5. Amyotrophic Lateral Sclerosis and Other Neurological Conditions

hnRNPA2/B1, hnRNPA1, and fused in sarcoma (FUS) are RBPs often associated with neurological diseases and, therefore, it is of the utmost importance to maintain RBP physiological levels in the nervous system. Patients with motor neuron disorders, such as amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy (SMA), among others, present mutations in genes encoding these RBPs. ALS is the commonest motor neuron disorder in adults, causing a gradual loss of upper and lower motor neurons and, eventually, fatal paralysis. The causes of ALS are yet to be understood, whereas most cases are sporadic and only 10% are hereditary [21]. There is a link between hnRNPA1 mutations and multisystem proteinopathies (MSP), a group of pleiotropic

neurodegenerative disorders that includes ALS [22]. Furthermore, this protein, alongside hnRNPA2/B1, seems to be depleted in the brains of AD patients, and misfolding and fibrilization of this protein have been associated with the disease [23][24]. Decreased levels of hnRNPA2/B1 and hnRNPA1 in the entorhinal cortex of patients have been implicated in the pathogenicity of sporadic AD [21]. These data correlate cholinergic neuron loss with reduced hnRNP levels and missplicing, which might explain some cognitive deficits observed in AD. Mutations in FUS have also been linked to neurological diseases, such as frontotemporal lobar degeneration (FTLD), essential tremor, and Huntington's disease [25][26]. It is important to notice that nearly every protein discussed above is involved in RNA transport and somehow in RNA splicing, thus, motor and nerve cells are also prone to be affected by dysregulations in these processes [21]. There is little information about the importance of IRES-mediated translation initiation in the development of these neurological conditions; however, the proteins related to such conditions act as ITAFs for a wide range of IRESs, meaning that their function may be compromised or dysregulated, either enhanced or inhibited and, for that reason, creating an imbalance in protein homeostasis that eventually affects the onset and development of the aforementioned conditions. Understanding the pathophysiological role of such RBPs will provide new prospects for research and the eventual development of RNA-based therapies targeting these diseases.

3. Muscular Atrophies

3.1. Ischemic Cardiomyopathy (Lymphangiogenesis Regulation)

Hypoxia is a key component of the tumour microenvironment and induces critical changes in tumour cell metabolism, angiogenesis and lymphangiogenesis [27]. However, hypoxia also constitutes major stress in other pathologies, such as ischemic pathologies, in which artery occlusion leads to hypoxic conditions, and then angiogenesis is promoted as a cellular response to fight the lack of oxygen and nutrients in cells. It has been shown that (lymph)angiogenesis is also induced by hypoxia and mediated at both transcriptional and post-transcriptional levels [27][28]. VEGF-A and FGF2, major angiogenic factors, and the lymphangiogenic growth factor VEGF-C are all induced by hypoxia through a translational mechanism [27]. As is already mentioned above, several IRESs have been identified in the mRNAs of (lymph)angiogenic growth factors from the FGF and VEGF families, suggesting that the activation of angiogenesis and lymphangiogenesis during stress might be highly controlled via IRES-mediated translation [28]. VEGF-A has pro-lymphangiogenic properties and its induction under hypoxia occurs in both physiological states and pathological conditions, such as ischemia or tumour development [29]. During hypoxia, VEGF-A IRES activity is positively regulated by MAPK3 kinase and hnRNPL, and inhibited by DEAD-box RNA helicase 6, all acting as its ITAFs [27]. On the other hand, VEGF-C induces endothelial cell proliferation, migration, and survival, and, during tumour growth and under hypoxia in vitro, VEGF-C IRES activity was demonstrated to be up-regulated [27][30]. Furthermore, VEGF-D over-expression correlates with an increase in lymphatic vessel growth (tumour lymphangiogenesis) and lymphatic metastasis [27]. It appears to exist two waves of IRES activation in response to hypoxia: a first response phase corresponding to early hypoxia, in which IRESs from (lymph)angiogenic growth factor mRNAs are activated, while a second response includes "non-angiogenic" c-myc IRES, which is activated in late hypoxia [28]. Vasohibin 1 (VASH1), angiogenesis- and stress-related protein, has already been described for its expression in endothelial cells and HL-1 cardiomyocytes. Vash1 mRNA translation is highly induced in early hypoxia and leads to a strong expression of VASH1, whose knockdown down-regulates earliest-induced IRESs, like FGF1, proposing this protein as a new ITAF in cardiomyocytes. Thus, under hypoxia, VASH1 is an activating ITAF of FGF1 and VEGF-D IRESs, but in normoxia, it acts as an inhibitor. This suggests that VASH1 interacts with different partners in the IRESome or that exist different VASH1 isoforms, implying that the main response to early hypoxia in cardiomyocytes is at the translation level [28]. All these results are crucial for a better understanding of the acute stress response in the ischemic heart. Since the role of hypoxia

in gene expression regulation has been mostly analysed in conditions of tumoral hypoxia, in which angiogenesis promotes the formation of abnormal vessels with a lack of function, it is important to study the response to hypoxia in the context of ischemic diseases. Indeed, HL-1 cardiomyocytes respond to hypoxia very early, whereas various human tumour cell lines require a longer time of exposure to hypoxia for IRES-dependent translation to be stimulated [28].

3.2. Myogenesis Regulation

Although this is not a pathology per se, myogenesis regulation influences cell proliferation and differentiation of several cells, such as cardiomyocytes, due to different IRESs and ITAFs related to myogenesis, with further influence in cardiac diseases or cardiomyopathies. While FGF1 and FGF2 inhibit myoblast differentiation, FGF1 also activates such a process, thanks to a transcription-translation coupling mechanism [31]. There are four promoters (A, B, C, and D) that drive transcription of FGF1: while promoter A is specifically active in the heart, skeletal muscle, and kidney, and promoter B in the brain, C and D are inducible and related to cell proliferation. As an outcome of eIF4E sequestering by 4E-BP-1 [31], cap-dependent translation initiation is impaired during the early stages of myoblast differentiation, which results in the specific activation of FGF1 IRES A through the myoblast differentiation process [32]. hnRNPM and $p54^{nrb}$/NONO act together to activate FGF1 IRES-mediated translation in a promoter-dependent manner, by binding to promoter A and IRES A [31][32]. This specific activation of FGF1 mRNA accumulation and stability, due to hnRNPM and $p54^{nrb}$ binding, is correlated with the induction of differentiation but is very weak during cardiomyocyte proliferation. $p54^{nrb}$/NONO and hnRNPM are also important in myogenesis, as they are needed for myotube differentiation from myoblasts, which suggests that both proteins may work as activator ITAFs of FGF1 IRES, despite no evidence of the direct interaction of $p54^{nrb}$ and hnRNPM with the RNA [32]. All in all, FGF1 expression is controlled by the promoter and the translational regulating factors during myoblast proliferation and differentiation [32]. On the other hand, several circRNAs are involved in several muscular processes, such as myoblast proliferation and differentiation, and muscular development [33][34]. It remains to be deciphered the role of circRNA IRES-mediated translation in these muscular processes. This can be a great basis on which to develop therapeutic strategies for muscular disorders.

3.3. Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most prevalent inherited neuromuscular disorder, with a prevalence of 1 in 3500 male births [35]. DMD develops due to deletions/mutations in the dystrophin gene, which prevents the production of full-length dystrophin molecules in skeletal muscle fibres. There have already been developed ASOs to correct splicing and restore the dystrophin levels in DMD patients [36][37]. Utrophin, the autosomal homologue of dystrophin, presents a high structural and functional similarity with the latter [38]. Utrophin A is the isoform expressed in skeletal muscles primarily in post-synaptic regions of the sarcolemma and its increased expression was identified in regenerating skeletal muscles. [39]. However, utrophin A mRNA levels did not increase concomitantly, suggesting that the increase in protein levels might be caused by changes in protein stability or translation efficiency, including the possibility of an IRES-dependent translation mechanism driving its expression [39]. Using bicistronic reporter vectors, it was demonstrated that the utrophin A 5′ UTR demonstrated no IRES activity in intact muscles. Indeed, the 5′ UTR of utrophin A causes a translation inhibition in skeletal muscle fibres under control conditions, whereas in regenerating muscles there is an IRES activation that accounts for utrophin A protein expression [40][41]. There is evidence that, in vivo, cap-independent translation driven by the utrophin A IRES occurs exclusively in skeletal muscles [39]. eEF1A2 (one of the two eEF1A isoforms) interacts with the utrophin A 5′ UTR in the same regions that can drive cap-independent translation in C2C12 myoblasts [39]. Mice that do not express functional eEF1A2 show motor neuron and muscle degeneration, which eventually leads to premature death [39]. However, eEF1A2 might not be the only protein required for skeletal muscle-specific utrophin A

IRES activity [39]. For instance, FGF2 improves regeneration when injected into the muscles of mice, whereas insulin growth factor (IGF) 1 receptor expression is up-regulated in muscle regeneration. Of note is that FGF2 5′ UTR contains an IRES and IGF-1 translation is also IRES-dependent [42]. In this regard, muscle regeneration may be considered a "cellular stress" that promotes IRES-mediated translation [41]. Given all the data, the up-regulation of endogenous levels of utrophin in muscle fibres of affected patients could functionally outweigh the absence of dystrophin and, thus, be used as a possible DMD treatment [43]. Over-expression of utrophin in muscle fibres of a DMD mouse model has been shown to alleviate the dystrophic pathology, proving how the regulation of utrophin expression could contribute to important therapeutic advances [41].

4. Other Specific Diseases

4.1. Diamond-Blackfan Anaemia

Diamond-Blackfan anaemia (DBA) is normochromic macrocytic anaemia characterised by the reduced erythroid precursors in the bone marrow, which is mostly diagnosed in infants less than one year of age, yet, recently, some cases have been diagnosed in adult patients [44]. About 50% of DBA patients have skeletal deformities, such as thumb malformations and growth retardation. In 55% of patients, DBA is associated with mutations in genes encoding ribosomal proteins, causing their haploinsufficiency and loss of function, ultimately impairing general translation [45]. An imbalance in the synthesis of ribosomal proteins activates p53, to which erythroblasts are extremely sensitive, inhibits cell proliferation, and may affect the translation initiation of specific transcripts important for erythroid differentiation, suggesting that DBA-associated severe anaemia is caused by a p53-dependent mechanism [45]. Knockdown of 40S ribosomal protein S19 (Rps19) in haematopoietic progenitors decreases the colony-forming capacity of erythroid progenitors, while in mouse foetal liver-derived erythroblasts impairs their proliferation, but not their differentiation [45]. Also, the knockdown of both Rps19 and Rpl11 resulted in phenotypical changes in erythroblasts during proliferation and differentiation [45]. Furthermore, reduced expression of Rps19 or Rpl11 repressed the translation of two essential transcripts for erythropoiesis, Bag1 and Csde1, which are both translated from an IRES and tightly up-regulated in erythroid cells [45]. Csde1 is an RNA-binding factor that controls IRES-mediated translation, despite no regulation of Bag1 mRNA [45]. Protein levels of Bag1 and Csde1 in erythroblasts from DBA patients are also low, although RNA expression is not affected [45]. The reduction of Csde1 expression inhibits both proliferation and maturation of erythroblasts, while the complete loss of Bag1 expression strongly impairs erythropoiesis and its reduction makes erythroblasts less prone to enter the terminal differentiation program [45]. This indicates that a reduction in Bag1 and Csde1 expression results in severe DBA due to a cooperative effect between them. All in all, the overall DBA phenotype seems to be caused by a combination of p53 activation and a defective mRNA translation [45]. Since p53 translation may be mediated through an IRES, it is plausible to assume that dysregulation of p53 IRES-mediated translation accounts for the development of the disease. In this regard, new therapies targeting p53 IRES would impair its translation and therefore reduce the erythroblast sensitivity to p53 activation. Following the same line, therapies modulating the expression of p53 IRES regulatory ITAFs would contribute to regulating erythroblast sensitivity.

4.2. Diabetes

The insulin receptor (INR) and insulin-like growth factor (IGF) receptor pathways are essential for the evaluation and response to nutrient availability, also playing an important role in cellular proliferation regulation and cell size determination. Prolonged exposure of cells to insulin induces insulin receptors (INR) down-regulation via internalization and enhanced protein degradation, which causes an imbalance and can lead to type 2 diabetes in humans and other associated diseases [46]. It was confirmed the existence of a functional IRES on the INR 5′ UTR, which is

strongly stimulated in the presence of PTB1 and nPTB, and slightly less stimulated in the presence of hnRNPK and PTB2. The 5′ UTR of IGF-1R mRNA also contains an IRES, as does the Drosophila insulin/IGF-like receptor (dINR) mRNA, which binds to HuR, a stability factor that inhibits translation, and hnRNPC, which enhances IRES-mediated translation and competes with HuR for the binding site [46]. There are differences in the location and sequence of the INR and IGF-1R IRESs, strongly suggesting different mechanisms of regulation, and distinct dependence on cell type and density [46]. Insulin itself could also stimulate IRES activity. Both INR and IGF-1R are expressed in the nervous system and have been correlated to important roles in neuronal development and protection from and/or promotion of age-related neurodegenerative diseases [46]. In Drosophila, the signalling cascade of insulin receptors activates the oncogenic protein kinase Akt, stimulating the modification and posterior phosphorylation of mTOR protein, which, in turn, inactivates the translation initiation inhibitor eIF4E-binding protein (d4E-BP) [47]. During high nutrient and high insulin-like peptide presence, d4E-BP is phosphorylated and inactive, unable to interact with eIF4E. This favours effective translation of many cellular transcripts no matter what mechanism of initiation is used. In contrast, in nutrient deficiency conditions or the absence of insulin, d4E-BP become dephosphorylated and active, inhibiting cap-dependent translation, and endorsing a selective translation of IRES-containing transcripts, such as dINR [47]. INR IRES may simply function to maintain the expression level of INR, fighting the inhibition of cap-dependent initiation under such conditions, including, perhaps, in some differentiated cells. However, in the case of the IGF-1R, there is evidence that regulation of expression does occur at a translational level [46]. All this information could provide new insight into insulin-resistant type 2 diabetes development.

All the mentioned pathologies and their related IRESs and ITAFs are summarised in **Table 1**. By looking at the information gathered in the table, there is a missing link between the knowledge of IRES and their ITAF regulation and the use of RNA-based therapies to specifically target IRES elements or their regulating ITAFs. Again, the use of IRES-based vectors to express proteins that allow the rescue of some of the corresponding wild-type phenotypes is missing for many diseases other than cancer.

Table 1. Summary of different groups of diseases, other than cancer, caused by IRES-mediated translation initiation misregulation. Here are listed the internal ribosome entry sites (IRESs) and IRES trans-acting factors (ITAFs) correlated to each pathology. The existing IRES-related RNA-based therapies for each pathology are also included.

Pathologies	IRES-Containing Transcripts	Related ITAFs	Tested RNA-Based Therapies	References
Spinocerebellar ataxia type 6	CACNA1A	n.i. *	miRNA-based therapy	[2][3]
Fragile X syndrome	fmr1	hnRNPQ	n.i. *	[7]
Alzheimer’s disease	p53 (p44 isoform)	APP (AICD), nucleolin	n.i. *	[11]

	Pathologies	IRES-Containing Transcripts	Related ITAFs	Tested RNA-Based Therapies	References
Neurodegenerative diseases	Parkinson's disease	HIF-1α	PINK1	Antisense oligo nucleotide reducing the expression of α-synuclein pathogenic protein	[19][20]
	Amyotrophic lateral sclerosis		Related RBPs: hnRNPA2/B1, hnRNPA1, FUS	n.i. *	[21]
Muscular atrophies	Ischemic cardiomyopathy	VEGFA, VEGFC, FGF1	hnRNPL, VASH1	n.i. *	[27][28]
	Myogenesis regulation	FGF1/FGF2	hnRNPM, $p54^{nrb}$	n.i. *	[32][48]
	Duchenne muscular dystrophy	utrophin A	eEF1A2	IRES over-expression by small molecules	[39][49]
Other diseases	Diamond-Blackfan anaemia	Bag1/Csde1, p53	Rps19, Rpl11	n.i. *	[45]
	Diabetes	INR/IGF-1R	PTBP1, HuR, hnRNPC	miRNA-based therapy	[46][47]

* n.i.: no information available.

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