REVIEW

The importance of pseudouridylation: human disorders related to the fifth nucleoside

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Abstract

Pseudouridylation is one of the most abundant RNA modifications in eukaryotes, making pseudouridine known as the "fifth nucleoside." This highly conserved alteration affects all non-coding and coding RNA types. Its role and importance have been increasingly widely researched, especially considering that its absence or damage leads to serious hereditary diseases. Here, we summarize the human genetic disorders described to date that are related to the participants of the pseudouridylation process.

Keywords Pseudouridylation · Pseudouridine · RNA modification

Introduction

The variability of the phenotype is primarily explained by genomic sequence changes. Accordingly, Mendelian disorders are generally caused by single nucleotide variations of the coding sequence. Nevertheless, epigenetic modifications largely contribute to the phenotype variability, and its severe forms can be even disease-causing (Araki and Mimura 2018, Cullell, Soriano-Tárraga et al. 2022, Derakhshan et al. 2022, Ojaimi, Banimortada et al. 2022, Pierce and Black 2022). Besides DNA methylation and histone modifications, RNAs are also subjects to epigenetic modifications that have serious impact on their function, implying a potential role in disease development (Han and Phizicky 2018; Ontiveros et al. 2019; Barbieri and Kouzarides 2020; Haruehanroengra et al. 2020; Destefanis et al. 2021, Kumari, Groza et al. 2021). Almost 200 different types of RNA modifications associated with 175 human diseases have thus far been identified according to the MODOMICS database (Boccaletto et al. 2022).

All types of RNAs, including transfer RNAs (tRNA), ribosomal RNAs (rRNA), messenger RNAs (mRNA) and other non-coding RNAs, undergo modifications during their maturation (Boccaletto et al. 2022). Nuclear and mitochondrial tRNAs are the subjects of the most diverse modifications. The tRNA and rRNA modifications were studied most extensively, because of their highest expression rate. Nevertheless, the modifications of the mRNA are also of special importance as they may directly affect the translation (Schwartz 2016; Helm and Motorin 2017; Zhao et al. 2017; Linder and Jaffrey 2019).

One of the most abundant RNA modifications in eukaryotic cells is pseudouridylation: the transformation of uridine to its other isoform called pseudouridine (Ψ).(Fig. 1). Some specific pseudouridylation sites are already present in archaebacteria, eubacteria and in the organelles of eukaryotic cells, such as mitochondria and chloroplasts (Charette and Gray 2000). Based on the conservation of these sites, they seem to be essential modifications, but no lethal or severe phenotypes were found related to most of them in yeasts or eubacteria. In these species, their major role is supposed to be the fine tuning of the RNA structure (Charette and Gray 2000). In mammals, the role of RNA pseudouridylation is supposed to be more remarkable. Inducible pseudouridylation of mammalian mRNA has a stimuli-specific pattern, which may help the cell in adaptation to stress situations. Heat shock and H_2O_2 treatment activate hundreds of different inducible pseudouridylation sites in mRNAs that are implicated in transport- and telomere- or chromatin-related functions, respectively (Li et al. 2015).

Most of the uridine-pseudouridine transformations occur in tRNAs, rRNAs and snRNAs (Charette and Gray 2000;

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Fig. 1 Isomerisation of uridine, schematic figure
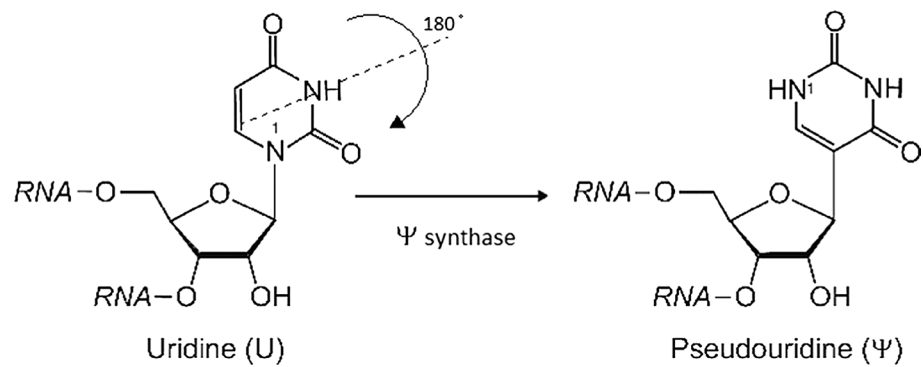
Spenkuch et al. 2014), but as described recently, it can also be found in mRNAs (Carlile et al. 2014; Lovejoy et al. 2014; Schwartz et al. 2014; Li et al. 2015). More than 100 specific uridines are known to be pseudouridylated in rRNAs, which maintain the proper functioning, folding and conformational stability of the rRNAs, their associations with ribosomal proteins, and thus ensure the catalytic activity of the ribosome (Decatur and Fournier 2002; King et al. 2003; Liang et al. 2009; Natchiar et al. 2017; Abou Assi et al. 2020). The modified uridines are typically located in evolutionarily conserved regions close to functional domains (Natchiar et al. 2017). Alteration of rRNA pseudouridylation thus directly influences the interactions with tRNAs and mRNAs, modifying translational efficiency, gene expression patterns and levels (Jack et al. 2011; Bastide and David 2018).

Two main types of enzymes catalyze the pseudouridylation. Stand-alone pseudouridine synthases (PUSs) — that are classified into six families (TruA, TruB, TruD, RsuA, RluA, PUS10) (Hamma and Ferré-D'Amaré 2006; Roovers et al. 2006; Rintala-Dempsey and Kothe 2017) — can recognize directly, without a guide RNA, the target uridine(s), and implement the modification by themselves (Hamma and Ferré-D'Amaré 2006). While bacteria use only stand-alone PUSs, eukaryotes also possess H/ACA small nucleolar ribonucleoprotein complexes for this purpose. This latter type of pseudouridine synthase necessitates a unique guide RNA specific for the target uridine and four core proteins: non-histone protein 2 (NHP2), nucleolar protein 10 (NOP10), glycine-arginine-rich protein 1 (GAR1) and the catalytically active dyskerin (DKC1), the sequence of which is similar to that of the TruB PUS family members (Lafontaine et al. 1998; Ramamurthy et al. 1999; Spedaliere et al. 2000; Kiss et al. 2004; Khanna et al. 2006; Penzo and Montanaro 2018). The H/ACA box small-nucleolar RNAs (snoRNA, "SNORA") guide the enzymatic protein complex to the substrate via site-specific complementary base-pairing at the target site (Ganot et al. 1997; Sumita et al. 2005; Henras et al. 2008; McMahon et al. 2015; Rintala-Dempsey and Kothe 2017; Czekay and Kothe 2021). The

rRNA pseudouridylation is primarily mediated by the H/ACA snoRNP complex: Knockdown of dyskerin decreases it by more than half (Schwartz et al. 2014). Nevertheless, at least 76 so-called orphan snoRNAs are known in human that are not complementary to any rRNAs (Ono et al. 2010; Gong et al. 2017), suggesting that they guide the modifications of mRNAs or other RNA classes (Kishore and Stamm 2006; Chu et al. 2012; Kishore et al. 2013; Bieth et al. 2015; Falaleeva et al. 2016). Site-specific synthases that modify mRNAs are also found among stand-alone PUSs (TRUB1, PUS7 and PUS1) (Li et al. 2015; Safra et al. 2017; Carlile et al. 2019). PUS enzymes are encoded in humans by 13 genes, out of which four (*PUS1, PUS3, PUS7* and *DKC1*) are associated with genetic diseases (Bykhovskaya et al. 2004; Shaheen et al. 2016; de Brouwer et al. 2018; Borghesi et al. 2022).

Besides pseudouridylation, these enzymes also mediate other processes. Stand-alone PUSs may function as RNA chaperons or as part of multiprotein complexes, as their absence causes more severe phenotypes in bacteria and yeasts than the lack of some pseudouridines per se (Grosshans et al. 2001; Ishitani et al. 2003; Hamma and Ferré-D'Amaré 2006). The H/ACA box small nucleolar ribonucleoprotein complexes also participate in the cleavage of the precursor of 18S rRNA and in maintaining telomerase activity (Heiss et al. 1998; Hamma and Ferré-D'Amaré 2006). Dyskerin is associated with — besides the H/ACA snoRNAs—telomerase RNA, which also contains a H/ACA RNA motif and serves as template to telomere elongation (Mitchell et al. 1999a, b).

Despite being an ubiquitous process, the deficiency in pseudouridylation leads to organ-specific defects. The description of the related human disorders (Summary in Table 1.) (Heiss et al. 1998; Ruggero et al. 2003; Montanaro et al. 2006; Jonkhout et al. 2017) and unraveling their pathophysiology may help to understand what makes these organs more susceptible than others to the defect of the most abundant RNA modification.

Human diseases related to DKC1, dyskerin

Secondary to the Xq28 localization of the *DKC1* gene, its mutations cause X-linked disorders (Devriendt et al. 1997; Heiss et al. 1998; Mitchell et al. 1999a, b). Most of the related disorders are recessive: Males and heterozygous females with extremely skewed X-inactivation are affected (Alder et al. 2013). There are, however, exceptions: the *DKC1* p.E206K-related cataracts and hearing impairment are transmitted in a dominant fashion: Heterozygous females are typically affected (Balogh et al. 2020).

As dyskerin contributes to various processes, such as pseudouridylation of different types of RNA, telomere lengthening and RNA maturation, the molecular mechanisms of most of the associated disorders are challenging to differentiate.

Dyskeratosis congenita (DC) (Mitchell et al. 1999a, b; Vulliamy et al. 2001; Alder et al. 2013; AlSabbagh 2020), the disease that gave dyskerin its name, results from telomere shortening. Mutations of several genes, some of which are implicated only in telomere maintenance but not in pseudouridylation, cause DC, indicating that the pathophysiology is independent of the pseudouridylation defect.

Telomeres, located at the end of chromosomes, protect their integrity and structure. Their length and stability are maintained by complexes of various proteins and RNAs. The main participants of the telomerase complex are: TERT: the reverse transcriptase enzyme; TERC: the RNA template for the telomere elongation; and dyskerin, NHP2, NOP10 and GAR1: the proteins responsible for the stability of the telomerase complex. Several additional regulators, protectors, and repair molecules are not detailed here, but the failure of either of them can lead to DC with various severity and different inheritance manner (autosomal dominant, autosomal recessive, X-linked) (Tummala et al. 2018; Garus and Autexier 2021; Dorgaleleh et al. 2022). Though it is genetically heterogeneous, mutations of *DKC1*, with X-linked inheritance fashion, are its most common causes. The DC-related *DKC1* mutations are *C*- and *N*-terminal missense mutations which affect less conserved regions and are implicated in guide RNA binding, but some missense variants in the TruB domain were also described (Aalfs, van den Berg et al. 1995; Knight et al. 1999a, b; Dokal 2000; Kiss et al. 2004; Trahan et al. 2010; Balogh et al. 2020). Without any alteration in its coding sequence, DC can also develop as a result of a promoter mutation and decreased dyskerin expression (Knight et al. 1999a, b; Salowsky et al. 2002; Parry et al. 2011).

In general, the early symptoms of DC represent the triad of mucocutaneous features (reticular skin hyperpigmentation, oral leukoplakia and nail dystrophy) typically not presenting before late childhood. Bone marrow failure develops later and gives rise to opportunistic infections, anemia, thrombocytopenia, and, as a consequence, internal bleeding events. There is a high risk of pulmonary fibrosis, which, together with malignancies and opportunistic infections, are responsible for the early mortality. Life expectancy is 20–50 years. Most of the severely affected organs and tissues (skin, nail, bone marrow) require a high proliferation rate (Dokal 2000; Gu et al. 2008; Dorgaleleh et al. 2022).

The p.S121G substitution in the TruB domain (pseudouridine synthase motif) of dyskerin was found in a 15-year-old patient with metachronous rectal cancer and bone marrow failure without other typical DC symptoms (Watanabe et al. 2019). The same variant of dyskerin was previously reported in HH (Hoyeraal-Hreidarsson) syndrome, a severe form of DC characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, and occasionally, enteropathy (Aalfs, van den Berg et al. 1995; Knight et al. 1999a, b). Along this line, another TruB domain substitution (p.R158W) was also described in HH syndrome (Knight et al. 2001; Vulliamy et al. 2006), suggesting that TruB domain substitutions cause more severe DC phenotypes than the *N*- and *C*-terminal substitutions.

DKC1 and *NOP10*-related nephrotic syndrome, cataracts, hearing impairment and enterocolitis

Dyskeratosis and HH syndrome already represented a significant pleiotropy of *DKC1* mutations. Nevertheless, a novel syndrome was recently described in two families with either a *DKC1* mutation (p.E206K) or a *NOP10* homozygous mutation (p.T16M) (Balogh et al. 2020). The affected children are asymptomatic during the first months of life, but stop growing in late infancy, develop cataracts, hearing impairment, diarrhea, nephrotic syndrome and later bone marrow failure. The majority of patients died during the first three years of life due to opportunistic infections, long before the potential appearance of DC-related mucocutaneous features. Some organ involvements are transmitted in a dominant fashion: Heterozygous females also develop cataracts and hearing impairment, typically in the second decade of life. The phenotype of a girl with a highly skewed X inactivation was similarly severe to that of the males, but no bone marrow failure or diarrhea was associated, indicating the rescue effect of cells expressing the wild type X and allowing her to reach adulthood.

Intriguingly, the two affected amino acids, DKC1 E206 and NOP10 T16, are known to interact with each other in the ribonucleoprotein complex (Rashid et al. 2006), and both pathogenic substitutions disrupt the catalytic pseudouridylation pocket, detaching the catalytic D125 of dyskerin from the uridine of the substrate RNA (Balogh et al. 2020). Though the telomeres of the affected patients are shortened, similarly to DC and HH syndrome, the highly different

clinical presentation suggests different underlying patho-physiology. Accordingly, decreased pseudouridine levels were found in the patients' rRNA. Zebrafish *dkc1* mutants recapitulated the human phenotype and showed reduced 18S pseudouridylation, ribosomal dysregulation and a cell-cycle defect in the absence of telomere attrition. This novel disorder is thus the consequence of defective snoRNP pseudouridylation and ribosomal dysfunction (Balogh et al. 2020).

Tumor predisposition

Patients with DC are susceptible to malignancies (Bellodi et al. 2010). Loss of dyskerin has a negative impact on the translation of specific mRNAs with IRES (internal ribosomal entry site) elements. Many antiapoptotic proteins belong to this group, where the IRES elements foster their translation under stress conditions, and help the survival of the cell (Holcik and Sonenberg 2005). The p27 tumor suppressor is one of them: its inappropriate expression predisposes to pituitary tumor (Slingerland and Pagano 2000; Bellodi et al. 2010). The *DKC1* p.S485G somatic mutation was identified in a patient with recurring pituitary tumor. The p.S485G dyskerin was found less stable and active, the 18S rRNA pseudouridylation as well as the p27 quantity decreased with a preserved expression at the mRNA level (Bellodi et al. 2010). The p.H259P variant of dyskerin was also identified in pituitary adenoma with lower P27 protein levels (Martins et al. 2016). A similar telomere independent mechanism was described in breast cancer, affecting the translation of the p53 tumor suppressor (Montanaro et al. 2010). These findings together suggest a tumor suppressor role for dyskerin. Even though, *DKC1* variants are rarely found in sporadic malignancies (Penzo et al. 2013).

Nevertheless, the role of dyskerin in tumor development is contradictory. Dyskerin overexpression was described in breast- (Montanaro et al. 2006, 2008; Elsharawy et al. 2020), and prostate cancer (Sieron et al. 2009; Stockert et al. 2019, 2021) and malignant glioma (Miao et al. 2019). High *DKC1* expression levels are associated with worse prognosis in these malignancies (Miao et al. 2019; Elsharawy et al. 2020). In vitro knock-down experiments in glioma cell lines indicate the role of DKC1 in proliferation, migration and invasion (Miao et al. 2019). In addition, dyskerin expression was found to be regulated by N-Myc and c-Myc oncogenes, and its downregulation resulted in reduced proliferation of neuroblastoma cells, which process was independent from the telomere length and p53 level (O'Brien et al. 2016). Dyskerin is often regarded also as an oncogene, but its role and the underlying molecular mechanisms are still to be elucidated.

Along this line, elevated pseudouridine level in blood or urine was suggested to be a potential biomarker of several malignancies (Seidel et al. 2006; Patejko et al. 2018):

breast (Zheng et al. 2005), colorectal (Feng et al. 2005), oesophageal (Masuda et al. 1993), gallbladder (Jiao et al. 2014), prostate (Stockert et al. 2021), ovarian (Chen et al. 2012; Zeleznik et al. 2020), small cell lung cancer (Tamura et al. 1986a, b; Tamura et al. 1987), hepatocellular carcinoma (Tamura et al. 1986a, b; Amuro et al. 1988), leukemia (Li et al. 1992) and lymphoma (Rasmuson and Björk 1983; Masaki et al. 2006).

The role of dyskerin in tumor development seems to depend on the specific tumor type.

Plasma and urine pseudouridine levels as potential biomarkers were also suggested in cardiovascular diseases, such as heart failure or cardiac hypertrophy. The role of pseudouridines in these conditions is not yet elucidated, but presumably the mitochondrial function and oxidative phosphorylation may be impaired via mitochondrial RNA modifications (Razavi et al. 2020; Wu et al. 2021).

The double-edged role of dyskerin, acting potentially both as tumor suppressor or oncogene, is intriguing. Sustained telomerase activity due to dyskerin overexpression or altered expression of pro- and anti-apoptotic factors secondary to dyskerin dysfunction can contribute to tumor progression. A minor isoform of dyskerin (Iso3) with cytoplasmic localization was found to have a role in oxidative metabolism. Its overexpression might also contribute to cancer progression by protecting cells from oxidative stress and apoptosis (Angrisani et al. 2018). On the other hand, loss-of-function of dyskerin seems to inhibit cell proliferation and tumor progression. Accordingly, cells expressing the mutant allele in heterozygous females with p.E206K undergo natural selection and X-inactivation tends to be skewed toward the mutant allele by the second decade of life (Balogh et al. 2020). Furthermore, the immortalized cell line established from the leukocytes of heterozygous females expressed exclusively the wild-type allele (our unpublished data). Autophagy and heat shock response have been similarly found to have such a dual role in cancer (Santagata et al. 2011; Chavez-Dominguez et al. 2020; Cyran and Zhitkovich 2022). As the stress response pathways are also associated with dyskerin functions (Li et al. 2015) and tumor progression (Santagata et al. 2011; Chavez-Dominguez et al. 2020; Cyran and Zhitkovich 2022), their causality remains to be explored in the double-edged relationship between dyskerin in cancer.

Human diseases related to stand-alone pseudouridine synthases

PUS1

MLASA (mitochondrial myopathy, lactic acidosis, sideroblastic anemia)(Inbal et al. 1995) is a rare autosomal

Table 1 Gene mutations in relation to pseudouridylation that result in human disorders

Gene	Encoded Protein	Variant	Symptoms	Inheritance	References
DKC1 (CBF5, DKCX)	dyskerin	N- and C-terminal missense mutations, Middle region p. S280R; PUA domain missense mutations	Dyskeratosis congenita Hoyeraal–Hreidarsson syndrome	X-linked recessive	Savage and Niewisch (1993), Aalfs, van den Berg et al. (1995), Knight et al. (1999a, b), Knight et al. (1999a, b), Dokal (2000), Knight et al. (2001), Koziel et al. (2011), Lim et al. (2014)
		p.S121G (TruB domain)	Metachronous rectal cancer Hoyeraal-Hreidarsson syndrome	X-linked	Aalfs, van den Berg et al. (1995), Knight et al. (1999a, b), Watanabe et al. (2019)
		p.R158W (TruB domain)	Hoyeraal-Hreidarsson syndrome	X-linked	Knight et al. (2001), Vulliamy et al. (2006)
		p.E206K	Males: nephrotic syndrome, cataracts, sensorineural deafness, enterocolitis, early lethality; Females: cataracts, sensorineural deafness	X-linked, dominant	Balogh et al. (2020)
		p.H259P (not proved to be the disease causing)	Pituitary tumor	Somatic	Martins et al. (2016)
		p.S485G			Bellodi et al. (2010)
NOP10	NOP10	p.T16M	Nephrotic syndrome, cataracts, sensorineural deafness, enterocolitis, early lethality	Autosomal recessive	Balogh et al. (2020)
PUS1	PUS1	Q101R	MLASA	Autosomal recessive	Kasapkara Ç, Tümer et al. (2017)
		p.R116W			Bykhovskaya et al. (2004), Zeharia et al. (2005)
		p.I163Lfs*4			Cao et al. (2016)
		p.E220*			Fernandez-Vizarra et al. (2007)
		p.R295Q			Cao et al. (2016)
		p.R295W	Congenital sideroblastic anemia, diarrhea, microcephaly, short stature, late-onset moderate muscle weakness	Autosomal recessive	Metodiev et al. (2015)

Table 1 (continued)

Gene	Encoded Protein	Variant	Symptoms	Inheritance	References
PUS3	PUS3	p.R19*	facial dysmorphia, seizures, developmental delay, hearing loss, delayed bone age, brain developmental disorders	Autosomal recessive	Fang et al. (2020)
		p.C114R	Congenital anencephaly, hypoplasia of cerebellum, ankle contracture, abnormal heart morphology	Autosomal recessive	Guo et al. (2020)
		p.R116S fs*2	Microcephaly, kidney agenesis, dysmorphic features, hypotonia, congenital heart defect, respiratory distress	Autosomal recessive	Borghesi et al. (2022)
		p.R166Q	Intellectual disability, renal abnormalities, diffuse WMD, dysmorphic features	Autosomal recessive	de Paiva et al. (2019)
		p.T208fs*	Facial dysmorphia, seizures, developmental delay, hearing loss, delayed bone age, brain developmental disorders	Autosomal recessive	Fang et al. (2020)
		p.R280*	Congenital anencephaly, hypoplasia of cerebellum, ankle contracture, abnormal heart morphology	Autosomal recessive	Guo et al. (2020)
		p.L366P	Intellectual disability, renal abnormalities, diffuse WMD, dysmorphic features	Autosomal recessive	de Paiva et al. (2019)
		p.S394Cfs*18	Intellectual disability, microcephaly, hypotonia, seizures, vision and hearing loss, global developmental delay	Autosomal recessive	Abdelrahman et al. (2018), Froukh et al. (2020)
		p.R435*	Severe intellectual disability, microcephaly, failure to thrive, short stature, epilepsy, growth deficiency, strabismus, gray sclera, hypotonia	Autosomal recessive	Torres et al. (2014), Shaheen et al. (2016), Alfares et al. (2017)

Table 1 (continued)

Gene	Encoded Protein	Variant	Symptoms	Inheritance	References
PUS7	PUS7	T30Lfs*20	Intellectual disability, speech delay, short stature, microcephaly, aggressive behavior	Autosomal recessive	de Brouwer et al. (2018)
		T110Rfs*4	Intellectual disability, speech delay, short stature, microcephaly, aggressive behavior, facial dysmorphia, hearing loss	Autosomal recessive	Shaheen et al. (2019)
		T397M	Intellectual disability, speech delay, short stature, microcephaly, aggressive behavior, facial dysmorphia, hearing loss, hypoglycemia, hyperuricemia, axonal neuropathy, ataxia	Autosomal recessive	Han et al. (2022)
		c.398+1G>T splice site variant			
		R450*	Intellectual disability, speech delay, short stature, microcephaly, aggressive behavior	Autosomal recessive	de Brouwer et al. (2018)
		15 exon del			
		D503Y	Intellectual disability, speech delay, short stature, microcephaly, aggressive behavior, facial dysmorphia, hearing loss	Autosomal recessive	Shaheen et al. (2019)
Mitochondrial tRNA^{Glu}		m.14692A>G	Maternally inherited diabetes and deafness	Maternal	Wang et al. (2016)

recessive, oxidative phosphorylation disorder, characterized primarily by muscle and bone marrow defects leading to exercise intolerance and anemia. Cognitive impairment, skeletal and dental abnormalities, delayed motor milestones, cardiomyopathy, dysphagia and respiratory insufficiency can be associated. Loss of function of the *YARS2* gene, encoding the mitochondrial tyrosyl-tRNA synthase, results in a similar phenotype. It remains to be explored how the defect of the pseudouridine synthase PUS1 disrupts the oxidative phosphorylation. PUS1, a member of the TruA stand-alone pseudouridylation synthase family, is necessary for the pseudouridylation of cytoplasmic and mitochondrial tRNAs. Its R116W substitution in the highly conserved catalytic center of the protein was the first reported causal variant in MLASA (Bykhovskaya et al. 2004; Zeharia et al. 2005). Since then, several other loss of function mutations have been described (listed in Table 1.) resulting the same (Fernandez-Vizarra et al. 2007; Cao et al. 2016, Kasapkara Ç, Tümer et al. 2017), or a similar disorder. (Metodiev et al. 2015) In a mouse model, it was shown that the rate of the muscle fibers expressing myosin heavy chain IIB and IIA is altered in the PUS1 null mutants, resulting in an altered muscle metabolism, and causing a very similar phenotype to the human (Mangum et al. 2016, Kasapkara Ç, Tümer et al. 2017).

Recently, PUS1 overexpression was found in breast cancer, and its knockdown was proved to suppress tumor proliferation and invasion in breast cancer cell lines (Fang et al. 2022).

PUS3

Intellectual disability is usually caused by chromosomal rearrangements or single gene mutations. Several tRNA modification enzymes were found defected indicating that brain development is especially sensitive to tRNA dysfunction (Ropers 2010; Torres et al. 2014; Shaheen et al. 2016; Abdelrahman et al. 2018; de Paiva et al. 2019; Borghesi et al. 2022). During their maturation tRNAs undergo several post-transcriptional modifications, which stabilize their structure and function and prevent translational frameshifting via stabilizing the codon-anticodon base pairing. Hypomodified tRNAs are often degraded, and the imbalances of tRNA pool may affect protein synthesis (Phizicky and Hopper 2010; Torres et al. 2014, Pereira, Francisco et al. 2018).

PUS3, a TruA family member is a general pseudouridine synthase of tRNAs, the alterations of which cause global developmental delay/intellectual disability (GDD/ID), microcephaly, short stature, severe hypotonia, gray sclera and severe syndromic features. The p.R435* mutation truncates the protein in the *C*-terminal region which is highly conserved in mammals. The mutation abolishes the isomerization of the U_{39} at least in six different tRNAs (Shaheen

et al. 2016). Along this line, the p.S394Cfs*18 mutation was also described to be disease causing and triggering complete degradation of the mRNA by nonsense-mediated decay (Abdelrahman et al. 2018). The p.R166Q and p.L366P variants were reported to cause also renal involvement (de Paiva et al. 2019). The reported variants are listed in Table 1.

PUS7

PUS7, similarly to PUS3, targets several tRNAs and mRNAs. The catalytic domain of the TruD family member enzyme is located to its C-terminal. The phenotype of patients with PUS7 loss of function mutations is very similar to those with PUS3 variants. Intellectual disability, short stature, and microcephaly are common. Aggressive behavior was reported in most of the cases. The altered enzymes seem to lose the isomerization capacity of the U_{13} of at least ten cytosolic tRNAs. In addition, dysregulation of general protein translation also follows (de Brouwer et al. 2018; Shaheen et al. 2019; Han et al. 2022). The reported mutations can be found in Table 1.

Mutation of the target U in a human disease

Besides enzyme function loss, the substitution of the target uridine may also inhibit the process, which, in case of a cardinal uridine site, can result in a phenotype by itself. Wang et al. found that the substitution of the U_{55} in the mitochondrial tRNA^{Glu} by cytosine (m.14692A > G) results in maternally inherited diabetes and deafness (MIDD). U_{55} pseudouridylation is a very conserved and essential step in the maturation of mitochondrial tRNA^{Glu}. In absence of the $\Psi_{55,}$ the tRNA^{Glu} becomes unstable and due to its structural alterations it cannot bind properly to the components of the translational machinery. As a consequence, the mitochondrial protein translation becomes hampered, and the ATP synthesis and the mitochondrial membrane potential will be reduced (Wang et al. 2016).

Hypothetical therapeutic usage

Due to the methodological development, hundreds of mRNA pseudouridylation sites were recently detected (Cerneckis et al. 2022). A modified stop codon (UAA, UAG, UGA) to ΨAA, ΨAG or ΨGA results in readthrough in yeast. It is tempting to speculate about its potential use in human nonsense mutation-caused disorders, i.e., to pass the premature termination codon in a directed way and thus translate the whole peptide (Mort et al. 2008; Karijolich and Yu 2011; Fernandez et al. 2013; Adachi and Yu 2020). Targeted pseudouridylation with designed guide RNAs would change the

STOP codon to serine or threonine (ΨAA, ΨAG); tyrosine or phenylalanine (ΨGA) (Cerneckis et al. 2022).

This hypothetical method was studied by Nir et al. (2022) and they made the following conclusions: snoRNA-mediated pseudouridylation can occur on mRNA targets, but at very low levels, the snoRNA complementary region should be longer than required in rRNAs and the natural intron cleveage is an important part of the process, that should be considered at the experimental design. Very recently two parallel methods were published (Adachi, Pan et al. 2023; Song et al. 2023), where the pseudouridylation of premature termination codons restored the translation at a low but promising level.

In vivo studies have been performed only in yeast to date (Karijolich and Yu 2011; Huang et al. 2012; Fernandez et al. 2013).

Conclusion

Pseudouridylation, the most abundant modification of RNAs, has been found implicated in several human disorders. While the underlying pathophysiology is being unraveled, its potential in therapeutic interventions is to be explored.

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