# The Implication of mRNA Degradation Disorders on Human DISease: Focus on DIS3 and DIS3-Like Enzymes

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**Abstract**

RNA degradation is considered a critical post-transcriptional regulatory checkpoint, maintaining the correct functioning of organisms. When a specific RNA transcript is no longer required in the cell, it is signaled for degradation through a number of highly regulated steps. Ribonucleases (or simply RNases) are key enzymes involved in the control of RNA stability. These enzymes can perform the RNA degradation alone or cooperate with other proteins in RNA degradation complexes. Important findings over the last years have shed light into eukaryotic RNA degradation by members of the RNase II/RNB family of enzymes. DIS3 enzyme belongs to this family and represents one of the catalytic subunits of the multiprotein complex exosome. This RNase has a diverse range of functions, mainly within nuclear RNA metabolism. Humans encode two other DIS3-like enzymes: DIS3L (DIS3L1) and DIS3L2. DIS3L1 also acts in association with the exosome but is strictly cytoplasmic. In contrast, DIS3L2 acts independently of the exosome and shows a distinctive preference for uridylated RNAs. These enzymes have been shown to be involved in important cellular processes, such as mitotic control, and associated with human disorders like cancer. This review shows how the impairment of function of each of these enzymes is implicated in human disease.

## 4.1 Introduction

RNA is a labile molecule, by its chemical nature, and a plethora of events and factors ensure its protection or decay. The termini of cytoplasmic mRNAs are usually protected by a 7-methyl guanosine ($m^7GpppG$) at the 5′ end cap, and by a long terminal poly(A) tail at the 3′ end, which promotes the association of Poly(A) Binding Proteins (PABPs) (Fig. 4.1a). These RNA modifications in the ends of the mRNA molecules warrant their stability. Specific protein factors

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associate with each of the structures in the mRNA extremities, and its physical interaction holds the mRNA in a circular conformation [1, 2]. This closed-loop mRNA structure is recognized by specific translation initiation complexes [2–5]. When a specific RNA is no longer required for the cellular metabolism, the molecule is signaled for degradation through a number of highly regulated steps (Fig. 4.1a).

## 4.2 Mechanisms of Cytoplasmic mRNA Degradation in Humans

The stability of mRNAs depends on intrinsic features of its sequence and on the cellular demands for the protein it encodes. For instance, specific features such as AU rich elements (AREs) that consist on stretches of adenine and uracil nucleo-
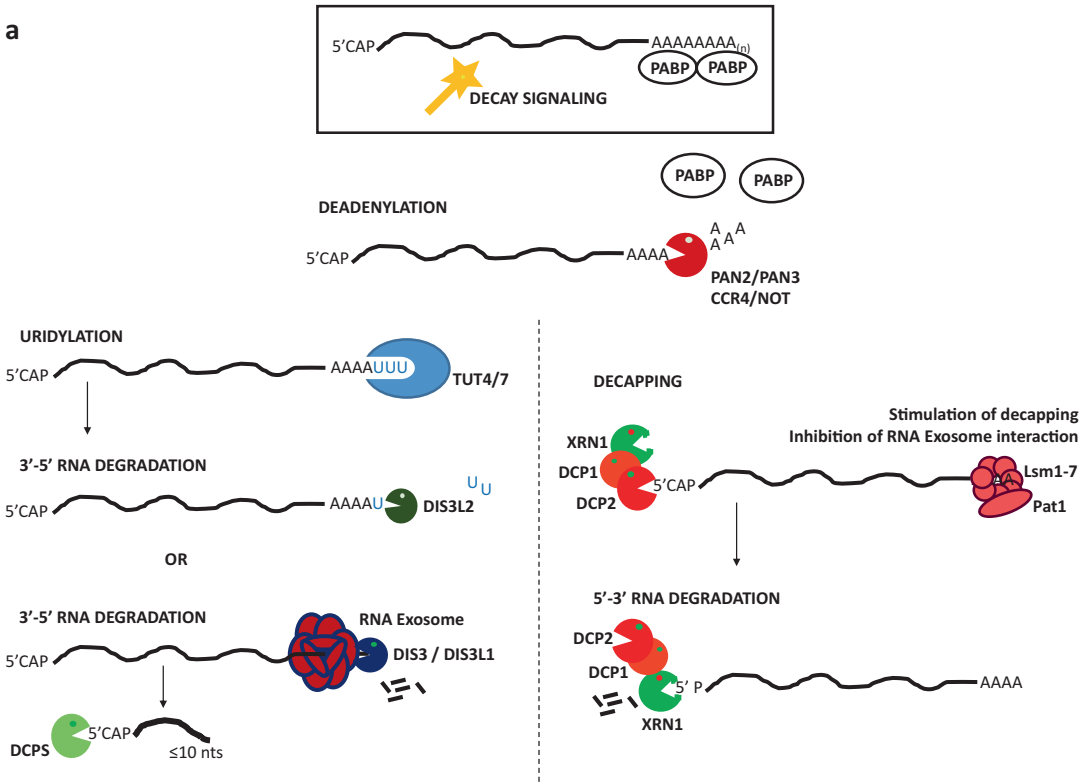
**Fig. 4.1** (**a**) Overview of RNA degradation pathways in humans. RNA transcripts first undergo removal of the 3′ poly(A) tail through a deadenylation step (performed by PAN2/PAN3 complex followed by the CCR4/NOT). Following this step, the RNA becomes vulnerable and can be degraded 3′-5′ by the Exosome-DIS3/DIS3L1 or by DIS3L2 (that prefers uridylated RNA substrates), independently of the exosome. Following deadenylation, RNAs can also be decapped by DCP1/DCP2 (removal of the 5′-cap), a step stimulated by Lsm1–7–Pat1 complex, exposing the transcripts to the 5′-3′ exoribonuclease XRN1. (**b**) Correlation between DIS3 enzymes and human disease. Scheme of human diseases that have been related with overexpression or dysfunction of DIS3, DIS3L1 and DIS3L2. Dashed arrows correspond to correlations that need further confirmation. Each enzyme-disease association represented in the picture is developed in the main text, with the respective references
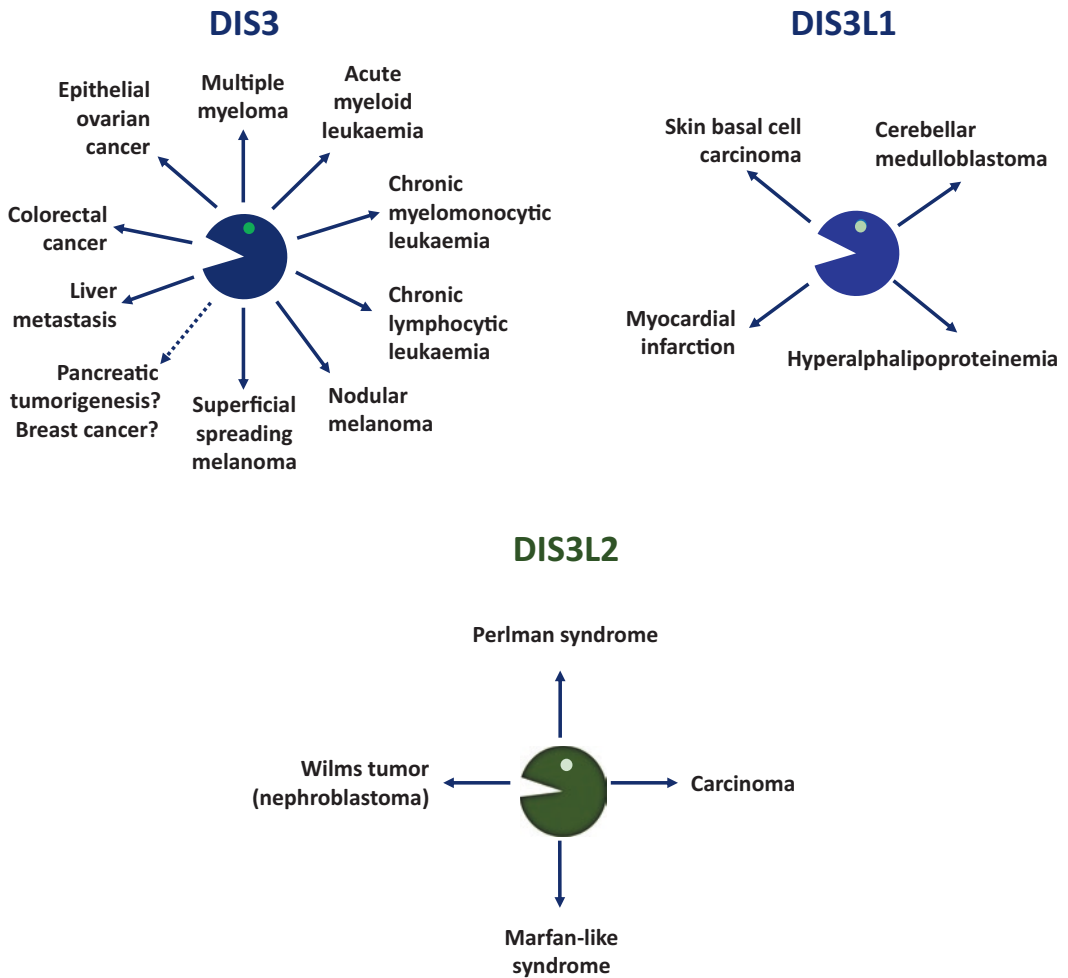
**b**



**DIS3**

Epithelial ovarian cancer
Multiple myeloma
Acute myeloid leukaemia
Colorectal cancer
Chronic myelomonocytic leukaemia
Liver metastasis
Chronic lymphocytic leukaemia
Pancreatic tumorigenesis? Breast cancer?
Superficial spreading melanoma
Nodular melanoma

**DIS3L1**

Skin basal cell carcinoma
Cerebellar medulloblastoma
Myocardial infarction
Hyperalphalipoproteinemia

**DIS3L2**

Perlman syndrome
Wilms tumor (nephroblastoma)
Carcinoma
Marfan-like syndrome

**Fig. 4.1**  (continued)

tides within the 3′UTR of some mRNAs [6–9] are able to promote or protect RNA from degradation [10–12]. The 3′UTR of the mRNAs can also contain other specific sequences that target its decay trough the binding of regulatory microRNAs (miRNAs) [13–15].

Under particular circumstances, like in cellular quality control mechanisms, endonucleolytic cleavage can directly disrupt the closed-circle RNA conformation and trigger subsequent decay, from either of the RNA extremities ([16, 17]; see other chapters in this volume for details on quality control mechanisms). Though, in general, mRNAs must be first deadenylated or decapped

to enable the access of exoribonucleases and trigger degradation.

For many cytoplasmic mRNAs, decay starts with the shortening or removal of the poly(A) tail. Deadenylation releases the PABPs that protect the (A) tail and leaves the 3′ end exposed to exoribonucleases. This is considered the main event that signals mRNAs for degradation from the 3′ end. The length of the poly(A) tail depends on the organism, but the regulation of its extension is always a dynamic process that involves the concerted action of poly(A) polymerases (PAPs) and poly(A) specific 3′ exonucleases (deadenylases). This allows the fine-tuning con-

trol of mRNA stability. Eukaryotic genomes encode a wide variety of deadenylases [18]. In humans, initial deadenylation is performed by the PAN2/PAN3 complex (reviewed in [19–21]) followed by the action of the CCR4/NOT complex (Fig. 4.1a) [22–26].

Following deadenylation, degradation can alternatively proceed from the 5′ end requiring the prior removal of the 5′-cap of the mRNA. The deccaping process is regulated by a plethora of activators and inhibitors [27–29]. One of these, the Lsm1–7-Pat1 complex, preferentially binds the shortened 3′-terminal adenosine extensions of the deadenylated mRNAs to stimulate decapping and inhibit exosome attachment [30, 31]. The reaction products of decapping enzymes are the 5′ $m^7$GDP cap and an unprotected 5′ monophosphate RNA that is then accessible for XRN1 processive and complete degradation [32, 33]. Decapping process commits RNAs to 5′-3′ degradation since XRN1 directly interacts with the decapping enzymes (DCP1/DCP2) [32] (Fig. 4.1a).

In contrast to the 5′-3′ pathway, where XRN1 is the only known cytoplasmic 5′-3′ exoribonuclease, there are several options in the degradation from the 3′ end. After initial deadenylation, the multisubunit RNA exosome complex may further degrade the shortened oligo(A) tail and proceed with the 3′-5′ degradation into the mRNA body. Exosome activity depends on the presence of specific cofactors, called "superkillers" or Ski proteins that regulate its activity. Human homologs of the Ski family – SKIV2L (Ski2), TTC37 (Ski3) and WDR61 (Ski8), associate in the Ski complex [26, 34]. The remaining mRNA fragment with its 5′-cap ($m^7$GpppG) is hydrolyzed by the scavenger decapping enzyme (DCPS) [35]. DCPS is a $m^7$G-specific pyrophosphatase that shows specificity towards RNA fragments not longer than 10 nts (Fig. 4.1a) [36–38].

DIS3 enzyme is the essential catalytic subunit of the exosome. The human genome encodes three members of DIS3 family: DIS3, DIS3L1 and DIS3L2 enzymes and their characteristics and specifications will be described therein in this chapter. While both DIS3 and DIS3L1 interact with the exosome ring, in different cellular locations [39–43], DIS3L2 does not. The enzyme represents a 3′-5′ RNA decay pathway alternative to degradation by XRN1 and the exosome [44, 45]. DIS3L2 shows a distinctive preference towards uridylated substrates, which prompted the discovery of new roles for 3′-uridylation in cytoplasmic mRNA decay. It was proposed that the requirement of deadenylation as an mRNA decay signal can be overcome trough 3′ oligouridylation of transcripts. It can either stimulate decapping and consequent degradation in the 5′-3′ direction, through Lsm1–7/Pat1 binding, or directly activate 3′-5′ DIS3L2 dependent degradation [46] (Fig. 4.1a). The importance of this uridylation-dependent pathway in bulk mRNA degradation was highlighted by the substantial technological progresses in RNA analysis in the recent years. Novel approaches as TAIL-seq revealed that 3′ end mRNA modifications such as urydilation, cytidylation or guanylation are also frequent [47]. The use of oligo(dT)-based priming methods, due to the long poly(A) tails in the 3' end of eukaryotic mRNAs, had previously underestimated its presence.

In general, regulation of gene expression in eukaryotic cells occurs through multiple parallel, partially redundant, mRNA decay pathways. This is further illustrated by the multiplicity of enzymes, which are able to catalyze the same reaction, and their functional redundancy. RNases' activity is also important for RNA surveillance and processing. Their high degree of conservation in different organisms and the specific phenotypes following their individual loss suggest defined roles within the cell.

Beyond the advances on the mechanisms whereby these enzymes affect cellular processes, structural information is crucial to explain the mechanism of action and exact function of the protein alone or in the context of a multiprotein complex. Structural changes across species provide insight into the evolution and conservation of the protein architecture. In fact, important findings over the last years have shed new light onto the mechanistic details of RNA degradation by members of the RNase II/RNB family of exoribonucleases, including DIS3 enzymes [45, 48–56]. A phylogenetic comparison of different Dis3 homologues in eukaryotes indicates a clear division of

three Dis3-like protein families, with the Dis3 group being the most conserved and the Dis3L1 and Dis3L2 groups being more divergent [45].

A growing number of publications associate DIS3-enzymes with several human diseases [57–60]. In this review we will try to sum up the mechanistic and structural details of these RNase II-like enzymes, and how human disorders can result from the associated defects.

### 4.2.1   DIS3

DIS3 (defective in sister chromatid joining) gene is located on human chromosome 13 (q22·1), and it encodes for a highly conserved ribonuclease (also known as Rrp44 in yeast) that contains both 3′-5′ exoribonuclease and endoribonuclease activities [40, 42, 61]. This RNase constitutes an essential catalytic subunit of the exosome [62]. This multiprotein complex is composed of a catalytically inert ring-shaped 9-subunit core with a prominent central channel and associated catalytic subunits [61, 63–65]. The composition of its catalytic subunits varies accordingly to cellular localization. Exosome-associated DIS3 enzyme exists mainly in the nucleus, where it acts on a vast array of different RNAs, and has a diverse range of functions in RNA metabolism including processing, maturation and quality control [66–71].

The specific domains of DIS3 dictate its degradation preferences. DIS3 is composed by two cold shock domains (CSD1 and CDS2), followed by an RNB and an S1 domain. Both CSDs and the S1 domain contribute to RNA binding. At the N-terminal region, it contains a PilT N-terminal (PIN) domain [40, 42, 51, 72] and also a CR3 motif involved in the binding of DIS3 to the exosome [73]. Both RNB and PIN domains are responsible for RNA degradation activity. The RNB catalytic domain is a hallmark of the RNase II protein family, and confers to DIS3 the ability to cleave RNA in a highly processive manner [51, 61, 74]. Arraiano's lab contributed to the resolution of the crystal structure of the family prototype, *E. coli* RNase II, and to its extensive functional characterization [49–51]. This was an important breakthrough in the understanding of

the mechanism of action of this ubiquitous family of proteins. Moreover, the determination of the electron microscopy structure of yeast Rrp44 (Dis3) suggested that the RNA recruitment mechanism is conserved [75]. The knowledge acquired by these model organisms was crucial for the construction of the 3D model of human DIS3. Its exoribonuclease activity is dependent on four conserved aspartic acid residues (D488, D487, D485, D489) that coordinate two magnesium ions in the catalytic center [61, 63]. The RNB active site in DIS3 is responsible to hydrolyze single-stranded RNA (ssRNA) in a 3′–5′ direction, releasing one nucleotide at a time and leaving an end product of 4 nts [51, 70, 76–78]. Only ssRNAs with a minimum length of 7 nts can be cleaved [76]. Dis3/Rrp44 is also able to unwind and digest structured RNAs as long as there is an unstructured region of ~4 nts at the 3′ terminus [79].

The PIN domain in DIS3 confers the ability to cleave RNA endoribonucleolytically [40, 42, 43]. PIN-like domains constitute a widespread superfamily of nucleases with representatives in all kingdoms of life [80, 81]. Combination of endoribonuclease and exoribonuclease activities is a widespread feature of RNA-degrading machines from bacteria to humans [48]. Both DIS3 activities cooperate with each other in the degradation of RNA molecules [40, 42, 43, 82]. The PIN domain is able to cleave circular and linear ssRNAs, preferentially with a 5′ monophosphate [40, 42]. Its active site is composed of four acidic amino acids essential for endoribonuclease activity (E97, D69, D177 and D146) that coordinate two divalent metal ions, and $Mn^{2+}$ is the preferred ion for its activity [40, 42]. It was proposed that the role of the PIN domain is to assist in the release of RNA substrates that are stalled at sites with strong secondary structures [83]. Besides its endoribonuclease activity, the PIN domain has also a structural role, being necessary for DIS3 association with the core exosome [39, 42, 43, 84].

Like in other organisms, such as *Drosophila* and yeast, human DIS3 is essential for survival [85, 86]. This RNase together with the exosome complex play a crucial role in maintaining the fidelity of gene expression. In the nucleus, the

exosome-associated DIS3 is involved in the degradation of a vast range of RNAs, including protein-coding RNAs, stable RNA species such as ribosomal RNA (rRNA), transfer RNA (tRNAs) and small nucleolar RNAs (snoRNAs), introns, long non-coding RNAs (ncRNAs), miRNAs and also unstable RNAs products, like Promoter Upstream Transcripts (PROMPTs) [87].

An impaired RNA surveillance system can compromise RNA homeostasis, having detrimental consequences for multiple biological processes, which may result in malignancy [88]. Indeed, an increasing number of publications have associated dysregulation of DIS3 with human disease, namely cancer (reviewed by [60, 89]). Sequencing data have identified *DIS3* gene as one of the most frequently mutated genes in multiple myeloma (on average in 11–18.5% of patients) [58, 90–93]. This constitutes the second most frequent hematologic tumor after lymphomas [94]. In multiple myeloma patients, DIS3 mutations were detected in highly conserved regions along PIN, CSD2, RNB and S1 domains [58, 90, 91, 93]. However, the mutations in the RNB domain seem to be more prevalent for the development of the disease [58, 90, 91], and contain mutational hotspots (D488, E665 and R780) [90, 93].

Tomecki and co-workers have studied Multiple myeloma mutations that abolish or cause dysfunction, without total inactivation, of DIS3 RNB activity *in vitro* [85]. The results indicated that the point mutations D487N, S477R, G766R and R780K cause significant aberrations on exoribonucleolytic activity. DIS3 amino acid changes with significantly decreased activity *in vitro* gave rise to a slower cellular proliferation rate in HEK293-derived cells [85]. DIS3-mutations such as R780K, an amino acid involved in RNA binding, also revealed an abnormal RNA metabolism, with accumulation of 5.8S processing intermediates, tRNAs, RNA polymerase III transcripts and PROMPTs [85].

Szczepińska and co-workers also observed a major role of DIS3 in maintaining RNA polimerase II transcriptome homeostasis and in the regulation of PROMPTs [87]. PROMPTs are transcribed in reverse orientation to most active protein coding genes, and cover ~1% of the human genome [72, 95]. Although the biological role of PROMPTs has yet to be elucidated, there are evidences that these RNAs could serve important functions in human cells [96]. For instance, the PROMPT HIF2PUT was suggested to be a novel regulator of osteosarcoma, the most common primary bone malignancy [96]. The observation that PROMPTs were the most prominent targets of DIS3 (>50-fold increase in DIS3 mutant cells), indicates that there are no alternative pathways for their decay, making their connection with DIS3 and disease necessary to be explored.

Multiple myeloma associated mutations were also mapped on PIN domain, showing only small effects on cell growth [85]. However, when mutations in PIN and RNB domains are combined, a synergistic effect in the proliferation and metabolic activity is observed in human cells [85, 87].

Human cells bear two DIS3 isoforms that differ in the size of the PIN domain. Isoform 1 encodes a full-length PIN domain, whereas the PIN domain of isoform 2 is shorter and misses a segment with conserved amino acids [52]. A study by Robinson and co-workers [52] anticipated that different ratios of the two PIN isoforms could be characteristic of several haematological cancers, namely Multiple Myeloma [52]. Isoform 1 was found in higher levels than isoform 2 in Multiple Myeloma patient samples and all cancer cell lines tested [52]. Contrastingly, healthy donors and Acute Myeloid Leukemia and Chronic Myelomonocytic Leukaemia patients have similar levels of both isoforms. Regarding leukaemias, in Acute Myeloid Leukemia (a cancer of the myeloid line of blood cells) missense mutations in DIS3 account for 4% of patients and were all found in the RNB domain [59]. In patients with Chronic Lymphocytic Leukemia (a monoclonal disorder characterized by a progressive accumulation of abnormal lymphocytes) DIS3 *locus*, 13q22, is often deleted [97]. This evidence together with DIS3 mutations in several cancers, suggest that DIS3 may function as a tumor suppressor gene.

Increased levels of DIS3 mRNA and protein have also been proposed as one of the causes of other types of cancer. This is the case of epithelial ovarian cancer in which DIS3 was observed to be

significantly up-regulated in plasma from patients in the late stage of the disease (FIGO III/IV) [98]. The majority of cancer deaths are due to metastasis of neoplastic cells from the primary tumor to distant organs. Metastasis is thus the most important factor that determines bad prognoses for cancer patients. In 1997, DIS3 was reported to have a 38-fold higher expression in primary tumors and metastatic cells from patients with colorectal cancers and liver metastases (compared to adenomas) [99]. The same study has classified DIS3 as an oncogene, being positively correlated with the incidence of metastasis and consistent with its involvement in the regulation of mitosis in *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Drosophila* [66, 100–102]. Other studies also reported a significant overexpression of DIS3 in colorectal carcinomas (compared to adenomas) [103, 104]. This overexpression could be explained by an amplification of the DIS3 *locus*, 13q22, frequently observed in colorectal cancer.

DIS3 was also found to be differentially expressed in melanoma cells [105]. Specifically, in superficial spreading melanoma cells, DIS3 has a reduced expression, contrarily to nodular melanoma cells, where DIS3 is overexpressed (compared to normal melanocytes) [105] However, in 2013, a wide-genome analysis resultant from five melanoma microarrays datasets did not recognize DIS3 as a melanoma biomarker [106]. DIS3 has also a role in pancreatic tumorigenesis and breast cancer, however their linkage has to be further explored [107, 108].

All examples presented here strongly suggest that both DIS3 overexpression and lack of function can lead to the manifestation of different cancers. This seems contradictory, but it is in agreement with the fact that DIS3 may function as either oncogene or tumor suppressor. Some genes are known to have both functions, and recently it was reported that most of these genes are transcription factors or kinases that can regulate transcription positively and negatively [109]. Since RNases are responsible to control post-transcriptionally gene expression, it is not surprising that DIS3 would also have a dual function. For instance, DIS3 is known to facilitate the maturation of the tumor suppressor *let-7* miRNA. When the levels of the mature *let-7* miRNA are reduced, translation of oncogenes (MYC and RAS) increases, enhancing tumorigenesis [110]. Human DIS3 also appear to function in the Ran signaling pathway required for nuclear import of proteins [111]. Ran was associated to cancer progression and has been investigated as a target for cancer therapy. In sum, the precise and dual role of DIS3 in cancer is not fully understood lacking further investigation.

### 4.2.2  DIS3L1

Human DIS3 and DIS3L1 have a similar domain composition, however only the first has an active endoribonuclease domain. Two important residues (E97 and D146N) are absent in DIS3L1 PIN domain rendering it inactive. The aspartic acid D146 is the most conserved in the PIN domains and its single mutation is reported to abolish its activity *in vivo* and *in vitro* [43, 112, 113]. The E97 is not strictly conserved across the PIN-domain family [112].

Both human DIS3 and DIS3L1 associate with the exosome ring. In contrast to the mainly nuclear localization of DIS3, DIS3L1 is strictly cytoplasmic [57, 72, 114]. The stable association of DIS3L1 with the cytoplasmic exosome suggests that it acts in concert with the core of the exosome in the degradation of cytoplasmic RNAs. One of the RNA substrates degraded by the exosome-associated DIS3L1 is the 28S rRNA. The degradation proceeds through polyadenylated intermediates, which accumulate upon DIS3L1 knockdown [115, 116]. DIS3L1 was also implicated in the degradation of intermediary products generated by DNA-based antisense oligonucleotides (ASOs), as part of the RNA surveillance machinery. These agents recruit RNase H1 that after endonucleolytic cleavage of the ASO-targeted mRNAs generates both a 5′ and a 3′ fragments. DIS3L1 appears to be involved, together with the exosome, on the 3′-5′ exoribonucleolytic degradation of the cytoplasmic upstream cleavage products [117].

Less is known about DIS3L1 association with human disease, but there are a few reports showing its implication on diverse pathologies, directly or indirectly related with its function over specific substrates. It was suggested that DIS3L1 could be implicated in the regulation of steady state levels of Y RNAs, an abundant class of small non-coding RNAs with a role in a range of cellular processes, such as RNA quality control, DNA replication, cellular stress responses and histone mRNA processing [118, 119]. Several enzymes are involved in Y RNAs maturation, both on its 3′ end adenylation and subsequent trimming. Poly(A)-specific ribonuclease (PARN) is one of the enzymes involved on its 3′ end processing and, in its absence, oligo(A) tails are degraded by the exoribonuclease DIS3L1. It was seen that PARN mutations cause a severe form of dyskeratosis congenita (DC), a telomere biology disorder characterized by dysplastic nails, lacy reticular pigmentation of the upper chest and/or neck, and oral leukoplakia [120]. The loss of PARN reduces the levels of human Y RNAs. At the same time, low levels of Y RNAs intensify the effect of PARN depletion on telomere maintenance, leading to the same severe DC phenotype. PARN seems to be responsible to stabilize Y RNAs by removing the oligoadenylated tails that recruit DIS3L1 for degradation [121]. Moreover, it was recently demonstrated that PARN is also involved in miRNAs stabilization by removing their oligo(A) tails – the signal for the recruitment of the cytoplasmic exonucleases DIS3L1 or DIS3L2. Therefore, upon PARN knockdown there is decrease in miRNAs' levels, namely several that target p53 mRNA (a gene that plays a central role in cancer). This was the missing link to explain the p53 accumulation that is observed in PARN-defective patients [122].

DIS3L1 also seems to play a role in cancer, similarly to DIS3 (see above) and DIS3L2 (see bellow) homologues. The Hedgehog (Hh) pathway controls cell proliferation and differentiation in response to a gradient of secreted Hh ligands, and its aberrant activation can promote tumorigenesis. The transcriptional factor Zfx is a common cell-intrinsic regulator of diverse Hh-induced tumors. *hDIS3L1*, the human gene encoding DIS3L1 was identified as direct transcriptional target of Zfx, in the context of skin basal cell carcinoma (BCC) and cerebellar medulloblastoma (MB) models *in vivo* and *in vitro* [123].

Two independent exome sequencing studies (technique that sequences all protein-coding genes in a genome) have reported an association of *hDIS3L1* with cardiac risk. First, in a study to identify genetic variants that confer susceptibility to myocardial infarction (MI) in the Asian population (Korean individuals), several single nucleotide polymorphisms (SNPs) on *hDIS3L1* gene were associated with MI risk. However, how the gene influences MI pathogenesis would have to be determined and confirmed in other ethnic populations [124]. In another study, novel SNPs were also identified in the *hDIS3L1* gene of individuals with Hyperalphalipoproteinemia (HALP). This condition of high-density lipoprotein cholesterol (HDL) levels is inversely correlated with coronary heart disease (CHD), and *hDIS3L1* gene was identified as a candidate gene associated with HALP [125].

### 4.2.3   DIS3L2

Human DIS3L2 is the third member of the RNase II/RNB family of enzymes. This protein is a processive 3′-5′ exoribonuclease (mainly cytoplasmic) able to degrade structured RNA molecules, as long as they possess a 2 nt 3′ overhang as a "landing platform" [44, 45]. Unlike its family counterparts (DIS3 and DIS3L1), DIS3L2 lacks the PIN and the CR3 domains on its structure, both necessary for the interaction with the exosome complex [39, 43, 45, 72, 73].

In mammalian cells, DIS3L2 is involved in miRNA maturation and in the decay of numerous RNA-species, namely bulk mRNA, ARES and ncRNAs [126–129]. Several studies associated DIS3L2 with a degradation pathway that relies on the addition of untemplated uridines to several classes of RNAs, in a process called uridylation [44, 45, 127, 129–132]. This process was reported for the first time in *S. pombe* [133], in which Dis3L2 was shown to degrade uridylated poly(A)-containing mRNAs [45]. Later

on, uridylation was found to be widespread and to have a decisive impact on RNA′s fate. There is a negative correlation between the addition of short (1–4) uridine residues with mRNA stability [47, 130]. Oligo(U) tailed mRNAs are recognized by Lsm1–7 complex stimulating 3′-5′ degradation by DIS3L2, however it can also trigger decapping by DCP2 allowing 5′-3′ degradation by XRN1 [44, 45, 129, 130, 133–135]. DIS3L2 crystal structure unveiled the DIS3L2 RNA pathway, revealing three uracil-specific zones that explain how DIS3L2 recognizes, binds and processes preferentially oligo(U)-tailed RNAs [132].

The uridylation process is achieved by proteins termed uridyltransferases (TUTases). Humans have seven TUTases that are strictly cytoplasmic, except TUTase-1 (TUT1) that can also be found in mitochondria [133, 136]. Two TUTases were implicated in mRNA uridylation at the 3′ end, TUT4 and TUT7 [130]. In the same study, Lim and colleagues showed that these TUTases were able to sense the length of the poly(A) tail, and preferentially uridylate mRNAs with a tail ranging between 0–25 As. On the contrary, PABPs preferentially bind longer poly(A) tails protecting them from the action of TUT4/7 [130].

This so called TUT-DIS3L2 mRNA decay mechanism was found to prevail in cells under apoptosis. Apoptosis is the most common physiological program of cell death, which plays a vital role in pathogen immune defense, removal of damaged cells, cancer surveillance and cancer therapy effectiveness [137]. Thomas and colleagues [138] observed in human apoptotic cells, that apoptosis triggers global mRNA decay, and the RNA products generated are 3′-uridylated by TUT4/7 and subsequently degraded by DIS3L2. Knockdown of the exoribonuclease inhibits mRNA decay and suppresses cell death; conversely, DIS3L2 overexpression enhances apoptosis, supporting that mRNA decay is a hallmark of cell death [138]. Recently, Liu and colleagues [139], brought another player to this process, a mitochondrial exoribonuclease called PNPT1 that evolved from bacterial PNPase [48, 139, 140]. The work performed in human colon cancer cells, demonstrated that, upon apoptosis trig-

gering, PNPT1 and DIS3L2 act in the same pathway. PNPT1 is released from mitochondria, and starts to degrade RNA from the 3′ end. PNPT1 stops whenever it encounters an obstacle (e.g. ribosome, RNA-binding protein or highly structured sequence), being the RNAs further degraded by the TUT-DIS3L2 pathway [139].

DIS3L2 is also involved in the regulation of let-7 miRNA expression in pluripotent cells, establishing a role of this enzyme in cell differentiation [128, 131, 132, 141]. Indeed, let-7 pre-miRNA biogenesis is one of the best characterized DIS3L2-mediated pathways. miRNAs from the let-7 family function as tumor suppressors and are involved in stem cell renewal [128, 131]. In undifferentiated cells, the expression of let-7 miRNAs is blocked by Lin28, a pluripotency factor that also functions as an oncogene in several cancers [142]. This RNA-binding protein binds to let-7 precursors and promotes their uridylation by TUT4/7. These RNA precursors are thus marked for DIS3L2 degradation, leading to inhibition of let-7 biogenesis.

A recent study has also found a role of DIS3L2 in nonsense-mediated decay (NMD), a quality control pathway that degrades aberrant and physiological mRNAs to maintain cellular homeostasis (as discussed in Chap. 3). In this context, DIS3L2 acts over 3′ ends of NMD decay intermediates that were previously subject to uridylation (L. Romão, personal communication and [143]).

The involvement of DIS3L2 in such cellular important processes, like apoptosis, cell differentiation and RNA quality control (NMD) anticipates its role in human disease. In fact, this RNase has been related with several human disorders. DIS3L2 is associated with Perlman syndrome, which is a rare congenital overgrowth disease [57, 144]. Children affected with Perlman syndrome display macrocephaly, facial abnormalities, neurodevelopmental delay, fetal gigantism, kidney abnormal enlargement and high neonatal mortality. These children also present nephroblastomatosis, an important precursor for Wilms' tumor, a kidney cancer also known as nephroblastoma. Astuti et al. [57] demonstrated that the affected children have germline mutations con-

sistent with DIS3L2 loss of function. DIS3L2 mutations were also associated with Wilms tumor susceptibility [57, 144]. It has been recently suggested that regulation of the growth-promoting gene, insulin growth factor 2 (IGF2), by DIS3L2, could be the link between this RNase and Wilms tumorigenesis [145]. Interestingly, Gregory RI and colleagues have found that DIS3L2 has no effect on the steady state mRNAs levels in DIS3L2-deficient cell lines and knockout mouse kidneys. Instead, it rather specifically perturbs endoplasmic reticulum (ER)-mediated translation (R.I. Gregory, personal communication).

Besides its well documented role in Perlman syndrome and Wilms' tumor, DIS3L2 was also found to be mutated in 3–6% of carcinomas [57, 146]. Also, DIS3L2 has been associated with a Marfan-like syndrome with skeletal overgrowth [147]. The Marfan syndrome is a disorder of the connective tissue that causes high mortality for untreated patients, mainly due to aortic complications [148]. Patients in which DIS3L2 gene was affected showed skeletal overgrowth and malformations, including severe scoliosis (abnormal curvature of the spine), arachnodactyly (long, slender fingers, curvature of the hands and feet) and mild syndactyly (interdigital webbing) [147, 149, 150].

## 4.3 Concluding Remarks

RNA degradation is a set of highly regulated steps that maintain cellular integrity and homeostasis. DIS3-enzymes act over a panoply of RNA substrates in eukaryotic cells and it is clear their role in human disease, namely in cancer development and progression. In this chapter, we explored the consequences of DIS3-enzymes impairment on the physiology of human cells. From this group of proteins, the most well-characterized is DIS3, however its role in cancer is not completely understood. Less is known about the mechanism of action and specific RNA targets of its homologs DIS3L1 and DIS3L2. The molecular mechanisms that link both proteins with disease are still unexplored. Despite the

progress that has been made, there is still much work to perform in order to completely understand how DIS3-enzymes regulate cellular pathways, and how they are related with disease progression. Clinical medicine will certainly benefit from this kind of fundamental research.

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