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DIS3L2: Unveiling a New Player in Tumorigenesis, with a Key Role in Colorectal Cancer

Juan F. García-Moreno[1,2], Paulo Matos[1,2,#], Luísa Romão[1,2,#,*]

[1]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, 1649-016 Lisboa, Portugal

[2]Faculdade de Ciências, BioISI – Instituto de Biossistemas e Ciências Integrativas, Universidade de Lisboa, 1749-016 Lisboa, Portugal

[#]Co-senior authors

[*]Correspondence should be addressed to Luísa Romão, luisa.romao@insa.min-saude.pt

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Abstract

DIS3L2 is a 3′-5′ exoribonuclease that recognizes and degrades uridylated transcripts in an exosome-independent manner and participates in several RNA degradation pathways, such as the nonsense-mediated mRNA decay, or the surveillance of aberrant structured non-coding RNAs. Although some studies have linked DIS3L2 to tumorigenesis and cancer-related processes, its exact role in the development and progression of cancer has remained unclear since the discovery of DIS3L2's ribonuclease activity a decade ago. While some authors have reported evidence of a tumor suppressor role for this exoribonuclease, other studies have shown DIS3L2 as a driver of tumorigenesis. Although differences in tissue type and methodologic approaches may somewhat account for the opposing findings, a recent study from our group further supports a pro-tumorigenic role for DIS3L2, this time in promoting colorectal cancer (CRC) progression. Indeed, proper DIS3L2 expression was proven essential to maintain key tumorigenic properties in CRC cells, including cell proliferation and invasion. Here, we summarize the current state of knowledge regarding the impact of DIS3L2 in cancer, namely in colorectal cancer. The collected data unveils DIS3L2 as a novel putative therapeutic target in cancer that warrants further investigation.

Keywords: Colorectal cancer, Uridylation, DIS3L2, AZGP1, mTOR, Cell viability, Cell migration, Cell invasion

Introduction

DIS3-like 3'-5' exoribonuclease 2, DIS3L2, belongs to the highly conserved RNase II/RNB family and has been a subject of considerable research since in 2012, Astuti et al. identified germline mutations in the *DIS3L2* gene among patients with Perlman syndrome, an autosomal recessive congenital overgrowth condition associated with Wilms tumor predisposition [1]. This finding has sparked interest in further exploring the cellular role of DIS3L2 and its potential implications in the pathogenesis of Perlman syndrome and related conditions. DIS3L2 consists of two cold-shock domains (CSD domains) at the N-terminus followed by a ribonuclease II domain (RNB domain) that confers enzymatic activity for catalyzing RNA degradation, and a carboxy-terminal S1 domain [2]. In contrast to DIS3L2, its two other human paralogs, DIS3 and DIS3L1, exhibit an additional structural feature, the PilT N-terminus (PIN) domain, which allows them to function as the catalytic subunits of the exosome complex [3-5]. The absence of this domain makes DIS3L2 the only major eukaryotic 3′-5′ exoribonuclease with the ability to degrade its substrates in an exosome-independent manner [2,6]. Several immunolocalization studies have demonstrated the cytoplasmic localization of DIS3L2, where it exerts its ribonuclease activity [1,2,6]. Astuti and colleagues documented for the first time its cytoplasmic ribonuclease activity in the human embryonic HEK293 cell line [1]. Later, in 2013, Malecki et al., conducted a deeper characterization of the cytoplasmic mRNA degradation pathway performed by DIS3L2 in *Schizosaccharomyces pombe* (*S. pombe*), detecting

an exosome-independent catabolic activity [6]. This important finding was also corroborated in human cells [2]. However, a recent report from Xing et al., using immunofluorescence and confocal microscopy, unveiled that DIS3L2 resides in both cytoplasm and nucleus [7]. Moreover, the authors highlight a distinctive role for nuclear DIS3L2, wherein it is involved in the regulation of alternative splicing [7].

In the cytoplasm, DIS3L2 is enrolled in an uridylation-dependent transcript degradation. Its action was described for the first time in *S. pombe*, in which DIS3L2 was observed to execute its ribonuclease activity over uridylated poly(A)-containing mRNAs [6]. Since then, several structural and biochemical studies have demonstrated that oligo-uridylation at the 3'-end serves as a recognition signal for DIS3L2 to bind and process its targets [6,8-11]. Furthermore, a mechanistic approach into the manner by which DIS3L2 identifies uridylated substrates unveiled an extensive network of uracil-specific motifs that allows the RNA molecule to navigate a path to the active site of DIS3L2's RNB domain [12]. After these findings, 3'uridylation has been reported as a pivotal post-transcriptional modification in the metabolism of different types of RNAs in the cell [10,11,13]. In human cells, three uridylyl transferases (TUT1, TUT4 and TUT7) have been reported to mediate uridylation at the 3'-end of specific RNAs. Largely localized within the nucleus, TUT1 mediates the oligo-uridylation required for U6 snRNA maturation, playing a key role in the correct functioning on the spliceosome [14]. On the other hand, TUT4 and TUT7 (TUT4/7) were identified as the two uridylyl transferases responsible for catalyzing uridine (U)-tailing at the 3'-end of cytoplasmic mRNAs, as documented by Lym and colleagues [15]. The authors observed that TUT4/7 induces uridylation over deadenylated transcripts that still preserve a tail ranging between 0 to 25 adenines. Besides targeting mRNAs, TUT4/7 also participates in the regulatory pathway of various classes of RNAs, such as miRNA biogenesis, surveillance of defective ncRNAs and decay of histone mRNAs at the end of the S phase [10,16-19].

DIS3L2 plays a vital role in several biological and physiological processes, including RNA quality control, as documented in the nonsense-mediated mRNA decay (NMD) pathway, and for aberrant structured non-coding RNAs [8-10], cell proliferation and tissue growth [20-22], cell division [1], cell differentiation [11,13] and apoptosis [23,24]. Hence, an abnormal functioning of DIS3L2's ribonuclease activity is expected to take part in the etiology of numerous human disorders, as evidenced by its association with the Perlman syndrome and Wilms tumors. Although some studies have linked DIS3L2 to tumorigenesis and cancer-related processes, its exact role in the development and progression of cancer remains unclear. Below, we review the current data evidencing a putative role for DIS3L2 in cancer, with an emphasis on recent findings by our group that implicate this exoribonuclease in the development of colorectal cancer (CRC).

The Dual Role of DIS3L2 in Cancer

While the literature on DIS3L2's role in cancer remains limited, some studies have demonstrated its association with vital biological processes directly related to tumorigenesis, particularly in certain tumor types like Wilms tumor. The latter is a rare kidney cancer, typically diagnosed in childhood, that stands as the first tumor type associated with lower DIS3L2 expression. Genome-wide autozygosity mapping conducted in families affected by the Perlman syndrome led to the identification of germline loss-of function mutations in the *DIS3L2* gene [1]. Interestingly, the authors also observed that patients with this syndrome show higher predisposition to Wilms tumorigenesis and identified complete and partial *DIS3L2* depletion in up to 30% of sporadic Wilms tumors. Transcriptome analysis in DIS3L2-null nephron progenitor cells from mice, which serves as a model for Wilms tumor research, unveiled a novel target of DIS3L2, the insulin growth factor 2 (*IGF2*) [25]. IGF2 possesses growth-promoting activity and has been identified as a driver of Wilms tumorigenesis [26], implying that it could potentially induce Wilms tumor development in a DIS3L2-deficient context. Moreover, the authors reported that DIS3L2-deficient mice exhibit phenotypes that resemble key Perlman syndrome features, strengthening the association between the occurrence of this syndrome and Wilms tumors. Consequently, these findings suggest that DIS3L2 could function as tumor suppressor in this rare kidney cancer, opening new avenues in the development of targeted treatments.

Astuti et al., showed that DIS3L2's knockdown (KD) in cervical cancer HeLa cells resulted in chromosomal abnormalities, mitotic errors, as well as cell death phenotypes [1]. In contrast, the same study also reported that lack of DIS3L2 expression in HeLa cells leads to an increased anchorage independent colony growth. Interestingly, an RNA-seq experiment performed by Lubas and colleagues in DIS3L2-depleted HeLa cells, revealed gene ontology (GO) enrichment of upregulated mRNAs coding for proteins related to cell-cycle regulation and DNA packaging, supporting the involvement of DIS3L2 in cell division and chromosomal stability [2]. These results prompted further research on the impact of DIS3L2 on cell proliferation and its role in the cell cycle. Cell proliferation plays a crucial role in several biological processes, for instance, during organogenesis, tissue homeostasis and repair or disease progression, especially in cancer, in which a sustained proliferative signaling constitutes a robust hallmark [27-29]. As reported by Towler et al., proper DIS3L2 expression is crucial for regulating cell proliferation in the wing imaginal discs of *Drosophila melanogaster* since its knockdown leads to wing overgrowth due to an increased cell proliferation rate [21]. In a more recent study, the authors performed an RNA-seq experiment on DIS3L2-null mutant wing discs and identified the imaginal disc growth factor 2 (Idgf2) as the DIS3L2 target responsible for promoting wing overgrowth [22]. In addition,

the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway was indicated as the pro-proliferative pathway by which DIS3L2 potentially controls cell proliferation and tissue growth. These insights may lead to innovative strategies for addressing disorders characterized by abnormal cell growth, such as the Marfan-like syndrome, a skeletal overgrowth condition associated with a chromosomal translocation that results in partial depletion of the *DIS3L2* gene [20], similarly to that observed in the Perlman syndrome.

Nonetheless, a growing body of literature illustrates several DIS3L2's pro-oncogenic activities in other tumorigenic contexts. A well-known example is the impact that uridylation and DIS3L2 exert in the highly conserved LIN28/let-7 pathway (**Figure 1A**). In this pathway, the RNA-binding protein LIN28 binds the let-7 miRNA precursor (pre-let-7) and recruits TUT4/7, promoting pre-let-7's 3'-end oligo-uridylation [16,19,30]. Uridylated pre-let-7 impedes DICER processing and triggers degradation by DIS3L2, thereby playing an important role in the downregulation of mature let-7 miRNA abundance [10,12,13]. Through this mechanism, LIN28 not only acts as a pluripotency factor responsible for maintaining an undifferentiated and proliferative state in stem cells [31,32], but can also operate as an oncoprotein in the context of various human malignancies [33,34]. Conversely, let-7 miRNA plays a pivotal role in the regulation of embryonic cell differentiation and can function as a tumor suppressor by silencing several human oncogenes [35-37]. The use of transcriptomic analysis employing the

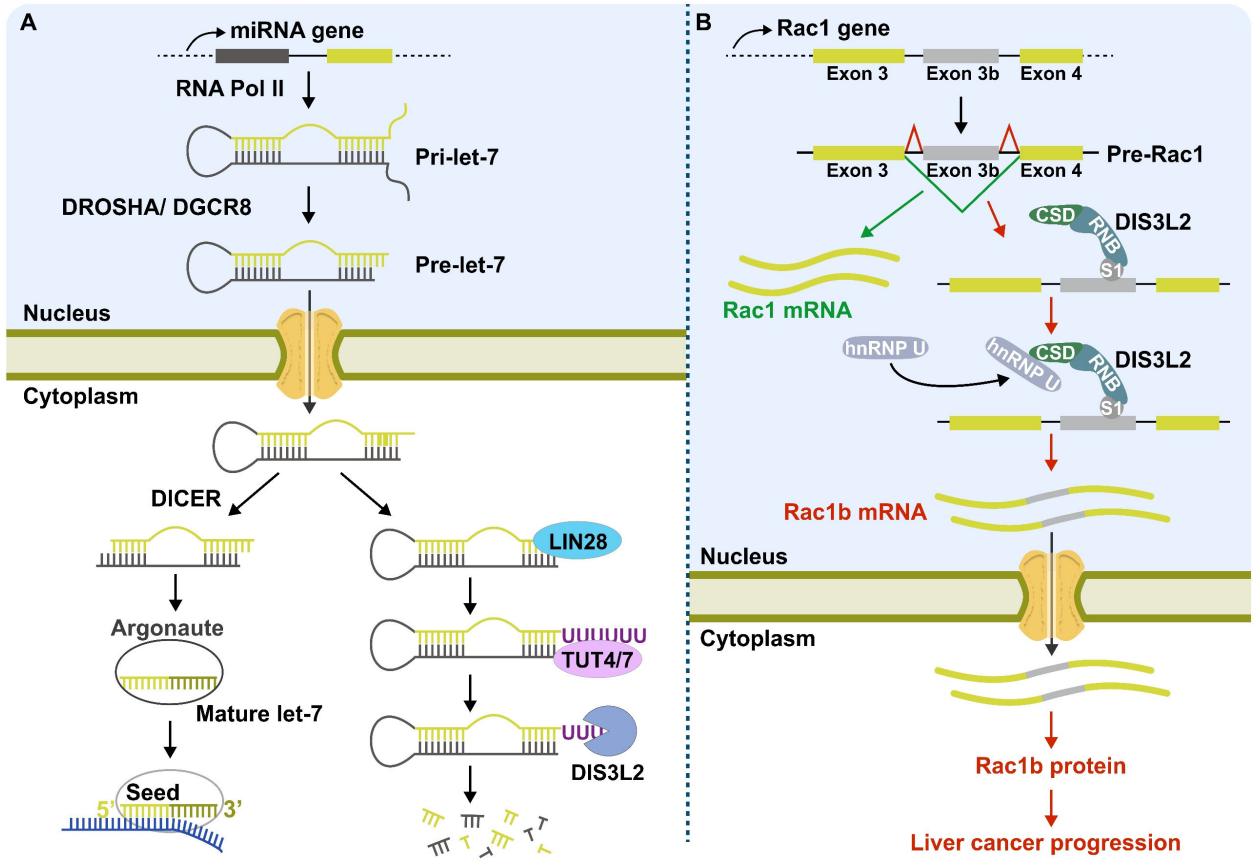

Figure 1. Pathways implicating DIS3L2 in cancer development. (A) Working model of DIS3L2's role in the biogenesis of let-7 miRNA. The RNA polymerase II (RNA Pol II) transcribes let-7 pri-miRNA (pri-let-7) which is further processed by DROSHA/DGCR8 complex into pre-let-7. Once in the cytoplasm, pre-let-7 is cleaved by DICER, yielding a mature miRNA (on the left). Then, mature let-7 is loaded into the RNA-induced silencing complex (RISC), which triggers Argonaute-mediated cleavage of the complementary miRNA target. On the bottom right, it is represented the suppression of let-7 biogenesis by the coordinated action of RNA-binding protein LIN28, terminal uridylyl transferases 4 and 7 (TUT4/7) and DIS3L2. LIN28 binds pre-let-7 and recruits TUT4/7, triggering uridylation at the 3'-end, which inhibits DICER-mediated processing. Next, the exoribonuclease DIS3L2 recognizes and degrades uridylated pre-let-7, leading to the derepression of several human oncogenes. **(B)** Schematic model for DIS3L2 involvement in Rac1 alternative splicing. DIS3L2 binds to exon 3b of pre-Rac1 by its S1 domain and interacts with the splicing factor hnRNP U through its cold-shock domain. The interplay between DIS3L2 and hnRNP U leads to the inclusion of exon 3b, resulting in the production of the oncogenic variant Rac1b, which promotes liver cancer progression.

Cancer Cell Line Encyclopedia (CCLE) database, revealed a positive correlation between DIS3L2 and LIN28 expression among a broad spectrum of tumor cell types [38]. Moreover, clustering analysis of expression patterns revealed that LIN28-positive cancer cells displayed not only high expression of DIS3L2, but also of uridylation factors and multiple endogenous let-7 targets [39]. When the expression analysis of these molecules was extended to human hepatocellular carcinoma (HCC) samples, using transcriptomic data from The Cancer Genome Atlas (TCGA) database, a positive correlation emerged between DIS3L2, TUT4 and LIN28, which had been found in the CCLE dataset [39]. These findings, along with the known tumor suppressor role of let-7 in multiple malignancies [35–37], supported a pro-tumorigenic role for DIS3L2 through inhibition of let-7 biogenesis.

Remarkably, Xing and colleagues also documented a pro-oncogenic role for DIS3L2 in HCC, but through its regulation of alternative splicing, independently of its ribonuclease activity in the cytoplasm [7]. These authors reported for the first time a nuclear localization of wild type DIS3L2, a phenomenon not previously observed in other studies, except for a version of DIS3L2 lacking exon 6, which was found in both the cytoplasm and the nucleus of HeLa cells [1]. The distinct cellular distribution of DIS3L2 may be attributed to the different cell types used in each study, suggesting that the subcellular localization of this protein occurs in a cell-type-dependent manner. Disruption of splicing patterns are frequent events in cancer, and certain splicing isoforms can endow tumor cells with advantageous properties, including pro-proliferative signaling, evasion of growth suppressors, inhibition of programmed cell death, and activation of metastatic properties [40]. Interestingly, DIS3L2 interacts with the heterogeneous nuclear ribonucleoprotein (hnRNP) U through its cold-shock domains [7]. hnRNP U is an RNA-binding protein widely involved in alternative splicing by regulating U2 snRNP maturation in the nucleus [41]. The interplay between DIS3L2 and hnRNP U controls alternative splicing of the small GTPase RAC1 pre-mRNA, inducing the inclusion of exon 3b, and subsequent production of the oncogenic splicing variant, RAC1B, which promotes HCC progression (**Figure 1B**). Moreover, immunohistochemistry analysis on clinical HCC samples revealed that DIS3L2 expression positively correlates with that of RAC1B as well as with HCC progression [7].

The discovery of this novel DIS3L2 function in alternative splicing, as well as, which of the nuclease domains were implicated in this nuclear process, provides novel targets for the development of small molecule drugs specifically aimed at inhibiting DIS3L2's capacity to promote the RAC1B splice variant. The identification of new druggable sites disrupting DIS3L2's interaction with either hnRNP U or its targeted pre-mRNA holds special interest for rational drug design, since targeting this tumor-associate alternative splicing event mediated by DIS3L2 could still preserve its ribonuclease activity in the cytoplasm, thus reducing the likelihood of potential side effects. Another promising therapeutic approach is the development of splice-switching antisense oligonucleotides (ASOs) to rescue mis-splicing. ASOs have the ability to modulate splicing by preventing splicing factors from binding to the targeted pre-mRNA (steric blocking), leading to exon skipping, as reported in a mouse model for Duchenne muscular dystrophy where ASO intervention avoids the inclusion of an exon containing deleterious nonsense mutations [42,43]. The use of splice switching ASOs to prevent inclusion of exon 3b could disrupt DIS3L2's binding to RAC1 pre-mRNA, leading to the production of its wild type splice isoform.

DIS3L2 Functions as an Oncoprotein in Colorectal Cancer

DIS3L2 is overexpressed in colorectal cancer and predicts a poor prognosis

Our group has recently published a comprehensive investigation into the functional networks of DIS3L2 in the context of CRC [44]. Analysis of DIS3L2 gene expression in a cohort of 434 patients, using transcriptomic data from the TCGA database, showed that DIS3L2 is overexpressed in CRC samples compared to non-cancerous colonic tissues. In addition, Kaplan-Meier analysis showed that higher DIS3L2 expression correlates with worse prognosis in patients affected by advanced CRC, namely stages III and IV. Similar results were documented by Xing et al. in HCC, in which both DIS3L2 mRNA and protein expression levels are significantly higher in HCC tissues compared to adjacent non-tumorigenic liver tissues [7]. Moreover, the authors observed a gradual increase in DIS3L2 expression from TNM stage I to IV, as well as a higher expression in patients with shorter survival times than those with lower DIS3L2 expression. These findings indicate that DIS3L2 expression plays an important role in metastatic stages of CR and HC carcinomas, wherein tumor cells spread beyond their original location.

Interference with DIS3L2 expression disrupts key tumorigenic properties

GO analysis of upregulated transcripts following DIS3L2 depletion in SW480 CRC cells revealed enrichment in genes directly associated with the negative regulation of cell proliferation, while downregulated transcripts were mostly enriched in genes associated with the promotion of cell cycle progression [44]. These observations suggested that the absence of DIS3L2 favors a lower proliferative state in this tumorigenic context. Indeed, metabolic assays showed that depletion of DIS3L2 substantially impaired cell proliferation in the poorly differentiated CRC cell lines, SW480 and HCT116 (**Figure 2A**). In contrast, the impact of DIS3L2 KD on well-differentiated Caco-2 and HT-29 cells, as well as in the non-

transformed colonic NCM460 cell line, is minimal or null. Similar results were reported in HCC cells, in which silencing of DIS3L2 results in lower cell proliferation [7]. On the other hand, the suppression of DIS3L2 expression in the embryonic cell line HEK293 has been documented to enhance cell proliferation [22]. The different impacts observed upon DIS3L2 depletion depending on the cell line indicate that this protein may act in a cell type-specific manner to regulate cell proliferation.

Cell migration is a crucial cellular process during cancer metastasis, since cell motility is a rate-limiting step during tumor cell dissemination to local and distant sites from the primary tumor [45]. The coordination of different cellular events is required to achieve directed cell migration, including protrusion, chemotaxis, and contractility [46]. Mirroring the results observed after DIS3L2 KD on cell proliferation, we demonstrated that depletion of DIS3L2 also repressed the migration of SW480 cells [44]. Moreover, depletion of DIS3L2 impaired both collective cell motility as well as individual migratory and invasive behavior of SW480 and HCT116 cells (**Figure 2B**). On the other hand, migration of Caco-2, HT-29 and NCM460 cells, does not respond to the knockdown of DIS3L2 [44]. These results indicate that the role of DIS3L2 in CRC may manifest only at later oncogenic stages, where it appears to play an essential role in sustaining both a pro-proliferative transcriptional context and an invasive cellular behavior. Interestingly, we found that overexpression of DIS3L2 does not elicit significant alterations in the cell viability and migration of CRC cells [44]. These results are in contrast to those obtained by Xing et al. after overexpressing DIS3L2 in HCC cells [7], indicating that DIS3L2 pro-oncogenic effects in these two oncogenic contexts may involve different mechanisms, such as its involvement in alternative splicing within the nucleus or its role as a cytoplasmic exoribonuclease. This will require further investigation in the future.

The function of DIS3L2 is inherent to transcript uridylation at the 3'-termini by TUT4/7. Interestingly, the transcriptome landscape in the CRC cell line SW480, is deeply disrupted upon DIS3L2 KD, which contrasts with the lower perturbation detected in the absence of DIS3L2 and uridylation, as showed in a hierarchical cluster analysis of the transcriptome [44]. In addition, the number of differentially expressed genes (DEGs) in DIS3L2-depleted SW480 cells exceeds more than twice the number of DEGs after triple KD of DIS3L2 together with TUT4 and TUT7. This phenomenon extends to the decrease observed in the cell viability of SW480 and HCT116 cells after DIS3L2 KD, which is fully reverted in the absence of DIS3L2 and uridylation. We also showed that mRNA accumulation of specific DIS3L2 substrates in DIS3L2-depleted SW480 cells is abolished after the triple KD of DIS3L2+TUT4/7. These findings indicate that the cellular impact of DIS3L2 in the cell is uridylation-dependent, which suggests that uridylation might protect DIS3L2-targeted mRNAs from alternative degradative pathways. However, further research is necessary to confirm whether DIS3L2 is the only ribonuclease responsible for the

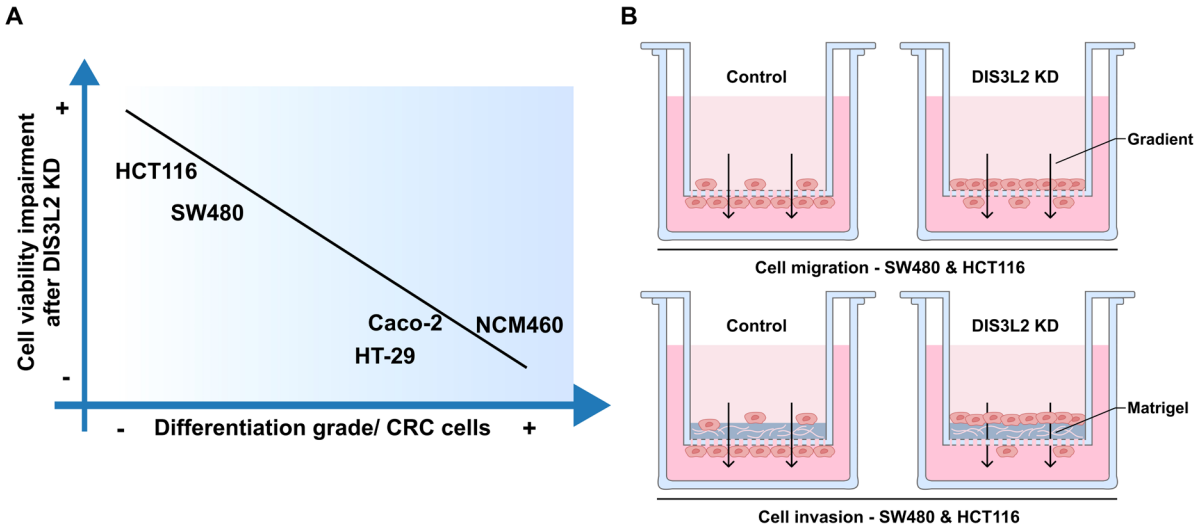

Figure 2. DIS3L2's impact on key colorectal cancer (CRC) properties. (A) Graphical representation of DIS3L2 knockdown (KD) impact on cell viability of CRC cells. Cell viability of highly dedifferentiated CRC cells (HCT116 and SW480) is significantly impaired after DIS3L2 KD, while that of well-differentiated CRC cells (Caco-2 and HT-29) and non-transformed colonocytes (NCM460) experiences a minimal change. **(B)** Experimental representation of DIS3L2 KD impact on chemotactic cell migration (top) and invasion (bottom) of CRC cells. Depletion of DIS3L2 in the highly dedifferentiated CRC cell lines SW480 and HCT116 results in a substantial repression of their migration and invasion abilities.

degradation of uridylated transcripts and to explore potential compensatory mechanisms that could be triggered in the absence of uridylation in the cell.

DIS3L2 knockdown leads to suppression of the mTOR signaling pathway

Several studies have documented the involvement of DIS3L2 in apoptosis, cell differentiation, division, and proliferation [1,13,22,23,44]; however, the mechanisms by which DIS3L2 regulates these cellular processes remains poorly characterized in most cases. In the context of CRC, our group has identified the mTOR pathway, as a key signaling pathway controlled by DIS3L2, that regulates cell proliferation [44]. The mTOR signaling pathway plays a key role in the regulation of cell growth and survival in both physiological and pathological conditions, especially in cancer [47]. The central player in this pathway is the mTOR protein, a serine/threonine kinase that captures and integrates intracellular and extracellular signals from the nutrient and energy status [48]. It takes part in two distinct complexes, each with different structures and biological functions: the mammalian target of rapamycin complex 1 (mTORC1) and the mammalian target of rapamycin complex 2 (mTORC2). mTORC1 consists of mTOR, mLST8, DEPTOR, Raptor, AKT1S1/PRAS40 and possesses two main downstream effectors, p70 ribosomal S6 kinase (p70S6K) and the eukaryotic initiation factor 4E-binding protein (4E-BP), that regulate protein synthesis, cell cycle progression, and cell growth [49] by modulating the levels of key regulator proteins such as cyclin D1, which is essential for the promotion of G1/S cell cycle progression [50] (**Figure 3**).

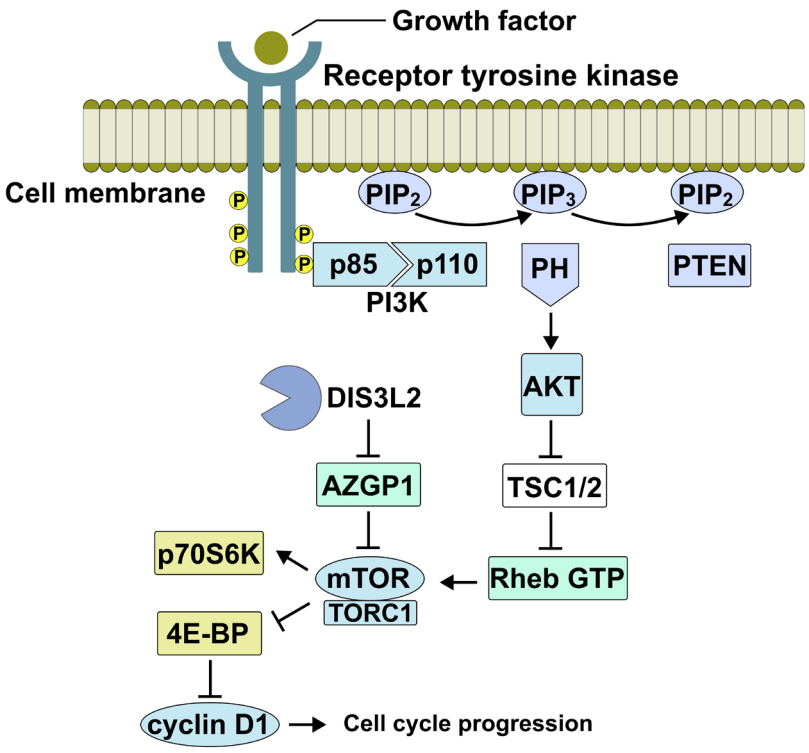

Figure 3. Schematic overview of DIS3L2's role in the mTOR signaling pathway. A growth factor binds the extracellular domain of a receptor tyrosine kinase at the cell membrane leading to its activation and autophosphorylation of tyrosine residues, which promotes recruitment of the amino-terminal domain of p85, the regulatory subunit of PI3K. The latter blocks the inhibition of p110 (catalytic subunit of PI3K) and induces the formation of the p85-p110 heterodimer to the lipid phosphatidylinositol-4,5-bisphosphate (PIP2), at the plasma membrane. Active PI3K phosphorylates PIP2, leading to the production of the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3). Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) function as a lipid phosphatase, removing the phosphate group in position 3 of the inositol ring from PIP3, therefore negatively regulating PI3K signaling. PIP3 recruits a subset of pleckstrin homology (PH)-domain containing proteins to the membrane, which induces AKT activation. AKT phosphorylates other cytoplasmic proteins, including TSC2 leading to disruption of the TSC2/TSC1 GAP activity over Rheb-GTP. Rheb-GTP activates mTOR, whereas the exoribonuclease DIS3L2 is directly involved in the degradation of AZGP1 mRNA, which ends in lower AZGP1 protein levels, reducing the inhibitory activity of AZGP1 over mTOR. Therefore, DIS3L2 functions as an activator of mTOR, which, in turn, regulates protein synthesis, cell cycle entry and cell proliferation by activating p70S6K and inactivating 4E-BP. mTOR phosphorylation of 4E-BP induces derepression of cyclin D1 mRNA translation, resulting in higher levels of cyclin D1, which promotes cell cycle progression.

The mTOR signaling pathway was highlighted by a KEGG pathway analysis for upregulated genes upon DIS3L2 KD in SW480 cells [44]. Most strikingly, we found AZGP1, a well-known mTOR inhibitor [51-53], highly upregulated in DIS3L2-depleted SW480 cells. The mTOR inhibitory activity of AZGP1 has been reported in different cancer types, and its overexpression is known to interfere with key CRC cell properties, such as a sustained proliferation, migration, and invasion, via suppression of the mTOR signaling pathway [51]. After verifying that DIS3L2 was directly involved in the degradation of AZGP1 mRNA, we confirmed that the depletion of DIS3L2 resulted in decreased mTOR and 4E-BP phosphorylation, in agreement with mTORC1 pathway inhibition. Consistently, we also found cyclin D1 protein levels significantly decreased after DIS3L2 depletion. Cyclin D1 together with its two binding partners, cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), constitute active complexes that phosphorylate and inhibit the retinoblastoma protein (RB), which promotes cell cycle progression [54,55], and high levels of cyclin D1 have been described in most human cancers, including CRC [56,57]. Collectively, these findings suggest that the AZGP1/mTOR/cyclin D1 axis might play a role in mediating DIS3L2's pro-proliferative function in CRC cells. Nonetheless, additional research is needed to gain a more comprehensive understanding of the interplay between this pathway and the tumor phenotypic changes associated with DIS3L2.

Conclusions

DIS3L2 attracted much of research interest since its 3'-5' exoribonuclease activity was confirmed in 2012 by Astuti and colleagues [1]. Most of the research related to this ribonuclease concerns its involvement in several degradation pathways, however whether it acts as a driver or suppressor of tumorigenesis has remained unclear. Notably, we recently documented an oncogenic role for DIS3L2 in CRC, in which its overexpression associates with more advanced disease stages and unfavorable prognosis [44]. Knockdown of DIS3L2 disrupts transcriptome homeostasis and impairs viability, migration, and invasion abilities of poorly differentiated CRC cells. While suppression of cell viability associated with DIS3L2 KD has been reported in other cancer types, such as HCC, the impact of this ribonuclease on cell migration and invasion of highly dedifferentiated CRC cells had never been described. Moreover, our study was the first to establish a link between DIS3L2 and the mTOR signaling pathway, leading to CRC cell survival and proliferation. Also potentially relevant is the observation that DIS3L2 depletion does not significantly impact well-differentiated CRC cells and non-transformed colonocytes, which makes this endonuclease an attractive target for the development of novel therapeutic approaches in advanced CRC.

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Author Contributions Statement

Juan Fernández García-Moreno wrote and prepared the original draft; Luísa Romão and Paulo Matos supervised, reviewed, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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