Modulation and function of Pumilio proteins in cancer

Isabelle Leticia Zaboroski Silva [1], Arissa Andreina Kohata [1], Patrícia Shigunov [*]

Laboratory for Basic Biology of Stem Cells (LABCET), Instituto Carlos Chagas - FIOCRUZ-PR, Curitiba, Paraná 81830-010, Brazil

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ABSTRACT

Post-transcriptional regulation is involved in tumorigenesis, and in this control, RNA-binding proteins are the main protagonists. Pumilio proteins are highly conserved RNA-binding proteins that regulate many aspects of RNA processing. The dysregulation of Pumilio expression is associated with different types of cancer. This review summarizes the roles of Pumilio 1 and Pumilio 2 in cancer and discusses the factors that account for their distinct biological functions. Pumilio levels seem to be related to tumor progression and poor prognoses in some kinds of tumors, such as lung, pancreatic, prostate, and cervical cancers. Pumilio 1 is associated with cancer proliferation, migration, and invasion, and so is Pumilio 2, although there are contradictory reports regarding the latter. Furthermore, the circular RNA, circPUM1, has been described as a miRNAs sponge, regulating miRNA involved in the cell cycle. The expression and function of Pumilio proteins depend on the fine adjustment of a set of modulators, including miRNAs, lncRNAs, and circRNAs; this demonstrates that Pumilio plays an important role in tumorigenesis through a variety of regulatory axes.

1. Introduction

Post-transcriptional regulation of gene expression plays a crucial role in many critical processes of cancer development and progression, such as apoptosis, proliferation, migration, or invasion. This regulation, which is known as RNA metabolism, can be mediated by RNA-binding proteins that control many aspects of RNA processing, as well as RNA splicing, polyadenylation, capping, modification, transport, localization, translation, and stability (reviewed by [1]). The Pumilio (Pum)/Puf family comprises highly conserved RNA-binding proteins in most eukaryotic organisms. Pum is involved in diverse biological processes,

such as embryonic development, functioning and development of the nervous system, stem cell and germ cell maintenance, rRNA processing, ribosome biogenesis, as well as chemotactic cell movement (reviewed by [2]).

The Pum family shares a conserved RNA-binding domain (RBD) that comprises eight repeated motifs that are denominated Puf repeats (PUM-HD, Pumilio homology domain). The RBD binds with great affinity and specificity to 8–10 nt regulatory sequences predominantly found in the 3′ untranslated regions (UTRs) of mRNAs [3–5]. Translation and mRNA stability are controlled by the interaction of trans-acting regulators with cis-acting RNA elements. The consensus binding site for proteins closely

related to Pum, 5'UGUAHAUA, herein referred to as the Pumilio Recognition/Response Element (PRE), is well conserved (reviewed by [6]) (Fig. 1A). Target downregulation is brought about via deadenylation, inhibition of translation initiation, and combinatorial activity involving miRNA (reviewed by [2,7]).

Mammals have two cytoplasmic canonical proteins, PUM1 and PUM2, which contain eight α-helical repeats that bind to the RNA bases of single-stranded RNA (reviewed by [6]) (Fig. 1B-C). PUF binding sites
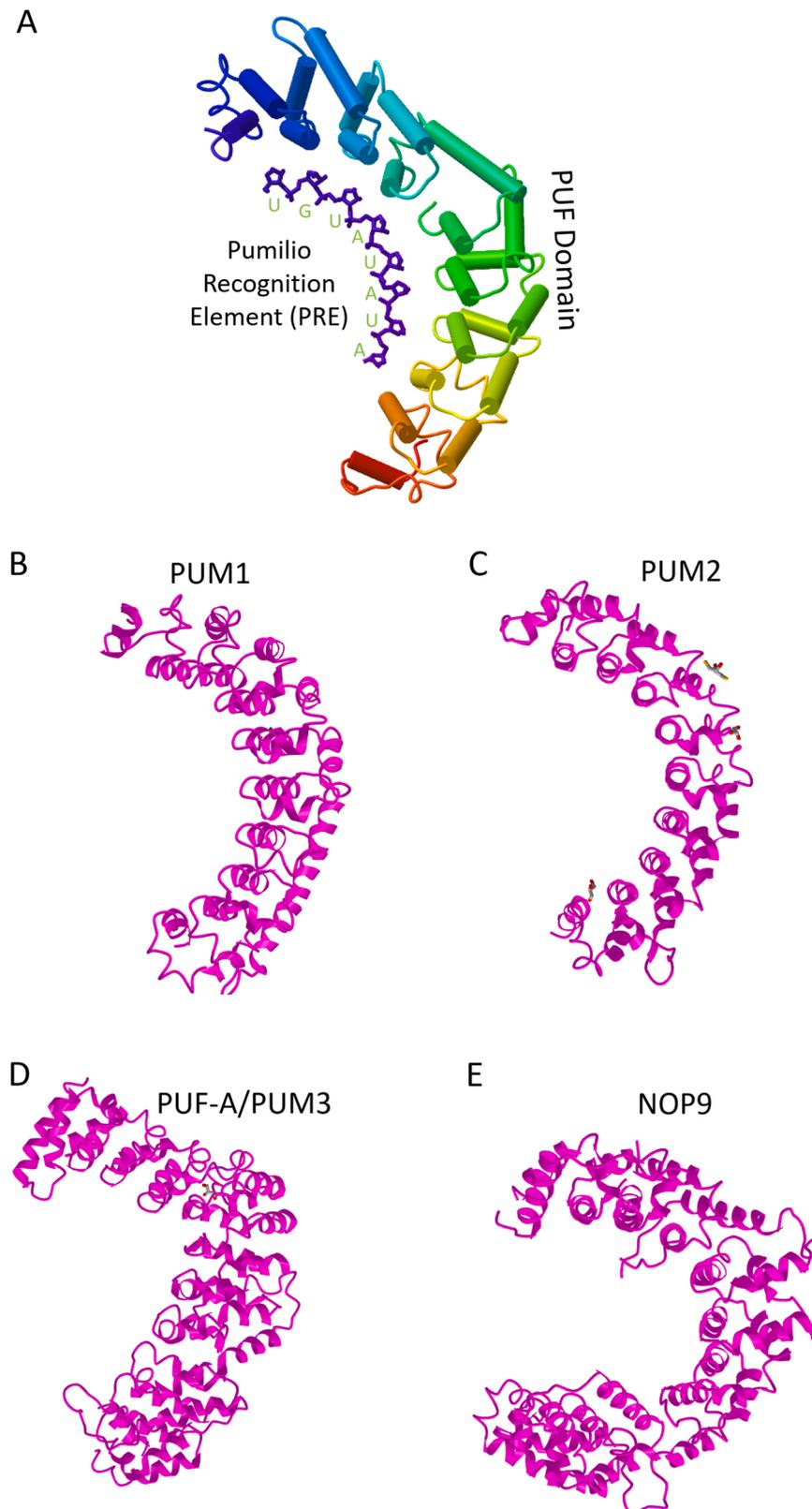
Fig. 1. Crystal Structure of Pumilio homology domain interacting with RNA (A) and of different proteins of Pumilio family: (B) PUM1 (human, PDB: 1M8Z), (C) PUM2 (mouse, PDB: 3GVT), (D) PUF-A/PUM3 (human, PDB: 4WZR), and (E) NOP9 (*Saccharomyces cerevisiae*, PDB: 5WTX) [15,70–74].

are prevalent in the transcriptome, and hundreds of mRNAs copurify with individual Pum [8–13]. PUF-A/PUM3 and Nop9 are unusual members of this family since they contain eleven α-helical repeats, with predominantly nucleolar localization, being involved in ribosome biogenesis [14–16] (Fig. 1D-E).

Several mRNAs involved in neurological disorders, cancer, and cardiovascular diseases were found to be targets of Pum, thereby indicating its influence on the genetic regulation of such diseases [17]. The dysregulated expression of Pum proteins can inhibit repair processes and replication of DNA and lead to chromosomal instability and tumorigenesis [18]. The expression levels of PUM1 and PUM2 are significantly altered in 17 types of cancer tissues, which result in the dysregulation of their target mRNAs [19]. These targets encode factors responsible for processes such as proliferation, apoptosis, and cell cycle, also involved in cancer [19]. This review discusses the findings regarding the Pum protein family in different types of cancer; it also identifies common regulatory axes that affect proliferation, migration, and invasion.

2. The role of Pumilio 1 in proliferation, migration, and invasion in different cancers

PUM1 protein levels are being associated with cellular processes that favored cancer development in different types of cancer and it seems to be associated with patient prognosis (Table 1). PUM1 protein levels were higher in pancreatic ductal adenocarcinoma tissues than they were in the adjacent tissues; they were significantly associated with the stages of TNM and with the overall survival time, and therefore indicate a correlation between a high PUM1 expression and a poor prognosis [20]. In vitro and in vivo assays showed that PUM1 knockdown inhibited cell proliferation, migration, invasion, and epithelial–mesenchymal transition (EMT) and also promoted apoptosis in MIA PaCa-2 and PANC-1 cells [20]. Dai and collaborators demonstrated that triptolide enhances the sensitivity of pancreatic cancer cells to TRAIL, a promising anticancer drug, by activating autophagy via PUM1 downregulation

[21]. PUM1-silencing enhanced TRAIL-induced suppression of proliferation as well as the promotion of apoptosis of pancreatic cancer cells [21]. The PUM1 expression level is high in colon tumors and metastatic tumor tissues when compared to the lower PUM1 expression in the normal colon tissues found through immunohistochemical analyses [22]. PUM1 overexpression is associated with increased cell proliferation, increase in the number of colonies, cell migration, and size and number of spheroids [22]. PUM1 was upregulated in cetuximab-resistant colon cancer cells, and PUM1 suppression inhibited their proliferation [23]. Immunohistochemistry also revealed that PUM1 levels in ovarian cancer tissues were higher than in normal tissues [24]. PUM1 downregulation suppresses viability, proliferation, migration, and invasion of ovarian carcinoma cells [24]. By studying 101 prostate samples from "The Cancer Genome Atlas" database, Li and collaborators found that PUM1 mRNA was significantly higher in prostate carcinoma than in benign tissues [25]. PUM1 downregulation in prostate cancer cells decreased proliferation, induced apoptosis, and repressed prostate tumorigenesis in vivo [25]. All this suggests that PUM1 could be an oncogene that promotes the growth and proliferation of pancreatic, colon, ovarian, and prostate cancer cells.

Long non-coding RNAs (lncRNAs) are non-coding RNAs with over 200 nt in length; their role in cancers and many other diseases has been highlighted [26]. lncRNAs serve as a "molecular sponge" or "molecular scaffold" for RNA binding proteins to regulate the expression of downstream genes. LncRNA activated by DNA damage (NORAD) plays an essential role in DNA protection and chromosomal stability [27]; by sequestering PUM proteins, NORAD represses the stability and translation of targets mRNAs [18]. In breast cancer tissues and lineage cells, NORAD and PUM1 were upregulated, while miR-323A-3p is downregulated [28]. When NORAD is overexpressed or miR-32–3a is downregulated, PUM1 levels enhance, suggesting a regulatory axis for viability, migration, and invasion of cancer cells, involving lncRNA and miRNA for PUM1 regulation [28]. Moreover, miR-411–5p regulates the proliferation, invasion, metastasis, and apoptosis of non-small cell lung

Table 1
Comparative table showing studies on PUM1 and circPUM1 in different types of cancer.

Type of cancer	Expression analysis	Regulatory axis	Prognosis	Ref.
Breast cancer	PUM1 protein and mRNA	NORAD/ miR-323a-3p	High NORAD expression was correlated with clinical stage (associated with high PUM1 expression).	[28]
Ovarian cancer	PUM1 protein and mRNA	STAT3, BCL2, MMP2, and VEGFA	–	[24]
Prostate cancer	PUM1 protein and mRNA	CDKN1B	Overexpression of PUM1 (and downregulation of CDKN1B) is associated with poor prognosis in prostate cancer patients.	[25]
Pancreatic cancer	PUM1 protein and mRNA	–	–	[21]
Pancreatic ductal adenocarcinoma	PUM1 protein and mRNA	PERK/eIF2/ATF4	Patients with high PUM protein expression tended to have a more advanced TNM stage and short survival time.	[20]
Colon cancer	PUM1 protein and mRNA	DXX5	–	[23]
Colon cancer	PUM1 protein and mRNA	–	–	[22]
Non-small cell lung cancer	PUM1 protein and mRNA	miR-411–5	–	[29]
Non-small cell lung cancer	CircRNA PUM1	miR-590–5p/ METTL3	Patients with high circPUM1 expression showed a shorter overall survival than those with low circPUM1 expression.	[38]
Renal cell carcinoma	CircRNA PUM1	FABP7 and MEK/ERK pathway, miR-340–5p	High expression of FABP7 indicates poor clinical outcomes and survival (associated with high expression of CircPUM1).	[40]
Ovarian cancer	CircRNA PUM1	NF-kB/miR-615–5p and MMP2/miR-6753–5p	–	[33]
Endometrial cancer	CircRNA PUM1	miR-136/NOTCH3	–	[34]
Lung adenocarcinoma	CircRNA PUM1	miR-326	–	[32]
Polycystic ovary syndrome	CircRNA PUM1	miR-760	–	[35]
Hepatocellular carcinoma	CircRNA PUM1	miR-1208	–	[36]
Pancreatic cancer	CircRNA PUM1	miR-200c-3p	–	[39]
Papillary thyroid cancer	CircRNA PUM1	miR-21–5p/ MAPK1	The overall survival was lower in patients with high circPUM1 expression than patients with low expression, showing that circPUM1 could predict a poor prognosis.	[37]

cancer by target regulating PUM1 [29]. The sum of regulatory elements acting on the mRNA and PUM1 proteins enables its function to undergo finer adjustments which are essential for balancing different processes.

## 2.1. Circular RNA PUM1 (CircPum1) in different cancers

Covalently linked circular RNAs (circRNAs) are generated by precursor mRNA back-splicing of exons of thousands of genes in eukaryotes and have also been associated in cancer development (reviewed by [30]). The functions of circRNAs consist of sequestration of microRNAs or proteins, adjustment of transcription and splicing, and still protein translation (reviewed by [31]). Interestingly, circPUM1 has been described for promoting different types of cancer, such as ovarian, polycystic ovary, endometrial, hepatocellular carcinoma, papillary thyroid, pancreatic, renal cell carcinoma, and non-small cell lung cancer [32–40] (Fig. 2 and Table 1). The induction of circPUM1 knockdown decreased proliferation, migration, and invasion, but increased apoptosis, levels of pro-apoptotic proteins (cleaved caspases-3), and secretion of proinflammatory factors (TNF-α, IL-6, and IL-8) in trophoblast cells [41]. CircPUM1 is significantly upregulated in both lung adenocarcinoma cell lines and tissues [32]. The silencing of circPUM1 impaired the proliferation, migration, and invasion capacity and increased apoptosis in A549 cells [32]. In vivo, circPUM1 silencing inhibits tumorigenesis [32]. Even the intraperitoneal injection of circPUM1-knockout tumor cells in nude mice resulted in a decrease in the tumor's metastatic ability [33]. circPUM1 could increase the development of hepatocellular carcinoma tumors and regulate the expression of EMT-related proteins [36]. The in vivo silencing of circ-PUM1 also impeded tumorigenesis of papillary thyroid cancer [37]. Therefore, the PUM1 gene expresses the canonical transcript, which will be translated into an RNA-interacting protein; it also expresses circRNA, which plays a regulatory role as an RNA molecule. The functions of both converge in the promotion of cancer.

Circular RNA PUM1 has been reported as a sponge of miRNAs that regulates the expression of proteins that play a role in the cell cycle, apoptosis, and invasion processes. In lung adenocarcinoma, CircPUM1 could sponge miR-326 and promote the expression of its downstream proteins: Cyclin D1 and Bcl-2 [32]. In ovarian cancer, Circular RNA PUM1 upregulates the expression of nuclear factor kappa B (NF-κB) and

MMP2 by sponging miR-615–5p and miR-6753–5p [33]. CircPUM1 promotes the progression of polycystic ovary syndrome by sponging miR-760 [35]. In endometrial cancer, circRNA PUM1 promotes migration and invasion by functioning as a molecular "sponge" by binding to miR-136 and upregulating miRNA's target gene, NOTCH3 [34]. CircPUM1 could promote the development of hepatocellular carcinoma by up-regulating the expression of MAP3K2 by sponging miR-1208 [36]. CircPUM1 knockdown in papillary thyroid cancer was partly ascribed to MAPK1 downregulation by upregulating miR-21–5p. In non-small cell lung carcinoma, circRNAPUM1 promoted tumor growth and glycolysis by sequestering miR-590–5p and up-regulating METTL3 [38]. Circ-PUM1 activates the PI3K/AKT signaling pathway by sponging miR-200c-3p and promotes the progression of pancreatic cancer [39]. CircPUM1 upregulates the FABP7 expression by competitively binding with miR-340–5p and then activating the MEK/ERK pathway, thus promoting the progression of clear cell renal cell carcinoma [40]. Since 2018, circPUM1 has been studied and its expression has been associated with the promotion of different types of cancer, however, the regulatory axes discovered in these studies have been inconsistent (Table 1). Of the nine works discussed in this review, each article reported isolated regulatory axes, which shows the need for further studies on the function of circPUM1 and its regulation. In addition to its regulatory role as an RNA-binding protein, the PUM1 gene encodes a circPUM1 that regulates miRNAs. How the circularization of the PUM1 transcript is regulated remains unclear. However, what is clear is that the PUM1 function as RBPs or as a miRNA sponge favors cancer progression.

3. Role of Pumilio 2 in proliferation, migration, and invasion in different cancers

PUM2 seems to be associated with proliferation, migration, and invasion of tumor cells (Fig. 3 and Table 2). Wang and collaborators (2019) reported that PUM2 knockdown decreased cell proliferation, viability, migration, and invasion and also increased apoptosis in two glioblastoma lineages, U87 and U251 [42]. The authors also reported an increase in PUM2 levels in glioblastoma samples in comparison with healthy tissue and pointed out that PUM2 could be modulating a cell cycle regulator known as BTG1, previously described as a tumor suppressor [42]. Clinical samples showed that circRBM33 levels were
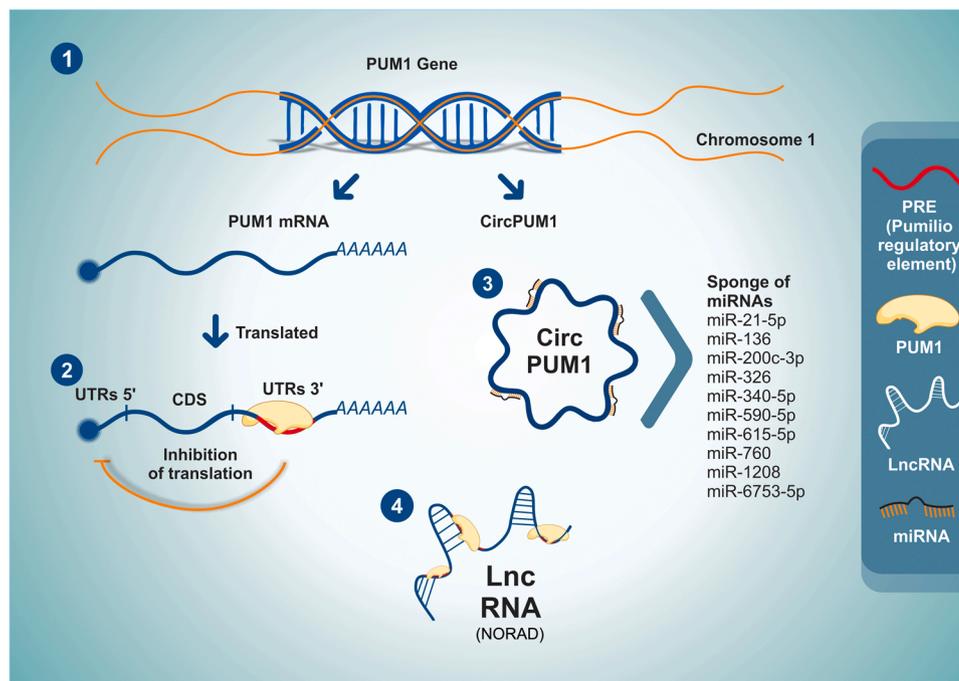
Fig. 2. Scheme of PUM1 function in cancer. (1) Transcriptional pathway of the PUM1 gene, synthesizing PUM1 mRNA and circPUM1. (2) PUM1 protein modulated inhibition of target mRNAs. Here, we demonstrate the specific binding between PUM1 and PRE (Pumilio regulatory element, 5'UGUAHAUA) sequence, which is enriched in the 3' untranslated regions (UTRs 3') of the mRNAs. Consequently, PUM1 downregulation targets may occur via deadenylation, inhibition of translation initiation, or combinatorial activity involving miRNA. (3) Role of CircPUM1 on the regulation of miRNAs. CircPUM1 may act as a sponge for the miRNAs described. Thus, CircPUM1 may downregulate the action of the miRNA. (4) LncRNA may act by sequestering PUM1 proteins and consequently upregulating the expression of PUM1 targets indirectly, as described for NORAD modulation of PUM1. Graphic Designer: Wagner Nagib.
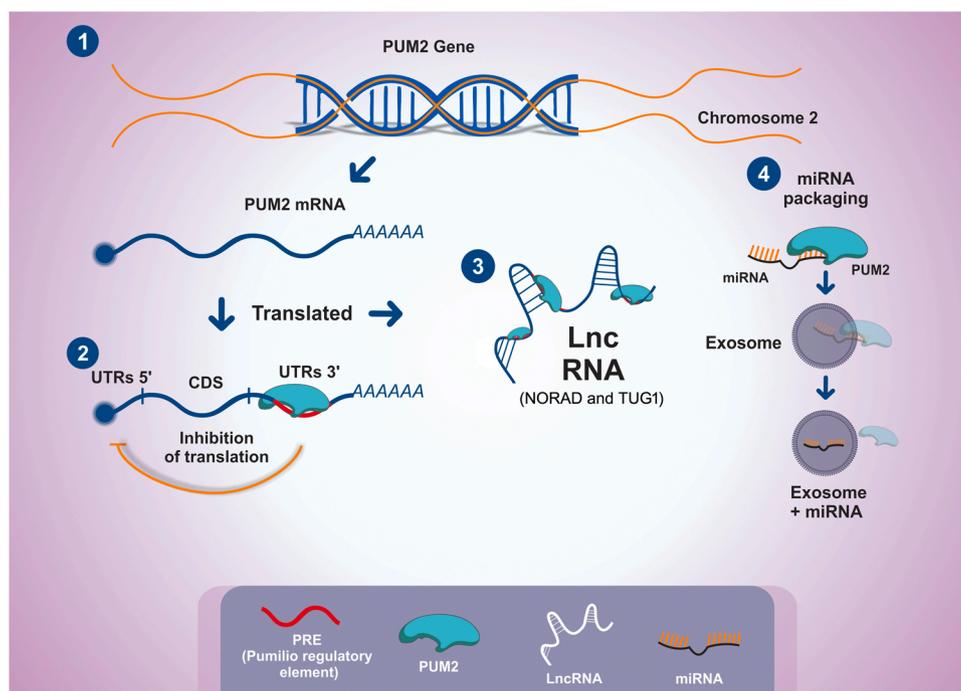
Fig. 3. Scheme of PUM2 function in cancer. (1) Transcriptional pathway of the PUM2 gene, synthesizing PUM2 mRNA. (2) PUM2 protein modulated inhibition of target mRNAs. Here, we demonstrate the specific binding between PUM1 and PRE (Pumilio regulatory element, 5'UGUAHAUA) sequence, which is enriched in the 3' untranslated regions (UTRs 3') of the mRNAs. Consequently, PUM2 downregulation targets may occur via deadenylation, inhibition of translation initiation, or combinatorial activity involving miRNA. (3) LncRNA may act by sequestering PUM2 proteins and, consequently, upregulating PUM2 targets indirectly. PUM2 interacts with lncRNAs NORAD and TUG1. Besides, circRBM33 and lncTTN-AS1 are sponges for miRNAs that regulate PUM2. (4) PUM2 participate in miRNA (miR-103a-3p and miR-130) packaging into exosomes in cancer cells. Graphic Designer: Wagner Nagib.

increased in cervical cancer tissues compared with the adjacent non-cancer tissues [43]. Ding and collaborators also demonstrated that circRBM33 acts as a sponge for miR-758–3p, a miRNA that regulates PUM2 expression. The overexpression of miR-758–3p decreased cellular proliferation, invasion, and migration, and increased apoptosis; these effects are overturned with PUM2 overexpression. Knockdown of circRBM33 reduced PUM2 levels in vitro and this effect was reversed by miR-758–3p inhibition. Lastly, tumor cells with circRBM33 knockdown that were injected into mice resulted in PUM2 downregulation and decreased tumor size. Once circRBM33 is upregulated in cervical cancer, miR-758–3p levels are downregulated, leading to increased PUM2 levels, which could be associated with the tumor's progression [43]. Thus, circular RNAs exert a modulating effect on Pum expression by acting in the sequestration of miRNAs.

Since PUM2 seems to be regulated by circRNA, it may also be regulated by lncRNAs. Clinical samples of cervical cancer tissues showed an upregulation of TUG1, a lncRNA, and PUM2, which corresponded with the TNM staging [44]. In vitro TUG1 overexpression in the HeLa and SiHa cervical cancer lineages promoted cell viability and migration; PUM2 levels were increased [44]. The increase in cell viability caused by TUG1 overexpression was overturned when cells were treated with a siPUM2, indicating that TUG1 is involved in cell viability and migration through its interaction with PUM2 [44]. TTN-AS1, which is another lncRNA, was upregulated in endometrial cancer tissues compared with adjacent non-cancer samples and in vitro in endometrial HEC1A and Ishikawa cell cancer lineages [45]. TTN-AS1 knockdown reduced cell proliferation, migration, and invasion while promoting apoptosis [45]; this lncRNA interacts with miR-376a-3p, a miRNA that targets PUM2 [45]. PUM2 levels were increased in the endometrial cancer tissues; they were negatively correlated with miR-376a-3p and positively correlated with TTN-AS1 [45]. Mice injected with HEC1A shTTN-AS1 cells showed a decrease in tumor size and PUM2 levels, indicating that PUM2 is regulated by TTN-AS1 via miR-376a-3p [45]. These results show that PUM2 is essential for cell viability, migration, and invasion since apparently PUM2 is upregulated by different pathways, including circRBM33, TUG1, and TTN-AS1.

In contrast, Hu and collaborators (2018) showed downregulated PUM2 in osteosarcoma samples and also demonstrated that its over-expression in MG63 and Saos2 osteosarcomas lineages leads to a

decrease in cell viability and affects EMT, thus hindering cell migration [46]. In bladder cancer, PUM2 is regulated by NORAD [47]. NORAD was upregulated in clinical tumor samples compared with normal adjacent tissues, and this expression seems to be positively correlated with the cancer stages [47]. NORAD knockdown in stage IV lineage cells TSSCUP promoted apoptosis and decreased proliferation, while PUM2 levels were increased [47], which shows the dual role of PUM2 in tumor progression depending on the cancer type. In another study, Castracani and collaborators (2020) showed that PUM2 expression is negatively correlated with HMOX1, a gene that was upregulated in clinical samples of glioblastoma. Its expression was induced using a drug called hemin, which was associated with increased cell proliferation and migration in two glioblastoma lineages, A172 and U87-MG [48]; this indicates that PUM2 could be associated with low tumor proliferation in bladder cancer. One explanation for these opposed effects is the wide range of PUM targets: in some kinds of tumors, PUM2 could regulate targets that promote tumorigenesis, whereas, in other types of cancer, PUM2 could regulate targets that inhibit cancer progression (see "Pumilio 1 and Pumilio 2 targets and regulatory axes in cancer" section).

Clinical samples showed that PUM2 expression was significantly increased in both luminal B type breast cancer tissues(n = 10) and triple-negative tissues (n = 16) in comparison with adjacent tissues, and negatively correlated with the overall survival and relapse-free survival of breast cancer patients [49]. Conversely, Tao and collaborators showed that the mRNA expression of PUM2 was significantly decreased in both luminal A type breast cancer tissues (n = 20) and triple-negative type tissues (n = 20) in comparison with adjacent healthy tissues [50]. Although both studies were conducted in China, the discrepancy could still be due to genetic bias. Variations in the collection of the tumor samples and the normal adjacent tissues could also be another possible explanation for the discrepancy. Tao and collaborators report having followed the WHO recommendations for the classification of breast cancer tumors. Moreover, Smialek and collaborators showed that among the 17 types of cancer evaluated, PUM2 was overexpressed in almost all of the samples, except for the ovarian and uterus cancer tissues, where the RNA expression level was lower than what was found in the healthy tissues [19].

Exosomes are extracellular cargo vessels that transport miRNAs and other materials to surrounding cells. Cancer exosomes perform cell-

Table 2
Comparative table showing different studies on PUM2 and PUM1/PUM2 in different types of cancer.

Type of cancer	Expression analysis	Regulatory axis	Prognosis	Ref.
Cervical cancer	PUM2 protein and mRNA	cRBM22/ miR-758–3p	Shorter overall survival in patients with high expression of circRBM33. cir-cRBM33 modulated tumor progression through regulation of PUM2 expression via sponging miR-758–3p.	[43]
Cervical cancer	PUM2 protein and mRNA	TUG1	Worse overall survival in patients with a high level of PUM2 (increased by overexpression of TUG1).	[44]
Endometrial cancer	PUM2 protein and mRNA	TTN-AS1/ miR-376a-3p	A high level of TTN-AS1 (and consequently high levels of PUM2) was associated with shorter overall survival, as well as tumor size, FIGO stage, and lymph node metastasis).	[45]
Ovarian cancer	PUM2 protein and mRNA	–	USP46 could be used as a predictor of drug resistance in patients. Downregulation of USP46 indicates cistein-resistance.	[59]
Bladder cancer	PUM2 protein and mRNA	NORAD	Patients with high NORAD expression (and PUM2 downregulation) were more likely to develop higher tumor stage and histologic grade and were significantly associated with worse overall survival.	[47]
Osteosarcoma	PUM2 protein and mRNA	–	PUM2 expression was positively correlated with the metastasis-free survival of OS patients.	[46]
Breast cancer	PUM2 protein and mRNA	SCAMP1	–	[50]
Breast cancer	PUM2 protein and mRNA	miR-376a	PUM2 expression is negatively correlated with survival, relapse-free survival, and metastasis-free survival.	[49]
Glioblastoma	PUM2 protein and mRNA	–	–	[42]
Glioblastoma	PUM2 mRNA (microarray)	HMOX1 and FBXW11	–	[48]
Non-small cell lung cancer	PUM2 protein and mRNA	–	–	[52]
Non-small cell lung cancer	PUM2 protein and mRNA	–	–	[53]
Seminoma	PUM1/PUM2 protein and mRNA	–	–	[58]
Bladder carcinoma	PUM1/PUM2 protein and mRNA	Nos	–	[57]
Myeloid leukemia	PUM1/PUM2 protein and mRNA	–	–	[55]
Non-small cell lung cancer		miR-340	miR-340 expression was significantly	[56]

Table 2 (*continued*)

Type of cancer	Expression analysis	Regulatory axis	Prognosis	Ref.
	PUM1/PUM2 protein and mRNA		lower in patients at more advanced than earlier stages of disease (what would cause the upregulation of PUM1/2).	

independent microRNA biogenesis and promote tumorigenesis [51]. Recently, PUM2 has been associated as a mediator/facilitator of miRNA packaging into exosomes in cancer cells [52,53]. Pum2 facilitated miR-103a-3p and miR-130a packaging into cancer-associated fibroblast-derived exosomes. Pum2 promoted cisplatin resistance by facilitating exo-miR-103a-3p and exo-miR130a secretion in non-small cell lung cancer cells [52,53]. PUM2 may play an essential role in packaging miRNA 103a-3p and 130a into exosomes, thereby indirectly impacting the abundance and function of miRNA-130a in exosomes. PUM2 was described as being associated with Argonaute proteins which is the core component of the miRNA-induced silencing complex (RISC) [54]. Dicer and Ago2, which are key components of miRNA processing, are also present inside cancer cell exosomes [51]. The mechanism surrounding PUM2 facilitation of miRNA packaging into exosomes is not yet fully understood. More studies are required in order to understand the mechanism of miRNA packaging in exosomes by PUM2, along with the possible role of RISC in the process.

4. Role of Pumilio 1 and Pumilio 2 in different cancers

The simultaneous expression of PUM1 and PUM2 was evaluated in a few types of cancer. For instance, studies with myeloid leukemia demonstrated high levels of PUM1 and PUM2 mRNA in three of the five acute myeloid leukemia cell sample groups compared to the control [55]. Knockdown of PUM1 or PUM2 showed inhibition of proliferative abilities in all of the six acute myeloid leukemia samples tested, while experiments carried out on human and murine hematopoietic stem/-progenitor cells indicated the importance of PUM1 and PUM2 in growth [55]. In non-small cell lung cancer, overexpression of miR-340 inhibited cell cycle progression and potentially induced apoptosis by downregulation of PUM1 and PUM2 (although PUM1 demonstrated a superior inhibition effect than PUM2), which can be explained by the downregulation of the cell cycle inhibitor p27. The results indicate that PUM1 and PUM2 can be involved in the post-transcriptional control of cell cycle inhibitors (aside from p27) and positive regulators, respectively [56]. Deficiency of PUM1 or PUM2 also induced loss of hematopoietic stem/progenitor cell survival, expansion, and clonogenic properties, demonstrating that Pum proteins play an essential role in tumor cell growth. In bladder cancer, the overexpression of PUM1 or PUM2 (in combination with a zinc finger RBP Nanos) was associated with the downregulation of E2F3, an oncogene with strong proliferative potential that is usually overexpressed or dysregulated in cancer [57]. This negative correlation between PUM2 and E2F3 is also shown by the knockdown of NORAD in the same type of cancer. The knockdown of NORAD decreased proliferation in the TSSCUP cell line while upregulating PUM2 and downregulating E2F3 [47]. Nonetheless, experiments with PUM1 and PUM2 showed a different effect on seminoma. The overexpression of PUM1 and PUM2 in a human TCam-2 seminoma cell line significantly reduced cell cycle progression by keeping cells in the G0/G1 phase. This overexpression was also involved with the downregulation of the SPIN1 mRNA level but did not affect SPIN3 [58]. These divergent results show that PUM1 and PUM2 proteins may have different roles depending on the cancer type.

5. Pumilio 1 and Pumilio 2 targets and regulatory axes in cancer

Pum proteins have thousands of mRNA targets, and the set of mRNAs expressed varies between cell types; it is no different in cancer cells. Several studies have evaluated the role of Pum proteins and their targets in different types of cancer cells [20,21,23–25,28,42,46,49,50,52,53, 55–59]. The knockdown of PUM1 and PUM2 inhibited the proliferation of acute myeloid leukemia cells and hematopoietic stem/progenitor cells. The underlying mechanism of inhibition can be explained by the decrease of FOXP1 mRNA and protein, which induce the expression of p21 and p27 cell cycle inhibitors [55]. In non-small cell lung carcinoma, miR-222/221 and p27 were also related to the knockdown of PUM1 or PUM2 [56]. As in the other cases, the knockdown of PUM1 or PUM2 increased the p27 level, but the double knockdown did not present any additional effect. Furthermore, the downregulated expression of p27 was correlated with the overexpression of miR-222/221, even though silenced PUM1 reversed this effect while silenced PUM2 reversed it only partially [56]. Finally, miR-222/221 and p27 were associated with miR-340, which downregulates PUM1 and PUM2, inhibiting cell cycle progression and inducing cell apoptosis. Altogether, these results suggest that overexpression miR-340 leads to PUM1 and PUM2 downregulation and, consequently, p27 accumulation by modulation of miR-222/221-mediated inhibition [56]. In pancreatic cancer, the enhanced sensitivity of TRAIL caused by a reduced PUM1 level was also associated with p27. PUM1 reduction leads to an increased p27 expression, driving TRAIL-induced autophagy activation [21]. This evidence suggests a strong correlation between the Pum proteins and the cell cycle regulation. Considering the cancer types cited in this paragraph (myeloid leukemia, non-small lung carcer, and pancreatic cancer), all were related to the expression of p21 and/or p27. Indeed, the increased level of these cell cycle inhibitors was positively associated with inhibition of proliferation. Furthermore, inhibition was associated with the knockdown of PUM1 and PUM2.

Meanwhile, other results indicate the correlation between PUM overexpression and the inhibition of cancer progression. In seminoma, the reduction of cell cycle progression was associated with PUM1 and PUM2 overexpression when targeting SPIN1. In this case, it was proposed that the overexpression of PUM1 and PUM2 downregulated the expression of SPIN1, a protein related to apoptotic and cycle control effects [58]. In bladder cancer, the knockdown of PUM1 or PUM2 increased while the overexpression decreased E2F3 levels in the TCCSUP cell line. Hence, E2F3 is likely to be sensitive to PUM regulation. In addition, the results indicated that PUM proteins cooperated with miRNAs to repress E2F3. The aforementioned interaction between PUM proteins and E2F3 may cause conformational changes in the E2F3 structure; this is favorable to miRNA modulated repression [57], once PUM/Nanos and miR-503 overexpression strongly enhanced the F3 repression compared with either of them on their own [57].

The PUM1 targets identified are quite diverse. In colon cancer cells with acquired resistance to cetuximab, the PUM1 overexpression has been positively associated with DDX5, a cell proliferation inducer [23]. In PUM1 knockdown experiments, the results showed decreased mRNA and protein expression of DDX5 [23]. In prostate cancer, the knockdown of PUM1, which is associated with an increase in cell growth rate, upregulated the CDKN1B protein, an inhibitor of cell cycle progression. Interestingly, the increase in the protein level is not related to mRNA increase. Therefore, the opposite, which is the overexpression of PUM1, favors the progression of cancer by decreasing CDKN1B expression [25]. Finally, four PUM1 targets were identified in ovarian cancer: STAT3, BCL2, MMP2, and VEGFA. The PUM1 downregulation decreased protein expression in all targets involved, whether it be tumor cell survival and proliferation, anti-apoptotic effects, tumor invasion and metastasis, and cell proliferation[24]. In pancreatic ductal adenocarcinoma, PUM1 knockdown activated the PERK/eIF2/ATF4 signaling pathway [20]. The PERK inhibitor diminished the in vitro effect of PUM1 knockdown on cell migration, invasion, and EMT [20]. On the other hand,

NORAD/miR-323a-3p/PUM1/eIF2 functions through an axial relationship in breast cancer [28]. As previously mentioned, in pancreatic adenocarcinoma and breast cancer, PUM1 and eIF2 belong to the same regulatory axis, which can also be explored in other types of cancer.

Similarly, several PUM2 targets were identified. In osteosarcoma, PUM2 overexpression decreased cell viability and enhanced stability and expression of STARD13 mRNA, which is recognized as a tumor suppressor in other types of cancer. The regulation of STARD13 is mediated by the competitive relation between miR-590–3p and miR-9, and the PUM2 expression may inhibit the osteosarcoma progression by repressing the RhoA/Rock pathway [46]. In contrast, the knockdown experiments showed that the depletion of PUM2 suppressed cell proliferation and led to increased mRNA and BTG1 protein levels. Therefore, BTG1, which is poorly expressed in tumors, was negatively regulated by PUM2. Furthermore, in this particular case, BTG1 mRNA showed a prolonged half-life [42].

In addition, PUM2 promotes cellular stemness in breast cancer via its competitive binding to neuropilin-1 (NRP-1) mRNA with miR-376a [49]. PUM2 overexpression inhibited the malignant biological behaviors of breast cancer cells [50]. The growth of xenograft tumors in nude mice was significantly inhibited by the silencing of SCAMP1-transcript variant 2 coupled with PUM2 overexpression. PUM2 inhibited the expression of INSM1 by binding to its 3′-UTR, and this inhibitory effect was amplified by the knockdown of SCAMP1-transcript variant 2 [50]. INSM1 transcriptionally inhibited SASH1 expression. SASH1 overexpression inhibited the malignant biological behaviors of breast cancer cells by inhibiting the activity of the PI3K/AKT signaling pathway. Tumor growth was inhibited in vivo by the silencing SCAMP1-transcript variant 2 in combination with PUM2 overexpression [50].

In non-small cell lung cancer, the modulation of PUM2 expression was evaluated in two different studies, both concerning cisplatin resistance. Initially, PUM2 overexpression increased the miR-103–3p level and decreased Bak1, suggesting that Bak1 suppresses cell apoptosis and PUM2 overexpression promotes cisplatin resistance [52]. Conversely, PUM2 knockdown, which is associated with smaller tumor weights and slower growth, decreased the exosomal level of miRNA-130-a [53]. The cisplatin resistance was also evaluated for ovarian cancer; in this case, PUM2 was upregulated, and USP46 was downregulated [59]. USP46 may be a PUM2 target since the latter regulates a set of ubiquitin-specific peptidase, including USP1, USP12, USP21, USP32, USP33, USP42, USP53, USP6, and USP8 [9]. The USP46 downregulation is associated with increased cell viability and inhibited apoptosis [59]. These results may provide clues for investigating the underlying mechanism of resistance conferred to cisplatin.

Human Pum have hundreds of mRNA targets, so we selected all Pum targets described in the articles in Tables 1 and 2 (total=17 Pum targets) for a more detailed analysis of this group of genes. Here, we analyze the gene networks, ontology, and biological processes of the elements modulated by Pum proteins in cancer cells (DDX5, USP46, CDKN1B, E2F3, STAT3, STARD13, BCL2, SCAMP1-TV2, MMP2, INSM1, VEGFA, BTG1, PERK, HMOX1, EIF2A, NRP-1, and ATF4) (Fig. 4). Analysis of the genetic networks of the elements regulated by Pum proteins allowed us to observe three well-defined and connected groups (blue, green, and red), especially displaying known interactions determined by experimental and curated databases (Fig. 4A). Of the ten most significant terms in the gene ontology analysis of elements regulated by Pum proteins, four terms are related to proliferation, three terms relate to cell differentiation and development (tube morphogenesis, angiogenesis, and tissue development), two terms are concern oxygen response, and one term is related to mRNA transcription (Fig. 4B). Interestingly, the terms related to the response to reduced oxygen levels and angiogenesis are well related since hypoxia represents a non-physiological level of oxygen tension, a common phenomenon in most malignant tumors (reviewed by [60]). Tumor-hypoxia leads to advanced but dysfunctional vascularization and attainment of epithelial-to-mesenchymal transition phenotype, resulting in cell mobility and metastasis [60]. Hypoxia alters

A
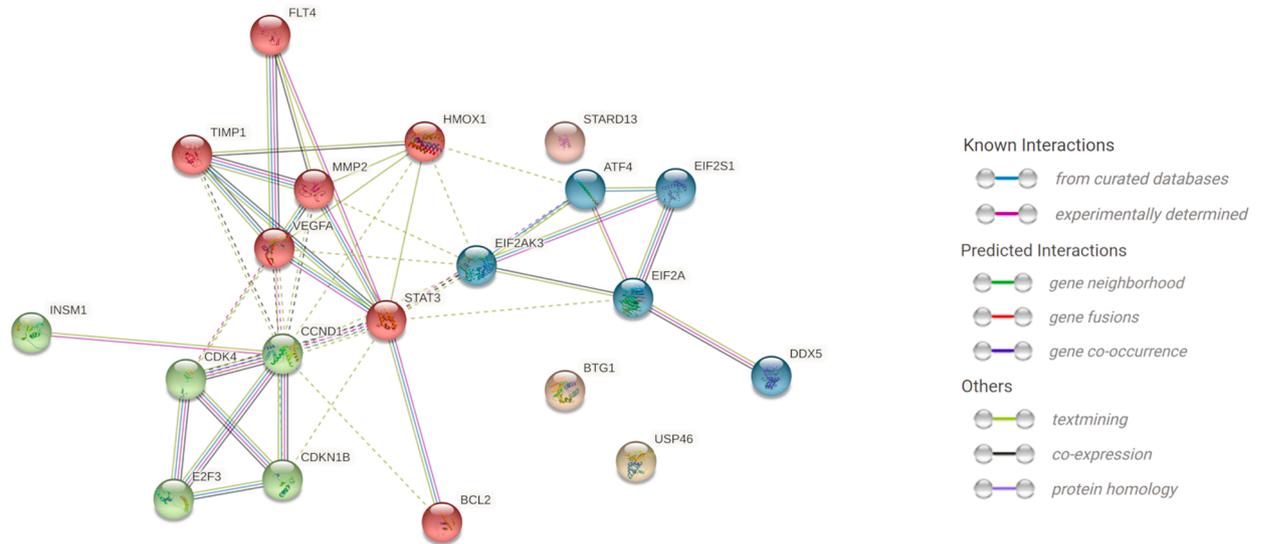
B
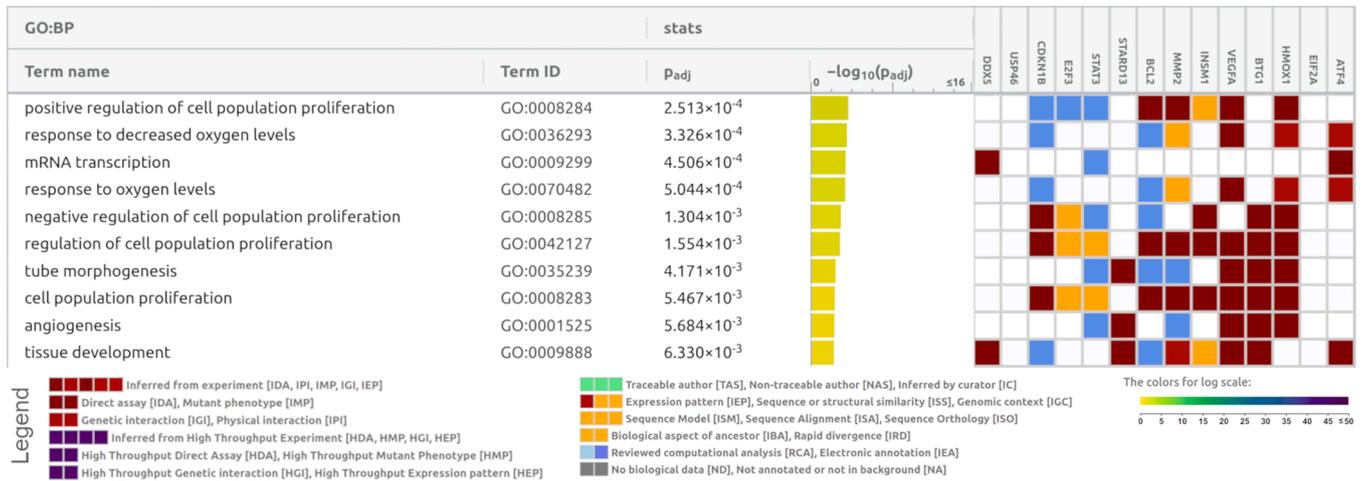
C
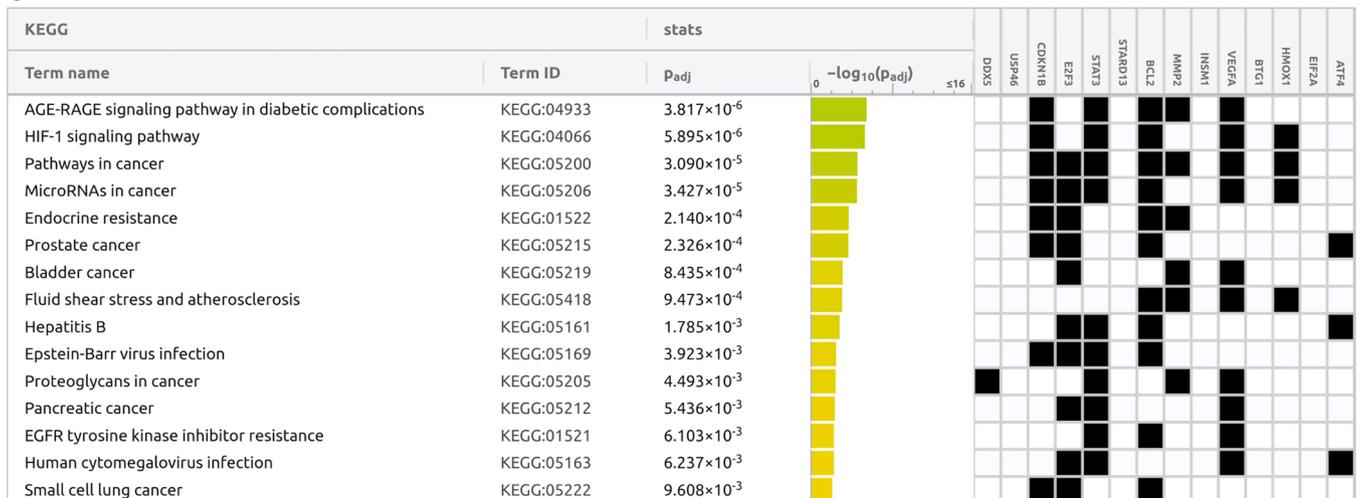
Fig. 4. The network of genes modulated by PUM1 and PUM2 in cancer was established by String (https://string-db.org/) and resulted in several interactions with three well-defined clusters (A). The ontology gene determined by gProfiler (https://biit.cs.ut.ee/gprofiler/gost,[75]) presents the biological processes in which these genes participate, with great emphasis on the regulation of proliferation (B). The biological pathways determined by KEGG of genes modulated by PUM1 and PUM2 in cancer (C).

the metabolism of cancer cells and contributes to therapy resistance by inducing cell quiescence [60]. It also gives rise to induces several complex intracellular signaling pathways, such as the major hypoxia-inducible factor (HIF) pathway. The biological pathways determined by KEGG of genes modulated by PUM1 and PUM2 in cancer

hold the HIF signaling pathway as the second most significant (Fig. 4C). Still, among the 15 most significant terms, seven are related to biological cancer pathways (pathway in cancer, miRNAs in cancer, prostate cancer, bladder cancer, proteoglycans in cancer, pancreatic cancer, and small cell lung cancer). Interestingly, the term endocrine resistance appears
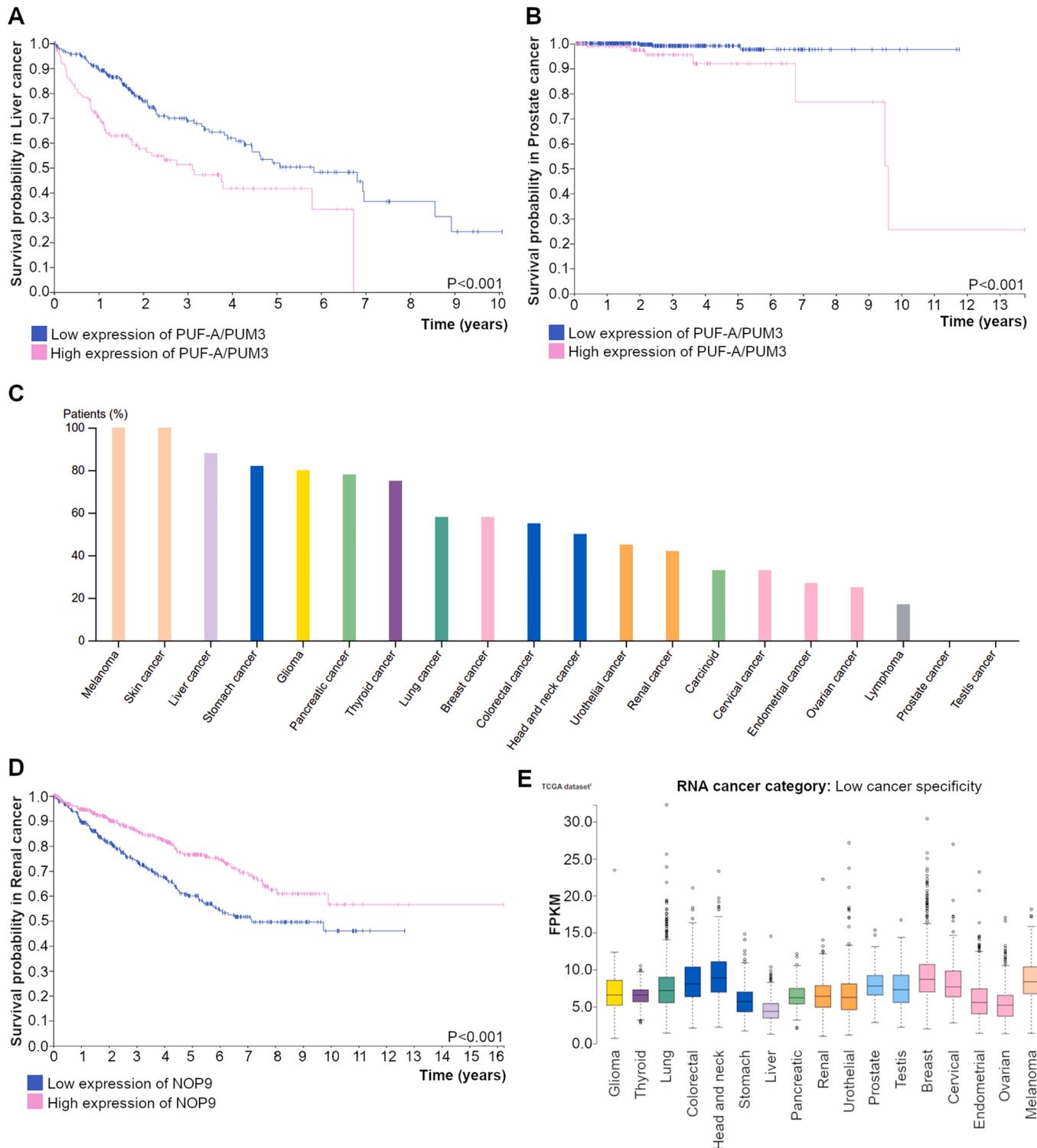
Fig. 5. PUF-A/PUM3 and NOP9 expression in cancer. (A-B) Kaplan-Meier plots for liver and prostate cancers where high expression of PUF-A/PUM3 has significant (p < 0.001) unfavorable with patient survival. (C) PUF-A/PUM3 protein expression summary in different cancers. Y-axis indicates the percentage of patients with high and medium protein expression levels (maximum 12 patients). (D) Kaplan-Meier plots for renal cancer where high expression of NOP9 has a significant (p < 0.001) association with patient survival. (E) RNA-seq data in 17 cancer types are reported as median FPKM (number Fragments Per Kilobase of exon per Million reads), generated by "The Cancer Genome Atlas" (TCGA). Reference: Human Protein Atlas proteinatlas.org [76].

among the biological pathways of PUM1 and PUM2 targets in cancer. Endocrine therapy for blocking the estrogen receptor pathway is highly effective; however, its usefulness is limited by common intrinsic and acquired resistance (reviewed by [61]). Curiously, two terms were related to viral infection (Epstein-Barr and human cytomegalovirus). Epstein-Barr is known for being the first human virus to be directly implicated in carcinogenesis (reviewed by [62]), and human cytomegalovirus is highly prevalent in glioblastomas and breast cancers [63,64]. This suggests that Pum proteins regulate genes associated with proliferation, endocrine resistance, angiogenesis, hypoxia, and viral infection in cancer cells.

6. Non-canonical Pumilio family proteins: PUF-A/PUM3 and Nop9 in cancer cells

Human PUF-A (also known as PUM3) is an RNA and DNA-binding protein that participates in the nucleolar processing of 7–5.8 S rRNA [65]. PUF-A/PUM3 is a clinical indicator for breast cancer progression [66]. The expression of PUF-A/PUM3 is positively associated with the clinical stages of breast cancer biopsies. The presence of PUF-A/PUM3 also affected the in vitro tumorigenicity of breast cancer cells. RNA-IP showed an association of PUF-A/PUM3 with RBBP4/RbAp48 and DDX3 in MDA-MB-231 cells [65]. PUF-A/PUM3 ablation reduces cell proliferation and transwell invasion in HeLa cells [65]. The phosphorylation of PUF-A on Y259 residue is essential for PUF-A's protein stability and clonogenic formation of HeLa cells [65]. Corroborating this, data from "The Cancer Genome Atlas" shows PUF-A/PUM3 as prognostic, indicating that high expressions are unfavorable in liver and prostate cancers (Fig. 5A-C). PUF-A/PUM3 is one of the top ten predictors of sensitivity to olaparib (cancer treatment drug) [67]. PUF-A/PUM3 mRNA expression is negatively correlated with olaparib IC50 for 20 types of cancer [67]. PUM3 is known to interact with PARP1 by binding to its catalytic domain and inhibiting its poly-ADP-ribosylation activity [68]. This interaction is relevant because olaparib also binds to the catalytic domain of PARP1 to inhibit the catalytic activity of PARP1 [67]. Despite the few studies regarding PUF-A/PUM3 in cancer, findings show an unfavorable correlation between high expression of PUF-A/PUM3 and patient survival/tumor progression in breast, liver, and prostate cancer (Fig. 5A-B) [65,66].

Nop9, the other non-canonical PUM family protein, recognizes structured and single-stranded RNA elements of preribosomal RNA [14] and is an essential factor in the processing of preribosomal RNA [69]. Data from "The Cancer Genome Atlas" shows that Nop9 is prognostic: high expression is favorable in renal cancer, and RNA expression has low cancer specificity (Fig. 5 D-E). Further studies are needed to find out if there is any relationship between Nop9 and cancer.

7. Conclusions

PUM1 protein and circPum1 are highly associated with the proliferative potential of several types of cancer, as shown in hepatocellular carcinoma, renal cell carcinoma, pancreatic, colon, ovarian, prostate, polycystic ovary, endometrial, papillary thyroid, and non-small cell lung cancers. Besides the in vitro analyses that correlated the growth rate, migration, and invasion abilities of cancer cells with PUM1 or circPUM1, the in vivo assays have shown that their silencing promotes tumor inhibition. However, the role of PUM2 in cancer is controversial. Experiments with glioblastoma lineages, cervical and endometrial cancer have found that PUM2 is associated with cell viability, migration, and invasion capacity and acts in favor of tumor progression. Furthermore, in non-small cell lung cancer, PUM2 acts as a treatment-resistance promoter. PUM2 seems to be an inhibitor of cancer progression in osteosarcoma, glioblastoma, and bladder cancer. Contradictory studies have shown that PUM2's function may differ, depending on the cancer type or individual genetics. The role of the PUM targets or axes involving PUM protein is mainly related to the cell cycle inhibitors p27 and p21.

Although other targets such as E2F3 are commonly associated with tumorigenesis, most recent studies have identified several other targets which have been described in this article. Furthermore, these discoveries may provide different targets with therapeutic implications that need to be investigated along with the relation between PUM proteins and miRNA and/or partners.

CRediT authorship contribution statement

I.Z.S., A.A.K., and P.S., conceptualization; A.A.K., literature research; P.S., preparation of illustrations; I.Z.S., A.A.K., and P.S., preparation of the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors report no potential conflicts of interest or financial interests.

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