PUM and Cancer

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PUM1 and PUM2 are RNA-binding Pumilio proteins, form ribonucleoprotein networks controlling the accessibility of hundreds of mRNAs for translation, in a variety of human tissues. Hence, PUMs exemplify one of the mechanisms safeguarding the cellular proteome. PUM1 and PUM2 expression is disturbed in cancer, resulting in dysregulation of their target mRNAs. These targets encode factors responsible for processes usually affected in cancer, such as proliferation, apoptosis, and the cell cycle.

Keywords: cancer ; mRNA ; PUM proteins ; post-transcriptional gene regulation ; PTGR ; 3’ Untranslated Re-gions ; 3’UTR

1. Introduction

Although growing evidence indicates that dysregulation of post-transcriptional gene expression plays a highly important role in cancer, except for microRNAs, it has been disregarded. The post-transcriptional gene expression regulation (PTGR) encompasses mostly RNA processing, which takes place beyond transcription, starting in the nucleus and continuing in the cytoplasm [1]. The main nuclear RNA processing steps consist of the 7-methylguanosine cap synthesis on the 5′-end of pre-mRNA, generation of the 3′-end by cleavage and polyadenylation, including alternative polyadenylation (APA), and a multistep splicing reaction that removes introns and produces mature mRNA molecules. Those nuclear RNA processing events are mediated by a multitude of RNA-binding proteins (RBPs) that associate with the nascent mRNA [2]. It is well-known now that a variety of RBPs are dysregulated in different cancer types (for review see [3]). Pumilio proteins, PUM1 and PUM2 that represent well-described examples of RBPs playing a role in PTGR in metazoans, including humans (for review see [4]) as well as in plants [5]. There is accumulating evidence now that expression of PUM proteins is dysregulated in several cancers (for review see [4]). PUM proteins contain a C-terminal highly conserved PUF domain, which binds to mRNA molecules. That domain consists of eight tandem imperfect repeats of 36 amino acids (aa). These repeats form a curved structure, each of them contacting one base within a PUM-binding element (PBE) – an eight-nucleotide conserved motif UGUAHAUW, located mostly in the 3′untranslated region (3’UTR) of

target mRNAs [6-9]. While the original *Drosophila melanogaster* Pum protein is unique, there are several Pum proteins that exist in yeast, *Caenorhabditis elegans* and other model organisms. There are two *PUM* genes in humans on chromosome 1 and 2 called *PUM1* and *PUM2*, respectively and they encode PUM1 and PUM2 proteins (for review see [4,10]). The rate of the overall aa similarity between PUM1 and PUM2 protein is 83%, with the PUF-domain being 91% identical [11]. Binding of PUM proteins with their co-factors in most cases directs a given mRNA towards repression, leading mostly to degradation. This binding however, may also stabilize mRNA, activate translation and/or storage in specific subcellular compartments (for review see [4]). It has been demonstrated that mRNA repression by PUM proteins consists of promoting deadenylation [12-14] and in some cases also decapping [15]. Deadenylation is followed mostly by degradation [16,17], which is in line with the finding that the presence of PBEs is closely associated with lower mRNA stability [18-20]. This phenomena was confirmed by studies in several human cell lines such as U2OS, HEK293 and HeLa [21-23]. The process of deadenylation is based on PUM interaction with components of the deadenylation Ccr4-Not (CNOT) complex [13,17,24].

2. PUM proteins expression and activity in cancer tissues

PUMs are involved in the control of a myriad of biological functions such as hematopoiesis, neurogenesis and gametogenesis while their dysfunction contributes to several diseases, which is in line with their ubiquitous pattern of expression, as they were identified in many human tissues.

Notably, there is emerging field of research concerning involvement of PUM proteins in specific types of cancer. The accumulated data show that the expression levels of *PUM1* and *PUM2* are significantly altered in 17 types of cancer tissues. Notably, in the majority of cancer tissues, PUM2 RNA expression was considerably higher than PUM1 expression (for review see [10]). Interestingly, in almost all of the samples, PUM1 level is increased, compared to healthy tissues (except adrenal gland and bladder cancers). It has also been demonstrated that PUM proteins control the level of several mRNAs encoding proteins involved in processes which are often disrupted in cancerogenesis such as apoptosis, proliferation and the cell cycle [25,26].

2.1. PUM proteins in leukaemia

Haematopoiesis is the process of differentiation of haematopoietic stem cells (HSCs) residing in the bone marrow, giving rise to various types of blood cells. Leukaemia does occur when cancer-driving mutations cause conversion of some HSCs to leukaemia stem cells (LSCs), which abnormally proliferate and grow in size, resulting in bone marrow malfunction. New evidence suggests that PUM1 and PUM2 play vital regulatory roles in the maintenance and proliferation of the normal human and mouse SCs [27,28]. Furthermore, it has been demonstrated that both PUMs are overexpressed in a majority of acute myeloid leukaemia (AML) samples, as well as in the cell-lines derived from that pathogenic samples. Importantly, PUM1 and PUM2 influence the cell cycle, proliferation, and apoptosis of the normal human and mouse HSCs, as well as the AML cells [27]. PUMs induce those effects by activating the expression of FOXP1 transcription factor mediated by direct binding PBE motifs located in the 3’UTR of FOXP1 mRNA. This PUM-mediated FOXP1 activation suppresses the expression of cell cycle inhibitors, such as CDKN1B, thereby promoting proliferation [27].

2.2. PUM proteins in seminoma

Seminoma is the most frequent type of testis germ cell tumours among western populations, with the highest incidence in men at the reproductive age and being more and more frequent [29]. In the TCam-2 seminoma cell line it has recently been demonstrated that PUM1 and PUM2 regulate several mRNAs functionally linked to cancer. Among such PUM1 and PUM2-repressed targets, there is mRNA encoding SPINDLIN1 (SPIN1), known for its role in mammalian gametogenesis. This spindle-binding protein is necessary for the meiotic progression of germ cells, while an abnormal overexpression of SPIN1 is associated with human ovarian cancers and was observed in some other cancer cell lines [16,30-32]. SPIN1 protein is expressed in TCam-2 cells and its overexpression caused a significant increase in proliferation and a reduced level of apoptosis. Those two features indicated that SPIN1 plays a proto-oncogenic role in TCam-2 cells. Besides, PUM1 and PUM2 repress a SPIN1 homologue called SPIN3, whereas its overexpression elicits a decrease in proliferation and an increase in apoptosis of TCam-2 cells [25]. Taken together, in this study SPIN1 demonstrated proto-oncogenic properties, while SPIN3 showed tumour suppressor features. Moreover, PUM1 itself (but not PUM2) strongly stimulated

apoptosis and moderately slowed down the cell cycle progression, suggesting that PUM1, similarly to SPIN3, plays a role of a tumour suppressor in TCam-2 cells. Altogether, by acting as SPIN1 and SPIN3 repressors, PUM proteins might promote a normal human male germ cell apoptotic status and thus prevent cancer [25].

In TCam-2 cells, PUMs also cause repression of mRNA encoding kinesin KIF18A [26]. This kinesin exists on positively charged ends of microtubules in the vicinity of kinetochore and it regulates dynamics of mitotic cell divisions. The knockout of Kif18a in mouse causes mitotic arrest and apoptosis of the male germ cells resulting in infertility. This kinesin is an important regulator of the cell cycle and apoptosis in the human germ cells as well [33,34]. Notably, it also plays a role of a proto-oncogene, as it is overexpressed in many cancer types. It has been reported that KIF18A positively influences TCam-2 cell proliferation, downregulates apoptosis, and promotes the cell cycle progression, these effects being opposite to the effects of PUMs. Therefore, repression by PUM proteins may represent one of the mechanisms affecting KIF18A level in regulating proliferation, the cell cycle, and apoptosis in TCam-2 cells [26].

3. PUM1 and PUM2 protein binding differences in cancer cells

To get a comprehensive insight into the importance of PUM-controlled PTGR in cancer, as well as into potential functional differences between PUM proteins, a direct identification of PUM-bound RNAs by protein-RNA co-immunoprecipitation (co-IP) followed by RNA-Seq analysis were performed in TCam-2 cells. It was found that about 90% of PUM-regulated targets were different for PUM1 and PUM2, and nearly 100% of all identified targets contained PBEs, thus validating these results [35]. The high numbers of PUM1 and PUM2-specific targets that have been identified in those studies was puzzling, considering that these proteins recognize the same PBE motif. A global immunoprecipitation (IP) and mass spectrometry (MS)-based identification of PUM1- and PUM2-binding proteins showed that PUM1 and PUM2 interact with mostly different groups of proteins, a majority of them representing RBPs. Combinatorial analysis of RIP, RNA-Seq, MS, as well as motif enrichment analysis for RNA-binding proteins in PUM1 and PUM2 mRNA targets, revealed that PUM1 and PUM2 form separate ribonucleprotein networks [35]. They resemble so-called “regulons” or “posttranscriptional operons”, as previously suggested for RBPs that recognize specific motifs in RNAs [36]. According to those networks, PUM1 and PUM2 may cooperate with varied protein cofactors to regulate separate mRNA target sub-pools responsible for unique pathways. These findings concerning PUM1 and PUM2 mRNA targets and their functional relations in TCam-2 cells are of interest and should be validated in patients suffering from testis germ cell tumours and several type of cancers affecting other human tissues.

3. Conclusions and Prospects

Although it has become clear that PUM proteins are important players in human health and disease, their role in cancer has not been sufficiently explored and several important issues should be addressed in the future studies. First, it is unclear whether *PUM* gene mutations in the patients may underlie at least some types of cancer. Such research has not been reported so far and seems crucial to assess the role of PUM proteins in tumorigenesis. Second, it is vital to explore the mechanisms of PUM expression at the transcriptional as well as posttranscriptional level in normal tissues and dysregulation of those mechanisms in different types of cancers. Third, it must be explained how such dysregulation affects the structure and dynamics of PUM1 and PUM2 interactomes in the course of carcinogenesis. Finally, it is necessary to assess the impact of PUM dysregulation on the cell cycle in cancer tissues and on processes that are often disturbed in cancer, like proliferation and apoptosis. Such studies would be valuable since they may provide new candidate targets for future therapies. It also emphasizes the importance of future studies to get a more complete picture of the role of PUM proteins in different types of cancer. Such studies may result in identification of novel targets for future cancer therapies.

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References

1. Kojima, S.; Shingle, D.L.; Green, C.B. Post-transcriptional control of circadian rhythms. *J Cell Sci* **2011**, *124*, 311-320, doi:10.1242/jcs.065771.
2. Singh, G.; Pratt, G.; Yeo, G.W.; Moore, M.J. The Clothes Make the mRNA: Past and Present Trends in mRNP Fashion. *Annu Rev Biochem* **2015**, *84*, 325-354, doi:10.1146/annurev-biochem-080111-092106.

3. Pereira, B.; Billaud, M.; Almeida, R. RNA-Binding Proteins in Cancer: Old Players and New Actors. *Trends Cancer* **2017**, *3*, 506-528, doi:10.1016/j.trecan.2017.05.003.
4. Goldstrohm, A.C.; Hall, T.M.T.; McKenney, K.M. Post-transcriptional Regulatory Functions of Mammalian Pumilio Proteins. *Trends Genet* **2018**, *34*, 972-990, doi:10.1016/j.tig.2018.09.006.
5. Zhang, C.; Muench, D.G. A Nucleolar PUF RNA-binding Protein with Specificity for a Unique RNA Sequence. *J Biol Chem* **2015**, *290*, 30108-30118, doi:10.1074/jbc.M115.691675.
6. Murata, Y.; Wharton, R.P. Binding of pumilio to maternal hunchback mRNA is required for posterior patterning in Drosophila embryos. *Cell* **1995**, *80*, 747-756, doi:10.1016/0092-8674(95)90353-4.
7. Zamore, P.D.; Williamson, J.R.; Lehmann, R. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA* **1997**, *3*, 1421-1433.
8. Edwards, T.A.; Pyle, S.E.; Wharton, R.P.; Aggarwal, A.K. Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell* **2001**, *105*, 281-289, doi:10.1016/s0092-8674(01)00318-x.
9. Wang, X.; Zamore, P.D.; Hall, T.M. Crystal structure of a Pumilio homology domain. *Mol Cell* **2001**, *7*, 855-865, doi:10.1016/s1097-2765(01)00229-5.
10. Smialek, M.J.; Ilaslan, E.; Sajek, M.P.; Jaruzelska, J. Role of PUM RNA-Binding Proteins in Cancer. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13010129.
11. Spassov, D.S.; Jurecic, R. Cloning and comparative sequence analysis of PUM1 and PUM2 genes, human members of the Pumilio family of RNA-binding proteins. *Gene* **2002**, *299*, 195-204, doi:10.1016/s0378-1119(02)01060-0.
12. Olivas, W.; Parker, R. The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J* **2000**, *19*, 6602-6611, doi:10.1093/emboj/19.23.6602.
13. Goldstrohm, A.C.; Hook, B.A.; Seay, D.J.; Wickens, M. PUF proteins bind Pop2p to regulate messenger RNAs. *Nat Struct Mol Biol* **2006**, *13*, 533-539, doi:10.1038/nsmb1100.
14. Goldstrohm, A.C.; Seay, D.J.; Hook, B.A.; Wickens, M. PUF protein-mediated deadenylation is catalyzed by Ccr4p. *J Biol Chem* **2007**, *282*, 109-114, doi:10.1074/jbc.M609413200.
15. Blewett, N.H.; Goldstrohm, A.C. A eukaryotic translation initiation factor 4E-binding protein promotes mRNA decapping and is required for PUF repression. *Mol Cell Biol* **2012**, *32*, 4181-4194, doi:10.1128/MCB.00483-12.
16. Morris, A.R.; Mukherjee, N.; Keene, J.D. Ribonomic analysis of human Pum1 reveals cis-trans conservation across species despite evolution of diverse mRNA target sets. *Mol Cell Biol* **2008**, *28*, 4093-4103, doi:10.1128/MCB.00155-08.
17. Van Etten, J.; Schagat, T.L.; Hrit, J.; Weidmann, C.A.; Brumbaugh, J.; Coon, J.J.; Goldstrohm, A.C. Human Pumilio proteins recruit multiple deadenylases to efficiently repress messenger RNAs. *J Biol Chem* **2012**, *287*, 36370-36383, doi:10.1074/jbc.M112.373522.
18. Jiang, P.; Singh, M.; Coller, H.A. Computational assessment of the cooperativity between RNA binding proteins and MicroRNAs in Transcript Decay. *PLoS Comput Biol* **2013**, *9*, e1003075, doi:10.1371/journal.pcbi.1003075.
19. Sharova, L.V.; Sharov, A.A.; Nedorezov, T.; Piao, Y.; Shaik, N.; Ko, M.S. Database for mRNA half-life of 19 977 genes obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic stem cells. *DNA Res* **2009**, *16*, 45-58, doi:10.1093/dnares/dsn030.
20. Schwanhausser, B.; Busse, D.; Li, N.; Dittmar, G.; Schuchhardt, J.; Wolf, J.; Chen, W.; Selbach, M. Global quantification of mammalian gene expression control. *Nature* **2011**, *473*, 337-342, doi:10.1038/nature10098.
21. Tichon, A.; Gil, N.; Lubelsky, Y.; Havkin Solomon, T.; Lemze, D.; Itzkovitz, S.; Stern-Ginossar, N.; Ulitsky, I. A conserved abundant cytoplasmic long noncoding RNA modulates repression by Pumilio proteins in human cells. *Nat Commun* **2016**, *7*, 12209, doi:10.1038/ncomms12209.
22. Bohn, J.A.; Van Etten, J.L.; Schagat, T.L.; Bowman, B.M.; McEachin, R.C.; Freddolino, P.L.; Goldstrohm, A.C. Identification of diverse target RNAs that are functionally regulated by human Pumilio proteins. *Nucleic Acids Res* **2018**, *46*, 362-386, doi:10.1093/nar/gkx1120.
23. Cottrell, K.A.; Chaudhari, H.G.; Cohen, B.A.; Djuranovic, S. PTRE-seq reveals mechanism and interactions of RNA binding proteins and miRNAs. *Nat Commun* **2018**, *9*, 301, doi:10.1038/s41467-017-02745-0.
24. Lau, N.C.; Kolkman, A.; van Schaik, F.M.; Mulder, K.W.; Pijnappel, W.W.; Heck, A.J.; Timmers, H.T. Human Ccr4-Not complexes contain variable deadenylase subunits. *Biochem J* **2009**, *422*, 443-453, doi:10.1042/BJ20090500.
25. Janecki, D.M.; Sajek, M.; Smialek, M.J.; Kotecki, M.; Ginter-Matuszewska, B.; Kuczynska, B.; Spik, A.; Kolanowski, T.; Kitazawa, R.; Kurpisz, M., et al. SPIN1 is a proto-oncogene and SPIN3 is a tumor suppressor in human seminoma. *Oncotarget* **2018**, *9*, 32466-32477, doi:10.18632/oncotarget.25977.
26. Smialek, M.J.; Kuczynska, B.; Ilaslan, E.; Janecki, D.M.; Sajek, M.P.; Kusz-Zamelczyk, K.; Jaruzelska, J. Kinesin KIF18A is a novel PUM-regulated target promoting mitotic progression and survival of a human male germ cell line. *J Cell Sci* **2020**, *133*, doi:10.1242/jcs.240986.
27. Naudin, C.; Hattabi, A.; Michelet, F.; Miri-Nezhad, A.; Benyoucef, A.; Pflumio, F.; Guillonneau, F.; Fichelson, S.; Vigon, I.; Dusanter-Fourt, I., et al. PUMILIO/FOXP1 signaling drives expansion of hematopoietic stem/progenitor and leukemia cells. *Blood* **2017**, *129*, 2493-2506, doi:10.1182/blood-2016-10-747436.

28. Spassov, D.S.; Jurecic, R. Mouse Pum1 and Pum2 genes, members of the Pumilio family of RNA-binding proteins, show differential expression in fetal and adult hematopoietic stem cells and progenitors. *Blood Cells Mol Dis* **2003**, *30*, 55-69, doi:10.1016/s1079-9796(03)00003-2.
29. Rajpert-De Meyts, E.; McGlynn, K.A.; Okamoto, K.; Jewett, M.A.; Bokemeyer, C. Testicular germ cell tumours. *Lancet* **2016**, *387*, 1762-1774, doi:10.1016/S0140-6736(15)00991-5.
30. Yue, W.; Sun, L.Y.; Li, C.H.; Zhang, L.X.; Pei, X.T. [Screening and identification of ovarian carcinomas related genes]. *Ai Zheng* **2004**, *23*, 141-145.
31. Gao, Y.; Yue, W.; Zhang, P.; Li, L.; Xie, X.; Yuan, H.; Chen, L.; Liu, D.; Yan, F.; Pei, X. Spindlin1, a novel nuclear protein with a role in the transformation of NIH3T3 cells. *Biochem Biophys Res Commun* **2005**, *335*, 343-350, doi:10.1016/j.bbrc.2005.07.087.
32. Li, Y.; Ma, X.; Wang, Y.; Li, G. miR-489 inhibits proliferation, cell cycle progression and induces apoptosis of glioma cells via targeting SPIN1-mediated PI3K/AKT pathway. *Biomed Pharmacother* **2017**, *93*, 435-443, doi:10.1016/j.biopha.2017.06.058.
33. Czechanski, A.; Kim, H.; Byers, C.; Greenstein, I.; Stumpff, J.; Reinholdt, L.G. Kif18a is specifically required for mitotic progression during germ line development. *Dev Biol* **2015**, *402*, 253-262, doi:10.1016/j.ydbio.2015.03.011.
34. Fonseca, C.L.; Malaby, H.L.H.; Sepaniac, L.A.; Martin, W.; Byers, C.; Czechanski, A.; Messinger, D.; Tang, M.; Ohi, R.; Reinholdt, L.G., et al. Mitotic chromosome alignment ensures mitotic fidelity by promoting interchromosomal compaction during anaphase. *J Cell Biol* **2019**, *218*, 1148-1163, doi:10.1083/jcb.201807228.
35. Smialek, M.J.; Ilaslan, E.; Sajek, M.P.; Swiercz, A.; Janecki, D.M.; Kusz-Zamelczyk, K.; Wozniak, T.; Kotecki, M.; Handschuh, L.; Figlerowicz, M., et al. Characterization of RNP Networks of PUM1 and PUM2 Post-Transcriptional Regulators in TCam-2 Cells, a Human Male Germ Cell Model. *Cells* **2020**, *9*, doi:10.3390/cells9040984.
36. Keene, J.D.; Tenenbaum, S.A. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol Cell* **2002**, *9*, 1161-1167, doi:10.1016/s1097-2765(02)00559-2.
