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The Retinoblastoma Binding Protein 6 Family is Essential for Embryonic Development and Carcinogenesis

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Abstract

Regulators of the tumour suppressor protein, p53 are promising anti-cancer drug candidates. The retinoblastoma binding protein 6 (RBBP6), a p53 negative regulator, has emerged as an important player in carcinogenesis and is essential for both invertebrate and vertebrate embryonic development. It is a multi-domain protein associated with key cellular processes such as cell cycle control and apoptosis. The mouse orthologue enhances the activity of the prototypical p53 negative regulator, Mouse Double Minute 2. The biological functions of RBBP6 include cell proliferation, cell differentiation, cell survival and angiogenesis. Consistent with these features, RBBP6 is implicated in several cancers as a critical factor and offers opportunities for development of new approaches to diagnosis and treatment. Currently, there are no drug candidates targeting RBBP6. There are, however, experimental as well as approved drugs that target RBBP6 interactors. In this review, rigorous analysis unveils important gaps that require urgent attention and RBBP6 is evaluated as a potential drug target.

Keywords: RBBP6; p53; pRb; Carcinogenesis; Embryonic development

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Introduction

The multi-domain retinoblastoma binding protein 6 (RBBP6) belongs to a distinct eukaryotic family and is absent in prokaryotes. It is essential for both vertebrate and invertebrate embryonic development. The molecular functions of RBBP6 include nucleic acid metabolism (replication, transcription and pre-mRNA splicing), E3 ubiquitin ligase activity and interaction with p53 and the retinoblastoma protein (RB1). Evidence shows that the murine orthologue enhances the E3 ligase activity of Mouse Double Minute (MDM2), the prototypical p53 negative regulator

We analyse the structures of the RBBP6 gene and protein using raw data published in databases and in literature. Furthermore, we examine the role RBBP6 plays in carcinogenesis and its potential as a diagnostic tool and therapeutic target.

Studied vertebrate, invertebrate and plant orthologues include the mouse (variously known as P2P-R or PACT (**p**53 **A**ssociated **C**ellular **P**rotein-**T**estis-derived)), *Drosophila melanogaster* (SNAMA), *Caenorhabditis elegans* (RBPL-1) and *Saccharomyces cerevisiae* (Mpe-1) [1]. Overall, the RBBP6 family has, through evolution, gained complexity. In mammals, many isoforms are generated from a single gene.

The Structure of the RBBP6 Gene and its Molecular and Biological Functions

The RBBP6 gene has 17 introns that are alternatively spliced to produce four transcripts (Figure 1A). It has been reported that RBBP6 is regulated by two promoters [2]. The vertebrate and invertebrate *p53* genes also generate multiple isoforms by alternative splicing, alternative initiation of translation, and by alternative promoter usage [3,4]. Our analysis suggests that all four transcripts have the same transcriptional start site and untranslated region. There is no evidence of a transcript produced by an alternative promoter.

All members of the RBBP6 family possess an N-terminal Domain With No Name (DWNN) with a ubiquitin-like three dimensional fold [5,6]. This domain can exist in humans as a single, independent domain. Immediately downstream DWNN, is a zinc-finger and a RING (Really Interesting New Gene) domain. This combination is called the DWNN catalytic module (DCM) [7]. Downstream

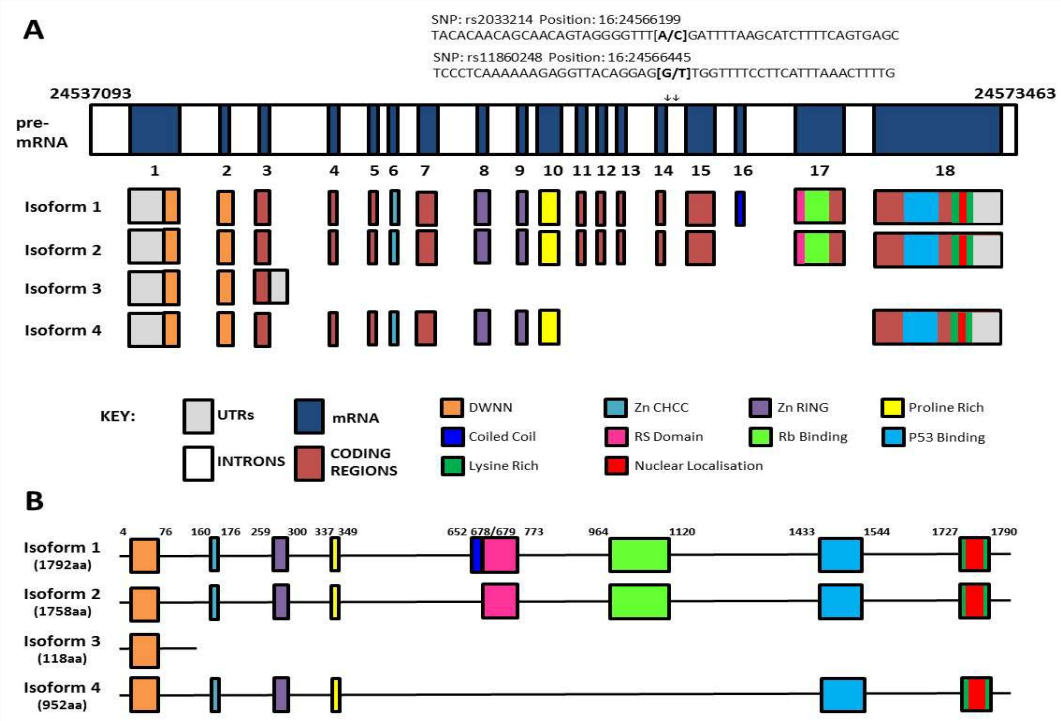

Figure 1: Organization of the *RBBP6* gene and domain organization of its protein product. **A**. **Gene structure and configuration of exons in the spliced transcripts.** The locations of SNPs in intron 14 associated with glioma are indicated. Pre-mRNA sequences that are landmark for splicing reactions with their associated binding proteins are shown. Location of exons are shown in the different RBBP6 isoforms **B. *RBBP6* isoforms and domains.** Arrangement of several domains in RBPP6. Domains contained in each isoform are shown.

the DCM, RBBP6 consists of a panoply of domains and motifs that may include the p53-binding domain, the retinoblastoma protein (Rb)-binding domain, the serine/arginine (SR)-region, a coiled-coil, a lysine-rich region at the C-terminal end and a nuclear localisation signal (Figure 1B). RBBP6 binds on co-proteins; p53 and Rb1 [8,9] as well as the Y-box-binding protein 1 (YB-1), a multifunctional transcription factor that is crucial for proliferation, differentiation and apoptosis [10]. At the molecular level, the activities of Yb-1 have an impact on DNA repair, mRNA transcription, splicing, translation and mRNA stability [11]. Thus RBBP6 belongs to a small subset of proteins that bind both tumour suppressor proteins. One study reports that the C-terminal end (amino acid 1215 – 1404) is in fact a topoisomerase 1 domain [12]. This has, however, not been pursued in such that there is no experimental evidence. RBBP6 is thus implicated in many biological processes and influences many cellular functions and effects (Figure 2).

Certain similarities between RBBP6 and Mdm2 attract attention to their roles. Firstly, both proteins bind to and act as negative regulators of p53 [5,8]. Secondly, they possess E3 ligase activity and catalyse ubiquitination of substrates such as p53 [10]. Importantly, both RBBP6 and Mdm2 null mutants die during embryogenesis suggesting that each is essential for development. Both null mutants can be rescued by concomitant deletion of p53. In *rbbp6*$^{-/-}$*p53*$^{-/-}$, the lethal phenotype is partially rescued albeit with severe developmental defects while *mdm2*$^{-/-}$*p53*$^{-/-}$ double mutants are born viable and develop normally. Even a heterozygous p53 background does not rescue the *mdm2* null phenotype. These results support an intimate functional relationship between Mdm2 and p53 [13-15]. By the same token a similar relationship between p53 and RBBP6 can be inferred. These findings suggest that RBBP6 has Mdm2-independent activities that are crucial for development.

There are four known RBBP6 isoforms (Figure 1B). Experimental evidence reveals differential expression of isoform 1 (approximately 250kDa) and isoform 3 (approximately 13 kDa) both having influence on carcinogenesis. The expression patterns of isoform 2 and 4 have not been elucidated.

RBBP6 has been detected in the nucleus, nucleoli and in the cytoplasm at different cell cycle stages [16]. Its role in cell cycle control is rather complex with some isoforms exhibiting distinct molecular functions. When isoform 1 is depleted, reduced cell proliferation is observed [12]. On the other hand, isoform 3 (which comprised the DWNN only) is downregulated in some cancers suggesting an anti-apoptotic role. It appears to control G_2/M phase as depletion slows down G_2/M cell cycle arrest [17]. The contrasting roles played by these isoforms may be explained by events occurring during pre-mRNA processing where isoform 3 is found to inhibit the full-length by competitive binding to the polyadenylation cleavage complex. The mechanism by which this interaction occurs appears to be via the cleavage stimulatory factor (CstF46), and is independent of RNA-binding. Notably, the DCM is sufficient for 3′ end cleavage during polyadenylation but DWNN and the rest of the module possess distinct functions. DWNN is responsible for binding to CstF while the zinc finger and RING domain are required for RNA-binding. Consequently, Isoform3 inhibits full length RBBP6 by competitive binding to CstF [18].

The multi-domain structure of RBBP6 portrays a protein that links molecular activities with cellular processes that are crucial for the cell cycle. For instance, in addition to binding to p53 and Rb1, RBBP6 catalyses ubiquitination and subsequent proteasomal degradation

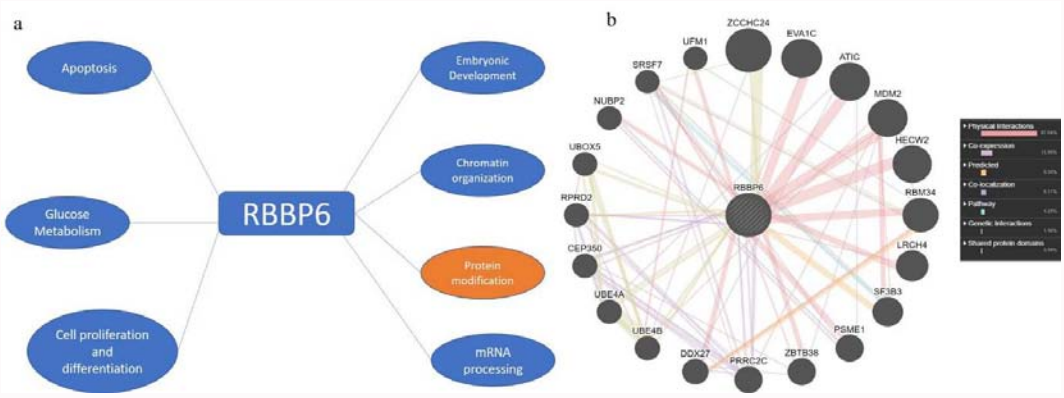

Figure 2: Biological processes and RBBP6 molecular interactions. A) Biological processes in which RBBP6 is involved. Blue coloured boxes indicate processes that have been experimentally shown, whereas orange is yet to be proven. B) RBBP6 molecular interactions are shown. The diagram was generated using Gene Mania. Interacting molecules are shown as nodes and the type of interaction or association is indicated by coloured edges as indicated in the colour key (insert).

of the transcriptional repressor protein ZBTB38. This leads to the depletion of the replication factor MCM10 and slowing down of the replication fork movement [19]. Disturbance of this system could have adverse impact on genome stability. A study on Saos2 cells shows that the full-length murine orthologue, known as P2P-R, promotes cell cycle progression and that its overexpression causes cell cycle arrest at prometaphase and mitotic apoptosis independently of both p53 and Rb. Furthermore, these activities occur in the absence of transcription [20]. Thus, RBBP6 may induce apoptosis independently of p53 and transcription by influencing a mitochondrial pathway. Indeed, probable mitochondrial localization was observed. Moreover, another study shows that an antibody to the *Drosophila* orthologue, immunoprecipitates the voltage-dependent anion channels (VDAC) localized on the outer mitochondrial membrane (OMM) [7]. VDAC is a pivotal regulator of global mitochondrial function and is at the frontier of cell life and death [21]. Physiologically, it facilitates the exchange of metabolites (e.g. succinate, glutamate etc.,) and anions between mitochondria and the cytosol. It is induced by wild type p53 to form high molecular weight multimers in the OMM to facilitate release of apoptogenic molecules [22]. VDAC closure is associated with mitochondrial outer membrane permeabilization (MOMP), reactions leading to the release of cytochrome c and apoptosis [23]. The precise manner by which RBBP6 interacts with VDAC is not clear.

The mitotic spindle assembly checkpoint protein (MAD2L2), was identified in a yeast two hybrid screen as an interactor with RBBP6 forming part of a network of proteins involved in cell death, cell differentiation, cell proliferation and protein modification [24]. MAD2L2 controls mitotic check point [25]. It acts in complex with other proteins to inhibit Cdc20 directly, preventing it from activating the anaphase-promoting complex (APC), a ubiquitin ligase that is important for initiation of chromosome segregation [26,27]. This implies that RBBP6 acts at the crucial step when chromosome segregation occurs in mitosis and in meiosis. Failure of this could result in aneuploidy.

RBBP6 is also found at nuclear matrix attachment regions (MARs) indicating involvement in chromatin organization where it recruits p53 and Rb, a member of the serine-arginine subfamily of protein kinases known as SRPK1a and matrix attachment factors, SAF-B and nucleolin [28]. These features further reinforce the role RBBP6 plays in chromatin organization.

Differential expression of RBBP6 has been observed in several cancers including colon [29,30], lung [31], breast [32], cervical [33], oesophageal [12], colorectal [34], brain [35], stomach [36], pancreatic [37], prostrate [38] cancers and in susceptibility to glioma [35] (Table 1). Accumulating evidence shows that the various isoforms possess unique functions. Isoform 3 is a cell cycle regulator required at the G2/M checkpoint and has anti-proliferative effects, because its overexpression stabilizes p53 and inhibits growth. Interestingly, the cell cycle arrest agent, As_2O_3, induces the expression of both isoforms 1 and 3 at G2/M in human Embryonic Kidney 293 (HEK 293) cells. However, unlike isoform 1, depletion of isoform 3 inhibits G2/M cell cycle arrest, suggesting that the two isoforms perform contrasting functions. The mechanism by which RBBP6 affects the cell cycle checkpoints is not clear but some evidence point to probable interactions with p53 and pRB. In addition to the specific cyclin-dependent kinases at checkpoints, p53 controls both the G1/S and G2/M phases since its transcriptional target, p21, inhibits the cyclin-dependent kinases at G1/S checkpoint as well as the cdc2 kinase at G2/M [39]. pRb, on the other hand, is the key regulator of the G2/M boundary and its depletion leads to G2 arrest [40]. Since RBBP6 has the uncommon characteristic of interacting with both p53 and Rb1, it probably influences both phases of the cell cycle. Generally, experimental evidence shows that isoform 3 is deregulated in tumours but abundantly expressed in tumour-associated normal tissue [17].

A study on the Chinese Han population, whereby all the common tagging single nucleotide polymorphisms (SNPs) in RBBP6 were genotyped, revealed that two of them were associated with either low grade glioma or the highly aggressive glioma multiforme [35]. These SNPs (rs2033214 and rs11860248) are located in intron 14 (Figure 1A). So far, the precise molecular underpinnings of these phenotypes have not been elucidated. However, the location of intron 14 upstream of exons that encode key RBBP6 functional domains such as the p53- and Rb-binding and RS domains implies that a truncated transcript resulting from these mutations would have an impact on cellular activities.

SNP rs2033214 occurs in a potential branch-point sequence (CUAAAU) and rs11860248 is at the 5′ end of the poly-pyrimidine tract (PPT). These SNPs may have an impact on splicing pathways. Defective splicing pathways have serious pathological consequences, with 15% of mutations implicated in hereditary disease affecting pre-mRNA splicing. Often, these are point mutations in exonic splicing

Table 1: Status of RBBP6 in various pathological conditions.

Role	Indicator	Reference
Cancer		
Colon cancer	Overexpressed	(Chen et al., 2013; Dlamini et al., 2016)
Lung cancer	Upregulated	(Motadi et al., 2011)
Breast cancer	Upregulated	(Moela et al., 2014)
Cervical cancer	Upregulated	(Ledwaba and Dlamini, 2005)
Oesophageal cancer (Rbbp6, PACT,P2P-R)	Genetic mutation, Upregulated	(Mehlo and Dlamini, 2007) (Li et al., 2007) (Yoshitake et al., 2004)
Colorectal cancer	Overexpressed	(Wiese et al., n.d.)
Glioma	Genetic variations (SNPs)	(Hu et al., 2014)
Gastric cancer	Upregulated	(Yashiro et al., 2015)
Prostrate cancer	Overexpressed	(Singh et al., 2006)
Biomarker		
Colon cancer	Overexpressed	(Chen et al., 2013)
Gastric cancer	Upregulated	(Yashiro et al., 2015)
Risk factor		
Familial myeloproliferative neoplasms	Germline mutations	(Harutyunyan et al., 2016)
Other Conditions		
Ovarian endometriosis	Upregulated	(Arimoto et al., 2003)
Neurological disorders	Duplicated	(Howell et al., 2013)
Myelodysplastic syndromes	Mutated	(Liu et al., 2015)
Embryonic lethality (PACT, SNAMA)	Absent	(Hull et al., 2015; Li et al., 2007)(Mather et al., 2005)
Infertility (RBPL-1)	Absent	(Huang et al., 2013)
Cell cycle progression during cancer (P2P-R)	Absent	(Gao and Scott, 2003)
Inability to synthesize nutrients	Absent	(Huang et al., 2013)

enhancers [41]. The branch-point, polypyrimidine tract and 3′ AG dinucleotide facilitate the committing step in splicing through binding of the branch point binding protein or splicing factor 1 (SF1) and the 65 kDa subunit of U2 auxiliary factor ($U2AF^{65}$) [42-44]. Since alternative splicing has a remarkable role in the brain [45], an appreciable number of brain-specific disorders associated with splicing defects can be expected. This tissue specific pre-mRNA splicing has been observed in gliomas. The point mutations in the polypyrimidine tract may alter initial stages of spliceosome assembly by interfering with PTB binding. The polypyrimidine tract binding protein (PTB) or hnRNP mediates neuron-specific splicing switch by competing with U2AF for the*cis*-element, 5′ UUCUCU 3′. This regulates either exon selection or skipping [46,47]. Overexpression of PTB seen in neoplastic transformation of glial cells leads to the development of glioblastoma multiforme tumours [48].

Recently, five germline RBBP6 mutations were identified in individuals with predisposition to familial myeloproliferative neoplasms and absent in the general population [34]. At least three of these mutations are in the p53-binding domain and the rest in the adjacent downstream region. The mutations exhibit low penetrance which is attributed to influence by stochastic factors such as somatic mutations or germline mutations in the Janus kinase 2 (*JAK2*) GGCC haplotype and a SNP in the telomerase reverse transcriptase (*TERT*) genes since these mutations have an additive effect on the MPN risk found in *rbbp6* carriers.

Overexpression of RBBP6 in colon cancer patients is associated with poor prognosis. Using quantitative real-time PCR, Western blot analysis and immunohistochemistry it was determined that simultaneous increase in RBBP6 and mutant *Tp53* results in death of a patient within a short period. Consequently, it is proposed that this relationship is predictive of colon cancer. Importantly, RBBP6 was also found to be an independent prognostic factor for overall and disease- free survival [29].

RBBP6 in Embryonic Development

RBBP6 is essential for both vertebrate and invertebrate development since null mutants die as embryos [5,13]. New evidence shows that it maybe involved in anterior-posterior patterning. Firstly, the promoter region of the *Snama* possesses putative transcription factor binding sites for genes involved in anterior posterior patterning [7]. Secondly, mouse embryos lacking the *pact* gene display slowed development and die before embryonic stage E7.5, with more extensive apoptosis when compared to wild type littermates. These embryos can be partially rescued by simultaneous deletion of *p53* and develop past gastrulation but with obvious defects along the anterior-posterior axis [13]. This phenotype is similar to but more severe than the $mdm2^{-/-}p53^{-/-}$phenotype [15,49]. The severity of the $pact^{-/-}$ phenotype suggests a more extensive role for RBBP6 in normal physiology.

RBBP6 is also implicated in embryonic development of the frog. This is based on expression profiles observed in key developmental stages of *Engystomopspustulosus* [50], suggesting a potential role in cell differentiation. At the blastula stage, RBBP6 is expressed highly in the animal cap which represents the presumptive ectoderm. During early neurulation RBBP6 transcripts are restricted to the presumptive eye region and later throughout the neural tube. The expression

profile of the frog RBBP6 suggests that it is required in tissues of ectodermal origin and in eye development. Similarly, SNAMA is essential for cell proliferation and survival in the morphogenetic furrow of the developing eye [51].

Although its role in differentiation is unclear RBBP6 is one of the proteins with more than three–fold expression in the pluripotent Human Embryonic stem cell RNA [52]. These findings reinforce the fact that it is required for cell differentiation. The pre-implantation embryo undergoes critical biological changes including, termination of female meiosis, chromatin reorganization and absence of apoptosis. Thus, the molecular functions of RBBP6 correlate with this critical period. RBBP6 is expressed at early stages of human development and is identified as an "early riser": being just detectable in the oocyte and highly upregulated at 2 – 8 cell stages. This expression is sensitive to α-amanitin indicating embryonic transcription [53]. These findings suggest that RBBP6 is required early in human development and may be involved in cell differentiation since it is highly expressed when pluripotency-related genes are normally upregulated. The Taqman assay used in this study would have identified either isoform 1 or 2 but not isoform 3 and 4 since the probe spans exons 13 and 14.

RBBP6 as a Biological Marker

A biomarker is defined by the National Institute of Health as a molecule found in body fluids, blood or tissues that may be employed as a predictor of a normal, an abnormal process or disease. Biomarkers may be useful in pathological contexts including; diagnosis (especially early detection of disease), assessment of response to treatment, identification of targets for therapy, prevention (assessing risk to disease and as "surrogates" or substitutes) and use as clinical endpoints [54].

RBBP6 was identified as a potential biomarker for oesophageal, colon and gastric cancers. It is a potential stem cell marker for gastric cancer as well as an independent prognostic factor for distant metastasis and advanced clinical stage because overexpression correlates with metastasis and tumour invasiveness (36). The current view is that RBBP6 expression levels may be predictive and prognostic indicators of gastric cancer.

RBBP6 may also be an independent prognostic factor for the survival of colon cancer patients. It's elevated levels in cancerous colon tissue are associated with advanced stage and metastatic tumours. When RBBP6 is overexpressed, patients have a low survival rate [29]. The limitation of this study is in the use of the Abcam antibody as it is not clear which isoform is detected. We assume that this was Abcam (Cat # ab55787) as it detects a 38 kDa protein consistent with information in the manufacturer's brochure. However, there is no isoform that matches this size. Since the antibody was raised against the region spanning amino acids 1582 – 1692, isoforms 3 and 4 are excluded. It probably represents either isoform 1 or 2.

Using cDNA microarray analysis comprising 9216 genes and 26 oesophageal cancer patients, RBBP6 (referred to in that article as PP-RP) was identified as an oesophageal tumour associated antigen (TAA) associated with HLA-A24. It was characterized by immunocytochemical staining and xenograft rejection and found to have higher expression in cancerous tissue in contrast to normal tissue. Furthermore, RBBP6 contains 10 epitopes recognized by HLA-A24-restricted cytotoxic T lymphocytes. Hence, adoptive transfer of RBBP6-specific cytotoxic T lymphocytes caused rejection of lung cancer xenograft in an HLA-A24-resctricted manor. However, it might be more useful to immunotherapy and vaccination than to diagnosis, since it is a nuclear protein. Although RBBP6 is expressed in certain normal tissues such as testis and placenta, it has not been detected in many others such as lung, spleen, kidney, lymph node, brain and liver [12].

RBBP6 as a Drug Target

There are three broad considerations to be taken when evaluating a drug target. Firstly, the biochemical pathway associated with it must be studied sufficiently to decide if the target is essential for the cell. This is important because issues of toxicity or safety are crucial in drug discovery and development. Many drugs fail during development for this key pharmacokinetic parameter [55]. Secondly, the target should be potentially or actually drugable. It must possess potential binding sites for putative drugs, especially for small molecules whose interactions have an impact on molecular function that is measurable *in vitro* and *in vivo*? Thirdly, for rational drug design, a three dimensional structure of the target or its homologue must be determined.

Current research suggests that RBBP6 is involved in important biochemical pathways. The three-dimensional structures of DWNN and the RING domain have been investigated and published [6,56]. A three dimensional structure of the p53-binding domain is possible by molecular modelling if a suitable template is found. This domain is attractive in the drug discovery process because its perturbation is likely to affect apoptosis and have an impact on carcinogenesis.

Currently, there are no RBBP6 targeting drugs that are either approved or at an experimental stage. There are drugs that target molecules associated with RBBP6 in DRUGSURV, a resource for repositioning of approved and experimental drugs in oncology based on patient survival information derived from clinical cancer expression datasets [57]. These data can be used to search for lead molecules with potential for development into therapeutic drugs. In all, the space is open for discovery of drugs that target RBBP6.

Conclusion

The RBBP6 family is emerging as an important player in embryonic development and in carcinogenesis. This review brings into focus the contrasting roles of the various isoforms. The independent DWNN module (isoform 3) is anti-proliferative and is down-regulated in cancers while the longer ones promote cell proliferation and are overexpressed in cancers. Evidence suggests that isoform 3 is an inhibitor of the longer ones.

This review exposes key areas that require more research; precise elucidation of the functions of the various isoforms and the tertiary structure of the p53- and Rb-binding domains. Biochemical features of these proteins suggest that it is a potential drug target and biological marker.

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References

1. Ntwasa M. The retinoblastoma binding protein 6 is a potential target for therapeutic drugs. Biotechnol Mol Biol Rev. 2008; 3: 24–31.

2. Pretorius A, Kaur M, Wamalwa M, February M, Essack M, Bajic V, et al. Functional analysis and characterization of the human RBBP6 promoters

based on a combination of molecular biology and in silico approaches provide additional evidence for RBBP6 role in apoptosis. JBio. 2013; 1: 2251-3159.

3. Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Alexandra Diot, Xirodimas DP, et al. p53 isoforms can regulate p53 transcriptional activity. Genes Dev. 2005; 19: 2122–2137.

4. Khoury MP, Bourdon JC. p53 isoforms: An intracellular microprocessor? Genes & Cancer. 2011; 2: 453-465.

5. Mather A, Rakghotho M, Ntwasa M. SNAMA, a novel protein with a DWNN domain and a RING finger-like motif: A possible role in apoptosis. Biochim Biophys Acta. 2005; 1727: 169-176.

6. Pugh D, AB E, Faro A, Lutya P, Hoffmann E, Rees DJ. DWNN, a novel ubiquitin-like domain, implicates RBBP6 in mRNA processing and ubiquitin-like pathways. BMC Struct Biol. 2006; 6: 1.

7. Hull R, Oosthuysen B, Cajee U-F, Mokgohloa L, Nweke E, Antunes R, et al. The Drosophila Retinoblastoma Binding Protein 6 family member has two isoforms and is potentially involved in embryonic patterning. Int J Mol Sci. 2015; 16: 10242-10266.

8. Simons A, Melamed-Bessudo C, Wolkowicz R, Sperling J, Sperling R, Eisenbach L, et al. PACT: cloning and characterization of a cellular p53 binding protein that interacts with Rb. Oncogene. 1997; 14: 145-155.

9. Witte MM, Scott RE. The proliferation potential protein-related (P2P-R) gene with domains encoding heterogeneous nuclear ribonucleoprotein association and Rb1 binding shows repressed expression during terminal differentiation. Proc Natl AcadSci USA. 1997; 94: 1212-1217.

10. Chibi M, Meyer M, Skepu A, G. Rees DJ, Moolman-Smook JC, Pugh DJR. RBBP6 Interacts with multifunctional protein YB-1 through its RING finger domain, leading to ubiquitination and proteosomal degradation of YB-1. J Mol Biol. 2008; 384: 908-916.

11. Lyabin DN, Eliseeva IA, Ovchinnikov LP. YB-1 protein: functions and regulation. Wiley Interdisciplinary Reviews: RNA. 2014; 5: 95-110.

12. Yoshitake Y, Nakatsura T, Monji M, Senju S, Matsuyoshi H, Tsukamoto H, et al. Proliferation potential-related protein, an ideal esophageal cancer antigen for immunotherapy, identified using complimentary DNA microarray analysis. Clin Cancer Res. 2004; 10: 6437-6448.

13. Li L, Deng B, Xing G, Teng Y, Tian C, Cheng X, et al. PACT is a negative regulator of p53 and essential for cell growth and embryonic development. Proc Natl Acad Sci USA. 2007; 104: 7951-7956.

14. de Oca Luna RM, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. Nature. 1995; 378: 203-206.

15. Jones SN, Roe AE, Donehower LA, Bradley A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. Nature. 1995; 378: 206-208.

16. Gao S, White MM, Scott RE. P2P-R protein localizes to the nucleus of interphase cells and the periphery of chromosomes in mitotic cells which show maximum P2P-R immunoreactivity. J Cell Physiol. 2002; 191: 145-154.

17. Mbita Z, Meyer M, Skepu A, Hosie M, Rees J, Dlamini Z. De-regulation of the RBBP6 isoform 3/DWNN in human cancers. Mol Cell Biochem. 2012; 362: 249-262.

18. Di Giammartino DC, Li W, Ogami K, Yashinskie JJ, Hoque M, Tian B, et al. RBBP6 isoforms regulate the human polyadenylation machinery and modulate expression of mRNAs with AU-rich 3′ UTRs. Genes Dev. 2014; 28: 2248-2260.

19. Miotto B, Chibi M, Xie P, Koundrioukoff S, Moolman-Smook H, Pugh D, et al. The RBBP6/ZBTB38/MCM10 axis regulates DNA replication and common fragile site stability. Cell Reports. 2014; 7: 575-587.

20. Gao S, Scott RE. P2P-R protein overexpression restricts mitotic progression at prometaphase and promotes mitotic apoptosis. J Cell Physiol. 2002; 193: 199-207.

21. Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N. VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Asp Med. 2010; 31: 227-285.

22. Wolff S, Erster S, Palacios G, Moll UM. p53's mitochondrial translocation and MOMP action is independent of Puma and Bax and severely disrupts mitochondrial membrane integrity. Cell Res. 2008; 18: 733-744.

23. Lemasters JJ, Holmuhamedov E. Voltage-dependent anion channel (VDAC) as mitochondrial governator—thinking outside the box. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2006; 1762: 181-190.

24. Wang J, Yuan Y, Zhou Y, Guo L, Zhang L, Kuai X, et al. Protein interaction data set highlighted with human Ras-MAPK/PI3K signaling pathways. J Proteome Res. 2008; 7: 3879-3889.

25. Cahill DP, da Costa LT, Carson-Walter EB, Kinzler KW, Vogelstein B, Lengauer C. Characterization of MAD2B and other mitotic spindle checkpoint genes. Genomics. 1999; 58: 181-187.

26. Yu H. Regulation of APC–Cdc20 by the spindle checkpoint. Curr Opin Cell Biol. 2002; 14: 706-714.

27. Pfleger CM, Salic A, Lee E, Kirschner MW. Inhibition of Cdh1–APC by the MAD2-related protein MAD2L2: a novel mechanism for regulating Cdh1. Genes Dev. 2001; 15: 1759-1764.

28. Scott RE, Giannakouros T, Gao S, Peidis P. Functional potential of P2P-R: A role in the cell cycle and cell differentiation related to its interactions with proteins that bind to matrix associated regions of DNA? J Cell Biochem. 2003; 90: 6-12.

29. Chen J, Tang H, Wu Z, Zhou C, Jiang T, Xue Y, et al. Overexpression of RBBP6, alone or combined with mutant TP53, is predictive of poor prognosis in colon cancer. PLoS ONE. 2013; 8: e66524.

30. Dlamini Z, Rupnarain C, Naicker S, Hull R, Mbita Z. Expression analysis and association of RBBP6 with apoptosis in colon cancers. J Mol Hist. 2016; 47: 169-182.

31. Motadi LR, Bhoola KD, Dlamini Z. Expression and function of retinoblastoma binding protein 6 (RBBP6) in human lung cancer. Immunobiology. 2011; 216: 1065-1073.

32. Moela P, Choene MMS, Motadi LR. Silencing RBBP6 (Retinoblastoma Binding Protein 6) sensitises breast cancer cells MCF7 to staurosporine and camptothecin-induced cell death. Immunobiology. 2014; 219: 593-601.

33. Mthembu NN, Motadi LR. Apoptotic potential role of Agave palmeri and Tulbaghia violacea extracts in cervical cancer cells. Mol Biol Rep. 2014; 41: 6143-6155.

34. Harutyunyan AS, Giambruno R, Krendl C, Stukalov A, Klampfl T, Berg T, et al. Germline RBBP6 mutations in myeloproliferative neoplasms. Blood. 2016; 122: 267.

35. Hu D, Zhang S, Zhao Y, Wang S, Wang Q, Song X, et al. Association of genetic variants in the retinoblastoma binding protein 6 gene with the risk of glioma: a case-control study in a Chinese Han population. J Neurosurg. 2014; 121: 1209-1218.

36. Morisaki T, Yashiro M, Kakehashi A, Inagaki A, Kinoshita H, Fukuoka T, et al. Comparative proteomics analysis of gastric cancer stem cells. PLoS ONE. 2014; 9: e110736.

37. Thalappilly S, Suliman M, Gayet O, Soubeyran P, Hermant A, Lecine P, et al. Identification of multi-SH3 domain-containing protein interactome in pancreatic cancer: A yeast two-hybrid approach. Proteomics. 2008; 8: 3071-3081.

38. Singh J, Manickam P, Shmoish M, Natik S, Denyer G, Handelsman D, et al. Annotation of androgen dependence to human prostate cancer-associated genes by microarray analysis of mouse prostate. Cancer Lett. 2006; 237: 298-304.

39. Agarwal ML, Agarwal A, Taylor WR, Stark GR. p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proceedings of the National Academy of Sciences. 1995; 92: 8493-8497.

40. Niculescu AB, Chen X, Smeets M, Hengst L, Prives C, Reed SI. Effects of p21Cip1/Waf1 at both the G1/S and the G2/M cell cycle transitions: pRb is a critical determinant in blocking DNA replication and in preventing endoreduplication. Mol Cell Biol. 1998; 18: 629-643.

41. Cáceres JF, Kornblihtt AR. Alternative splicing: multiple control mechanisms and involvement in human disease. Trends Genet. 2002; 18: 186-193.

42. FÖRch P, Merendino L, MartÍNez C, ValcÁRcel J. Wu S, Romfo CM, et al. U2 small nuclear ribonucleoprotein particle (snRNP) auxiliary factor of 65 kDa, U2AF65, can promote U1 snRNP recruitment to 5′ splice sites. Biochem J. 2003; 372: 235-240.

43. Wu S, Romfo CM, Nilsen TW, Green MR. Functional recognition of the 3′ splice site AG by the splicing factor U2AF35. Nature. 1999; 402: 832-835.

44. Hastings ML, Krainer AR. Pre-mRNA splicing in the new millennium. Curr Opin Cell Biol. 2001; 13: 302-309.

45. Licatalosi DD, Darnell RB. Splicing regulation in neurologic disease. Neuron. 2006; 52: 93-101.

46. Ashiya M, Grabowski PJ. A neuron-specific splicing switch mediated by an array of pre-mRNA repressor sites: evidence of a regulatory role for the polypyrimidine tract binding protein and a brain-specific PTB counterpart. RNA. 1997;3(9):996-1015.

47. Valcárcel J, Gebauer F. Post-transcriptional regulation: The dawn of PTB. Curr Biol. 1997; 7: R705-R708.

48. Jin W, McCutcheon IE, Fuller GN, Huang ES-C, Cote GJ. Fibroblast growth factor receptor-1 α-exon exclusion and polypyrimidine tract-binding protein in glioblastoma multiforme tumors. Cancer Res. 2000; 60: 1221-1224.

49. Luna RMdO, Wagner DS, Lozano G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. Nature. 1995; 378: 203-206.

50. Romero-Carvajal A, Sáenz-Ponce N, Venegas-Ferrín M, Almeida-Reinoso D, Lee C, Bond J, et al. Embryogenesis and laboratory maintenance of the foam-nesting túngara frogs, genus Engystomops (= Physalaemus). Dev Dyn. 2009; 238: 1444-1454.

51. Jones C, Reifegerste R, Moses K. Characterization of Drosophilamini-me, a gene required for cell proliferation and survival. Genetics. 2006; 173: 793-808.

52. Zeng X, Miura T, Luo Y, Bhattacharya B, Condie B, Chen J, et al. Properties of pluripotent human embryonic stem cells BG01 and BG02. Stem Cells. 2004; 22: 292-312.

53. Vassena R, Boué S, González-Roca E, Aran B, Auer H, Veiga A, et al. Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. Development. 2011; 138: 3699-3709.

54. De Gruttola VG, Clax P, DeMets DL, Downing GJ, Ellenberg SS, Friedman L, et al. Considerations in the evaluation of surrogate endpoints in clinical trials: Summary of a National Institutes of Health Workshop. Control Clin Trials. 2001; 22: 485-502.

55. Hughes JP, Rees S, Kalindjian SB, Philpott KL. Principles of early drug discovery. Br J Pharmacol. 2011; 162: 1239-1249.

56. Kappo MA, AB E, Hassem F, Atkinson RA, Faro A, Muleya V, et al. Solution Structure of RING Finger-like Domain of Retinoblastoma-binding Protein-6 (RBBP6) Suggests It Functions as a U-box. J Biol Chem. 2012; 287: 7146-7158.

57. Amelio I, Gostev M, Knight RA, Willis AE, Melino G, Antonov AV. DRUGSURV: a resource for repositioning of approved and experimental drugs in oncology based on patient survival information. Cell Death Dis. 2014; 5: e1051.