



Review Article

R2TP/PAQosome as a promising chemotherapeutic target in cancer

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ABSTRACT

R2TP/PAQosome (particle for arrangement of quaternary structure) is a novel multisubunit chaperone specialized in the assembly/maturation of protein complexes that are involved in essential cellular processes such as PI3Ks (phosphatidylinositol 3-kinase-like kinases) signaling, snoRNP (small nucleolar ribonucleoprotein) biogenesis, and RNAP II (RNA polymerase II) complex formation. In this review article, we describe the current understanding of R2TP/PAQosome functions and characteristics as well as how the chaperone complex is involved in oncogenesis, highlighting DNA damage response, mTOR (mammalian target of rapamycin) pathway as well as snoRNP biogenesis. Also, we discuss its possible involvement in HNSCC (head and neck squamous cell carcinoma) including OSCC (oral squamous cell carcinoma). Finally, we provide an overview of current anti-cancer drug development efforts targeting R2TP/PAQosome.

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1. Background of R2TP and PAQosome

1.1. Discovery of R2TP as an Hsp90-interacting complex in yeast

Heat shock protein 90 (Hsp90) is a molecular chaperone that activates and stabilizes a wide range of client proteins by collectively working with its co-chaperones [1]. In 2005, Houry and co-workers performed a high-throughput proteomics study to identify Hsp90 interacting proteins including co-chaperones, regulators, and its substrates in yeast *Saccharomyces cerevisiae* [2]. They identified 198 distinct proteins that physically interact with Hsp90 directly or indirectly in yeast. They found two highly-conserved novel interactors of Hsp90, which they named Pih1 (protein interacting with Hsp90) and Tah1 (tetratricopeptide repeat-containing protein associated with Hsp90). Subsequently, they identified that the both Pih1 and Tah1 physically and functionally associate with essential AAA+ (ATPase associated with diverse cellular activities) family of ATPases, Rvb1 and Rvb2, having conserved Walker A and Walker B motifs involved in ATP binding and hydrolysis. The complex was named R2TP (Rvb1-Rvb2-Tah1-Pih1) (Fig. 1A). Rvb1 and Rvb2 form heterohexameric ring and Pih1-Tah1 heterodimer interact with the Rvb1-Rvb2 complex. Furthermore, they showed that R2TP controls box C/D snoRNP maturation upon different nutrient conditions in yeast [3,4].

1.2. Identification of PAQosome in human: R2TP associated with Prefoldin-like complex

In 2007, Coulombe group identified a network of novel protein complexes associating with RNAP II in human [5]. The protein interaction network included RUVBL1/Pontin, RUVBL2/Reptin, PIH1D1/NOP17, an uncharacterized protein FLJ21908 containing helix-turn-helix tetratricopeptide repeat (TPR) motifs, which was named RPAP3 (RNAP II-associated protein 3), as well as components of Prefoldins and a WD40 repeat protein WDR92/Monad. RUVBL1/Pontin, RUVBL2/Reptin, PIH1D1/NOP17, and RPAP3 correspond to Rvb1, Rvb2, Pih1, and Tah1 in yeast, respectively. In 2008, RPAP3 was also identified as WDR92/Monad interacting protein [6]. Intriguingly, RPAP3 has a longer C-terminal domain compared to the yeast Tah1 (Fig. 2). The C-terminal domain of RPAP3 has been shown to interact with RUVBL1-RUVBL2 hexamer [7]. Two isoforms of RPAP3 are present in human cells: RPAP3-iso1 and RPAP3-iso2. The RPAP3-iso2 is a splicing variant of RPAP3 with deletion of exon 12. We showed that only RPAP3-iso1 interacts with and stabilizes PIH1D1 [8]. Maurizy et al. determined the structure of RPAP3 C-terminal domain and showed that it directly binds to the RUVBL1-RUVBL2 heterohexamer [7]. Of note, RPAP3-iso1 mainly localizes in cytoplasm while RPAP3-iso2 is mostly nuclear, suggesting that the alternative splicing of RPAP3 could regulate the complex formation of R2TP and its localization and function. In 2009, Coulombe group identified R2TP/Prefoldin-like complex, which is composed of R2TP (RUVBL1, RUVBL2, RPAP3, and PIH1D1) and Prefoldin-like complex (PFDN2, PFDN6, PDRG1, UXT, URI1)

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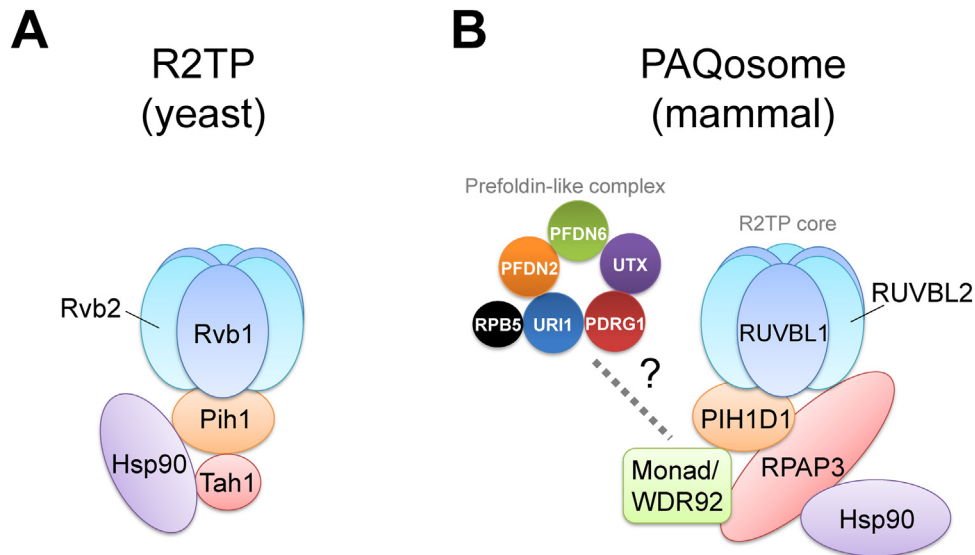


Fig. 1. Schematic representation of R2TP and PAQosome. (A) R2TP complex in yeast. R2TP is composed of Rvb1, Rvb2, Tah1, and Pih1 and is associated with Hsp90. (B) PAQosome in mammal. PAQosome is composed of R2TP core: RuvBL1/Pontin, RuvBL2/Reptin, RPAP3, and PIH1D1, URI1 prefoldin-like complex: URI1, PFDN2, UTX, PFDN6, and PDRG1, and URI1-associated protein RPB5, and RPAP3-associated protein Monad/WDR92.

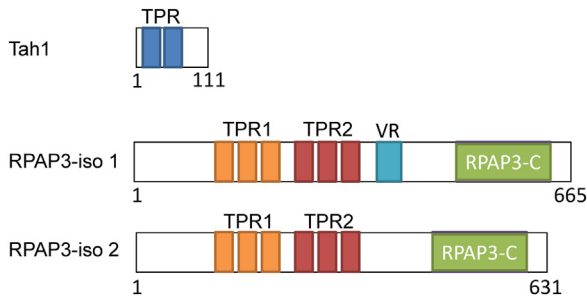


Fig. 2. Primary structure of Tah1 in yeast and its human ortholog RPAP3. Tah1 contains a single TPR domain with two TPR motifs. RPAP3 has two isoforms: RPAP3-iso1 and -iso2. The RPAP3-iso2 is a splicing variant of RPAP3 without variable region (VR) corresponding to exon 12. RPAP3-C terminal domain (RPAP3-C) is conserved in RPAP3-like proteins.

and its associated proteins (RPB5/POLR2E and WDR92/Monad), by using TAP-tagged RPAP3 affinity purification [9]. In 2018, Houry et al. proposed to rename this large multisubunit chaperone complex as PAQosome (Fig. 1B) [10]. WDR92/Monad associates with several subunits of the Prefoldin-like module, suggesting that the RPAP3-binding protein WDR92/Monad is possibly responsible for the interaction between R2TP and Prefoldin-like module to form PAQosome [11]. It has been shown that URI1 stabilizes RPB5 and PDRG1 but not RUVBL1 and RUVBL2 [12].

1.3. The targets of R2TP/PAQosome

Since over a decade of the discovery, a number of researchers have revealed that R2TP core complex and PAQosome are involved in chaperoning quaternary structure formation such as assembly and maturation of multiprotein complexes. R2TP targets U4 and U5 snRNPs (small nuclear ribonucleoprotein), and snoRNPs [4,11,13,14]. PAQosome targets RNAP II, PIKKs complexes such as mTOR, ATR (ATM and Rad3-related), ATM (Ataxia Telangiectasia Mutated) and SMG-1 (Suppressor with Morphological effect on Genitalia 1), MRN complex (Mre11-Rad50-Nbs1), TSC complex (TSC1-TSC2-TBC1D7), and axonemal dynein arm [11,15–19].

1.4. Possible R2TP targets in head and neck squamous cell carcinoma (HNSCC)

Genome-wide studies have revealed a number of key regulators and potential therapeutic targets in oncogenesis including HNSCC. For example, Gonzalez-Perez et al. analyzed somatic mutations in various types of cancer samples obtained from 31 different projects such as International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) [20,21]. They summarized the result in IntoGen-mutation platform (<https://www.intogen.org/search>) which allows us to search significant cancer driver genes conveniently [22]. 167 mutational cancer driver genes in HNSCC were identified, including targets of R2TP: mTOR, ATM, and ATR. Also, Gong et al. analyzed snoRNA expression in more than 10,000 samples across 31 different types of cancers, and identified 46 clinically relevant snoRNAs. They found that the overexpression of DKC1, NOP58, and NOP56 in more than five cancer types, and also DKC1 is overexpressed in HNSCC [23].

Therefore, we describe the function of R2TP/PAQosome in mTOR and ATM/ATR signaling as well as snoRNP biogenesis, and discuss how its function is related to oncogenesis.

2. Oncogenesis-related function of R2TP/PAQosome

2.1. Function in mTOR

PIKKs are key signal transducers that are involved in diverse cellular processes such as nutrient signaling, DNA damage response, mRNA surveillance pathway as well as chromatin remodeling [24]. There are six PIKKs in mammals: mTOR, ATM, ATR, SMG1, DNA-PKcs (DNA-dependent Protein Kinase Catalytic Subunit), and TRRAP (Transformation/Transcription domain-Associated Protein). It has been identified that PAQosome is required for the stability of PIKK complexes through interacting with TELO2 (Telomere maintenance 2), which is a HEAT-repeat containing protein [25]. PIH1D1 directly binds to the CK2-phosphorylated TELO2 and forms TELO2-PAQosome, and the TELO2 also associates with and stabilizes PIKKs, especially mTOR and SMG1, suggesting that TELO2 is an adaptor protein for targeting the substrates of PAQosome [22].

mTOR coordinates the environmental signals such as nutrients and growth factors with intracellular metabolism and cell

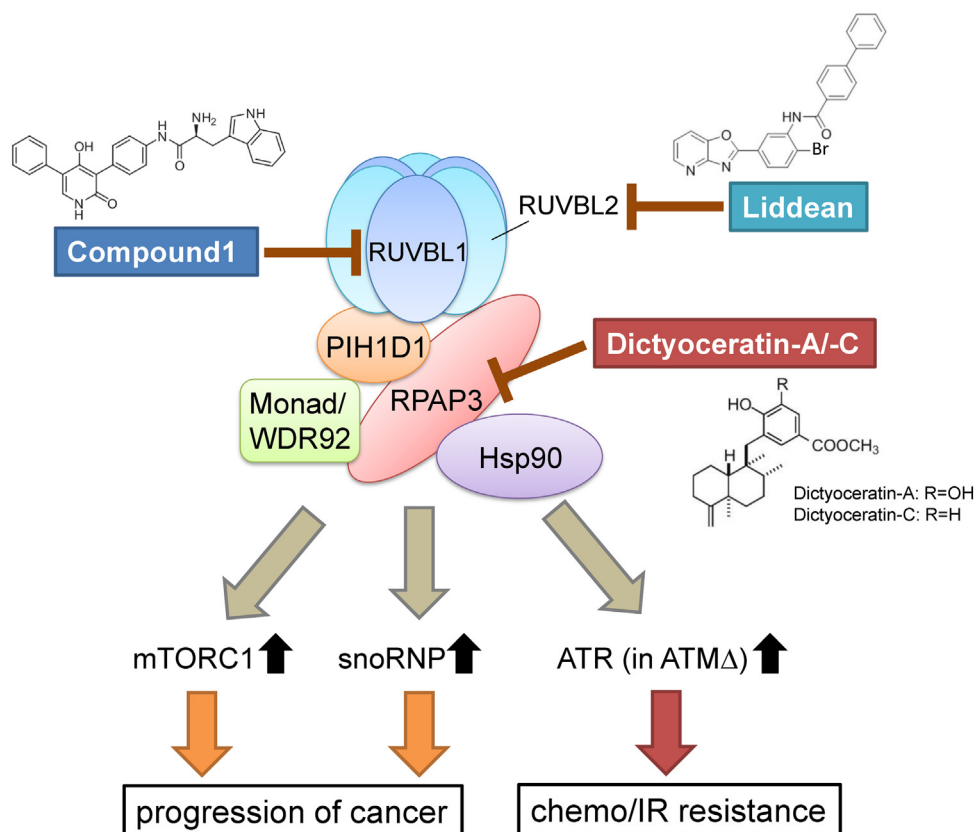


Fig. 3. Currently developed inhibitors of R2TP core components. Compound 1, Liddean, and Dictyoceratin-A/-C interact with RUVBL1, RUVBL2, and RPAP3, respectively [46,47,50]. R2TP/PAQosome promotes cancer development by chaperoning mTORC1 and snoRNP, and it may confer chemotherapy and ionizing radiation resistance to ATM-deficient cancers by activating ATR.

growth. mTOR is involved in many cellular processes including protein, lipid and nucleotide synthesis, and autophagy [26]. It has been shown that dysregulation of mTOR pathway causes various types of cancers including HNSCC [27]. There are two distinct mTOR complexes: mTORC1 and mTORC2. mTORC1 is composed of mTOR, Raptor, GβL/mLST8, PRAS40, and Deptor. mTORC2 includes mTOR, mLST8, Deptor, Rictor, GβL/mLST8, mSIN1, and Protor 1/2. Previously, we analyzed how R2TP is involved in mTOR complex assembly. We identified that PIH1D1 specifically interacts with mTORC1 but not with mTORC2, and we found that its interaction is required for mTORC1 activity [28]. mTORC1 is known to positively regulate the pre-rRNA expression, and our PIH1D1 knockdown experiment in cancer cells showed the significant decrease of the pre-rRNA levels, suggesting that the activity of mTORC1 is controlled by the chaperoning function of R2TP/PAQosome. Kim et al. showed that RUVBL1-RUVBL2-TELO2-TTI1-TTI2 complex is also involved in mTORC1 dimerization and lysosomal localization [29]. They found that the mRNA expression of R2TP genes are significantly higher in the breast and colorectal carcinomas. We also observed that the protein levels of R2TP are significantly upregulated in OSCC-derived cell lines (Kiguchi unpublished data). Given that mTOR protein stability is maintained by R2TP/PAQosome association through TELO2, high levels of R2TP might stabilize overexpressed-mTOR and contribute to the malignancy of cancer (Fig. 3).

2.2. Function in DNA damage response (DDR)

Coordination of the cell-cycle progression and the DNA metabolism including DNA replication and DNA repair as well as DNA checkpoint are essential for the maintenance of genome sta-

bility. ATM and ATR belong to PIKK family and are the key regulator of DDR [30]. Upon DNA damage stress, ATM and ATR are recruited to damage sites, and they activate Chk2 (Checkpoint Kinase 2) and Chk1 (Checkpoint Kinase 1) for determining the cell fate. The ATM activation is regulated by MRN complex [31]. MRN associates with DSBs (DNA double-strand breaks), and is involved in the localization of ATM to the site of DSBs and activates ATM kinase. Recently, it has been revealed that the MRN complex is one of the targets of R2TP [32]. Phosphorylated-MRE11, a component of MRN complex, directly binds to R2TP through PIH1D1. This MRE11-PIH1D1 interaction is regulated by CK2 (Casein Kinase 2) phosphorylation of the serines 558/561 and 688/689 in MRE11 C-terminus. Depletion of the PIH1D1 destabilizes MRE11 and leads to a defect in homologous recombination upon DNA damage. These results suggest that R2TP regulates the activity of MRN complex in the DDR pathway through stabilizing MRE11.

As aforementioned, PIKKs family proteins interact with R2TP complex through CK2 phosphorylated-TELO2. The CK2 phosphosite mutant of TELO2, which is not able to bind R2TP/PAQosome but retains interaction with PIKKs, destabilizes ATM and ATR [22]. ATM/ATR-regulated DNA damage response protects normal cells from oncogenesis. At an early stage of the oncogenesis, activated-oncogenes induce the cells to replication stress and results in formation of DNA DSBs [33,34]. In the absence of ATM activity, DDR pathway is defective and leads the precancerous cells to malignancy. Deficiency in ATM is observed in approximately 10% of human cancers [35]. Intriguingly, in ATM-deficient/decreased OSCCs, ATR-Chk1 pathway is upregulated, and they are resistant to ionizing radiation as well as chemotherapeutic drugs [36–38]. Given that R2TP/PAQosome are involved in DDR through ATM/ATR,

it could progress this hyperactive ATR-Chk1 pathway in ATM-deficient cells (Fig. 3).

2.3. Function in snoRNP biogenesis

SnoRNP biogenesis is one of the most important biological processes since it is required for the processing of pre-ribosomal RNA (pre-rRNA) in ribosome biogenesis [14]. There are two types of snoRNPs, box C/D and box H/ACA snoRNPs, and are required for site-specific 2'-O-methylation and pseudouridylation of pre-rRNA, respectively, which are required for pre-rRNA processing [14]. The box C/D snoRNPs are composed of box C/D snoRNAs and snoRNPs: NOP58, NOP56, SNU13, and FBL, whereas the box H/ACA snoRNPs consist of box H/ACA snoRNAs and snoRNPs: NOP10, NHP2, GAR1, and DKC1. R2TP assists in the assembly/maturation of the both box C/D and box H/ACA snoRNPs, reviewed previously [14,39].

Cancer cells generally require vigorous ribosome biogenesis to produce high levels of protein for their proliferation and growth. It has been reported that some of the snoRNAs and snoRNPs expression are coordinately upregulated in various types of cancers. For example, box H/ACA and DKC1 are upregulated in breast cancer, and box H/ACA, NOP10 and GAR1 are increased in HNSCC, suggesting that hyperactive snoRNP biogenesis is strongly required for the survival of cancer cells [23,40–42]. Given that the snoRNP complexes need R2TP for their assembly, R2TP expression could be coordinately upregulated in cancer cells. The mRNA and/or protein levels of RUVBL1 and RUVBL2 are increased in hepatocellular carcinoma, colon cancer, and breast cancer [43,44]. Previously, we have shown that PIH1D1 is overexpressed in several breast cancer cell lines compared to normal epithelial cells [28]. Also, we found that the R2TP protein levels are significantly upregulated in OSCC compared to normal oral epithelial cells (Kiguchi unpublished data). These facts suggest that R2TP/PAQosome contributes to cancer progression through activating snoRNP biogenesis (Fig. 3).

3. R2TP as a chemotherapeutic target for cancer

As mentioned above, a number of accumulating research suggests that R2TP/PAQosome is tightly linked with oncogenesis, and its inhibition could decrease proliferation activity of the cancer cells. Therefore, the multisubunit chaperone complex as well as its components could be promising candidates for cancer chemotherapy (Fig. 3).

3.1. RUVBL1 inhibitor: compound 1

Elkaim et al. performed a molecular docking approach for virtually screening of the RUVBL1 inhibitors. They selected four compounds (compound 1–4) possessing 4-hydroxy-2-pyridone and 4-hydroxy-2-quinolone, by *in silico* screening, and chemically synthesized these compounds [45,46]. Compounds 1 and 2 showed significantly reduced ATPase activity of RUVBL1 but 3 and 4 did not. Furthermore, compound 1 showed anti-proliferative effect on KB, MCF7, HCT116 and HL60 cell lines, and also induced cell death of HL60 cells at 50 μ M after 24 h of the treatment.

3.2. RUVBL2 inhibitor: Liddean

RUVBL2 inhibitor was screened *in silico* by targeting Walker A site [47]. One compound out of 4.4 million compounds of virtual chemical library was predicted to sit deep in the Walker ADP/ATP binding pocket, and its bromo-analog was synthesized, named Liddean. Liddean stabilizes RUVBL2 more effectively than ADP and promotes a higher ordered oligomeric state of RUVBL2. Intriguingly, Liddean also stabilizes p53-RUVBL2 complex by modifying the binding activity of the RUVBL2 to the substrates. The treatment

of HCT116 cells with Liddean dissociates intracellular interactions of RUVBL1-RUVBL2 as well as p53-RUVBL2.

3.3. RPAP3 inhibitors: dictyoceratin-A and -C

RPAP3 inhibitor was isolated from sea sponge living in the Indonesian sea. Arai and the colleagues isolated eight sesquiterpene phenol/quinones including dictyoceratin-A and -C from *Dactylospongia elegans* by searching for differentiation-inducing compounds in human chronic myelogenous leukemia, K562 cells [48]. Subsequently, they found that the dictyoceratin-A and -C showed hypoxia-selective proliferative inhibitory activity against prostate cancer cells, DU145 [49]. Recently, they identified that RPAP3 is the target of dictyoceratin-A and -C [50]. When DU145 cells were grown in hypoxic condition, the protein levels of HIF-1 α (Hypoxia-Inducible Factor), which is a key regulator of oxygen homeostasis involved in tumor proliferation in hypoxia, were significantly elevated. And also, HK2 (Hexokinase 2) and VEGF (Vascular endothelial growth factor), which are regulated by HIF-1 α upon hypoxic condition, were upregulated. However, the treatment of the cells with dictyoceratin-A or -C reduced the HIF-1 α , HK2, and VEGF expression levels without affecting the RPAP3 levels in hypoxic condition. Furthermore, overexpression of RPAP3 in DU145 cells negated the inhibitory effect of dictyoceratin-A on cell proliferation in hypoxia, indicating that dictyoceratin-A specifically inhibits the RPAP3 activity in hypoxic condition. The interacting region of dictyoceratin-A and -C in RPAP3 was found to be amino acids 109–231, which overlaps with TPR1 domain (amino acids 133–234) that binds HSP90 C-terminal MEEVD motif tighter than the another TPR domain, TPR2 (amino acids 282–383). Therefore, dictyoceratin-A and -C could directly inhibit the interaction between RPAP3 and HSP90.

4. Conclusion

After the discovery of the R2TP/PAQosome, its characteristics and roles in cellular processes have been gradually revealed. Also, several lines of evidence strongly suggest the possible role of R2TP/PAQosome in oncogenesis including HNSCC/OSCC. However, it remains unclear how this molecular chaperone complex contributes to oncogenesis. Currently, research groups and pharmaceutical companies are searching and developing inhibitors of R2TP/PAQosome as a promising chemotherapeutic target for cancers. In parallel, further investigation will be required for better understanding of its chaperoning function in oncogenesis.

Conflict of interest

We declare no conflicts of interest associated with this manuscript.

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