Ring finger protein 146/Iduna is a Poly (ADP-ribose) polymer binding and PARsylation dependent E3 ubiquitin ligase

Zhi-dong Zhou,1 Christine Hui-shan Chan,2 Zhi-cheng Xiao4,5 and Eng-King Tan1,3,*

1National Neuroscience Institute; 2Department of Neurology; Singapore General Hospital; 3Duke-NUS Graduate Medical School; Singapore; 4Institute of Molecular and Clinical Medicine; Kunming Medical College; Kunming City, China; 5Monash Immunology and Stem Cell Laboratories; Monash University; Clayton, VIC Australia

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Recent findings suggest that Ring finger protein 146 (RNF146), also called Iduna, have neuroprotective property due to its inhibition of Parthanatos via binding with Poly(ADP-ribose) (PAR). The Parthanatos is a PAR dependent cell death that has been implicated in many human diseases. RNF146/Iduna acts as a PARsylation-directed E3 ubiquitin ligase to mediate tankyrase-dependent degradation of axin, thereby positively regulates Wnt signaling. RNF146/Iduna can also facilitate DNA repair and protect against cell death induced by DNA damaging agents or γ-irradiation. It can translocate to the nucleus after cellular injury and promote the ubiquitination and degradation of various nuclear proteins involved in DNA damage repair. The PARsylation-directed ubiquitination mediated by RNF146/Iduna is analogous to the phosphorylation-directed ubiquitination catalyzed by Skp1-Cul1-F-box (SCF) E3 ubiquitin complex. RNF146/Iduna has been found to be implicated in neurodegenerative disease and cancer development. Therefore modulation of the PAR-binding and PARsylation dependent E3 ligase activity of RNF146/Iduna could have therapeutic significance for diseases, in which PAR and PAR-binding proteins play key pathophysiologic roles.

Ring finger proteins contain ring fingers, which are considered to be the functional module for E3 ubiquitin ligase activity.1,2 Ring finger protein 146 (RNF146), a novel PARsylation-directed ring finger E3 ubiquitin ligase, is located at 6q22.1-q22.33 of human chromosome.3 RNF146 encodes a protein of 359 amino acids with a predicted molecular weight of 39.8 kDa. The molecular structure of RNF146 contains one N-terminus C3HC4 ring finger domain (35–77 aa) as well as one WWE domain (91–167 aa).3 The poly(ADP-ribose) (PAR) binding motif (144–167 aa) at the tail of WWE domain of RNF146 is involved in various important functions. The detailed molecular structure of RNF146 is illustrated in Figure 1. This PAR-binding protein was recently demonstrated to protect against Parthanatos and function as PARsylation-directed E3 ubiquitin ligase to ubiquitinate PARsylated substrates.3-5 Here, we provide a concise summary of these novel functions of RNF146 and discuss the potential pathophysiological and therapeutic significance of novel functions of RNF146 for human diseases.

RNF146, a PAR-Binding Dependent Neuroprotective Inhibitor of Parthanatos

Parthanatos is a special form of cell death dependent on poly(ADP-ribose) polymerase-1 (PARP-1) activation which has been recently identified and defined via combination of names of PARP-1 and Thanatos, the Greek personification of death and mortality.6,7 In Parthanatos, stimulus induces rapid activation of PARP-1 in nucleus which functions to synthesize PAR polymer.6,7 The synthesized PAR polymer can be used for post-translational modifications of protein residues.8 However the accumulated free PAR polymer synthesized in nucleus can translocate to cytoplasm where it binds with apoptosis inducing factor (AIF) and promotes AIF release from mitochondria outer membrane.6,7,9 The AIF then translocates back to nucleus where it induces degradation of DNA and executes the death sentence for cells.6,7 The Parthanatos has been implicated in the pathogenesis of many human diseases such as inflammation, diabetes, neurodegenerative disease, heart attack, stroke and ischemia reperfusion injury.6,7

Recently, RNF146 has been identified to be the first novel endogenous inhibitor of Parthanatos to rescue neurons from glutamate excitotoxicity in brain.4 Furthermore the neuroprotective function of RNF146 was dependent on its binding with PAR in cytosol.4 The authors named RNF146 the Iduna, a mythological Norwegian goddess who guards a tree full of golden apples used to restore health to sick and injured gods.4 They showed that the expression level of RNF146 in mouse cortical neuronal cultures increases significantly after NMDA challenge.4 They further showed that RNF146 protects neurons against NMDA induced toxicity in a PAR binding dependent manner.3 The protection of RNF146 has been verified in in vitro and in vivo models. Previous studies demonstrated that binding of PAR with AIF was the key event to induce AIF release from the cytosolic side of the mitochondrial outer membrane and functions to trigger Parthanatos.10 Thus RNF146 functions as a PAR binding protein.
Nevertheless, these new findings have potential therapeutic applications targeting human disorders where Parthanatos is a key player. The possible neuroprotective mechanism of RNF146 against Parthanatos is illustrated in Figure 2.

RNF146 has recently been shown to be a PARsylation dependent E3 ubiquitin ligase. Therefore it is possible that RNF146 promotes ubiquitination and degradation of AIF after PAR binding with AIF. However, Andrabi et al. showed that overexpression of RNF146 can preemptively bind with PAR in cytoplasm, therefore RNF146 prevents interaction of PAR with AIF in mitochondria and abrogates AIF release and subsequent induction of Parthanatos. One limitation of these findings was that a synthesized peptide based on PAR binding sequence of RNF146 (144–167 aa) was used to confirm the binding activity of this sequence with PAR in vitro. It isn’t clear whether this short synthesized peptide is enough to protect cells against Parthanatos similar to full length RNF146.

Figure 1. Molecular structure of RNF146. The RNF146 is a novel ring finger E3 ubiquitin ligase with 359 amino acids. The molecular structure of RNF146 contains one N-terminus C_HC, ring finger domain (35–77 aa, 7 cysteine and 1 histidine residues involved in ring finger formation is highlighted in red) as well as one WWE domain (91–167 aa, the highly conserved 2 tryptophan and 1 glutamic acid, which WWE domain is named after, are highlighted in red). The PAR binding motif (144–167 aa) is at the C-terminus tail of WWE domain of RNF146 (highlighted in blue at the end of WWE sequence).

Figure 2. Potential molecular mechanism for RNF146 induced neuroprotection against Parthanatos. Extensive extra-cellular stimulus can activate PARP-1/2 in nucleus. The activated PARP-1/2 can lead to production of large sum of PAR in nucleus. These PAR can translocated to cytoplasm where can bind with AIF in outer membrane of mitochondria (left). The binding of PAR with AIF can trigger the release of AIF from mitochondria (left). The AIF then translocates to nucleus and induces Parthanatos of cells (left). However in the presence of RNF146, the PAR will bind to RNF146 preemptively, hereby prevent the binding of PAR with AIF in mitochondria (right). The abrogation of binding between PAR and AIF by RNF146 will inhibit Parthanatos and contribute to cell rescue (right).
RNF146 without RING domain still can partially block glutamate induced excitotoxicity. This protective effect by RNF146 without RING domain is weaker compared with the protective effect by wild type RNF146. Furthermore the protective effect by RNF146 without RING domain is likely to be dependent on the overexpressed level of RNF146 without RING domain that is far above endogenous level of RNF146 in cells. Further research to determine if the total AIF protein levels can be increased by RNF146 RNAi and decreased by overexpression of RNF146 will be useful.

**RNF146, a PAR-Binding Dependent PARsylation-Directed E3 Ubiquitin Ligase that Positively Regulates Wnt Signaling**

The Wnt signaling pathway is important to normal development and cancer. In the absence of Wnt ligand, the β-catenin binds with degradation complex including GSK-3β, CKI, APC and axin. In degradation complex, GSK-3β and CKI phosphorylate β-catenin. After being phosphorylated, the β-catenin can be recognized by β-Trcp and finally contributes to phosphorylation-directed ubiquitination of β-catenin by Skp1-Cul1-F-box (SCF) E3 ubiquitin complex and gets degraded. However in the presence of Wnt ligand, the binding of Wnt ligand to its receptor can disrupt the degradation complex and spares β-catenin from phosphorylation. The spared β-catenin then enters nucleus and activates Wnt-targeted genes.

As axin is the β-catenin receptor can disrupt the degradation complex and spares β-catenin in the presence of Wnt ligand, the binding of Wnt ligand to its receptor can disrupt the degradation complex and spares β-catenin from phosphorylation. The spared β-catenin then enters nucleus and activates Wnt-targeted genes.

Recent studies demonstrated that inhibition of tankyrase activity can stabilize axin and downregulate Wnt signaling. It was proposed that tankyrase could lead to PARsylation of axin and promote ubiquitination and degradation of axin in cells. RNF146 was confirmed to be the novel PARsylation-directed E3 ubiquitin ligase to induce ubiquitination of PAR modified axin and positively regulate Wnt signaling. It was found that tankyrases catalyzed transferring of PAR to residue of axin (PARslation). After PARsylation of axin, RNF146 binds with PARsylated axin via the PAR binding motif of RNF146. RNF146 then functions as a PARsylation-directed E3 ligase to recruit E2 ubiquitin-conjugating enzyme and contributes to ubiquitination of PARsylated axin. This leads to downregulation of axin level, dissociation of degradation complex for β-catenin, increased level of β-catenin and upregulation of Wnt signaling in cells. Thus RNF146 positively regulates Wnt signaling via its novel PARsylation-directed E3 ubiquitination and degradation of axin. The mechanisms for positive regulation of Wnt signaling via tankyrase-dependent PARsylation and ubiquitination of axin by RNF146 are summarized in Figure 3. RNF146 was also shown to promote PARsylation-directed ubiquitination and degradation of tankyrases. However the tankyrase can PARsylate RNF146, which subsequently ubiquitinates PARsylated RNF146, leading to RNF146 degradation.

So far proteins BLZF1 and CASC3 have also been shown to be substrates targeted by tankyrase and RNF146 for degradation. BLZF1 is required for normal Golgi structure and for protein transport from the endoplasmic reticulum (ER) through the Golgi apparatus to the cell surface. BLZF1 is upregulated during retinoid-induced maturation of NB4 promyelocytic leukemia. It has been suggested that BLZF1 might act as a transcription factor, or a coregulator, involved in either cell growth control and/or maturation. On the other hand, CASC3 protein plays a role in the stress response by participating in cytoplasmic stress granule assembly and by favoring cell recovery following stress. CASC3 has been implicated in breast cancer development and metastasis, as well as gastric cancers. Furthermore recent findings by Kang et al. demonstrated that RNF146 is a PARsylation dependent E3-ligase and it plays a role in DNA damage and repair. Therefore physiological functions of RNF146 concerning its PARsylation-directed ubiquitination of targeted PARsylated substrates might be involved in complicated cellular processes.

The identification of RNF146 as a PARsylation-directed E3 ligase establishes a novel molecular paradigm that links tankyrase-dependent PARsylation to ubiquitination and degradation of proteins. The mechanism of PARsylation-directed ubiquitination of degradation substrates is similar to that of phosphorylation-directed ubiquitination of substrates by SCF E3 ubiquitin complex. In SCF induced ubiquitination, the C-terminal of scaffolding protein Culin1 binds with ROC1 (small ring finger protein), while the N-terminal of Culin1 binds with SKP1 which further recruits F-box proteins (FBP) as the recognizing protein to bind with phosphorylated protein substrates. Then ROC1 recruits E2 and contributes to ubiquitination of phosphorylated substrates. However for RNF146 induced PARsylation-directed ubiquitination of axin, RNF146 directly recognizes PARsylated substrates via its PAR-binding motif in WWE domain. The RNF146 can further recruit E2 ubiquitin-conjugating enzyme via its ring finger domains, and contribute to ubiquitination of PARsylated substrates. Therefore the PARsylation-directed ubiquitination of substrates emerges as another post-translational modification markers directed ubiquitination of protein substrates, besides the phosphorylation-directed ubiquitination of substrates. The catalytic mechanisms of phosphorylation-directed ubiquitination of substrates and PARsylation-directed ubiquitination of substrates are summarized in Figure 4.

**RNF146, a PAR-Binding Dependent PARsylation-Directed E3 Ubiquitin Ligase in DNA Damage and Repair**

The role of PARP-1 catalyzed PARsylation, which is vital to DNA repair, has been extensively studied for decades and detailed mechanism of PARsylation related DNA repair has been summarized in several well-written review papers. In brief, after detection of DNA damage by PARP-1, the PARP-1 can be activated. The activated PARP-1 synthesizes PAR at the DNA damage site and transfers PAR to amino acid residues of various nuclear proteins including PARP-1 itself, PARP-2, XRCC-1, Aurora B, DNA pol β, Topo I, II, DNA pol α, H1,
Figure 3. Molecular mechanism for RNF146 induced positive regulation of Wnt signaling. In the absence of Wnt ligand, the β-catenin binds with degradation complex including GSK-3β, CK1, APC and axin. In the degradation complex, GSK-3β and CK1 phosphorylate β-catenin sequentially. After phosphorylation, the β-catenin can be degraded via phosphorylation-directed ubiquitination (A). However tankyrase (PARP-5) can PARsylate axin. After PARsylation of axin, RNF146 can bind with PARsylated axin via PAR-binding motif of RNF146. Then RNF146 function as PARsylation-directed E3 ubiquitin ligase and contribute to degradation of axin. This leads to downregulation of axin level, dissociation of degradation complex for β-catenin and increased β-catenin level. The β-catenin can enter nucleus and activate Wnt targeted genes (B).

Recent findings by Kang et al. demonstrated that RNF146 is a PARsylation dependent E3-ligase and it played a role in DNA damage and repair.5 In their report, they verified that RNF146 has PARsylation dependent E3 ligase activity.5 Furthermore they showed that RNF146 can translocate to nuclear after cellular injury and can bind to a number of nuclear proteins that are either PARsylated or bind with PAR.5 These RNF146 interacting nuclear proteins include PARP-1, PARP-2, nucleolin, DNA ligase III, Ku70, Ku86, XRCC1 and histones.5 These nuclear proteins are all essential to PARsylation dependent DNA repair.25-27 They also demonstrated that PAR binding is required for RNF146 ubiquitination and degradation of these nuclear proteins.5 Dependent on the PAR binding and ubiquitin E3 ligase activity of RNF146, RNF146 facilitates DNA repair and protects against cell death induced by the DNA damaging agent and promotes cell survival after γ-irradiation.5

Mortusewicz et al. reported that the PAR degrading enzyme PAR glycohydrolase (PARG) is recruited to DNA damage sites through PAR- and PCNA-dependent mechanisms.28 Previous studies have shown that PARG is required for efficient repair of single- and double-strand breaks and oxidized bases of DNA strands.29-31 It is known that exorbitant activation of PARP-1 can deplete cellular ATP and lead to cell death.25 Therefore to strictly control PAR signals in cells seems to be an indispensable process...
The Roles of RNF146 in Cancer Development and Neurological Disorders

Two recent findings suggest a potential link between RNF146 and cancer development.32,33 A genome-wide association study (GWAS) identified a risk locus at chromosome 6q22.33 for breast cancer.3 Two candidate genes in the 6q22.33 region include ECHDC1 and RNF146.32 The subsequent study on Jewish Ashkenazi breast cancer high-risk women demonstrated that an intron sequence variation was detected in 4/105 genotyped patients in the third intron of ECHDC1 gene.33 No other sequence variations in the genomic regions of RNF146 and ECHDC1 genes were found in any of studied participants.33 However recent findings by Campa et al. showed no association of one single-nucleotide polymorphisms (SNP) of RNF146 (rs2180341) with breast cancer risk based on studies of breast cancer samples from population not involving Ashkenazi Jews.34 The finding that RNF146 can positively regulate Wnt signaling via PARsylation dependent degradation of axin provides new supporting evidences for the potential roles of RNF146 in mammary cancer development.35,36 An association between Wnt signaling and breast cancer has been established by previous studies in references 35–38. Furthermore BLZF1 and CASC3, the two new candidate genes in the 6q22.33 region, were found in any of studied participants.35

The Potential Novel Functions of Other PAR-Binding Proteins

On the other hand, investigators have found that RNF146 is upregulated early in brains of Alzheimer disease (AD) patients, compared with aged controls.42 They used subtractive hybridization of transcripts from human frontal cortex, which degenerates in the late stage of AD, against transcripts of the inferior temporal cortex, which is influenced in the early stage of AD.42 Their findings suggest that RNF146 might function early in the progression of AD.42 However it is not clear whether the early increased expression of RNF146 in AD brain contributes to neurodegeneration of AD or just a protective response to neurons injury in early AD. This will be a research area worth exploring in the future.

In a unbiased proteomic screen for PAR-binding proteins, hundreds of PAR-binding proteins have been identified.43 These PAR-binding proteins are found to be involved in DNA damage signaling and DNA repair, modification of chromatin structure, regulation of transcription, replication, RNA metabolism, RNA splicing, protein synthesis, cell death and cell cycle and mitosis.43 It is possible that some of these PAR binding proteins might also have similar functions as RNF146 and might also act as inhibitors of Parthanatos or function as PARsylation-directed E3 ligases.

Cullin1 was found to have PAR binding activity.43 Furthermore overexpression of Cullin1 could promote cell proliferation.44 The increased Cullin1 has been associated with cancer development.
Therefore it was suggested that Cullin1 might have E3 ubiquitin-protein ligase activity with WWE domain and HECT domains.47 The DTX family proteins have also been verified to have E3 ubiquitin ligase activity with WWE and ring finger domains.48 The TRIP12 is a probable E3 ubiquitin ligase with WWE and HECT domains. Therefore it is highly possible that these proteins can function as PARylation-directed E3 ubiquitin ligases similar to RNF146. The molecular structures of DTX1, DTX2, DTX4, TRIP12 and HUWE1 with respective domains are illustrated in Figure 6.

Future Perspectives for Potential Applications of PAR-Related Strategies

The free PAR can induce AIF release from mitochondria and mediate Parthanatos of cells. However RNF146 can bind with PAR in cytosol and can abrogate PAR induced Parthanatos.4 Therefore, in principle, any agents that function along the pathway of PAR induced Parthanatos may have therapeutic potential. Agents that can disrupt nucleus translocation of AIF, interfere with the binding between PAR and AIF or directly bind with PAR as well as agents that can promote degradation of PAR or inhibit PAR production (PARP-1 inhibitors) might function as cellular protective agents against Parthanatos. Therefore screening for nature or synthetic compounds which could regulate PAR induced Parthanatos might present a promising therapeutic approach in the future. On the contrary, PAR mimics with longer lifespan or PARP-1 activators might become effective therapeutic agents for cancer therapy as they can activate Parthanatos and promote cell death. On the other hand, as binding of PAR with RNF146 is requested to activate RNF146 PARylation dependent E3 ligase activity, small-molecule PAR mimics that can activate RNF146 E3 ligase activity might be utilized as Wnt signaling activators for bone anabolism. Small-molecule PAR mimics that can bind with RNF146 but function to abrogate RNF146 E3 ligase activity might be applied to block Wnt-dependent tumor growth.

However some caveats need to be considered in the therapeutic application of PAR-related strategies. First, PAR-binding proteins have been implicated in cancer, thus this potential link needs to be fully investigated before they can be considered as therapeutic targets for other diseases. Second, the PAR-binding proteins have complicated and essential functions in cells, especially for repair of DNA, synthesis of DNA, DNA directed RNA synthesis, therefore the PAR-related strategies should also be proven to be safe from serious disturbance of these intracellular functions,
Table 1. The putative PAR-binding motif in RNF146, DTX-1, DTX2, DTX4, TRIP12 and HUWE1

| Sites | PAR-binding motif | Proteins |
|------|------------------|----------|
| 151 - 170 | MVQYRRNEHRRRKRKIDII | RNF146 |
| 81 - 100 | QFRDQTGMYPVRRNEDPS | DTX-1 |
| 161 - 180 | RGTRRRLRRRLWPLT | DTX-1 |
| 161 - 180 | RGTRRRLRRRLWPLT | DTX-1 |
| 161 - 180 | RGTRRRLRRRLWPLT | DTX-1 |
| 91 - 110 | TMRAVRRHLFPQHSSAPRGRGV | DTX-2 |
| 161 - 180 | QTNKTSFCCR-SVRRQAGPPY | DTX-2 |
| 71 - 80 | QSMNQFRDGTGLR-PYRRNY | DTX-4 |
| 141 - 160 | GQINQTOGRORVVRLDLI | DTX-4 |
| 141 - 160 | GQINQTOGRORVVRLDLI | DTX-4 |
| 811 - 830 | YSESKKDDAPAQLMKEDPEL | TRIP12 |
| 821 - 840 | AQLMKEDPELAKSFIKTLFG | TRIP12 |
| 151 - 170 | MVQYRRNEHRRRKRKIDII | RNF146 |
| 1641-1660 | AWKSGETSVRFTAGRRYTV | HUWE1 |

Consensus: 
\[
[HKR]_1- X_2- X_3- [AIQVY]_4- [KR]_5- [AILV]_6- [FILPV]_8
\]

The putative PAR-binding motif of these proteins is acquired based on the consensus sequence for PAR-binding motif: [HKR], \( X_2- \), [AIQVY], \( [KR]_5- \), [AILV], [FILPV]. More than one predicted PAR-binding motif according to the consensus sequence can be discerned in some proteins.

Figure 6. Molecular structures of DTX1, DTX2, DTX4, TRIP12 and HUWE1. The molecular structure of DTX1, DTX2, DTX4, TRIP12 and HUWE1 containing WWE domains, ring finger domains, HECT domain as well as other domains or motif is illustrated in different color.
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