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Tankyrase promotes primary precursor miRNA processing to precursor miRNA

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ABSTRACT

Tankyrases (TNKS and TNKS2) are members of poly(ADP-ribose) polymerase (PARP) family proteins. Tankyrase has multiple ankyrin repeat cluster (ARC) domains, which recognize the tankyrase-binding motifs in proteins including the telomeric protein, TRF1 and Wnt signal regulators, AXINs. However, the functional significance of tankyrase interaction with many other putative binding proteins remains unknown. Here, we found that several proteins involved in microRNA (miRNA) processing have putative tankyrase-binding motifs and their functions are regulated by tankyrase. First, chemical inhibition of tankyrase PARP activity downregulated the expression levels of precursor miRNAs (pre-miRNAs) but not primary precursor miRNAs (pri-miRNAs). A subsequent reporter assay revealed that tankyrase inhibitors or PARP-dead mutant tankyrase overexpression repress pri-miRNA processing to pre-miRNA. Conversely, a PARP-1/2 inhibitor, olaparib, did not affect pri-miRNA processing. Tankyrase ARCs bound to DGCR8 and DROSHA, which are essential components for pri-miRNA processing and have putative tankyrase-binding motifs. These observations indicate that tankyrase binds to Microprocessor, DGCR8 and DROSHA complex and modulates pri-miRNA processing to pre-miRNA.

1. Introduction

Tankyrases (tankyrase-1/TNKS and tankyrase-2/TNKS2, also known as PARP5a/ARTD5 and PARP5b/ARTD6) are members of the poly(ADP-ribose) polymerase (PARP) enzyme family, which have PARP catalytic domains and poly(ADP-ribosyl)ate various proteins [1,2]. Tankyrase-1/TNKS and tankyrase-2/TNKS2 are thought to be functionally redundant because their double knockout mice exhibit embryonic lethality but their single knockout mice do not [3]. Structurally, tankyrases have an ankyrin repeat domain, a sterile alpha motif (SAM) and a C-terminal catalytic PARP domain [4], and the ankyrin repeats form five conserved ankyrin repeat clusters (ARCs). Tankyrases bind to their target proteins through ARCs [5,6]. In those target proteins, tankyrase-binding motifs are the eight-amino acid consensus sequence: Rxx(small hydrophobic amino acids)G/P(D plus some other tolerated amino acids)G(no P)(preferable D/E, not preferable R/K) by fluorophore-conjugated peptide (FP) binding assay [7]. Tankyrase-binding proteins, including TRF1, AXIN, PTEN, AMOT, TNKS1BP1 and MERIT40, have been reported in the last two decades and it has become apparent that tankyrases are involved in telomere maintenance, promotion of Wnt/β-catenin signaling, proliferation of cancer cells, activation of Yes associated protein 1 (YAP1), cancer cell invasion and DNA damage response through the above-mentioned proteins [8–13].

Protein translation is finely regulated to control cell proliferation, differentiation and survival through microRNAs (miRNAs). MicroRNA genes are transcribed as primary precursor miRNAs (pri-miRNAs) by RNA polymerase II (Pol II) in the nucleus [14]. The pri-miRNAs are clopped to precursor miRNAs (pre-miRNAs) by Microprocessor, which comprises DROSHA, a double-stranded ribonuclease (RNase) III, and DiGeorge syndrome critical region 8 (DGCR8), a double-stranded RNA-binding protein [15,16]. The pre-miRNAs are transferred from the nucleus to the cytoplasm by exportin 5 (XPO5) and cleaved to mature miRNAs by DICER, an RNase III [17,18].

In the present study, we focused on the critical molecules that regulate miRNA processing in the list of proteins that have putative tankyrase-binding motifs [7]. As a potential linkage between

Abbreviations: ARC, ankyrin repeat cluster; FN, Flag-tagged with nuclear localization signal; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HE, PARP dead H1184A/E1291A mutant; PARP, poly(ADP-ribose) polymerase; pre-miRNA, precursor miRNA; primary precursor miRNA, pri-miRNA; TBP, TATA-box binding protein; TNKS, tankyrase; WT, wild-type.

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poly(ADP-ribosyl)ation and miRNA processing, stress granule-associated PARPs cause poly(ADP-ribosyl)ation of the AGO/miRNA complex and suppress the AGO/miRNA complex [19]. The stress granule associated PARPs contain tankyrase, which binds to AGO; however, whether tankyrase modulates the function of AGO remains unknown [19]. DICER binds to tankyrase, but its physiological significance is unknown [20]. In this study, we found that DGCR8 and DROSHA also have putative tankyrase-binding motifs, in addition to AGO and DICER, and addressed the actual binding of tankyrase with Microprocessor, DGCR8/DROSHA complex, and its functional significance in miRNA processing.

2. Materials and methods

2.1. Cell culture and chemical inhibitors

HeLa, HEK293T and A549 cells were maintained in DMEM high glucose medium (Nacalai Tesque, Kyoto, Japan) with 10% heat-inactivated fetal bovine serum (FBS). MKN1 and COLO-320DM cells were maintained in RPMI1640 medium (Nacalai Tesque) with 10% FBS. These cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). G007-LK and olaparib were obtained from Selleck (Houston, TX, USA). IWR-1 was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). RK-287107 was reported previously [21].

2.2. Plasmids

Flag epitope and nuclear localization signal (FN)-tankyrase 1-H1184A/E1291A (PARP-dead mutant)/pLPC (FN-TNKS-HE) was described previously [22]. LexA-ARC I to V on pLPC vectors were described previously [5]. Flag-DGCR8 and Flag-DROSHA were cloned from HEK293T and mouse MEF cDNA, respectively, and inserted into pcDNA3 (Invitrogen, Carlsbad, CA, USA).

2.3. Quantitative reverse transcription (qRT)-PCR

Total RNAs were prepared with miRNeasy Mini Kit (Qiagen, Hilden, Germany). Small RNAs including precursor and mature miRNAs were purified from the total RNAs using RNeasy MinElute Cleanup Kit (Qiagen) and the residual fraction contained large RNAs including pri-miRNAs. The cDNA of small RNAs was synthesized using miScript II RT Kit (Qiagen), and that of large RNAs using SuperScript III First-Strand (Thermo Fisher Scientific, Waltham, MA, USA). Expression levels were quantified using real-time PCR analysis using Hs_miR-17_2 miScript Primer Assay (Qiagen), Hs_miR-17_PR_1 miScript Precursor Assay (Qiagen), Hs_RNU6-2_11 miScript Primer Assay (Qiagen) and miScript SYBR Green PCR Kit (Qiagen) (Fig. 1A) and FastStart Universal SYBR Green Master (Roche, Indianapolis, IN, USA) with the LightCycler 480 Real-Time PCR System. The primer sequences used are described in the Supplementary Table.

2.4. Primary precursor miRNA (pri-miRNA) processing assay

The pmirGLO vector was obtained from Promega (Madison, WI, USA). To obtain the vectors for pri-miRNA processing assay, primary precursor let-7a1 or miR-30a sequences were inserted between Luc2 and the poly(A) signaling sequence. HeLa cells were transfected with indicated vectors and subjected to the pri-miRNA processing assay using the Dual-Glo Luciferase Assay System (Promega).

2.5. Western blot analysis and immunoprecipitation assay

Cells were harvested and the cell lysates were prepared with a lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet-40) containing 0.125 mM dithiothreitol and 2% (v/v) of a protease inhibitor cocktail (Nacalai Tesque). For immunoprecipitation, the cell lysates were prepared with a lysis buffer without dithiothreitol and after clearing with centrifugation, the supernatants were incubated with the indicated antibodies for 4 h and incubated with Protein G Sepharose 4 Fast Flow (GE Healthcare, Chicago, IL, USA) or Protein A Agarose beads (Cell Signaling Technology, Danvers, MA, USA) for another 1 h at 4 °C. The beads were washed five times with cell lysis buffer. Western blot analysis was performed as previously described [21]. The primary antibodies used were anti-Flag (M2; Sigma, St. Louis, MO, USA, 0.4 μg/mL), anti-tankyrase-1/2 (H350; Santa Cruz Biotechnology, 0.4 μg/mL), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (6C5; Fitzgerald, Acton, MA, USA, 0.33 μg/mL), anti-LexA (2–12; Santa Cruz Biotechnology, 0.2 μg/mL), anti-DROSHA (D28B1; Cell Signaling Technology, 1:1000) and anti-DGCR8 (ab90579; Abcam, Cambridge, UK, 0.5 μg/mL).

3. Results

3.1. Tankyrase promotes primary precursor miRNAs processing to precursor miRNAs in HeLa cells

According to Guettler et al., several miRNA-processing proteins, such as DROSHA, DGCR8, XPO5, AGO and DICER, have putative tankyrase-binding motifs [7]. To investigate the functional involvement of tankyrase in miRNA processing, we first quantitated the expression levels of primary precursor miR-17 (pri-miR-17), precursor miR-17 (pre-miR-17) and mature miR-17–5p, which is a major product of pre-miR-17, in HeLa cells treated with the tankyrase-specific PARP inhibitors G007-LK and IWR-1. Consequently, pri-miR-17 expression levels were not altered but those of pre-miR-17 and mature miR-17–5p decreased after treatment with tankyrase inhibitors (Fig. 1A). Next, we investigated whether the other pri-miRNAs also decreased. Upon treatment with tankyrase inhibitors, the levels of five arbitrarily selected pri-miRNAs were not affected, except for pri-let-7a1, which was downregulated by IWR-1 (Fig. 1B). Conversely, pre-miRNAs of miR-15a, miR-15b, miR-23a and let-7a1 were significantly decreased by tankyrase inhibitors, whereas pre-miR-21 was not decreased (Fig. 1B). To monitor the activity of pri-miRNA processing to pre-miRNA, we next inserted pri-let-7a1 or pri-miR-30a sequence between PGK promoter-driven Luc2 and poly(A) signaling sequence (Fig. 1C). In this reporter system, Luc2 expression increases when the pri-miRNA processing is suppressed (Fig. 1C, *bottom left panel*); however, it decreases when the pri-miRNA processing is promoted (Fig. 1C, *bottom right panel*). SV40 promoter-driven Rluc expression was also monitored as internal control, which is independent of the pri-miRNA processing. Consequently, relative Luc2 enzyme activities were marginally but statistically significantly elevated by the tankyrase inhibitors G007-LK and RK-287107 (Fig. 1D). Conversely, olaparib, a PARP1/2 inhibitor, did not affect Luc 2 activity. These observations indicate that tankyrase PARP activity, but not that of PARP1/2, is involved in pri-miRNA processing promotion.

3.2. Tankyrase PARP activity is necessary to promote pri-miRNA processing

To investigate whether tankyrase PARP activity is necessary to regulate pri-miRNA processing, we examined pri-miRNA and pre-miRNA expression levels in HeLa cells transiently transfected

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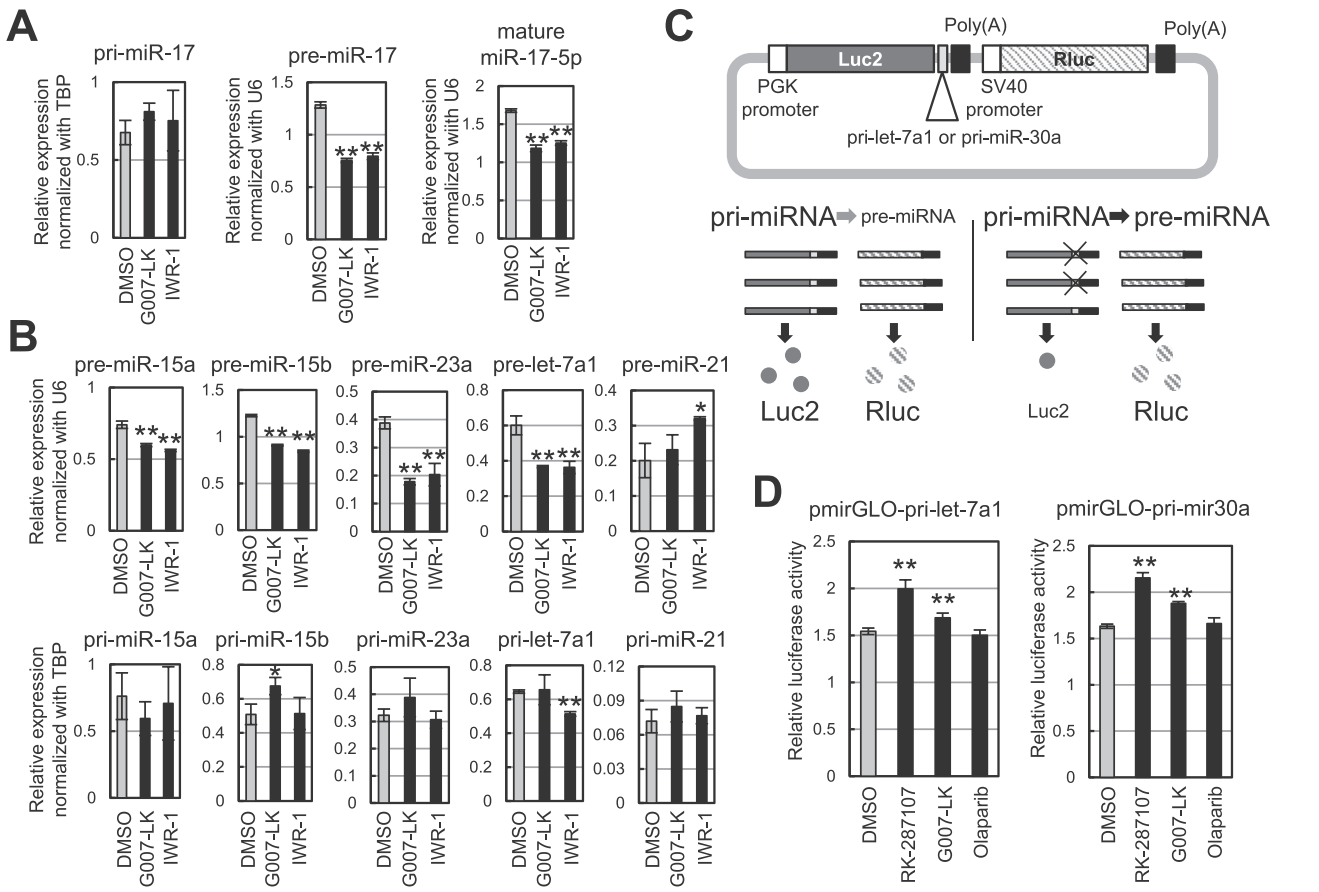

Fig. 1. Tankyrases promote pri-miRNA processing to pre-miRNA in HeLa cells. (A) The expression levels of primary precursor, precursor and mature forms of miR-17 were examined in HeLa cells after treatment with 5 μM G007-LK or 10 μM IWR-1 for 16 h. *TBP*: TATA-box binding protein used as an internal control for large RNAs. *U6*: U6 small nuclear RNA used as an internal control for small RNAs. (B) The expression levels of primary precursor and precursor forms of the indicated miRNAs were examined in HeLa cells after treatment with 5 μM G007-LK or 10 μM IWR-1 for 16 h. (C) Scheme of the pri-miRNA processing assay using the pmirGLO reporter system. (D) Pri-miRNA processing assay. HeLa cells were transfected with the indicated vectors before treatment with 1 μM of RK-287107, G007-LK or olaparib for 24 h. *Error bar* indicates standard deviation. $^{**}P < 0.01$, $^{*}P < 0.05$ by two-sided Student's paired *t*-test.

with Flag-tagged PARP-dead tankyrase with nuclear localization signal sequence at the N terminus (FN-TNKS-HE). Because endogenous tankyrases are expressed in both cytoplasm and nucleus and exogenous tankyrase preferentially localizes to the cytoplasm, nuclear localization signal tagging to the exogenous gene is necessary to enhance the tankyrase function in the nucleus [23]. While neither the expression levels of pri-miR-15b nor pri-let-7a1 were affected, the respective pre-miRNA levels were significantly decreased by FN-TNKS-HE overexpression (Fig. 2A and B). These observations suggest that FN-TNKS-HE exerts a dominant negative effect on pri-mRNA processing. Therefore, we next performed a pri-miRNA processing assay as in Fig. 1C using HeLa cells transiently transfected with the wild-type tankyrase (FN-TNKS-WT) or FN-TNKS-HE. Consequently, luciferase activity decreased when the wild-type but not PARP-dead tankyrase was overexpressed in the cells (Fig. 2C and D). These observations suggest that tankyrase promotes pri-miRNA processing in its PARP activity-dependent manner.

3.3. Tankyrase binds to DROSHA and DGCR8

In the Microprocessor complex, DGCR8 has an RNA-binding domain and binds to pri-miRNAs, whereas DROSHA, a ribonuclease, binds to the pri-miRNA-bound DGCR8 and processes the pri-miRNA to pre-miRNA in the nucleus [15,16]. Because both DGCR8 and DROSHA have tankyrase-binding consensus sequences (Fig. 3A) [7], we investigated whether tankyrase actually interacts with DROSHA and DGCR8. Tankyrase binds to its partner proteins through five ARCs [5,6]. Therefore, to first examine whether the tankyrase ARCs bind to DGCR8, we co-transfected HeLa cells with each of the LexA-tagged ARCs (LexA-ARC I to V) and Flag-tagged DGCR8 expression vectors. Immunoprecipitation followed by Western blot analysis revealed the co-immunoprecipitated LexA-ARC I, IV and V proteins with Flag-tagged DGCR8 (Fig. 3B). We could not confirm that ARC II and III interact with DGCR8 because LexA-ARC II and III bands were overlapped with the background signals of the IgG bands. We also detected co-immunoprecipitated LexA-ARCs I, IV and V with Flag-tagged DROSHA, although the ARC V binding to DROSHA was weaker than those of ARCs I and IV (Fig. 3C). Next, we examined whether full-length tankyrase binds to DGCR8 and DROSHA. HeLa cells were transfected with FN-TNKS-HE or mock vectors, and the cell lysates were subjected to immunoprecipitation with anti-Flag antibody. We detected co-immunoprecipitation of the endogenous DGCR8 and DROSHA with FN-TNKS-HE (Fig. 3D). It has been reported that some poly(-ADP-ribosyl)ated proteins by tankyrase are ubiquitinated and led to proteasomal degradation [20,24,25]. Thus, we examined the protein levels of endogenous DGCR8 and DROSHA in HeLa cells after treatment with G007-LK. As shown in Fig. 3E, G007-LK had no effect on DGCR8 and DROSHA protein levels (Fig. 3E). Furthermore,

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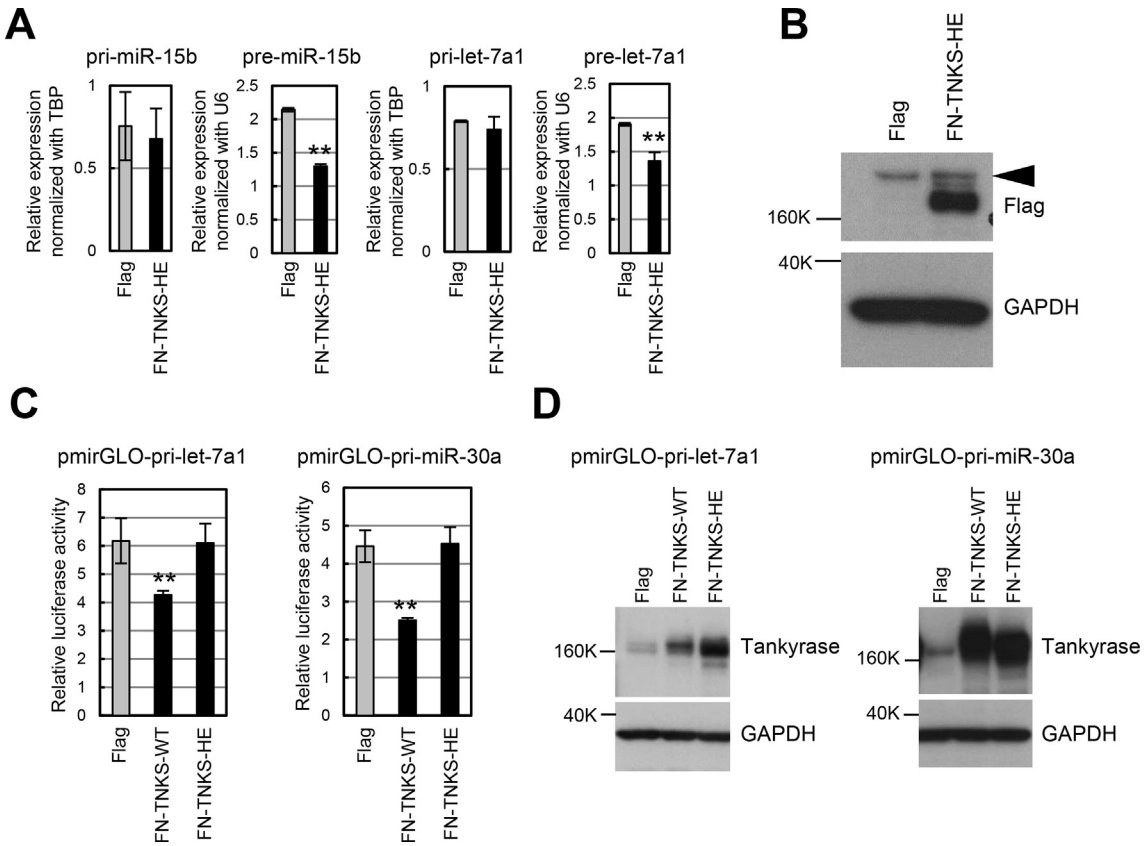

Fig. 2. Tankyrase PARP activity is necessary to promote pri-miRNA processing. (A) The expression levels of primary precursor and precursor forms of the indicated miRNAs were examined in HeLa cells under the exogenous expression of PARP-dead tankyrase. *FN-TNKS-HE*, Flag-tagged PARP-dead tankyrase with nuclear localization signal sequence. *TBP*: TATA-box binding protein used as an internal control for large RNAs. *U6*: U6 small nuclear RNA used as an internal control for small RNAs. (B) The protein expression levels detected by Western blot analysis in the same batch as in (A). *Arrowhead* indicates non-specific bands. (C) Pri-miRNA processing assay. HeLa cells were transfected with the indicated vectors. *FN-TNKS-WT*, Flag-tagged wild-type tankyrase with nuclear localization signal sequence. (D) The protein expression levels of the same batch as in (C). *Error bar* indicates standard deviation. $^{**}P < 0.01$ by two-sided Student's paired *t*-test.

G007-LK had no effect on DGCR8 binding to DROSHA (Fig. 3F). These observations indicate that tankyrase binds to DROSHA and DGCR8 but does not affect their protein levels or the complex formation.

3.4. Tankyrase promotes pri-miRNA processing to pre-miRNA in HEK293T and A549 cells

Because the cell-based experiments above were performed with HeLa cells, we further examined whether the tankyrase-mediated promotion of pri-miRNA processing is widely observed in other cell lines derived from different tissues, including colorectal cancer COLO-320DM, gastric cancer MKN1, embryonic kidney HEK293T and lung cancer A549. Tankyrase inhibitors significantly decreased pre-miRNAs but not pri-miRNAs in A549 cells and HEK293T (Fig. 4A and B). However, tankyrase inhibitors did not repress the pri-miRNA processing in MKN1 and COLO-320DM cells (Fig. 4C and D). These observations indicate that tankyrase is functionally involved in pri-miRNA processing in a cell context-dependent manner.

In addition to pri-miRNA cleavage, DGCR8 is involved in degradation of telomerase RNA component (TERC) in the nucleoli [26]. To investigate whether tankyrase is involved in TERC degradation, we examined TERC expression levels in tankyrase inhibitor-treated HeLa and A549 cells. Tankyrase inhibitors did not affect TREC expression, which indicates that tankyrase is not involved in TERC degradation (Fig. 4E).

4. Discussion

According to Guettler et al., many proteins involved in miRNA biogenesis have putative tankyrase-binding motifs, although the functional relevance has not been addressed [7]. While AGO and DICER, both of which are essential to produce mature miRNA in the cytoplasm, also have putative tankyrase-binding motifs, we found that tankyrase inhibitors decrease pre-miR-17 and mature miR-17−5p expression to comparable levels (Fig. 1A). These observations indicate that tankyrase inhibitors repress pri-miRNA processing (that is, production of pre-miRNAs from pri-miRNAs) but not pre-miRNA maturation (production of mature miRNAs from pre-miRNAs). Thus, we focused on the former event of miRNA-processing in the nuclei. We have optimized the concentrations of tankyrase inhibitors by Western blot analysis: because tankyrase auto-poly(ADP-ribosyl)ates and destabilizes itself, tankyrase stabilization (that is, accumulation) can be used as a pharmacodynamic biomarker of tankyrase inhibitors (Fig. 3F; data not shown) [8,13].

Tankyrase has five conserved subdomains, ARCs I to V [5,6]. Our results suggest that DGCR8 binds to ARC I more preferentially than ARC IV and V, whereas DROSHA binds to ARC I and ARC IV more preferentially than ARC V (Fig. 3B and C). Among these five ARCs, ARC V is the most important for the poly(ADP-ribosyl)ation of its binding protein, TRF1, and telomere elongation [5]. These observations suggest that tankyrase poly(ADP-ribosyl)ates DGCR8 and DROSHA less efficiently, if at all, compared with TRF1.

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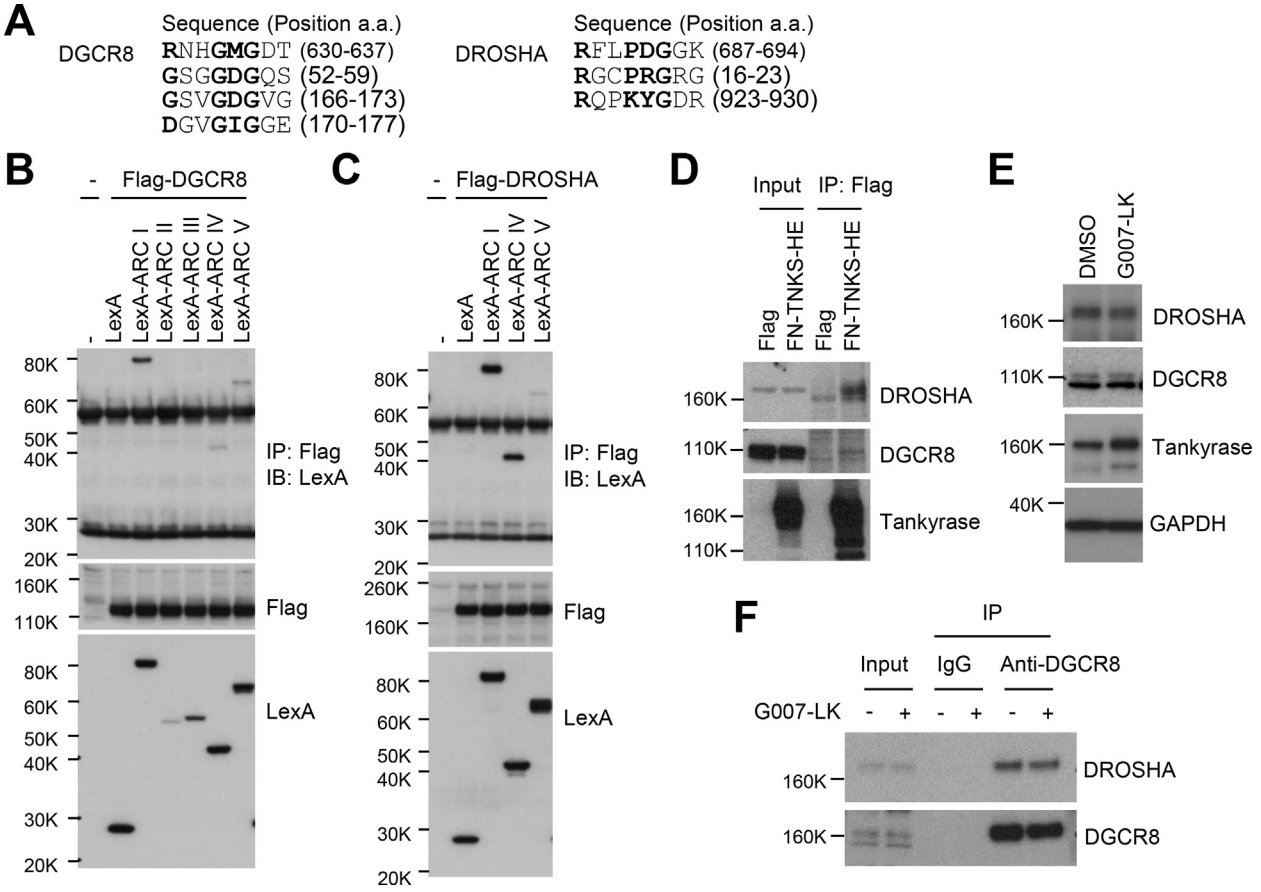

Fig. 3. Tankyrase binds to DROSHA and DGCR8. (A) Putative tankyrase-binding motif sequences in DGCR8 and DROSHA. *Number* indicates the position of amino acid residue. (B) Tankyrase ANK repeat cluster domains (ARCs) bind DGCR8. HeLa cells were transiently transfected with LexA-ARCs and Flag-DGCR8 expression vectors, and the cell lysates were immunoprecipitated as indicated. (C) ARCs bind DROSHA. HeLa cells were transiently transfected with LexA-ARCs and Flag-DROSHA expression vectors and the cell lysates were immunoprecipitated as indicated. (D) Exogenous tankyrase binds to endogenous DROSHA and DGCR8. HeLa cells were transiently transfected with FN-TNKS-HE expression vector, and the cell lysates were immunoprecipitated as indicated. Input: 2%. (E) DROSHA and DGCR8 protein levels in HeLa cells after treatment with 1 μM G007-LK for 6 h. The lower bands in the anti-DGRC8 blot are non-specific. (F) Effect of a tankyrase inhibitor on the interaction between endogenous DROSHA and DGCR8. HeLa cells were treated with 3 μM G007-LK for 4 h, and the cell lysates were subjected to immunoprecipitation experiments. IP: immunoprecipitation, IB: immunoblotting.

While we detected the binding of the ectopically expressed tankyrase with the endogenous DGCR8 and DROSHA (Fig. 3D), we failed to detect binding between the endogenous tankyrases and endogenous DGCR8 or DROSHA (our unpublished observations). This might be not only because of the potency of the antibodies used, but also because interaction between tankyrase and its binding partners is transient.

While our results indicate that tankyrase PARP activity is involved in pri-miRNA processing (Figs. 1 and 2), we did not detect poly(ADP-ribosyl)ated DGCR8 or DROSHA (our unpublished observations). Axin is a tankyrase target and its poly(ADP-ribosyl)ation by tankyrase leads to the E3 ubiquitin-protein ligase, RNF146-mediated ubiquitination and proteasomal degradation [25]. While Axin protein level is upregulated by tankyrase inhibitor-mediated stabilization [8], neither DGCR8 nor DROSHA protein was increased by tankyrase inhibitors (Fig. 3F), which suggests that DGCR8 and DROSHA are not subjected to tankyrase-mediated destabilization. Quantitative proteomic analysis has been reported using SW480 cells treated with XAV939, a tankyrase inhibitor [27]; however, neither DGCR8 nor DROSHA increased or decreased after XAV939 treatment. Intriguingly, it has been reported that DICER, which also has putative tankyrase-binding motifs, is accumulated in tankyrase-1 and -2 double knockout cells, although the physiological significance of this accumulation has not been addressed [20].

TRF1 is a negative regulator of telomere length maintenance. When poly(ADP-ribosyl)ated by tankyrase, TRF-1 is released from telomeres [4,23]. While this is a good example that tankyrase-mediated poly(ADP-ribosyl)ation causes dissociation of molecular complexes, tankyrase inhibitor had no effect on the interaction between DGCR8 and DROSHA (Fig. 3F). Instead, tankyrase inhibitors downregulated pri-miRNA processing to pre-miRNA, which suggests that tankyrase-mediated poly(ADP-ribosyl)ation of Microprocessor, the DGCR8/DROSHA complex, might alter the interaction between DGCR8 and pri-miRNA or the ribonuclease activity of DROSHA.

Our results showed that tankyrase inhibitors reduced pri-miRNA processing to pre-miRNA in HEK293T and A549 cells, but not in COLO-320DM and MKN1 cells. Among these cell lines, we and others reported that COLO-320DM cells are sensitive to the antiproliferative effect of tankyrase inhibitors [21,28,29]. The survival and growth of COLO-320DM cells depend on the Wnt/β-catenin signaling. Tankyrase inhibitors induce accumulation of Axin, which in turn downregulates Wnt/β-catenin signaling in COLO-320DM cells [29]. Wnt/β-catenin pathway is not activated at the steady state, that is, without exogenous Wnt ligands in HEK293T cells that have intact Wnt/β-catenin signaling components [21]. These observations suggest that Wnt/β-catenin signaling is not

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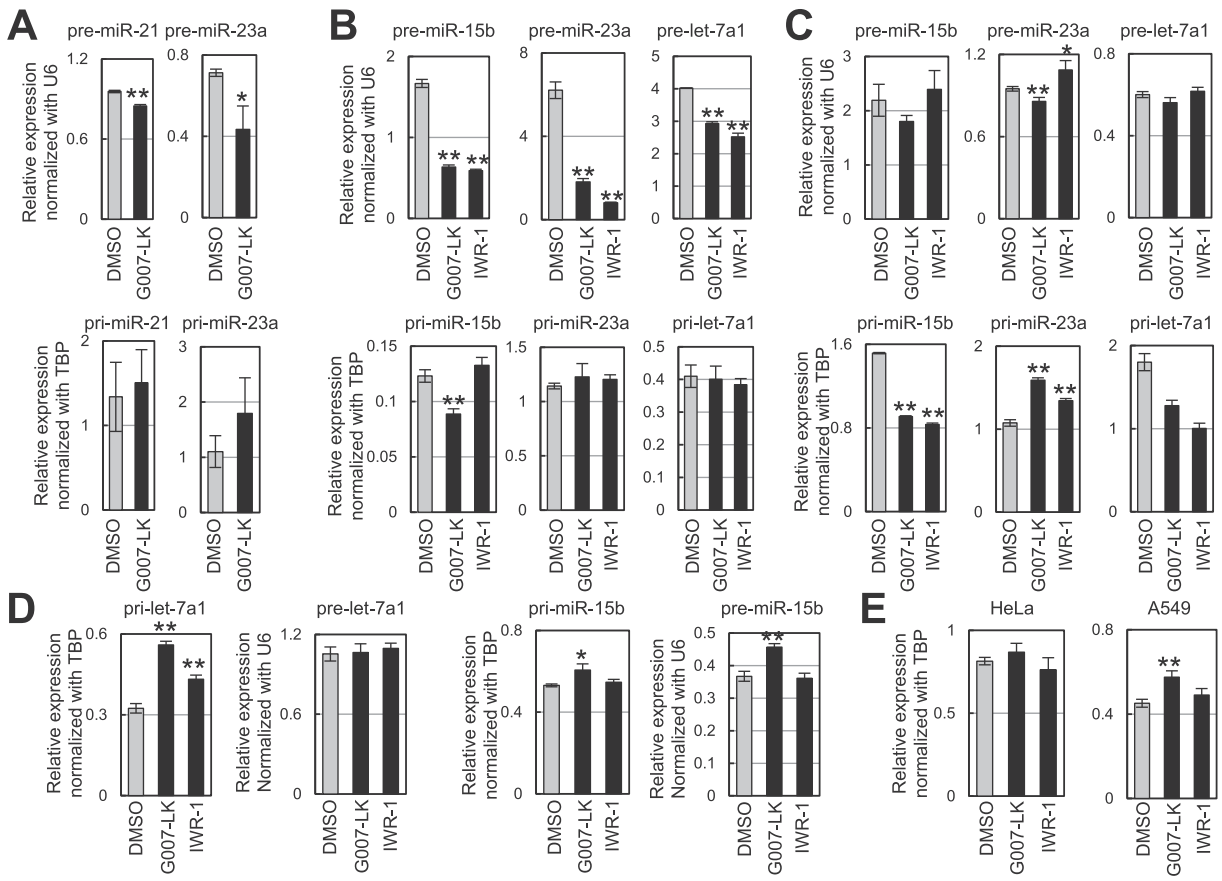

Fig. 4. Tankyrase promotes pri-miRNA processing to pre-miRNA in HEK293T and A549 cells. (A) Expression levels of primary precursor and precursor forms of the indicated miRNAs in HEK293T cells were examined after treatment with 1 μM G007-LK for 16 h. *TBP*: TATA-box binding protein used as an internal control for large RNAs. *U6*: U6 small nuclear RNA used as an internal control for small RNAs. (B) Expression levels of primary precursor and precursor forms of the indicated miRNAs in A549 cells were examined after treatment with 5 μM G007-LK or 10 μM IWR-1 for 16 h. (C) Expression levels of primary precursor and precursor forms of the indicated miRNAs in MKN1 cells were examined after treatment with 5 μM G007-LK or 10 μM IWR-1 for 16 h. (D) Expression levels of primary precursor and precursor forms of the indicated miRNAs in COLO-320DM cells were examined after treatment with 0.1 μM G007-LK or 4 μM IWR-1 for 16 h. Because of the high sensitivity of this cell line to tankyrase inhibitors, the drug concentrations used were lower than in cases of other cell lines. (E) Expression levels of TERC in HeLa and A549 cells after treatment with 5 μM G007-LK or 10 μM IWR-1 for 16 h. *Error bar* indicates standard deviation. $^{**}P < 0.01$, $^{*}P < 0.05$ by two-sided Student's paired *t*-test.

involved in modulation of pri-miRNA processing by tankyrase.

It has been reported that the stress granule contains several PARPs, including tankyrase-1, which interact with and poly(ADP-ribosyl)ate AGO family proteins and suppress AGO/miRNA complex upon stress in the cytoplasm [19]. Here we have demonstrated that tankyrase inhibitors decrease pri-miRNA processing and that tankyrase binds DGCR8 and DROSHA, which suggests that tankyrase modulates miRNA biogenesis in the nucleus. Our present findings will give a new insight into the molecular mechanisms for poly(ADP-ribosyl)ation-mediated regulation of miRNA biogenesis.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.11.191.

References

[1] S.J. Hsiao, S. Smith, Tankyrase function at telomeres, spindle poles, and beyond, Biochimie 90 (2008) 83–92.
[2] H. Seimiya, The telomeric PARP, tankyrases, as targets for cancer therapy, Br. J. Canc. 94 (2006) 341–345.
[3] Y.J. Chiang, S.J. Hsiao, D. Yver, et al., Tankyrase 1 and tankyrase 2 are essential but redundant for mouse embryonic development, PLoS One 3 (2008) e2639.
[4] S. Smith, I. Giriat, A. Schmitt, et al., Tankyrase, a poly(ADP-ribose) polymerase at human telomeres, Science 282 (1998) 1484–1487.
[5] H. Seimiya, Y. Muramatsu, S. Smith, et al., Functional subdomain in the ankyrin domain of tankyrase 1 required for poly(ADP-ribosyl)ation of TRF1 and telomere elongation, Mol. Cell. Biol. 24 (2004) 1944–1955.
[6] H. Seimiya, S. Smith, The telomeric poly(ADP-ribose) polymerase, tankyrase 1, contains multiple binding sites for telomeric repeat binding factor 1 (TRF1)

and a novel acceptor, 182-kDa tankyrase-binding protein (TAB182), J. Biol. Chem. 277 (2002) 14116–14126.
[7] S. Guettler, J. LaRose, E. Petsalaki, et al., Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease, Cell 147 (2011) 1340–1354.
[8] S.M. Huang, Y.M. Mishina, S. Liu, et al., Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling, Nature 461 (2009) 614–620.
[9] N. Li, Y. Zhang, X. Han, et al., Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth, Genes Dev. 29 (2015) 157–170.
[10] T. Ohishi, H. Yoshida, M. Katori, et al., Tankyrase-binding protein TNKS1BP1 regulates actin cytoskeleton rearrangement and cancer cell invasion, Cancer Res. 77 (2017) 2328–2338.
[11] H. Seimiya, Y. Muramatsu, T. Ohishi, et al., Tankyrase 1 as a target for telomere-directed molecular cancer therapeutics, Cancer Cell 7 (2005) 25–37.
[12] W. Wang, N. Li, X. Li, et al., Tankyrase inhibitors target YAP by stabilizing angiomotin family proteins, Cell Rep. 13 (2015) 524–532.
[13] K. Okamoto, T. Ohishi, M. Kuroiwa, et al., MERIT40-dependent recruitment of tankyrase to damaged DNA and its implication for cell sensitivity to DNA-damaging anticancer drugs, Oncotarget 9 (2018) 35844–35855.
[14] Y. Lee, M. Kim, J. Han, et al., MicroRNA genes are transcribed by RNA polymerase II, EMBO J. 23 (2004) 4051–4060.
[15] A.M. Denli, B.B. Tops, R.H. Plasterk, et al., Processing of primary microRNAs by the Microprocessor complex, Nature 432 (2004) 231–235.
[16] R.I. Gregory, K.P. Yan, G. Amuthan, et al., The Microprocessor complex mediates the genesis of microRNAs, Nature 432 (2004) 235–240.
[17] E. Lund, S. Guttinger, A. Calado, et al., Nuclear export of microRNA precursors, Science 303 (2004) 95–98.
[18] J.E. Park, I. Heo, Y. Tian, et al., Dicer recognizes the 5' end of RNA for efficient and accurate processing, Nature 475 (2011) 201–205.
[19] A.K. Leung, S. Vyas, J.E. Rood, et al., Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm, Mol. Cell 42 (2011) 489–499.
[20] A. Bhardwaj, Y. Yang, B. Ueberheide, et al., Whole proteome analysis of human tankyrase knockout cells reveals targets of tankyrase-mediated degradation, Nat. Commun. 8 (2017) 2214.
[21] A. Mizutani, Y. Yashiroda, Y. Muramatsu, et al., RK-287107, a potent and specific tankyrase inhibitor, blocks colorectal cancer cell growth in a preclinical model, Cancer Sci. 109 (2018) 4003–4014.
[22] T. Ohishi, T. Hirota, T. Tsuruo, et al., TRF1 mediates mitotic abnormalities induced by Aurora-A overexpression, Cancer Res. 70 (2010) 2041–2052.
[23] S. Smith, T. de Lange, Tankyrase promotes telomere elongation in human cells, Curr. Biol. 10 (2000) 1299–1302.
[24] W. Chang, J.N. Dynek, S. Smith, TRF1 is degraded by ubiquitin-mediated proteolysis after release from telomeres, Genes Dev. 17 (2003) 1328–1333.
[25] Y. Zhang, S. Liu, C. Mickanin, et al., RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling, Nat. Cell Biol. 13 (2011) 623–629.
[26] S. Macias, R.A. Cordiner, P. Gautier, et al., DGCR8 acts as an adaptor for the exosome complex to degrade double-stranded structured RNAs, Mol. Cell 60 (2015) 873–885.
[27] Y.E. Kim, H.J. Jeon, D. Kim, et al., Quantitative proteomic analysis of 2D and 3D cultured colorectal cancer cells: profiling of tankyrase inhibitor XAV939-induced proteome, Sci. Rep. 8 (2018) 13255.
[28] T. Lau, E. Chan, M. Callow, et al., A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth, Cancer Res. 73 (2013) 3132–3144.
[29] N. Tanaka, T. Mashima, A. Mizutani, et al., APC mutations as a potential biomarker for sensitivity to tankyrase inhibitors in colorectal cancer, Mol. Cancer Ther. 16 (2017) 752–762.

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