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Tankyrase-1 assembly to large protein complexes blocks its telomeric function

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ABSTRACT

Tankyrase-1 poly(ADP-ribosyl)ates the telomere-binding protein TRF1. This post-translational modification dissociates TRF1 from telomeres, enhancing telomerase-mediated telomere elongation. Tankyrase-1 multimerizes via its sterile alpha motif domain, but its functional implication remains elusive. Here, we found that excessive amounts of tankyrase-1 form punctate nuclear foci. This focus formation depends on both homophilic multimerization and heterophilic protein–protein interaction. These foci are functionally dormant because they do not efficiently release TRF1 from telomeres. Consistently, hyper-overexpression of tankyrase-1 attenuates its ability to elongate telomeres. These observations suggest that tankyrase-1 assembly to large protein complexes masks its telomeric function.

Structured summary:
MINT-7987689, MINT-7987677: *Tankyrase-1* (uniprotkb:O95271) and *TRF1* (uniprotkb:P54274) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)
MINT-7987977: *Tankyrase-1* (uniprotkb:O95271) *physically interacts* (MI:0915) with *TRF1* (uniprotkb:P54274) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7987998: *Tankyrase-1* (uniprotkb:O95271) *physically interacts* (MI:0915) with *Tankyrase-1* (uniprotkb:O95271) by *anti tag coimmunoprecipitation* (MI:0007).

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1. Introduction

Tankyrase-1 poly(ADP-ribosyl)ates (PARsylates) a telomeric repeat-binding factor 1 (TRF1) [1]. PARsylated TRF1 dissociates from telomeres, resulting in an "open" state of telomeres. Thus, tankyrase-1 overexpression in human cells enhances telomere access of telomerase, which in turn elongates telomeres [2]. Since unusual maintenance of telomere length against the DNA end replication problem is an essential factor for cancer cell immortality, tankyrase-1 has been postulated as a target for cancer therapy [3,4].

Tankyrase-1 has five domains called ankyrin repeat cluster (ARC) I to V (see Fig. 3A), each of which works as an independent TRF1-binding unit [5,6]. Among them, ARC V is essential for TRF1 PARsylation [6]. Meanwhile, tankyrase-1 has a sterile alpha motif (SAM) domain, which contributes to protein multimerization [7,8]. Actually, tankyrase-1 forms large protein complexes (>2000 kDa) via the SAM domain both in vitro and in intact cells. In the cytoplasm, tankyrase-1 multimerization seems to be required for further assembly of punctate protein foci and larger vesicles, which presumably affect the apical secretion pathway [8]. However, the functional significance of tankyrase-1 multimerization in telomere length regulation is unknown. Here, we screened compounds that induce tankyrase-1 multimerization (i.e., nuclear focus formation), and found that hyper-overexpression of exogenous tankyrase-1 by histone deacetylase (HDAC) inhibitors leads to its focus formation in SAM- and ARC-dependent manners. Importantly, these foci were functionally inactive. This study demonstrates a novel regulation mechanism of tankyrase-1, which is distinct from modulation of the poly(ADP-ribose) polymerase (PARP) catalytic activity.

2. Materials and methods

2.1. Plasmids

The FLAG-nuclear localization signal (NLS)–tagged tankyrase-1 (FN-tankyrase-1) has been described [2]. Because more than half

Abbreviations: 3AB, 3-aminobenzamide; ARC, ankyrin repeat cluster; FN, FLAG tag and nuclear localization signal; HDAC, histone deacetylase; PARP, poly(ADP-ribose) polymerase; PARsylation, poly(ADP-ribosyl)ation; SAM, sterile alpha motif; TRF1, telomeric repeat-binding factor 1; TSA, trichostatin A; VPA, valproic acid

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of endogenous tankyrase-1 resides outside the nucleus, NLS tagging to the exogenous gene allowed us to enhance the tankyrase-1 function exclusively in the nucleus [2]. FN-tank-ΔARC III-V and FN-tank-PARP-dead were constructed as described [6,9]. FN-tank-ΔSAM was generated by PCR with pLPC/FN-tankyrase-1 (Fig. 3A).

2.2. Fluorescence microscopy

Immunofluorescence staining was performed as described [10]. In brief, cells were transfected with each vector. After incubation for 20 h, cells were fixed with 2% paraformaldehyde and permeabilized with 0.5% Nonidet P-40. Cells were stained with anti-FLAG (M2) and anti-TRF1 (5747) [6]. For immuno-FISH, the permeabilized cells were hybridized with Cy3-PNA $(CCCTAA)_3$ [3] and stained with anti-FLAG.

2.3. Western blot analysis

Western blot analysis was performed as described [5] with anti-tankyrase-1 (H-350, Santa Cruz Biotechnology, Santa Cruz, CA), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (10R-G109a), or anti-Myc (A-4, Santa Cruz Biotechnology).

2.4. Co-immunoprecipitation

TNE lysates [5] were incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich, St. Louis, MO) for 1 h. After washing with the PARP reaction buffer [1], the bead-bound proteins were subjected to western blot analysis.

2.5. Southern blot analysis

FN-tankyrase-1-overexpressing HTC75 cells (HTC75/FN-tankyrase-1) [6] were treated with 125 nM trichostatin A (TSA) for up to 130 PD. Telomeric restriction fragments (TRF), which correspond to telomeric and subtelomeric DNAs, were detected by Southern blot analysis [6].

3. Results

3.1. HDAC inhibitors induce non-telomeric foci of exogenous tankyrase-1

Tankyrase-1 overexpression in the nuclei of HeLa I.2.11 cells eliminates the nuclear TRF1 dots, reflecting dissociation of TRF1 from telomeres and degradation of the protein (Fig. 1A, *top and middle*) [2,11]. In these cells, the exogenous tankyrase-1 exhibited flat nucleoplasmic distribution (Fig. 1A, *middle*). Treatment with 3-aminobenzamide (3AB, a PARP inhibitor) caused tankyrase-1 foci by tethering tankyrase-1 to the TRF1 dots (Fig. 1A, *bottom*) [3]. Thus, tankyrase-1 inhibition can be visualized by its focus formation.

To examine the mechanism for tankyrase-1 inhibition in intact cells, we screened the Screening Committee of Anticancer Drugs (SCADS) inhibitor kit, consisting of 95 function-defined inhibitors [12], for a compound that induces the tankyrase-1 foci. We found that HDAC inhibitors, Scriptaid and TSA, induce the foci (Fig. 1B, *Exp. 1*). This focus formation was also observed upon treatment with other structurally related (CHAHA) and unrelated [apicidin, valproic acid (VPA), MS-275, HC-toxin] HDAC inhibitors (Fig. 1B, *Exp. 2*; Supplementary Fig. 1), indicating that this effect was derived from HDAC inhibition. While these inhibitors blocked the TRF1 release from telomeres, the tankyrase-1 foci did not colocalize with TRF1 or telomeres (Fig. 1C and 2). These results suggest that HDAC inhibitors block the tankyrase-1 transgene in a different way than PARP inhibitors.

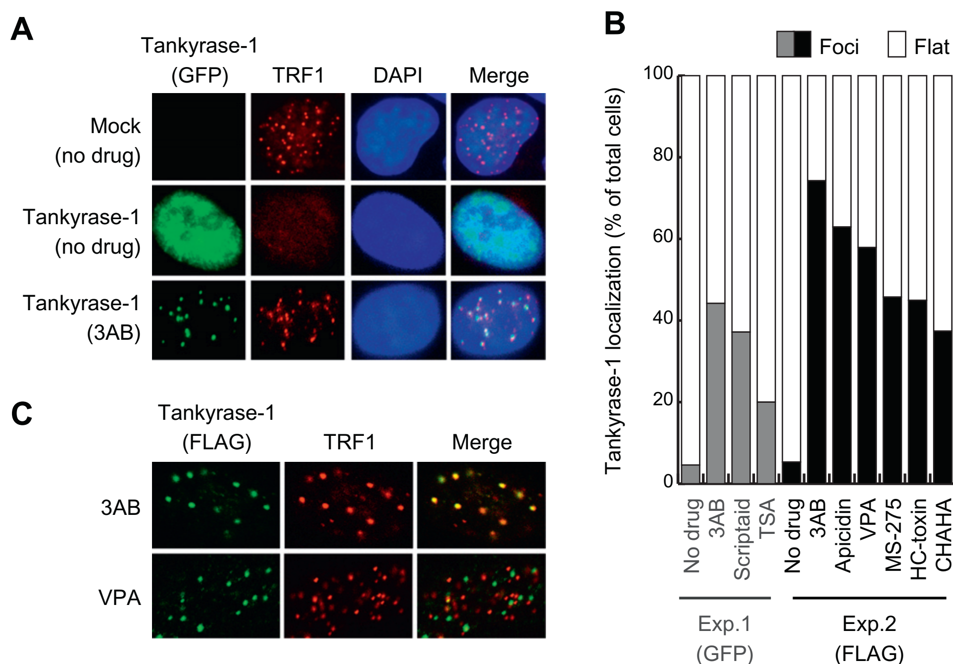

Fig. 1. Focus formation of exogenous tankyrase-1 by HDAC inhibitors. (A) Tankyrase-1–mediated dissociation of TRF1 from telomeres. HeLa I.2.11 cells were transfected with enhanced green fluorescence protein (EGFP)–FN-tankyrase-1 and incubated with or without PARP inhibitor 3AB (3 mM) for 18 h. Active tankyrase-1 exhibited flat nucleoplasmic distribution with the loss of telomeric TRF1 dots whereas 3AB induced telomeric co-localization of tankyrase-1 with TRF1. (B) Tankyrase-1 focus formation by HDAC inhibitors. Cells were transfected with EGFP–FN-tankyrase-1 (*Exp. 1*) or FN-tankyrase-1 (*Exp. 2*) and incubated with 3 mM 3AB, 1 μM Scriptaid, 100 nM TSA, 5 μM apicidin, 5 mM VPA, 5 μM MS-275, 0.5 μM HC-toxin, or 0.5 μM CHAHA for 18 h. These transgenes were expressed under the CMV promoter. More than 80 transfected cells were analyzed for each compound. (C) Confocal microscopy confirmed that tankyrase-1 foci by 3AB were telomeric whereas those by VPA were non-telomeric.

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