CellPress OPEN ACCESS

Trends in Molecular Medicine

Review

PARsylation-mediated ubiquitylation: lessons from rare hereditary disease Cherubism

Yoshinori Matsumoto [1,2,*] and Robert Rottapel [1,3,4,5,6,*]

Modification of proteins by ADP-ribose (PARsylation) is catalyzed by the poly (ADP-ribose) polymerase (PARP) family of enzymes exemplified by PARP1, which controls chromatin organization and DNA repair. Additionally, PARsylation induces ubiquitylation and proteasomal degradation of its substrates because PARsylation creates a recognition site for E3-ubiquitin ligase. The steady-state levels of the adaptor protein SH3-domain binding protein 2 (3BP2) is negatively regulated by tankyrase (PARP5), which coordinates ubiquitylation of 3BP2 by the E3-ligase ring finger protein 146 (RNF146). 3BP2 missense mutations uncouple 3BP2 from tankyrase-mediated negative regulation and cause Cherubism, an autosomal dominant autoinflammatory disorder associated with craniofacial dysmorphia. In this review, we summarize the diverse biological processes, including bone dynamics, metabolism, and Toll-like receptor (TLR) signaling controlled by tankyrase-mediated PARsylation of 3BP2, and highlight the therapeutic potential of this pathway.

Highlights

Tankyrase (PARP5)-mediated PARsylation is a post-translational protein modification that creates a recognition site for the E3-ubiquitin ligase ring finger protein 146 (RNF146), leading to ubiquitylation and proteasomal degradation of its substrates, such as the adaptor protein SH3-domain binding protein 2 (3BP2).

Missense mutations in *SH3BP2*, which are associated with the autosomal dominant disorder Cherubism, are clustered in the binding motif for tankyrase and uncouple 3BP2 from tankyrase-mediated negative regulation. Stabilization of 3BP2 due to Cherubism mutations results in the activation of SRC and SYK kinases in osteoclasts, leading to craniofacial dysmorphic features in patients with Cherubism.

Other tankyrase substrates, including the tumor suppressor genes *AXIN*, *AMOT*, *PTEN*, and *LKB1*, suggest that tankyrase inhibitors have potential therapeutic benefit in selected tumors. However, recent *in vivo* studies show that mice lacking tankyrase in the myeloid-monocytic lineage develop severe systemic inflammation, demonstrating potential toxicities associated with tankyrase inhibition.

PARsylation-mediated ubiquitylation: updated research history

PARsylation is a reversible post-translational protein modification in which covalently linked PAR polymers are added to lysine, aspartic acid, and glutamic acid residues of substrates. PARsylation is hydrolyzed by PAR hydrolase or PAR glycohydrolase (PARG) [1–3]. The **PARP** family (see Glossary) has 18 members, which share a conserved PARP catalytic domain [4–7]. PARP1 and PARP2 are required components of the non-homologous end joining (NHEJ) repair pathway, recruiting DNA repair proteins to sites of single-stranded breaks. Inhibition of PARP1 manifests synthetic lethality with tumors that are deficient in homologous recombination [4–6].

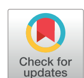
PARP5a (**tankyrase 1**; TNKS1) and its paralog **PARP5b** (**tankyrase 2**; TNKS2) trigger proteasomal-mediated degradation of protein substrates whereby PARsylation creates a recognition site for the recruitment of the E3-ubiquitin ligase **RNF146**, leading to ubiquitylation and proteasomal degradation of target proteins. This has been best exemplified by tankyrase-mediated degradation of the adaptor protein **3BP2** [8,9] and β-catenin inhibitor AXIN [10]. It has been further shown that mutations in 3BP2 specifically uncouple tankyrase binding and modification of 3BP2 by PARsylation, revealed as the pathogenic mechanism underlying **Cherubism**, a rare hereditary syndrome characterized by severe craniofacial developmental defects in children [8,11]. In this review, we summarize the roles of the tankyrase-3BP2 pathway in controlling bone homeostasis, metabolism, and immune activation.

3BP2 is required for ABL kinase activation and osteoblast differentiation

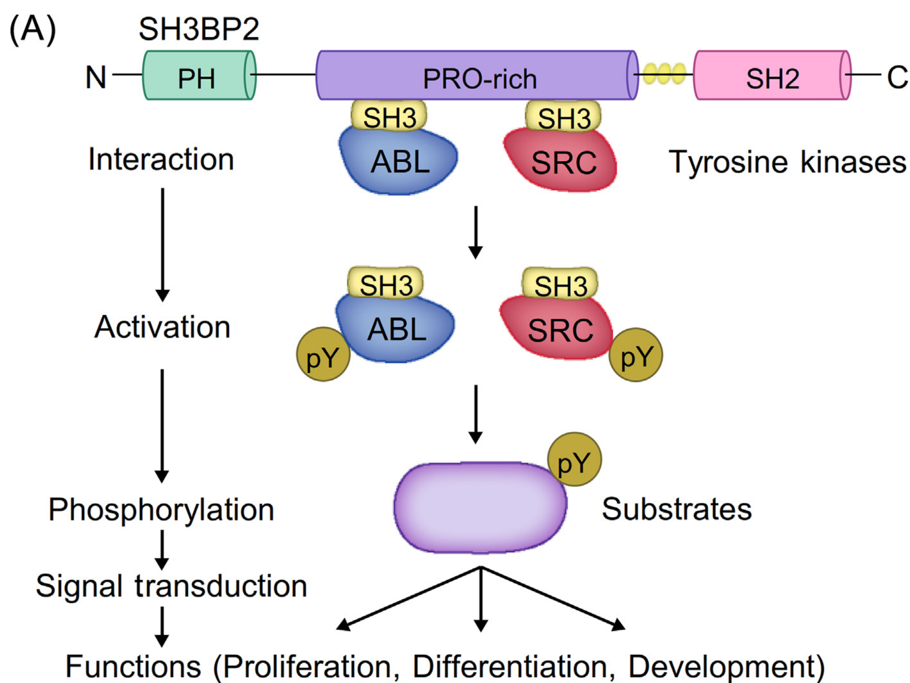
The adapter protein 3BP2 was originally identified as an Abelson murine leukemia viral oncogene homolog 1 (ABL) kinase SH3 domain-binding protein [12]. 3BP2 contains an N-terminal phospholipid-binding pleckstrin homology (PH) domain, a central proline-rich (PR) SH3 binding region, and a C-terminal binding SRC-homology 2 (SH2) domain [13] (Figure 1A). 3BP2 nucleates a

[1]Princess Margaret Cancer Center, University Health Network, University of Toronto, Toronto, ON M5G 1L7, Canada
[2]Department of Nephrology, Rheumatology, Endocrinology and Metabolism, Okayama University Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama 700-8558, Japan
[3]Department of Medicine, University of Toronto, Toronto, ON M5S 1A8, Canada
[4]Department of Medical Biophysics, University of Toronto, Toronto, ON M5S 1A8, Canada
[5]Department of Immunology, University of Toronto, Toronto, ON M5S 1A8, Canada

© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).



CellPress
OPEN ACCESS



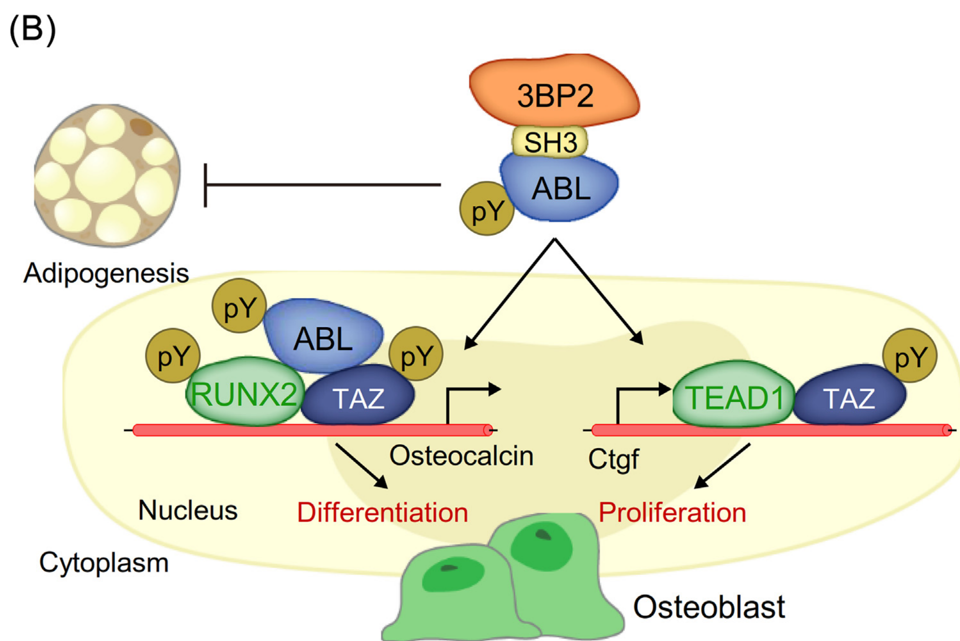


Figure 1. Structure and general functions of SH3-domain binding protein 2 (3BP2). (A) Schematic of the structure of 3BP2, which contains an N-terminal phospholipid-binding pleckstrin homology (PH) domain, a central proline-rich (PR) region, and a C-terminal phosphotyrosine-binding SRC-homology 2 (SH2) domain. 3BP2 binds to the SH3 domain of

(Figure legend continued at the bottom of the next page.)

[6]Division of Rheumatology, St. Michael's Hospital, Toronto, ON M5B 1W8, Canada

*Correspondence:
ymatsumoto@okayama-u.ac.jp
(Y. Matsumoto) and
Robert.rottapel@uhnresearch.ca
(R. Rottapel).

signaling complex including ABL, SRC, VAV, PLCγ, and SYK. Engagement of the ABL and SRC SH3 domains by the PR region of 3BP2 enforces an open active configuration of these kinases [14]. *Sh3bp2*$^{-/-}$ *mice were generated* to further elucidate the normal function of 3BP2 in controlling bone homeostasis. 3BP2 is an ABL-binding protein, as revealed by knockout studies showing that 3BP2 is essential for normal osteoblast differentiation; thus, the skeletal system in *Sh3bp2*$^{-/-}$ mice was found to be osteopenic. These data suggest that 3BP2 and ABL are on a common genetic pathway regulating osteoblast function [15]. 3BP2 is required for ABL kinase activation in primary calvarial osteoblast progenitors in *in vitro* culture and re-expression of constitutively active ABL rescued defective osteoblastogenesis observed in *Sh3bp2*$^{-/-}$ cells. To elucidate the function of ABL in osteoblasts, it was shown that ABL is required for the assembly and activation of the RUNX2-TAZ osteoblast lineage master transcription factor complex and for the formation of the TAZ-TEAD complex required for osteoblast expansion, while active ABL suppresses adipogenesis [16] (Figure 1B).

3BP2 is required for SRC activation and osteoclastogenesis

Observations of the ability of macrophages derived from 3BP2-knockout mice showed that they failed to form normal osteoclasts when cultured in the presence of RANK ligand (RANKL) and M-CSF. Moreover, macrophages lacking 3BP2 were unable to activate SRC in response to osteopontin or integrin signaling [15]. Thus, 3BP2 is necessary for macrophage fusion required for multinucleated osteoclast differentiation and the formation of the resorption lacunae needed for bone resorption.

Single missense mutations in *SH3BP2* cause Cherubism

Cherubism, an autosomal dominant disorder, was originally reported by Jones *et al.* in 1950 [11] and named to describe the 'heavenly gaze' of affected children, reminiscent of the cherubs depicted in Raphael's Sistine Madonna (1512). Patients with Cherubism experience formation of destructive boney cystic lesions of the mandibular, zygomatic maxillary bones, and bones of quadrangular pyramidal cavern of the eye socket (Figure 2A). Failure of teeth to erupt or only partially erupt and displaced tooth eruption are due to cystic bone lesions. Teeth roots do not find hold in the bone lesions and/or are resorbed; thus, patients frequently have severe periodontal disease. Of note, bone destruction observed in patients with Cherubism occurs only in craniofacial bones, and this clinical manifestation generally progresses until puberty, followed by spontaneous partial regression [11]. Histological analysis of the boney cysts observed in patients with Cherubism shows the presence of multinucleated osteoclast-like giant cells, influx of inflammatory cells, and fibrosis. The diagnosis of Cherubism is made based on the presence of clinical manifestations and radiographic and histological findings.

The genetic analysis of patients with Cherubism identified single missense mutations in *SH3BP2* on chromosome 4p16.3 [17–19]. Most Cherubism mutations have been reported to affect one of three amino acids within the hexapeptide sequence (RSPPDG) between the PH and SH2 domains in exon 9 of *SH3BP2* [19] (Figure 2A). Genetically engineered mice carrying a single *Sh3pb2* mutant allele found in patients with Cherubism (P416R, equivalent to P418R in humans) show phenotypic similarity to the human syndrome [20]. Homozygous Cherubism knock-in mice

nonreceptor tyrosine kinases, including Abelson murine leukemia viral oncogene homolog 1 (ABL) and SRC through its PR motif and enforces an open active configuration of these proteins, leading to kinase activation and subsequent phosphorylation and functional modification of their substrates. (B) Schematic showing that 3BP2-mediated ABL kinase activation potentiates the assembly and activation of the RUNX2-TAZ master transcription factor complex required for osteoblastogenesis, and the formation of the TAZ-TEAD complex required for osteoblast expansion. By contrast, 3BP2-mediated ABL activation suppresses adipogenesis.

Glossary

Cherubism: rare hereditary syndrome associated with severe craniofacial developmental defects in children. Cherubism arises from missense mutations in *SH3BP2*, which stabilize the steady-state protein levels of the adapter protein 3BP2.

Poly(ADP-ribose) polymerase (PARP): superfamily that catalyzes the addition of ADP-ribose (PARsylation) from NAD+ to its substrates, leading to modification of their function and metabolism. PARP1 inhibitors have been developed for the treatment of breast and ovarian cancers carrying mutations in *BRCA1/2*. To date, out of 18 enzymes, PARP1, 2, 5a, and 5b similarly modify their substrates with PAR polymers.

Ring finger protein 146 (RNF146): RING-domain E3-ubiquitin ligase that contains a RING domain and WWE domain, through which it recognizes iso-ADP-ribose in PARsylated proteins, leading to conformation changes in, and activation of, the RING domain. This allosteric switch increases the enzymatic activity of RNF146 and catalyzes the formation of a K48-linked polyubiquitin chain, which leads to degradation of its substrates by the 26S proteasome.

SH3-domain binding protein 2 (3BP2): adaptor protein, originally identified as an Abelson murine leukemia viral oncogene homolog 1 (ABL) kinase SH3 domain-binding protein; essential for activation of substrates including ABL, SRC, VAV, and SYK.

Tankyrase 1/2 (TNKS1/TNKS2; PARP5a/PARP5b): members of the PARP family, which catalyze the addition of ADP-ribose from NAD+ to its substrates. TNKS1 was originally identified as a repressor of the telomerase inhibitor TRF1/TERF1 and TNKS2 as a binding partner to Grb14. Double knockout of *Tnks1* and *Tnks2* in mice is embryonic lethal, whereas knockout of either *Tnks1* or *Tnks2* results in only mild phenotypes, demonstrating that tankyrases share some functional redundancy. Tankyrases share an 85% amino acid identity and contain N-terminal five ankyrin repeat clusters (ARC1–5), a sterile-alpha motif (SAM) domain mediating tankyrase oligomerization, and a C-terminal PARP catalytic domain.

Toll-like receptors (TLRs): recognize pathogen-associated molecular patterns (PAMPs) derived from various

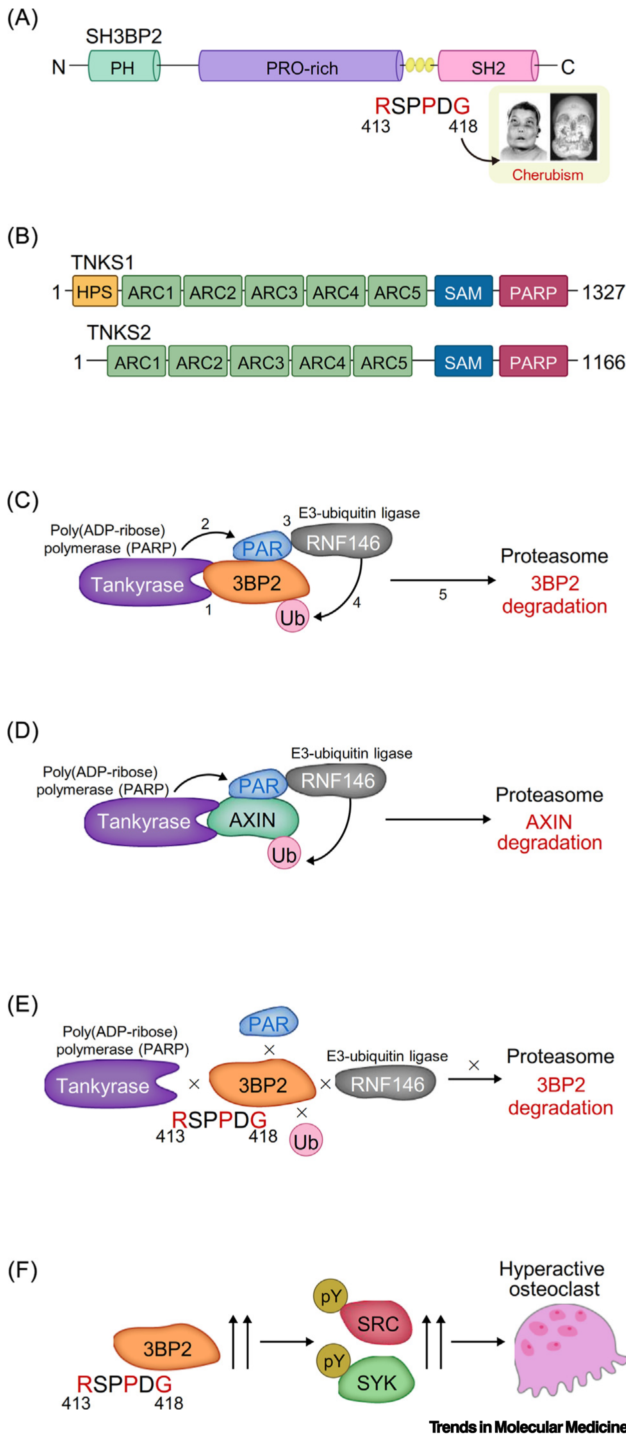


Figure 2. Mechanism of the SH3-domain binding protein 2 (3BP2) degradation pathway and Cherubism. (A) Single missense mutations in *SH3BP2* cause Cherubism. Mutations are generally located within a hexapeptide upstream of the SH2 domain. (B) Domain organization of human tankyrase (TNKS)-1 and TNKS2. Tankyrases comprise the C-terminal catalytic poly(ADP-ribose) polymerase, the sterile-alpha motif (SAM) domain, and the ankyrin domain divided into five ankyrin domain clusters (ARC1–5), which mediate the recognition of, and binding to, substrates. TNKS1 also contains an HPS region with unknown function. (C) Schematic showing that tankyrases bind to (1) and PARsylate (2) 3BP2, which creates a recognition site for E3-ubiquitin ligase ring finger protein 146 (RNF146) (3), leading to 3BP2 ubiquitylation (4) and subsequent proteasomal degradation (5). (D) Both 3BP2 and AXIN are similarly ubiquitylated and degraded in a tankyrase-mediated PARsylation-dependent manner. (E) 3BP2 missense Cherubism mutations within the tankyrase-binding site disrupt the interaction of 3BP2 with tankyrase, leading to the accumulation of 3BP2. (F) Accumulated 3BP2 leads to hyperactivation of SRC, SYK, and VAV in macrophages, which results in hyperosteoclastogenesis through activation of the tyrosine kinase signaling pathway. Images in (A) reproduced from [19].

microbes and nucleic acids. Activated TLRs interact with the adaptor protein myeloid differentiation factor 88 (MyD88), which results in phosphorylation of IL1 receptor-associated kinase 1 (IRAK-1) and subsequent phosphorylation and proteasomal degradation of inhibitor κBα (IκBα). NF-κB, which is sequestered in the cytoplasm by IκBα, then translocates to the nucleus and binds to the promoter region of the target genes associated with inflammation. Dysregulated TLR signaling enhances the severity of septic shock and inflammatory disorders, including rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease.

carrying two alleles of P416R have systemic inflammation with elevated levels of TNFα and osteopenia due to highly active osteoclasts [20], demonstrating that 3BP2 functions as a checkpoint for the production of inflammatory cytokines and osteoclast activity.

Tankyrase and RNF146 regulate the steady-state expression of 3BP2

To clarify the molecular mechanism by which 3BP2 mutations cause Cherubism, a yeast two-hybrid system with full-length 3BP2 was used as bait to identify 3BP2-binding proteins from a hematopoietic cell library; a paralog of TNKS1, TNKS2, was identified as a potential 3BP2-binding partner [8]. TNKS1 and TNKS2 belong to the PARP family, the 18 members of which share a conserved PARP catalytic domain [4–7]. Tankyrase uses NAD+ as a substrate and releases nicotinamide following the transfer of ADP-ribose onto substrate proteins. TNKS1 was originally identified as a repressor of the telomerase inhibitor TRF1/TERF1 through ubiquitylation-dependent degradation [21,22], involved in telomere elongation [23] and control of mitosis [24]. In mice, single knockout of either *Tnks1* or *Tnks2* shows mild phenotypes [25–29], while double knockout of *Tnks1* and *Tnks2* is embryonic lethal, demonstrating that tankyrases are functionally redundant. Tankyrases share 85% amino acid identity [30] and contain N-terminal five ankyrin repeat clusters (ARC1–5) [9,31], a sterile-alpha motif (SAM) domain mediating tankyrase oligomerization [32] and a C-terminal PARP catalytic domain [21,33,34] (Figure 2B). Structural analysis using NMR spectroscopy revealed that the catalytic activity of TNKS1 and TNKS2 is controlled by the formation of extended homo- and heterofilamentous structures [35]. ARC domains 1, 2, 4, and 5 share strong structural similarity and bind to tankyrase substrates through an optimal recognition sequence, REAGDGEE [9]. The hexapeptide sequence RSPPDG in the interdomain region of 3BP2 binds to tankyrase ARC4 an affinity of 5 μM [9]. Recruitment of 3BP2 to tankyrase enables its PARsylation, which triggers ubiquitylation by the E3-ligase RNF146 and its subsequent proteasomal-mediated degradation (Figure 2C). RNF146 contains a WWE domain, which recognizes iso-ADP-ribose and alleviates repression of the catalytic activity of the RING domain [36,37]. Y156 and R157 in the WWE domain are required for PAR binding [38]. Activation of the RNF146 RING domain triggers the formation of K48-linked polyubiquitin chains on substrate proteins [37,39]. Thus, RNF146 is a PAR-regulated E3-ligase [39]. It contains several tankyrase-binding motifs and is itself a tankyrase substrate, as well as being PARsylated [40]. Similarly, tankyrase is a substrate of RNF146 and is ubiquitylated. Tankyrase and RNF146 form a large degradation complex that, when activated, controls the expression of both proteins and engulfs tankyrase substrates, such as 3BP2 or AXIN, in a proteasomal degradation pathway [8,10] (Figure 2D).

Cherubism mutations uncouple 3BP2 from the negative regulation of tankyrase

Cherubism mutations disrupt binding to the preformed substrate recognition site on the surface of the tankyrase ARC domains, resulting in the loss of 3BP2 PARsylation and proteasomal degradation (Figure 2E). Thus, Cherubism mutant 3BP2 leads to increased steady-state expression, which potently activates the 3BP2 signaling module containing SRC and ABL kinases, and associated enzymes VAV and SYK [8,9]. The accumulation of 3BP2 protein and hyperactivation of SRC, SYK, and VAV in macrophages drives accelerated osteoclastogenesis, leading to bone loss *in vivo* and the excessive production of inflammatory cytokines (Figure 2F). Thus, Cherubism mutations result in a hypermorphic gain-of-function alteration in 3BP2 and explain the autosomal dominant mode of inheritance of the syndrome.

RANKL signaling regulates RNF146 expression during osteoclastogenesis

The 3BP2 E3-ubiquitin ligase, RNF146, has a role in controlling osteoclast formation. Studies of osteoclastogenesis in genetically engineered mice lacking RNF146 due to LysM-Cre deletion in the myeloid monocytic lineage showed that these mice had osteopenia resulting from accelerated osteoclastogenesis [41]. *In vitro* studies showed that RNF146 is a critical switch controlling SRC activation and β-catenin signaling during osteoclastogenesis. RANKL activates NF-κB, and NF-κB directly represses the transcription of RNF146, leading to stabilization of 3BP2 and subsequent activation of SRC and SYK required for osteoclast differentiation

CellPress
OPEN ACCESS

(Figure 3A). In addition, repression of RNF146 by RANKL stabilizes AXIN1 [41], thereby inhibiting the osteoclast suppressive effect of β-catenin signaling [42–44] (Figure 3B).

RNF146 is required for osteoblast differentiation, osteocalcin secretion, and β-islet development

To determine the role of RNF146 selectively in the osteoblast lineage, RNF146 was deleted using Osterix-Cre recombinase. Mice with RNF146-deleted osteoblasts display a phenotype similar to cleidocranial dysplasia (CCD) [45,46], an autosomal dominant disorder characterized by a calvarial closure defect and short stature mainly due to impairment of intramembranous bone formation by osteoblasts [47]. Loss of RNF146 in calvarial osteoblasts resulted in stabilization

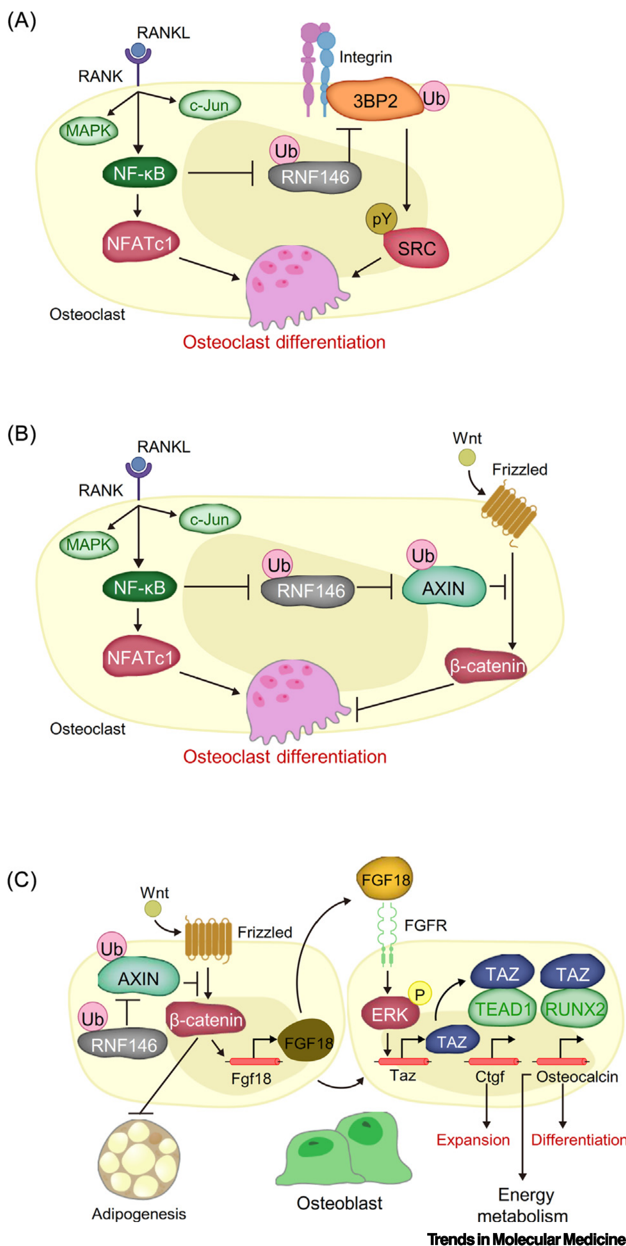


Figure 3. PARsylation-mediated ubiquitylation of SH3-domain binding protein 2 (3BP2) and AXIN by ring finger protein 146 (RNF146) controls bone and energy metabolism. (A) Schematic showing that RANK ligand (RANKL) integrates multiple pathways controlling osteoclastogenesis through repression of RNF146. In macrophages, RANKL transcriptionally represses RNF146 through activation of NF-κB, leading to stabilization of 3BP2 and subsequent activation of SRC-mediated osteoclastogenesis. (B) In addition to stabilization of 3BP2 (A), repression of RNF146 by RANKL leads to stabilization of AXIN, which triggers osteoclastogenesis through inhibition of β-catenin. (C) Schematic showing that RNF146 controls bone, glucose, and lipid metabolism through regulation of the Wnt/β-catenin and Hippo pathways. In osteoblasts, loss of RNF146 causes stabilization of AXIN1, a negative regulator of the Wnt/β-catenin pathway, which results in suppression of β-catenin-induced *Fgf18* expression. Reduction of *Fgf18* expression fails to induce ERK-mediated *TAZ* expression, which results in disruption of the formation of TAZ with RUNX2 and TEAD, leading to defective osteoblast differentiation and expansion, respectively. Additionally, mice with RNF146-deficient osteoblasts show fatty bone marrow and are glucose intolerant due to enhanced adipogenesis and defective insulin secretion as a consequence of reduced osteoblast-mediated osteocalcin production.

of AXIN1 and suppression of β-catenin signaling. Diminished β-catenin signaling in RNF146-deficient osteoblasts resulted in defective *Fgf18* expression, a growth factor required for osteoblast formation (Figure 3C). Loss of *Fgf18* expression in turn led to failure to induce expression of TAZ, which forms a transcriptional complex with RUNX2 and TEAD1, required for osteoblast differentiation and expansion, respectively [47] (Figure 3C). Mutations in RUNX2 cause CCD [48–53], demonstrating that RNF146 and RUNX2 share a common biochemical and genetic pathway.

Deletion of RNF146 in osteoblasts resulted in perturbations of mesenchymal cell lineage determination, leading to reduced osteoblast formation while favoring enhanced adipogenesis in the bone marrow. Moreover, failure of osteoblast formation resulted in reduced secretion of the osteoblast factor osteocalcin, which is involved in pancreatic β-islet cell differentiation. Deletion of RNF146 in the osteoblast lineage not only manifested profound defects in skeletal formation, but also led to defective insulin secretion secondary due to β-islet atresia [54], resulting in a glucose-intolerant state (Figure 3C).

Recent studies reported that SOX9, a master transcription factor controlling chondrogenesis [55–57], is regulated by tankyrase-mediated PARsylation [58]. Tankyrase inhibitors ameliorated osteoarthritis in mice, and transplantation of tankyrase-depleted mesenchymal stem cells resulted in regeneration of cartilage damage [58], suggesting the tankyrase-SOX9 axis as a therapeutic target for the treatment of osteoarthritis.

The tankyrase-3BP2 pathway regulates TLR signaling

Mice carrying two alleles of the Cherubism mutations develop lethal autoinflammation with activated myeloid cell infiltration into the skin and visceral organs associated with high serum levels of inflammatory cytokines [8,20]. **TLRs** [59–61] on macrophages derived from Cherubism mice secrete high levels of TNFα and IL6 in response to their ligands [8,20,62,63], suggesting that the 3BP2 signaling module regulates TLR signaling. Mice lacking both Tnks1 and Tnks2 in the myeloid monocytic lineage develop severe multiorgan autoinflammation and succumb to inflammatory bowel disease [64]. Macrophages derived from these tankyrase-deficient mice produce high levels of TNFα and IL6 cytokines in response to TLR2 ligands. 3BP2-mediated activation of SRC and SYK leads to phosphorylation of a regulatory tyrosine (tyrosine 647) in the TIR domain of TLR2, which potentiates its signaling and activation of NF-κB (Figure 4), explaining the hyperactivity of TLR2 signaling in the absence of tankyrase [64]. These data suggest that inflammation in the gut in tankyrase-deficient mice is due to dysregulated TLR signaling in response to commensal bacteria.

3BP2 is required for neutrophil activation

Neutrophils derived from 3BP2-deficient mice fail to polarize their actin cytoskeleton or migrate in response to a gradient of the chemotactic peptide, fMLF, and fail to adhere, crawl, and emigrate out of the vasculature in response to fMLF superfusion [65]. 3BP2 is required for optimal activation of SRC family kinases, small GTPase Rac2, and neutrophil superoxide anion production, and for *Listeria monocytogenes* clearance *in vivo*. The functional defects observed in 3BP2-deficient neutrophils may partially be explained by the failure to fully activate the VAV1 guanine nucleotide exchange factor (GEF) and properly localize the P-Rex1 GEF at the leading edge of migrating cells [65]. Thus, 3BP2 is required for G protein-coupled receptor-mediated neutrophil function [65,66].

The roles of 3BP2 in B cell and T cell functions

The 3BP2 SH2 domain binds to the tyrosine phosphorylated cytoplasmic tail of the B cell co-stimulatory molecule CD19 [67,68], suggesting that B cell co-stimulation through CD19 is mediated, in part, by the 3BP2 signaling module [69]. 3BP2-deficient mice exhibit increased splenic

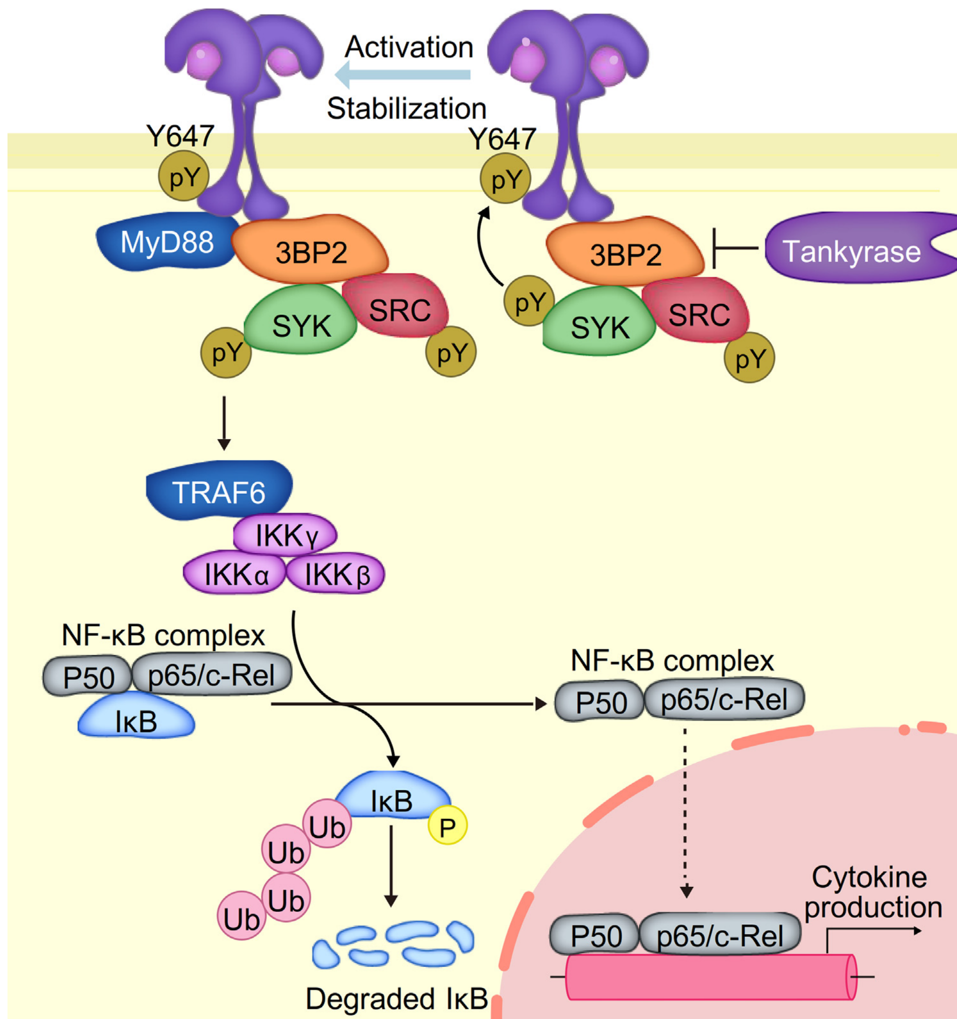


Figure 4. PARsylation-mediated ubiquitylation of SH3-domain binding protein 2 (3BP2) controls the innate immune system. Schematic showing that tankyrase controls cytokine production and inflammation. In macrophages, 3BP2-mediated activation of SRC and SYK leads to phosphorylation of a regulatory tyrosine (tyrosine 647) in the TIR domain of Toll-like receptor (TLR)-2, which activates the downstream signaling pathway through phosphorylation of inhibitor κBα (IκBα), an inhibitory component of NF-κB, leading to its proteasomal degradation, release of active NF-κB to the nucleus, and induction of cytokine production.

marginal-zone B cells [70] and reduction of peritoneal $CD5^+$ B1 B cells and a thymus-independent type 2 (TI-2) antigen response [69,71,72]. 3BP2-deleted B cells demonstrate defective proliferation and cell survival following cross-linking of the B cell receptor (BCR) as a consequence of impaired SYK phosphorylation [69]. B cells lacking 3BP2 showed enhanced BCR-induced death via a caspase 3-dependent apoptotic pathway compared with wild-type cells [69]. Shukla *et al*. found that engagement of BCR induces 3BP2 phosphorylation. They identified that tyrosine 183 in 3BP2 is a major site of SYK phosphorylation, which creates a docking site for VAV1 and PLC-γ2 required for the activation of NFAT in B cells [74].

3BP2 is a component of the T cell receptor (TCR) signaling complex and binds to LCK, ZAP-70, PLCγ1, LAT, and Grb2 in *in vitro* biochemical studies [73]. Therefore, the 3BP2 signaling complex

recapitulates many signaling components proximal to the TCR. 3BP2 is expressed at a low level in resting T cells and its expression is induced following TCR and CD28 co-stimulation [75]. Successful antiviral responses require the sustained activation and expansion of CD8+ T cells for periods that far exceed the time limit of physical T cell interactions with antigen-presenting cells (APCs). The expanding CD8+ T cell pool generates the effector and memory cell populations that provide viral clearance and long-term immunity, respectively. 3BP2 is recruited to cytoplasmic microclusters and nucleates a signaling complex that facilitates MHC:peptide-independent activation of signaling pathways downstream of the TCR [75]. Induction of the adaptor molecule 3BP2 sends a second wave of TCR-like signals [75] that is critical for sustaining CD8+ T cell proliferation and regulating effector and memory differentiation [76–79].

Tankyrase controls the expression of multiple tumor suppressor proteins

Ninety percent of human colorectal tumors are driven by the active Wnt/β-catenin signaling pathway caused by the mutations in the oncogene *CTNNB1*, the tumor suppressor genes encoding adenomatous polyposis coli (*APC*) or AXIN. β-catenin is negatively regulated by a destruction complex that includes AXIN [80], glycogen synthase kinase 3β (GSK-3β), APC, and the E3-ubiquitin ligase β-TrCP [80]. AXIN1 and AXIN2 contain two tankyrase-binding motifs that mediate a bivalent interaction with the ARC domains of tankyrase [83,84]. AXIN can be PARsylated by tankyrase, which triggers ubiquitylation by RNF146 and proteasome-mediated degradation (Figure 2D). Tankyrase-mediated suppression of AXIN expression leads to activation of β-catenin signaling [10,85]. Tankyrase inhibitors were developed to restore AXIN expression as a strategy to suppress the Wnt signaling pathways in colon cancer.

Tankyrase regulates the expression levels of other tumor suppressors, including PTEN, angiomotin (AMOT), and LKB1. PTEN contains a single canonical tankyrase-binding site at the N-terminal region and is PARsylated by tankyrase. PTEN contains three lysine residues, Lys342, Lys344, and Lys349, which are ubiquitylated by RNF146 [86]. Knockdown of tankyrase in HCT116 and RKO cells impaired cell proliferation and xenograft tumor formation, and antagonized AKT phosphorylation. Tankyrases are upregulated and are negatively correlated with PTEN status in colon tumors [86].

Tankyrase promotes tumorigenesis through activation of the Hippo pathway oncoprotein Yes-associated protein (YAP) [87–89], which is associated with the epithelial–mesenchymal transition (EMT) and activation of the transcription factor TEAD, leading to cell proliferation and invasion [90–92]. Inhibition of tankyrase-mediated ubiquitylation stabilizes AMOT family proteins [93–95], which interact with, and sequester, YAP in the cytoplasm, leading to cytoplasmic sequestration of YAP and a reduction in its activity and tumorigenesis [96].

The LKB1/AMPK pathway has an important role in tumor suppression, and its inhibition is associated with several human cancers. AMPK activation by LKB1 is tightly regulated by tankyrase-mediated PARsylation and subsequent K63- but not K48-linked ubiquitination by RNF146. The expression levels of tankyrase negatively correlate with phosphorylated-AMPK levels and poor survival in patients with lung cancer. LKB1 activation by tankyrase inhibitors induces AMPK activation and suppresses tumorigenesis [97].

Breast tumor associated gene 1 (BRCA1) regulates homologous recombination, while PARP1 controls NHEJ DNA repair pathways. Both proteins recruit accessory proteins that participate in repair of DNA at damaged sites [98,99]. PARP1 inhibitors have shown clinical utility for the treatment of breast and ovarian cancers in tumors carrying mutations in *BRCA1/2* [4,100–102]. PARP1 is a tankyrase substrate that is degraded following RNF146-mediated ubiquitylation

[37]. Tankyrase thereby controls PAPR1 protein expression levels necessary for the fine-tuning of the DNA repair process. Chemotherapy-induced DNA damage leads to increased PARP1 expression and PARP1-mediated PARsylation of a tumor suppressor protein bromodomain-containing protein 7 (BRD7), which increases cancer cell resistance to DNA-damaging agents by the repression of BRD7 expression following RNF146-medaited ubiquitylation. One of the functions of the PARP1/2 inhibitor olaparib is to restore the expression of BRD7 and sensitivity of BRD7-positive cancer cells to chemotherapeutic drugs [103].

In view of these studies, PARP-mediated ubiquitylation promotes tumorigenesis through regulation of the different substrates and, therefore, PARP-specific inhibitors have been investigated as a potential therapeutic target for cancer therapy [81,104].

Tankyrase represses the 3BP2 signaling pathway required for mouse models of rheumatoid arthritis, lupus, and hepatic steatosis

In vivo analysis of two rheumatoid arthritis (RA) mouse models using either the human TNFα-transgenic (hTNF-Tg) or collagen-induced arthritis (CIA) has shown the association between 3BP2 expression and development of RA. Bone erosion observed in hTNF-Tg mice was ameliorated in *Sh3bp2*$^{-/-}$hTNF-Tg mice, and 3BP2 deficiency suppressed induction of arthritis through reduction of autoantibody production in the CIA model [105]. By contrast, heterozygous Cherubism mutant mice showed exacerbation of bone loss with increased osteoclast formation in the hTNF-Tg arthritis or CIA mouse model [106,107], demonstrating that 3BP2 expression controls osteoclast activity and the production of inflammatory cytokines that could contribute to the pathogenesis of RA.

Systemic lupus erythematosus (SLE) is a polygenic systemic autoimmune disease characterized by the production of autoantibodies and immune complexes that lead to tissue inflammation and damage. MRL/MpJ-*Fas*$^{lpr/lpr}$ mice carry a loss-of-function mutation in the death receptor *Fas/CD95* in the MRL/MpJ background and are a classic mouse model of systemic autoimmunity and autoinflammation seen in lymphoproliferative monogenic disorder. These mice exhibit early mortality, develop anti-double-stranded DNA (dsDNA) antibodies, and have nephritis, splenomegaly, and lymphadenopathy. The role of 3BP2 was examined by intercrossing 3BP2-deficient mice with C57BL/6J (B6) mice carrying *Fas*$^{lpr/lpr}$ [108]. 3BP2 deficiency significantly improved the splenomegaly and glomeruloproliferative changes observed in the B6.*Fas*$^{lpr/lpr}$ mice and reduced serum anti-dsDNA antibody levels. B cell lineage-specific deletion of 3BP2 in the B6.*Fas*$^{lpr/lpr}$ background did not improve the lupus-like phenotype or the production of autoantibodies. Importantly, 3BP2 deficiency reduced the number of dendritic cells in the spleen. These data suggest that the lupus-like phenotype observed in B6.*Fas*$^{lpr/lpr}$ mice is contingent on the role of 3BP2.

Hepatic steatosis is a common disorder of liver injury associated with metabolic syndrome and insulin resistance and can predispose patients to hepatocellular cancer. Chronic hepatic inflammation and injury can lead to fatty infiltration of the liver and hepatocyte dysfunction. Innate immune cells, including macrophages and neutrophils, are frequently present in the liver with fatty infiltration and can be activated by pathogen- and damage-associated molecular pattern receptors (PAMPs and DAMPs), which can lead to inflammation and fibrosis. Luci *et al*. examined the role of the 3BP2 signaling module in animal models of hepatic steatosis [109]. They found that the hepatic expression of 3BP2 and SYK correlated with metabolic steatohepatitis severity in mice. 3BP2 deficiency and SYK deletion in myeloid cells were protective against liver inflammation, tissue damage, and subsequent fibrosis. Silencing 3BP2 or SYK in primary hepatocytes decreased the expression of inflammatory cytokines, including TNFα, IL6, IL1β, and CCL2. By contrast, Cherubism mice developed severe hepatitis and liver fibrosis following a high-fat diet

(HFD) or methionine choline-deficient (MCD) diet. SYK expression is elevated in the liver of patients with hepatic steatosis and correlates with the severity of disease. These data demonstrate the central role of the 3BP2 signaling module in modulating hepatic inflammation, injury, and fibrosis in models of hepatic steatosis [109].

The role of tankyrase in regulating the antiviral interferon-sensing apparatus

Xu *et al*. recently showed that tankyrase regulates the viral RNA-sensing protein virus-induced signal adaptor (VISA), also known as MAVS, to control interferon response to viral infection. Tankyrase translocates to mitochondria and interacts with the mitochondrial adaptor protein VISA through a canonical tankyrase-binding motif RNPDGG. Tankyrase PARsylates VISA at Glu137, which creates a binding site for RNF146, resulting in the ubiquitylation and degradation of VISA. Pharmacological inhibition or genetic knockout of tankyrase increases the RNA virus-triggered induction of interferon effector genes, which impair viral replication. Tnks1- or Tnks2-deficient mice produced higher levels of type I interferons after encephalomyocarditis virus infection and reduced virus loads in the brain and lungs of knockout animals. This study demonstrates an essential role for PARsylation in virus-triggered innate immune signaling and the role of tankyrase in buffering the production of inflammatory cytokines [110].

The role of tankyrase-mediated PARsylation in modulating neuronal stress granule formation

Amyotrophic lateral sclerosis (ALS) is associated with the redistribution of RNA-binding protein TDP43 from the nucleus to cytoplasm in response to stress pathways. Cytoplasmic TDP43 can accumulate in stress granules in response to short-term stress, where it is sequestered from being modified by phosphorylation. Stress granule assembly occurs via condensation of RNA–protein complexes into dynamic liquid droplets, a process called liquid–liquid phase separation (LLPS). Importantly, PAR polymers promote LLPS and stress granule formation. McGurk *et al*. showed that the TDP43 nuclear localization sequence (NLS) contains two PAR-binding motifs, and that TDP43 binds directly to PAR polymers [111]. Moreover, they showed that the ability of TDP43 to bind to PAR polymers determined its ability to undergo LLPS and be recruited to stress granules. By contrast, N-terminal truncated mutant forms of TDP-43 associated with ALS were unable to bind to PAR, and failed to undergo LLPS or be recruited to stress granules. Therefore, the N-terminal region of TDP-43 is important for the recruitment of the protein to stress granules and suggests that, in ALS, stress-granule recruitment of TDP-43 is impaired. McGurk showed in a *Drosophila* genetic screen that downregulation of tankyrase increased nuclear and decreased cytoplasmic levels of TDP43 and mitigated TDP43-induced degeneration of the nervous system. The authors then showed that the tankyrase inhibitor XAV939 [85] markedly reduced the formation of stress-induced cytoplasmic foci of TDP43 without altering the formation of stress granules. These data show that tankyrase is a key regulator of the partitioning of TDP43 into stress fibers and that inhibition of tankyrase activity mitigates ALS-associated degeneration by reducing the cytoplasmic localization of TDP43 and subsequent neurodegeneration.

Proteomic analysis elucidates new tankyrase substrates

Proteomic analysis has identified new potential uses for tankyrase-binding proteins [9,112,113] (Table 1, Key table). Tankyrase-binding motif-containing proteins, which are increased following Tankyrase inhibition, are associated with Wnt signaling, glucose transport, miRNA processing, Notch signaling, and Hippo signaling. The Rab11 GTPase proteins, required for cytokinesis, ciliogenesis, and lumenogenesis, are activated by Rab11-specific GEFs SH3BP5 and SH3BP5L. Tankyrase-mediated ubiquitylation of SH3BP5 and SH3BP5L by RNF146 suppresses Rab11a-mediated epithelial cyst lumen formation, demonstrating a role for tankyrase

Clinician's corner

Although no diagnostic criteria of Cherubism have been established, detecting single allele mutations of exon 9 in *SH3BP2* is helpful to confirm the diagnosis in ~80% of patients clinically diagnosed in the presence of clinical manifestations and radiographic and histologic findings. Mutations in Pro418 (to Leu, Arg, or His) are the most common, while other mutations are also observed in Arg415 (to Pro or Gln) and Gly420 (to Glu or Arg). Approximately 20% of Cherubism patients who present with clinical features may be caused by mutations in other genes that are associated with the 3BP2 signaling pathway. Candidate genes include loss-of-function mutations in *TNKS1*, *TNKS2*, and *RNF146*.

Genetic studies demonstrate the association between 3BP2 and various biological processes, including bone metabolism and inflammation. Bone is a highly dynamic organ that constantly undergoes remodeling regulated by osteoblast-mediated bone formation and osteoclast-mediated bone resorption. RUNX2 is a master transcription factor for osteoblast differentiation, and TAZ, a transcriptional co-activator, interacts with RUNX2 and activates its transcriptional activity. 3BP2-mediated ABL kinase activation induces RUNX-TAZ complex formation, which results in bone formation. In contrast to osteoblasts, osteoclasts arise from the macrophage lineage following M-CSF and RANKL stimulation. Normal osteoclastogenesis requires 3BP2-mediated SRC activation.

3BP2-mediated cytokine production is controlled through activation of TLR signaling. More than ten TLRs have been identified in humans. TLR2 signaling is controlled by 3BP2-mediated phosphorylation of its TIR domains. Genetic knockout of tankyrase leads to hyperactivation of TLR2 as a result of hyperactivation of SRC and SYK kinases and phosphorylation of TLR2, which results in the production of cytokines, such as IL6 and TNFα.

Wnt/β-catenin signaling is highly conserved and controls cell-fate determination, tissue homeostasis, and organogenesis. β-catenin is negatively

in regulating epithelial morphogenesis and polarity [114]. In *Drosophila*, tankyrase is a positive regulator of the JNK signaling pathway, whereby it interacts with, and PARsylates, JNK, resulting in the addition of K63-ubiquitin chains that activate JNK kinase, required for stress tolerance, energy homeostasis, and lifespan prolongation [115].

regulated by a multiprotein destruction complex involving AXIN, GSK3β, CK1, and APC. Mutations in β-catenin or APC, which lead to the stabilization of β-catenin, can drive colon tumor formation. Tankyrase PARsylates AXIN and stimulates its destruction, thereby potentiating the β-catenin pathway. Tankyrase inhibitors designed to stabilize AXIN have been developed to antagonize β-catenin signaling.

Therapeutic potential of targeting tankyrase and the 3BP2 signaling module

The first tankyrase inhibitor, XAV939, was identified in a screen to suppress the Wnt/β-catenin pathway through stabilization of AXIN [85]. Subsequently, numerous tankyrase inhibitors have been developed that target the NAD-binding site, the adenosine binding pocket, or both [34].

New inhibitors designed to disrupt the interaction of tankyrase with its substrates in the ARC domain-binding groove have recently been developed. This concept is based on elucidation of the interaction between the fourth ARC domain of tankyrase and 3BP2 hexapeptide, provided by the co-crystal structure [9]. These peptide interaction-blocking inhibitors show potential to suppress the Wnt/β-catenin pathway [116]. The therapeutic potential of tankyrase inhibitors to treat human tumors driven by β-catenin is contingent on a functionally intact destruction complex involving AXIN, APC, and GSK3β. Unfortunately, many colon tumors arise from mutations in APC, AXIN, or β-catenin itself, which renders them impervious to regulation from the destruction complex. Thus, tankyrase inhibitors would be of little value in these patients.

One of the concerns facing the deployment of tankyrase inhibitors is any resulting adverse side effects [117], given that genetic studies have shown that depletion of Tnks1 and Tnks2 in the myeloid lineage leads to severe inflammatory bowel disease and systemic autoinflammation [64]. TNKS1 and TNKS2 are involved in the nervous system and are detected in the soma and neurites in hippocampal neurons. The tankyrase inhibitor XAV939 inhibits neurite outgrowth and synapse formation through stabilization of AXIN and subsequent suppression of the Wnt/β-catenin pathway in neurons [118]. Thus, these studies suggest that tankyrase inhibitors could have unexpected neuronal toxicities.

Additionally, pharmacological inhibition of tankyrase may affect bone metabolism. Diminished β-catenin signaling in RNF146-deficient osteoblasts results in defective osteoblastogenesis through suppression of β-catenin-induced *Fgf18* expression [47] (Figure 3C) and deletion of RNF146 in macrophages leads to increased osteoclastogenesis [41] (Figure 3B), both of which trigger bone loss through inhibition of β-catenin. Sclerostin, a negative regulator of the Wnt/β-catenin pathway, is now the therapeutic target for osteoporosis [119], demonstrating that long-term use of tankyrase inhibitors may cause bone loss due to suppression of osteoblastogenesis but enhancement of osteoclastogenesis.

Although the development of tankyrase inhibitors has garnered considerable interest given the pleiotropic pathways that it regulates in cancer, clinical deployment of these inhibitors has been challenging. There are no current clinical trials studying tankyrase inhibitors.

Regulation of tankyrase

The upstream regulatory mechanisms controlling Tankyrase activity have also been investigated (Table 1). GDP-mannose 4,6-dehydratase (GMD), which is a cytoplasmic protein required for the first step of fucose synthesis, interacts with, and sequesters, TNKS1 in an inactive stable form [120]. The Wnt inhibitor kinase inhibitor 4 (WIKI4) inhibits TNKS2 enzymatic activity [121], and prostate-associated gene 4 (PAGE4), which is selectively expressed in stromal prostate fibroblasts, acts as a tissue-specific tankyrase inhibitor by competitively binding to its substrate AXIN1. Both WIKI4 and PAGE4 inhibit AXIN1 degradation, which results in the

Key table

Table 1. Functions of substrates regulated through PARsylation-mediated ubiquitylation

Regulation	Substrate(s)	Function of substrates	Refs
TNKS1/2			
Activation	JNK	Required for lifespan, stress tolerance and energy homeostasis in *Drosophila*	[115]
Destabilization	TRF1	Telomere binder and negative regulator of telomere elongation	[21,22]
	AXIN1/2	Tumor suppressor through regulation of Wnt signaling pathway	[10,85]
		Activate osteoclastogenesis through regulation of Wnt signaling pathway	[41]
		Suppress osteoblastogenesis through regulation of Wnt signaling pathway	[47]
		Suppress neurite outgrowth and synapse formation through regulation of Wnt signaling pathway	[118]
	3BP2	Adaptor protein in tyrosine kinase signaling; mutated in Cherubism	[8,9,19]
		Required for both osteoblastogenesis and osteoclastogenesis	[15,16]
		Activates TLR signaling and cytokine production	[20,62–64]
		Required for neutrophil activation	[65]
		Required for proliferation and survival of both B and $CD8^+$ T cells	[69,75]
		Exacerbates collagen-induced arthritis	[106,107]
		Deletion of 3BP2 improves SLE	[107]
		Pathogenesis of metabolic steatohepatitis	[109]
	RNF146	E3-ubiquitin ligase for AXIN1/2 and 3BP2	[8,10]
	SOX9	Master transcription factor of chondrogenesis	[58]
	PTEN	Tumor suppressor through regulation of AKT signaling pathway	[86]
	AMOT	Tumor suppressor through regulation of YAP activity	[96]
	LKB1/AMPK	Tumor suppressor	[97]
	PARP1	Required for DNA repair	[37]
	SH3BP5	Required for cyst lumen formation	[114]
	VISA	Critical component in antiviral IFN-β signaling pathways	[110]
Stabilization	TDP43	Causes amyotrophic lateral sclerosis and frontotemporal degeneration	[110]
Unknown	GMD	Sequesters TNKS1 in inactive stable form	[120]
PARP1			
Destabilization	BRD7	Tumor suppressor	[103]

suppression of Wnt/β-catenin signaling [120]. The ubiquitin-specific protease 34 (USP34), which is a component of the β-catenin destruction complex, deubiquitylates AXIN to antagonize tankyrase-mediated PARsylation and RNF146 ubiquitylation to inactivate the Wnt/β-catenin pathway [121]. These studies demonstrate that tankyrase-mediated PARsylation and subsequent proteasomal degradation of its substrates is controlled under a variety of distinct physiological states.

Concluding remarks

Since the original cloning of TNKS1 as a regulator of the telomerase complex and TNKS2 as a regulator of the adapter protein Grb14, there has been a growing appreciation of the importance of tankyrase-mediated PARsylation coupled to the RNF146 E3-ubiquitin ligase. These studies

Outstanding questions

Recent research identified the structural determinant for tankyrase substrate recognition. However, not every protein with canonical tankyrase-binding motifs is PARsylated, ubiquitylated, and destroyed. What are the fundamental rules that determine the fate of PARsylated proteins mediated by tankyrase: destroyed by RNF146, inactivated, or activated?

Recent cryo-electron microscopy structural studies showed how tankyrase catalytic and noncatalytic functions depend on its filamentous polymerization. How is tankyrase assembly and activity regulated by upstream signaling pathways?

What are the biochemical and biological differences between TNKS1 and its paralog TNKS2?

Many of the clinical questions regarding the natural history of Cherubism remain. Why does bone destruction occur in craniofacial bones?

Do patients with Cherubism have more widespread osteopenia?

Why does Cherubism manifest in children at age 2–5 years, progress until puberty, and then spontaneously regress?

Genetically engineered models of Cherubism manifest widespread autoinflammation and autoimmunity with the production of autoantibodies. What is the immune dysfunction present in patients with Cherubism?

PARP inhibitors have been developed for tumors with homologous recombination defects. Some PARP inhibitors can inhibit tankyrase activity. Is there any evidence of perturbation of tankyrase-regulated pathways in patients treated with PARP inhibitors?

CellPress
OPEN ACCESS

highlighted a previously underappreciated role for reversible PARsylation as a form of post-translational modification in the cytoplasmic compartment that is distinct from the well-established role of PAR modification in the nucleus catalyzed by PARP1 and PARP2 during DNA repair. Numerous studies have contributed to the emerging view of the pleiotropic biological processes that tankyrases participate in, including the control of inflammatory pathways, bone dynamics, metabolic control, suppression of tumor suppressor pathways, control of polarity and epithelial morphogenesis, telomere and mitotic spindle function, and the regulation of stress granules in neurons. We have highlighted the importance of the tankyrase-3BP2 pathway as first exemplified in Cherubism, a rare human developmental syndrome. Recent cryo-electron microscopy structural studies have shown how tankyrase catalytic and noncatalytic functions depend on its filamentous polymerization [124], while there remains much still to understand especially regarding the upstream regulatory pathways controlling tankyrase activation and the biochemical and biological differences between TNKS1 and its paralog TNKS2. Many of the clinical questions of why, for example, bone destruction occurs in craniofacial bones in Cherubism, remain. The development of potent and highly selective tankyrase inhibitors will serve as chemical probes to address some of these questions (see Outstanding questions).

Declaration of interests

None declared by authors.

References

1. Luo, X. and Kraus, W.L. (2012) On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev.* 26, 417–432
2. Bonfiglio, J.J. *et al.* (2017) Serine ADP-ribosylation depends on HPF1. *Mol. Cell* 65, 932–940
3. Gibson, B.A. *et al.* (2016) Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. *Science* 353, 45–50
4. Ke, Y. *et al.* (2019) The role of PARPs in inflammation-and metabolic-related diseases: molecular mechanisms and beyond. *Cells* 8, 1047
5. Bock, F.J. and Chang, P. (2016) New directions in poly(ADP-ribose) polymerase biology. *FEBS J.* 283, 4017–4031
6. Gupte, R. *et al.* (2017) PARPs and ADP-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes Dev.* 31, 101–126
7. Gibson, B.A. and Kraus, W.L. (2012) New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat. Rev. Mol. Cell Biol.* 13, 411–424
8. Levaot, N. *et al.* (2011) Loss of Tankyrase-mediated destruction of 3BP2 is the underlying pathogenic mechanism of cherubism. *Cell* 147, 1324–1339
9. Guettler, S. *et al.* (2011) Structural basis and sequence rules for substrate recognition by Tankyrase explain the basis for cherubism disease. *Cell* 147, 1340–1354
10. Zhang, Y. *et al.* (2011) RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat. Cell Biol.* 13, 623–629
11. Jones, W.A. *et al.* (1950) Cherubism–familial fibrous dysplasia of the jaws. *J. Bone Joint Surg. Br.* 32-B, 334–347
12. Ren, R. *et al.* (1993) Identification of a ten-amino acid proline-rich SH3 binding site. *Science* 259, 1157–1161
13. Cicchetti, P. *et al.* (1992) Identification of a protein that binds to the SH3 region of Abl and is similar to Bcr and GAP-rho. *Science* 257, 803–806
14. Deckert, M. and Rottapel, R. (2006) The adapter 3BP2: how it plugs into leukocyte signaling. *Adv. Exp. Med. Biol.* 584, 107–114
15. Levaot, N. *et al.* (2011) 3BP2-deficient mice are osteoporotic with impaired osteoblast and osteoclast functions. *J. Clin. Invest.* 121, 3244–3257
16. Matsumoto, Y. *et al.* (2016) Reciprocal stabilization of ABL and TAZ regulates osteoblastogenesis through transcription factor RUNX2. *J. Clin. Invest.* 126, 4482–4496
17. Tiziani, V. *et al.* (1999) The gene for cherubism maps to chromosome 4p16. *Am. J. Hum. Genet.* 65, 158–166
18. Mangion, J. *et al.* (1999) The gene for cherubism maps to chromosome 4p16.3. *Am. J. Hum. Genet.* 65, 151–157
19. Ueki, Y. *et al.* (2001) Mutations in the gene encoding c-Abl-binding protein SH3BP2 cause cherubism. *Nat. Genet.* 28, 125–126
20. Ueki, Y. *et al.* (2007) Increased myeloid cell responses to M-CSF and RANKL cause bone loss and inflammation in SH3BP2 'cherubism' mice. *Cell* 128, 71–83
21. Smith, S. *et al.* (1998) Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* 282, 1484–1487
22. Chang, W. *et al.* (2003) TRF1 is degraded by ubiquitin-mediated proteolysis after release from telomeres. *Genes Dev.* 17, 1328–1333
23. Smith, S. and de Lange, T. (2000) Tankyrase promotes telomere elongation in human cells. *Curr. Biol.* 10, 1299–1302
24. Canudas, S. *et al.* (2007) Protein requirements for sister telomere association in human cells. *EMBO J.* 26, 4867–4878
25. Haikarainen, T. *et al.* (2014) Tankyrases: structure, function and therapeutic implications in cancer. *Curr. Pharm. Des.* 20, 6472–6488
26. Hsiao, S.J. *et al.* (2006) Tankyrase 2 poly(ADP-ribose) polymerase domain-deleted mice exhibit growth defects but have normal telomere length and capping. *Mol. Cell. Biol.* 26, 2044–2054
27. Chiang, Y.J. *et al.* (2006) Generation and characterization of telomere length maintenance in tankyrase 2-deficient mice. *Mol. Cell. Biol.* 26, 2037–2043
28. Yeh, T.Y. *et al.* (2009) Hypermetabolism, hyperphagia, and reduced adiposity in tankyrase-deficient mice. *Diabetes* 58, 2476–2485
29. Chiang, Y.J. *et al.* (2008) Tankyrase 1 and tankyrase 2 are essential but redundant for mouse embryonic development. *PLoS ONE* 3, e2639
30. Kaminker, P.G. *et al.* (2001) TANK2, a new TRF1-associated poly(ADP-ribose) polymerase, causes rapid induction of cell death upon overexpression. *J. Biol. Chem.* 276, 35891–35899
31. De Rycker, M. *et al.* (2003) Vertebrate tankyrase domain structure and sterile alpha motif (SAM)-mediated multimerization. *Biochem. J.* 372, 87–96

32. De Rycker, M. and Price, C.M. (2004) Tankyrase polymerization is controlled by its sterile alpha motif and poly(ADP-ribose) polymerase domains. *Mol. Cell. Biol.* 24, 9802–9812
33. Hsiao, S.J. and Smith, S. (2008) Tankyrase function at telomeres, spindle poles, and beyond. *Biochimie* 90, 83–92
34. Zamudio-Martinez, E. *et al.* (2021) Tankyrases as modulators of pro-tumoral functions: molecular insights and therapeutic opportunities. *J. Exp. Clin. Cancer Res.* 40, 144
35. DaRosa, P.A. *et al.* (2016) Structural insights into SAM domain-mediated tankyrase oligomerization. *Protein Sci.* 25, 1744–1752
36. He, F. *et al.* (2012) Structural insight into the interaction of ADP-ribose with the PARP WWE domains. *FEBS Lett.* 586, 3858–3864
37. Wang, Z. *et al.* (2012) Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination. *Genes Dev.* 26, 235–240
38. Kang, H.C. *et al.* (2011) Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14103–14108
39. DaRosa, P.A. *et al.* (2015) Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. *Nature* 517, 223–226
40. DaRosa, P.A. *et al.* (2018) Structural basis for tankyrase-RNF146 interaction reveals noncanonical tankyrase-binding motifs. *Protein Sci.* 27, 1057–1067
41. Matsumoto, Y. *et al.* (2017) RANKL coordinates multiple osteoclastogenic pathways by regulating expression of ubiquitin ligase RNF146. *J. Clin. Invest.* 127, 1303–1315
42. Albers, J. *et al.* (2013) Canonical Wnt signaling inhibits osteoclastogenesis independent of osteoprotegerin. *J. Cell Biol.* 200, 537–549
43. Otero, K. *et al.* (2012) TREM2 and beta-catenin regulate bone homeostasis by controlling the rate of osteoclastogenesis. *J. Immunol.* 188, 2612–2621
44. Wei, W. *et al.* (2011) Biphasic and dosage-dependent regulation of osteoclastogenesis by beta-catenin. *Mol. Cell. Biol.* 31, 4706–4719
45. Mundlos, S. (1999) Cleidocranial dysplasia: clinical and molecular genetics. *J. Med. Genet.* 36, 177–182
46. Marie, P. and Sainton, P. (1898) Sur la dysostose cléido-cranienne héréditaire. *Rev. Neurol.* 6, 835–838
47. Matsumoto, Y. *et al.* (2017) Ubiquitin ligase RNF146 coordinates bone dynamics and energy metabolism. *J. Clin. Invest.* 127, 2612–2625
48. Mundlos, S. *et al.* (1997) Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. *Cell* 89, 773–779
49. Lee, B. *et al.* (1997) Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. *Nat. Genet.* 16, 307–310
50. Kobayashi, T. and Kronenberg, H.M. (2014) Overview of skeletal development. *Methods Mol. Biol.* 1130, 3–12
51. Ducy, P. *et al.* (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* 89, 747–754
52. Komori, T. *et al.* (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 89, 755–764
53. Ott, C.E. *et al.* (2010) Deletions of the RUNX2 gene are present in about 10% of individuals with cleidocranial dysplasia. *Hum. Mutat.* 31, E1587–E1593
54. Lee, N.K. *et al.* (2007) Endocrine regulation of energy metabolism by the skeleton. *Cell* 130, 456–469
55. Ng, L.J. *et al.* (1997) SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse. *Dev. Biol.* 183, 108–121
56. Lefebvre, V. *et al.* (1998) A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. *EMBO J.* 17, 5718–5733
57. Wright, E. *et al.* (1995) The Sry-related gene Sox9 is expressed during chondrogenesis in mouse embryos. *Nat. Genet.* 9, 15–20
58. Kim, S. *et al.* (2019) Tankyrase inhibition preserves osteoarthritic cartilage by coordinating cartilage matrix anabolism via effects on SOX9 PARylation. *Nat. Commun.* 10, 4898
59. Takeuchi, O. *et al.* (1999) Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 11, 443–451
60. Kawai, T. and Akira, S. (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650
61. Mogensen, T.H. (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev.* 22, 240–273
62. Yoshitaka, T. *et al.* (2014) Enhanced TLR-MYD88 signaling stimulates autoinflammation in SH3BP2 cherubism mice and defines the etiology of cherubism. *Cell Rep.* 8, 1752–1766
63. Prod'Homme, V. *et al.* (2015) Cherubism allele heterozygosity amplifies microbe-induced inflammatory responses in murine macrophages. *J. Clin. Invest.* 125, 1396–1400
64. Matsumoto, Y. *et al.* (2022) Tankyrase represses autoinflammation through the attenuation of TLR2 signaling. *J. Clin. Invest.* 132, e140869
65. Chen, G. *et al.* (2012) The 3BP2 adapter protein is required for chemoattractant-mediated neutrophil activation. *J. Immunol.* 189, 2138–2150
66. Strathmann, M.P. and Simon, M.I. (1991) G alpha 12 and G alpha 13 subunits define a fourth class of G protein alpha subunits. *Proc. Natl. Acad. Sci. U. S. A.* 88, 5582–5586
67. Songyang, Z. *et al.* (1994) Specific motifs recognized by the SH2 domains of Csk, 3BP2, fps/fes, GRB-2, HCP, SHC, Syk, and Vav. *Mol. Cell. Biol.* 14, 2777–2785
68. Wang, Y. *et al.* (2002) The physiologic role of CD19 cytoplasmic tyrosines. *Immunity* 17, 501–514
69. Chen, G. *et al.* (2007) The 3BP2 adapter protein is required for optimal B-cell activation and thymus-independent type 2 humoral response. *Mol. Cell. Biol.* 27, 3109–3122
70. Kraal, G. *et al.* (1995) Expression of the mucosal vascular addressin, MAdCAM-1, on sinus-lining cells in the spleen. *Am. J. Pathol.* 147, 763–771
71. Lane, P.J. *et al.* (1986) Differences in the recruitment of virgin B cells into antibody responses to thymus-dependent and thymus-independent type-2 antigens. *Eur. J. Immunol.* 16, 1569–1575
72. Martin, F. and Kearney, J.F. (2000) B-cell subsets and the mature preimmune repertoire. Marginal zone and B1 B cells as part of a 'natural immune memory'. *Immunol. Rev.* 175, 70–79
73. Deckert, M. *et al.* (1998) Adaptor function for the Syk kinases-interacting protein 3BP2 in IL-2 gene activation. *Immunity* 9, 595–605
74. Shukla, U. *et al.* (2009) Tyrosine phosphorylation of 3BP2 regulates B cell receptor-mediated activation of NFAT. *J. Biol. Chem.* 284, 33719–33728
75. Dimitriou, I.D. *et al.* (2018) Timed regulation of 3BP2 induction is critical for sustaining $CD8^+$ T cell expansion and differentiation. *Cell Rep.* 24, 1123–1135
76. Kaech, S.M. and Ahmed, R. (2001) Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat. Immunol.* 2, 415–422
77. van Stipdonk, M.J. *et al.* (2001) Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat. Immunol.* 2, 423–429
78. Conley, J.M. *et al.* (2016) T cells and gene regulation: the switching on and turning up of genes after T cell receptor stimulation in CD8 T cells. *Front. Immunol.* 7, 76
79. Sherman, E. *et al.* (2013) Super-resolution characterization of TCR-dependent signaling clusters. *Immunol. Rev.* 251, 21–35
80. Zeng, L. *et al.* (1997) The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* 90, 181–192
81. Lehtio, L. *et al.* (2013) Tankyrases as drug targets. *FEBS J.* 280, 3576–3593
82. Kikuchi, A. (1999) Roles of Axin in the Wnt signalling pathway. *Cell. Signal.* 11, 777–788
83. Eisemann, T. *et al.* (2016) Tankyrase-1 ankyrin repeats form an adaptable binding platform for targets of ADP-ribose modification. *Structure* 24, 1679–1692
84. Morrone, S. *et al.* (2012) Crystal structure of a Tankyrase-Axin complex and its implications for Axin turnover and Tankyrase substrate recruitment. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1500–1505
85. Huang, S.M. *et al.* (2009) Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 461, 614–620

86. Li, N. *et al.* (2015) Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth. *Genes Dev.* 29, 157–170
87. Halder, G. and Johnson, R.L. (2011) Hippo signaling: growth control and beyond. *Development* 138, 9–22
88. Pan, D. (2010) The hippo signaling pathway in development and cancer. *Dev. Cell* 19, 491–505
89. Zhao, B. *et al.* (2010) The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. *Genes Dev.* 24, 862–874
90. Jiao, S. *et al.* (2014) A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. *Cancer Cell* 25, 166–180
91. Kapoor, A. *et al.* (2014) Yap1 activation enables bypass of oncogenic Kras addiction in pancreatic cancer. *Cell* 158, 185–197
92. Zhang, W. *et al.* (2014) VGLL4 functions as a new tumor suppressor in lung cancer by negatively regulating the YAP-TEAD transcriptional complex. *Cell Res.* 24, 331–343
93. Chan, S.W. *et al.* (2011) Hippo pathway-independent restriction of TAZ and YAP by angiomotin. *J. Biol. Chem.* 286, 7018–7026
94. Wang, W. *et al.* (2011) Angiomotin-like proteins associate with and negatively regulate YAP1. *J. Biol. Chem.* 286, 4364–4370
95. Zhao, B. *et al.* (2011) Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. *Genes Dev.* 25, 51–63
96. Wang, W. *et al.* (2015) Tankyrase inhibitors target YAP by stabilizing angiomotin family proteins. *Cell Rep.* 13, 524–532
97. Li, N. *et al.* (2019) Tankyrase disrupts metabolic homeostasis and promotes tumorigenesis by inhibiting LKB1–AMPK signalling. *Nat. Commun.* 10, 4363
98. Benjamin, R.C. and Gill, D.M. (1980) Poly(ADP-ribose) synthesis in vitro programmed by damaged DNA. A comparison of DNA molecules containing different types of strand breaks. *J. Biol. Chem.* 255, 10502–10508
99. Satoh, M.S. *et al.* (1994) Dual function for poly(ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry* 33, 7099–7106
100. Sonnenblick, A. *et al.* (2015) An update on PARP inhibitors–moving to the adjuvant setting. *Nat. Rev. Clin. Oncol.* 12, 27–41
101. Ledermann, J.A. and Pujade-Lauraine, E. (2019) Olaparib as maintenance , treatment for patients with platinum-sensitive relapsed ovarian cancer. *Ther. Adv. Med. Oncol.* 11, 1–18
102. Ledermann, J. *et al.* (2012) Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N. Engl. J. Med.* 366, 1382–1392
103. Hu, K. *et al.* (2019) Poly(ADP-ribosyl)ation of BRD7 by PARP1 confers resistance to DNA–damaging chemotherapeutic agents. *EMBO Rep.* 20, e46166
104. Mehta, C.C. and Bhatt, H.G. (2021) Tankyrase inhibitors as antitumor agents: a patent update (2013 - 2020). *Expert Opin. Ther. Pat.* 31, 645–661
105. Mukai, T. *et al.* (2015) Loss of SH3 domain-binding protein 2 function suppresses bone destruction in tumor necrosis factor-driven and collagen-induced arthritis in mice. *Arthritis Rheumatol.* 67, 656–667
106. Mukai, T. *et al.* (2014) SH3BP2 cherubism mutation potentiates TNF-alpha-induced osteoclastogenesis via NFATc1 and TNF-alpha-mediated inflammatory bone loss. *J. Bone Miner. Res.* 29, 2618–2635
107. Mukai, T. *et al.* (2014) SH3BP2 gain-of-function mutation exacerbates inflammation and bone loss in a murine collagen-induced arthritis model. *PLoS ONE* 9, e105518
108. Kawahara, K. *et al.* (2021) SH3BP2 deficiency ameliorates murine systemic lupus erythematosus. *Int. J. Mol. Sci.* 22, 4169
109. Luci, C. *et al.* (2022) SYK-3BP2 pathway activity in parenchymal and myeloid cells is a key pathogenic factor in metabolic steatohepatitis. *Cell Mol. Gastroenterol. Hepatol.* 13, 173–191
110. Xu, Y.R. *et al.* (2022) Tankyrases inhibit innate antiviral response by PARylating VISA/MAVS and priming it for RNF146–mediated ubiquitination and degradation. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2122805119
111. McGurk, L. *et al.* (2020) TDP-43, a protein central to amyotrophic lateral sclerosis, is destabilized by tankyrase-1 and -2. *J. Cell Sci.* 133, jcs245811
112. Li, X. *et al.* (2017) Proteomic analysis of the human tankyrase protein interaction network reveals its role in pexophagy. *Cell Rep.* 20, 737–749
113. Bhardwaj, A. *et al.* (2017) Whole proteome analysis of human tankyrase knockout cells reveals targets of tankyrase-mediated degradation. *Nat. Commun.* 8, 2214
114. Chandrakumar, A.A. *et al.* (2021) Tankyrase regulates epithelial lumen formation via suppression of Rab11 GEFs. *J. Cell Biol.* 220, e202008037
115. Li, P. *et al.* (2018) Tankyrase mediates K63-linked ubiquitination of JNK to confer stress tolerance and influence lifespan in *Drosophila*. *Cell Rep.* 25, 437–448
116. Xu, W. *et al.* (2017) Macrocyclized extended peptides: inhibiting the substrate-recognition domain of tankyrase. *J. Am. Chem. Soc.* 139, 2245–2256
117. Asano, Y. *et al.* (2023) Pharmacologic inhibition of PARP5, but not that of PARP1 or 2, promotes cytokine production and osteoclastogenesis through different pathways. *Clin. Exp. Rheumatol.* Published online January 23, 2023. http://dx.doi.org/10.55563/clinexprheumatol/qf55h8
118. Mashimo, M. *et al.* (2022) Tankyrase regulates neurite outgrowth through poly(ADP-ribosyl)ation-dependent activation of beta-catenin signaling. *Int. J. Mol. Sci.* 23, 2834
119. Cosman, F. *et al.* (2016) Romosozumab treatment in postmenopausal women with osteoporosis. *N. Engl. J. Med.* 375, 1532–1543
120. Bisht, K.K. *et al.* (2012) GDP-mannose-4,6-dehydratase is a cytosolic partner of tankyrase 1 that inhibits its poly(ADP-ribose) polymerase activity. *Mol. Cell. Biol.* 32, 3044–3053
121. James, R.G. *et al.* (2012) WIKI4, a novel inhibitor of tankyrase and Wnt/ss–catenin signaling. *PLoS ONE* 7, e50457
122. Koirala, S. *et al.* (2020) Tissue-specific regulation of the Wnt/beta-catenin pathway by PAGE4 inhibition of tankyrase. *Cell Rep.* 32, 107922
123. Lui, T.T. *et al.* (2011) The ubiquitin-specific protease USP34 regulates axin stability and Wnt/beta-catenin signaling. *Mol. Cell. Biol.* 31, 2053–2065
124. Pillay, N. *et al.* (2022) Structural basis of tankyrase activation by polymerization. *Nature* 612, 162–169