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SUMOylation regulation of ribosome biogenesis: Emerging roles for USP36

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Ribosome biogenesis is essential for cell growth, proliferation, and animal development. Its deregulation leads to various human disorders such as ribosomopathies and cancer. Thus, tight regulation of ribosome biogenesis is crucial for normal cell homeostasis. Emerging evidence suggests that posttranslational modifications such as ubiquitination and SUMOylation play a crucial role in regulating ribosome biogenesis. Our recent studies reveal that USP36, a nucleolar deubiquitinating enzyme (DUB), acts also as a SUMO ligase to regulate nucleolar protein group SUMOylation, thereby being essential for ribosome biogenesis. Here, we provide an overview of the current understanding of the SUMOylation regulation of ribosome biogenesis and discuss the role of USP36 in nucleolar SUMOylation.

KEYWORDS

USP36, ribosome biogenesis, SUMOylation, ubiquitin, deubiquitination

Introduction

Ribosome biogenesis is a complex multi-step cellular process for making the ribosome, including the synthesis of ribosomal RNA (rRNA) and ribosomal proteins, rRNA processing, and the assembly of the mature ribosome subunits in the nucleolus and subsequent transport into the cytoplasm (Rodnina and Wintermeyer, 2009). It requires coordinated and fine-tuned transcription catalyzed by all three RNA polymerases (RNA Pol I, II and III). RNA Pol I-driven transcription of the rRNA precursor (pre-rRNA) from multiple copies of the rDNA genes is considered as a rate-limiting step (Moss and Stefanovsky, 2002; Grummt, 2003; Chedin et al., 2007). Pre-rRNA contains 18S, 5.8S, and 28S rRNA species separated by internal transcribed spacers, ITS1 and ITS2, and flanked by 5′ and 3′ external transcribed spacers (5′-ETS and 3′ETS). These transcribed regions are removed by a series of cleavage steps in the nucleolus to generate mature rRNA species that are also extensively modified by methylation and pseudouridylation. These processing steps are meticulously regulated by a series of ribosome biogenesis factors, including small nucleolar RNAs (snoRNAs) and rRNA processing enzymes, ensuring accurate processing of pre-rRNA (Ashcroft et al., 2000; Baßler and Hurt, 2019; Bohnsack and Bohnsack, 2019; Singh et al., 2021; Dorner et al., 2023; Vanden Broeck and Klinge, 2023). 5S rRNA is transcribed by RNA pol III in the nucleoplasm and translocated into the nucleolus to be assembled into the large ribosome subunit. Concurrently, ribosomal proteins are assembled into ribosomal subunits by interacting with rRNA molecules through sequential and intricate assembly processes, which also require auxiliary factors

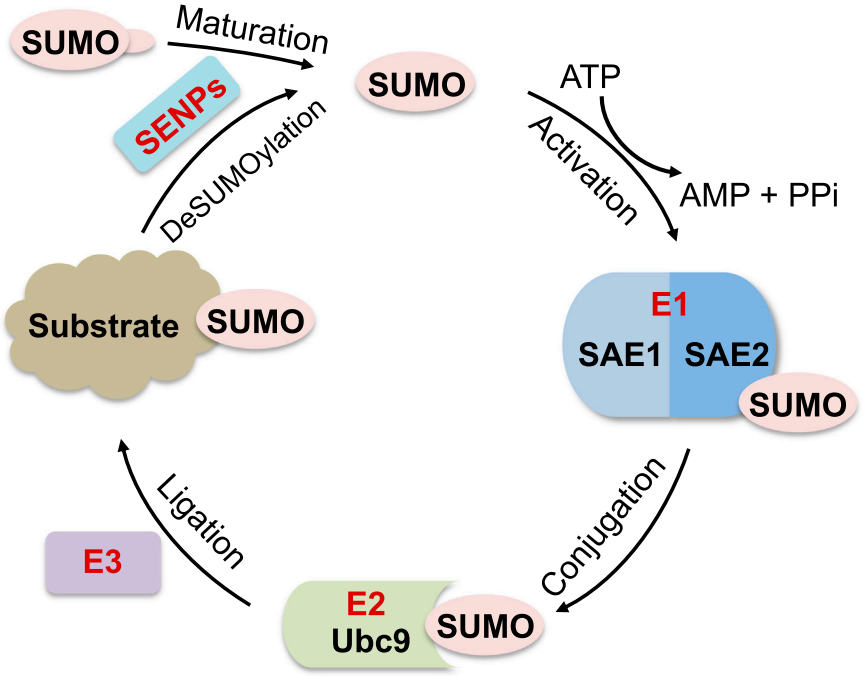

FIGURE 1
Diagram of SUMOylation pathway. SUMO precursors are cleaved by SUMO proteases (SENPs) to generate mature SUMOs with di-glycine at their C-terminus, which forms a thioester bond with the heterodimeric SUMO E1 (SAE1/SAE2) in an ATP-dependent manner. Activated SUMO is then transferred to SUMO E2 (Ubc9) also via forming a thioester bond with Ubc9. Finally, Ubc9 conjugates SUMO to substrates, which is facilitated by a SUMO E3. SUMOylated protein substrates can be deSUMOylated by SUMO proteases.

such as chaperones and transport factors for accurate assembly and quality control. Once assembled, ribosomal subunits are ferried to the cytoplasm through nuclear pore complexes, where large and small subunits reunite to form functional ribosomes (Greber, 2016).

Ribosome biogenesis is essential for normal cell growth and proliferation. Defects in ribosome biogenesis are associated with a group of diseases collectively called ribosomopathies, including Diamond-Blackfan anemia (DBA), Dyskeratosis congenita (DKC), Treacher Collins syndrome (TCS), etc (Dai and Lu, 2008; Teng et al., 2013; Raiser et al., 2014; Danilova and Gazda, 2015; Mills and Green, 2017), whereas aberrant over-activation of ribosome biogenesis and translation is tightly linked to human cancer, thus being a therapeutic target (Bywater et al., 2012; Quin et al., 2014; Brighenti et al., 2015; Gentilella et al., 2015; Derenzini et al., 2017; Sulima et al., 2017; Pelletier et al., 2018). Therefore, it is crucial to understand how ribosome biogenesis is regulated during normal cell homeostasis and how it is deregulated in human diseases. Indeed, diverse cell growth or suppression signaling pathways have been shown to regulate ribosome biogenesis, such as mTOR, RAS, MYC oncogenic signaling, and p53, RB, and PTEN tumor suppressors (Dai et al., 2007; Dai and Lu, 2008). Recent studies including ours have shown that SUMOylation plays a critical role in regulating ribosome biogenesis. In this mini-review, we summarize the current understanding of SUMO regulation of ribosome biogenesis.

SUMOylation and its role in ribosome biogenesis

SUMOylation is a posttranslational modification of proteins by covalent conjugation of small ubiquitin-like modifiers (SUMOs), which plays a crucial role in the regulation of many cellular processes, including transcription, RNA splicing and processing, chromatin dynamics, DNA replication, DNA damage repair, cell cycle control, as well as ribosome biogenesis (Geiss-Friedlander and Melchior, 2007; Bergink and Jentsch, 2009; Finkbeiner et al., 2011a; Cubenas-Potts and Matunis, 2013; Jentsch and Psakhye, 2013; Raman et al., 2013; Chymkowitch et al., 2015a; Eifler and Vertegaal, 2015; Enserink, 2015; Nuro-Gyina and Parvin, 2015; Sarangi and Zhao, 2015). Mammals express three main SUMO isoforms, SUMO1-SUMO3. SUMO2 and SUMO3 are 97% identical (referred to as SUMO2/3) and each shares 45% sequence identity with SUMO1 (Kamitani et al., 1997; Kamitani et al., 1998a; Kamitani et al., 1998b; Muller et al., 2001; Geiss-Friedlander and Melchior, 2007). Like ubiquitination, SUMOylation occurs through sequential reactions involving a heterodimeric SUMO-activating enzyme SAE1/SAE2 (E1), a single SUMO-conjugating enzyme Ubc9 (E2) and a SUMO ligase (E3) in an ATP-dependent manner (Muller et al., 2001; Geiss-Friedlander and Melchior, 2007; Hay, 2013). In the final step, Ubc9 transfers SUMO to substrate acceptor lysine (Lys, K) residues via an isopeptide linkage, which is facilitated by SUMO E3s (Muller et al., 2001; Geiss-Friedlander and Melchior, 2007; Hay, 2013) (Figure 1). The SUMO acceptor Lys is often present within a

conserved ΨKxE motif, where Ψ is a large hydrophobic amino acid and x is any amino acid (Rodriguez et al., 2001; Sampson et al., 2001; Geiss-Friedlander and Melchior, 2007; Gareau and Lima, 2010). Unlike ubiquitination which requires a ubiquitin (Ub) E3, SUMO E1 and Ubc9 can mediate SUMOylation, albeit with less efficiency, in the absence of SUMO E3. Also, while there are over 600 ubiquitin E3s in human (Deshaies and Joazeiro, 2009; Metzger et al., 2012), only a small number of SUMO E3 have been identified that are classified into three categories: a family of SP-RING-containing proteins including *PIAS/siz family members* (PIAS1, PIAS3, PIASxα, PIASxβ and PIASy (Kahyo et al., 2001; Sachdev et al., 2001; Nakagawa and Yokosawa, 2002; Nishida and Yasuda, 2002; Schmidt and Muller, 2002)) and Nse2 (Andrews et al., 2005; Potts and Yu, 2005; Berkholz et al., 2014), the nuclear pore Ran Binding Protein 2 (*RanBP2*) (Pichler et al., 2002; Reverter and Lima, 2005), and the ZNF451 family proteins (ZNF451-1, ZNF451-2, ZNF451-3) (Cappadocia et al., 2015; Eisenhardt et al., 2015; Varejao et al., 2020). In addition, a few other proteins possess SUMO E3-like activity such as Pc2, RSUME, TRIM28, SLX4 (Kagey et al., 2003; Morita et al., 2005; Weger et al., 2005; Carbia-Nagashima et al., 2007; Pungaliya et al., 2007; Oh et al., 2010; Pelisch et al., 2010; Chu and Yang, 2011; Yang et al., 2011; Oh and Chung, 2013; Guervilly et al., 2015; Neo et al., 2015; Ouyang et al., 2015; Yamashita et al., 2016) and Cajal body marker protein Coilin (Lett et al., 2023). SUMOylation is also a reversible modification. A group of deSUMOylating enzymes or SUMO proteases, including SENP1-3, SENP5-7, DESI-1, DESI-2, and USPL1, catalyze the removal of SUMO from substrates (Hickey et al., 2012; Kunz et al., 2018). Therefore, SUMOylation is highly dynamic in cells.

The SUMO modification facilitates and/or stabilizes protein-protein interaction through SUMO binding to the SUMO-interacting motif (SIM) in other proteins (Gareau and Lima, 2010; Jentsch and Psakhye, 2013). SIMs are characterized by a short stretch of core hydrophobic amino acids (V/I)x(V/I)(V/I) flanked by acidic residues (Song et al., 2004; Hannich et al., 2005; Song et al., 2005; Kerscher, 2007). SUMOylation can also interfere with protein-protein interactions through steric hindrance (Moldovan et al., 2006) or compete with other lysine-directed modifications like acetylation or ubiquitination (Desterro et al., 1998). Consequently, SUMOylation can regulate protein localization, trafficking, stability and activity (Gareau and Lima, 2010; Jentsch and Psakhye, 2013). Interestingly, emerging evidence suggests that SUMOylation tends to target an entire group of functionally and physically connected proteins, called protein group SUMOylation (Psakhye and Jentsch, 2012; Jentsch and Psakhye, 2013), such as DNA damage repair proteins (Psakhye and Jentsch, 2012), yeast septins (Johnson and Blobel, 1999; Johnson and Gupta, 2001; Takahashi et al., 2001), telomeres (Potts and Yu, 2007; Ferreira et al., 2011; Hang et al., 2011) and ribosome biogenesis-related proteins (Panse et al., 2006; Finkbeiner et al., 2011a; Finkbeiner et al., 2011b; Castle et al., 2012). Multiple SUMO-SIM interactions contribute to the formation and stabilization of the multi-protein complexes (Psakhye and Jentsch, 2012; Jentsch and Psakhye, 2013).

SUMOylation has been shown to play a vital role in regulating ribosome biogenesis (Han et al., 2018). It was initially found that many ribosome biogenesis factors and pre-ribosomal particles are modified by SUMO and the SUMOylation of pre-ribosomal particles in the nucleus and subsequent deSUMOylation at the nuclear pore complex (NPC) is necessary for efficient ribosome biogenesis and export in yeast (Panse et al., 2006). Recent proteomic studies found that ribosome biogenesis-related proteins are one of the major groups of SUMOylated proteins (Vertegaal et al., 2004; Matafora et al., 2009; Amente et al., 2012; Hendriks et al., 2014). In human, several nucleolar proteins critical for rRNA synthesis, processing and ribosome maturation, such as nucleophosmin (NPM) (Tago et al., 2005; Liu et al., 2007), nucleolin (Zhang et al., 2015) and Las1L (Finkbeiner et al., 2011b; Castle et al., 2012), are modified by SUMOylation. Interestingly, proteins (e.g., Nop58, Nhp2 and DKC1) in small nucleolar ribonucleoprotein (snoRNP) complexes that are responsible for rRNA 2′-O-ribose methylation and pseudouridylation critical for rRNA processing (Mannoor et al., 2012; Watkins and Bohnsack, 2012; Lui and Lowe, 2013; Dupuis-Sandoval et al., 2015) are modified by SUMO (Matic et al., 2010; Westman et al., 2010). SUMOylation of Nop58 has been shown to be essential for its efficient binding to snoRNAs (Westman et al., 2010). Similarly, components of the RNA degradation rixosome or Rix1 complex (PELP1, TEX10, WDR18, LAS1L, NOL9, MDN1, and SENP3) critical for ribosome biogenesis (Raman et al., 2016; Chen et al., 2018; Vanden Broeck and Klinge, 2023) and gene silencing (Shipkovenska et al., 2020; Zhou et al., 2022; Zhou et al., 2023), are also modified by SUMOylation. PELP1 SUMOylation is critical for ribosome maturation by promoting the recruitment of MDN1, an AAA ATPase that removes assembly factors from the pre-60S particles (Chen et al., 2018). On the other hand, the removal of SUMO via deSUMOylation is also important for ribosome biogenesis. DeSUMOylation of NPM, a nucleolar protein required for rRNA processing (Savkur and Olson, 1998; Itahana et al., 2003), by SENP3, is critical for 28S rRNA maturation and the subsequent nucleolar export of the 60S pre-ribosomal subunit (Haindl et al., 2008). Depletion of SENP3 inhibits rRNA processing reminiscent of the NPM knockdown (Yun et al., 2008). Likewise, deSUMOylation of LAS1L and PELP1 by SENP3 is essential for these proteins partitioning from the nucleoplasm to nucleolus (Finkbeiner et al., 2011b; Castle et al., 2012), and deSUMOylation of PELP1 is needed to release both MDN1 and PELP1 from pre-ribosomes (Raman et al., 2016). Thus, balanced levels of SUMOylation and deSUMOylation of ribosome biogenesis factors are critical for ribosome biogenesis and maturation in the nucleus.

SUMOylation also regulates rDNA transcription. Genome-wide binding of SUMO pathway proteins in both human fibroblast cells and yeast showed that SUMO is widely distributed in pro-growth genes, including ribosomal protein genes (RPGs), tRNA genes and rDNA (Neyret-Kahn et al., 2013; Chymkowitch et al., 2015b). However, there are conflicting results regarding how SUMOylation regulates the transcription of these ribosome biogenesis-related genes. SUMOylation of Rap1 has been shown to stimulate the recruitment of basal transcription factors to promote RPG transcription in yeast (Chymkowitch et al., 2015b). Consistently, the recruitment of SUMO isopeptidase Ulp2 to rDNA promotes rDNA silencing by opposing the degradation of silencing protein Tof2 and Net1 mediated by the SUMO-targeted ubiquitin ligase (STUBL) Slx5:Slx8 (Gillies et al., 2016; Liang et al., 2017). Deletion of Ulp2 leads to the increased expression of rRNA, snoRNAs, RP genes, and ribosome biogenesis factors (Ryu et al.,

2018). Yet, other studies have suggested that SUMOylation negatively regulates rRNA transcription by mediating rDNA silencing. Both SUMO and Ubc9 are required for efficient repression of interrupted rDNA units and variable expression of intact rDNA. Disruption of the SUMO pathway leads to uniformly high expression of individual rDNA in single cells (Luo et al., 2020). Similarly, inhibiting SUMOylation by knockdown of Ubc9 has been shown to promote gene transcription resulting in senescence in human fibroblast cells (Neyret-Kahn et al., 2013). SUMOylation-induced inhibition of upstream-binding factor (UBF) and c-Myc expression was shown to be primarily responsible for the repression of rDNA transcription (Peng et al., 2019). Interestingly, accumulation of Tof2 due to the loss of the SUMO ligase Siz1 can also result in increased rDNA recombination (Abraham et al., 2019). rDNA is highly dynamic and vulnerable to excess recombination events. Also, rDNA damage repair via homologous recombination requires transient release of rDNA from the nucleolus that is controlled by SUMOylation of RAD52 (Torres-Rosell et al., 2007) and the CLIP-cohibin complex (Capella et al., 2021) in yeast. Thus, SUMOylation can prevent overactivation of rDNA recombination. Further studies are needed to clarify the cell context-dependent and dynamic role of SUMOylation in regulating rDNA transcription, recombination and damage repair.

USP36, a nucleolar deubiquitinating enzyme

USP36 was initially identified by sequence alignment and differential display in ovarian cancer cell lines as a ubiquitously expressed deubiquitinating enzyme (DUB) (Li et al., 2008). USP36 contains a ubiquitin-specific protease (USP) domain at its N-terminus and a nucleolar localization signal (NoLS) at its C-terminus (Endo et al., 2009a; Endo et al., 2009b), the rest of the protein are largely unstructured (Reed et al., 2015). Early work by Komada group has revealed that USP36 is a nucleolar DUB implicated in nucleolar structure and function in ribosomal biogenesis by deubiquitinating NPM and fibrillarin (Endo et al., 2009a; Endo et al., 2009b). Subsequently, USP36 has been shown to deubiquitinate and stabilize the RNA helicase DHX33 (Fraile et al., 2018). Also, human USP36 can restore the stability and levels of Rpa190, the largest subunit of RNA Pol I, pre-rRNA processing, and cell growth of yeast strain with the deletion of the yeast ortholog Ubp10 (Thevenon et al., 2009), suggesting that USP36 may regulate the ubiquitination and stability of human RNA Pol I. Knockdown of USP36 results in the morphological changes in the nucleolus and defects in rRNA synthesis and processing (Endo et al., 2009b; Fraile et al., 2018; Ryu et al., 2021; Chen et al., 2023). Thus, USP36 plays a critical role in the regulatory network of ribosome biogenesis and it is not surprising that homozygous deletion of the USP36 gene in mice is embryonic lethal (Fraile et al., 2018; Ryu et al., 2021). Furthermore, we identified that USP36 deubiquitinates c-Myc and stabilizes c-Myc in the nucleolus (Sun et al., 2015a; Sun et al., 2015b). c-Myc is considered as a master regulator of ribosome biogenesis as it promotes transcription by all three classes of RNA Polymerases (van Riggelen et al., 2010). The role of USP36 as a c-Myc DUB in promoting cell growth has been further demonstrated in a number of model systems including *Drosophila*, in which a nucleolar isoform of *Drosohpila* USP36 (dUSP36) stabilizes dMYC and is essential for cell growth and *drosophila* development (Liu et al., 2019; Zhang et al., 2019; Thevenon et al., 2020; Deng et al., 2022). In addition, USP36 has been shown to deubiquitinate and stabilize N-Myc (MYCN) in neuroblastoma cells (Juvvuna et al., 2021). Thus, by regulating c-Myc, USP36 also coordinates ribosome biogenesis with cell cycle progression. Together, it appears that the major cellular function of USP36 is to contribute to ribosome biogenesis in the nucleolus. Supporting this notion is that our recently purified USP36-associated protein complex was enriched ribosome biogenesis-related nucleolar proteins (Chen et al., 2023).

In addition to its role in ribosome biogenesis, USP36 also exerts notable effects in other cellular processes by deubiquitinating various substrates (Table 1), including cell survival, gene expression, and apoptosis, underscoring its multifunctionality in cells (Zhu et al., 2021). Like its yeast homolog Ubp10 (Gardner et al., 2005) and *Drosophila* homolog dUSP36/Scny (Buszczak et al., 2009), USP36 is a histone H2B deubiquitinating enzyme and regulates histone H2B modifications and thus gene transcription (DeVine et al., 2018). *Drosophila* USP36 interacts with immune deficiency (IMD) and prevents K63-polyubiquitinated IMD accumulation while promoting IMD degradation, thereby preventing constitutive immune activation (Thevenon et al., 2009). Deletion of dUSP36 also suppresses cell growth by inducing autophagy (Taillebourg et al., 2012). USP36 interacts with chromodomain helicase DNA binding protein 7 (CHD7) and deubiquitinates and stabilizes CHD7, resulting in increased expression of SOX9 to promote neuroblastoma growth (Mondal et al., 2018). USP36 also stabilizes mitochondria SOD2 (Kim et al., 2011) to protect cells from ischemia-induced apoptosis (Liu et al., 2019). Recently, it has also been shown that USP36 deubiquitinates and stabilizes the DNA damage-tolerant polymerase PrimPol, thus regulating chemotherapy response (Yan et al., 2020). USP36 has been shown to deubiquitinate and stabilize SNAIL2, thus promoting epithelial-mesenchymal transition (EMT) (Qiu et al., 2021). Likewise, USP36 deubiquitinates and stabilizes the nucleolar SNAIL1 to promote ribosome biogenesis and tumor cell survival under ribotoxic stress and contributes to chemoresistance (Qin et al., 2023). USP36 stabilizes YAP and regulates the Hippo signaling in esophageal squamous carcinoma cells (Zhang et al., 2022) and TAZ activation (Wang et al., 2022). USP36 regulates the levels of PKM2 to promote aerobic glycolysis (Wu et al., 2023). By deubiquitinating and stabilizing the m6A demethylase ALKBH5, USP36 regulates its function in gene expression and glioblastoma progression (Chang et al., 2023). USP36 also deubiquitinates ALR (augmenter of liver regeneration) and may play a role in the transition of the steatotic hepatocytes into malignant hepatocellular carcinoma (HCC) (Zheng et al., 2023). Thus, USP36 may play a crucial role in diverse cellular processes beyond ribosome biogenesis.

USP36 acts as a novel SUMO ligase

Intriguingly, we recently found that USP36 acts as a novel SUMO ligase to promote nucleolar protein SUMOylation (Ryu et al., 2021). USP36 has all SUMO E3 characteristics: 1) It binds to Ubc9; 2) It binds to SUMO; 3) It promotes SUMOylation of

TABLE 1 List of the DUB and SUMO ligase substrate proteins regulated by USP36.

Name	Mode of action by USP36	References
DUB substrates		
NPM	Deubiquitinates and stabilizes NPM and regulates ribosome biogenesis, nucleoli structure and cell growth	Endo et al. (2009b)
Nucleolin	Deubiquitinates and stabilizes Nucleolin and regulates ribosome biogenesis, nucleoli structure and cell growth	Endo et al. (2009b)
DHX33	Deubiquitinates and stabilizes the DEAH-box RNA helicase DHX33, playing a role in rRNA processing, translation and cell survival	Fraile et al. (2018)
c-Myc	Deubiquitinates and stabilizes c-Myc and promotes its transactivation activity and role in cell growth and proliferation	Sun et al. (2015a), Liu et al. (2019), Zhang et al. (2019), Thevenon et al. (2020), Deng et al. (2022)
MYCN	Deubiquitinates and stabilizes MYCN and regulates neuroblastoma cell growth	Juvvuna et al. (2021)
PrimPol	Deubiquitinates K29-linked polyubiquitination of the DNA damage tolerant polymerase PrimPol and increases its stability, playing a role in cell response to DNA replication stress	Yan et al. (2020)
Snail1	Deubiquitinates and stabilizes Snail1 in the nucleolus, promoting ribosome biogenesis and tumor cell survival in response to ribotoxic stress	Qin et al. (2023)
Snail2	Deubiquitinates and stabilizes Snail2, mediating epithelial-mesenchymal transition (EMT) in glioblastoma	Qiu et al. (2021)
YAP	Deubiquitinates and stabilizes YAP and promotes esophageal squamous carcinoma cell growth and progression via the Hippo/YAP signaling	Zhang et al. (2022)
DOCK4	Deubiquitinates and stabilizes DOCK4, a guanine nucleotide exchange factor, and promotes Wnt/β-catenin signaling and EMT induced by high glucose of diabetic renal tubular epithelial cells	Zhu et al., (2021)
CHD7	Deubiquitinates and stabilizes CHD7, promoting SOX9 expression and neuroblastoma cell growth	Mondal et al. (2018)
SOD2	Deubiquitinates and stabilizes mitochondria SOD2 to protect cells from ischemia-induced apoptosis	Kim et al. (2011), Liu et al. (2019)
PKM2	Deubiquitinates and stabilizes PKM2 and regulates aerobic glycolysis in breast cancer cells	Wu et al. (2023)
ALKBH5	Deubiquitinates and stabilizes the m^6A demethylase ALKBH5 and regulates the stemness and tumorigenesis of glioblastoma	Chang et al. (2023)
ALR	Deubiquitinates and stabilizes ALR and regulates the mitochondrial integrity	Zheng et al. (2023)
H2B	Deubiquitinates H2Bub1 and regulates gene transcription	DeVine et al. (2018)
SUMO substrates		
Nop58, Nop56	SUMOylates Nop58 and Nop56 and promotes Nop58 binding to Box C/D snoRNAs, playing a role in pre-rRNA processing	Ryu et al. (2021)
Nhp2, DKC1	SUMOylates Nhp2 and DKC1 and promotes Nhp2 binding to Box H/ACA snoRNAs, playing a role in pre-rRNA processing	Ryu et al. (2021)
EXOSC10	SUMOylates the RNA exosome catalytic subunit EXOSC10 and promotes its binding to rRNA and RNA exosome activity in rRNA processing	Chen et al. (2023)
DGCR8	SUMOylates DGCR8 and plays a role in regulating microRNA biogenesis	Li et al. (2023)

multiple proteins including components of snoRNPs in cells; 4) It SUMOylates multiple substrates, including Nop58, Nhp2 and PARP1, *in vitro* using recombinant proteins; 5) The full USP36's SUMO E3 activity requires the SUMO interaction with the backside of Ubc9 (Ryu et al., 2021), as in the case of PIAS1 and ZNF451 family SUMO ligases (Cappadocia et al., 2015; Eisenhardt et al., 2015; Koidl et al., 2016; Eisenhardt et al., 2019). Mutating either Phe 22 of Ubc9 or Asp 63 of SUMO2, which disrupts the SUMO-Ubc9 backside interaction, markedly inhibited USP36's activity to promote SUMOylation *in vitro* (Ryu et al., 2021). These data suggest that USP36 possesses a SUMO ligase activity, arguing that it acts as an adaptor protein to increase Ubc9 function. The SUMO ligase activity is located at the N-terminal domain which overlaps with the USP domain. Interestingly, mutating the DUB catalytic Cys 131 (C131) that inactivates USP36's DUB activity also abolished its SUMO ligase activity (Ryu et al., 2021). We did find that mutating the DUB catalytic triad residue His 382 to Ala (H382A) abolishes its DUB, but not SUMO E3, activity (Ryu

et al., 2021). However, mutant(s) specifically inactivating SUMO E3, but not DUB activity, are yet to be identified. Thus, further structural analyses are warranted to delineate the mechanism of the SUMO ligase activity and the crosstalk between its DUB activity and SUMO E3 activity. Nevertheless, to the best of our knowledge, USP36 is the only nucleolar SUMO ligase and the only protein with DUB and SUMO ligase dual functions identified so far.

Roles of USP36 in nucleolar SUMOylation

We showed that USP36 mainly promotes the nucleolar protein SUMOylation (Ryu et al., 2021), suggesting that its SUMO ligase function is linked to its role in regulating nucleolar dynamics and ribosome biogenesis (Table 1). Our initial proteomic analysis identified Nop58, a component of Box C/D snoRNP complex, as one of the proteins whose SUMOylation is increased by overexpression of USP36. We subsequently revealed that Nop56 from Box C/D snoRNP and Nhp2 and DKC1 from Box H/ACA snoRNP complex are all SUMOylated by USP36 (Ryu et al., 2021), supporting the notion that SUMOylation occurs in multiple proteins in protein complexes. Consistent with early studies showing that Nop58 SUMOylation regulates its binding to snoRNAs, we also showed that USP36-mediated SUMOylation promotes Nop58 binding to snoRNAs, implying that SUMO modification of the snoRNP complex may stabilize the complex and facilitate its conformation favoring snoRNA binding. We then purified USP36-associated protein complex that enriches ribosome biogenesis-related proteins, including all the ten components of the nucleolar RNA exosome complex. We found that USP36 interacts with the RNA exosome by binding to the exoribonuclease protein EXOSC10 (Chen et al., 2023) and SUMOylates EXOSC10 at Lys 583. We showed that USP36 SUMOylation of EXOSC10 promotes its binding to rRNA and the activity of the RNA exosome in rRNA processing, underscoring its role in maintaining rRNA biogenesis and cellular growth. Additionally, the perturbation of ribosome biogenesis (ribosomal stress) reduces USP36 levels and results in a noticeable reduction in EXOSC10 SUMOylation. Therefore, USP36 acts as a nucleolar SUMO E3 ligase to SUMOylate EXOSC10 and promotes the functionality of RNA exosomes within the realm of ribosome biogenesis. It would be interesting to test whether USP36 also promotes group SUMOylation of core exosome subunits and its adaptor proteins such as TRAMP complexes (Lubas et al., 2011; Kilchert et al., 2016; Zinder and Lima, 2017).

Notably, it appears that the levels and stability of both snoRNP and RNA exosome complex proteins are not significantly regulated by USP36, suggesting that at least under normal cell growth conditions, USP36 SUMO ligase activity may play a major role in regulating the targeting of these complexes to rRNA and thereby facilitating rRNA processing compared to its DUB activity. Further supporting the role of these regulations, we showed that USP36 is critical for multiple steps of rRNA processing, including RNA exosome-mediated 5.8S rRNA maturation as well as the maturation of both 18S and 28S rRNA. Knockdown of USP36 results in the accumulation of pre-rRNAs and reduction of 21S, 12S and 7S rRNA intermediates. Interestingly, we observed that USP36 strongly binds to pre-rRNA (Chen et al., 2023). The RNA-binding property of USP36 has also been shown by others wherein it binds to long noncoding RNAs (lncRNAs) (Mondal et al., 2018) and microRNAs (Treiber et al., 2017; Li et al., 2023). Indeed, it plays a role in microRNA biogenesis by binding to the microprocessor complex and mediating DGCR8 SUMOylation in the nucleolus (Li et al., 2023). These studies suggest that USP36 itself might be an RNA-binding protein. Further studies are warranted to examine whether USP36 binds to these RNAs directly or indirectly through its interacting partner proteins.

Our proteomic analysis revealed that USP36 associates with a large group of nucleolar proteins involved in ribosome biogenesis (Chen et al., 2023), suggesting that USP36 may mediate group SUMOylation of additional protein complexes in the nucleolus and possibly regulates SUMOylation-ubiquitination crosstalk. By doing so, USP36 may act as a nucleolar regulation hub controlling nucleolar dynamics and protein-RNA complex formation, stability, and activity in ribosome biogenesis.

Together, it is evident that USP36, an established DUB, is not solely confined to this role, as it also plays a pivotal role in orchestrating the SUMOylation of the nucleolar proteins involved in ribosome biogenesis, thereby extending the regulatory landscape of USP36 and shedding light on its multifaceted involvement in cellular processes.

USP36 is upregulated in cancers and could be a therapeutic target

An increasing body of research highlights the aberrant expression of USP36 in various cancers and its crucial role in tumor development and growth. USP36 has been shown to be overexpressed in various human cancers, including breast cancer (Sun et al., 2015a; Wu et al., 2023), ovarian cancer (Yan et al., 2020), esophageal squamous cell carcinoma (Zhang et al., 2022), gastric cancer (Wang et al., 2022), hepatocellular carcinoma (HCC) (Sun et al., 2022), lung cancer (Deng et al., 2022), neuroblastoma (Juvvuna et al., 2021), and glioblastoma (Chang et al., 2023). High expression of USP36 correlates with poor patient prognosis in these tumors. Consistently, the knockdown of USP36 suppresses cancer cell proliferation and xenograft tumor growth in various tumor types (Sun et al., 2015a; Juvvuna et al., 2021; Wang et al., 2022; Chang et al., 2023). These studies also established the regulation of an array of target proteins by USP36 via deubiquitination, whose expression is correlatively upregulated in these cancers, including MYC, EMT factor SNAILs (Qiu et al., 2021; Qin et al., 2023), YAP (Zhang et al., 2022) and TAZ (Wang et al., 2022) in Hippo signaling, PrimPol in DNA replication and replication stress (Chang et al., 2023), and PKM2 in glycolysis (Wu et al., 2023). It will be interesting to test whether these substrates are also regulated by USP36's SUMO ligase activity. Intriguingly, it is observed that high USP36 expression positively correlated with both macrophage infiltration levels and multiple immune checkpoint molecules in HCC tissues (Sun et al., 2022). These studies, together with the established role of USP36 in ribosome biogenesis shed light on a potential role for USP36 in tumor growth and progression and thus as a promising therapeutic target in cancer.

Selective USP36 DUB inhibitor is currently not available. Future efforts are thus warranted to develop USP36 DUB-specific inhibitors. Particularly, USP36 possesses DUB/SUMO ligase dual activities and both activities positively regulate cell growth and proliferation. Thus, targeting both DUB and SUMO ligase activities would have extra benefit and this is likely, as we have observed that inactivating USP36's DUB activity by mutating the DUB catalytic Cys 131 also abolishes its SUMO ligase activity. Developing inhibitors specifically targeting USP36's SUMO ligase activity would be even more promising as this could avoid the non-specific effects of DUB inhibitors. Cancer cell proliferation and survival often require sustained oncogenic signaling called oncogene addiction, as well as elevated ribosome biogenesis and protein translation. Therefore, inhibiting ribosome biogenesis would be synthetic lethal to these cancer cells addictive to oncogenic signals, providing a therapeutic window to overcome USP36's essential role in normal cell growth. Supporting the therapeutic role of USP36, ribosome biogenesis has emerged as a key therapeutic target. Early chemotherapeutic compounds such as actinomycin D, etoposide, and 5-fluorouracil inhibit ribosome biogenesis by blocking rRNA synthesis and/or inhibiting rRNA processing (Ferreira et al., 2020). The small molecule CX-5461 is a potent selective inhibitor of RNA Pol I transcription by targeting the interaction between SL-1 transcription factor complex and RNA Pol I (Drygin et al., 2011; Bywater et al., 2012; Haddach et al., 2012). Recent phase I clinical trial showed that CX-5461 has anti-tumor activity against advanced hematologic cancers (Khot et al., 2019). In addition, inhibiting USP36 would also have therapeutic effects via targeting cellular pathways depicted above beyond ribosome biogenesis. A recent study reported that a natural product extracted from *Baccharis coridifolia*, OST-01, has anti-leukemic activity by targeting c-MYC via down-regulating USP36, although it is not clear whether USP36 is the direct target of OST-01 (Kang et al., 2024). Future studies will delve deeper into the feasibility of USP36 as a potential therapeutic target, offering novel insights for the advancement of cancer treatment strategies.

Perspective

The exploration of USP36's multifaceted roles in ribosome biogenesis has yielded valuable insights into its intricate functions on cell growth and proliferation. As we consider the implications of these findings, several exciting perspectives and potential avenues for future research emerge. Identifying additional substrates in the nucleolus and deciphering their influence on various cellular processes beyond ribosome biogenesis would delve into the broader landscape of USP36-mediated SUMOylation. Elucidating the mechanistic details of how USP36 promotes malignancy and its interactions with specific oncogenic pathways could pave the way for the development of targeted therapies that disrupt these processes and hinder tumor growth. Structural and mechanistic characterization of USP36 to differentiate its DUB activity from SUMO ligase activity would help develop DUB-specific, SUMO ligase-specific, or DUB/SUMO ligase dual inhibitors. Investigating potential crosstalk between USP36 and other cellular pathways could integrate into broader cellular networks, such as DNA repair, cell cycle control, and autophagy, providing new perspectives on its role in maintaining cellular homeostasis. In conclusion, the multifaceted roles of USP36 in ribosome biogenesis, SUMOylation, and cancer have set the stage for exciting future research endeavors.

Author contributions

YY: Writing–original draft. YL: Writing–review and editing. RS: Writing–review and editing. X-XS: Writing–original draft, Writing–review and editing. M-SD: Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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