Role of p97/VCP (Cdc48) in genome stability

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INTRODUCTION

Genome stability is a prerequisite for cell survival, cancer prevention, and control of aging (Papanicolaou-Chromak and Peterson, 2013). The genome is constantly attacked by various reactive oxygen species from endogenous sources. In addition to endogenous sources, DNA lesions can also be generated by a variety of exogenous sources, such as ionizing radiations (IR), the ultraviolet light (UV), and many chemical agents, some of which are products of our industrialized society. It is estimated that the mammalian genome accumulates thousands of DNA lesions every day that disturb DNA synthesis and cell division, two essential processes in genome amplification, preservation and transition to the next generation (Jackson and Bartek, 2009). Failure to maintain genome stability leads to chromosomal aberrations, gene mutations, and cancer or cell death. To maintain genomic stability, cells respond to DNA damage by activating a spatiotemporal signaling pathway known as the DNA damage response (DDR; Ciccia and Elledge, 2010). Following DNA damage, the DDR drives cell cycle checkpoints and initiates DNA repair or induces apoptosis if the genome is severely damaged. Although DDR after double-strand break (DSB) has been widely investigated and is considered as a DDR model, other types of DNA lesions can cause activation of lesion-specific DDRs that signal and recruit appropriate sensor, transducer, and effector proteins (Jackson and Bartek, 2009). For example, unrepaired DNA lesions that enter S phase encounter a serious problem during DNA replication. When the DNA replication fork approaches these lesions, the cell activates the DNA damage tolerance (DDT) pathway, which enables survival by activating the translesion DNA synthesis (TLS) pathway (Friedberg, 2005). The TLS pathway enables recruitment of translesion DNA polymerases (DNA pols), which can bypass bulky DNA lesions and ensure continuous DNA synthesis. Activation of lesion-specific DDR and appropriate DNA repair mechanisms or DDT ensures cell survival and prevents genomic instability and cancer. DDR and DDT are controlled by various post-translational modifications (PTMs) in which ubiquitination and sumoylation play an essential role (Bergink and Jentsch, 2009; Al-Hakim et al., 2010; Ulrich and Walden, 2010; Bekker-Jensen and Madlani, 2011; Lehnmann, 2011b; Pakkala and Jentsch, 2012). Ubiquitination and sumoylation regulate timely assembly and dis-assembly of various DNA repair and genome caretaker molecules. Disturbances in this tight regulation could cause severe defects in the DDR and lead to genomic instability, which has been demonstrated in certain types of breast and ovarian cancers and patients with RIDDLE syndrome (Blundred and Stewart, 2011; Lipkowitz and Weissman, 2011). Mutations in BRCA1, an E3 ubiquitin ligase involved in DDR, can result in breast and ovarian cancers, while mutations in the DDR-related E3 ligase RNF168 can cause RIDDLE syndrome, which is characterized by radiation hyper-sensitivity, immunodeficiency, dysmorphic features, and learning difficulties. Because many chromatin-associated DDR proteins are tightly bound to sites of DNA damage and are often protected from degradation, spatiotemporal turnover and degradation are facilitated by the ubiquitin-proteasome system (UPS; Dianov, 2011; Levy-Bard et al., 2011; Ramadan and Meirang, 2011). It is still not known how the UPS and its largest component, the proteasome, approach, remove and degrade tightly bound protein complexes on chromatin. Discovery of a p97 function related to...
In recent years, ubiquitin-dependent molecular chaperone p97 has emerged as an essential regulator of the UPS (ubiquitin-dependent protein degradation) pathway. This ATPase/UFD activity, p97 extracts (segregates) polyubiquitinated proteins from diverse cellular locations and presents them for proteasome degradation. p97 is also involved in degradation of highly folded ubiquitinated soluble proteasome substrates and thus functions as unfoldase (Beskow et al., 2009). Recently elucidated functions of p97 in several DNA metabolic processes indicate that the protein is a constitutive component of various essential chromatin-related processes in the cell cycle, DNA replication and repair, mitosis, and transcription. However, the function of p97 as it relates to genome stability has not yet been established.

In this review, we discuss various p97 functions in chromatin-related processes essential for maintenance of genome stability and attempt to establish p97 as a genome caretaker. We also present a large body of evidence suggesting that proteaseduced chromatin stress (PICHROS) induces genotoxic stress, ultimately leading to genome instability.

**UBIQUITIN-DEPENDENT MOLECULAR CHAPERONE p97**

Molecular chaperone p97, also known as VCP in vertebrates, Cdc48 in S. cerevisiae, Cdc48 in C. elegans, TER94 in Drosophila, and VAT in archaeabacteria, is a class II member of the ATPase associated with diverse cellular activities (AAA) ATPases (Woodman, 2003; Halaswani and Lutterbach, 2010; Jentsch and Rumpf, 2007; Stölz et al., 2011; Meyer et al., 2012; Verma et al., 2013). p97 is highly conserved from archaeabacteria to humans and is one of the most abundant proteins in the cytoplasm and nucleoplasm (Peters et al., 1999). p97 is a homohexameric barrel-like molecular machine composed of the following domains: N-terminal, two ATPase cassette (D1 and D2) and a C-terminal domain (Figure 1A). The N-terminal region of p97 binds a myriad of substrates primarily embedded in different cellular structures, such as the endoplasmic reticulum (ER), ribosomes, membrane vesicles, spindles, or chromosomes. After it is bound to the substrate, p97 uses ATP hydrolysis in the D2 domain to stimulate major intrinsic conformational changes (Woodman, 2003) that are transmitted throughout the entire p97 molecule to the N-terminal domain (Mouiller et al., 2002; Li et al., 2012). Intrinsic conformational changes allow p97 to remodel and thus extract (segregate activity) bound substrates from diverse cellular structures or protein complexes. Beside its segregase activity from different cellular structures, p97 serves as unfoldase in processing of highly folded soluble proteasome substrates (Beskow et al., 2009). The majority of p97 substrates are ubiquitinated, and ubiquitin serves as a major regulator of p97 function (Yeung et al., 2008; Hanzelmann et al., 2011). It is a highly conserved scaffold protein involved in diverse pathways and substrates is governed by different p97 adaptor protein (VCP) or Cdc48, a central element of the UPS and is involved in degradation of highly folded ubiquitinated soluble proteasome substrates and thus functions as unfoldase (Beskow et al., 2009). Recently elucidated functions of p97 in several DNA metabolic processes indicate that the protein is a constitutive component of various essential chromatin-related processes in the cell cycle, DNA replication and repair, mitosis, and transcription. However, the function of p97 as it relates to genome stability has not yet been established.

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In addition to its function in the UPS, in the last few years p97 function emerged in maintenance of cellular homeostasis by regulating two closely related processes, autophagy and endosomal trafficking (Ju et al., 2009; Tesse et al., 2010; Ritz et al., 2011). p97 plays an essential role in maturation of ubiquitin-coating autophagosomes, suggesting its function in autophagic degradation of ubiquitinated substrates. The focus of this review is the
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FIGURE 1 | p97 and its three core adaptors regulate diverse cellular functions. (A) Domain structure of p97 monomer: N-terminal region (yellow), L1 and L2 linker regions (gray), two ATPase cassettes D1 and D2 (green), and the C-terminal tail (blue). (B) p97 homohexamer forms a barrel-like structure that mutually binds one of three core adaptor complexes, Npl4−Ufd1 heterodimer, UBXD1 or p47, and regulates diverse cellular functions as indicated in white boxes. ERAD, endoplasmic reticulum-associated degradation; RAD, ribosomal-associated degradation; CAD, chromatin-associated degradation.

function of p97 in CAD and genome stability. For details on p97 function in autophagy and endosomal trafficking, see elsewhere (Ju and Weihl, 2010b; Bug and Meyer, 2012).

CHROMATIN-ASSOCIATED PROTEIN DEGRADATION AND PROTEIN-INDUCED CHROMATIN STRESS

Chromatin is a large protein-integration platform in which structural, dynamic, and spatiotemporal association with proteins involved in diverse processes, such as transcription, DNA replication, DNA repair, and cell division, must be tightly regulated. Nearly every protein recruited to chromatin during these processes that are essential for genome maintenance require timely removal or disassembly. Various components of the UPS and proteasome are tightly associated with chromatin, particularly when an increased protein turnover rate is needed, as in cases of DNA damage (Levy-Barda et al., 2011; Ramadan and Meiering, 2011; Butler et al., 2012). CAD plays an essential role in maintaining genome integrity and cellular homeostasis (Figure 2). Although chromatin is a key site of protein turnover necessary for genome stability, CAD has recently emerged as an important component of chromatin metabolism (Acs et al., 2011; Levy-Barda et al., 2011; Meiering et al., 2011; Feng and Chen, 2012; Galanty et al., 2012; Gudjonsson et al., 2012; Mallette et al., 2012; Mattiroli et al., 2012; Ramadan, 2012; Yin et al., 2012). Many proteins that operate on chromatin are tightly bound to the chromatin structure or chromatin-associated processes and are even considered insoluble. In addition to the physical presence of proteasomes in the vicinity of chromatin, mechanical force is needed to remodel and disassemble
Chromatin-associated protein degradation (CAD) regulated by p97–Ufd1–Npl4. Chromatin-bound proteins (S represents any known p97 substrate on chromatin) are polyubiquitinated and sumoylated by specific E3 ubiquitin and SUMO ligases. The p97−Ufd1−Npl4 complex is recruited to polyubiquitinated substrates by a ubiquitin-binding domain in Npl4 or Ufd1. ATP hydrolysis, located in the D2 ATPase cassette, induces conformational changes in the p97 molecule to remodel and release chromatin-bound substrates. Extracted substrates are either degraded by proteasomes or recycled. The p97−Ufd1−Npl4 complex maintains CAD and prevents PICHROS.

Discovery of the ubiquitin-dependent molecular segregase p97 as an integral part of CAD sheds new light on how cells disassemble proteins from chromatin (Ramadan, 2012). Inactivation of p97 by RNAi or p97 segregase mutants in various systems (e.g., yeast and humans) causes K48-polyubiquitinated p97 substrates to accumulate on chromatin, leading to PICHROS. PICHROS negatively affects downstream events that involve accumulated substrates, such as DNA replication, DNA repair, mitosis, and transcription (Figure 3; Ramadan et al., 2007; Franz et al., 2011; Meerang et al., 2011; Raman et al., 2011; Verma et al., 2011). To our knowledge, the first identified chromatin-associated substrate of p97 was Aurora B (Ramadan et al., 2007). Since this discovery, information regarding removal of chromatin substrates by ubiquitin-dependent p97 activity has rapidly progressed (Wilcox and Laney, 2009; Acs et al., 2011; Franz et al., 2011; Meerang et al., 2011; Raman et al., 2011; Davis et al., 2012; Ghosal et al., 2012; Mosbech et al., 2012). The importance of CAD function in genome stability was further confirmed by discovery of K48 ubiquitinated substrates orchestrated by E3 ubiquitin ligase RNF8 and p97 at sites of DNA damage (Meerang et al., 2011). Understanding the function of CAD in genome stability has rapidly progressed (see above). Tight collaboration between ubiquitination and sumoylation at sites of DNA damage plays a role in CAD. The evolutionary conserved SUMO-targeted E3 ubiquitin ligase (STUbL) RNF4 in mammals, Sk5–Sk6 in budding yeast and Rfp1/2–Sk6 in fission yeast is recruited to sumoylated substrates at sites of DNA damage (Prudden et al., 2007; Galanty et al., 2012; Yin et al., 2012).
FIGURE 3 | Protein-induced chromatin stress (PICHROS). As indicated in Figure 2, chromatin-bound substrates are polyubiquitinated, but cannot be removed due to inactivation of p97. Accumulation of polyubiquitinated substrates on chromatin causes PICHROS that severely disturbs, mostly inhibits, essential DNA metabolic processes, such as DNA replication, transcription, or DNA repair, and leads to genome instability.

STUbL polyubiquitines SUMO-modified proteins and primes them for proteasomal degradation. Inactivation of STUbL causes hyperaccumulation of SUMO conjugates and SUMO-induced cell toxicity. In human cell lines, RNF4 is recruited to sumoylated substrates and polyubiquitines DSB-induced factors, such as mediator of DNA damage checkpoint 1 (MDC1) and replication factor A (RPA). Consequently, polyubiquitinated MDC1 and RPA are removed from sites of damage in a proteasome-dependent manner (Shi et al., 2008; Galanty et al., 2012). RNF4-dependent MDC1 and RPA turnover allow recruitment of downstream factors, such as BRCA2 and Rad51, essential for efficient DNA repair. Inactivation of RNF4 also causes persistence of other factors at sites of damage, such as E3 ubiquitin ligases RNF8 and RNF168 and DNA damage signaling factor 53BP1 (Yin et al., 2012). Two additional E3 ubiquitin ligases, UBR5 and TRIP12, are involved in CAD and protection from PICHROS (Gudjonsson et al., 2012). UBR5 and TRIP12 regulate turnover of RNF168, the primary E3 ubiquitin ligase that ubiquitines histones after DSBs. Depletion of UBR5 and TRIP12 causes hyperaccumulation of RNF168 and a significant increase in ubiquitin conjugates, which stimulates widespread accumulation of ubiquitin-dependent factors 53BP1 and BRCA1. Deregulation of spatiotemporal SUMO- and ubiquitin-dependent protein turnover may cause hyperaccumulation of various proteins on chromatin, consequently leading to PICHROS and genome instability. In the following text, we will focus on p97-dependent CAD and PICHROS directly...
related to genome stability. We will also elaborate the function of p97 in cell cycle, which may indirectly affect genome stability.

**p97 IN THE CELL CYCLE**

Cell cycle progression can be simplified into two main processes, DNA replication (S phase) and segregation of replicated chromosomes into two daughter cells (M phase). The G1 and G2 phases are intercalated to ensure that all requirements necessary for safe DNA replication and segregation are achieved. The transition from one cell cycle phase to another is controlled by numerous mechanisms to ensure correct cell division. Cyclin-dependent kinases (CDKs) play a central role in cell cycle progression. The activity of CDKs is coordinated by transcription and ubiquitin-dependent degradation of different cell cycle-specific cyclins, CDK inhibitors, and phosphatases (Malumbres and Barbacid, 2009). Additionally, checkpoint mechanisms activated upon DNA damage ensure the quality of DNA during replication and segregation (Branzei and Foiani, 2009).

The Cdc48 gene (p97 homolog) was identified in the first genetic screen for cell division cycle (cdc) mutants in yeast (Moir et al., 1982). Cdc48 generally attaches to the ER, but relocates in the nucleus after phosphorylation in a cell cycle-dependent manner (Madeso et al., 1998). Several observations in yeast and other organisms have revealed that Cdc48 is crucial for normal cell cycle progression and associated genomic stability (Mouysset et al., 2008; Drischel et al., 2009). In yeast, mutations in the Cdc48 gene cause delayed G1/S transition and G2/M arrest. In C. elegans embryos, depletion of Cdc-48 or one of its cofactors, Ufd-1 or Npl-4, causes delays in S phase progression due to activation of replication checkpoints (Mouysset et al., 2008). p97 or Ufd1–Npl4 inactivation also leads to delayed progression through anaphase and exit from mitosis in human cell lines and Xenopus egg extracts (Ramadan et al., 2007; Dobrynin et al., 2011). These p97-defective phenotypes clearly demonstrate the crucial function of p97 in different phases of the cell cycle. p97 is important for protein degradation, and cell cycle progression requires removal of degraded proteins. Determining the role of p97 in regulating these processes is important for understanding how protein recycle/degradation affects the normal cell cycle.

**p97 IN G1/S TRANSITION**

Many cell fate decisions are determined in the G1 phase of the cell cycle. A key question is whether or not to proliferate (replicate). When the cellular environment is favorable, cells initiate the division cycle. The cellular commitment to replicate the genome and divide is known as the restriction point. After passing the restriction point, cells switch from mitogen-dependent growth in early G1 to growth factor-independent progression in S phase, which is controlled by the retinoblastoma protein (pRb) and the cdk4/cyclin D complex. Cdk4/cyclin D hyperphosphorylates pRb, which consequently releases E2F transcription factors that activate transcription of several regulatory genes necessary for G1/S transition and S phase progression, such as cyclin E and cyclin A. The activity of cdk4/cyclin D needs to be tightly regulated (Malumbres and Barbacid, 2009).

Mutations in the Cdc48 gene delay G1/S transition in budding yeast (Fu et al., 2003). Cdc2M/Cln, a yeast cyclin/cyclin important for G1/S transition controls the execution of Start (a yeast cell cycle commitment point equivalent to the restriction point in mammalian cells). Far1p is a Cdc28/Cln inhibitor and its degradation is needed for G1/S progression. Cdc48 physically interacts with ubiquitinated Far1p and stimulates its degradation. The defect in G1/S transition after Cdc48 inactivation was shown to be due to persistence of the Cdc28/Cln inhibitor Far1p. In contrast to normal cells in which Far1p is degraded following release from G1 arrest, Cdc48 mutant cells accumulated ubiquitinated Far1p. G1/S delay could be rescued following mutations in both Cdc48 and Far1p genes, clearly suggesting that Cdc48 is required for Far1p degradation. Although no data are available, a similar p97-dependent process could exist in higher eukaryotes in which CDK activity is also regulated by CDK inhibitors. In addition to Far1p degradation, Cdc48 and Npl4 complex controls G1/S transition via cell wall integrity pathway mechanisms in yeast (Hsieh and Chen, 2011). The mechanisms by which Cdc48 controls cell wall integrity have not been determined, although Cdc48 appears to regulate Mpk1 activity, which is a MAP kinase family member important for cell wall integrity, in response to stress conditions, including heat shock.

**p97 IN DNA REPLICATION AND S PHASE**

To divide and preserve an intact genome, cells must tightly regulate DNA replication. DNA synthesis occurs in the S phase, but preparation starts in late mitosis and G1 by loading a pre-replicative complex (pre-RC) at each origin of replication. Some pre-RCs are active, while others remain dormant. Pre-RCs consist of an origin recognition complex (ORC), cell division control protein (Cdc6), chromatin licensing and replication factor (Cdt1), and the minichromosome maintenance (MCM) helicase complex. Pre-RC is activated by phosphorylation to recruit essential replication factors, such as MCM-10, CDC-45, and the Go-Ichi-Ni-San complex (GINS), to form a pre-initiation complex (pre-IC), which recruits DNA primase and polymerases to initiate DNA synthesis. After the origins fire, the pre-RC factors are removed or inhibited to prevent re-replication of the genome during the same cell cycle. Various obstacles during DNA synthesis, such as secondary DNA structures, DNA-protein complexes or damaged bases, can stall the fork or lead to fork collapse. To ensure that the DNA replication fork can handle all of these challenges, cells activate an intra-S phase checkpoint (Branzei and Foiani, 2010).

p97 function includes regulation of DNA replication and progression through the S phase (Mouysset et al., 2008). Depletion of Cdc-48 (p97) or its adaptors Ufd-1 or Npl-4 in C. elegans significantly delays progression through the S phase due to activation of at1 (ATR) and the Chkl-dependent intra-S phase checkpoint. Depletion of Cdc-48, Ufd-1, or Npl-4 reduces the number of nucleoli and total amount of DNA and increases the number of chromosomal bridges in C. elegans embryos. Depletion of intra-S phase checkpoint kinases completely restores the delay in S phase progression in Cdc-48−, Npl-4−, or Ufd-1-defective cells, but could not restore the number of nuclei, DNA content or decrease the number of chromosomal bridges. Recent work by...
two independent groups provided mechanistic insight into the role of p97−Ufd1−Npl4 in DNA replication (Franz et al., 2011; Raman et al., 2011). The research groups showed that p97 regulates Cdt1 chromatin turnover and stability via two distinct pathways, (i) a UV lesion-related pathway (Raman et al., 2011) and (ii) firing and elongation of the replication fork (Franz et al., 2011). In the UV lesion-related pathway, p97 regulates destruction of Cdt1 at sites of UV-induced DNA damage. After UV damage, nucleotide excision repair (NER) machinery recognizes distorted DNA (thymine dimers) and excises damaged DNA strands 25–30 nucleotides in length. This gap is repaired by DNA polymerization in proliferating cell nuclear antigen (PCNA)- and E3 ubiquitin ligase Cul4–DDB2-dependent manners. Cdt1 associates with PCNA to initiate DNA replication at sites of damage, but must be tightly regulated to prevent re-replication. After initiation of DNA synthesis, PCNA-bound Cdt1 is polyubiquitinated by Cul4–DDB2, extracted from chromatin in a p97−Ufd1−Npl4-dependent manner and presented to the proteasome for final degradation (Raman et al., 2011). p97 directly controls gap-filling DNA synthesis after UV damage. In the second pathway, p97 controls DNA replication by extracting polyubiquitinated Cdt1 from chromatin under physiological conditions in C. elegans and a Xenopus egg extract. Inactivation of the p97−Ufd1−Npl4 complex stabilizes Cdt1 on interphase and mitotic chromatin. Consequently, Cdc45 and GINS are stabilized on chromatin-bound Cdt1 and could interfere with DNA replication fork elongation (Franz et al., 2011). Cdc-48/p97 depletion leads to accumulation of Cdt1 on chromatin in C. elegans embryos, Xenopus egg extract and human cell lines. Re-expression, a typical phenotype caused by Cdt1 overexpression, was not observed in p97/Cdc-48 depleted cells. In contrast, two independent research studies showed that inactivation of p97 caused G2/M arrest in human embryonic kidney cell line (HEK293) and decreased the total DNA content in C. elegans (Moury et al., 2008; Raman et al., 2011). This is diametrically different from a typical re-replication phenotype, which involves an increased amount of total DNA characterized by elevated Cdt1 protein levels (Terra and Dutta, 2008; Ramanathan and Ye, 2011). The molecular mechanisms involving p97 regulates DNA replication cannot be simply attributed to extraction and stability of Cdt1 from chromatin. However, the evolutionarily conserved function of p97−Ufd1−Npl4 in DNA replication has been demonstrated (Moury et al., 2008; Dutschel et al., 2009; Franz et al., 2011; Raman et al., 2011). p97 most likely regulates multiple steps during DNA synthesis. This hypothesis could be further supported by direct physical interactions with several replicative helicases, such as the Werner protein and HJ-M (Bloom helicase homolog, Partridge et al., 2003; Indig et al., 2004; Caruso et al., 2008).

p97 IN MITOSIS

Mitosis is characterized by profound changes in cell physiology that allow nuclear envelope disassembly and separation of genetic material into two daughter cells. Spatiotemporal coordination of complex mitotic processes depends on phosphorylation and ubiquitination events that are crucial for genome integrity. At the end of mitosis, inhibition/degradation of several kinases, such as cyclin B and Aurora B, leads to spindle disassembly, cytokinesis, chromatin decondensation, and nuclear envelope reformation (Carriona et al., 2012). Aurora B was the first p97 substrate discovered in chromatin (Ramadan et al., 2007). In Xenopus egg extracts and C. elegans, p97/Cdc-48−Ufd1−Npl4 binds polyubiquitinated Aurora B and extracts it from mitotic chromatin. p97-dependent removal of Aurora B allows chromatin decondensation and nuclear envelope formation during exit from mitosis. A similar mechanism was observed in human cell lines, although regulation of Aurora B activity by p97 initiates much earlier during mitosis (Dobrynin et al., 2011). Depletion of Ufd1 or Npl4 causes an increase in Aurora B activity, which leads to defects in chromosomal alignment in anaphase, resulting in missegregated chromosomes and multi-lobed nuclei. These results establish p97−Ufd1−Npl4 as a negative regulator of Aurora B activity, which regulates multistep processes in chromosome dynamics during mitosis. Similarly, Cdc-48 in C. elegans was shown to be required for proper condensation and segregation of mitotic chromosomes by controlling AIR-2/Aurora B (Sasagawa et al., 2012). Cdc-48 is required for localization of AIR-2 at regions between homologous chromosomes in meiosis I. In the absence of Cdc-48, higher levels of AIR-2 increase phosphorylation of its substrates over the entire length of the chromosomes, leading to defective chromosome segregation.

In contrast to the p97−Ufd1−Npl4 core complex, which extracts and inactivates Aurora B in mitosis, another p97 core complex (p97−P47) balances Aurora B activity in mitosis using a different mechanism (Cheng and Chen, 2010). The Cdc-48−Shp1 complex (p97−P47 in metazoans) facilitates nuclear localization of Glc7, the yeast ortholog of protein phosphatase-1 (PP1), which counteracts Aurora B kinase activity. Inactivation of the Cdc-48−Shp1 complex causes cell cycle arrest in metaphase due to a defect in bipolar attachment of the kinetochore that activates the spindle checkpoint.

In addition to Aurora B regulation, Cdc48/p97 is required for proper spindle disassembly at the end of mitosis (Cao et al., 2003; Xenopus egg extracts containing the dominant negative form of p97 (p97QQ) or depleted of Ufd1 or Npl4 cofactors were unable to disassemble the spindle and reform interphase microtubules (MTs) and remained in a mitotic state. Cdc48/p97−Ufd1−Npl4 specifically interacts with spindle assembly factors XMAP215, TPX2, and Plx1 at the exit of mitosis, promoting its sequestration in the cytoplasm or extraction from MTs. This function of p97 was further confirmed in yeast where p97 (Cdc48) is required for degradation of spindle assembly factors Ase1 and Cdc5.

In conclusion, two p97 core complexes, p97−Ufd1−Npl4 and p97−P47, coordinate Aurora B activity in chromatin to allow proper chromosomal alignment, segregation, decondensation, and nuclear envelope formation at mitotic exit. In addition to direct effects on mitotic chromatin, the p97−Ufd1−Npl4 complex also regulates spindle disassembly at the end of mitosis.

p97 IN DNA DAMAGE

To cope with DNA damage, preserve genetic information for the next generation and survive, cells have evolved a variety of DNA repair mechanisms specific for different types of damage, which are orchestrated by DDR and DDT (Nyberg et al., 2002; Jackson et al., 2003).
and Bartek, 2009; Curtin, 2012). Although p97 phosphorylation after IR and physical interaction with BRCA1 predicted the role of p97 in DNA repair more than a decade ago, its function in DNA repair remained a mystery (Zhang et al., 2000; Livingstone et al., 2005). The molecular mechanism of p97 emerged in DSB repair, TLS and transcription-coupled NER (TC-NER) only recently (Acs et al., 2011; Meerang et al., 2011; Verma et al., 2011; Davis et al., 2012; Ghosal et al., 2012; Mosbech et al., 2012). Whether p97 is involved in other DNA repair pathways remains to be discovered.

**DVC1 LINKS p97 TO TRANSLESION DNA SYNTHESIS**

DNA is particularly vulnerable to damage during DNA replication. Some base adducts may escape detection by repair proteins before progression through the S phase. During replication, these lesions cannot be accommodated at the active sites of replicative DNA pols and stall progression of the DNA replication fork (Lehmann, 2011a). Stalled replication forks activate DDR pathways and recruits translesional DNA pols, a mechanism known as TLS (Ullrich, 2007; Sale et al., 2012). TLS uses a DNA pol switch mechanism, modulated by the ubiquitination status of PCNA, in which replicative DNA pols are exchanged for translesion DNA pols (Lehmann, 2011b).

Translesion DNA synthesis is initiated by monoubiquitination of PCNA by the E3 ubiquitin ligase Rad18. Specialized translesion DNA pols are recruited to monoubiquitinated PCNA at stalled replication forks to promote bypass of damaged DNA. However, replication through a lesion site often requires the sequential action of two DNA pols in which one inserts a nucleotide opposite the damage (DNA pol eta) and the other extends from the inserted nucleotide (DNA pol zeta), as is observed in wild-type C. elegans but not in human cells, which correlates to about 50 endogenous DSBs per cell cycle (Bennett et al., 1993). It is estimated that approximately 1% of endogenous single-strand DNA breaks convert to DSBs during S phase in mammalian cells, which correlates to about 50 endogenous DSBs per cell cycle (Vilenchik and Knudson, 2003). DSBs also occur as a consequence of certain medical interventions related to diagnostics or therapy (e.g., X-ray). To prevent genome instability, ubiquitination and sumoylation have emerged as an essential PTM in regulating DDR and DSBR.

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A wide variety of DNA helix-distorting lesions, such as UV-induced photodimers, thymine dimers and DNA adducts induced by chemicals in transcribed DNA, block progression of the RNA pol II complex. Blocking transcription at sites of DNA damage represents a serious obstacle for DNA replication and leads to DNA replication fork collapse. To prevent DNA replication fork collapse at sites of stalled transcription and avoid apoptosis, cells activate TC-NER, a subpathway of NER. TC-NER rapidly removes lesions from the transcribed strand and allows transcription to continue (Fousteri and Mullenders, 2008; Lehmann, 2011a). Upon induction of DNA damage by UV or UV-mimetic drugs, thymine dimers form, transcription stalls, and the TC-NER subpathway is activated. Stalled transcription recruits E3 ubiquitin ligase Cul3 to ubiquitinate the RNA pol II complex and prime it for proteasomal degradation (Chen et al., 2007; Ribar et al., 2007). It is hypothesized that the stalled DNA pol II complex shields DNA lesions and prevents access by the NER machinery. As a result, the complex must be removed and degraded. The Cdc48\(\text{Npl4}\) complex together with Ubx4 and Ubx5 (human homologous UBXD9 and UBXD7) play an important role in UV-dependent turnover of the stalled RNA pol II complex in yeast (Verma et al., 2011). The Cdc48\(\text{Npl4}\)-subcomplex is recruited at sites of UV-induced damage and facilitates CR of Rpb1, the largest RNA pol II subunit. The stalled RNA pol II complex also stimulates increased proteasome recruitment at sites of UV lesions that form a tight complex with p97\(\text{Cdc48}\). These results suggest tight cooperation between p97\(\text{Cdc48}\) and proteasomes at sites of lesions, in the earliest step of TC-NER. Beside its involvement in the upstream step of TC-NER, p97 also operates in the gap-filling DNA synthesis, which is the final step of both NER subpathways, TC-NER and global genome-NER (GG-NER; Raman et al., 2011). During gap synthesis, p97 promotes segregation and degradation of PCNA-bound Cdt1 to prevent re-replication (Raman et al., 2011; Ramanathan and Ye, 2011). Together, these results suggest that p97 operates at different levels in both NER subpathways. It would be interesting to see whether and how p97 operates in early steps of GG-NER.

**p97 and Diseases Related to Genome Stability**

p97 has been implicated in the pathogenesis of many human diseases, including breast, colorectal, lung, prostate and pancreatic cancers, chronic obstructive pulmonary disease and severe emphysema, systemic sclerosis, amyotrophic lateral sclerosis (ALS; Johnson et al., 2010). IBMPFD is an autosomal dominant negative inherited degenerative disorder due to a single missense mutation in p97 in humans. There are several families with IBMPFD worldwide, but the mutations are primarily located in the N-terminal region, the linker region between the N-terminal and D1 areas and the D1 region (Weihl et al., 2009). IBMPFD is characterized by disabling weakness, osteolytic lesions consistent with Paget’s disease of bone and several types of neurodegenerative disorders (Vij, 2008; Haines, 2010; Min et al., 2011). Inclusion body myopathy associated with Paget’s disease of bone and frontotemporal dementia (IBMPFD) is the only disorder that has been directly linked to p97 dysfunction to date (Kimonis et al., 2008). p97 mutations have been linked to 2% of isolated familial amyotrophic lateral sclerosis (ALS; Johnson et al., 2010). IBMPFD is an autosomal dominant negative inherited degenerative disorder due to a single missense mutation in p97 in humans. There are several families with IBMPFD worldwide, but the mutations are primarily located in the N-terminal region, the linker region between the N-terminal and D1 areas and the D1 region (Weihl et al., 2009). IBMPFD is characterized by disabling weakness, osteolytic lesions consistent with Paget’s disease of bone and frontotemporal dementia. Accumulation of cytoplasmic and nuclear ubiquitin-positive aggregates in the tissues of patients with IBMPFD suggests that p97 processing of ubiquitinated substrates is the key mechanism involved in pathogenesis. Because p97 processes substrates for lysosomal and proteasomal degradation pathways, it is not clear which pathway
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Altomare et al., 2009). IκB kinase (IKK) (potential inhibitor of the pro-survival function of nuclear factor kappa B (NFκB)) or HIF1α (promoter of tumor angiogenesis and metastases; Arai et al., 2002; Ramadan et al., 2007; Alexandru et al., 2008; Dobrynin et al., 2011). Elevated p97 expression correlates with the progression, prognosis, and metastatic potential of many cancers (Yamamoto et al., 2003, 2004a,b,d). For example, high levels of p97 protein correlate with colorectal carcinomas (Yamamoto et al., 2004c). Low p97 expression levels have been observed in adenomas, while high levels have been shown in all metastatic tumors. Patients with tumors that express high levels of p97 show a higher recurrence rate and poorer disease-free periods and overall survival compared to patients with tumors that have low p97 expression, suggesting that high levels of p97 indicate a poor prognosis. Similar to colorectal cancer, p97 is overexpressed in non-small cell lung carcinomas (Yamamoto et al., 2004b). All of the data described are based on a correlation between p97 protein levels and cancer development and progression. Whether elevated p97 expression increases the degradation of growth inhibitory proteins or elevated expression is a cellular response to protein-induced stress in cancer is not known (Haines, 2010). However, inhibition of p97 by a small molecule significantly reduced NSCLC tumor growth in in vitro and in vivo models (Valle et al., 2011). This suggests that increased p97 levels may be directly responsible for tumorigenesis.

There is no evidence that mutations in p97 are related to cancer. Considering its ubiquitin-dependent functions in diverse processes related to proliferation and maintenance of protein homeostasis, one could speculate that cancer cells rely on intact p97 function.

Because p97 is essential for cell survival, alterations in its adaptors would be expected to cause cancer. Haplinsufficiency of the FAI1 gene, a member of the UBX family of adaptors, was observed in 30% of cervical carcinomas and 12.5% of mantle-cell lymphomas (Hidalgo et al., 2003; Iba et al., 2009). FAF1 protein levels are downregulated in gastric carcinomas and a large percentage of mesotheliomas (Froling-Poulsen et al., 2003; Albhame et al., 2009). p97 and the FAI1 adaptor likely play important roles in cancer development, although clear molecular mechanisms have not been elucidated.

Direct evidence of p97 involvement in genome stability and cancer development was recently emerged by the discovery of a novel premature aging syndrome due to a homologous mutation in the p97 adaptor DVC1. DVC1 recruits p97 to stalled replication forks and is essential in preventing mutagenesis. A DVC1 mutation in novel premature aging syndrome eliminated recruitment of p97 to stalled replication forks, which consequently induced DNA replication fork collapse and an increased level of chromosomal aberrations (our unpublished results, D. Lessel et al., submitted).

CONCLUSION

As one of the most abundant cellular proteins, p97 is essential for cell development, proliferation, and growth. p97 has recently emerged as a central element in the ubiquitin system involved in both proteasomal and lysosomal degradation pathways as well as ubiquitin-dependent, degradation-independent processes. A central function of p97 enzymatic activity is to convert its own ATPase activity into remodeling activity to release (extract) and process a myriad of ubiquitinated substrates from various cellular locations. p97 plays an essential role in protein homeostasis and protection from protein stress due to accumulation of short-lived, misfolded, old, and damaged proteins. The diversity of p97 related to a myriad of ubiquitinated substrates in a variety of cellular processes is governed by multiple adaptors. An adaptor hierarchy based on a second tier of p97 adaptors primarily from the UBX family controls three core p97 adaptor complexes, p97–UBX–UBX–UBX, and p97–UBX–UBX. Although the role of p97 in the cell cycle and DNA repair has been known for several decades, its function in genome stability is beginning to emerge (Figure 4). Elucidation of CAD orchestrated by p97 in DNA replication, DNA repair and discovery of DVC1 has finally established the role of p97 in genome stability. This was further demonstrated by discovery of a novel premature aging syndrome in a human due to a homologous mutation in the p97 adaptor DVC1. Although p97 overexpression correlates with tumor progression, inactivation of p97 or its adaptors leads to genome instability. These facts support the hypothesis that tight regulation of the p97 system is essential for genome stability and protection against cancer. Understanding how p97 protects cells from PICHROS and its function in DNA replication, repair, recombination, mitosis and the cell cycle will be essential for fully understanding the role of p97 in genome stability, aging and cancer. Many questions await further investigation, such as the identification of p97 substrates, adapters, and pathways that contribute to genome stability. p97 has a broad range of adaptors many of which possess ubiquitin-binding domains. This strongly suggests that p97 most probably binds and modulates variety of ubiquitinated substrates, besides the ones already known and described in this review, which are essential for genome stability. Hence, we have to identify other CAD-related substrates of p97, as well as the composition of p97-adaptor for specific substrates related to genome stability. Although it seems simple, the second tier of p97-adaptors and E3-ubiquitin ligases for many established pathways and substrates is still unknown, as indicated...
FIGURE 4 | Role of p97 in genome stability. The circle represents a summary of p97 functions in diverse cellular processes, from yeast to human, essential for maintenance of genome stability. Key players from different species are abbreviated with human homologues (e.g., yeast Cdc48 and Ubx5 is equivalent to human p97 and UBXD7). The p97 homohexamer is centrally located. The first ring (blue) represents two core adaptor complexes, Ufd1-Npl4 and p47. The second ring (orange) represents the next tier of p97 adaptors that direct the function of p97 core complexes. DVC1 directs \( p97^{-1}Ufd1^{-Npl4} \) function toward TLS, and UBXD7 and UBXD9 direct \( p97^{-1}Ufd1^{-Npl4} \) toward stalled transcription and TC-NER. The third ring (gray) represents E3 ubiquitin ligases that ubiquitinate p97-substrates. The fourth ring (green) represents p97-substrates that must be remodeled by p97 to avoid PICHROS and prevent genome instability. The fifth ring (white) represents different cellular processes in which p97 plays an essential role for maintenance of genome stability. PP1, protein phosphates 1/Glc7 in yeast; CDK inhibitor, cyclin-dependent kinase inhibitor Far1p in yeast; L3MBTL1, polycomb protein that contains malignant brain tumor (MBT) domain; CDT1, chromatin licensing and DNA replication factor 1; SET8, histone methyltransferase; XMAP215, processive microtubule polymerase; TPX2, microtubule associated protein; PLX1, polo-like kinase; DNA pol \( \delta \) and \( \eta \), DNA polymerases \( \delta \) and \( \eta \).

by question marks in Figure 4. In addition to its role in numerous cellular processes (Figure 4, fifth ring), p97 most probably also plays a role in other ubiquitin-controlled pathways and processes that directly regulate genome stability, such as base excision repair, mismatch repair, DNA damage checkpoints, and apoptosis. Finally, understanding the complex function of p97, its adaptors and substrates in genome stability might directly help to develop a new and more efficient anti-cancer drug(s). This speculation is based on effects of bortezomib (Velcade), a proteasome inhibitor currently used for treatment of multiple myeloma and mantle-cell lymphoma and fascinating results showing that p97 inhibitor significantly reduced NSCLC tumor growth in vitro and in vivo models (Valle et al., 2011). Taken together, one anticipates interesting and dynamic time ahead in investigating the function of p97 in the context of genome stability and cancer therapy.

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REFERENCES

Asai, T., Tomit, Y., Nakatsuka, S., Alexandru, G., Graumann, J., Smith, B. J., Aladjem, M. I., Latterich, M., Boehm, H., Okumura, T., and Jentsch, S. (2009).

Bennett, C. B., Lewis, A. L., Baldwin, K. B., Bekker-Jensen, S., and Mailand, N. (2009).

Bentley, C., Salomons, F. A., Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 ATPase VCP/p97 signals via de-ubiquitination of Fanconi anemia factor 1 in abnormally induced meiosis in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A. 108, 2320–2325.

Bentley, C., Salomons, F. A., Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 ATPase VCP/p97 signals via de-ubiquitination of Fanconi anemia factor 1 in abnormally induced meiosis in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A. 108, 2320–2325.

Bentley, C., Salomons, F. A., Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 ATPase VCP/p97 signals via de-ubiquitination of Fanconi anemia factor 1 in abnormally induced meiosis in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A. 108, 2320–2325.
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Johnson, E. S., Ma, P. C., Ota, I. M., and Ju, J. S., and Weihl, C. C. (2010a). Kimonis, V. E., Fulchiero, E., Vesa, Lange, S. S., Takata, K., and Wood, J. Cell Biol. 41, 1661–1668.

Weihl, C. C. (2010b). p97/VCP at the intersection of inclusions as a cause of familial ALS. Trends Biochem. Sci. 35, 565–572.

Inclusion-body myopathy, Paget’s disease of bone and frontotemporal dementia review of a unique disorder. Biochim. Biophys. Acta 1782, 125–129.

Johnson, E. S., Baloh, R. H., et al. (2009). Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. J. Cell Biol. 187, 475–484.

Shao, Z., et al. (2011). Involve-ment of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. Proc. Natl. Acad. Sci. U.S.A. 108, 2489–2494.

Vessey, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., et al. (2010). Alaskan-Finn mutation in C1orf124 is associated with familial ALS. Nat. Genet. 42, 1169–1175.

Moiard, F., Vissers, J. H., Van Dijk, Mallette, F. A., Mattiroli, F., Cui, and Weinert, T. A. (2002). DNA damage modulates nucleolar morphology. Cell Cycle 1, 865–878.

Marko, D., Falchier, E., Vasa, J., and Watts, G. (2000). VCP disease associated with a neurological syndrome of frontotemporal dementia and parkinsonism. Biochim. Biophys. Acta 1523, 125–129.

Leyry-Balas, A., Leventhal, Y., Davis, A. J., Chung, Y. M., Esener, J., Mao, Z., et al. (2011). Impulse-ment of the nuclear proto-oncogene A20gamma in the cellular response to DNA double-strand breaks. Cell Cycle 10, 4300–4310.

Li, G., Huang, C., Zhao, G., and Weiner, J. (2012). Dual recruitment of Cdc48 (p97)-UVD-Nip1 ubiquitin-selective degrage-ase by small ubiquitin-like modifier protein (SUMO) and ubiquitin in SUMO-targeted ubiquitin ligase-mediated genome stability functions. J. Biol. Chem. 287, 28010–28019.

Nervy, B., McKelson, R. J. P., Putnam, C. W., and Weinert, T. A. (2012). A genome-wide survey of DNA strand breaks. Trends Genet. 28, 3211–3216.

Partridge, J. J., Lopere, I. O, T., Latt, terich, M., and Iqbal, F. K. (2013). DNA damage modulates nucleolar interaction of the Werner protein with the AAA ATPase p97/VCP. Mol. Biol. Cell 24, 4221–4229.

Peiris, J. M, Walsh, M. J. and Franks, W. W. (1990). An abundant and ubiq-uitous homo-oligomeric ring-shaped ATPase particle related to the putative vesicle fusion proteins Sec5p and Sec9p. EMBO J. 9, 1757–1762.

Priddl, J., Poberaj, S., Raffa, G., Slaw, D. A., Perry, J. J., Tainer, J. A., et al. (2007). SUMO-targeted ubiquitin ligase in genome stability. EMBO J. 26, 8409–8419.

Pukkila, J., and Jentsch, S. (2012). Pro-tein group modification and repair in the SUMO pathway as exemplified in DNA repair. Cell 151, 807–820.

Ramanathan, K., Bruckert, R., Spig, M., Poppy, O., Ruest, T., Gotta, M., et al. (2007). Cdc48/p97 protein refor-mation of the nucleus by extracting the lens keratos B from keratin. Nature 430, 1258–1261.

Raman, K., and Meisinger, M. (2011). Degradation-linked ubiquitin signal and proteasome are integral compo-nents of DNA double strand break repair: new perspectives for anti-cancer therapy. FEBS Lett. 580, 2868–2875.

Raman, M., Haines, G. C., Walter, J. C., and Harper, J. W. (2011). A genome-scale protein identi-ty screen identifies p97 as an essential regulator of DNA damage-dependent ERD1 destruction. Mol. Cell 44, 72–84.

Ramanathan, H. N., and Ye, Y. (2011). Revoking the cellular license to replic-ate: yet another AASS agonist. Mol. Cell 44, 5–6.

Rohat, B., Prakash, L., and Prakash, S. (2007). ERAT and UKL1 are required along with E3L for RNA polymerase II polyadenylation and degrada-tion in DNA-damaged yeast cells. Mol. Cell Biol. 27, 3211–3220.

Stolz, A., Hilt, W., Buchberger, A., Al-Hakim, A. K., Kolas, N. K., Miller, J., and Wolf, D. H. (2011). Cdc48: a component of the ubiquitin proteasome system at the intersection of the autophagy and the ubiquitin proteasome system. Autophagy 6, 283–285.

Julius, S., Balogh, D., Hjuhn, L., Buervens, Z., Villani, M. A., Zhuang, Z., et al. (2012). Characterization of human Spar-tan/Ctr124, an ubiquitin-PH interacting regulator of DNA dam-age tolerance. Nat. Cell Biol. 40, 10793–10800.

Kim, M. S., Macheda, Y., Ushida, A. A., Weihl, M. J. P., Fang, Y. P., and Macheda, Y. I. (2013). Regulation of centrosome duplication by the AAA ATPase Ctr124. Biochim. Biophys. Acta 1823, 125–129.

Kimonis, V. E., Falchier, E., Vasa, J., and Watts, G. (2000). VCP disease associated with a neurological syndrome of frontotemporal dementia and parkinsonism. Biochim. Biophys. Acta 1523, 125–129.

Klopstock, P., Ewans, C. A., Forster, A., Zhang, X., and Freemont, P. S. (2012). Regulation of p97 in the ubiquitin-proteasome system by the UPS protein family. Biochim. Biophys. Acta 1825, 125–129.

Lange, S. S., Takan, K., and Wood, R. D. (2011). DNA polymerases and cancer. Nat. Rev. Cancer 11, 96–110.

Lehmann, A. R. (2011a). DNA poly-merases and repair synthesis in NER in human cells. DNA Repair (Amst). 10, 103–124.

Lehmann, A. R. (2011b). Ubiquitin-family modifications in the replica-tion of DNA damage. FEBS Lett. 585, 2772–2779.
power machine in protein degradation. Trends Biochem. Sci. 36, 515–523.

Tate, J. K., and Dutta, A. (2008). Human Cdc48 lacking the evolutionarily conserved region that interacts with BRCA2 is capable of inducing re-replication. J. Biol. Chem. 283, 6817–6825.

Tronc, E., Salomons, F. A., Vooi, J., Bott, L. C., Kremers, V., Yao, T. P., et al. (2010). VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. Annu. 8, 217–227.

Ulrich, H. D. (2007). Conservation of DNA damage tolerance pathways from yeast to humans. Biochim. Ac. 75, 1336–1337.

Ulrich, H. D., and Wüller, H. (2010). Ubiquitination signalling in DNA replication and repair. Nat. Rev. Mol. Cell Biol. 11, 479–489.

Václavík, C. W., and Deshaies, R. J. (2013). Critical role of VCP/p97 in the pathogenesis and progression of non-small cell lung carcinoma. PLoS ONE 8,e29073. doi: 10.1371/jour- nal.pone.0029073.

Vera, R., Oania, R. S., Kolawa, V. E., et al. (2009). Valosin-containing protein (VCP) is a common regulator: ubiquitin takes command of an AAA ATPase. Autophagy 5, 515–523.

Vera, R., Oania, R., Fang, R., Smith, Y. N., and Deshaies, R. J. (2011). Cdc48/p97 promotes degradation of aberrant nascent polyadenylate bound to the ribosome. eLife 2:e00388. doi: 10.7554/eLife.00388.

Vereeken, J., and Knudson, A. G. (2010). VCP/p97 is essential for the ribonuclease Drosophila. Proc. Natl. Acad. Sci. U.S.A. 107, 12071–12076.

Velorich, M. M., and Knudson, A. G. (2011). Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. Proc. Natl. Acad. Sci. U.S.A. 108, 12071–12076.

Weihl, C. C., Pestronk, A., and Kimonis, V. E. (2009). Valosin-containing protein (VCP) is strongly associated with progression of gastric carcinoma. J. Clin. Oncol. 27, 1432–1438.

Wilcox, A. J., and Laney, J. D. (2009). A ubiquitin-selective AAA ATPase mediates transcriptional switching by remodeling a repression/promoter DNA complex. Nat. Cell Biol. 11, 1401–1406.

Woodman, P. G. (2003). VCP, a protein coping with multiple identities. J. Cell Sci. 116, 4283–4296.

Wu, Y. (2006). Diverse functions with a common regulator: ubiquitin takes command of an AAA ATPase. J. Struct. Biol. 156, 29–40.

Xiong, H. O., Kloppsteck, P., Niwa, H., and Yamauchi, T., et al. (2003). Expression of valosin-containing protein in colorectal carcinomas as a predictor for disease recurrence and prognosis. Clin. Cancer Res. 10, 651–657.

Yamamoto, S., Tomin, A., Hoshida, Y., Takiguchi, S., Fujiwara, Y., Yasuda, T., et al. (2005). Expression level of valosin-containing protein (VCP) as a prognostic marker for glioblastoma multiforme. Cancer Genetics 155, 201–205.

Yamamoto, S., Tomin, A., Hoshida, Y., Takiguchi, S., Fujisawa, Y., Yasuda, T., et al. (2005). Expression level of valosin-containing protein is strongly associated with progression of gastric carcinoma. Clin. Cancer Res. 11, 5588–5595.

Yin, Y., Seifert, A., Chua, J. S., Martin, J. F., Goldowska, F., and Hay, R. T. (2012). SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. Genes Dev. 26, 1196–1208.

Zhang, H., Wang, Q., Kajino, K., and Grams, M. I. (2008). VCP, a weak ATPase involved in multiple cellular events, interacts physically with BRCA1 in the nucleus of living cells. DNA Cell Biol. 19, 235–236.

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