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A Role for VCP/p97 in the Processing of Drug-Stabilized TOP2-DNA Covalent Complexes

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ABSTRACT

DNA topoisomerase II (TOP2) poisons induce protein-DNA crosslinks termed TOP2-DNA covalent complexes, in which TOP2 remains covalently bound to each end of an enzyme-induced double-strand DNA break (DSB) via a 5′-phosphotyrosyl bond. Repair of the enzyme-induced DSB first requires the removal of the TOP2 protein adduct, which, among other mechanisms, can be accomplished through the proteasomal degradation of TOP2. VCP/p97 is a AAA ATPase that utilizes energy from ATP hydrolysis to unfold protein substrates, which can facilitate proteasomal degradation by extracting target proteins from certain cellular structures (such as chromatin) and/or by aiding their translocation into the proteolytic core of the proteasome. In this study, we show that inhibition of VCP/p97 leads to the prolonged accumulation of etoposide-induced TOP2A and TOP2B complexes in a manner that is epistatic with the proteasomal pathway. VCP/p97 inhibition also reduces the etoposide-induced phosphorylation of histone H2A.X, indicative of fewer DSBs. This suggests that VCP/p97 is required for the proteasomal degradation of TOP2-DNA covalent complexes and is thus likely to be an important mediator of DSB repair after treatment with a TOP2 poison.

SIGNIFICANCE STATEMENT

TOP2 poisons are chemotherapeutic agents used in the treatment of a range of cancers. A better understanding of how TOP2 poison–induced DNA damage is repaired could improve therapy with TOP2 poisons by increasing TOP2 poison cytotoxicity and reducing genotoxicity. The results presented herein suggest that repair of TOP2-DNA covalent complexes involves the protein segregase VCP/p97.

Introduction

DNA topoisomerase II (TOP2) is an important enzyme that mediates topological changes in DNA, including the unwinding of supercoils and the decatenation of sister chromatids. This is achieved by passing an intact double helix of DNA through a double-strand DNA break (DSB) introduced by the enzyme. TOP2 forms a covalent 5′-phosphotyrosyl linkage with each end of the DNA break, resulting in a transient intermediate of the TOP2 reaction cycle termed the TOP2-DNA covalent complex. TOP2-DNA covalent complexes are rapidly reversed after strand passage by religation of the DSB. However, TOP2 poisons such as etoposide and mitoxantrone inhibit religation of the DSB and stabilize TOP2-DNA covalent complexes, which accumulate on DNA. TOP2-DNA complexes obstruct elongating DNA and RNA polymerases, leading to the arrest of replication and transcription, and further processing of trapped TOP2-DNA complexes also leads to the liberation of protein-free DSBs. Therefore, timely repair of TOP2-DNA complexes is crucial for the maintenance of genome stability.

TOP2 is a large protein, and it is well understood that, in the presence of TOP2 poisons, TOP2-mediated DSB remains concealed within the TOP2-DNA complex until processing of the TOP2 adduct occurs (Martensson et al., 2003). Although a number of processing pathways have been described, one major mechanism involves the proteolysis of TOP2. This results in a protein-free DSB linked to short phosphotyrosyl peptides, which can then be repaired by the 5′-phosphodiesterase TDP2 prior to DSB repair (Cortes Ledesma et al., 2009; Gao et al., 2014). TOP2-DNA complexes can be degraded by the proteasome in a ubiquitin-dependent manner (Mao et al., 2001; Alchanati et al., 2009; Swan et al., 2020). Indeed, inhibition of the proteasome (or ubiquitination) increases levels of etoposide-stabilized TOP2-DNA complexes (Mao et al., 2001; Zhang et al., 2006; Fan et al., 2008; Alchanati et al., 2009; Tammaro et al., 2013; Lee et al., 2016; Swan et al., 2020) and reduces the activation of etoposide-induced DNA damage response proteins, including histone H2AX, RPA, and p53 (Zhang et al., 2006; Swan et al., 2020). It has been proposed that TOP2-DNA complexes can be repaired directly by TDP2 through the remodeling of TOP2, which is mediated by the ZATT SUMO ligase when TOP2 is SUMOylated and the proteasome is inhibited (Schellenberg et al., 2017). This pathway has been observed only in the

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ABBREVIATIONS: DSB, DNA double-strand break; H2AX, histone H2A.X; γH2AX, S-139 phospho-histone H2A.X; siRNA, small interfering RNA; TARDIS, trapped in agarose DNA immunostaining; TOP2, DNA topoisomerase 2; TOP2A, DNA Topoisomerase IIα; TOP2B, DNA topoisomerase IIβ; VCP, valosin-containing protein.

absence of a functional proteasome, further exemplifying the importance of proteolysis in the removal and repair of TOP2-DNA complexes (Lee et al., 2018). In addition, upstream SUMOylation may also be required for efficient ubiquitination and targeting of stalled TOP complexes to the proteasome (Sun et al., 2020) Aside from TOP2 proteolysis, TOP2 may be removed by cleavage of the DNA containing the TOP2 adduct, involving the nuclease activity of Mre11, which is stimulated by CtIP (Neale et al., 2005; Hartsuiker et al., 2009; Lee et al., 2012).

In addition to the proteasome, another protease has recently been shown to aid in the proteolysis of TOP1- and TOP2-DNA complexes (Balakirev et al., 2015; Lopez-Mosqueda et al., 2016; Stingele et al., 2016; Vaz et al., 2016; Maskey et al., 2017). SPRTN (Wss1 in yeast) is a replication-associated metalloprotease implicated in the degradation of a number of protein-DNA adducts (Duxin et al., 2014; Stingele et al., 2014). SPRTN forms a complex with TOP1 and TOP2 in vivo (Vaz et al., 2016), and depletion of SPRTN increases the sensitivity to etoposide and prevents the degradation of TOP2A-DNA complexes (Lopez-Mosqueda et al., 2016). Interestingly, Fielden et al. (2020) show that the degradation of TOP1-DNA complexes by SPRTN requires the ATPase activity of VCP/p97. ATP binding to VCP/p97 induces a major conformational change and subsequent unfolding of the target protein, which then may or may not be degraded. Indeed, VCP/p97 is known to be a key mediator of the ubiquitin-proteasome system in which protein unfolding facilitates the translocation of target proteins into the narrow proteolytic core of the 26S proteasome (Beskow et al., 2009; van den Boom et al., 2016; van den Boom and Meyer, 2018). Similarly, it is suggested that the ATPase activity of VCP/p97 enables the remodeling of TOP1, which facilitates the proteolytic cleavage of TOP1-DNA complexes by SPRTN (Fielden et al., 2020). Furthermore, Wei et al. reported an increase in levels of ubiquitinated TOP2 in yeast after inactivation of temperature-sensitive Cdc 48 (the yeast homolog of VCP/p97).

VCP/p97 is implicated in the extraction of protein complexes from various cellular structures. Although perhaps best known for its role in endoplasmic reticulum–associated degradation, VCP/p97 also enables the removal of protein complexes from chromatin (chromatin-associated degradation). This includes protein complexes that are otherwise tightly bound to DNA, such as stalled RNA polymerase II (Verma et al., 2011; Lafon et al., 2015; He et al., 2017) and the sterically trapped Ku70/80 complex (van den Boom et al., 2016). In the current study, we investigate the potential role of VCP/p97 in the removal of drug-stabilized TOP2-DNA covalent complexes and their subsequent processing to protein-free DSBs. We show that both the degradation of TOP2-DNA complexes and the appearance of etoposide-induced DSBs is reduced upon chemical inhibition or siRNA knockdown of VCP/p97.

Materials and Methods

Cell Culture and Reagents. K562 cells were maintained at 37°C, 5% CO_2, in RPMI medium containing 10% FBS and 5% (v/v) penicillin-streptomycin. Cells are regularly checked for mycoplasma. Etoposide and Benzyl*N*-[(2*S*)-4-methyl-1-[[(2*S*)-4-methyl-1-[[(2*S*)-4-methyl-1-oxopentan-2-yl]amino]-1-oxopentan-2-yl]amino]-1-oxopentan-2-yl]carbamate (MG132) were purchased from Sigma-Aldrich (Dorset, UK). 3-(3-(Cyclopentylthio)-5-(((2-methyl-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)oxy)methyl)-4H-1,2,4-triazol-4-yl)pyridine (NMS-873) was obtained from Sigma-Aldrich (Missouri), VCP/p97 siRNA was purchased from Qiagen (siRNA ID GS7415, containing four siRNAs: SI03019681, SI03019730, S103149657, and S104350444; Maryland).

Trapped in Agarose DNA Immunostaining Assay. TARDIS assays were performed essentially as described previously (Willmore et al., 1998; Cowell and Austin, 2018; Swan et al., 2020). K562 cells were plated at 2×10^5 cells/ml and incubated overnight before drug treatment. TOP2 antibodies used were 4566-TOP2A and 4555-TOP2B (Atwal et al., 2019) or ubiquitin (FK2; Merck Millipore). Nuclear ghosts were counterstained with Hoechst 33258. Hoechst and immunofluorescent images were captured using an Olympus IX-81 epifluorescence microscope (10× objective) fitted with an Orca-AG camera (Hamamatsu) and suitable narrow band filter. Slides were scored automatically as described previously (Atwal et al., 2019) using Volocity 6.3 software (PerkinElmer Inc.). Statistical analysis was performed using GraphPad Prism 8.2 (Perkin Elmer, San Diego, CA). Each TARDIS experiment contained an additional 100 μM etoposide treatment that was used for normalization. Median integrated fluorescence values per nucleus were calculated, and these were converted to a percentage of the median obtained for 100 μM etoposide, and the mean of the medians ± S.D. from replica experiments were calculated. Bar charts represent the mean values, and individual median values are plotted for each replicate as blue-lined white circles.

γH2AX Immunofluorescent Assays. Cell seeding and immunofluorescence were carried out as described previously (Swan et al., 2020). Quantitative immunofluorescence analysis was performed as for the TARDIS assay.

Western Blotting. Western blotting was carried out as described previously (Swan et al., 2020) using mouse monoclonal anti-VCP/p97 antibody (ab11433; Abcam). Blots were developed on film or using a LI-COR C-DiGit Chemiluminescence Western Blot Scanner.

Data Analysis. This is an exploratory study. Statistical analysis was performed using Graph Pad Prism 8.2. The details of tests performed are given in figure legends. For signifying P values, * refers to $P < 0.05$, ** refers to $P < 0.01$, *** refers to $P < 0.001$, and **** refers to $P < 0.0001$. Error bars in bar charts represent S.D. values. Sample sizes (numbers of replicate experiments) were specified in advance of data acquisition based on prior knowledge of the characteristics of the assays involved and anticipating occasional lost or failed samples.

Results

Chemical Inhibition of VCP/p97 Slows Removal of Etoposide-Induced TOP2A and TOP2B Covalent Complexes. A number of ubiquitinated proteins are now known to be removed from DNA by VCP/p97, including stalled RNA polymerase (Verma et al., 2011), Ku70/80 (van den Boom et al., 2016), and the polycomb protein L3MBTL1 (Acs et al., 2011). The potential role of VCP/p97 in the processing of etoposide-induced TOP2-DNA covalent complexes was first investigated using the TARDIS assay. TARDIS is an immunofluorescence-based technique that allows the quantification of TOP2 covalently bound to DNA (TOP2-DNA covalent complexes) on a single-cell basis (Willmore et al., 1998; Cowell and Austin, 2018). The TARDIS assay was performed in the presence or absence of NMS-873, a small-molecule inhibitor of VCP/p97 (Magnaghi et al., 2013), or the proteasome inhibitor MG132 for comparison.

Cells were treated for 2 hours with etoposide alone or etoposide in combination with NMS-873. After 2 hours of continuous drug exposure, etoposide was removed from the culture medium and cells were incubated for a further 2 hours in the

presence of 5 μM NMS-873 or DMSO. Cells were collected at 0, 0.5, 1, and 2 hours after etoposide removal, and levels of TOP2A- and TOP2B-DNA complexes were measured using the TARDIS assay. Where cells were incubated with the proteasome inhibitor MG132, levels of TOP2A-DNA complexes were significantly increased at 0.5, 1, and 2 hours after etoposide removal compared with DMSO-treated cells, and levels of TOP2B-DNA complexes were significantly increased at 0.5 hours, as previously shown (Lee et al., 2016; Swan et al., 2020). Levels of TOP2A- and TOP2B-DNA complexes were also significantly increased 0 hours after etoposide removal when cells were maintained in media containing 5 μM NMS-873 (Fig. 1A) compared with DMSO-treated cells. Levels of TOP2A-DNA complexes remained significantly increased at 0.5 and 1 hours after etoposide removal in the presence of NMS-873, whereas TOP2B-DNA complexes returned to normal levels by 0.5 hours after etoposide removal. This suggests that inhibition of VCP/p97 reduces the removal of drug-stabilized TOP2A- and TOP2B-DNA complexes from chromatin, similar to proteasome inhibition.

When etoposide-treated cells were cotreated with both NMS-873 and MG132, there was no additional increase in levels of TOP2A- or TOP2B-DNA complexes at any time point tested, suggesting that VCP/p97 and the proteasome operate via the same pathway. This correlated with a significant increase in the levels of ubiquitinated TOP2-DNA complexes as measured by ubiquitin TARDIS (Fig. 1B), which may be due to reduced removal of ubiquitinated TOP2 from DNA.

VCP/p97 Is Involved in the Processing of TOP2-DNA Complexes to Protein-Free DSBs. Removal of the TOP2 protein adduct is required for liberation of the TOP2-induced DSB and the subsequent recognition of the break by DNA damage response machinery. In response to a DNA double-strand break, the histone variant H2AX becomes phosphorylated, and this leads to the recruitment of repair factors such as BRCA1 and 53BP1. Inhibition of the proteasome reduces the etoposide-induced phosphorylation of histone H2AX (Lee et al., 2016; Swan et al., 2020), consistent with the requirement for the proteasome in the processing of TOP2-DNA complexes to protein-free DSBs that invoke the DNA damage response.

To determine whether VCP/p97 is involved in the processing of etoposide-induced TOP2-DNA complexes to protein-free DSBs, the γH2AX assay was performed in the presence or absence of NMS-873. Levels of etoposide-induced γH2AX were significantly reduced with NMS-873 treatment after 1 and 2 hours of continuous drug incubation but returned to normal levels at 4 hours (Fig. 1C). This suggests that the appearance of etoposide-induced DSBs is partly VCP/p97-dependent.

Effect of VCP/p97 siRNA on the Processing of TOP2-DNA Covalent Complexes. The role of VCP/p97 was further investigated by siRNA knockdown of VCP/p97. This led to a reduction in the level of p97 protein in knockdown cells to ∼17% of that in control cells (Fig. 2A). VCP/p97 knockdown cells were then treated with etoposide for up to 4 hours, and levels of γH2AX were measured after 0, 1, 2, and 4 hours of continuous etoposide exposure. Consistent with the effect of NSM-873, siRNA knockdown of VCP/p97 reduced the appearance of etoposide-induced DSBs after 1, 2, and 4 hours of etoposide treatment (Fig. 2B), supporting the notion that the removal of etoposide-induced TOP2-DNA complexes, and the subsequent appearance of protein-free DSBs, is partly VCP/p97-dependent.

In addition, the TARDIS assay was used to investigate the effect of VCP/p97 knockdown on levels of TOP2-DNA complexes. VCP/p97 knockdown cells and control cells were treated with etoposide for 2 hours, followed by incubation in etoposide-free medium for a further 2 hours. Levels of TOP2-DNA complexes after etoposide washout were then measured at 0, 0.5, 1, and 2 hours using the TARDIS assay. As previously shown after chemical inhibition of VCP/p97, levels of TOP2B-DNA complexes were significantly increased in VCP/p97 knockdown cells after 2 hours of exposure to etoposide (0 hours after etoposide removal; Fig. 2C) but reduced to control levels after etoposide removal. In contrast, the levels of TOP2A-DNA complexes were not significantly affected by VCP/p97 siRNA knockdown either after 2 hours of continuous etoposide exposure or after etoposide removal. It is unclear why VCP/p97 knockdown affected levels of TOP2B but not TOP2A in the TARDIS assay. Notably, the knockdown of VCP/p97 was incomplete in these cells (Fig. 2A), which may account for the normal processing of TOP2A-DNA complexes.

Discussion

VCP/p97 is a AAA ATPase implicated in the SPRTN-dependent degradation of TOP1-DNA complexes in human and yeast cells. VCP/p97 is known to facilitate the unfolding of target proteins, which may lead to their proteolytic degradation. VCP/p97 promotes the proteasomal degradation of many protein complexes on DNA, including RNAP II and Ku70/80 (Verma et al., 2011; van den Boom et al., 2016). Here, we show that inhibition of VCP/p97 slows the processing of etoposide-induced TOP2-DNA complexes to protein-free DSBs.

We show that inhibition or siRNA knockdown of VCP/p97 reduces etoposide-induced histone H2AX phosphorylation. As VCP/p97 is not required for the appearance of irradiation-induced γH2AX foci (Meerang et al., 2011), this implies a specific role for VCP/p97 in the induction of etoposide-induced DSBs. We propose that VCP/p97 is required for the removal of TOP2 adducts from DNA. For example, VCP/p97 may facilitate the extraction of TOP2, thereby improving the efficiency of TOP2 degradation by the proteasome or SPRTN. In the current study, inhibition of VCP/p97 increased levels of TOP2A- and TOP2B-DNA complexes on chromatin in a manner that was epistatic to the proteasomal pathway. Although it is unclear how VCP/p97 facilitates the removal of covalently bound TOP2 from DNA, it is plausible that unfolding of the covalently attached TOP2 protein may facilitate the translocation of TOP2 into the catalytic core of proteasomes present on chromatin (Verma et al., 2011). Alternatively, VCP/p97 could be involved in the disassembly of TOP2 dimers in which only one TOP2 protomer is covalently attached to the DNA (i.e., etoposide-induced single-stranded DNA breaks). It was estimated that only 2% to 3% of etoposide-induced breaks are DSBs, with the remainder consisting of single-stranded DNA breaks (Bromberg et al., 2003; Muslimovic et al., 2009; Yu et al., 2017).

It is important to note that, like proteasome or ubiquitin-activating enzyme inhibition, inhibition of VCP/p97 is reported to deplete levels of nuclear ubiquitin (Xu et al., 2004; Dantuma et al., 2006; Heidelberger et al., 2018). Therefore, although the VCP/p97-dependent processing of drug-

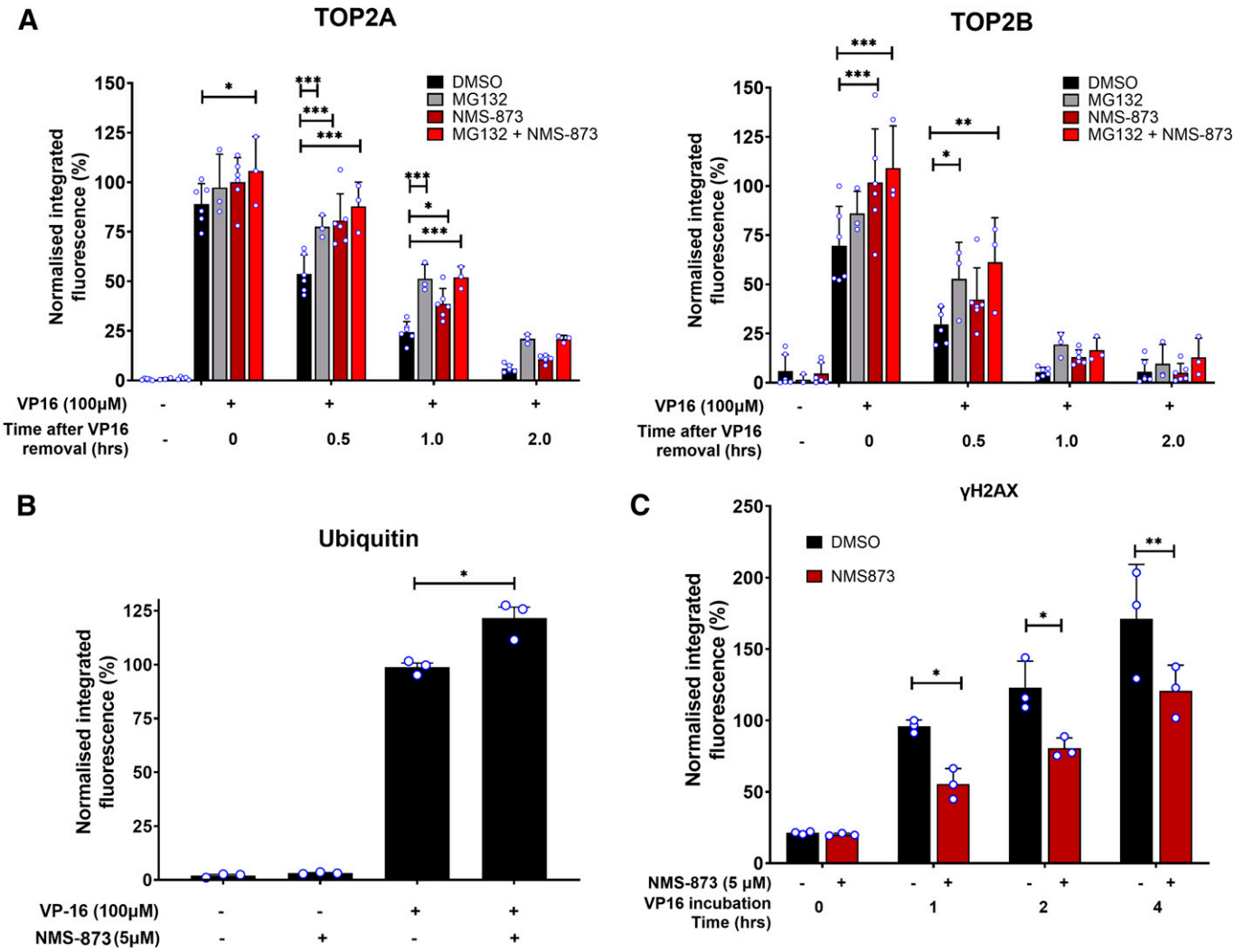

Fig. 1. Effect of the VCP/p97 inhibitor NMS-873 on the processing of etoposide-induced TOP2-DNA complexes to DSBs. (A) The TARDIS reversal assay was used to measure levels of TOP2-DNA complexes in K562 cells treated with 100 μM etoposide (VP-16) alone or in combination with 5 μM NMS-873 (or MG132 for comparison). After 2 hours, etoposide was removed from the culture medium, and cells were incubated for up to 2 hours in etoposide-free medium containing DMSO, MG132, or NMS-873. Cells were collected at 0, 0.5, 1, and 2 hours after etoposide removal. Averages are normalized to a 2-hour 100 μM etoposide control, and statistical analysis was performed by two-way ANOVA and Bonferroni post hoc test. (B) K562 cells were treated with 100 μM etoposide (VP-16) alone or in combination with 5 μM NMS-873 for 2 hours. Cells were processed as per the TARDIS assay and probed with anti-ubiquitin antibody (clone FK2). All values were normalized to a 100 μM VP-16 control, and statistical comparisons were made by unpaired *t* test. (C) K562 cells were treated with 10 μM etoposide (VP-16) alone or in combination with 5 μM NMS-873 for up to 4 hours, and protein-free DSBs were measured by γH2AX assay. Statistical significance was determined by two-way ANOVA and Bonferroni post hoc test. Averages were normalized to a 1-hour 10 μM etoposide positive control. For each graph, bars represent mean values ± S.D.; individual values (medians of fluorescence value per cell from individual replicates) are shown as blue-lined circles.

stabilized TOP2-DNA complexes was epistatic with the proteasomal pathway, we cannot exclude the possibility that the observed proteasome- and VCP/p97- dependent processing of TOP2-DNA complexes is due to inhibition of another ubiquitin-dependent (but proteasome-independent) pathway. For example, ubiquitination regulates the binding of SPRTN to DNA (Stingele et al., 2016), and the proteolysis of TOP2 by SPRTN is increased by the addition of free ubiquitin (but not SUMO-2) in vitro (Lopez-Mosqueda et al., 2016). Therefore, it is plausible that the VCP/p97-dependent pathway described here could be required for the SPRTN-dependent processing of TOP2-DNA complexes. Like SPRTN and the proteasome, VCP/p97 itself can interact directly with ubiquitinated proteins via ubiquitin interacting motifs and ubiquitin adaptor proteins like Ufd1 and Npl4 (Meyer et al., 2000). For instance, VCP/p97 is recruited to stalled replication forks via monoubiquitinated PCNA, where it facilitates translesion synthesis through the extraction and subsequent SPRTN-dependent degradation of translesion polymerase Pol eta (Mosbech et al., 2012). It has also been suggested that SPRTN could serve as a second proteolysis step to further reduce the size of large peptides resulting from the proteasomal degradation of large protein-DNA adducts (Larsen et al., 2019). Further studies are required to investigate the potential involvement of VCP/p97 in the SPRTN-dependent proteolysis of TOP2-DNA complexes.

Extraction of chromatin-associated proteins by VCP/p97 can also lead to protein inactivation without subsequent degradation. For example, the VCP/p97-dependent extraction of ubiquitinated Aurora B from chromatin leads to the inactivation of Aurora B upon exit from mitosis, allowing the decondensation of chromatin

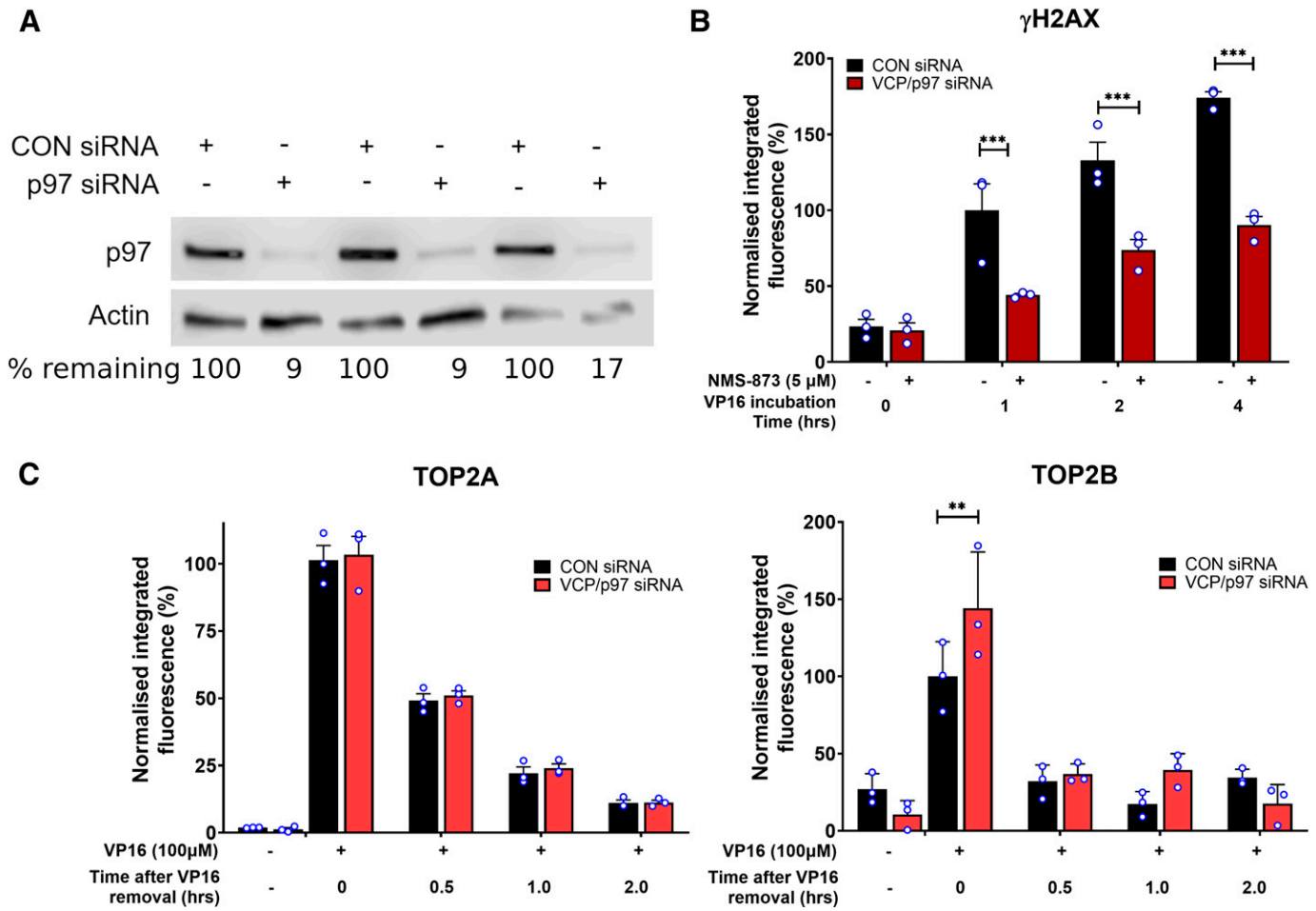

Fig. 2. siRNA knockdown of VCP/p97 and the effect on TOP2-DNA complex processing to DSBs. (A) VCP/p97 siRNA knockdown from triplicate experiments was tested by Western blot. Each replicate represents the cells used in γH2AX and TARDIS experiments shown in (B) and (C). (B) Cells treated with VCP/p97 siRNA or control siRNA (CON) were exposed continuously to 10 μM etoposide for up to 4 hours, and levels of protein-free DSBs were measured using the γH2AX assay. (C) VCP/p97 siRNA or control (CON) siRNA cells were treated with 100 μM etoposide for 2 hours followed by 2 hours incubation in etoposide-free media. The TARDIS assay was used to measure levels of TOP2A- and TOP2B-DNA complexes at 0, 0.5, 1, and 2 hours after etoposide removal. Statistical significance was determined by two-way ANOVA and Bonferroni post hoc test.

and nuclear envelope formation (Ramadan et al., 2007). It is therefore plausible that VCP/p97 could mediate the extraction of TOP2 from chromatin to regulate TOP2-specific functions. VCP/p97 is required for the activation of other factors, which may indirectly affect TOP2 function, such as NRF1. NRF1 activates transcription via a direct interaction with PARP1, which potentially leads to the recruitment of the PARP1/DNA-PK/Ku80/Ku70/TOP2B-containing protein complex to promoters. NRF1 copurifies with the PARP1/DNA-PK/Ku80/Ku70/TOP2B-containing protein complex (Hossain et al., 2009). The data presented herein suggest that TOP2-DNA complexes can be removed by a VCP/p97-dependent mechanism, which may lead to the proteolysis of TOP2 adducts by the proteasome. This is one of many reported pathways that have been shown to facilitate the removal of trapped TOP2-DNA complexes from DNA and subsequent DSB repair. Redundancy between repair pathways increases the timeliness of DNA repair, in which the ability to respond rapidly to DNA damage is crucial for genome stability. Choice of processing pathway may depend on various factors such as the availability or proximity of specific repair proteins. For example, the proteasomal pathway may occur where proteasomes are already present on nearby chromatin, such as those involved in the repair of stalled RNAP II after collision with protein adducts, including TOP2-DNA complexes. Alternatively, pathway choice may be determined by the availability of modifying proteins such as ubiquitinating enzymes. For example, Hu et al. (2020) show that the deubiquitination of VCP/p97 substrates is required for their translocation through the VCP/p97 central pore, and re-ubiquitination may then be required to redirect those proteins to the proteasome for degradation. Pathway choice may also depend on the context or structure of the DNA adduct, as suggested for SPRTN (Li et al., 2019; Reinking et al., 2020).

VCP/p97 has been implicated in the processing of various protein adducts on DNA, including those sterically trapped or tightly associated with chromatin. With increasing understanding of the diverse functions of VCP/p97 in the cell and dysfunctions in many diseases, there are now a number of VCP/p97 inhibitors in development, including CB 5083, which is currently in phase I clinical trials (Zhou et al., 2015; Rycenga et al., 2019). We show that inhibition of VCP/p97 increases levels of etoposide-induced TOP2-DNA complexes and reduces levels of potentially leukemogenic protein-free DSBs. This is a new and important observation that suggests that cotreatment with a VCP/p97 inhibitor may increase the cytotoxicity of TOP2 poisons while also reducing TOP2 poison genotoxicity. Future investigations could determine whether

combinations of VCP/p97 inhibitor and etoposide increase cytotoxicity while reducing translocations.

Authorship Contributions

Participated in research design: Swan, Cowell, Austin.

Conducted experiments: Swan.

Wrote or contributed to the writing of the manuscript: Swan, Cowell, Austin.

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