MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 5, e1796179 (3 pages)
https://doi.org/10.1080/23723556.2020.1796179

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Ser^{784} phosphorylation: a clinically relevant enhancer of VCP function in the DNA damage response

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ABSTRACT

Valosin-containing protein (VCP) is essential for proteostasis during many cellular processes. However, it remains uncertain how its diverse functions are selectively regulated. We recently showed that DNA damage-induced Ser^{784} phosphorylation specifically increases VCP function for the DNA damage response and significantly influences the survival of chemotherapy-treated breast cancer patients.

ARTICLE HISTORY
Received 8 July 2020
Revised 9 July 2020
Accepted 10 July 2020

KEYWORDS
Proteostasis; DNA damage response; chemotherapy; phosphorylation; prognostic biomarker

The evolutionarily conserved DNA damage response (DDR) consists of intimately connected cellular processes which collectively maintain genome stability and determine cell fate. Many DDR events are orchestrated by proteostatic changes influencing protein level and localization in a spatiotemporally choreographed manner. An important aspect of proteostasis during DDR is proteasomal degradation of lysine 48 (K48)-linked polyubiquitinated proteins. This is best understood on chromatin, especially around DNA damage sites, where rapid protein reorganization takes place to enable DNA repair and checkpoint signaling[1]. Studies in the last decade have demonstrated that efficient degradation of many chromatin-associated proteins depends on VCP (Valosin-Containing Protein), an essential and highly abundant AAA+ (ATPases Associated with diverse cellular Activities) ATPase.[2] Functioning as a barrel-shaped homo-hexamer, VCP utilizes energy from ATP hydrolysis within its central ATPase domains to extract and unfold polyubiquitinated proteins from various organelles and cellular structures (e.g., endoplasmic reticulum, endosome, chromatin, ribosome) to facilitate their turnover.[3] Most substrates interact with the N-terminal domain of VCP indirectly through ubiquitin-binding cofactors such as the dimeric NPL4 (Nuclear Protein Localization protein 4) and UFD1 (Ubiquitin recognition Factor in ER-associated Degradation 1) complex (Figure 1a).

Given the involvement of VCP in diverse proteostatic processes, it is reasonable to speculate that mechanisms may exist to selectively modulate its activity in response to specific cellular stresses. Upon DNA damage, VCP undergoes phosphorylation at Ser^{784} in its C-terminal tail (Figure 1a). This was first discovered by Livingstone et al. in 2005 using a cross-reacting phospho-CHK2 antibody[4] and subsequently confirmed in 2007 by unbiased proteomic profiling of phosphatidylinositol 3-kinase-related kinases (PIKK) substrates in response to ionizing radiation and ultraviolet treatments.[5,6] Ser^{784} can be phosphorylated by all three master kinases in the PIKK family (Ataxia-Telangiectasia Mutated, ATM; Ataxia Telangiectasia and Rad3-related, ATR; DNA-dependent Protein Kinase, DNA-PK) in response to diverse genotoxic agents and treatments. These observations suggested early on that Ser^{784} phosphorylation of VCP likely plays an important role in DDR. However, this theory remained untested for 15 years until our recent study.[7]

Similar to Livingstone et al.[4] we serendipitously detected pSer^{784}-VCP in human breast cancer samples using a cross-reacting phospho-PFN1 antibody. Motivated by the chemotherapy-dependent correlation with patient survival, we identified the unknown nuclear antigen of the phospho-PFN1 antibody to be pSer^{784}-VCP. Using a custom-generated pSer^{784}-VCP-specific monoclonal antibody, we confirmed the identity of the DNA damage-induced nuclear antigen of the cross-reacting antibody in cell lines and tissues. Interestingly, Ser^{784} phosphorylation is a relatively late and lingering DDR event and detected both at DNA damage foci and within soluble nucleoplasm. Subsequent characterization of VCP knockdown and rescue cell lines revealed that Ser^{784} phosphorylation is important for DNA damage repair and PIKK-dependent checkpoint signaling, and cells expressing the phospho-resistant VCP(S784A) mutant are hypersensitive to a broad range of genotoxic agents including the poly (ADP-ribose) polymerase (PARP) inhibitors. Thus, our data for the first time uncover the functional significance and clinical relevance of Ser^{784} phosphorylation of VCP.[7]

On a mechanistic level, our study suggests that the functional importance of Ser^{784} phosphorylation for DDR is due, at least partially, to the increased VCP activity in chromatin-associated degradation. We found that VCP knockdown causes significant K48-polyubiquitin buildup on chromatin which can be fully rescued by the phospho-mimetic (S784D, serine^{784} to aspartate) but not phospho-resistant (S784A, serine^{784} to alanine) VCP mutants. In comparison, the rescuing abilities of these two VCP mutants with regard to the buildup of nucleoplasmic K48-polyubiquitin do not differ significantly.[7] These findings are

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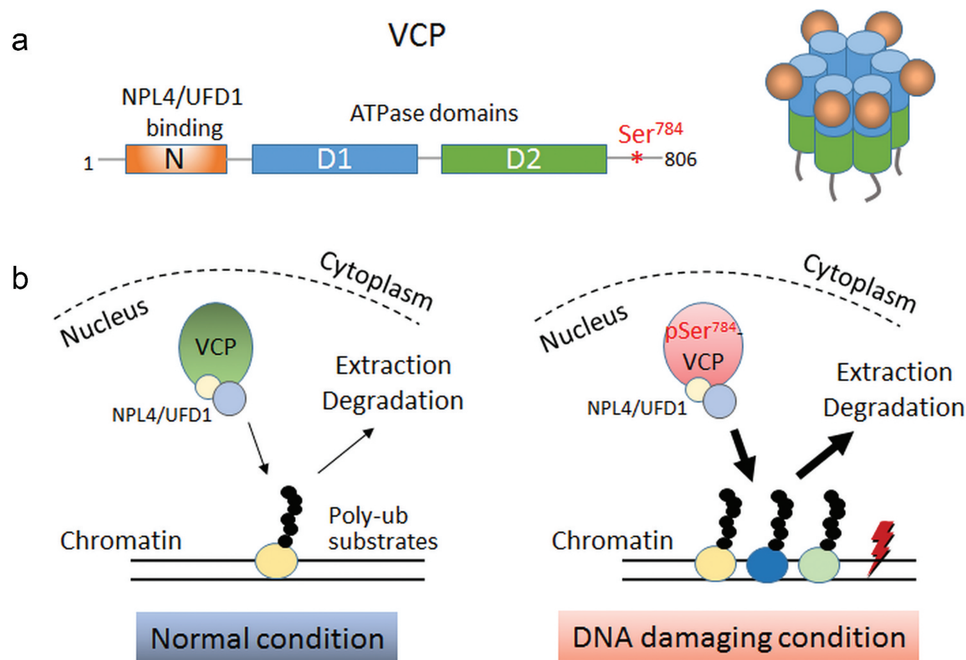

Figure 1. DNA damage-induced Ser^{784} phosphorylation selectively increases VCP (valosin-containing protein) activity for chromatin-associated protein degradation. (a) Schematics showing the domain structure of monomeric VCP (left) and 3D structure of a functional VCP hexamer (right). The N-terminal domain of VCP interacts with the majority of ubiquitin-binding cofactors such as NPL4 (Nuclear Protein Localization protein 4) and UFD1 (Ubiquitin recognition Factor in ER-associated Degradation 1). D1 and D2 are the central ATPase domains. Ser^{784} is located in the structurally disordered C-terminal tail of VCP. (b) Working model depicting the selective increase of nuclear VCP activity by DNA damage-induced Ser^{784} phosphorylation with regard to chromatin-associated protein degradation. In the absence of DNA damage, unphosphorylated VCP extracts its chromatin-associated poly-ubiquitinated substrates at a normal rate. Under DNA-damaging conditions, Ser^{784} phosphorylation turns VCP into a more efficient protein segregase presumably to extract more chromatin-associated substrates that are functionally important for DNA damage response.

consistent with the well-known importance of VCP for chromatin-associated degradation during DDR and suggest that Ser^{784} phosphorylation may be a selective functional accelerator in this regard (Figure 1b). However, the potential effects of Ser^{784} phosphorylation on soluble nuclear proteins cannot be ruled out. Our finding that Ser^{784} phosphorylation increases the level of HIF1α,[7] a soluble nuclear substrate of VCP[8] despite its lack of obvious connection to DDR, raises the interesting possibility that Ser^{784} phosphorylation may differentially affect VCP activity toward protein substrates in a context-dependent fashion. Future research to identify and characterize off-chromatin nuclear substrates of VCP will be necessary to test this theory, and further improve our understanding of the functional involvement of VCP in DDR.

Another interesting finding in our study is that Ser^{784} phosphorylation, within the C-terminal tail of VCP, decreases its interaction with N-domain-binding cofactors NPL4/UFD1 and K48-polyubiquitins,[7] indicative of long-range inter-domain conformational change. Notably, an early report by Klein et al. identified several AKT (AK strain Transforming, also known as protein kinase B) phosphorylation sites in VCP (Ser^{352}, Ser^{746}, and Ser^{748}), and reported that an increase in cellular AKT activity decreases VCP/polyubiquitin interaction.[9] Thus, phosphorylation of VCP may be a general mechanism to release substrates in response to different stimuli. In the case of Ser^{784} phosphorylation, it is tempting to speculate that it triggers the release of chromatin-associated substrates from VCP to speed up their proteasomal degradation and enables faster recycling of VCP for more rounds of protein extraction.

In addition to mechanistic insights, our study also reveals for the first time cancer relevance of pSer^{784}-VCP. By staining three independent tissue microarrays consisting of >3000 breast tumor samples, we observed a significant correlation between high pSer^{784}-VCP levels and poor patient survival in a chemotherapy-dependent fashion, particularly for those in the triple-negative subgroup[5]. Given that baseline pSer^{784}-VCP (trigged by endogenous DNA damage) in pre-treatment samples was used in our analyses, we most likely have underestimated the true prognostic value of pSer^{784}-VCP which will be reexamined by future experiments using drug-induced pSer^{784}-VCP levels. Collectively, our study has two important therapeutic implications. First, low pSer^{784}-VCP levels may be used as a novel predictive biomarker to select patients who can benefit from genotoxic chemotherapies. Second, tumors containing high levels of pSer^{784}-VCP may benefit from chemo-sensitization by PIKK inhibitors many of which are in clinical development.[10]

Disclosure of potential conflicts of interest

Provisional patent has been filed for the monoclonal pSer^{784}-VCP antibody as a predictive biomarker for cancer chemotherapies.

Funding

This work was funded by National Cancer Institute (5R01CA181671); Alvin J. Siteman Cancer Center; Longer Life Foundation;Susan G. Komen Foundation [CCR14300139].

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