Cell Cycle 9:7, 1227-1228; April 1, 2010; © 2010 Landes Bioscience

HERCulean giant orchestrates ubiquitin-mediated signaling on damaged chromosomes

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Key words: DNA damage, ubiquitylation signaling, sumoylation, HERC2, RNF8, RNF168, DNA repair, chromatin modulation

Abbreviations: DDR, DNA damage response; DSB, double strand break; ATM, ataxia telangiectasia mutated

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Genome maintenance requires coordination of DNA damage signaling, repair, cell cycle checkpoints, chromatin remodeling and cell death mechanisms to ensure proper organismal development and disease-free homeostasis.[1] Such demanding orchestration of the multifaceted cellular DNA damage response (DDR) relies on post-translational protein modifications and dynamic recruitment of damage sensors, signaling, chromatin-modifying and DNA repair complexes at the DNA lesions. In response to DNA double strand breaks (DSBs), arguably the most toxic type of DNA lesions, ATM kinase-mediated phosphorylations are critical, in concert with recently identified ubiquitylation-mediated[2-5] and emerging sumoylation-mediated[6-8] signaling (**Fig. 1**).

The DSB-signaling ubiquitylation cascade involves three consecutively recruited E3 ubiquitin ligases, RNF8, RNF168 and BRCA1, respectively. RNF8 and RNF168 operate with the E2 ubiquitin-conjugating enzyme UBC13 to ubiquitylate H2A(X)-type histones, and likely additional substrates, to facilitate local chromatin modulation and recruitment of repair factors such as 53BP1 and BRCA1/BARD1, thereby promoting cell survival in response to genotoxic stress.[2-5] Pathophysiological relevance of this pathway is illustrated by the intimate link of DSB signaling and repair with tumorigenesis, aging, neurodegenerative and immunodeficiency disorders,[1,8,9] including the recent discovery of *RNF168* mutation as the cause of the so-called Riddle syndrome.[5] Most recently, HERC2, a HECT domain-containing protein of unknown function has been identified as a novel component of this complex pathway and important regulator of ubiquitylation signaling at DSB lesions.[10]

HERC2 is remarkable already for its size, since with 4834 amino acid residues and relative molecular mass of 528,000, it is a real giant,[11-13] even compared to large proteins such as ATM or BRCA2 involved in DSB response. The *HERC2* gene encompasses astonishing 93 exons that, apart from the genomically uncharacterized titin, places HERC2 in the company of type VII collagen (118 exons) and perlecan (94 exons), the largest characterized genes whose number of exons may represent the upper limit that a gene can have. Based on homology with HERC1, and the presence of three RCC1-like domains and the C-terminal HECT domain, HERC2 is a putative ubiquitin ligase, possibly involved in vesicular trafficking. Apart from such speculations and some intriguing links of *HERC2* gene mutations with certain diseases (see below), the biological function(s) of this giant protein remained unknown. Whatever its physiological role(s), however, the exceptionally high degree of sequence identity between mouse and human HERC2,[11-13] suggests that even minor changes of the amino acid sequence could functionally disable the protein.

So, what is the new evidence that implicates HERC2 in response to genotoxic stress? The idea of HERC2 as a DDR signaling component was inspired by the physical interaction, enhanced upon ionizing radiation, between HERC2 and the ubiquitin ligase RNF8,[10] (**Fig. 1**). RNF8 homo-dimerizes and through phosphoprotein interactions of the FHA domain forms a bridge between the upstream factor MDC1 and the C-terminal threonine 4827 of HERC2, phosphorylated by ATM and the other major apical DDR kinases ATR and DNA-PK.[10] Importantly, HERC2 itself becomes recruited to sites of DSBs, it is required for proper ubiquitin-mediated signaling by RNF8 and RNF168, and for efficient accrual of key DNA repair factors 53BP1 and BRCA1 at the damaged chromosomes.

Despite contribution of direct catalytic function of HERC2 as ubiquitin ligase cannot be excluded, the data[10] overall indicate that HERC2 represents a novel class of assembly factor that dictates the specific interaction at DSBs of RNF8 with UBC13, rather than with other E2 enzymes, known to interact with RNF8 in other cellular settings. In addition, HERC2 appears to facilitate also the interplay of RNF168 with UBC13, and to stabilize RNF168,[10] (**Fig. 1**). The concept of HERC2 as a specificity factor to promote the RNF8-UBC13 interaction in DSB signaling is supported by the fact that a RNF8-UBC13 fusion protein can rescue the ubiquitin-mediated DSB signaling in the absence of HERC2.[10] Given the multiple roles of E3 ubiquitin ligases, and their promiscuous partnerships with multiple E2s under diverse conditions, it is tempting

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Submitted: 02/03/10; Accepted: 02/03/10
Previously published online: www.landesbioscience.com/journals/cc/article/11411
Comment on: Bekker-Jensen S, et al. Nat Cell Biol 2010; 12:80–6.

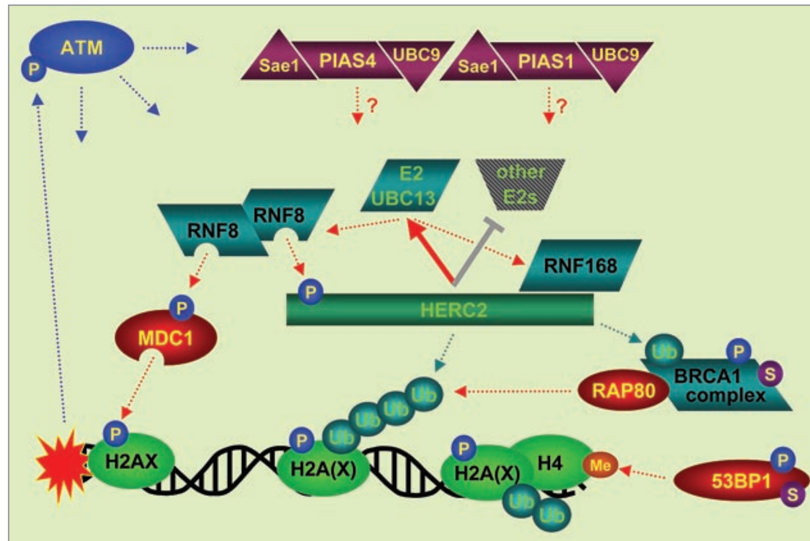

Figure 1. HERC2 coordinates assembly of the ubiquitylation machinery at sites of DNA damage. Activation of ATM kinase induced by DSBs leads to phosphorylation (P) of histone H2AX, MDC1, and other proteins, which initializes protein interactions (red arrows) at DSB-flanking chromatin. Dimeric E3 ubiquitin ligase RNF8 bridges phosphorylated MDC1 and HERC2. By stabilizing another E3 ubiquitin ligase, RNF168, and by selectively promoting the RNF8-Ubc13 interaction, HERC2 enables ubiquitylation of histones H2A(X) (Ub, green arrows) and Ub-dependent accrual of DNA repairs factors such as the BRCA1 complex and 53BP1, the latter recruited to exposed methylations (Me) of histone H4. Speculative role of HERC2 in assembly of the sumoylation (S) machinery: E1 (Sae1), E2 (UBC9) and E3 (PIAS1 and PIAS4) SUMO ligases, is indicated.

to speculate that assembly/specificity factors analogous to HERC2 in DDR, may well be identified for other ubiquitylation-regulated cellular responses. Such legitimacy control over E2-E3 interactions might help, for example, to avoid harmful consequences of excessive DNA recombination if active RNF8-UBC13 complexes formed at undamaged chromatin during DNA replication. Whether HERC2 plays any role as an assembly factor for the sumoylation cascade recently identified to closely interact with the RNF8/RNF168 ubiquitylation pathway[6-8] (**Fig. 1**) remains to be established.

Genetic alterations of *HERC2* have been implicated in neurobehavioral pathologies, the Prader-Willi and Angelman syndromes in humans, and the 'runty, jerky, sterile' phenotype in mice, respectively.[11-13] The exciting task now is to examine whether these features, and the observed enhanced sensitivity to some infections and reported susceptibility to ulcerative colitis in individuals with *HERC2*-defective variants[14] may reflect impaired genome maintenance, as predicted from the role of HERC2 in DDR. In addition to sterility, immunological and neurological phenotypes, all consistent with a genome maintenance function, HERC2 is a plausible candidate for a novel tumor suppressor. The latter possibility is at least consistent with ulcerative colitis as a premalignant condition, and the observed silencing of HERC2 gene promoter in some adenomas. Given the size of HERC2, testing these hypotheses will provide challenges of truly HERCulean proportions.

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Acknowledgements

Authors' work was supported by Grant Agency of the Czech Republic (project 301/08/0353), Academy of Sciences of the Czech Republic (projects AV0Z50520514 and IAA501370902), Czech Ministry of Education (projects MSMT6198959216 and MSMT CZ.1.05/2.1.00/01.0030); Danish Research Council, Danish National Research Foundation, Lundbeck Foundation (R13-A1287), and European Community 7th Framework Programme (projects TRIREME, GENICA and INFLA-CARE).

References

1. Jackson SP, Bartek J. Nature 2009; 461:1071-8.
2. Stewart GS. Cell Cycle 2009; 8:1532-8.
3. Panier S, Durocher D. DNA Repair 2009; 8:436-43.
4. Doil C, et al. Cell 2009; 136:435-46.
5. Stewart GS, et al. Cell 2009; 136:420-34.
6. Galanty Y, et al. Nature 2009; 462:935-41.
7. Morris JR, et al. Nature 2009; 462:886-91.
8. Bartek J, Hodny Z. Cancer Cell 2010; 17:9-11.
9. Bartek J, et al. Cell Cycle 2007; 6:2344-7.
10. Bekker-Jensen S, et al. Nat Cell Biol 2010; 12:80-6.
11. Nicholls RD, Knepper JL. Annu Rev Genomics Hum Genet 2001; 2:153-75.
12. Lehman AL, et al. Proc Natl Acad Sci USA 1998; 95:9436-41.
13. Ji Y, et al. Hum Mol Genet 1999; 8:533-42.
14. Weersma RK, et al. Amer J Gastroenterol 2009; 104:630-8.