Cell Cycle 12:24, 3716–3717; December 15, 2013; © 2013 Landes Bioscience

Stoichiometry of ubiquitin-binding proteins directs DSB repair

Comment on: Helchowski CM, et al. Cell Cycle 2013; 12:3749-58; PMID:24107634; http://dx.doi.org/10.4161/cc.26640

Christopher J Bakkenist[1] and Cyrus Vaziri[2]; [1]Departments of Radiation Oncology and Pharmacology and Chemical Biology; University of Pittsburgh School of Medicine; Hillman Cancer Center; Pittsburgh, PA USA; [2]Department of Pathology; University of North Carolina Chapel Hill; Chapel Hill, NC USA; Email: bakkenistcj@upmc.edu and cyrus_vaziri@med.unc.edu; http://dx.doi.org/10.4161/cc.26963

DNA damage is an ever-present challenge to genome stability and cell viability. Responses to DNA damage assume particular significance in the clinic, where the majority of cancer patients are treated with ionizing radiation (IR) and systemic DNA damaging agents at doses intended to kill malignant cells while sparing normal cells.

Following the induction of DNA double-strand breaks (DSBs), a large number of proteins implicated in DNA damage signaling and repair rapidly associate in distinct nuclear microenvironments that are believed to be focused on the incident lesions.[1] The hierarchical assembly of DNA damage-responsive proteins at DSBs has been well studied through analyses of IR-induced nuclear foci (IRIF), and the kinetics of IRIF assembly and resolution have been presumed to correlate with DNA damage signaling and repair. IRIF assembly is driven by a cascade of reversible posttranslational modifications that include ubiquitylations on DSB-flanking chromatin.[2,3] RNF8, a RING-type E3 ubiquitin ligase, plays a key proximal role in DNA damage sensing and repair and is rapidly associated in IRIF, where it polyubiquitinates type 2A histones. RNF8-mediated histone H2A ubiquitylation generates binding sites for the ubiquitin binding domain (UBD) of RNF168, an unrelated RING-type E3 ubiquitin ligase. RNF168 further increases the ubiquitylation state of DSB-flanking chromatin, facilitating the association of additional repair factors, including 53BP1, and the ubiquitin ligases BRCA1 and RAD18.

Most DSBs are repaired by non-homologous end joining (NHEJ) or homologous recombination (HR), and the choice between these pathways is determined at the level of 5′ end resection, which is believed to commit DSB repair to the HR pathway. 53BP1, a principal component of IRIF and DSB-induced foci, blocks the resection of chromatid breaks during class switch recombination, thereby suppressing HR and promoting NHEJ.[4,5] The stoichiometry of DNA damage signaling and repair proteins associated at IRIF is thus a potentially important and incompletely understood determinant of the selection of DSB repair pathway and, ultimately, DNA damage tolerance. In this issue, Helchowski et al.[6] provide new and cautionary insight into the biological significance of IRIF. These investigators show that RAD18 overexpression dramatically impairs 53BP1 and RAP80-BRCA1 association at IRIF. Remarkably, a NLS-GFP-RAD18 fragment comprising minimally the UBD and a short juxtaposed "LRM" targeting sequence[7] is sufficient to inhibit 53BP1 association at IRIF and concomitantly increase HR. This disruption of 53BP1 association at IRIF appears to be caused by the selective and competitive binding of overexpressed RAD18 UBD-LRM to as-yet-unidentified ubiquitylated proteins on DSB-flanking chromatin. The impairment of 53BP1 and RAP80-BRCA1 association at IRIF in RAD18-overexpressing cells did not affect DNA damage signaling, overall rates of DSB repair, or radiosensitivity. The long-term consequences were confined to the redirection of DSB repair to favor the HR mechanism.

The results of Helchowski[6] extend previous studies showing that ectopic expression of full-length RNF169 (an RNF168 paralog that recognizes polyubiquitin structures but does not itself contribute to DSB-induced chromatin ubiquitylation) impaired 53BP1 and RAP80-BRCA1 association but not H2AX phosphorylation at IRIF.[7,8] The impaired 53BP1 and RAP80-BRCA1 association at IRIF in cells overexpressing RNF169 required an intact UBD (but not E3 ubiquitin ligase activity) and caused reduced NHEJ efficiency and increased HR efficiency without impacting radiosensitivity. Together, these studies demonstrate that overexpressed UBDs can change the stoichiometry of proteins associated at IRIF and thereby redirect DSB repair from NHEJ to HR.

These data are extremely provocative: while IRIF have been used as correlatives for many studies of DNA damage signaling and repair, to our knowledge, these recent studies are the first to directly and deliberately manipulate the stoichiometry of IRIF components without knockdown or knockout and elicit robust cellular phenotypes.

The study by Helchowski[6] is also a cautionary tale that illustrates how commonly used protein probes of DNA damage signaling and repair (such as GFP-tagged DNA repair proteins) can perturb the very systems they are designed to study, leading to artifacts and misinterpretation. However, the practice of medical oncology requires that malignant cells be selectively perturbed. The demonstration that the selection of DNA repair pathway can be manipulated with a relatively short polypeptide suggests a clinical opportunity to inhibit NHEJ in malignant cells with acquired mutations in HR genes, with the expectation of achieving selective malignant cell killing. (**Fig. 1**)

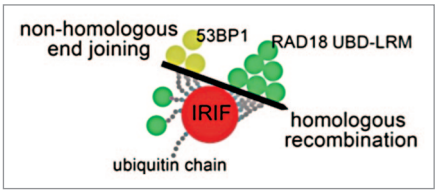

Figure 1. The stoichiometry of the ubiquitin-binding proteins RAD18 and RNF169 associated at ionizing radiation-induced foci impacts 53BP1 association and the selection of DSB repair mechanism.

References

1. Maser RS, et al. Mol Cell Biol 1997; 17:6087-96; PMID:9315668
2. Lukas J, et al. Nat Cell Biol 2011; 13:1161-9; PMID:21968989; http://dx.doi.org/10.1038/ncb2344
3. Jackson SP, et al. Mol Cell 2013; 49:795-807; PMID:23416108; http://dx.doi.org/10.1016/j.molcel.2013.01.017
4. Bunting SF, et al. Cell 2010; 141:243-54; PMID:20362325; http://dx.doi.org/10.1016/j.cell.2010.03.012
5. Bothmer A, et al. J Exp Med 2010; 207:855-65; PMID:20368578; http://dx.doi.org/10.1084/jem.20100244
6. Helchowski CM, et al. Cell Cycle 2013; 12:3749-58; PMID:24107634
7. Panier S, et al. Mol Cell 2012; 47:383-95; PMID:22742833; http://dx.doi.org/10.1016/j.molcel.2012.05.045
8. Poulsen M, et al. J Cell Biol 2012; 197:189-99; PMID:22492721; http://dx.doi.org/10.1083/jcb.201109100