Deciphering the DNA damage histone code

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The histone H2A variant H2AX is important in the cellular response to DNA double strand breaks (DSBs) and is required to maintain genomic integrity and suppress malignant transformation.[1] H2AX is phosphorylated on serine (S) 139 to form γ-H2AX in chromatin around DNA breakage sites.[1] This post-translational modification (PTM) of H2AX creates binding sites for MDC1 and other proteins to facilitate their retention around DSBs, which appears critical for DNA repair and checkpoint activation.[1] Additional PTMs of H2AX, such as lysine (K) 5 acetylation (2), K119 ubiquitylation,[3,4] and tyrosine (Y) 142 phosphorylation,[5,6] have been implicated in modulation of the DNA damage response (DDR). Despite many studies, the mechanisms by which γ-H2AX and other PTMs of H2AX function in the cellular response to and repair of DSBs.

In a previous issue of *Cell Cycle*, Xie et al. report the identification of novel H2AX PTMs and evaluate the impact of these and previously identified PTMs upon aspects of the DNA damage response.[7] Mass spectrometry of human H2AX isolated from mouse embryonic stem (ES) cells that were either untreated or exposed to 50 Gy of ionizing radiation (IR) revealed constitutive threonine (T) 120 and S121 phosphorylation, and IR-induced K35 acetylation and T110 phosphorylation.[7] Experiments with $H2ax^{-/-}$ ES cells expressing wild-type or mutant H2AX cDNAs suggest that T101 and S139 phosphorylation and K36 acetylation each are regulated independently and critical for IR resistance.[7] However, only S139 phosphorylation was required for the retention of MDC1 and 53BP1 in chromatin following DNA damage, as well as for homologous recombination (HR) mediated repair of I-Sce1 DSBs induced within an integrated reporter.[7] Radiation induced K5 acetylation and K119 ubiquitylation were observed; however Y142 phosphorylation was not detected.[7] Xie et al. also observed no difference between the abilities of wild-type and Y142F H2AX to support IR-induced MDC1 focus formation and promote HR,[7] despite prior studies showing impairment of MDC1 binding and modulation of apoptosis/survival decisions.[5,6] Moreover, although H2AX serves critical functions in nonhomologous end-joining (NHEJ) during antigen receptor gene rearrangements,[8] Xie et al. were unable to demonstrate a role for H2AX in NHEJ-mediated repair of I-Sce1 DSBs induced within an integrated reporter.[7]

The work of Xie et al.[7] provides important contributions to our understanding of how H2AX regulates the DDR and DSB repair. Their findings suggest that T101 phosphorylation and K36 acetylation of H2AX each facilitates cellular IR resistance through mechanisms distinct from MDC1-dependent H2AX functions in HR-mediated DSB repair. These PTMs may create unique binding sites for DSB response/repair proteins, influence the ability of MDC1 or other factors to bind γ-H2AX, and/or generate charged amino acids that alter local chromatin structure. The differences in the observed contributions of H2AX Y142 phosphorylation between the experiments of Xie et al.[7] and others[5,6] may reflect cell/tissue specific modulation of the DDR and/or the relative abundance of H2AX in chromatin in relation to histone H2A and other H2A variants. Considering that H2AX distribution throughout the genome varies and can be influenced by transcription, cell cycle phase, and differentiation,[9] the inability of Xie et al. to assay H2AX functions in NHEJ may be a consequence of the location of reporter integration. In addition, since a 50% reduction in H2AX expression leads to a ten fold reduction in DSB-induced γ-H2AX densities around DSBs,[9] modest differences in the levels of ectopically expressed wild-type and mutant H2AX proteins within and among studies may have influenced conclusions. Other less obvious factors also could account for discrepancies between this and other published analyses of H2AX PTMs.

The new report by Xie et al.[7] reveal that the H2AX-dependent cellular response to DSBs is far more complicated and less well understood than previously appreciated. Their results indicate that many future studies will be required to elucidate how multiple H2AX PTMs interact with each other, as well as with PTMs of other nearby histones, to coordinate the DDR. Such experiments should include gene-targeted mutation of H2ax so that physiologic regulation of H2AX expression by the H2ax promoter and 3'UTR is maintained. Future studies also will need to consider the cellular, chromosomal, and genomic contexts of DSBs, as well as the manner by which these DNA breaks are induced.

References

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